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TITLE: The Power of Comparative Physiology: Evolution, Integration and Applied

PRINCIPAL INVESTIGATOR: Martin Frank, Ph.D.

CONTRACTING ORGANIZATION: American Physiological Society
Bethesda, Maryland 20814-3991

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

This application provided support for an American Physiological Society conference entitled "The Power Of Comparative Physiology: Evolution, Integration And Applied", held August 24-28, 2002 in San Diego, CA. The fundamental goal of the conference was to bring together comparative biologists who utilize a diversity of approaches including molecular, cellular, organ, and organismal physiology/biochemistry, functional morphology, biomechanics and biophysics, ecology and evolutionary biology to understand physiological processes and traits. The meeting highlighted the accomplishments that have occurred since the last large comparative meeting and more importantly, provided a forum to showcase new directions and approaches. Many of these directions and approaches were relevant to the mission of USAMRMC and included Cellular And Molecular Responses To Depressed Metabolism And Low Temperature, Mitochondrial Responses To Environmental Challenges and Opportunity, Polar Molecular Biology, Integration Of Motor Function: Mechanisms That Reduce Energy Cost Or Enhance Performance, Diving Physiology, The Design Of Artificial Muscle And Robots, Physiological And Genetic Responses To Environmental Stress, and Acclimatization To Hypoxia.

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Introduction

This application provided support for an American Physiological Society conference entitled "The Power Of Comparative Physiology: Evolution, Integration And Applied", scheduled for August 24-28, 2002 in San Diego, California. The fundamental goal of the conference was to bring together comparative biologists who utilize a diversity of approaches including molecular, cellular, organ, and organismal physiology/biochemistry, functional morphology, biomechanics and biophysics, ecology and evolutionary biology to understand physiological processes and traits. The meeting highlighted the accomplishments that have occurred since the last large comparative meeting and more importantly, provided a forum to showcase new directions and approaches.

The specific aims of the proposal included:

1. To convene an internationally recognized interdisciplinary group of investigators to explore the rapid changes that comparative physiology has undergone as a result of the incorporation of a variety of new tools and technologies into the discipline;
2. To promote widespread participation of young scientists with an emphasis on women and under-represented minorities, through a travel award program;
3. To interest new investigators and students in pursuing research using comparative approaches to understand physiological processes and traits.

Body

Since it's founding in 1887 by five noted scientists, the American Physiological Society (APS) has been devoted to fostering basic and applied scientific information. Through these activities, the Society has grown to approximately 10,600 members and continues to make major contributions to the progress of science and the advancement of biological and biomedical knowledge.

The Society sponsors one general scientific meeting in the spring that is devoted to the dissemination of newly acquired scientific information. The Spring Experimental Biology Meeting is held with other societies that are members of the Federation of American Societies for Experimental Biology (FASEB). The April 2001 meeting attracted over 11,000 scientists and exhibitors with physiological scientists contributing more than 2,600 papers in 175 sessions. In addition, there were 53 APS-sponsored symposia involving some 250 speakers and 16 invited lecturers.

The Society also regularly sponsors up to two other meetings, called the APS Conferences, each year. In 2002, APS sponsored two conferences. From February 20-23, 2002, APS held a conference in San Francisco entitled "Physiological Genomics of Cardiovascular Disease: From Technology to Physiology" and in August the Society sponsored this conference entitled "The Power Of Comparative Physiology: Evolution, Integration And Applied."

The Society is also a major publisher of journals and books on physiology. The journals of the Society include the *American Journal of Physiology*, the *Journal of Applied Physiology*, the *Journal of Neurophysiology*, and *Physiological Reviews*. All of the journals are currently available online with the assistance of HighWire Press. The Society, in conjunction with the International Union of Physiological Sciences, also publishes a trends-type journal, entitled *News in Physiological Sciences* (*NIPS*). The Society recently launched a new journal, *Physiological Genomics*, which appears online prior to print.

In planning for this conference, the organizers were drawing upon the American Physiological Society's strong commitment to comparative physiology. In the Fall of 1990 in Orlando, Florida, the APS sponsored a meeting, largely organized by the Comparative Section centered on the theme *"In Search of Physiological Principles - The Use of Animal Diversity and Novel Technology."* Guest societies at this meeting included Society for Integrative and Comparative Biology (SICB, formerly known as American Society of Zoologists), Society for Experimental Biology (SEB), Canadian Society of Zoologists (CSZ) and the Comparative Respiratory Society. Based on the success of the Orlando meeting, it was clear that the APS provided an important service to the widely dispersed community of comparative physiologists and it was the hope of the comparative section that such a meeting would be sponsored on a regular basis (every four years).

In the Fall of 1994 in San Diego, California, the APS continued its sponsorship of a large comparative meeting, this time based on the theme *"Regulation, Integration, Adaptation: A Species Approach."* Guest societies at this meeting included SICB, SEB, CSZ and the German Society of Zoologists. The combined success of Orlando and San Diego indicated that the APS had assumed the leadership role in providing a home for and defining the future of comparative physiology.

The scheduling of this conference by APS reasserted APS' leadership role in defining comparative physiology as we enter the 21st century.

Comparative biology, through its diversity of investigative approaches, is an extremely powerful paradigm for studying physiology. It is the only approach to physiological research that, by its very nature, often seeks to understand physiological processes and traits over several different time domains. These domains range from investigating the proximal details of physiologic mechanisms to investigations that aim to understand and gain insights into ultimate causation, i.e. the evolutionary or adaptive significance of a physiological process or trait. Consequently, comparative biology utilizes a diversity of approaches including molecular, cellular, organ, and organismal physiology/biochemistry, functional morphology, biomechanics and biophysics, ecology and evolutionary biology. Within the past 10 years, comparative physiology has undergone rapid changes resulting from the incorporation of a variety of new tools and technologies. These include the many new and powerful tools of molecular biology, the use of remote sensing and physiological monitoring technologies, advances in microelectronics and

computers and the rigorous application of evolutionary theory. Singularly and in combination these approaches have resulted in a rethinking of many long held concepts and constructs in comparative physiology as well as the development of new syntheses.

The meeting highlighted the accomplishments that have occurred since the last large comparative meeting and more importantly, provided a forum to showcase new directions and approaches.

Guest societies included the Society for Integrative and Comparative Biology (formerly American Society of Zoologists), Society for Experimental Biology (SEB, UK), Canadian Society of Zoologists (CSZ, Canada), German Society of Zoologists (GSZ, Germany) and the Australian Society of Comparative Biochemistry and Physiology.

ORGANIZING COMMITTEE

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The conference was held over four-days, starting on Saturday evening and ending on Wednesday evening. The full schedule of sessions, including a listing of lectures, symposia, and poster presentations is included in the form of an appendix.

Reportable Outcomes and Conclusions

The APS returned to the Town & Country Resort and Conference Center in San Diego for its third APS Intersociety meeting focusing on comparative physiology entitled *"The Power of Comparative Physiology: Evolution, Integration, and Application"*. The meeting, held August 24-28, included six guest societies: the Society for Integrative and Comparative Biology (SICB), Society for Experimental Biology (SEB), Canadian Society of Zoologists (CSZ), German Society of Zoologists (GSZ), the European Society of Comparative Physiology and Biochemistry (ESCPB), and the Australian & New Zealand Society of Comparative Biochemistry and Physiology (ANZSCBP).

As Chair of the Organizing Committee, **James Hicks** (University of California, Irvine) created a 4-day meeting that incorporated five plenary lectures, 19 symposia and 21 poster sessions. Presenters covered topics as wide-ranging as: regulation, renal function, genetics, hypoxia, integration, motor function, metabolism, neurophysiology, microarrays, homeostasis, muscle physiology, environmental physiology, diving, physiological evolution, host-parasite interactions, artificial muscles and robots, hypoxia, developmental physiology and plasticity. The organisms studied were also quite diverse, ranging from invertebrates (like the fruit fly and cabbage looper) on up the evolutionary scale to frogs, fish, reptiles, birds and mammals.

The meeting attracted 554 registrants—31% (177) were female and 35% (196) represented young scientists; including 54 postdoctoral and 142 student registrants. 22% (123) were members of APS or one of the 6 guest societies; 10% (58) were nonmembers; 28% (156) were invited speakers or organizers. Of the total registrants, 19% (105) worked outside The Americas, 3% (18) in US government labs and 1% (8) in industry.

Registration Type	Total	Percent
Member (APS or Guest Society)	123	31%
Retired Member	5	>1%
Nonmember	58	10%
Postdoctoral	54	9%
Student	142	25%
Invited Speaker or Organizer	156	28%
Nonscientist Guest of Registrant	15	2%
Undergraduate	1	>1%
	554	

The meeting agenda was arranged to feature a morning plenary lecture, followed by 4-5 concurrent symposia, which were then followed by unopposed poster sessions. The poster sessions were designed to maximize interaction among participants and featured beer, wine and light snacks. These *Poster Socials*, were sponsored each day by one of the top four journals publishing comparative research: Sunday was sponsored by *Comparative Biochemistry and Physiology*, Monday by *Physiological and Biochemical Zoology*, Tuesday by the *Journal of Experimental Biology*, and Wednesday was sponsored by the *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology.*

In addition to the scientific sessions, several social activities were offered to attendees. The Opening Reception on Saturday evening was an informal reception held poolside at the famed Town & Country's Tiki Hut; Monday featured a special-purchase dinner at the Scripps Institute of Oceanography's Birch Aquarium, and; the last night, Wednesday, featured the Scholander Award Banquet and Lecture. The Scholander Award lecture was presented by **Barbara Block** (Hopkins Marine Station, Stanford University) entitled *"The Fire Inside: Saving Atlantic Bluefin Tuna"*.

Three awards for best abstract presentation by a graduate student were presented during the Scholander Banquet. Recipients and their respective awards were: **The Society of Integrative and Comparative Biology Young Investigator Award** presented to **John Zehmer**, Arizona State University for his presentation entitled *"Plasma membrane rafts of rainbow trout are subject to thermal acclimation"*; the **Society for Experimental Biology Young Investigator Award** presented to **Scott D. Kirkton**, Arizona State University for his presentation entitled *"Oxygen delivery problems may reduce jumping performance in larger locusts"*, and; the **Scholander Award**, sponsored by the **APS Comparative Physiology Section,** presented to **Todd E. Gillis**, Simon Fraser University for his presentation entitled *"Sequence mutations in teleost cardiac troponin C that are permissive of cardiac function at low temperatures"*. Each awardee received a cash prize and a one year complimentary subscription to the journal published by the sponsoring society.

From the outset, the inclusion young investigator participation was very important to the organizing committee who therefore designed a travel award program for graduate students and postdoctoral fellows. 55 travel grants were provided totaling over $30,000. Travel awardees that met application guidelines received partial travel reimbursement, complimentary registration and a ticket to the Birch Aquarium dinner event.

There were eleven recipients of the APS Porter Physiology Development Committee's Minority Travel Fellowship Award, supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institute of General Medical Sciences (NIGMS). The fellowship provides reimbursement of travel expenses and each recipient is matched with an APS member attending the meeting who offers guidance and makes introductions to other scientists. Recipients were: **Lee A. Aggison, Jr.**, Stillman College; **Thomas F. Gallegos**, New

Mexico State University; **Vallie M. Holloway**, Loyola University Medical Center; **Rafael Alejandro Leos**, New Mexico State University; **Marcy K. Lowenstein**, Florida International University; **Rudy M. Ortiz**, University of California, Santa Cruz; **Elizabeth S. Quintana**, New Mexico State University; **Luciana Oliveira Santos**, University of Utah; **LaTonia Marie Stiner**, Wright State University; **Vanessa I. Toney**, Brown University; and **Ruth A. Washington**, Stillman College.

In all, 291 abstracts were programmed into poster sessions. Of these 36% (107) were represented by female presenters and 23% (68) were from countries outside The Americas. Researchers working in industry comprised 1% (4); those from US government labs also represented 1% (4) of the total submissions.

The Society wishes to thank the members of the Intersociety Meeting Organizing Committee: **James Hicks**, Chair (University of California, Irvine), **Albert Bennett** (University of California, Irvine), **Barbara Block** (Hopkins Marine Station, Stanford University), **Steven C. Hand** (Louisiana State University, Baton Rouge), **Donald C. Jackson** (Brown University) and, **Stephen C. Wood** (VA Medical Center, Nashville).

The Society gratefully acknowledges the financial support provided through unrestricted educational grants received from: National Science Foundation, US Army Medical Research Acquisition Activity, US Department of the Navy, Office of Naval Research, and the Thomas Maren Foundation.

References

The Power of Comparative Physiology: Evolution, Integration and Application. *The Physiologist* Volume 45, Number 4, August 2002, Pages 249 – 380.

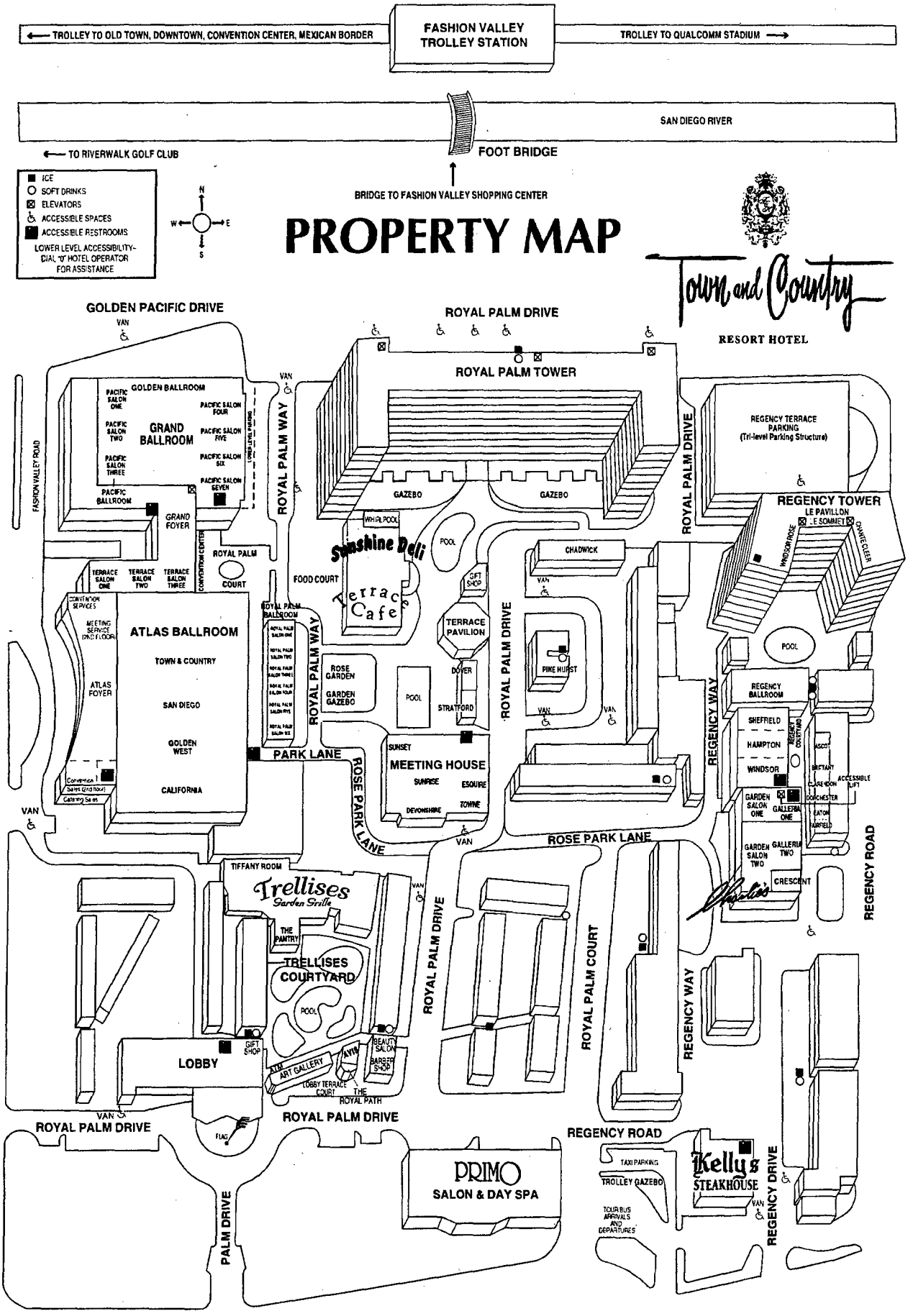
Appendices

1. The Power of Comparative Physiology: Evolution, Integration and Application
The Physiologist Volume 45, Number 4, August 2002.
Pages 249 – 380

An APS Intersociety Meeting

The Power of Comparative Physiology: Evolution, Integration, *and Application*

San Diego, California
August 24-28, 2002


TROLLEY TO OLD TOWN, DOWNTOWN, CONVENTION CENTER, MEXICAN BORDER
FASHION VALLEY TROLLEY STATION
TROLLEY TO QUALCOMM STADIUM
SAN DIEGO RIVER
TO RIVERWALK GOLF CLUB
FOOT BRIDGE
BRIDGE TO FASHION VALLEY SHOPPING CENTER
ICE
SOFT DRINKS
ELEVATORS
ACCESSIBLE SPACES
ACCESSIBLE RESTROOMS
LOWER LEVEL ACCESSIBILITY- DIAL "0" HOTEL OPERATOR FOR ASSISTANCE
PROPERTY MAP
Town and Country
RESORT HOTEL
GOLDEN PACIFIC DRIVE
ROYAL PALM DRIVE
ROYAL PALM TOWER
GOLDEN BALLROOM
GRAND BALLROOM
PACIFIC SALON ONE
PACIFIC SALON TWO
PACIFIC SALON THREE
PACIFIC SALON FOUR
PACIFIC SALON FIVE
PACIFIC SALON SIX
PACIFIC SALON SEVEN
PACIFIC BALLROOM
GRAND FOYER
FASHION VALLEY ROAD
ROYAL PALM WAY
LOWER LEVEL PARKING
CONVENTION CENTER
ROYAL PALM COURT
TERRACE SALON ONE
TERRACE SALON TWO
TERRACE SALON THREE
CONVENTION SERVICES
MEETING SERVICES (2ND FLOOR)
ATLAS BALLROOM
TOWN & COUNTRY
SAN DIEGO
GOLDEN WEST
CALIFORNIA
ATLAS FOYER
Convention Sales (2nd floor)
Catering Sales
ROYAL PALM BALLROOM
ROYAL PALM ONE
ROYAL PALM TWO
ROYAL PALM THREE
ROYAL PALM FOUR
ROYAL PALM FIVE
ROYAL PALM SIX
WHIRLPOOL
POOL
Sunshine Deli
FOOD COURT
Terrace Cafe
GAZEBO
GAZEBO
ROSE GARDEN
GARDEN GAZEBO
POOL
GIFT SHOP
TERRACE PAVILION
DOVER
STRATFORD
SUNSET
MEETING HOUSE
SUNRISE
ESQUIRE
DEVONSHIRE
TOWNE
CHADWICK
PINE HURST
PARK LANE
ROSE PARK LANE
ROSE PARK LANE
TIFFANY ROOM
Trellises
Garden Grille
THE PANTRY
TRELLISES COURTYARD
POOL
LOBBY
GIFT SHOP
ATM
ART GALLERY
AVIS
BEAUTY SALON
BARBER SHOP
LOBBY TERRACE COURT
THE ROYAL PATH
FLAG
ROYAL PALM DRIVE
ROYAL PALM DRIVE
ROYAL PALM DRIVE
ROYAL PALM COURT
REGENCY WAY
REGENCY WAY
REGENCY TERRACE PARKING (Tri-level Parking Structure)
REGENCY TOWER
LE PAVILLON
LE SOMMET
WINDSOR ROSE
CHANTECLEER
POOL
REGENCY BALLROOM
SHEFFIELD
HAMPTON
WINDSOR
REGENCY COURTYARD
ASCOT
BRITANY
CLARENDON
ACCESSIBLE LIFT
DORCHESTER
EATON
MARFIELD
GARDEN SALON ONE
GALLERIA ONE
GARDEN SALON TWO
GALLERIA TWO
CRESCENT
REGENCY ROAD
REGENCY ROAD
REGENCY DRIVE
PRIMO
SALON & DAY SPA
TAXI PARKING
TROLLEY GAZEBO
TOUR BUS ARRIVALS AND DEPARTURES
Kelly's
STEAKHOUSE
PALM DRIVE
VAN

The Power of Comparative Physiology: Evolution, Integration and Application

August 24-28, 2002—Town & Country Resort & Convention Center, San Diego, CA

TIME	SUNDAY, AUGUST 25	MONDAY, AUGUST 26	TUESDAY, AUGUST 27	WEDNESDAY, AUGUST 28
8:00-9:00 AM	**1.0** Plenary Lecture **G. Somero**	**13.0** Plenary Lecture **A. Cossins**	**25.0** Plenary Lecture **J.B. West**	**40.0** Plenary Lecture **R.B. Huey**
9:00 AM-1:00 PM	**2.0** The Power of Integration **G. Lauder**, Chair	**14.0** DNA Microarrays: Applications to Comparative Physiology **A. Gracey**, Chair	**26.0** Phylogenetic Approaches to Understanding Physiological Evolution **T. Garland, Jr.**, Chair	**41.0** Developmental Physiology: Plasticity and Constraints **D.T. Manahan/S.C. Hand**, Cochairs
	3.0 Polar Molecular Biology: Proteins and Enzymes at their Lower Temperature Extremes **D. Petzel**, Chair	**15.0** Homeostasis of Essential yet Toxic Metals **M. Grosell/N. Bury**, Cochairs	**27.0** The Comparative Physiology of Carbonic Anhydrase **K. Gilmour/S.F. Perry**, Cochairs	**42.0** Physiological and Genctic Responses to Environmental Stress **G. Hofmann/M. Feder**, Cochairs
	4.0 Integration of Motor Function: Mechanisms that Reduce Energy Cost and/or Enhance Performance **A.A. Biewener**, Chair	**16.0** Linking Muscle Genes to Structure and Physiology, a Comparative Approach **A. El Haj/ I. Johnston**, Cochairs	**28.0** The Influence of Comparative Physiology on Engineering: Neuro-muscular Biological Inspiration toward the Design of Artificial Muscle and Robots **R. Full, Jr.**, Chair	**43.0** Acclimatization to Hypoxia: Supply versus Demand Strategies **F.L. Powell**, Chair
	5.0 Cellular and Molecular Responses to Depressed Metabolism and Low Temperature **H. Carey/G. Florant**, Cochairs	**17.0** Mitochondrial Responses to Environmental and Physiological Challenge **C. Moyes**, Chair	**29.0** Relaxed Homeothermy **P. Frappell/P. Butler**, Cochairs	**44.0** Regulation of Vertebrate Renal Function: a Comparative Approach **W.H. Dantzler/E.J. Braun**, Cochairs
	6.0 Neuropeptides Integrating Physiological Processes in Invertebrates: an Evolutionary and Comparative Approach **K.H. Hoffman**, Chair	**18.0** Diving: Where have We Been, Where are We Going? **M.A. Castellini**, Chair **D.R. Jones/P.J. Butler**, Cochairs	**30.0** Host-parasite Interactions: a Comparative Approach **G. Filk**, Chair	
2:30-5:30 PM	**Poster Session & Social** Sponsored by the: ***Comparative Biochemistry and Physiology Journal***	**Poster Session & Social** Sponsored by the: ***Physiological and Biochemical Zoology Journal***	**Poster Session & Social** Sponsored by the: ***Journal of Experimental Biology***	**Poster Session & Social** Sponsored by the: ***American Journal of Physiology: Regulatory, Integrative and Comparative Physiology***
Evening Events	**Evening Free**	**Birch Aquarium Social** Scripps Institute of Oceanography **6:00-10:00 PM**	**Evening Free**	**49.0** Scholander Award Banquet featuring **Barbara Block** **6:00-10:00 PM**

2002 APS Intersociety Meeting
The Power of Comparative Physiology:
Evolution, Integration, and Application

APS Council

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James W. Hicks, Ph.D. (Chair)

Albert Bennett, Ph.D.	Steven C. Hand, Ph.D.	Stephen C. Wood, Ph.D.
Barbara Block, Ph.D.	Donald C. Jackson, Ph.D.	

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The Intersociety Meeting Organizing Committee and The American Physiological Society gratefully acknowledge financial support provided through unrestricted educational grants from:

National Science Foundation
U.S. Army Medical Research Acquisition Activity
U.S. Department of the Navy, Office of Naval Research
Thomas Maren Foundation
American Journal of Physiology: Regulatory, Integrative and Comparative Physiology
Comparative Biochemistry and Physiology Journal
Journal of Experimental Biology
Physiological and Biochemical Zoology Journal

GENERAL INFORMATION

Location:
Town & Country Resort and Convention Center, 500 Hotel Circle North, San Diego, California 92108, telephone: 800-772-8527 or 619-291-7131, Fax: 619-291-3584.

APS Registration Desk:
Town & Country Resort and Convention Center Atlas Foyer.

On Site Registration Hours:

Saturday, August 24	2:00 PM – 9:00 PM
Sunday, August 25	7:00 AM – 4:00 PM
Monday, August 26	7:30 AM – 4:00 PM
Tuesday, August 27	8:00 AM – 3:00 PM
Wednesday, August 28	8:00 AM – 3:00 PM

On Site Registration Fees:
The registration includes entry into all scientific sessions and exhibits, the Opening Reception, Scholander Banquet, and daily Poster Session Socials.

Type	Fee
Member	$310
Retired APS Member	$200
Nonmember	$360
Postdoctoral	$250
Student	$200
Spouse	$75

Payment Information:
Registrants may pay by check, money order or credit card (VISA, Master Card, or American Express). Checks and money orders must be payable to The American Physiological Society and drawn on a United States bank. Your name and full address should be typed or printed clearly on your check.

Member Registration:
Official guest society members may register at member rates provided they supply proof of their membership. Certification of membership may be provided in the form of a copy of the membership identification card or letter from the guest society headquarters. Official Guest societies are: American Physiological Society, Australian and New Zealand Society for Comparative Physiology and Biochemistry, Canadian Society of Zoologists, European Society of Comparative Physiology and Biochemistry, German Society of Zoologists, Society for Experimental Biology, and The Society for Integrative and Comparative Biology.

Postdoctoral Registration
Any person who has received a Ph.D. degree in physiology or a related field within four years of this meeting and as attested by the department head may register at the postdoctoral rate. **A statement signed by the department head must be presented at the time of registration.**

Student Registration:
Any student member or regularly matriculated student working toward a degree in one of the biomedical sciences is eligible to register at the student rate. Nonmember postdoctoral fellows, hospital residents and interns, and laboratory technicians do not qualify as students. **A Student identification card must be presented at the time of registration.**

Guest Registration:
Nonscientist, spouse or guest registrants may register for a fee of $75. The guest registration fee includes entry into the Exhibit Hall, Opening Reception, Poster Session Socials and the Scholander Banquet. Guest Registrants may **not** attend symposia or lectures.

Press:
Press badges will be issued at the APS Conference Registration Desk (located in the Atlas Foyer) only to members of the working press and freelance writers bearing a letter of assignment from an editor. Representatives of allied fields (public relations, public information, public affairs, etc.) may register as nonmembers in the registration area.

Audio/Video Taping of Sessions:
Audio or video taping of sessions is not permitted without prior and written approval of the The American Physiological Society and Conference Organizing Committee Chair.

Individuals Requiring Assistance:
Registrants with questions regarding special housing, transportation and auxiliary requirements should contact the APS Meeting Office, 9650 Rockville Pike, Bethesda, Maryland 20814-3998, telephone, 301-530-7010. **This meeting is accessible to all people.**

Continuing Medical Education (CME) Credit:
The Federation of American Societies for Experimental Biology (FASEB) is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians. Category I CME credits will be offered at this meeting. CME application forms will be available at the Conference Registration Desk. For the purposes of continuing medical education credits toward the American Medical Association Physician's Recognition Award, the APS Conference: The Power of Comparative Physiology: Evolution, Integration and Application is jointly sponsored by FASEB. There is a $35 application fee, payable upon submission of the form. For more information, contact the FASEB Office of Scientific Meetings and Conferences at 301-530-7010.

Program Objective:
The goal of this meeting is to bring together comparative biologists who utilize a diversity of approaches including molecular, cellular, organ and organismal physiology/ biochemistry, functional morphology, biomechanics and biophysics, ecology and evolutionary biology to understand physiological processes and traits. The meeting will highlight accomplishments that have occurred since the last large comparative meeting and, more importantly, will provide a forum to showcase new directions and approaches.

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Target Audience:
This meeting is intended for all scientists and professionals from different fields who share an interest in learning how advances in the field can aid in the study of comparative biology.

Message Center:
The message board will be located in the Atlas Foyer. Attendees should check for messages daily. Please suggest that callers who wish to reach you during the day leave a message with the APS Conference Registration Desk during registration hours: 619-291-3584 and ask for the APS Conference Registration Desk in the Atlas Foyer.

Social Events:

Opening Reception—Saturday, August 24, 7:00-9:00 PM, Tiki Hut Pavilion, poolside—Start the meeting off under the stars, munching and talking to colleagues at the famed Town & Country Resort Tiki Hut.

Afternoon Poster Session Socials—Sunday through Wednesday, 2:30-5:30 PM, Lower Level, Exhibit Hall—The poster sessions have been designed to enhance participation and interaction by featuring beer and wine with light snacks.

Reception at Birch Aquarium at Scripps—Separate-purchase event; cost $50—Monday, 6:00-10:00 PM, 2300 Expedition Way, La Jolla—Join us for a light dinner, cash bar and networking with your colleagues on Monday, August 26, 6:00-10:00 PM. Shuttle buses will depart the Resort at 5:30 PM. Ticket price includes entrance fee.

The Birch Aquarium—the interpretive center for Scripps Institution of Oceanography—is a unique and stimulating facility with a spectacular setting overlooking the Pacific Ocean. The mission of the Birch Aquarium, in brief, is to 1) provide ocean science education through creative exhibits and programs; 2) interpret Scripps Institution of Oceanography research, emphasizing the inter-disciplinary nature of the science used to study the Earth; and 3) to promote conservation through education and research. To lean more about the Aquarium visit their web site at: http://aquarium.ucsd.edu/index.html.
TICKETS MUST BE PURCHASED IN ADVANCE.

Scholander Lecture and Award Banquet—Wednesday, August 28, 6:00 PM, Grand Ballroom—All registrants are invited to attend the Wednesday evening banquet featuring the Scholander Lecturer, **Barbara Block**, Hopkins Marine Station, Stanford University who will present a talk entitled *"The Fire Inside: Saving Atlantic Bluefin Tuna"*. Prior to the lecture there will be a presentation of the Scholander Award winner. A cash bar reception is scheduled at 6:00 PM followed by dinner at 7:00 PM. **Each registrant must pick-up a complimentary dinner coupon by 10:00 AM on Monday, August 26th at the APS Conference Registration desk.**

San Diego Area:
Local information including locations of attractions, accommodations, shopping and dining are available on the San Diego Convention & Visitors Bureau website: http://www.sandiego.org.

Weather:
San Diego enjoys beautiful weather year round with an average daily temperature of 70°.

Airline Reservations:
United Airlines and US Airways are the official co-carriers for the meeting. Special discounted rates can be obtained by contacting the appropriate airline and referencing the identification code listed:
United Airlines: 800-521-4041, meeting ID code: 592SV
US Airways: 877-874-7687, Gold File Number: 20122236

Car Rental:
Alamo Rent-a-Car has been appointed the official car rental company for the meeting. Special discounted rates have been extended to all participants. Reservations may be made by calling **800-732-3232**. Be sure to identify yourself as an APS meeting attendee and refer to **Group ID #964592** and request **rate code GR.**

SATURDAY, AUGUST 24

ONSITE REGISTRATION
SAT. 2:00 PM-9:00 PM—ATLAS FOYER.

OPENING RECEPTION
SAT. 7:00-9:00 PM—TIKI HUT PAVILLION.

SUNDAY, AUGUST 25

Plenary Lecture

1.0 AN INTEGRATED VIEW OF PROTEIN ADAPTATION: FROM THE SEQUENCE TO THE "SOUP"
SUN. 8:00-9:00 AM—TOWN & COUNTRY RM.

Speaker: **George Somero**,
Stanford Univ., Hopkins Marine Station.

Symposium

2.0 THE POWER OF INTEGRATION
SUN. 9:00 AM-1:00 PM—TOWN & COUNTRY RM.

Chair: **George Lauder**

Part I: Integrating Across Levels of Analysis

9:00 **2.1** Genomics and Physiology: Integrative Studies of metabolism and Growth in Larvae. **Donal Manahan**, Univ. of Southern California.

9:25 **2.2** Endothermy in Fish: Thermogenesis, Ecology and Evolution. **Barbara Block**, Hopkins Marine Station, Stanford Univ.

9:50 **2.3** Selection Experiments: A Unique Tool for Integrating Morphology, Physiology and Behavior. **Ted Garland**, Univ. of California, Riverside.

Part II. Integrating across disciplines

10:15 **2.4** Genetics and Comparative Physiology: New Approaches to Understanding the Genetic Basis of Functional Traits. **Michelle Riehle**, Univ. of California, Irvine.

10:40 **2.5** Hydrodynamics and Comparative Physiology: Quantifying Fluid Motion to Understand How Animals Swim. **George Lauder**, Harvard Univ.

11:05 Break

11:20 **2.6** Mathematical and Mechanical Modeling: Insights into Organismal Function. **Sanjay Sane**, Univ. of California, Berkeley.

Part III. Integrating across species

11:45 **2.7** Paleontology, Physiology, and the Use of Phylogeny to Study the Evolution of Vertebrate Locomotion. **Stephen M. Gatesy**, Brown Univ.

12:10 **2.8** Biophysics of Avian Structural Coloration: Insights from a Comparative Analysis. **Richard Prum**, Univ. of Kansas.

12:35 **2.9** Comparative Analysis and Phylogeny as Tools for Testing Physiological Hypotheses about the Evolution of Endothermy in Fishes. **Kathy Dickson**, California State Univ., Fullerton.

Symposium

3.0 POLAR MOLECULAR BIOLOGY: PROTEINS AND ENZYMES AT THEIR LOWER TEMPERATURE EXTREMES
SUN. 9:00 AM-1:00 PM— SAN DIEGO RM.

Chair: **David Petzel**

9:00 **3.1** The Expression of Myoglobin in Hemoglobinless Antartic Fish. **Bruce Sidell**, Univ. of Maine.

9:30 **3.2** Antifreeze Proteins in Artic and Antarctic Fishes. **Arthur DeVries**, Univ. of Illinois.

10:00 **3.3** Evolution of AFGP Gene in Northern Cod Fish. **Chris Cheng**, Univ. of Illinois.

10:30 **3.4** Warm-Acclimation of Antarctic Trematomus bernacchii Decreases Gill Na/K-ATPase 3-Subunit Isoform Protein Expression without a Change in Isoform mRNA Expression. **Sierra Guynn**, Creighton Univ.

11:00 Break

11:15 **3.5** A Structural Basis of Protein Cold-Adaptation in Antarctic Fish? **Craig Marshall**, Univ. of Otago, Dunedin, New Zealand.

11:45 **3.6** Metabolic Rate Adjustments to Polar Cold: Whole Animal Phenomena-Molecular Explanations? **Hans Pörtner and Magnus Lucassen**, Alfred Wegner Inst. for Polar & Marine Res., Bemerhaven, Germany.

12:15 **3.7** The Nature of Antarctic Fish Biodiversity. **Joseph T. Eastman**, Ohio Univ.

Symposium

4.0 INTEGRATION OF MOTOR FUNCTION: MECHANISMS THAT REDUCE ENERGY COST AND/OR ENHANCE PERFORMANCE

SUN. 9:00 AM-1:00 PM—GOLDEN WEST RM.

Chair: **Andrew A. Biewener**

9:00 **4.1** Are the Functional Dynamics of Muscle Constrained by Architecture? **Andrew A. Biewener**, Harvard Univ.

9:30 **4.2** Diverse Mechanical Functions in a Single Muscle: How Muscles Change Function for Different Locomotor Demands. **Annette M. Gabaldón,** Oregon State Univ.

10:00 **4.3** Multiple Mechanical Functions of Muscles in Running Birds. **Richard L. Marsh**, Northeastern Univ.

10:30 **4.4** Patterns in Form, Muscle Function and Performance in Fish. **John Altringham**, Leeds Univ. U.K.

11:00 **4.5** Varying Dynamics of Muscle Function in Relation to Locomotor Performance. **Anna Ahn,** Concord Field Station, Harvard Univ.

11: 30 **4.6** Scaling of Insect Flight Muscle Efficiency. **Graham Askew**, Univ. of Leeds, U.K.

12:00 **4.7** Linking Muscle Function to Spring-Like Behavior of the Legs During Locomotion. **Claire Farley**, Univ. of Colorado, Boulder.

12:30 **4.8** Coordination, Muscle Work, and Efficacy in Human Vertical Jumping. **Maarten Bobbert**, Free Univ. of Amsterdam, The Netherlands.

Don't Forget to Pick-up your complimentary Banquet Ticket by 10:00 AM, Monday!!

Symposium

5.0 CELLULAR AND MOLECULAR RESPONSES TO DEPRESSED METABOLISM AND LOW TEMPERATURE

SUN. 9:00 AM-1:00 PM—CALIFORNIA RM.

Supported by an unrestricted educational grant from the United States Department of the Navy, Office of Naval Research.

Chairs: **Hannah Carey** and **Gregory Florant**

9:00 **5.1** Introduction. **Hannah Carey,** Univ. of Wisconsin, Madison.

9:05 **5.2** The Impact of Post-Genome Science on Comparative Physiology: Model Species and 'Bespoke' Solutions. **Andrew Cossins**, Univ. of Liverpool, U.K.

9:30 **5.3** Gene Expression Profiling of Aging and its Retardation by Caloric Restriction. **Tomas Prolla**, Univ. of Wisconsin, Madison.

9:55 **5.4** Molecular Determinants of the Hibernating Phenotype. **Sandra Martin,** Univ. of Colorado Sch. of Med.

10:20 **5.5** Mammalian Hibernation through the Eyes of mRNA and Protein Expression Profiling. **Matthew Andrews**, Univ. of Minnesota, Duluth.

10:45 **5.6** Insulin Signaling Pathways in Mammalian Hibernators. **Gregory Florant**, Colorado State Univ.

11:10 **5.7** Cellular Metabolic Responses to Hypoxia: Role of Mitochondria as the Cellular Site of O_2 Sensing. **Paul Schumacker**, Univ. of Chicago.

11:45 **5.8** Stress-Induced Signaling Pathways Associated with Depressed Metabolism and Low Temperature. **Hannah Carey**, Univ. of Wisconsin, Madision.

12:10 Enhanced Antioxidant Activity in the Longest-Living Rodent Species (*Heterocephalus glaber*). **Timothy O'Connor**, City College of New York, CUNY (**11.10**).

12:25 Evidence for a Cryoprotective Protein in Freeze-Tolerant Larvae of the Goldenrod Gall Fly, *Eurosta solidaginis*. **Nancy Pruitt**, Colgate Univ. (**11.4**).

12:40 Discussion

Symposium

6.0 NEUROPEPTIDES INTEGRATING PHYSIOLOGICAL PROCESSES IN INVERTEBRATES: AN EVOLUTIONARY AND COMPARATIVE APPROACH

SUN. 9:00 AM-1:00 PM —RM.

Chair: **Klaus H. Hoffmann**

9:00 **6.1** New Tricks From Old Animals: The Generation and Interpretation of Positional Information in *Hydra*. **Thomas Bosch**, Univ. of Kiel, Germany.

9:30 **6.2** Worms: Neural Simplicity and Neuropeptide Complexity. **Aaron Maule**, Queen's Univ. of Belfast, UK.

10:00 **6.3** Modulation of Neuropeptide Receptors by Gene-Related Peptides and Acid pH. **Paul Benjamin**, Univ. of Sussex, Brighton, UK.

10:30 **6.4** Post-Translational Modifications of the CHH/MIH/GIH Family of Sinus Gland Neuropeptide Hormones. Evolutionary Implications. **Alberto Huberman**, S. Zubirán Natl. Inst. of Med. Sci. and Nutrition, Mexico City, Mexico.

11:00 **6.5** Effects of Adipokinetic Hormones on Reproduction in Insects. **Matthias W. Lorenz**, Univ. of Bayreuth, Germany.

11:30 **6.6** Expression and Structure-Function Studies of Locust ITP: an Antidiuretic Neuropeptide Related to Several Major Crustacean Hormones. **John Phillips**, Univ. of British Columbia.

12:00 **6.7** Insect Adipokinetic Hormones: Release and Integration of Flight Energy Metabolism. **Dick Van der Horst**, Utrecht Univ., The Netherlands.

12:30 **6.8** Insect Allatostatin: Evolutionary Trends and Multifunctional Tasks. **Klaus H. Hoffmann and Gerd Gaede,** Univ. of Bayreuth, Germany and Univ. of Cape Town, South Africa.

Posters

7.0 SCHOLANDER/SICB/SEB AWARD COMPETITION

SUN.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

Board #

1 **7.1** Plasma membrane rafts of rainbow trout are subject to thermal acclimation. **J. K. Zehmer, A. M. Sanchez and J. R. Hazel**. Arizona State Univ., Tempe.

2 **7.2** The heat shock response in gastropods (Genus *Tegula*): from promoters to intertidal zonation. **L. Tomanek**. Stanford Univ., Pacific Grove.

3 **7.3** Effect of intermittent hypoxia on the estuarine teleost, *Gillichthys mirabilis*. **N.M. Aguilar**. Univ. of California, Irvine.

4 **7.4** Neurotransmitter receptors in NOS-expressing neurons of the rat glossopharyngeal nerve. **V.A. Campanucci, M. Zhang and C.A. Nurse**. McMaster Univ., Hamilton, Canada.

5 **7.5** Variation in oxygen sensitivity in insects of different size and age. **K.J. Greenlee and J.F. Harrison**. Arizona State Univ., Tempe.

6 **7.6** Regulation of the cardiovascular system of common carp (*Cyprinus carpio*) during severe hypoxia at three acclimation temperatures. **J.A.W. Stecyk and A. Farrell**. Simon Fraser Univ.

7 **7.7** Evaluation of Na^+, K^+ Cl^- and H^+ transport across the apical membrane in Malpighian (renal) tubule cells of *Rhodnius prolixus*. **J.P. Ianowski and M.J. O'Donnell**. McMaster Univ.

8 **7.8** The effects of amino acids on ion transport and fluid secretion in the Malpighian tubules of *Rhodnius prolixus*. **M.H. Hazel and M.J. O'Donnell**. McMaster Univ.

9 **7.9** Reduced GFR during gold acclimation of freeze-tolerant Cope's gray treefrog helps to conserve circulating cryoprotectant glycerol. **J.C. West and D.L. Goldstein**. Wright State Univ.

10 **7.10** Osmoregulation in avian nectarivores: an integrative approach. **T.J. McWhorter, C. Martinez del Rio and B. Pinshow**. Univ. of Arizona, Univ. of Wyoming and Ben-Gurion Univ. of the Negev, Israel.

Board#

11 **7.11** Saluretic actions of acutely elevated vasopressin in fasting northern elephant seals. **R.M. Ortiz, C.E. Wade, C.L. Ortiz and F. Talamantes**. Univ. of California, Santa Cruz and NASA-Ames Res. Ctr.

12 **7.12** Elimination of plant toxins: an explanation for dietary specialization in mammalian Herbivores? **J.S. Sorensen-Forbey, C.A.S. Turnbull and M.D. Dearing**. Univ. of Utah.

13 **7.13** Photoperiod-induced weight loss in lemmings is due to an increase in energy expenditure. **M.S. Johnson, M.L. Blaylock and T.R. Nagy**. Univ. of Alabama, Birmingham.

14 **7.14** Shunting in alligators: does it make a difference? **M.N. Gardner and D.R. Jones**. Univ. of British Columbia.

15 **7.15** Function of the hammerhead shark cephalofoil. **S.M. Kajiura**. Univ. of California, Irvine.

16 **7.16** Effects of feeding on strong ions and blood gases in *Varanus exanthematicus*. **L. Hartzler, A F. Bennett and J.W. Hicks**. Univ. of California, Irvine.

17 **7.17** The effects of pregnancy on ventilation and oxygen consumption in the lizard, *Tiliqua rugosa*. **S. Munns and C. Daniels**. Univ. of Adelaide, Australia.

18 **7.18** Swimming effects on metabolic recovery from anoxia in turtles. **D.E. Warren and D. C. Jackson**. Brown Univ.

19 **7.19** Molecular cloning of multi-drug resistant (MDR) transporter cDNAs in the cabbage looper, *Trichoplusia ni*. **M.R. Rheault, M. O'Donnell and C. Donly**. McMaster Univ. and Agriculture and Agri-Food Canada.

20 **7.20** Comparative effects of the anesthetics brevital and isofluorane on cardiovascular function in the turtle. **V.I. Toney, S.J. Warburton, D.C. Jackson, S. Carney and T. Wang**. Brown Univ., New Mexico State Univ., Tougaloo Col., Providence, RI and Aarhus Univ., Denmark.

21 **7.21** Does chronic hypoxia during postnatal development elicit long-lasting changes in chemosensitivity in rats? **R.W. Bavis, E.B. Olson, Jr., E.H. Vidruk and G.S. Mitchell**. Univ. of Wisconsin, Madison.

22 **7.22** Metabolic indicators in harbor seal muscle tissue. **L.K. Polasek and R. Davis**. Texas A&M Univ., Galveston.

Board#

23 **7.23** Fiber type composition in the swimming muscles of harbor seals (*Phoca vitulina*). **R.R. Watson and R.W. Davis**. Texas A&M Univ., Galveston.

24 **7.24** Chemosensitivity during sleep in the juvenile harbour seal (Phoca vitulina richardsi). **L.A. Skinner and W.K. Milsom**. Univ. of British Columbia.

25 **7.25** A longitudinal study of oxygen store development in nursing harbor seal pups. **C.A. Creelman, J.M. Burns and J.F. Schreer**. Univ. of Alaska and Univ. of Waterloo, Canada.

26 **7.26** Does titin contribute to the muscle spring? **T.E. Reich, P. Keim and S.L. Lindstedt**. Northern Arizona Univ.

27 **7.27** Biochemistry of steller sea lion muscle as it relates to development of dive physiology. **J.P. Richmond, J.M. Burns, L.D. Rea**. Univ. of Alaska and Alaska Dept. of Fish & Game, Anchorage.

28 **7.28** Ontogeny of diving bradycardia in bottlenose dolphins (*Tursiops truncatus*). **S.R. Noren**. Univ. of California, Santa Cruz.

29 **7.29** Hypothalamic thermosensitivity and body temperature set-point changes in hypoxic squirrels. **G.J. Tattersall andW.K. Milsom**. Univ. of British Columbia.

30 **7.30** Species and developmental differences in respiratory cold tolerance: hibernator versus non-hibernator. **B. Zimmer and W.K. Milsom**. Univ. of British Columbia.

31 **7.31** Oxygen delivery problems may reduce jumping performance in larger locusts. **S.D. Kirkton, G.S. Timmins, D. Hartung, J.A. Niska and J.F. Harrison**. Arizona State Univ. and Univ. of New Mexico.

32 **7.32** Cardiovascular changes induced by voluntary and mechanical ventilation in full term emu embryos (*Dromaius novaehollandiae*). **E.M. Dzialowski, S.J. Warburton, J.L. Black and W.W. Burggren**. Univ. of North Texas, Denton and New Mexico State Univ.

33 **7.33** Proteins in plastic and population variation in egg production in grasshoppers. **J.D. Hatle and S.A. Juliano**. Illinois State Univ.

34 **7.34** Molecular chaperone activity in ectothermic animals: temperature sensitivity of Hsc70 orthologues from perciform fishes. **S.P. Place and G.E. Hofmann**. Arizona State Univ.

Board#

35 **7.35** Acclimation-induced variability in the activation of heat shock transcriptional factor HSF1 in the goby *Gillichthys mirabilis*: implications for ecological plasticity in the heat shock response. **B.A. Buckley and G.E. Hofmann**. Arizona State Univ.

36 **7.36** Acclimation of eurythermality: a comparative analysis of cardiac and neural thermal tolerance in porcelain crabs from different thermal habitats. **J.H. Stillman**. Hopkins Marine Station, Stanford Univ.

37 **7.37** Metabolic adjustments to seasonal cold exposure in juvenile green turtles. **A.L. Southwood, C.A. Darveau and D.R. Jones**. Univ. of British Columbia.

38 **7.38** Index of biological compensation of temperature (Z-approach). **M.V. Zakhartsev, H.O. Portner and R. Blust**. Univ. of Antwerp, Belgium and Alfred Wegener Inst. for Polar & Marine Res., Bremerhaven, Germany.

39 **7.39** Muscular adaptation to cold exposure increases energetic cost of locomotion in monodelphis domesticaa mammal lacking brown adipose tissue. **P.J. Schaeffer and S.L. Lindstedt**. Washington Univ. and Northern Arizona Univ.

40 **7.40** Gene expression and cold adaptive phenotypes in *Caenorhabditis elegans*. **P. A. Murray, A.Y. Gracey and A.R. Cossins**. Univ. of Liverpool, U.K.

41 **7.41** Downregulated protein synthesis during mammalian hibernation: active and passive mechanisms. **F. Van Breukelen and S.L. Martin**. Univ. of Colorado, Denver.

42 **7.42** Sequence mutations in teleost cardiac troponin C that are permissive of cardiac function at low temperatures. **T.E. Gillis, C.D. Moyes and G.F. Tibbits**. Simon Fraser Univ. and Queens Univ.

43 **7.43** Snake venom: prey digestion from the inside out? **M.D. McCue**. Univ. of California, Irvine.

44 **7.44** Strategies of digestion: effects of age and diet quality on digestive efficiency and mean retention time in harbor seals. **S.J. Trumble and M.A. Castellini**. Univ. of Alaska, Fairbanks.

45 **7.45** Electrophysiological properties of the L-type Ca^{2+} current in cardiomyocytes from Pacific mackerel and Bluefin tuna. **H.A. Shiels, J. Blank, A.P. Farrell, and B.A. Block**. Univ. of Leeds, UK and Hopkins Marine Station, Stanford Univ.

Board#

46 **7.46** Exercise studies of mudskippers. **H.J. Lee, B.E. Simmons, J.M. Fenger, J.B. Graham**. UCSD.

47 **7.47** Sex vs. parthenogenesis: increased capacity for sustained locomotion at low temperature in parthenogenetic geckos. **M. Kearney, R. Wahl, and K. Autumn**. Univ. of Sydney, Australia and Lewis & Clark Col., Portland, OR.

48 **7.48** Allometric cascade: a multiple-causes model of body mass effects on metabolism. **C.A. Darveau, R.K. Suarez, R.D. Andrews and P.W. Hochachka**. Univ. of British Columbia, Univ. of California, Santa Barbara and Univ. of Alaska, Seward.

49 **7.49** Steady swimming muscle dynamics of the shortfin mako shark (*Isurus oxyrhincus*) and the leopard shark (*Triakis semifasciata*). **J. Donley and R.E. Shadwick**. Scripps Inst. of Oceanography and UCSD.

50 **7.50** Determination of mechanical equivalent of heat and functional capacity of metabolism of body. **Y. Cinar**. Univ. of A. Izzet Baysal, Duzce, Turkey.

51 **7.51** Cognitive influence on the physiology of diving in harbour seals (*Phoca vitulina*). **S.J. Thornton, G. Weingartner, R.D. Andrews, A. Zelichowska, P.W. Hochachka**. Univ. of Otago, Dunedin, New Zealand and Univ. of British Columbia.

52 **7.52** The oxidatively-stressed seal. **D.M. Bailey, B. Davies, T.P. Johnson, G.W. Davison, I.S. Young and M.A. Fedak**. Univ. of Glamorgan, UK, Queen's Univ. Belfast and The Sea Mammal Res. Unit, St. Andrews, UK.

Posters

8.0 THE POWER OF INTEGRATION

SUN.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

Board #

53 **8.1** Digestive enzyme activity in herbivorous and carnivorous prickleback fishes (Teleostei:Stichaeidae): ontogenetic and phylogenetic effects. **D.P. German, M.H. Horn and A.Gawlicka**. California State Univ., Fullerton.

Board#

54 **8.2** Histochemistry and enzyme histochemistry of the digestive system in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae). **A. Gawlicka, M.H. Horn and K.H. Kim**. California State Univ., Fullerton.

55 **8.3** What does it take to be a herbivore? Gut structure and function in three species of new world silverside fishes (Teleostei: Atherinopsidae) with different diets. **M.H. Horn, A. Gawlicka, E.A. Logothetis, A.M. Jones, J.W. Cavanagh, D.P. German and C.T. Freeman**. California State Univ. Fullerton, North Carolina Aquarium, Wilmington and Colorado State Univ.

56 **8.4** Simulation of the 6000-km migration run of European eel shows remarkably low energy costs. **V. Van Ginneken, E. Anthonissen and G. Van den Thillart**. Evol. & Ecol. Sci., Leiden, The Netherlands.

57 **8.5** Lactate processing in endothermic fishes: gluconeogenic enzyme activities in fast glycolytic myotomal muscle and liver of tunas and the short-fin mako shark. **J.M. Backey, S. Paul and K.A. Dickson**. California State Univ., Fullerton.

58 **8.6** Decrease in the degree of hyperkalemia caused by an acute lactic acid infusion. **K.S. Kamel, S. Cheema-Dhadli, C. Chong, M.A. Shafiee and M.L. Halperin**. St. Michael's Hospital, Univ. of Toronto.

59 **8.7** Temperature and the chemical composition of poikilotherms. **H.A. Woods, W. Makino, J. Cotner, S. Hobbie, J.F. Harrison, K. Acharya, J.J. Elser**. Univ. of Texas, Austin, Univ. of Minnesota, St. Paul and Arizona State Univ., Tempe.

60 **8.8** May we translate physiological data of rat mud therapy studies to human? **S. Korobov**. Lermontovskii Clin. Sanatorium, Odessa, Ukraine.

61 **8.9** Measuring lean, fat and total body masses of migrant birds with dual-energy X-ray absorptiometry. **C. Korine, I.G. Van Tets, S. Daniel and B.Pinshow**. Ben-Gurion Univ. of the Negev and Blaustein Inst. for Desert Res., Israel.

Posters

9.0 POLAR MOLECULAR BIOLOGY PROTEINS AND ENZYMES AT THEIR LOWER TEMPERATURE EXTREMES

SUN.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

Board #

62 **9.1** Changes in gill basolateral membrane composition and Na^+K^+ ATPase activity in Arctic char *Salvelinus alpinus* exposed to seawater. **J.S. Bystriansky and J.S. Ballantyne**. Univ. of Guelph.

63 **9.2** Osmoregulation and freezing avoidance in fertilized eggs of the antarctic naked dragon fish *Gymnocraco acuticeps*. **M. Marjanovic, B. Lawrence, N. Wright, J. Carlson and A.DeVries**. Eastern Illinois Univ., Charleston and Univ. of Illinois, Urbana-Champaign.

64 **9.3** Do high rates of protein degradation partially explain low growth rates in antarctic limpets? **K.P. Fraser, A. Clarke and L.S. Peck**. British Antarctic Survey, Cambridge, UK.

65 **9.4** Calcium binding of parvalbumin is conserved at normal physiological temperatures in antarctic and temperate teleost fishes. **T.S. Moerland, J.R. Erickson and B.D. Sidell**. Florida State Univ., Tallahassee and Univ. of Maine, Orono.

66 **9.5** Structure function studies of lens crystallins from cold adapted antarctic notothenioid fishes. **A.J. Kiss and A. DeVries**. Univ. of Illinois, Urbana-Champaign.

67 **9.6** Pancreatic expression of antifreeze protein is a common mechanism in all antifreeze-producing fish to prevent intestinal freezing. **J. Logue and C.C. Cheng**. Univ. of Illinois, Urbana-Champaign.

68 **9.7** The physiological cost of temperature adaptation in marine ectotherms. **A. Clarke and K.P.P. Fraser**. British Antarctic Survey and Cambridge, UK.

69 **9.8** Substrate specificity and structure of fatty Acyl CoA synthetase from notothenioid fishes. **T.J. Grove and B.D. Sidell**. Univ. of Maine, Orono.

70 **9.9** Mechanisms of LDH adaptation to seasonal temperature change in cod (*Gadus morhua*). **M.V. Zakhartsev and R. Blust**. Univ. of Antwerp, Belgium.

Posters

10.0 INTEGRATION OF MOTOR FUNCTION MECHANISMS THAT REDUCE ENERGY COST AND/OR ENHANCE PERFORMANCE

SUN.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

Board #

71 **10.1** Is the anterior, axial position of the red myotomal muscle in tunas associated with an increased locomotor performance? **C.A. Sepulveda, J.B. Graham, K.A. Dickson and H.E. Dowis**. UCSD and California State Univ., Fullerton.

72 **10.2** Thunniform swimming: muscle dynamics and mechanical power production by aerobic fibers of yellowfin tuna (*Thunnus albacares*). **R.E. Shadwick, D.A. Syme and S.L. Katz**. Scripps Institution of Oceanography and Univ. of Calgary.

73 **10.3** Manipulation of center of mass position in trotting quadrupeds. **D. Lee**. Univ. of Utah.

74 **10.4** Hind limb joint kinetics of the horse during jumping. **D.J. Dutto, D.F. Hoyt, S.J. Wickler, E.A. Cogger and H.M. Clayton**. California State Poly. Univ., Pomona and Michigan State Univ.

75 **10.5** EMG activity in forelimb and hind limb muscles during level and incline trotting in the horse. **D.F. Hoyt, S.J. Wickler, K.L. De La Paz and E.A. Cogger**. California State Poly. Univ., Pomona.

76 **10.6** Time of contact and muscle strain rates do not explain the energetics of the walk-trot transition in horses. **D.A.J. Johnsen, D.F. Hoyt, E.A. Cogger and S.J. Wickler**. California State Poly. Univ., Pomona.

77 **10.7** Mitochondria are calcium sinks in rodent extraocular muscle. **F.H. Andrade and C.A. McMullen**. Case Western Reserve Univ.

78 **10.8** Fascicle strain in an architecturally complex muscle in running birds. **J.A.Carr, C. Buchanan, D.J. Ellerby, H. Henry and R.L. Marsh.** Northeastern Univ.

89 **10.9** Mechanical function of a "hamstring" muscle in running guinea fowl. **D.J. Ellerby, R.L. Marsh, C. Buchanan, J. Carr and H. Henry**. Northeastern Univ.

80 **10.10** The effects of incline on the three-dimensional hindlimb kinematics of the arboreal lizard, *Chamaeleo calyptratus*. **T.E. Higham and B.C. Jayne**. Univ. of Cincinnati.

Board #

81 **10.11** *In vivo* length changes of the rat rectus femoris and vastus lateralis during treadmill locomotion. **R.J. Monti and A.A. Biewener**. Harvard Univ.

82 **10.12** Temperature-dependent plasticity of aerodynamic design in *Drosophila*: implications for kinematics and free-flight ability. **S.P. Roberts, M.R. Frazier, S.D. Kirkton and J.F. Harrison**. Univ. of Nevada, Las Vegas, Univ. of Washington and Arizona State Univ.

83 **10.13** Effects of load type and air temperature on the energetics of load carriage in the honeybee, *Apis mellifera*. **J.F. Harrison, E. Okoroh, E. Feuerbacher, J.H. Fewell and S.P. Roberts**. Arizona State Univ., Univ. of California, Berkeley and Univ. of Nevada Las Vegas.

84 **10.14** Forms of locomotion in the moon snail, *Euspira lewisii*. (Mollusca: gastropoda). **G.B. Bourne, P.R. Spackman, M. S. Newel**. Univ. of Calgary.

85 **10.15** Metabolite diffusion in giant muscle fibers of the spiny lobster *Panulirus argus*. **G.S. Adams, S.T. Kinsey and T.S. Moerland.** Univ. of North Carolina, Wilmington and Florida State Univ.

86 **10.16** Gender difference in running speed: humans versus horses and dogs. **P.L. Entin, D.A. Prante and E.E. Entin**. Northern Arizona Univ. and Aptima, Inc., Woburn, MA.

87 **10.17** Withdrawn.

88 **10.18** The evolution of tendon: morphology and material. **A. Summers**. Univ. of California, Irvine.

Posters

11.0 CELLULAR AND MOLECULAR RESPONSES TO DEPRESSED METABOLISM AND LOW TEMPERATURE

SUN.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

Board #

89 **11.1** Pharmacological anoxia and true anoxia result in two different whole-cell NMDAR current responses in cortical neurons from the western painted turtle. **L. Buck and D. Shin**. Univ. of Toronto.

Board #

90 **11.2** Stable isotope changes during fasting in pinnipeds. **K.A. Hobson, V.K. Stegall and L. Rea**. Prairie and Northern Wildlife Res. Ctr., Saskatoon and Alaska Dept. of Fish & Game, Anchorage.

91 **11.3** Changes in the apoptotic pathway in intestinal epithelial cells during hibernation. **C.C. Fleck and H.V. Carey**. Univ. of Wisconsin, Madison.

92 **11.4** Evidence for a cryoprotective protein freeze-tolerant larvae of the goldenrod gall fly, *Eurosta solidaginis*. **N.L. Pruitt.** Colgate Univ.

93 **11.5** Partial links between the seasonal acquisition of cold tolerance and desiccation resistance in the Goldenrod Gall Fly *Eurosta solidaginis*. **N.C. Ruehl, J.B. Williams and R.E. Lee, Jr.** Miami Univ., Oxford, OH.

94 **11.6** Consequences of starvation on metabolic rate and life history traits in the nematode, *Caenorhabditis elegans*. **W.A. Van Voorhies**. New Mexico State Univ.

95 **11.7** Proteomic analysis of brain and heart proteins in a hibernating mammal. **K.P. Russeth, C.M. Walker, M.M. Tredrea and M.T. Andrews**. Univ. of Minnesota, Duluth.

96 **11.8** Out cold: protein expression in liver of golden-mantled ground squirrels. **E. Epperson and S.L. Martin**. Univ. of Colorado Hlth Sci. Ctr. and Sch. of Med., Denver.

97 **11.9** Neuroendocrine control of hibernation in mammals: role of the HPA axis. **A.K. Shaw, C. Watschke, M.M. Tredrea and M. Andrews**. Univ. of Minnesota, Duluth.

98 **11.10** Enhanced antioxidant activity in the longest-lived rodent species (*Heterocephalus glaber*). **B. Andziak, R. Buffenstein and T.P. O'Connor**. City College of New York.

Poster

12.0 NEUROPEPTIDES INTEGRATING PHYSIOLOGICAL PROCESSES IN INVERTEBRATES: AN EVOLUTIONARY AND COMPARATIVE APPROACH

SUN.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

Board #

99 **12.1** Topical application of an insect neuropeptide on crickets (*Gryllus bimaculatus*). **M.W. Lorenz**. Univ. of Bayreuth, Germany.

100 **12.2** Interaction of molluscan cardioactive neuropeptides. **R.B. Hill, D.D. Brooks, T.J. Fort, L.P. Collis and H. Huddart**. Univ. of Rhode Island, Kingston, Univ. of Central Lancashire, UK, Univ. of Puerto Rico and Lancaster Univ., UK.

Going to the Birch Aquarium Monday night?

You MUST have a ticket to board the bus and to gain entry into the aquarium

Buses Depart 5:30 PM, Monday front entrance

MONDAY, AUGUST 26

Plenary Lecture

13.0 THE IMPACT OF POST-GENOME SCIENCE ON COMPARATIVE PHYSIOLOGY: MODEL SPECIES AND BESPOKE SOLUTIONS
MON. 8:00-9:00 AM—TOWN & COUNTRY RM.

Speaker: **Andrew Cossins**, Univ. of Liverpool.

Symposium

14.0 DNA MICROARRAYS: APPLICATIONS TO COMPARATIVE PHYSIOLOGY
MON. 9:00 AM-1:00 PM—TOWN & COUNTRY RM.

Chair: **Andrew Gracey**

9:00 Introduction.

9:05 **14.1** The Molecular Cascade Linking Cd Toxicity to Piscine Developmental Abnormalities. **Peter Kille**, Cardiff Univ.

9:40 **14.2** Metabolism and Microarray Analysis of Cardiac Gene Expression. **Doug Crawford**, Univ. of Missouri.

10:15 **14.3** Genomics Approaches for Understanding Adaptation. **Anthony Long**, Univ. of California, Irvine.

10:50 Break

11:15 **14.4** Gene Expression Associated with Diurnal Temperature Cycling in the Annual Killfish *Austrofundulus Limnaeus*. **Jason Podrabsky**, Hopkins Marine Station, Stanford Univ.

11:50 **14.5** Expression Profiling During Thermal and Hypoxic Acclimation in Common Carp. **Andrew Gracey**. Univ. of Liverpool, UK.

12:25 **14.6** A Common Gene Expression Program in the Response of Yeast Cells to Diverse Environmental Changes. **Audrey Gasch**, Lawrence Berkeley Natl. Lab.

Symposium

15.0 HOMEOSTASIS OF ESSENTIAL YET TOXIC METALS
MON. 9:00 AM-1:00 PM—SAN DIEGO RM.
Supported by the Society for Experimental Biology

Chairs: **Martin Grosell** and **Nicolas Bury**

9:00 Opening Remarks.

9:10 **15.1** How Copper Enters Cells: Roles of High Affinity Copper Transporters in Physiology and Development. **Dennis Thiele**, Univ. of Michigan.

9:40 **15.2** Heavy Metal Uptake and Sequestration in Lobster Hepatopancreatic Epithelial Cells and their Organelles. **Gregory Ahern**, Univ. of North Florida.

10:10 **15.3** Copper Homeostasis in Telost Fish. **Martin Grosell**, The August Krogh Inst., Denmark.

10:40 **15.4** Physiology, Toxicology, and Homeostasis of Silver in Fish and Aquatic Invertebrates. **Chris M. Wood**, McMaster Univ.

11:30 **15.5** Molecular Control of Zinc Transport in Fish. **Christer Hogstrand**, King's Col. London, UK.

12:00 **15.6** Uptake and Regulation of Iron in Telost Fish. **Nicolas Bury**, King's Col., London, UK.

12:30 **15.7** Bioavailability and Cellular Processing of Zinc in Fish Using *in vivo* and *in vitro* Approaches. **Ronny Blust**, Univ. of Antwerp, Belgium.

Symposium

16.0 LINKING MUSCLE GENES TO STRUCTURE AND PHYSIOLOGY, A COMPARATIVE APPROACH
MON. 9:00 AM-1:00 PM—GOLDEN WEST RM.

Chairs: **Alicia El Haj and Ian Johnston**

9:00 **16.1** Single Molecule Analysis and the Myosin Family of Molecular Motors. **James Spudich**, Stanford Univ.

9:30 **16.2** Effect of Temperature Acclimation on Structure and Thermal Stability of Myosin Isoforms in Carp Fast Skeletal Muscle. **Shugo Watabe**, Univ. of Tokyo.

10:00 **16.3** Genes Regulating Muscle Growth in Telost Fish and their Responses to Temperature Change. **Ian A. Johnston**, Gatty Marine Lab., Univ. of St. Andrews, U.

10:30 **16.4** Molecular Determinants of Cardiac Na^+-Ca^{2+} Exchanger Temperature Dependence. **Glen F. Tibbits**, Simon Fraser Univ.

11:00 Break

11:15 **16.5** Linking Temperature Related Shifts in Muscle Genotype and Phenotype to Whole Animal Physiology and Performance: A Crustacean Model. **Alicia J. El Haj**, Keele Univ., UK.

11:45 **16.6** Alternative Splicing, Muscle Contraction and Intraspecific Variation of Dragonfly Flight Muscle. **James Marden**, Pennsylvania State Univ.

12:15 **16.7** An Integrative Analysis of Myosin Function. **Sanford I. Bernstein**, San Diego State Univ.

12:45 Discussion

Symposium

17.0 MITOCHONDRIAL RESPONSES TO ENVIRONMENTAL AND PHYSIOLOGICAL CHALLENGE

MON. 9:00 AM-1:00 PM—CALIFORNIA RM.

Chair: **Chris Moyes**

9:00 **17.1** Origins of Variation in Mitochondrial Content of Vertebrate Muscle. **Chris Moyes**, Queen's Univ.

9:25 **17.2** Mitochondrial Reactive Oxygen Species Production. **Anne Murphy**, MitoKor Inc., San Diego.

9:50 **17.3** Mitochondrial Mechanisms in Cell Death. **John Lemasters**, Univ. North Carolina, Chapel Hill.

10:15 **17.4** Role of Mitochondrial Reactive Oxygen Species in Signaling in Endothelial Cells Undergoing Mechanical Strain. **Paul Shumacker**, Univ. of Chicago.

10:40 **17.5** Mitochondria: a Comparative Perspective on the Proton Leak and Membrane Bilayer. **Anthony Hulbert**, Univ. of Wollongong, Australia.

11:05 Break

11:45 **17.6** Role of Nitric Oxide and Mitochondria in Control of Firefly Flash. **June Aprille**. Univ. Richmond.

12:10 **17.7** Energy Metabolism and Insect Flight. **Raul Suarez**, Univ. of California, Santa Barbara.

12:35 **17.8** Mitochondrial Structure and Function in Relation to Exercise. **Hans Hoppeler**, Univ. of Berne, Switzerland.

Symposium

18.0 DIVING: WHERE HAVE WE BEEN AND WHERE ARE WE GOING?

MON. 9:00 AM-1:00 PM—PACIFIC BALLROOM

Cochairs: **Michael A. Castellini, David R. Jones and Patrick J. Butler**

9:00 **18.1** Introduction. **Michael A. Castellini,** Univ. of Alaska, Fairbanks.

9:05 **18.2** Diving Bradycardia: Reflexes, Reflexes Everywhere but No Time to Stop and Think? **David R. Jones**, Univ. of British Columbia.

9:30 **18.3** Behavioral Influences on Diving Energetics in Penguins. **Rory P. Wilson**, Univ. of Kiel, Germany.

10:00 **18.4** The Effect of Behavior on Physiological Dive Capacity in Marine Mammals: What Lies Beneath. **Terrie M. Williams**, Univ. of California, Santa Cruz.

10:30 **18.5** Physiology and Behavior of Free-Diving Penguins. **Paul J. Ponganis**, Scripps Inst. of Oceanography, UCSD.

11:00 **18.6** The Development of Diving Ability in Pinnipeds. **Jennifer M. Burns**, Univ. of Alaska.

11:30 **18.7** The Balance Between Hypoxia and Aerobic Metabolism in Seals During Diving. **Randall W. Davis**, Texas A&M Univ., Galveston.

12:00 **18.8** The Energetics of Diving and the Question of Metabolic Depression. **Russel D. Andrews**, Univ. of Alaska, Fairbanks.

12:30 **18.9** Diving Into the Future. **Patrick J. Butler**, Univ. of Birmingham, U.K.

Posters

19.0 SCHOLANDER/ SICB/SEB AWARD COMPETITION

MON.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

See session 7.0 for full listing.

Posters

20.0 DNA MICROARRAYS: APPLICATIONS TO COMPARATIVE PHYSIOLOGY

MON.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

Board #

53 **20.1** Down-regulation of metabolism in fish exposed to hypoxia and starvation. **C.Y. Hung, D.J. Randall and R.Kong**. City Univ. of Hong Kong.

54 **20.2** Evolution of desiccation resistance in laboratory populations of *Drosophila*. Physiological and molecular mechanisms. **C.H. Vanier and A.G. Gibbs**. Univ. of Arizona.

55 **20.3** Loading states modulate skeletal muscle gene profile. **M. Flück, S. Schmutz, M. Wittwer, M. Mayet-Sornay, D. Desplanches and H. Hoppeler**. Univ. of Berne, Switzerland and Univ. of Lyon, France.

56 **20.4** Proteome analysis of rainbow trout liver proteins: molecular responses to altered diet. **S. Martin, F. Medale, S. Kaushik and D. Houlihan**. Univ. of Aberdeen, UK and INRA, St. Pee Sur Nivelle, France.

56A **20.5** Production of a bespoke cDNA clone set for transcript screening of mammalian hibernation. **D. Williams, A. Gracey, S. Martin and A. Cossins**. Univ. of Liverpool, UK and Univ. of Colorado Sch. of Med., Denver.

Poster

21.0 HOMEOSTASIS OF ESSENTIAL YET TOXIC METALS

MON.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

Board #

57 **21.1** Transcriptome and proteome responses to zinc in fish. **S. Balesaria, C.N. Glover and C. Hogstrand**. King's College, London UK.

58 **21.2** Investigation of putative transporters responsible for zinc transport in the fish gill. **A. Qiu and C. Hogstrand**. King's College, London, UK.

59 **21.3** Long-term kinetic measurements of intracellular free zinc using the fluorescent probe FluoZin-3. **F.A.R. Muylle, D. Adriaensen, W. De Coen, J. Timmermans and R. Blust**. Univ. of Antwerp, Belgium.

60 **21.4** Copper accumulation and metallothionein induction in three freshwater fish during sublethal copper exposure. **G. De Boeck, T.T.H. Ngo, K. Van Campenhout and R. Blust**. Univ. of Antwerp, Belgium.

Posters

22.0 LINKING MUSCLE GENES TO STRUCTURE AND PHYSIOLOGY, A COMPARATIVE APPROACH

MON.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

Board #

62 **22.1** Variation in heavy chain myosin genes between stenothermal and eurythermal crustaceans: a link between phenotypic plasticity and genotype. **J. Rock, N.M. Whiteley, J.M. Holmes, J.L. Magnay, S.J. McCleary, S.Beech, G. Goldspink and A.J. El Haj**. Univ. of Wales, Keele Univ., and Univ. of London, UK.

63 **22.2** Myosin heavy chain isoform distribution and expression in lobster skeletal muscles. **S. Medler, D.L. Mykles**. Colorado State Univ., Ft. Collins.

64 **22.3** Kinetic differences between *Drosophila* muscle types: the fast wild type myosin versus a slow embryonic isoform expressed in *Drosophila* indirect flight muscle. **D.M. Swank, S.I. Bernstein, D. W. Maughan**. Univ. of Vermont and San Diego State Univ.

Board #

65 **22.4** Force generation and shortening velocity in canine extraocular and limb muscle fibers. **P.J. Reiser, M.P. Vitucci and J.A. Morrison**. Ohio State Univ.

66 **22. 5** Ca^{2+} transients activate calcineurin/NFATc1 and initiate fast-to-slow transformation. **G.Gros, N. Hanke, R.J. Scheibe, J.D. Meissner and H.-P. Kubis**. Med. Hochschule Hannover, Germany.

Posters

23.0 MITOCHONDRIAL RESPONSES TO ENVIRONMENTAL AND PHYSIOLOGICAL CHALLENGE

MON.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

Board #

67 **23.1** Preliminary characterization of a monocarboxylate transporter in isolated cardiac mitochondria from *Bufo marinus*. **J.M. Duerr**. George Fox Univ., Newberg, OR.

68 **23.2** Mechanisms of energy conservation in the liver of the overwintering frog, *Rana temporaria*. **E. Court and R. Boutilier**. Univ. of Cambridge, UK.

69 **23.3** Effects of temperature, magnesium and quinine on mitochondrial proton leak in teleost fishes. **A.G. Rosenberger and J.S. Ballantyne**. Univ. of Guelph.

70 **23.4** Intracellular PO_2 is not an important modulator of tissue oxygen consumption above the P_{50} of myoglobin in mouse skeletal muscle *in vivo*. **D.J. Marcinek, W.A. Ciesielski, K.E. Conley and K.A. Schenkman**. Univ. of Washington and Children's Hosp. and Regional Med. Ctr., Seattle.

71 **23.5** Changes in mitochondrial oxidative phosphorylation during insect metamorphosis. **M.E. Chamberlin**. Ohio Univ.

72 **23.6** Partial compensation of proton permeability in mitochondria and inner membrane liposomes from thermally acclimated trout. **M.F. Gerrits and J.R. Hazel**. Arizona State Univ.

73 **23.7** Bioenergetics of diapause in encysted embryos of the brine shrimp *Artemia franciscana*. **J.A. Reynolds, J.A. Covi and S.C. Hand**. Louisiana State Univ., Baton Rouge.

Board #

74 **23.8** RNA Synthesis and transcript stability in mitochondria from embryos of *Artemia franciscana* under conditions of anoxia-induced quiescence. **B.D. Eads and S.C. Hand**. Univ. of Wisconsin, Madison, Louisiana State Univ., Baton Rouge.

Posters

24.0 DIVING: WHERE HAVE WE BEEN AND WHERE ARE WE GOING?

MON.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors in attendance 2:30-5:30 PM

Board #

75 **24.1** Diving experience and the aerobic dive capacity of muskrats: does training produce a better diver? **R.A. MacArthur and K.L. Campbell**. Univ. of Manitoba.

76 **24.2** The functional significance of the cardiovascular dive response to routine diving in the harbor seal Pho*ca vitulina*. **N.M. Elliott, R.D. Andrews and D.R. Jones**. Univ. of British Columbia.

77 **24.3** Oxygen, carbon dioxide, and behavior: what are divers doing at the surface? **L.A. Cornick and M.A. Castellini**. Univ. of Alaska, Fairbanks.

78 **24.4** Identifying prey ingestion based on blubber levels of 20:1ω11 and 22:1ω11 fatty acids in free-ranging Steller sea lions (*Eumetopias jubatus*). **L.D. Rea**. Alaska Dept. of Fish & Game, Anchorage.

79 **24.5** Can terrestrial models of "body condition" be applied to a marine mammal? **M.A. Castellini, B. Fadely, J.M. Castellini, S.J. Trumble and T. Mau**. Univ. of Alaska, Fairbanks and Natl. Marine Mammal Lab., Seattle.

80 **24.6** Muscle blood flow and heart rate during sleep apnea in elephant seals. **T. Knower, D.H. Levenson and P.J. Ponganis**. UCSD.

81 **24.7** Seasonal and short-term effect of temperature on metabolic rate of the loggerhead turtle, *Caretta caretta*. **S. Hochscheid, F. Bentivegna and J.R. Speakman**. Stat. Zool. Anton Dohrn, Naples, Italy and Univ. of Aberdeen, UK.

82 **24.8** Fetal lung development in the elephant reflects the adaptations required for snorkeling in adult life. **J.B. West, Z. Fu, A.P. Gaeth and R. V. Short**. UCSD and Univ. of Melbourne, Australia.

Board #

83 **24.9** Aerobic capacity in the skeletal muscles of Weddell seals: key to longer dive durations? **S.B. Kanatous, R.W. Davis, R. Watson, L. Polasek, T.M. Williams and O. Mathieu-Costello**. Univ. of Texas Southwestern Med. Ctr., Dallas, Texas A&M Univ., Galveston, Univ. of California, Santa Cruz and UCSD.

84 **24.10** Overcoming buoyancy: surface descent in thick-billed murres (*Uria lomvia*). **J.L. Hamilton**. Brown Univ.

85 **24.11** The reflex control of heart rate during diving in lesser scaup ducks. **K. Borg and D.R. Jones**. Univ. of British Columbia.

86 **24.12** Heart rate, rate of oxygen consumption and abdominal temperature during diving in macaroni penguins. **J.A. Green, P.J. Butler, T.J. Woakes and I.L. Boyd**. Univ. of Birmingham and Univ. of St. Andrews, UK.

87 **24.13** Can diving optimality models predict adjustments in the diving behaviour of tufted ducks? **L. Halsey, P. Butler and T. Woakes**. Univ. of Birmingham, UK.

88 **24.14** Factors influencing the proximate composition of milk in a sub-polar otariid, *Callorhinus ursinus*. **M. E. Goebel and D. P. Costa**. NOAA/Antarctic Ecosystem Res. Div. and Univ. of California, Santa Cruz.

89 **24.15** Voluntary underwater submergence in conscious rats activates pre-sympathetic brainstem nuclei. **P. McCulloch**. Midwestern Univ.

90 **24.16** Antioxidant protection in marine birds and mammals. **T. Zenteno-Savín, R. Elsner and P.J. Ponganis**. Ctr. de Invest. Biol. del Noroeste, La Paz, Mexico, Univ. of Alaska, Fairbanks and UCSD.

TUESDAY, AUGUST 27

Plenary Lecture

25.0 INSIGHTS INTO RESPIRATORY MECHANICS: LESSONS FROM THE ELEPHANT

TUES. 8:00-9:00 AM—TOWN & COUNTRY RM.

Speaker: **John B. West**, UCSD

Symposium

26.0 PHYLOGENETIC APPROACHES TO UNDERSTANDING PHYSIOLOGICAL EVOLUTION

TUES. 9:00 AM-1:00 PM—TOWN & COUNTRY RM.

Chair: **Theodore Garland, Jr.**

9:00 **26.1** Introduction. **Theodore Garland, Jr.** Univ. of California, Riverside.

9:05 **26.2** What are Phylogenies and Why do they Matter? **Wayne P. Maddison**, Univ. of Arizona.

9:30 **26.3** Phylogenetically Based Statistical Methods: When, Why, and How to Use Them. **Theodore Garland, Jr.**, Univ. of California, Riverside.

9:55 **26.4** What are the Appropriate Tests of Mechanistic and Historical Explanations for Evolutionary Patterns? **Kellar Autumn**, Lewis & Clark Col., Portland, OR.

10:20 **26.5** A Phylogenetic Perspective on the Evolution of Vertebrate Surfactants. **Christopher B. Daniels**, Univ. of Adelaide, Australia.

10:45 Break

10:55 **26.6** Using Phylogenies to Understand the Evolution of Function and Behavior in Lizards. **Duncan J. Irschick**, Tulane Univ.

11:20 **26.7** The Evolution of Complex Systems: Oxygen Secretion in the Eye and Swim Bladder of Fishes. **Michael Berenbrink**, The Univ. of Liverpool.

11:45 **26.8** Use of Phylogenetic Information to Understand the Evolution of Anuran Thermal Biology. **Carlos Arturo Navas**, Univ. of São Paulo, Brazil.

12:10 **26.9** Evolutionary Physiology of Larks along Temperature and Moisture Gradients. **Joe Williams**, Ohio State Univ.

12:35 **26.10** Evolutionary Physiology of Habitat Transitions. **Carol E. Lee**, Univ. of Wisconsin, Madison.

Symposium

27.0 THE COMPARATIVE PHYSIOLOGY OF CARBONIC ANHYDRASE

TUES. 9:00 AM-1:00 PM—SAN DIEGO RM.

Supported by an unrestricted educational grant from the Thomas Maren Foundation.

Cochairs: **Katie Gilmour** and **Steve F. Perry**

9:00 **27.1** A Comparative Approach to Carbonic Anhydrase: The Work of Tom Maren. **Erik R. Swenson**, Univ. of Washington.

9:30 Carbonic Anhydrases in an Autotrophic Animal, the Symbiotic Tubeworm *Riftia pachyptila*. **Marie-Cecile De Cian**, CNRS-UPMC Britany, France. **(32.5)**

9:45 **27.2** Environmentally Mediated Expression of Carbonic Anhydrase in the Gills of Euryhaline Crustaceans. **Raymond P. Henry**, Auburn Univ.

10:15 Comparative Analysis of Carbonic Anhydrase in the Midgut of Different Species of Mosquito Larvae: Do Different Species Regulate their Midgut pH by the Same Mechanism? **Maria del Pilar Corena**, Univ. of Florida. **(32.1)**

10:30 **27.3** Comparative Molecular Physiology and Evolution of Vertebrate Carbonic Anhydrases. **Bruce Tufts**, Queen's Univ.

11:00 **27.4** The Critical Role of Carbonic Anhydrase in Calcium Homeostasis and Water Absorption in Marine Teleost Fish. **Rod Wilson and Martin Grosell,** Univ. of Exeter, UK and Univ. of Copenhagen, Denmark.

11:15 **27.5** Comparative Physiology of Pulmonary Carbonic Anhydrase. **Erich K. Stabenau and Thomas A. Heming**, Bradley Univ., Peoria and Univ. of Texas Med. Branch, Galveston.

11:45 **27.6** Physiological Functions of Extracellular Carbonic Anhydrases in Different Locations—Theoretical and Experimental Evidence. **Gerolf Gros**, Hannover Med. Hochschule, Germany.

Symposium

28.0 THE INFLUENCE OF COMPARATIVE PHYSIOLOGY ON ENGINEERING: NEUROMUSCULAR BIOLOGICAL INSPIRATION TOWARD THE DESIGN OF ARTIFICIAL MUSCLE & ROBOTS

TUES. 9:00 AM-1:00 PM—GOLDEN WEST RM.

Chair: **Robert Full, Jr.**

9:00 **28.1** Inspiration from Comparative Physiology in the Design of Artificial Muscles, Skeletons and Control Systems. **Robert J. Full**, Univ. of California, Berkeley.

9:30 **28.2** The Components of Muscle Power Output. **Robert K. Josephson**, Univ. of California, Irvine.

10:00 **28.3** Facilitating Control Using Intelligent Mechanics in Animals and Machines. **Reinhard Blickhan**, Friedrich-Schiller-Univ., Jena, Germany.

10:30 **28.4** The Myosin Heavy Chains: The Design of an Evolutionarily Constrained Molecular Motor. **Richard Lieber**, UCSD and VA Med. Ctr., San Diego.

11:00 Break

11:15 **28.5** Intelligent Transtibial Prostheses with Muscle-Like Actuators. **Glenn K. Klute**, VA Rehab R&D Ctr. Seattle and Univ. of Washington.

11:45 **28.6** Electro Active Elastomers as Artificial Muscle. **Roy Kornbluh**, SRI International, Menlo Park, CA.

12:15 **28.7** Dynamic Locomotion and Energetics of RHEX, A Six-Legged Robot. **Martin Buehler**, McGill Univ.

Symposium

29.0 RELAXED HOMEOTHERMY

TUES. 9:00 AM-1:00 PM—CALIFORNIA RM.

Cochairs: **Peter Frappell** and **Pat Butler**

9:00 **29.1** Relaxed Homeothermy in Hibernating Mammals. **Brian Barnes**, Univ. of Alaska, Fairbanks.

9:30 **29.2** Body Temperature and Metabolic Rate During Natural Hypothermia in Mammals. **Gerhard Heldmaier**, Philipps Univ., Marburg, Germany.

10:00 **29.3** Relaxed Homeothermy in Bats. **John Speakman**, Univ. of Aberdeen, UK.

10:30 **29.4** Regulated Decrease in Body Temperature (Anapyrexia) in Birds when Migrating and Foraging at Sea. **Pat Butler**, Univ. of Birmingham, UK.

11:00 **29.5** Behavioural Heterothermia. **Peter Frappell**, La Trobe Univ., Australia.

11:30 **29.6** The Role of Hyperthermia in the Water Economy of Birds and Mammals. **Irene Tieleman**, Univ. of Groningen, The Netherlands.

12:00 Hypothalamic Thermosensitivity and Body Temperature Set-point Changes in Hypoxic Squirrels. **Glenn Tattersall**, Univ. of Oulu, Finland. **(7.29)**

12:15 Fasting-induced Shallow Hypothermia in Birds: Effect of Repeated Fasts. **Esa Hohtola**, Univ. of British Columbia. **(34.5)**

Symposium

30.0 HOST-PARASITE INTERACTIONS: A COMPARATIVE APPROACH

TUES. 9:00 AM-1:00 PM—PACIFIC BALLROOM

Supported by the Society for Experimental Biology

Chair: **Gert Filk**

9:00 Welcome and Introduction.

9:15 **30.1** New Developments in our Understanding of Host-Parasite Interactions Between the Salmon Louse, *Lepeophteirus Salmonis* and its Hosts. **Stewart C. Johnson**, National Research Council, Halifax, Nova Scotia.

10:00 **30.2** Avian Coccidiosis: A host-Parasite Relationship to be Restored. **Arno N. Vermeulen**, Intervet International BV, Boxmeer, The Netherlands.

10:45 **30.3** Tick Modulation of Host Immunity: Immunobiology, Genomics, and Proteomics. **Francisco Alarcon-Chaidez**, Univ. of Connecticut Hlth. Ctr.

11:30 **30.4** The Pathophysiology in Piscine and Mammalian Haemoflagellate Diseases. **Patrick T.K. Woo**, Univ. of Guelph.

12:15 **30.5** Physiological Stress and Disease Resistance. **Gert Filk.** Univ. of Nijmegen, The Netherlands.

Posters

31.0 PHYLOGENETIC APPROACHES TO UNDERSTANDING PHYSIOLOGICAL EVOLUTION

TUES.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

1 **31.1** Interpopulational differences in behavior and exercise physiology in an anuran species. **F.B. Oliveira and C.A. Navas**. Univ. of São Paulo, Brazil.

2 **31.2** An objective ancestry test for fossil bones. **J.A. Mastropaolo**. California State Univ., Huntington Beach.

3 **31.3** A discussion of the "comparative method" and the mechanisms of correlated evolution. **W.I. Lutterschmidt and G.M. Sanford**. Sam Houston State Univ., Huntsville, TX.

4 **31.4** Delta-9-Desaturase—a complex evolutionary tale? **H. Evans, A.R. Cossins and A. Gracey**. The Univ. of Liverpool, UK.

5 **31.5** The phylogeny of paenungulates: a clue from bile salt composition. **L.R. Hagey**. Zoological Society of San Diego.

6 **31.6** Reproductive constraints on adaptive differences in escape performance among guppy populations. **C.K. Ghalambor and D.N. Reznick**. Univ. of California, Riverside.

7 **31.7** Rapid evolutionary changes in endurance and sprint speed in *Tropidurus* sister species: relationships with morphology and physiology. **T. Kohlsdorf, R.J ames, R.S. Wilson and C.A. Navas**. Univ. of Sao Paulo, Brazil, Coventry Univ., Coventry, UK and Univ. of Antwerp, Belgium.

Board #

8 **31.8** Aerobic capacity of South American stingless bees. **O.I. Françoso Jr. and J.E.P.W. Bicudo**. Univ. of São Paulo, Brazil.

9 **31.9** Post-hatching yolk consumption and stored energy reserves in hatchling snapping turtles, *Chelydra serpentina*. **M. S. Finkler and B.T. Kressley**. Indiana Univ., Kokomo.

10 **31.10** Metabolic costs of egg production: evidence for energy reallocation? **F. Vezina and T.D. Williams**. Simon Fraser Univ.

11 **31.11** Effects of meal type on postprandial calorigenesis in *Python molurus*. **M.D. McCue, A.F. Bennett, and J. W. Hicks**. Univ. of California, Irvine.

12 **31.12** Stomach pH and the cost of gastric digestion for the Burmese python. **S.M. Secor**. Univ. of Alabama, Tuscaloosa.

13 **31.13** Evolution of water conservation mechanisms in *Drosophila* Species. **A.G. Gibbs**. Univ. of Arizona.

Posters

32.0 THE COMPARATIVE PHYSIOLOGY OF CARBONIC ANHYDRASE

TUES.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

14 **32.1** Comparative analysis of carbonic anhydrase in the midgut of different species of mosquito larvae: do different species regulate their midgut pH by the same mechanism? **M. del Pilar Corena, J.K. Nayar, J.W. Knight, H. Zhong, C. Brock, C. Tu, T.J. Seron, and P.J. Linser**. The Whitney Lab., St. Augustine, FL, Univ. of Florida, Florida Med. Entomology Lab., Vero Beach, PHEREC-FAMU, Panama City and Univ. of Florida, Gainesville.

15 **32.2** Oyster Carbonic Anhydrase. **M.G. Hamilton amd M. Amatulli**. Fordham Col. at Lincoln Ctr., New York.

16 **32.3** The distribution and physiological significance of carbonic anhydrase in fish gills. **K.M. Gilmour and S.F. Perry**. Carleton Univ. and Univ. of Ottawa.

17 **32.4** Quantitation and expression of larval aedes aegypti midgut carbonic anhydrase. **T.J. Seron, J.D. Ochrietor, and P.J. Linser**. Univ. of Florida and The Whitney Lab, St. Augustine.

18 **32.5** Carbonic anhydrases in an autotrophic animal, the symbiotic tubeworm *Riftia pachyptila*. **M. De Cian, X. Bailly, S. Boulben, J. Strub, A. Van Dorsselaer and F. H. Lallier**. CNRS-UPMC, Britany, France and CNRS-ULP, UMR, Strasbourg, France.

Posters

33.0 THE INFLUENCE OF COMPARATIVE PHYSIOLOGY ON ENGINEERING: NEUROMUSCULAR BIOLOGICAL INSPIRATION TOWARD THE DESIGN OF ARTIFICIAL MUSCLE AND ROBOTS

TUES.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

19 **33.1** Biologically inspired self-evolving interfaces for the warfighter mission. **P. Gao, C. Harvey, S. Narayanan, L. Rothrock, C. Phillips, P. Smith, M. Haas, W. Nanry, S. Ogan, M. Buck, M. Deckard, A. Darisipudi, A. Seth and M.G. Wheatly**. Wright State Univ., Ohio State Univ., Air Force Res. Lab. & Air Force Institute of Tech., Dayton.

20 **33.2** Contribution of cytological studies of the intrinsic nerve plexus of the rat heart to the conception of artificial cardiac pacemakers. **J. Moravec and M.L. Moravec**. INSERM, Bron, France.

21 **33.3** Modulation of power output in cockatiels. **T.L. Hedrick, B.W. Tobalske and A.A. Biewener**. Harvard Univ. and Univ. of Portland.

22 **33.4** Dynamic properties of isolated gecko setal arrays. **S. Sponberg, A. Gassett, W. Hansen and K. Autumn**. Lewis & Clark Col., Portland, OR.

23 **33.5** Voltage clamping with digital signal processor based feedback control. **J. Wu, R.B. Hill, L.P. Collis and Y. Sun**. Univ. of Rhode Island, Kingston.

24 **33.6** The scaling of damping: importance for control. **A.M. Peattie, M.S. Garcia, A.D. Kuo, T. Libby, K. Meijer, P.C. Wang and R.J. Full**. Univ. of California, Berkeley and Univ. of Michigan.

Board #

25 **33.7** Compliant damped legs of arthropods inspire the design of robot legs. **D.M. Dudek, X. Xu, M.R. Cutkosky and R.J. Full**. Univ. of California, Berkeley and Stanford Univ.

Posters

34.0 RELAXED HOMEOTHERMY

TUES.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

26 **34.1** Does natural hypothermia improve the five performance of muskrats? **A.G. Hindle, R.W. Senkiw and R.A. MacArthur**. Univ. of Manitoba.

27 **34.2** Hibernating black bears retain skeletal muscle protein and strength. **T.D. Lohuis, P.A. Iaizzo and H.J. Harlow**. Univ. of Wyoming and Univ. of Minnesota, Minneapolis.

28 **34.3** Effects of pyrogen-induced fever on peak metabolic rates in the nine-banded armadillo (*Dasypus novemcinctus*). **J.G. Holmes**. Univ. of New Orleans.

29 **34.4** Functional significance of cold-induced fever. **P. Boily, F.M. Knight**. Univ. of New Orleans and Univ. of the Ozarks, Clarksville, AR.

30 **34.5** Fasting-induced shallow hypothermia in birds: effect of repeated fasts. **E. Hohtola, T. Pilto, M. Laurila and S. Saarela**. Univ. of Oulu, Finland.

31 **34.6** Body temperature profiles associated with muscle activity and strength retention in hibernating black bears. **H.J. Harlow, T.D. Lohuis and P.A. Iaizzo**. Univ. of Wyoming and Univ. of Minnesota.

32 **34.7** Torpor upregulates UCP2 and UCP3 in mouse tissues. **N. Stephens, G. Garber, H. Akeda-Yamazaki, P.D. Neufer, and S. Swoap**. Williams Col., Williamstown, MA, John B. Pierce Lab. Fndn. and Yale Univ.

33 **34.8** Thermal liability in the smallest marine mammal, the sea otter (*Enhydra lutris*. **L. Yeates and T.M. Williams**. Univ. of California, Santa Cruz.

34 **34.9** Metabolic depression, temperature regulation and pregnancy in hibernating black bears. **O. Toien, J. Blake, D. Grahn, H.C. Heller, D.M. Edgar and B.M. Barnes**. Univ. of Alaska, Fairbanks, Stanford Univ. and Hypnion Inc., Worcester, MA.

Board #

34a **34.10** Bigeye thresher sharks possess large orbital retina mirabilia and have a wide thermal niche. **K.C. Weng and B.A. Block.** Hopkins Marine Station, Stanford Univ.

Posters

35.0 BIOCHEMICAL ADAPTATIONS

TUES.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

35 **35.1** Comparison of plasma and red blood cell fatty acids as predictors of diet in captive harbor seals. **T.L. Mau, M.A. Castellini and J.M. Kennish**. Univ. of Alaska, Fairbanks and Univ. of Alaska, Anchorage.

36 **35.2** Effects of early nutritional supplementation of linoleic acid on memory. **V.M. Holloway, F. Close, E. Oriaku and M. Soliman**. Loyola Med. Ctr. and Florida A&M Univ.

37 **35.3** Numbers, longevity and dynamics of the free pulmonary macrophages (FRMs) in the chicken and the rat. **L.N. Nganpiep and J.N. Maina**. Univ. of the Witwatersrand, Parktown, South Africa.

38 **35.4** A further look into the Cheng-Prusoff equation for determination of dissociation constants. **H.C. Cheng.** Aventis Pharmaceuticals Inc.

39 **35.5** Fatty acid metabolism of rainbow trout: different preferential metabolism of palmitate and oleate. **J. Weber, G. Brichon and G. Zwingelstein**. Univ. of Ottawa and Univ. of Lyon, France.

40 **35.6** Putative convergent evolution of A_4-lactate dehydrogenase in *Chromis* species (Pomacentridae) from across the pacific: evidence for key sites in biochemical adaptation to temperature. **G.C. Johns and G.N. Somero**. Stanford Univ., Pacific Grove.

41 **35.7** Alterations in hepatic metabolism of sulfur-amino acids by ethanol in rats. **Y.C. Kim, S.K. Kim, Y.S. Jung, Y.R. Chae and J. M. Seo**. Seoul National Univ., Republic of Korea.

Board #

42 **35.8** Sugar preferences and enzyme activities in a frugivorous bird, the yellow-vented bulbul. **I.G. van Tets, A.K. Green, T.J. McWhorter and B. Pinshow**. Ben-Gurion Univ. of the Negev, Israel, Univ. of Wisconsin, Madison and Univ. of Arizona.

43 **35.9** Purification and characterization of alanine racemase from the muscle of black tiger prawn *Penaeus monodon*. **H. Abe and N. Yoshikawa**. Univ. of Tokyo.

44 **35.10** Cortisol metabolism and inter-population variation in glycolytic enzyme expression. **P.M. Schulte and L. DeKoning**. Univ. of British Columbia and Univ. of Waterloo, Canada.

45 **35.11** Responses to and tolerance of temperature extremes differ among phosphoglucose isomerase genotypes in a montane leaf beetle. **E.P. Dahlhoff and N.E. Rank**. Santa Clara Univ. and Sonoma State Univ., Rohnert Park, CA.

46 **35.12** Effects of temperature on locomotory performance of two species of California willow beetles. **D.M. McMillan, N.E. Rank, D.J. Irschick and E.P. Dahlhoff**. Santa Clara Univ., Sonoma State Univ., Rohnert Park, CA and Tulane Univ.

Posters

36.0 TEMPERATURE AND THERMOREGULATION

TUES.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

47 **36.1** Toxin ingestion: a behavioral adaptation of mammalian herbivores to cold? **L.O. Santos, J.S. Sorensen-Forbey, J.D. McLister and M.D. Dearing**. Univ. of Utah.

48 **36.2** Behavioral thermoregulation in the amphibious purple shore crab *Hemigrapsus nudus*. **I.J. McGaw**. Univ. of Nevada.

49 **36.3** Active regulation of brain temperature in yellowfin tuna. **K.E. Korsmeyer and R.W. Brill**. Hawaii Pacific Univ., Kaneohe and National Marine Fisheries Service, Honolulu.

Board #

50 **36.4** The relationship between body temperature, heart rate and rate of oxygen consumption in Rosenberg's goanna (*Varanus rosenbergi*) at various levels of activity. **T.D. Clark, P.J. Butler and P.B. Frappell**. La Trobe Univ., Melbourne, Australia and Univ. of Birmingham, UK.

51 **36.5** Correlations between energy metabolism, thermal environment, and activity in anuran amphibians from genus scinax (*Amphibia / Hylidae*). **J.E. Carvalho, F.R. Gomes, C.R. Bevier and C.A. Navas**. Univ. of São Paulo, Brazil and Colby Col., Waterville, ME.

52 **36.6** Modification of the physiological stress response in green sturgeon, acipenser medirostris: the influence of time of day and temperature. **S.E. Lankford, T.E. Adams and J.J. Cech, Jr.** Univ. of California, Davis.

53 **36.7** Direct observation of cooling in cerebral arterial blood in pigeons, *Columba livia*. **T.F. Gallegos and M.H. Bernstein**. New Mexico State Univ., Las Cruces.

54 **36.8** Diet and the evolution of thermoregulatory energetics in the woodrats *Neotoma albigula* (a generalist) and *Neotoma stephensi* (a specialist). **J.D. McLister, J.S. Sorensen-Forbey and M.D. Dearing**. Univ. of Utah.

55 **36.9** Measuring temperatures and heat flux from dolphins in the eastern tropical pacific: is thermal stress associated with chase and capture in the tuna purse-seine fishery? **D.A. Pabst, W.A. McLellan, E.M. Meagher, A.J. Westgate, M.D. Scott and K. Forney**. Univ. of North Carolina, Wilmington, Duke Univ., Inter-American Tropical Tuna Commission, La Jolla and National Marine Fisheries Service, Santa Cruz.

56 **36.10** Comparative physiology of heat production and its response to dehydration: is it connected to habits and habitats? **A. Haim, N. Palgi and S. Koon**. Univ. of Haifa–Oranim and Kiryat Tivon, Israel.

Posters

37.0 HEART AND CIRCULATION

TUES.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

57 **37.1** Cardiovascular responses of the terrestrial hermit crab *Coenobita clypeatus* to changes inbody position. **C.S. Knehr and C.L. Reiber**. Univ. of Nevada, Las Vegas.

58 **37.2** The effect of continuous and intermittent exercise and temperature on ghost crab heart rate. **R.B. Weinstein and M.F. Eleid**. Univ. of Arizona.

59 **37.3** Endothelial cells from the eel, *Anguilla rostrata*, a system to study the response to environmental changes. **R.A. Garrick, B.R. Woodin, R.L. Cox and J.J. Stegeman**. Fordham Univ. at Lincoln Center, NY and Woods Hole Oceanographic Inst.

60 **37.4** Effect of temperature on the sarcoplasmic reticulum Ca^{2+} ATPase from tuna hearts. **A.L. Fernandez, J.M. Morrissette, J.M. Blank and B.A. Block**. Hopkins Marine Station, Stanford Univ.

61 **37.5** Measurement of Ca^{2+} release transients in cardiac myocytes of tuna and mackerel using confocal microscopy. **J.M. Morrissette, S.H. Thompson and B.A. Block**. Hopkins Marine Station, Stanford Univ.

62 **37.6** Vascular anatomy of skipjack tuna gills. **H. Dewar, J.B. Graham, R.W. Brill and K.R. Olson**. Pfleger Inst. of Environ. Res., Oceanside, CA, Scripps Inst. of Oceanography, UCSD, Natl. Marine Fisheries Service, Southwest Fisheries Sci. Ctr., Honolulu and Indiana Univ. Sch. Med., Notre Dame.

63 **37.7** Transvascular and intravascular fluid transport in rainbow trout. **K.R. Olson, D.W. Kinney and D.W. Duff**. Indiana Univ. Sch. Med., Notre Dame.

64 **37.8** The β adrenergic receptor system of the rainbow trout. **T.W. Moon, J. Nickerson, S.G. Dugan and G. Drouin**. Univ. of Ottawa.

65 **37.10** The importance of preload on cardiac performance in bullfrogs and turtles. **S.J. Warburton, D.C. Jackson, V.I. Toney and T. Wang**. New Mexico State Univ., Brown Univ., and Aarhus Univ., Denmark.

66 **37.9** Stretched dog and pig femoral arteries relax to acetylcholine through different endothelium-dependent mediators. **N.E. Woodley and J.K. Barclay**. Ohio Northern Univ., and Univ. of Guelph.

Board #

67 **37.11** Delayed depolarization of the cog-wheel valve and pulmonary-to-systemic shunting in alligators. **D.A. Syme, K. Gamperl and D.R. Jones**. Univ. of Calgary, Memorial Univ. of Newfoundland, and Univ. of British Columbia.

68 **37.12** Regulation of systemic resistance and changes in blood flow distribution in the red-eared slider (*Trachemys scripta*) during anoxic submergence. **J.A.W. Stecyk, J. Overgarrd, T. Wang and A. Farrell**. Simon Fraser Univ. and Aarhus Univ., Denmark.

69 **37.13** Molecular diagnostic in long QT syndrome in Mexican patients. **H.M. Barajas, A.G. Ramírez, A. Cordero, R. Bloise and S. Priori**. Univ. of Guadalajara-CUSUR, Mexico, IMSS, Guadalajara, and Inst. of Molec. Cardiol., Pavia, Italy.

70 **37.14** Kidney of giraffes: hypertensive ruminants. **N.S.R. Maluf**. Cleveland, OH.

71 **37.15** Cardiac hormone as a protection against volume overload. **V. Tervonen, O. Vuolteenaho and M. Nikinmaa**. Univ. of Turku, Finland and Univ. of Oulu, Finland.

Posters

38.0 RESPIRATION AND ACID-BASE

TUES.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

72 **38.1** Laplace's law and the alveolus: a misconception of anatomy and a misapplication of physics. **H. Prange**. Indiana Univ., Bloomington.

73 **38.2** Comparison of oxygen carrying capacity of a new perfluorocarbon (PFC) blood substitute in rats breathing room air or 100% Oxygen. **R.M. Kiral, R.W. Nicora and D.P. Evitts**. Synthetic Blood International Inc., Costa Mesa.

74 **38.3** Avian intrapulmonary chemo-receptors: role of L-type calcium channels in CO_2 sensing. **S.X. Egan and S. C. Hempleman**. Northern Arizona Univ., Flagstaff.

75 **38.4** Central glutamatergic control of cardioventilatory function in catfish. **M.L. Burleson, J. Turesson, M. Hedrick and L. Sundin**. Univ. of Texas, Arlington, Goteborg Univ., Sweden and California State Univ, Hayward.

Board #

76 **38.5** Function of the postpulmonary septum in lung ventilation in *Varanus*. **T. Owerkowicz and J.W. Hicks**. Harvard Univ. and Univ. of California, Irvine.

77 **38.6** Pre-exercise inhalation of nedocromil sodium (an inflammatory/mast cell stabilizer) does not mitigate exercise-induced arterial hypoxemia in thoroughbred horses. **M. Manohar, T.E. Goetz, S. Humphrey and T. DePuy**. Univ. of Illinois, Urbana-Champaign.

78 **38.7** The physiology of overwintering in the common snapping turtle (*Chelydra serpentina)* and the softshell turtle (*Apalone spinifera*). **S.A. Reese, D.C. Jackson and G.R. Ultsch**. Univ. of Alabama, Tuscaloosa and Brown Univ.

79 **38.8** Anemia: a basis for the cost of reproduction? **T.D. Williams, W. Challenger, J. Christians, M. Evanson and F. Vezina**. Simon Fraser Univ.

80 **38.9** Cutaneous CO^{18} (and thus O_2) diffusing capacity decreases in response to dehydration in the toad, *Bufo Woodhouseii*. **W. W. Burggren and T. Z. Vitalis**. Univ. of North Texas, Denton and GeneMax Pharmaceuticals Inc., Vancouver, Canada.

81 **38.10** Effects of chronic cold and submergence on blood oxygen transport in hibernating map turtles. **L.A. Maginniss, S. A. Ekelund and G. R. Ultsch**. DePaul Univ. and Univ. of Alabama, Tuscaloosa.

82 **38.11** Modulation of periodic breathing by altered patterns of lung inflation in an amphibian, B*ufo marinus*. **S.G. Reid and N.H. West**. UCSD and Univ. of Saskatchewan.

Posters

39.0 OSMOTIC AND IONIC REGULATION

TUES.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

83 **39.1** Fluorescent measurement of calcium transport in crustacean cells. **F.P. Zanotto, M.G.W. Wheatly, P. Chavez-Crooker and G.A. Ahearn**. Univ. of São Paulo, Brazil, Wright State Univ., Univ. de Antofagasta, Casilla, Chile, and Univ. of North Florida.

84 **39.2** Expression of PMCA3 mRNA and protein in crustacean during molting. **P. Gao, L. Kelly, Z. Zhang and M.G. Wheatly**. Wright State Univ.

Board #

85 **39.3** Gill Na^+/H^+ exchangers (NHE) in marine and freshwater adapted fish. **J.B. Claiborne, S.L. Edwards, D. Gunning, N. Hair, B. Wall and A.I. Morrison-Shetlar**. Georgia Southern Univ., Statesboro.

86 **39.4** Regulatory volume decrease and increase in northern fur seal red blood cells. **H. Fujise, K. Nishiki, T. Fukuoka and K. Kohyama**. Azabu Univ., Sch. of Vet. Med., Sagamihara, Japan and Izu-Mito Sea Paradise, Numazu, Japan.

87 **39.5** Localization and molecular characterization of the crayfish NCX. **L.M. Stiner, Z. Zhang, P.Gao and M.G. Wheatly**. Wright State Univ.

WEDNESDAY, AUGUST 28

Plenary Lecture

40.0 PATTERNS OF SUCCESS AND OF DEATH IN HIMALAYAN MOUNTAINEERING

WED. 8:00-9:00 AM—TOWN & COUNTRY RM.

Speaker: **Raymond B. Huey**,
Univ. of Washington.

Symposium

41.0 DEVELOPMENTAL PHYSIOLOGY: PLASTICITY AND CONSTRAINTS

WED. 9:00 AM-1:00 PM–TOWN & COUNTRY RM.

Cochairs: **Donal T. Manahan** and **Steven C. Hand**

9:00 **41.1** Dual Purpose Genes and the Re-unification of Physiology and Development. **Robert E. Maxson**, Univ. of Southern California, Norris Hosp.

9:30 **41.2** Physiology of Marine Invertebrate Development: Starvation Survival and Metabolic Regulation. **Donal T. Manahan**, Univ. of Southern California.

10:00 **41.3** Temporary Suspension of Developmental Programs: Requirements and Mechanisms for Surviving Environmental Stress. **Steven C. Hand**, Louisiana State Univ., Baton Rouge.

10:30 **41.4** Functional Ontogeny of the Circulatory System in Fish. **Bernd Pelster.** Univ. of Innsbruck, Austria.

11:00 **41.5** Patterns of Gene Expression During Insect Diapuse. **David L. Denlinger**, Ohio State Univ.

11:30 **41.6** Developmental Constraints on the Evolution of Physiological Systems. **Timothy J. Bradley**, Univ. of California, Irvine.

12:00 **41.7** Oxygen Regulation in Crustacean Development. **Nora Terwilliger**, Oregon Inst. of Marine Biology, Univ. of Oregon.

Symposium

42.0 PHYSIOLOGICAL AND GENETIC RESPONSES TO ENVIRONMENTAL STRESS

WED. 9:00 AM-1:00 PM—SAN DIEGO RM.

Chairs: **Gretchen Hofmann** and **Martin Feder**

9:00 **42.1** Introduction. Environmental Stress: A Multifaceted Concept in Integrative Physiology. **Gretchen Hofmann**, Arizona State Univ.

9:05 **42.2** Adaptation to Stressful Conditions in *Drosophila*: Insights from a Broad and Multifaced Approach. **Ary Hoffmann**, La Trobe Univ., Australia.

9:30 **42.3** Heat Shock Proteins and the Stress Response: Transcriptional Regulation of HSP Genes. **Martin E. Feder**, Univ. of Chicago.

9:55 **42.4** Genomic Response of Yeast to Anaerobiosis. **Kurt Kwast**, Univ. of Illinois, Urbana-Champaign.

10:20 **42.5** Intracellular Osmotic Stress Signaling in Euryhaline Telosts: Role of 14-3-3. **Dietmar Kültz**, Whitney Labs, Univ. of Florida.

10:45 **42.6** Adaptations to Anhydrobiosis: Lessons from Nature. **John Crowe**, Univ. of California, Davis.

11:10 **42.7** Corticosterone and Inclement Weather: Mechanisms underlying Adaptive Behavioral Responses in Mountain Birds. **Creagh Breuner**, Univ. of Texas, Austin.

11:35 **42.8** The Scale of Stress: Time and Topography on Wave-Swept Shores. **Mark Denny**, Stanford Univ.

11:55 **42.9** Evolved Thermotolerance and the Expression of Heat Inducible Genes in Thermally Adapted *Escherichia coli*. **Michelle Riehle**, Univ. of California, Irvine.

12:20 **42.10** Ecological Consequences of Environmental Stress and Stress Resistance: Diving into Comorant Evolution from the Cretaceous to the Present. **Warren Porter**, Univ. of Wisconsin, Madison.

Symposium

43.0 ACCLIMATIZATION TO HYPOXIA: SUPPLY VS DEMAND STRATEGIES

WED. 9:00 AM-1:00 PM, GOLDEN WEST RM.

Chair: **Frank L. Powell**

9:00 **43.1** Comparative Physiology of Acclimatization to Hypoxia. **Frank L. Powell**, UCSD.

9:30 **43.2** Interactions of Thermal, Metabolic and Respiratory Control in Hypoxic Homeotherms. **William K. Milsom**, Univ. of British Columbia.

10:00 **43.3** Physiological Signals and Comparative Responses to Decreased Oxygen Supply. **Donna F. Boggs**. Eastern Washington Univ.

10:30 Break

10:45 **43.4** Effects of Hypoxia on Gene Expression: Evolutionary Origins and Functional Significance. **Randall S. Johnson**, UCSD.

11:15 **43.5** Molecular Mechanisms of Oxygen Sensing and Apoptosis in Mammalian Cells. **Navdeep S. Chandel**, Northwestern.

11:45 **43.6** Metabolic Responses to Intermittent and Chronic Hypoxia in Fishes. **Nancy M. Aguilar**, White Mountain Res. Station, UCSD and Univ. of California, Irvine.

12:15 Break

12:30 Regulation of Systemic Resistance and Changes in Blood Flow Distribution in the Red-eared Slider (*Trachemys scripta*) during Anoxic Submergence. **Jonathan Anthony William Stecyk**, Simon Fraser Univ. **(37.12)**

12:35 Does Chronic Hypoxia During Postnatal Development Elicit Long-Lasting Changes in Chemosensitivity in Rats? **Ryan W. Bavis**, Univ. of Wisconsin. **(7.21)**

12:40 Neurotransmitter Receptors in Nos-Expressing Neurons of the Rat Glossopharyngeal Nerve. **Veronica Andrea Campanucci**, McMaster Univ. (**7.4**)

12:45 Hypoxia Regulation of Gene Expression in Crustaceans: A Potential HIF-1 System. **Jennifer Mary Head,** Oregon Institute of Marine Biology, U. Oregon. (**47.14**)

12:50 Variation in Oxygen Sensitivity in Insects of Different Size and Age. **Kendra J Greenlee,** Arizona State Univ. (**7.5**)

Symposium

44.0 REGULATION OF VERTEBRATE RENAL FUNCTION: A COMPARTIVE APPROACH

WED. 9:00 AM-1:00 PM—CALIFORNIA RM.

Cochairs: **William H. Dantzler** and **Eldon J. Braun**

9:00 Introduction.

9:05 **44.1** Regulation of Renal Blood Flow and Glomerular Filtration. **Stanley Yokota**, West Virginia Univ. Sch. of Med.

9:35 **44.2** Regulation of Proximal and Distal Tubule. **William H. Dantzler**, Univ. of Arizona.

10:05 **44.3** Regulation of Water Movement. **Hiroko Nishimura**, Univ. of Tennessee.

10:35 **44.4** Regulation of Nitrogen Excretion. **Patrick J. Walsh**, Univ. of Miami.

11:05 Break

11:20 **44.5** Regulation of Renal and Lower Gastrointestinal Function: Role in Fluid and Electrolyte Balance. **Eldon J. Braun**, Univ. of Arizona.

11:50 **44.6** Regulation of Salt Gland and Renal Interactions. **Maryanne Hughes**, Univ. of British Columbia.

Posters

45.0 DEVELOPMENTAL PHYSIOLOGY: PLASTICITY AND CONSTRAINTS

WED.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

1 **45.1** Ontogeny of the cutaneous permeability barrier in hatchling king snakes. **H.B. Lillywhite, J.G. Menon, G.K. Menon and M.C. Tu**. Univ. of Florida, William Paterson Univ. of New Jersey, California Academy of Sci., San Francisco and National Taiwan Normal Univ., Taipei.

2 **45.2** The ontogeny of energy consumption in leatherback and olive ridley marine turtle hatchlings. **T.T. Jones, R.R. Reina and P.L. Lutz**. Florida Atlantic Univ., Boca Raton and Drexel Univ.

3 **45.3** Plasticity and constraints of grunion developmental timing. **K.L. Martin, E.A. Smyder and A.J. Walker.** Pepperdine Univ.

4 **45.4** Oxygen consumption and temperature in larvae of the Antarctic starfish *Odontaster validus*. **L.S. Peck and E. Prothero-Thomas**. British Antarctic Survey, Cambridge, UK.

5 **45.5** Changes in blood chemistry during hypoxic exposure in embryos of the domestic chicken. **D.A. Crossley II and J.W. Hicks**. Univ. of California, Irvine.

6 **45.6** Comparative locomotor function in turtles: can species differences in adult motor patterns be traced to juveniles? **R.W. Blob, E.L. Scanga, M.W. Westneat**. Clemson Univ. and Field Museum, Chicago.

7 **45.7** Non-skilled motor behavior lateralization during the early postnatal development in white rats. **M. Erlikh and A. Vol'nova**. St.-Petersburg State Univ., Russian Federation.

8 **45.8** Molt cycle changes in tissue-specific abundance of cryptocyanin and hemocyanin mRNA in the dungeness crab, *Cancer magister*. **N.B. Terwilliger, D.W. Towle and M. Ryan**. Oregon Inst. of Marine Biology, Univ. of Oregon and Mt. Desert Island Biol. Lab., Salsbury Cove, ME.

9 **45.9** Developmental expression and actions of corticotropin-releasing hormone in tadpoles of *Xenopus laevis*. **G.C. Boorse, K.A. Glennemeier and R.J. Denver**. Univ. of Michigan.

Board #

10 **45.10** Effect of photoperiod and melatonin on growth and development of neonatal gerbils (Meriones unguiculatus). **S.B. Chaplin, T.L. Kelly and S.C. O'Connell**. Univ. of St. Thomas, St. Paul, MN.

Posters

46.0 PHYSIOLOGICAL AND GENETIC RESPONSES TO ENVIRONMENTAL STRESS

WED.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

11 **46.1** Phosphoserine and other unusual osmolytes in deep-sea vesicomyid bivalves: correlations with depth. **P.H. Yancey, J. Fiess, H. Hudson, J. Hom and C. Kato**. Whitman College, Walla Walla, WA and JAMSTEC, Yokosuka, Japan.

12 **46.2** Environmental salinity reduction leads to increased abundance of $Na^+/K^+/2Cl^-$ cotransporter mRNA in gills of the blue crab *Callinectes sapidus*. **D.W. Towle, P. Peppin and D. Weihrauch**. Mt. Desert Island Biol. Lab., Salsbury Cove, ME and Univ. of Illinois, Chicago.

13 **46.3** Recovery of water, ion content, and energy stores following desiccation in *Drosophila melanogaster*. **D.G. Folk and T.J. Bradley**. Univ. of California, Irvine.

14 **46.4** Pelvic skin blood flow and water uptake in toads, *Bufo alvarius*. **A.L. Viborg and S.D. Hillyard**. August Krogh Institute Univ. of Copenhagen, Denmark and Univ. of Nevada, Las Vegas.

15 **46.5** Paracellular permeability and chemosensory function of toad skin. **S.D. Hillyard and E.H. Larsen**. Univ. of Nevada, Las Vegas, August Krogh Institute and Univ. of Copenhagen, Denmark.

16 **46.6** The role of NaK ATPase and V type H ATPase in ion transport in euryhaline mosquito larvae. **M.L. Patrick and S.S. Gill**. Univ. of California, Riverside.

17 **46.7** Species-specific variation in sulfide physiology between closely related vesicomyid clams. **S.K. Goffredi and J.P. Barry**. Monterey Bay Aquarium Res. Inst., Moss Landing, CA.

Board #

18 **46.8** Deleterious effects of mild overwintering temperatures on survival and potential fecundity of rose-galling Diplolepis wasps (Hymenoptera: Cynipidae). **R.E. Lee, Jr., J.B. Williams and J.D. Shorthouse**. Miami Univ., Oxford, OH and Laurentian Univ., Sudbury, Canada.

19 **46.9** Cross-tolerance in tidepool sculpins (*Oligocottus maculosus*): a strategy for life in the intertidal zone. **A.E. Todgham and G.K. Iwama**. Univ. of British Columbia and National Research Council, Halifax, Nova Scotia.

20 **46.10** Modulation of the stress response: effects of breeding stage, season and relationship to nest abandonment. **O.P. Love, F.Vezina, and T.D. Williams**. Simon Fraser Univ.

21 **46.11** Recent thermal history altered the thermal resistance and Hsp70 accumulation in tissues of the tidepool sculpin (*Oligocottus maculosus*) under acute heat stress. **K. Nakano and G.K. Iwama**. Univ. of British Columbia and National Research Council, Halifax, Nova Scotia.

22 **46.12** Extreme resistance to desiccation and microclimate related differences in cold-hardiness of overwintering gall wasps (Hymenoptera: Cynipidae) on roses in southern Canada. **J. Williams, J.D. Shorthouse and R.E. Lee, Jr.** Miami Univ., Oxford, OH, Laurentian Univ., Sudbury, Canada.

23 **46.13** Influence of thermal stress on rates of protein synthesis and metabolism in an intertidal crustacean. **N.M. Whiteley and L.S. Faulkner**. Univ. of Wales, Bangor, U.K.

24 **46.14** CO_2 release pattern in female *Culex tarsalis* and effect of age, flight, egg production and blood-feeding. **E.M. Gray**. Univ. of California, Irvine.

25 **46.15** Physiological and behavioral sensitivity to environmental stressors measured by changes in fish guild structure in urbanized streams. **D.S. Millican, W.I. Lutterschmidt and B. Deal**. Sam Houston State Univ. and Construction Engineering Res. Lab., Champaign, IL.

26 **46.16** Urine composition in water stressed cricetid rodents: sodium oxalate. **I. Vatnick, C. Korine, I. van Tets and B. Pinshow**. Widener Univ., Chester, PA and Ben-Gurion Univ. of the Negev, Israel.

Board #

27 **46.17** Characterization of very-low density lipoprotein particle size during avian egg production. **K.G. Salvante, M. Wallowitz, R.L. Walzem and T.D. Williams**. Simon Fraser Univ. and Texas A&M Univ., College Station.

28 **46.18** Molecular basis of angiogenetic disturbances in Baltic salmon early mortality syndrome. **K.A. Vuori, A. Soitamo, P.J. Vuorinen and M. Nikinmaa**. Univ. of Turku, Finland and Finnish Game and Fisheries Res. Inst., Helsinki, Finland.

29 **46.19** Neonates of the common map turtle (*Graptemys geographica*) overwinter terrestrially in northern Indiana: does hatchling cold hardiness influence geographic distribution? **P.J. Baker, J.P. Costanzo and R.E. Lee, Jr.** Miami Univ., Oxford, OH.

30 **46.20** Cold hardiness and desiccation resistance in hatchling *Emydoidea blandingii*. **S.A. Dinkelacker, J.P. Costanzo and R.E. Lee, Jr.** Miami Univ., Oxford, OH.

31 **46.21** Are physical factors facilitating marine species invasions? **C.E. Braby, G. N. Somero**. Stanford Univ., Pacific Grove.

32 **46.22** Characterization of oxidative stress in *Saccharomyces cerevisiae* mutants lacking superoxide dismutase. **K.M. O'Brien, R.P. Dirmeier, M.M. Engle and R.O. Poyton**. Univ. of Colorado, Boulder.

33 **46.23** Fluorescein transport in malpighian tubules of the cricket, *Acheta domesticus*: affinity and specificity characteristics. **R.M. Kauffman, A.K. Jenner and D.S.G. Neufeld**. Eastern Mennonite Univ., Harrisonburg, VA.

34 **46.24** Physiological responses, desaturase activity and fatty acid composition in milkfish (*Chanos chanos*) under cold acclimation. **S. Hsieh and C. Kuo**. National Pingtung Univ. of Sci. and Tech., Taiwan.

35 **46.25** The pathway to heat acclimation: does HIF-1 plays a role? A lesson from *C. elegans* mutants. **M. Horowitz, H. Jiang, J. Powell-Coffman, Z. Bromberg, J. Shleir, M. Treinin**. The Hebrew Univ., Jerusalem, Israel; Iowa State Univ.

Board #

36 **46.26** Stressor-dependent regulation of heat shock response in Zebrafish, *Danio rerio* **S. Airaksinen, C.M.I. Råbergh, A. Palomäki, A. Lahti, L. Sistonen, M. Nikinmaa**. Turku Centre for Biotechnology and Univ. of Turku, Finland.

37 **46.27** A Comparative study examining the utility of Hsp70 mRNA and protein in red blood cells as bio-indicators of acute and chronic temperature stress in the thermo-sensitive brook trout (*Salvelinus fontinalis*). **S.G. Lund, M.E.A. Lund and B.L. Tufts**. Queen's Univ., Kingston, ON, Canada.

38 **46.28** A Role for Hsp90 in the estrogenic response of juvenile rainbow trout (*Oncorhynchus mykiss*) to ß-Estradiol and 4-Nonlyphenol. **S. Currie and D.L. Chaput**. Mount Allison Univ., Sackville, NB, Canada.

39 **46.29** Phenostasis and patterns of growth: a framework from which to interpret adaptive capacity. **J.M. Szewczak**. Univ. of California, White Mountain Research Station, Bishop.

40 **46.30** Basal metabolic rate may not be related to body composition. **H.I. Ellis and J.R. Jehl, Jr.** USCD and Smithsonian Inst., Washington, D.C.

Posters

47.0 ACCLIMATIZATION TO HYPOXIA: SUPPLY VS. DEMAND STRATEGIES

WED.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM—7:00 PM
Authors present posters 2:30—5:30 PM

Board #

41 **47.1** Intracellular pH regulation of rainbow trout (Oncorhynchus- Mykiss) hepatocytes: hypoxia stimulates sodium/proton exchange. **E. Rissanen, A. Tuominen, A. Bogdanova and M. Nikinmaa**. Univ. of Turku, Finland and Univ. of Zurich, Switzerland.

42 **47.2** Depression of lipolysis in CARP; a possible hypoxia protection mechanism. **G. van den Thillart and G. Vianen, J. Zaagsma**. Leiden Univ., and the Univ. of Groningen, Netherlands.

Board #

43 **47.3** Hypoxia induces gross-morphological changes in crucian carp gills. **J. Sollid, P. De Angelis, K. Gundersen and G.E. Nilsson**. Institute of Biology, Oslo, Norway and Institute of Pathology, Oslo, Norway.

44 **47.4** Effect of hypoxia on fish: what role(s) does apoptosis play? **W.L. Poon and D. Randall**. City Univ. of Hong Kong.

45 **47.5** Developmental plasticity in tadpole shrimp: cardiac and respiratory responses to chronic hypoxic exposure. **C.L. Reiber and S. Harper**. Univ. of Nevada, Las Vegas.

46 **47.6** Effect of reproductive state and hypoxia on cardiovascular responses in the grass shrimp *Palaemonetes pugio*. **L.A. Jones, J.A. Guadagnoli and C.L. Reiber**. Univ. of Nevada, Las Vegas.

47 **47.7** Metabolic and thermal acclimation to hypoxia in rats. **S. Lacefield and D.F. Boggs**. Eastern Washington Univ.

48 **47.8** 2,3-DPG changes in horses, mules and burros with exposure to altitude. **M.J. Hurson, H.M. Greene, J.M. Szewczak and S.J. Wickler**. California State Polytechnic Univ. and UCSD, Bishop.

49 **47.9** Respiratory consequences of mouthbrooding and hypoxia in coral reef fish. **G.E. Nilsson and S. Ostlund-Nilsson**. Univ. of Oslo, Norway.

50 **47.10** Effects of hypoxia and epinephrine on erythrocytes of high-altitude acclimated pigeons, *Columba livia*. **E.S. Quintana and M.H. Bernstein**. New Mexico State Univ., Las Cruces.

51 **47.11** Properties of skeletal muscle in mice with an inherited capacity for hypoxic exercise tolerance. **G.S. Adams, J.D. Luedeke, M.H. Ernst, R.D. McCall and S.T. Kinsey**. Univ. of North Carolina, Wilmington.

52 **47.12** Amino acid sequences of the embryonic globin chains of a marsupial, the tammar wallaby (*Macropus eugenii*). **R.A. Holland, K.H. Gill, R.M. Hope, D. Wheeler, S.J. Cooper and A.A. Gooley**. Univ. of New South Wales, MacQuarie Univ., Adelaide Univ., South Australian Museum, Adelaide and Proteome Systems Limited, NSW, Australia.

53 **47.13** Inhibition of hypoxic pulmonary vasoconstriction reduces high altitude pulmonary edema in rats. **J.T. Berg, S. Ramanathan and E.R. Swenson**. Univ. of Hawaii, Honolulu and Univ. of Washington.

Board #

54 **47.14** Hypoxia regulation of gene expression in crustaceans: a potential HIF-1 system. **J.M. Head and N.B. Terwilliger**. Oregon Institute of Marine Biol., Univ. Oregon, Charleston.

55 **47.15** HIF-1alpha, erythropoietin and adaptation to excessive erythrocytosis. **M. Gassmann**. Univ. of Zurich, Switzerland.

56 **47.16** Acute and long-term neuroprotective responses to hypoxia in snail neurons. **P. Donohoe, E. Court and R. Boutilier**. Univ. of Cambridge, U.K.

57 **47.17** Microcalorimetric evidence of an oxyconformism in tissue metabolism of mammalian neonates. **D. Singer, A. Ince and B. Hallmann**. Univ. of Würzburg and Univ. of Göttingen, Germany.

Posters

48.0 REGULATION OF VERTEBRATE RENAL FUNCTION: A COMPARATIVE APPROACH

WED.—EXHIBIT HALL, LOWER LEVEL

Posters on display 8:00 AM – 7:00 PM
Authors present posters 2:30-5:30 PM

Board #

58 **48.1** Sipping human, gulping camel: the story behind future sweat. **M.L. Halperin, D.Z. Cherney, P.S. Aujla, D.N. Glick and M.A. Shafiee**. St. Michael's Hospital, Univ. of Toronto.

59 **48.2** Endothelin inhibits NaCl transport across the fish gill by release of nitric oxide and prostaglandin E. **D.H. Evans and P.M. Piermarini**. Univ. of Florida.

60 **48.3** Redistribution of body water and salt tolerance in wild ducks. **M. R. Hughes and D.C. Bennett**. Univ. of British Columbia.

61 **48.4** Distribution and possible function of aquaporin water channels in amphibian skin. **D.R. Powers, P.W. Gramenz, T.L. Baker and D.J. Kimberly**. George Fox Univ., Newberg, OR.

62 **48.5** Comparison of renal and salt gland function in three species of wild ducks. **D.C. Bennett and M.R. Hughes**. Univ. of British Columbia.

63 **48.6** Renal structure and function in *Notomys alexis* and *Mus musculus domesticus*. **J.R. Roberts and L.J. Gordge**. Univ. of New England, NSW, Australia.

Board #

64 **48.7** A novel, non-invasive electrophysiological technique for analysis of organic cation transport by isolated cells and tissues. **M.J. O'Donnell and M.R. Rheault**. McMaster Univ.

65 **48.8** Contribution of cytoskeletal elements to rapid fluid transport in insect malpighian tubules. **J.H. Spring and R. Hazelton-Robichaux**. Univ. of Louisiana, Lafayette and Louisiana State Univ., Eunice.

66 **48.9** Cell-to-lumen taurine efflux during net secretion by primary monolayer cultures of flounder renal epithelium. **S. Benyajati and J.L. Renfro**. Univ. of Oklahoma Hlth. Sci. Ctr. and Univ. of Connecticut.

Plenary Lecture

49.0 SCHOLANDER AWARD BANQUET LECTURE
WED.—8:00 PM, GRAND BALLROOM

Title: The Fire Inside: Saving Atlantic Bluefin Tuna.

Speaker: **Barbara Block,** Hopkins Marine Station, Stanford Univ.

EXHIBITS

Registrants are invited to visit the exhibits Sunday – Tuesday, August 25-27, 2:00 PM – 5:00 PM.

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The Power of Comparative Physiology: Evolution, Integration and Application
Abstracts of Invited and Contributed Presentations

1.0

AN INTEGRATED VIEW OF PROTEIN ADAPTATION: FROM THE SEQUENCE TO THE 'SOUP'

George N. Somero. Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950.

A conserved set of protein-based processes and structures is found in organisms adapted to wide ranges of environmental factors that influence protein stability and function (1). Adaptation to temperature, salinity, and hydrostatic pressure involves complementary changes in protein amino acid sequence and in the milieu—the 'soup'—within which proteins conduct their functions. Adaptive modification of the temperature sensitivities of enzymes, e.g., the muscle isoform of lactate dehydrogenase (A_4-LDH) can be achieved with only one to a few amino acid substitutions. Many of these adaptive changes in primary structure occur at sites that influence the conformational mobility of the protein. Adaptive modifications of the cellular 'soup' include selection of low-molecular-mass organic osmolytes that either have minimal influence on proteins or, in some cases, enhance protein stability in the face of physical and chemical stress. Influences of organic osmolytes are temperature-dependent, yet conserved among protein orthologs at their normal temperatures of function (2). Protein concentration may influence selection for protein stability, favoring low intrinsic stability for proteins that occur in protein-rich cellular compartments such as the mitochondrial matrix (3). (Supported by the National Science Foundation.)

REFERENCES:

1. Hochachka, P.W., and G.N. Somero. *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford University Press. 2002.
2. Fields, P.A., B.D. Wahlstrand, and G.N. Somero. Intrinsic versus extrinsic stabilization of enzymes. *Eur. J. Biochem.* 268: 4497-4505, 2001.
3. Lin, J.J., T.H. Yang, B.D. Wahlstrand, P.A. Fields, and G.N. Somero. Phylogenetic relationships and biochemical properties of the duplicated cytosolic and mitochondrial isoforms of malate dehydrogenase from a teleost fish, *Sphyraena idiastes*. *J. Mol. Evol.* 54: 107-117, 2002.

THE POWER OF INTEGRATION

2.1

GENOMICS AND PHYSIOLOGY: INTEGRATIVE STUDIES OF METABOLISM AND GROWTH IN LARVAE.

Donal T. Manahan, Dept. of Biol. Sciences, Univ. of Southern California, Los Angeles, CA 80089-0371 USA.

Growth is an example of the physiological integration of numerous and complex biological rate processes. We have used quantitative genetics together with physiological, biochemical and genomic analysis to study the mechanistic bases of differential growth rates in larvae. In collaboration with Dr. Dennis Hedgecock, we used controlled genetic crosses of the Pacific oyster (*Crassostrea gigas*) to produce larvae with different growth rates when cultured under identical environmental conditions. We conducted a range of physiological studies on these larvae, spanning several different levels of biological analysis – from whole-organism feeding rates, respiration rates, and rates of protein synthesis, to studies of protein and lipid class compositions, to activities of specific enzymes and nutrient transport kinetics. We have also extended these investigations by examining the patterns of gene expression in hybrid growth differences. Over 3 million larval cDNAs have been studied to identify the most important genes associated with hybrid vigor. Advantages of applying whole-organism and genomic approaches to the study of complex physiological processes will be reviewed.

REFERENCES:

Manahan, D.T.
Amino acid fluxes to and from seawater in axenic veliger larvae of a bivalve (*Crassostrea gigas*).
Mar. Ecol. Prog. Ser., 53: 247-255 (1989).
Culturing and development of methods for studying physiology of bivalve larvae.

Griffing B. 1990.
Use of controlled-nutrient experiment to test heterosis hypothesis.
Genetics 126:753-767 (1990).
Important method for analysis of heterosis in plants.

Hedgecock, D., D.J. McGoldrick, D.T. Manahan, J. Vavra, N. Appelmans, and B.L. Bayne.
Quantitative and molecular genetic analyses of heterosis in marine bivalve molluscs.
J. exp. Mar. Biol. Ecol., 203: 49-59 (1996).
Genetic approaches used to manipulate physiology in bivalve larvae.

2.2

ENDOTHERMY IN FISH: THERMOGENESIS, ECOLOGY AND EVOLUTION.

B. A. Block, Morrissette, J. M., Blank, J. M., Landiera, A. Hopkins Marine Station, Stanford University, CA.

Endothermy has evolved multiple times and in multiple forms in pelagic fishes. Tunas have a suite of physiological specializations including high metabolic rates, cardiac outputs and aerobic capacities associated with endothermy. Billfishes have independently evolved a unique form of cranial endothermy involving a thermogenic organ situated beneath the brain and close to the eyes. Telemetry and archival tag studies indicate that swordfish are able to maintain their cranial temperatures up to 14°C above that of the surrounding water and tunas maintain muscle and visceral temperatures up to 23°C above ambient. Our studies of endothermy at the cell and whole animal level provide insight into the physiological steps associated with the acquisition of endothermy in both lineages. In tunas, a key step in the physiological pathway toward the endothermic state may be the evolution of excitation-contraction coupling pathway in myocytes. The increased reliance on calcium induced calcium release may be required to increase heart rate. In the billfishes, the presence of a unique expression pattern of sarcoplasmic reticulum proteins associated with excitation thermogenic coupling in skeletal muscles facilitates heat production close to the brain and eyes. The physiological steps required for endothermy, and the increased ecological performance associated with the endothermic condition will be examined.

REFERENCES:

Blank, J.M., Morrisette, J.M., Davie, P.S. and B.A. Block. (2002). Effects of temperature, epinephrine and Ca on the hearts of yellowfin tuna (*Thunnus albacares*).
Morrisette, J., Xu, L., Meissner, G., and B. A. Block. 2000. Characterization of RYR-Slow, A ryanodine receptor specific to slow-twitch skeletal muscle. American J. Physiology. 279: R1889-R1898.
Block, B. A. 1994. Thermogenesis in Muscle. Annual Review of Physiology. 56: 535-577
Block, B. A., J. Finnerty. A. F. R. Stewart, and J. A. Kidd. 1993. Evolution of endothermy in fish: Mapping physiological traits on a molecular phylogeny. Science. 260: 210-214.

2.3

SELECTION EXPERIMENTS: A UNIQUE TOOL FOR INTEGRATING MORPHOLOGY, PHYSIOLOGY, AND BEHAVIOR. Theodore Garland, Jr., Dept. of Biology, Univ. of California, Riverside, CA 92521.

Organisms are highly structured entities, and functional interactions often span multiple levels of biological organization. At what level selection typically acts in nature is controversial, although a strong case can be made that behavior is often the most direct target. When selection does act on one or more traits, the expected amount and timing of correlated evolution in other traits is unclear, although various models have been proposed. Interspecific comparative studies usually lack sufficient temporal resolution to resolve such issues empirically, but selective breeding can allow direct study. Currently available technology permits selection to be imposed at any level and on virtually any trait of interest, and also allows monitoring of correlated responses at all levels of organization, ranging down to gene-expression profiling and ultimately identification of particular alleles that underlie the responses to selection. From an outbred base population of laboratory house mice (Hsd:ICR), we used artificial selection to produce 4 replicate lines that exhibit high voluntary wheel running (S) as compared with 4 randombred control lines (C). S and C lines have been found to differ for many other traits, including behavior (e.g., aggression, thermoregulatory nesting), regional brain activity, responses to psychoactive drugs, body size and composition, skeletal size and symmetry, muscle mass and insulin-stimulated glucose uptake, expression of anti-oxidant enzymes, body temperature, plasma corticosterone, and median lifespan. Some differences appear only with wheel access, either acutely or chronically (i.e., genotype-environment interaction). The overall pattern of correlated responses does not appear to match predictions of simple models.

REFERENCES:

Garland, T., Jr., Morgan, M.T., Swallow, J.G. , Rhodes, J.S., Girard, I., Belter, J.G. and Carter, P.A.
Evolution of a small-muscle polymorphism in lines of house mice selected for high activity levels. Evolution 56:in press, 2002.

Unexpected discovery of a major gene that almost halves hindlimb muscle mass, doubles aerobic capacity, shows altered myosin heavy chain expression, and is favored in the selected lines.

Girard, I., McAleer, M.W., Rhodes, J.S. and Garland, T., Jr.
Selection for high voluntary wheel running increases intermittency in house mice (*Mus domesticus*).
J. Exp. Biol. 204:4311-4320, 2001.

S mice run differently, and further increase in wheel running may be limited by a lack of heritable variation in $\dot{V}O_2max$.

Rhodes, J.S., Hosack, G.R., Girard, I., Kelley, A.E., Mitchell, G.S. and Garland, T., Jr.
Differential sensitivity to acute administration of cocaine, GBR 12909, and fluoxetine in mice selectively bred for hyperactive wheel-running behavior. Psychopharmacol. 158:120-131, 2001.

Results implicate altered dopamine function in S mice.

2.4

Genetics and comparative physiology: new approaches to understanding the genetic basis of functional traits

Riehle, M. M.
Department of Ecology and Evolutionary Biology
University of California Irvine, Irvine, CA 92697-2525

A multidisciplinary biological approach is integral to the investigation of the genetic basis of functional and adaptive traits. This type of investigation can be achieved by combining the power of comparative and evolutionary physiology with that of genetics and array technology. For a long while physiologists have used a candidate gene approach and genetic manipulations such as transgenics, gene knock-ins, gene knock-outs, and RNAi to investigate similar questions, and while these methods may be informative, they are inherently biased by previous investigations and previous knowledge. There are at least 2 distinct advantages to using large genome scans: (1) They are unbiased and allow the organism to tell the investigator what is important as opposed to the investigator testing only what they hypothesize is important (*i.e.* candidate genes). (2) Large genome scans are not an endpoint, but instead act as a funnel to filter out a large majority of genes while placing focus on a manageable set of genes.

While a fruitful interaction between comparative physiology and genetics appears desirable in principle, the study species of interest may present difficulties. Historically, most genetic and sequencing effort has been performed on model organisms, leaving these as the most amendable subjects for genetic characterization. However, for justifiable reasons, many comparative physiologists do not work on model organisms. There are three ways around this problem (1) Use a model organism as your study system and instead of studying intricacies of a given system concentrate more the on general adaptive mechanisms and patterns. (2) Use a non-model system that has a close relative which is a model system (*e.g.* a species of *Peromyscus* and the model *Mus musculus*). (3) Generate the genetic information yourself, thereby allowing you to look at the genetic basis of adaptation in any organism of interest. Examples of these different methods have been used to study the genetic and molecular basis of high temperature adaptation in bacteria and the physiological response to hypoxia in long jawed mudsuckers. The synergy of comparative physiology with genetics and genome technology, now the subject of entire symposia, is sure to lead comparative and evolutionary physiologists in many new directions. Supported by an NSF Predoctoral Fellowship, NSF DDIG, and a SICB Grant in Aid of Research to MMR.

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2.5

HYDRODYNAMICS AND COMPARATIVE PHYSIOLOGY: QUANTIFYING FLUID MOTION TO UNDERSTAND HOW ANIMALS SWIM.

George V. Lauder, Dept. of Organismic and Evolutionary Biology, Harvard University

Fish locomotion has served as a model system for the investigation of *in vivo* muscle function, the neural control of rhythmic movements in vertebrates, and the energetics of movement through a dense and viscous medium. But, until recently, we have been unable to quantify the forces exerted by the body and fins of fishes on the fluid environment. Analyses of fish locomotion have thus occurred in the absence of knowledge of force output which has so benefited studies of terrestrial locomotion. In the last four years, the technique of Digital Particle Image Velocimetry (DPIV) has become available from the field of experimental hydrodynamics. DPIV quantifies water flow patterns over body and fin surfaces and allows calculation of locomotor forces exerted on the water in the wake of freely-swimming fishes. DPIV has provided documentation of the structure and orientation of wake vortex rings, allowed calculation of force output from fins in x, y, and z dimensions, provided explanations for maximum swimming performance, and allowed testing of long-standing hypotheses about the effect of caudal fin shape on locomotor function. The infusion of a key technique from one discipline can have a dramatic effect on another, making it possible to test classic hypotheses about animal function that were previously impossible to address.

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2.6

Mathematical and mechanical modeling: insights into organismal function. Sanjay P. Sane & Michael H. Dickinson, Department of Integrative Biology University of California, Berkeley, CA 94720

Dynamically scaled mechanical models have served as powerful tools in the measurement of instantaneous forces and flow fields around flying and swimming organisms. Because they forge an easy link between kinematics and forces, such models are crucially important in the development of semi-empirical, quasi-steady models of flapping wings and fins, as well as validation of various computational fluid dynamic models. In this paper, we describe how dynamically scaled models have contributed to our knowledge of the fundamental principles underlying insect flight aerodynamics. We used a scaled model of Drosophila to build a large library of flapping kinematics and the corresponding aerodynamic forces. From these data, we show that in addition to the leading edge vortices during wing translation, unsteady forces are also generated by added mass, wing rotation, and the interception of the wake from a previous stroke. By incorporating these additional components into existing translational quasi-steady models of insect flight, we can substantially improve the instantaneous force predictions of the models. The corresponding flow visualization data allow us to correlate changes in the ambient flow fields with the forces on flapping insect wings, thereby allowing us a comprehensive insight into various lift generation mechanisms used by hovering insects. Such models, based on measured force coefficients, can be readily used to study maneuverability and control, as well as flight energetics.

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2.7

PALEONTOLOGY, PHYSIOLOGY, AND THE USE OF PHYLOGENY TO STUDY THE EVOLUTION OF VERTEBRATE LOCOMOTION. Stephen M. Gatesy, Dept. of Ecology and Evolutionary Biology, Brown Univ., Providence, RI 02912 USA

The locomotor system of birds (feathered flight, obligate bipedality) is dramatically different from that of even their closest living relatives, the crocodylians. Such marked distinction has made the evolution of the avian locomotor apparatus one of the most hotly debated transitions in the history of vertebrates. Extant diversity offers little toward efforts to reconstruct the ancestral precursors of birds, but fossil organisms provide direct evidence of such intermediates. Despite the inherent limitations of inferring physiology and behavior from often fragmentary skeletal remains, extinct taxa preserve combinations of primitive and derived features not seen in today's fauna.

Changes in locomotor structures through time can be traced using a cladistic hypothesis that relates living and extinct forms. This phylogeny acts as the primary constraint on the otherwise infinite number of scenarios that could be invoked. Model species are critical for understanding how living animals work, but major trends in the history of life are inferred most effectively through integration of all data within a phylogenetic framework. Supported by NSF.

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2.8

BIOPHYSICS OF AVIAN STRUCTURAL COLORATION: INSIGHTS FROM A COMPARATIVE ANALYSIS. **Richard O. Prum**, Dept. of Ecology and Evolutionary Biology, and Natural History Museum, University of Kansas, Lawrence, KS 66045

Although they are typically overlooked as physiological in origin, the structural colors of the avian integument are created by coherent scattering (i.e., constructive interference) of ambient light with nanoscale structures composed of collagen macrofibers, mucopolysaccarides, -keratin, melanin, and air. Three classes of structural color producing arrays can be recognized based on their physical organization: laminar, crystal-like, and quasi-ordered. Traditionally, color production by laminar and crystal-like arrays has been analyzed using thin film optics and Bragg's Law, respectively. Recently, we first recognized quasi-ordered arrays for the first time. To predict the reflectance spectrum from TEM micrographs of quasi-ordered arrays, we have developed a Fourier analysis tool. From comparative phylogenetic analyses of structurally colored avian tissues, we have documented many cases of evolutionary transitions among these classes of spatial organization in structurally colored bird feathers and skin: e.g. quasi-ordered to crystal-like, or crystal-like to laminar. Previous methods to analyze color production were designed for efficiency and accuracy assuming a single spatial organization, but these methods are mathematically incompatible with one another and cannot be used to analyze evolutionary transitions among different systems. The newly developed Fourier tool provides accurate predictions of the shape of the reflectance spectrum and its iridescence (i.e. changes in hue with angle of observation) from all three classes of structurally colored tissues, and constitutes the first available method to investigate the evolution of nanostructure classes and optical function within clades.

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2.9

COMPARATIVE ANALYSIS AND PHYLOGENY AS TOOLS FOR TESTING PHYSIOLOGICAL HYPOTHESES ABOUT THE EVOLUTION OF ENDOTHERMY IN FISHES. Kathryn A. Dickson, Department of Biological Science, California State University, Fullerton, CA 92834

Regional endothermy (the ability to use metabolic heat to maintain the temperature of certain tissues elevated above ambient temperature) has evolved independently among several fish lineages, including lamnid sharks, thresher sharks, billfishes, and scombrid fishes. Among the scombrid fishes, the endothermic tunas have been compared with their ectothermic sister taxa (bonitos and mackerels) to elucidate the evolutionary sequence of character state changes that have resulted in endothermy in this lineage. Among scombrids, only the tunas possess the following characteristics: axial, anterior aerobic locomotor muscle, vascular counter-current heat exchangers perfusing that muscle, an elevated standard metabolic rate, and use of the thunniform mode of swimming. Much has been learned about the evolution of endothermy in tunas from comparative analyses among scombrid fishes in the context of phylogenies based on morphological and molecular data. This approach is being extended to comparisons between endothermic sharks and other active sharks to assess the extent of convergent evolution among these distantly related lineages. To test current hypotheses, additional studies of differences among the 15 tuna species and studies of the sharks are needed.

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POLAR MOLECULAR BIOLOGY: PROTEINS AND ENZYMES AT THEIR LOWER TEMPERATURE EXTREMES

3.1

THE EXPRESSION OF MYOGLOBIN IN HEMOGLOBINLESS ANTARCTIC FISH. Bruce D. Sidell, School of Marine Sciences, University of Maine, Orono, ME 04469-5751.

The 16 members of the Family Channichthyidae (Antarctic icefishes) are unique among adult vertebrate animals in lacking the O_2-binding protein, hemoglobin (Hb). Until recently they also were thought to be devoid of the intracellular O_2-binding protein, myoglobin (Mb). We have found that 6 icefish species express Mb in heart [Mb(+)], while 10 others do not [Mb(-)].

Mapping the trait of Mb expression on the consensus phylogeny of this family reveals that loss of Mb expression has occurred by at least 4 independent events during the evolution of the family. Loss of Mb expression also has resulted from at least 3 discretely different mutational mechanisms in Mb(-) species. Superficially, these observations suggest that Mb is not of physiological importance at the severely cold body temperatures of these species.

A combination of isolated, perfused heart studies and O_2-binding kinetics of icefish Mb, however, clearly establishes that icefish Mb is functional at cold temperature and helps support enhanced mechanical performance of hearts, when present. Ventricular muscle from Mb(-) species shows features of both tissue and subcellular structure that are putatively adaptive in compensating for loss of Mb function and which closely resemble features in viable Mb-knockout mammalian models. Why such apparently deleterious traits as loss of Hb and Mb expression have persisted in icefish species is not resolved, but may ultimately be attributed to the unique environmental and ecological characteristics of the Southern Ocean.

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3.2

ANTIFREEZE PROTEINS IN ARCTIC AND ANTARCTIC FISHES. Arthur L. DeVries, Dept. of Animal Biology, University of Illinois, Urbana, IL 61801

High levels of blood antifreeze glycopeptides (AFGPs) are in part responsible for the extreme freezing avoidance observed in the Antarctic fish fauna. With Arctic fishes the antifreeze proteins (AFs) are both AFGPs and antifreeze peptides (AFPs). The AFs protect the polar fishes by absorbing to ice that occasionally enters the circulation and inhibits ice growth by binding to specific crystal planes altering their surface free energy. Growth inhibited crystals are sequestered in the spleen where they eventually disappear. Quantification of the micro solutes (NaCl) and the AFs indicate that together they account for all of the freezing point depression observed in the native serum of the Arctic fishes. With the Antarctic notothenids the contribution of the AFGPs and micro solutes account for only 60% of the freezing point depression. The remainder is due to the presence of an AFP which by itself exhibits low levels of antifreeze activity but in the presence of the high molecular AFGPs a synergistic effect occurs that is large enough to account for the all the antifreeze activity in the native serum. The AFs are found in the blood and the extra cellular fluids including the intestinal fluid however they are not present in the cytoplasm. Pancreatic secretion is the source of the intestinal fluid AFs and may as well be the source of the blood AFs.

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3.3

EVOLUTION OF AFGP GENE IN NORTHERN COD FISH. Chi-Hing C. Cheng, Chun Yang, and James Logue, Dept. of Animal Biology, University of Illinois, Urbana, IL61801.

Antifreeze proteins are a group of novel proteins that are highly diverse in sequences and structures, united only by their common function to bind to ice crystals and inhibit ice growth. Inherent in this protein diversity is the diverse evolutionary origins and molecular pathways that lead to the creation of these novel ice-binding proteins. New proteins (antifreeze proteins included) usually evolve from pre-existing proteins through gene duplication events followed by sequence divergence. The evolution of the Antarctic notothenioid AFGP gene included a novel mechanism - *de novo* amplification of a short sequence from its trypsinogen-like protease ancestor to form the coding region of the new protein. Evolutionary ancestry is commonly inferred through statistically significant similarities in the gene sequences. The northern cod fish (gadids) which are unrelated to Antarctic notothenioids have independently evolved very similar AFGPs from a different genomic origin, but thus far there are no similar sequences in the databases to infer the ancestry of the cod AFGP gene. Through extensive characterization of AFGP coding sequences from 8 gadid species, we have identified a potential molecular pathway by which the cod AFGP gene arose, which did not involved a pre-existing functional gene but only a short non-coding sequence

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3.4

Warm-acclimation of Antarctic *Trematomus bernacchii* decreases gill Na/K-ATPase α3-subunit isoform protein expression without a change in isoform mRNA expression.

Guynn[1], Sierra R., Scofield[2], Margaret A., Petzel[1], David H.
[1]Departments of Biomedical Sciences and [2]Pharmacology at Creighton University

Antarctic fish, living in -2°C waters, have a serum osmolality of ≈ 600mOsm/kg. Upon warm acclimation to 4°C, the serum osmolality of the Antarctic *T. bernacchii* decreases by 25% due to proportional losses of Na^+ and Cl^-. The ions are excreted due to a significant increase in gill Na/K-ATPase activity. Previously we have shown, using ouabain binding, that the increase in Na/K-ATPase activity is not due to an increase in enzyme number. Additionally, we have shown that *T. bernacchii* gill contains both the mRNA and protein of the Na/K-ATPase α1, α2 and α3-subunit isoforms which have different kinetic and physiologic properties. Therefore, we hypothesized that the increase in activity is due to a change in the gill Na/K-ATPase α-subunit isoform expression. Using real time RT-PCR, no significant change in mRNA expression was found with the gill α1, α2 or α3 isoforms with warm acclimation. We also used western blotting and isoform specific antibodies to measure the band density of the Na/K-ATPase α1, α2 and α3-subunit isoform proteins in cold and warm-acclimated *T. bernacchii* gills. The Na/K-ATPase α3-subunit isoform protein decreased ($p<0.05$) by 50% during warm acclimation. There were no significant changes in α1 or α2-subunit isoform proteins. The data suggests that warm acclimation of *T. bernacchii* regulates gill Na/K-ATPase α-subunit expression at the translational or post-translational level. The Na/K-ATPase α3-subunit isoform has a lower intracellular Na^+ affinity, increases $[Na^+]_{IC}$ when transfected into HeLa cells, and inhibits the activity of the α1 isoform. Our results suggest the warm-acclimated *T. bernacchii* downregulate the α3-subunit isoform, lowering their $[Na^+]_{IC}$ and increasing the activity of the existing Na/K-ATPase α1 or α2-subunit isoforms. Supported by NSF-OPP 9613738 and the Clare Boothe Luce Fellowship.

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3.5

A STRUCTURAL BASIS OF PROTEIN COLD-ADAPTATION IN ANTARCTIC FISH? C.J. Marshall, B.F. Anderson*, R.I. Fleming and C.A. Love. Department of Biochemistry, University of Otago, PO Box 56, Dunedin, New Zealand, *Institute of Molecular Biosciences, Massey University, Palmerston North, New Zealand

Changes in temperature have a profound effect on the biological reactions. Typically, a 10°C change in temperature is associated with a 2-fold alteration in reaction rate. It has been proposed that the resting metabolic rates of animals living in the cold show compensation for temperature-induced reductions in activity: a phenomenon called cold-adaptation. Recent work has shown that resting metabolic rates of cold animals do not show such cold-adaptation but it is much less clear if maximal activities are cold-adapted.

We have addressed this problem by investigation of the properties of lactate dehydrogenase (LDH) from Antarctic and temperate fish. The amount of LDH in different tissues and from different fish was determined. LDH was purified from white muscle and the kinetic properties over a range of temperatures established. The inferred amino acid sequence of each LDH was obtained by nucleic acid sequencing and the structures of a number of LDH were determined by X-ray crystallography.

Our structural analysis does not support local changes in enzyme flexibility but suggests that the structural basis of cold-adaptation is subtle and may involve alterations to the hydrophobic core of the molecule.

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3.6

METABOLIC RATE ADJUSTMENTS TO POLAR COLD: WHOLE ANIMAL PHENOMENA - MOLECULAR EXPLANATIONS?

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According to a recent hypothesis, the energy demanding capacity to withstand temperature fluctuations as well as the constraints and tradeoffs involved in thermal adaptation define macroscale biogeography of marine ectotherms and the limitation of each individual species or population to a characteristic thermal niche. The comparison of strictly stenothermal Antarctic and more or less eurythermal boreal to Arctic species is used to identify the mechanisms and limits of thermal adaptation, considering the much younger thermal history of Arctic compared to Antarctic fauna.

Functional studies indicate that adjustments in the capacities and thermal properties of mitochondrial (enhanced levels of aerobic enzymes and proton leakage) as well as cellular membrane functions (ion and pH regulation), reflect the higher energy costs of cold adaptation in eurytherms than in stenotherms. However, specific bottlenecks in the cold induced expression of enzymes in eurytherms exist, indicated by a comparison of the expression of cytochrome c oxidase and citric synthase in boreal (*Zoarces viviparus*) and Antarctic eelpout (*Pachycara brachycephalum*). The boreal eelpout also exhibited a cold induced increase in the transcription of Na^+/H^+-Antiporter (NHE) and Na^+/K^+-ATPase (ATN-A) genes, however, the data indicate that protein levels remained constant and that the adjustment of membrane properties may be a key in understanding the functional changes suitable to maintain ion and acid-base balance. Molecular strategies will be discussed that would allow Antarctic animals to minimize the cost of temperature adaptation, at the expense of extreme stenothermality in the cold.

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3.7

THE NATURE OF ANTARCTIC FISH BIODIVERSITY. Joseph T. Eastman, Department of Biomedical Sciences, Ohio University, Athens, OH 45701

There are several unusual aspects of the species and ecological diversity of fishes occupying the Antarctic continental shelf. The fauna is relatively small and unusual in composition, consisting of 216 species with higher taxonomic diversity restricted to 18 families. Notothenioids, liparids and zoarcids account for 87% of the fauna. Ninety-seven species of perciform notothenioids comprise 45% of the fauna. However at the highest latitudes on the shelf (73-77°S), benthic trawling reveals that notothenioids contribute 77% of the species diversity, 92% of the abundance and 91% of the biomass. There are probably 30-60 undescribed species in the Southern Ocean. The recent discovery in the Ross Sea of seven species thought to be endemic to West Antarctica raises the possibility that a larger than expected percentage of notothenioids have a circum-Antarctic distribution. Distance, currents, deep water and subzero temperatures isolate the waters of the Antarctic shelf from other shelf areas in the Southern Hemisphere. Historically the Antarctic shelf has been an insular evolutionary site for a variety of marine organisms including fishes. There has been a nearly complete replacement of the late Eocene fish fauna. Hence with little competition, notothenioids underwent considerable morphological and ecological diversification. In the shelf waters they form an adaptive radiation and possibly a species flock. I evaluate Antarctic notothenioids against criteria used in recognizing freshwater species flocks (Greenwood, 1984; Ribbink, 1984). Although monophyly is in question, notothenioids exhibit the disproportionate speciosity (3.6-fold more Antarctic than non-Antarctic species) and high endemism (97%) characteristic of a species flock. Notothenioids may be the first recognized example of a species flock of marine fish. Since these criteria were formulated with respect to freshwater fishes, I consider other features that might assist in identifying marine flocks.

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INTEGRATION OF MOTOR FUNCTION: MECHANISMS THAT REDUCE ENERGY COST AND/OR ENHANCE PERFORMANCE

4.2

Diverse Mechanical Functions in a Single Muscle: How Muscles Change Function for Different Locomotor Demands. Annette M. Gabaldón and Thomas J. Roberts. Dept. of Zoology, Oregon State University, Corvallis, OR 97331.

Running animals' muscles must perform diverse mechanical functions for different locomotor activities. Uphill running demands high mechanical energy production (positive work), whereas downhill running demands high mechanical energy absorption (negative work). To produce these different functions, animals must either activate specialized muscles or alter the mechanical function within single muscles. Our studies on hindlimb muscles in turkeys support the hypothesis that there are individual muscles with significant capacities for both mechanical energy production and mechanical energy absorption. Simultaneous measurements of muscle length (from sonomicrometry) and muscle force (from tendon strain gages) allow the calculation of muscle work during treadmill running in wild turkeys. Both the lateral gastrocnemius (LG) and peroneus muscles alter their mechanical function significantly with changes in running incline. During uphill running, both muscles act as a motor by increasing positive work, whereas in downhill running they act as a brake by increasing negative work. These muscles can be equally effective at absorbing work as they are at producing it. Surprisingly, the strategy used to alter mechanical function is fundamentally different in the two muscles. The LG length trajectory is altered from uphill to downhill running; it simply shortens more to produce positive work and lengthens more to produce negative work. In contrast, peroneus mechanical function is altered by changing the timing of force production, with relatively little change in length patterns. As a power producer in uphill running, peroneus generates high forces in late stance during shortening and only low forces in early stance during lengthening. As a power absorber in downhill running, high forces shift to early stance where lengthening occurs. These results show that runners utilize flexibility of mechanical function within single muscles to meet different locomotor demands. NIH AR46499.

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4.3

Multiple mechanical functions of muscles in running birds. Richard L. Marsh. Biology, Northeastern Univ. Boston, MA 02115. r.marsh@neu.edu

Two basic observations about running present a puzzle. First, animals running on the level perform no net work. Second, the metabolic cost of running increases with increasing speed. Because no net work is performed, the mechanical determinants of the cost of running, or running economy, are not obvious. Despite considerable interest in running economy, we currently know very little about the mechanical functions and energy consumption of the muscles used during running. C.R. Taylor and others hypothesized that running economy would be maximized if most muscles operated isometrically during running, and mechanical energy exchanges in each stride were accomplished by the storage and release of elastic energy. The pinnate ankle extensors of birds and mammals appear to operate in this manner during level running. However, data available on the muscles in the thigh present a more complex picture, with muscles displaying active lengthening, active shortening, and near isometric contractions. Some muscles perform work that does not appear externally because they work against co-contracting muscles that function to provide joint stability during critical phases of the stride. The amount of muscle mass devoted to various mechanical functions, and the metabolic cost of these functions is currently not known for any species. We are using running guinea fowl *Numida meleagris* to begin to fill this gap in our knowledge. Sonomicrometry and electromyography are being used to classify the mechanical functions of the bulk of the hind limb muscles. Muscle mass, electromyography, and blood flow measures are being used to assess the contribution of each muscle to energy use. Supported by NIH grant AR47337.

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4.4

Patterns in form, muscle function and performance in fish
John Altringham. Biology, University of Leeds, UK

Undulatory swimming in fish is powered by the segmented myotomal muscle. Sequential contraction of this muscle generates a backward travelling wave of lateral displacement of the body and caudal fin, pushing against the water to generate forward thrust. Given the enormous diversity in fish body form, kinematics and swimming performance, can we describe the way fish use their muscles to swim in terms of variations on a common theme? How do these variations relate to swimming performance? I will discuss experiments on muscle function in swimming fish, from my own and other laboratories, which can be used to address these questions. Two features in particular stand out. In virtually all species studied, muscle contraction kinetics become slower from anterior to posterior and there is a progressive phase lag between the onset of muscle activation and the strain cycle. We know enough to know that these features are important, but their full significance is not clear. I will suggest some possible explanations for these trends. Some fish are exceptions to these rules, notably at the extremes of body form and swimming mode, such as eels and tuna. These exceptions can tell us not only why these particular fish are different, but also provide clues about how all fish use their muscles to swim.

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4.5

Varying Dynamics of Muscle Function in Relation to Locomotor Performance. A. N. Ahn. Concord Field Station. Harvard University, Bedford, MA 01730.

The mechanical behavior of muscle during locomotion is often predicted from its anatomy, stimulation pattern, basic contractile properties, and the joint kinematics. Cockroaches and frogs provide model systems to examine these assumptions. In the cockroach leg, two muscles operating at the same joint are innervated by a single motor neuron. Direct measurements under *in vivo* running conditions reveal that the same neural signal and joint kinematics can result in different mechanical behaviors from two anatomically similar muscles with the same contraction kinetics, force-length properties, and force-velocity properties. In the frog semimembranosus (SM), *in vivo* segment length changes along the muscle's length were examined during hopping to link data addressing *in vitro* sarcomere behavior with *in vivo* muscle behavior. As expected, the proximal and mid segments of the SM shortened similarly. In contrast, the strain of the distal segment was lower and more variable, often lengthening before shortening. These differences in strain amplitude and pattern imply that adjacent segments along a parallel-fiber muscle can operate on different regions of their force-length and force-velocity relationships. Moreover, these *in vivo* segment strain patterns differ from the patterns of sarcomere heterogeneity seen *in vitro* in single fibers. A single neural input to two muscles within group and to adjacent segments along a muscle can result in variable motor output during locomotion.

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4:6

Scaling of insect flight muscle efficiency

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One of the most significant components of the daily energy budget of many animals is the energy expended during locomotion. Efficient conversion of metabolic energy into locomotor work is therefore of enormous importance. In all modes of locomotion, locomotor efficiency improves with increasing body size. To what extent is this scaling relationship determined by the scaling of myofibillar efficiency? We measured the mechanical power output and energy consumption of glycerinated fibres from the dorsolongitudinal flight muscle of several species of euglossine bees and bumblebees. Mechanical power output was determined using the work loop technique and ATP utilisation by a NADH-linked assay in which resynthesis of ATP utilised by the fibre was coupled to the oxidation of NADH. The mechanochemical coefficient (MC) was calculated from the ratio of the net power output to the rate of ATP hydrolysis was calculated and the myofibrillar efficiency as the ratio of MC to the free energy of ATP hydrolysis. Myofibrillar efficiency increased with increasing body mass and decreased with increasing wingbeat frequency, supporting the hypothesis that faster cycling muscles are less efficient than those that cycle at lower frequencies. The observed scaling of myofibrillar efficiency with body mass agrees with the improvement of overall efficiency with increasing body size.

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4.7

LINKING MUSCLE FUNCTION TO SPRING-LIKE BEHAVIOR OF THE LEGS DURING LOCOMOTION. Claire T. Farley, University of Colorado, Boulder CO 80309-0354.

A spring-mass model predicts the body's center of mass (COM) movements in running, trotting and hopping mammals. In the model, a linear "leg spring" represents stance leg mechanical behavior and a mass represents body mass. During the stance phase, the model's leg spring and an animal's leg follow a similar pattern, first shortening and then extending. The leg's muscles generate force in proportion to the leg length change, thereby mimicking a spring's force-displacement pattern. We found that a spring-mass model predicts a running human's COM trajectory to within 3.8%. Runners adjust leg stiffness to offset changes in elastic surface stiffness within a single step even if a change is unexpected. Because natural terrain dissipates energy, we examined how human hoppers adapt to changes in surface damping. On surfaces that dissipate up to 69% of the energy that they absorb, the leg's net work output increases to offset surface energy losses, thus maintaining a similar COM trajectory regardless of surface damping. On a heavily damped surface, the leg produces 2.4-fold more work during takeoff than it absorbs during landing by generating a greater force over a longer length change. In conclusion, on hard and elastic surfaces, runners adjust the stiffness of their spring-like legs to accommodate surface stiffness. On damped surfaces, the leg deviates from spring-like behavior and generates positive work to overcome surface energy losses. Supported by NIH R29AR44008.

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4.8

Coordination, muscle work, and efficacy in human vertical jumping.

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Vertical jump height is determined by the product of the total amount of work produced by the muscles during the push-off and the efficacy ratio, denoting which fraction of this work is converted into potential and vertical kinetic energy of the center of mass. The efficacy ratio cannot be unity, if only because at take-off some energy is inevitably present as rotational energy of the body segments, which does not contribute to jump height.

Analysis of work output and efficacy in vertical jumping allows for an interpretation of several aspects of the design of the musculoskeletal system and the pattern of segmental rotations observed *in vivo*. Efficacy potentially benefits from having the body segments with a large mass and moment of inertia located proximally. This potential benefit is realized *in vivo* by a proximo-distal contribution of segmental rotations to vertical acceleration of the center of mass: the push-off is initiated with a rotation of the upper body, and ends with a powerful plantar flexion. The latter helps to constrain the angular velocities of the heavy proximal segments at take-off and therewith the rotational energy in the system. Moreover, it helps to constrain the hip angular velocity and therewith the shortening velocity of the hip extensor muscles, which benefits the force and work output of these muscles. The plantar flexion can be powerful by virtue of elastic recoil of the long tendons of the calf muscles and also by virtue of the bi-articularity of gastrocnemius.

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5.2

The Impact of Post-Genome Science on Comparative Physiology: Model Species and 'Bespoke' Solutions

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Genome science has now come of age, with the more or less complete listing of genes in a few 'model' species. The resulting catalogue identifies novel genes and allows details of genome architecture and evolution to be addressed. More significantly, the expression of these genes and their encoded proteins can now be profiled at the level of the whole system, particularly in respect of specific phenotypic features and physiological responses. These well resourced 'model' species thus provide profound insights into fundamental mechanisms, many with direct relevance to species of comparative interest. However, the expression profiling techniques can also used to screen non-model species, the core requirement being a 'bespoke' collection of species-specific cDNA clones. Protein expression profiling by new mass spectrometric methods can also be used for non-model species. Together these screening methods offer new opportunities for mechanistic investigation, describing responses in unsurpassed detail and with broad if not total coverage of large-scale systems. Just as screening methods have assisted development biology, these transcript and protein profiling techniques will greatly enhance discovery of responses in comparative and environmental physiology.

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5.3

Gene expression profiling of aging and its retardation by caloric restriction. **Tomas Prolla**. University of Wisconsin- Madison.

To examine molecular events associated with aging and its retardation by caloric restriction (CR). we have employed high-density oligonucleotide arrays providing data on thousands of genes to define transcriptional patterns in two brain regions (cerebellum and neocortex), skeletal muscle, and cardiac muscle. Male C57BL/6 mice were either fed normally or subjected to CR. To investigate aging, 5 month (young adult) and 30 month-old normally fed mice were compared. To study CR, 30 month-old control and CR mice were compared. In both brain regions, aging resulted in a gene expression profile suggestive of a marked inflammatory response, oxidative stress and reduced neuronal plasticity and neurotrophic support. In the brain, CR selectively attenuated the age-associated induction of genes encoding inflammatory and stress responses. In skeletal muscle and heart, aging is also associated with specific transcriptional alterations that are prevented by CR. In addition to providing an improved understanding of the aging process, the use of DNA microarrays generates panels of hundreds of transcriptional biomarkers of molecular aging, providing a new tool to measure biological age on a tissue-specific basis. These studies suggest that genomic approaches may be useful in understanding the molecular basis of the aging process in experimental animals.

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5.4

MOLECULAR DETERMINANTS OF THE HIBERNATING PHENOTYPE. Sandra L. Martin, L. Elaine Epperson and Frank van Breukelen, Department of Cellular and Structural Biology and Program in Molecular Biology, University of Colorado School of Medicine, Denver, CO 80262

Hibernators are unique among mammals in their ability to attain, withstand and reverse low body temperatures. Hibernators repeatedly cycle between body temperatures near zero during torpor and 37°C during euthermy. How do these animals maintain cardiac function, cell integrity, blood fluidity and energetic balance during their prolonged periods at low body temperature, and avoid damage upon rewarming? Hibernation is often considered an example of a unique adaptation for low temperature function in mammals. Although such adaptation is apparent at the level of whole animal physiology, it is surprisingly difficult to demonstrate adaptation at the cellular or biochemical level that improves function in the cold and is unique to hibernators. Rather than adaptation for improved function in the cold, the key molecular adaptations of hibernation may lie in the ability to exploit the cold to depress most aspects of biochemical function, then to rewarm without damage, thereby restoring optimal function of all systems(1). For example, each torpor period is characterized by dramatic reductions in the rates of transcription (2) and translation (3) by a combination of active and passive mechanisms. Yet each of the numerous rewarmings that punctuate the hibernation season fully reactivate these and other biochemical processes. Some of the novel regulation that underlies hibernation occurs via differential modification or expression of proteins; several specific examples of these will be discussed, including proteins with roles in fatty acid metabolism and protein synthesis.

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5.5

MAMMALIAN HIBERNATION THROUGH THE EYES OF mRNA AND PROTEIN EXPRESSION PROFILING. Matthew T. Andrews[1,2], Kevin P. Russeth[2], Aubie K. Shaw[2] and Meaghan M. Tredrea[1], [1]Department of Biology, University of Minnesota Duluth and [2]Department of Biochemistry and Molecular Biology, University of Minnesota School of Medicine, 10 University Drive, Duluth, MN 55812.

The revolution in high-throughput screening technologies has brought genome-wide analysis of gene expression to "non-traditional" biological systems. Understudied plant and animal species with unique physiological and adaptive properties are now on the brink of being explored at a level of detail only previously seen with certain model organisms. Mammalian hibernation is one example of a complex physiological process that is now poised for such an investigation. In a state of deep hibernation, body temperature hovers a few degrees above 0°C, oxygen consumption holds at 1/30 to 1/50 of the aroused condition and heart rate can be as low as 3-10 beats/minute, compared to 200-300 beats/minute when the animal is awake and active. We have begun to characterize the genes responsible for regulating the physiological characteristics of hibernation in the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*, and are using high-throughput genomic and proteomic strategies to identify additional genes and gene products that control the hibernating phenotype. Our findings include gene products that play a role in metabolic rate reduction, fuel selection and low-temperature lipolysis during hibernation. This work was supported by the U.S. Army Research Office (Grant DAAD19-01-1-0014).

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5.6

INSULIN SIGNALING PATHWAYS IN MAMMALIAN HIBERNATORS. Gregory L. Florant, Susan F. Hudachek, Marguerite Kelher, and Scott A. Summers*. Depts. of Biology and *Biochemistry, Colorado State University, Ft. Collins, CO 80523.

Most mammals that hibernate develop many characteristics associated with obesity and insulin resistance. For example, in a three-month period (summer) preceding hibernation, the yellow-bellied marmot (*M. flaviventris*) nearly doubles its body mass, and most of the added mass is fat. We have measured several circulating factors during this weight-gain period including insulin, glucose, free fatty acids, and a blunted clearance of glucose following insulin injection. These observations indicate the development of insulin resistance during this time of year. Furthermore, we found a decrease in expression of the insulin receptor and insulin receptor substrate-1 in skeletal muscle and white adipose tissue. In both tissues there is a significant decrease in the expression of the beta-isoform of the serine/threonine kinase Akt/protein kinase B that coincides with the period of maximal insulin resistance. When marmots are at their maximal weight in autumn, they switch from a lipogenic state to a lipolytic one, hibernate, and voluntarily fast for nearly seven months (October to April). During this fast, blood insulin and glucose concentrations return to basal levels while they rely on fat stores for energy. Concurrently, Akt/Protein Kinase B expression increases in association with the regained insulin sensitivity. We conclude that studying the insulin signaling pathway will provide exciting insights into cellular and molecular responses during periods of low food intake as well as low temperature. Supported by an AHA grant (535669) to SAS and a NIH grant (537714) to GLF.

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5.7

Cellular metabolic responses to hypoxia: role of mitochondria as the cellular site of O_2 sensing. PT Schumacker, Dept. of Medicine, The University of Chicago, Chicago, IL 60637.

Hypoxia elicits adaptive responses at the organismal level, the tissue level, and at the cellular and molecular levels. Within cells, hypoxia increases the expression of glycolytic enzymes and membrane glucose transporters, while it also triggers metabolic hibernation. These responses are adaptive in that they protect ATP supply in the face of more severe O_2 deprivation. Hypoxia also triggers the secretion of Vascular Endothelial Growth Factor (VEGF) and the hormone erythropoietin, which help to augment tissue O_2 supply. Some of these responses are mediated by the activation of the transcription factor Hypoxia Inducible Factor-1 (HIF-1). In the lung, hypoxia triggers hypoxic pulmonary vasoconstriction, which enhances the efficiency of lung gas exchange. Collectively, these responses require an O_2 sensor and an increasing body of data implicates mitochondria as the cellular O_2 sensor responsible for triggering many of these adaptive responses. Hypoxia stimulates a paradoxical increase in mitochondrial ROS production, which is necessary and sufficient to induce a variety of diverse responses to hypoxia including HIF-1 activation, metabolic hibernation and hypoxic pulmonary vasoconstriction .
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5.8

STRESS-INDUCED SIGNALING PATHWAYS ASSOCIATED WITH DEPRESSED METABOLISM AND LOW TEMPERATURE. Hannah V. Carey, Department of Comparative Biosciences, University of Wisconsin School of Veterinary Medicine, Madison, WI 53706.

Each winter mammalian hibernators experience conditions typically associated with physiologic stress in non-hibernating species including drastic changes in body temperature, metabolism, respiration and blood flow. Work in several laboratories is revealing sites of physiologic stress and the defense mechanisms that are utilized to insure survival during and after the hibernation season. The low temperatures of torpor have the potential to exert hypothermic stress to cells due to alterations in membrane fluidity, ion channel function and cytoskeletal integrity, and membrane modifications that may counter these threats have been identified. The transitions into and out of the torpid state may also increase the risk for oxidative stress, especially during entrance and arousal when metabolic demand may exceed the delivery of oxygen and nutrients to tissues. Oxidative stress in hibernators has been detected in gut, brain, brown adipose tissue, liver and plasma, as evidenced by increased lipid peroxidation, urate production, antioxidant enzymes and protein ubiquitylation as well as altered glutathione redox balance, depending on tissue type. Induction of stress proteins and the stress-activated transcription factor, NF-κB occur in intestine of hibernating 13-lined ground squirrels. Transcriptional targets of NF-κB include immune activation and apoptosis, both of which are altered in the gut during hibernation. Taken together, these studies highlight the growing recognition that hibernators may represent a unique natural model for the evolution of endogenous defense mechanisms in response to naturally occurring stress.

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NEUROPEPTIDES INTEGRATING PHYSIOLOGICAL PROCESSES IN INVERTEBRATES: AN EVOLUTIONARY AND COMPARATIVE APPROACH

6.1

New tricks from old animals: the generation and interpretation of positional information in *Hydra*. Thomas C. G. Bosch. Zoological Institute, University Kiel, Germany

A major challenge in analysing position dependent cell differentiation is understanding the information when and where key regulatory genes will be expressed. In the simple freshwater polyp *Hydra*, peptides serve as important signaling molecules in development and differentiation. A systematic approach has revealed that *Hydra* contains several hundreds of peptide signaling molecules; some of them are neuropeptides, others are derived from epithelial cells. Moreover, in a nonbiased ("knowledge-independent") approach we have identified genes from *Hydra* encoding signal molecules and effector genes with no sequence similarity to genes in other organisms. When tested functionally, the novel genes were found to be essential for axial patterning and differentiation of *Hydra* specific characteristics. Experimental analyses of the *cis*-regulatory apparatus of these novel genes reveals target sites for novel *trans* acting factors. The discovery of novel and taxon-specific developmental genes in *Hydra* and other organisms may provide an answer to the question "How are taxon specific features encoded ?".

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6.2

Worms: neural simplicity and neuropeptide complexity. Aaron G. Maule *et al.* Parasitology Research Group, Queen's University Belfast, Belfast BT9 7BL, UK. David P. Thompson *et al.*, Pharmacia Animal Health, Kalamazoo, MI 49001, USA. Tim A. Day *et al.*, Iowa State University, Ames IA, 50011, USA.

Helminths or worms comprise 2 invertebrate phyla, the Nematoda (roundworms) and the Platyhelminthes (flatworms) although structurally they have little in common. They are often grouped together because their parasitic members represent a significant health and economic burden worldwide. With respect to helminth parasite control, motor function is a favoured target for anti-parasitic drugs and studies aimed at characterizing these targets have uncovered surprising complexity. This is especially evident in nematode neuropeptidergic systems that are well developed and functionally multifaceted. The nematode *Caenorhabditis elegans* has c.56 different neuropeptide-encoding genes representing at least 12 different peptide families. Amongst these peptides are families common to all helminths (FMRFamide-related peptides, FaRPs) and others that appear unique to each phylum. For example, flatworm neuropeptide F is an ortholog of the most abundant mammalian brain neuropeptide, neuropeptide Y, yet is absent from nematodes. The largest and most diverse peptide family in nematodes are the FaRPs, comprising 60 members encoded on 22 *C. elegans flp* genes. Although FaRPs occur throughout nematode nervous systems, the expression patterns of individual *flp* genes are often restricted, suggesting each peptide fulfils limited and specific roles. Comparative expression patterns show inter-species variation, highlighting the potential for inter-species functional differences. Although the majority of nematode FaRPs modulate motor activity, they have a limited range of physiological effects, indicating neuropeptide diversity in these worms is not mirrored by cognate receptor diversity. In contrast, only 4 FaRPs have been characterised from flatworms and a single myoexcitatory effect has been identified. Of particular interest has been the identification of cross-phyla activity for FaRPs, not only in flatworms and roundworms, but also in arthropods.

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6.3

Modulation of neuropeptide receptors by gene-related peptides and acid pH

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FMRFamide is known to activate a variety of ion channels in molluscan tissue and our aim was to examine whether responses to this peptide were modulated by other signalling molecules of physiological importance. SEQPDVDDYLRDVVLQSEEPLY ('SEEPLY') is encoded on the FMRFamide gene of the snail *Lymnaea* (Santama and Benjamin, 2000), and we examined its possible co-modulatory effects on a previously characterized Ca^{2+} channel shown to be present on isolated ventricular cells of the heart. We will show that SEEPLY alone has no effect but acts to modulate the ability of FMRFamide to open these Ca^{2+} channels. SEEPLY can both up- and down-regulate FMRFamide-activated Ca^{2+} channel activity depending on the initial $P_{(open)}$ response to FMRFamide (Brezden et al, 1999). A second kind of modulation will be described where acid pH modulates the effects of repetitive application of FMRFamide on a FMRFamide-gated Na^+ channel (FaNaC) that is present on a *Lymnaea* giant neuron (Perry et al, 2001). Acidification prevents the desensitizing of the FMRFamide-induced inward current and is consistent with the hypothesis that FaNaCs share a common ancestry with mammalian acid-sensing ion channels (ASICS).

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6.4

POST-TRANSLATIONAL MODIFICATIONS OF THE CHH/MIH/GIH FAMILY OF SINUS GLAND NEUROPEPTIDE HORMONES. EVOLUTIONARY IMPLICATIONS. A. Huberman, Dept. of Biochemistry, Instituto Nacional de Ciencias Médicas y Nutrición "S. Zubirán", Vasco de Quiroga #15, Tlalpan, Mexico City 14000, Mexico.

Peptidergic neurons of the crustacean X-organ, located in the medulla terminalis of the eyestalk, synthesize a family of neuropeptide hormones essential for the regulation of the physiology and metabolism. They are packaged into neurosecretory granules that through axonic migration are concentrated in a neurohemal organ, the sinus gland, from where they are released directly into the hemolymph by a process of exocytosis.

The family is comprised by the crustacean hyperglycemic hormone (CHH), the molt-inhibiting hormone (MIH), the gonad-inhibiting hormone (GIH), and the mandibular organ-inhibiting hormone (MOIH). They consist of 72-78 residues and always contain six conserved Cys residues that form three disulfide bonds. Among the post-translational modifications we find a N-terminal pyroglutamate residue, a C-terminal amidation and a most unusual isomerization of a L-Phe to a D-Phe residue in the third position of CHH and MIH isomorphs in crayfish and lobsters.

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6.5

Effects Of Adipokinetic Hormones On Reproduction In Insects

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Neuropeptides of the adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) family induce the mobilization of flight substrates such as lipids, carbohydrates and/or proline from energy reserves stored in the fat body. In addition to their energy-mobilizing function, peptides of this family have been shown to inhibit lipid synthesis in locusts, crickets, and mosquitoes. Therefore, these peptides might play an important role in the regulation of insect development and reproduction.

In female crickets, large amounts of lipids are required for a quantitatively normal egg production: four to five days after adult emergence, females of the Mediterranean field cricket, *Gryllus bimaculatus*, carry up to 800 ripe eggs in their ovaries, which is about 25-30% of the total body weight. This egg mass contains approximately 80 mg of lipids. During the first two days of adult life, female crickets synthesize large amounts of lipids which are stored in the fat body and mobilized during the following two days of maximal egg growth, leading to a dramatic increase in ovary size and a marked decrease in fat body lipid content.

The adipokinetic hormone of the cricket, Grybi-AKH, has been shown to effectively inhibit fat body lipid synthesis *in vitro*, but significant effects of injected or topically applied AKH *in vivo* could be also demonstrated. Repeated injections or topical applications of AKH inhibited the egg production of female crickets, demonstrating the capacity of AKH to interfere with reproductive events. The role of adipokinetic hormones in the regulation of insect reproduction and the possible use of AKH and AKH-analogs as insect control agents is discussed.

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6.6

EXPRESSION AND STRUCTURE-FUNCTION STUDIES OF LOCUST ITP: AN ANTIDIURETIC NEUROPEPTIDE RELATED TO SEVERAL MAJOR CRUSTACEAN HORMONES. John E. Phillips. Dept. Of Zoology, Univ. Of B.C. Vancouver, BC, V6T 1Z4., Canada.

Locust (*Schistocerca gregaria*) hindgut is responsible for water/ionic homeostasis, pH regulation, and nitrogen excretion. The dominant transport process is an electrogenic Cl-pump that drives K+ and fluid reabsorption and can be continuously monitored *in vitro* as a short-circuit current. This bioassay was used to identify the major stimulant (ITP) in nervous corpora cardiaca acting on the ileum. ITP is 72 amino acids (aa) long, terminally amidated, and has 3 disulphide bridges which are conserved relative to a large family of crustacean neurohormones that control molting (MIH), reproduction (VIH) and metabolism (CHH). ITP is the first member of this protein family to be synthesized and its cDNA expressed using a plasmid transfection vector. Both transient and stably transformed *Drosophila* Kc1 cells secrete a very active form of ITP (KcITP) that is cleaved from its propeptide correctly at the N-terminus (unlike baculovirus-infected Sf9 cells). This system has allowed us to use site-directed mutagenesis to identify specific amino acids at both the N- and C- termini that are essential for activation of the ITP receptor (ITP-R). Similar studies show that each of the disulphide bridges is required for full activity of KcITP. Inactive mutant forms of ITP were also tested for antagonistic activity on ITP-R stimulated with KcITP: 3 weak antagonists were found. There is as much evolutionary divergence of ITP homologues between major insect orders as from crustacean CHH. Using information from the Drosophila Genome Project, we cloned a *Drosophila* cDNA encoding a dipteran preproITP. There is also new evidence for ITP homologues in Coleoptera and Lepidoptera.. Alternate splicing of different C-termini produces a peptide (ITP-L) that acts as an ITP-R antagonist.

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6.7

Insect adipokinetic hormones: release and integration of flight energy metabolism. Dick J. Van der Horst. Department of Biochemical Physiology. Utrecht University. 3584 CH Utrecht, The Netherlands.

Insect flight involves mobilization, transport and utilization of endogenous energy reserves at extremely high rates. Peptide adipokinetic hormones (AKHs), synthesized and stored in neuroendocrine cells, integrate flight energy metabolism. The complex multifactorial control mechanism for AKH release in the locust includes both stimulatory and inhibitory factors. The AKHs are synthesized continuously, resulting in an accumulation of AKH-containing secretory granules. Additionally, secretory material is stored in intracisternal granules. This strategy allows the adipokinetic cells to comply with large variations in secretory demands; changes in secretory activity do not affect the rate of hormone biosynthesis.

AKH-induced lipid release from fat body target cells has revealed a novel concept for lipid transport in the circulatory system. Similar to sustained locomotion in mammals, insect flight activity is powered by oxidation of free fatty acids derived from endogenous reserves of triacylglycerol. The transport form of the lipid, however, is diacylglycerol (DAG), which is delivered to the flight muscles associated with lipoproteins. While DAG is loaded onto the multifunctional insect lipoprotein, high-density lipophorin (HDLp), multiple copies of the exchangeable apolipoprotein III associate reversibly with the expanding particle. The resulting low-density lipophorin (LDLp) specifically shuttles the DAG to the working muscles.

Many structural elements of the lipoprotein system in insects appear to be similar to that in mammals; the functioning of the insect lipoprotein in energy transport during flight activity, however, is intriguingly different.

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6.8

Insect allatostatin: evolutionary trends and multifunctional tasks

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Neuropeptides control key functions in the life cycle of insects. Factors that stimulate (allatotropins) or inhibit (allatostatins) the activity of the juvenile hormone (JH) producing corpora allata (CA) have been isolated from a variety of insects. The allatoregulating peptides can be classified into four groups: an allatotropin (Manse-AT) and an allatostatin (Manse-AS) from *Manduca sexta* that both seem to act as allatoregulators only in lepidopterans; the FGL-amide allatostatins, originally isolated from cockroaches which inhibit JH biosynthesis in cockroaches and crickets, and the W^2W^9-amides where allatostatic functions seems to be restricted to crickets. The finding of allatoregulating peptides in insect species where they do not act on the CA – and also in non-insect invertebrates – suggest other functions such as neuro- and myomodulatory or ecdysiostatic activity. Considerable progress has been made in the characterization of these neuropeptides and their genes, but also in the structure elucidation of their receptors. Our knowledge on the mechanisms of action of the peptides, however, is still poor.

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7.1

Plasma Membrane Rafts of Rainbow Trout are Subject to Thermal Acclimation.

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Rafts are cholesterol- and sphingolipid-enriched microdomains of the plasma membrane (PM) that organize many signal transduction pathways. Interactions between cholesterol and saturated lipids lead to patches of liquid ordered (L_o) membrane (rafts) phase separating from the remaining PM. Phase behavior is temperature sensitive and acute changes in temperature experienced by poikilotherms would be expected to perturb raft structure, necessitating an acclimatory response. An increase in PM cholesterol concentration with acclimation to elevated temperature has classically been explained in terms of fluidity homeostasis but may, instead, be required to stabilize raft structure. We present evidence in favor of this view using hepatocyte plasma membranes from rainbow trout acclimated to 5 and 20°C. Increases in cholesterol concentration associated with acclimation to 20°C were 3.4 times greater in raft compared to PM. Using detergent solubility as a probe of intermolecular interaction strength, and thus the tendency to form the L_o phase, we show that raft, but not PM or non-raft PM, show acclimatory compensation of this property. Furthermore, we present infrared spectroscopic evidence that the L_o phase melting temperature of rafts from 5°C-acclimated fish is very close to their acclimation temperature, emphasizing the need to restructure them during acclimation to elevated temperatures. This work was supported by NSF grant IBN 9816438.

7.2

The Heat Shock Response in Gastropods (Genus *Tegula*): From Promoters to Intertidal Zonation

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Temperate and subtropical species of the genus *Tegula* occupy widely differing thermal environments from the subtidal to the mid-intertidal zone. We investigated possible mechanisms at the biochemical and molecular level that could limit the thermal niche of these species. We focused on the heat-shock or stress response - which is characterized by the increased synthesis of heat-shock proteins (hsps) - because its properties have been shown to play an important role in setting the upper thermal limits of organisms. We studied interspecific variation in the response, its regulation and its importance in setting limits to distribution ranges. Interspecific differences in onset, maximum and cessation temperature of hsp70 synthesis as well as in endogenous levels of hsp70 and heat-shock transcription factor 1 (HSF1) under laboratory and field conditions correlate with vertical position along the subtidal to intertidal gradient. Quantifying endogenous levels of hsp70, hsp90 and HSF1 we found that acclimation-induced but not interspecific variation in the onset temperature of the response could be explained with a limited version of the 'cellular thermostat' model for the transcriptional regulation of hsp70 synthesis. Furthermore, differing levels of HSF1 in combination with variation in promoter sequences of hsp70 could be responsible for the interspecific differences in the stress responses.

7.3

Effect of Intermittent Hypoxia on the Estuarine Teleost, *Gillichthys mirabilis*

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Intertidal environments are subject to large fluctuations in oxygen tension driven by algal photosynthesis and respiration. The effects of acute hypoxia on fishes have been studied previously, but few have considered the physiological consequences of these daily oxygen oscillations. The hypothesis of this study was that daily exposure to moderate hypoxia pre-acclimates teleosts for severe hypoxia itself. Metabolic rate (VO_2) was measured in the estuarine teleost, *Gillichthys mirabilis*, under three experimental conditions: (1) 7 days of normoxia (Po_2 = 150mmHg), (2) 7 days of chronic moderate hypoxia (Po_2 = 30mmHg); and (3) 7 days of moderate intermittent hypoxia (12hr @ Po_2 = 150mmHg : 12hr@ Po_2 = 30mmHg). Each condition was followed by 12hr of acute severe hypoxia (Po_2 = 15mmHg), and 2hr normoxia. In the intermittently hypoxic group, VO_2 decreased by approximately 30% during moderate hypoxia, relative to normoxia each day, even though the resting normoxic VO_2 was variable from day to day. During severe hypoxia in all three groups, VO_2 decreased by approximately 50%, relative to resting VO_2, suggesting that the metabolic response to a given oxygen tension lacks plasticity. However, the ability to recover to resting or near resting VO_2 was variable between the groups (the normoxic group had recovered slowest). Thus, the physiological adjustments that occur during hypoxic exposure may be of greater importance for recovery than for acclimatization. (Funded by an NSF Post-doctoral Fellowship).

7.4

NEUROTRANSMITTER RECEPTORS IN NOS-EXPRESSING NEURONS OF THE RAT GLOSSOPHARYNGEAL NERVE

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Nitric oxide (NO) has been implicated as an important neurotransmitter involved in the efferent inhibition of carotid body (CB) O_2-chemoreceptors. An extensive plexus of NO synthase (NOS)-containing nerve fibers projects to the CB. Autonomic fibers from neurons located in the glossopharyngeal nerve (GPN) contribute to this plexus. In the present study we examined the properties of cultured GPN neurons that expressed NOS and innervated the CB, consistent with their involvement in chemoreceptor inhibition. In addition to possessing O_2-sensing mechanisms, these neurons were sensitive to a variety of neurotransmitters. ATP application evoked inward currents (EC_{50} ~ 2 M) that were partially inhibited by suramin (IC_{50} ~ 5 M). GPN neurons expressed positive $P2X_2$ and $P2X_3$ immunoreactivity (IR) and were surrounded by $P2X_7$-IR processes. Some neurons were sensitive to 5-HT (50 M) and displayed spontaneous activity that was reversibly inhibited by 5-HT receptor antagonists. In addition, other neurotransmitters such as DA (100 M) and ACh (50 M) modulated membrane potential in GPN neurons. Taken together these results suggest the involvement of diverse neurotransmitter pathway in the activation/modulation of the GPN inhibitory input to the rat CB. Supported by NSERC Canada, CIHR and The SIDS Foundation-Canada.

7.5

Variation in Oxygen Sensitivity in Insects of Different Size and Age

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A prominent theory asserts that larger insects may have difficulty with oxygen delivery, due to increased tracheal distances. To test this hypothesis, we exposed insects to decreasing atmospheric oxygen and measured CO_2 emission using flow-through respirometry. We determined the critical PO_2 for each group of insects as the PO_2 below which CO_2 emission decreases. We predicted that if larger insects had difficulty with gas exchange, they would have higher critical PO_2s. Contrary to the prediction, we found that in developing grasshoppers, critical PO_2 actually decreases with size due to the development of a ventilatory response to hypoxia characterized by increased abdominal pumping and tidal volumes. Also contrary to the prediction, we found that in growing caterpillars critical PO_2s did not vary with size, despite the lack of obvious ventilatory movements. However, these effects may have been due to development and not body size per se. Therefore, we also determined critical PO_2s for seven species of adult grasshoppers (mass range 0.2-6.5g). The largest species had the highest critical PO_2 and the smallest species had the lowest critical PO_2, as would follow from the prediction. However, when the largest species was removed, there was no clear relationship between size and critical PO_2. Together these data do not support the hypothesis that oxygen delivery is more difficult for larger insects, at least during rest. This work was supported by the National Science Foundation.

7.6

Regulation of the cardiovascular system of common carp (*Cyprinus carpio*) during severe hypoxia at three acclimation temperatures

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In vivo measurements of the cardiovascular responses of anoxia-tolerant teleosts to a prolonged exposure of severe hypoxia are limited. We present the first direct measurements of cardiovascular status during prolonged severe hypoxia in the anoxia-tolerant teleost, common carp (*Cyprinus carpio* L.), describing an appreciable cardiac depression and increased peripheral resistance at acclimation temperatures of 5, 10, and 15°C. Calculations of cardiac power output, however, reveal that despite the large cardiac down-regulation occurring with severe hypoxia, a Pasteur effect is required to maintain cardiac function. Serial intra-arterial injections of -adrenergic, cholinergic, purinergic, and -adrenergic antagonistic drugs during severe hypoxia revealed that autonomic control of the cardiovascular system was conserved at all three acclimation temperatures. Specifically, injection of the -antagonist phentolamine, which decreased systemic resistance and increased heart rate, illustrated that a strong -adrenergic tone and barostatic reflex were retained during severe hypoxia. Atropine injection after -adrenergic blockade did not further increase cardiac activity, while injection of the -adrenergic antagonist propranolol reduced cardiac status to routine hypoxic levels indicating a large -adrenergic tonus on the heart. Injection of the purinergic antagonist aminophylline in hypoxic fish had no cardiovascular effects, but increased respiration rate. Funded by NSERC and a SFU Graduate Fellowship awarded to J.A.W.S.

7.7

Evaluation of Na^+, K^+, Cl^- and H^+ transport across the apical membrane in Malpighian (renal) tubule cells of *Rhodnius prolixus*.

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The current model of serotonin-stimulated fluid secretion in *Rhodnius prolixus* Malpighian tubule cells proposes that transport of Na^+, K^+, Cl^- and osmotically-obliged water involves an H^+-ATPase, Na^+ (or K^+)/H^+ exchanger and Cl^- channels in the apical membrane, and bumetanide-sensitive Na^+:K^+:2Cl^- cotransport across the basolateral membrane. Thermodynamic feasibility of proposed transporters were studied by calculating electrochemical potentials for each ion across the apical membrane. Net electrochemical potentials for each proposed transporter were then calculated. For this, measurements of lumenal Na^+, K^+, Cl^- and H^+ and intracellular H^+ activities were performed using ion selective microelectrodes. Intracellular and lumenal of H^+ activity were measured using double-barreled ion-selective microelectrodes allowing simultaneous measurement of ion activity and membrane potential. Lumenal Na^+, K^+ and Cl^- activities were measured using single-barreled ion-selective microelectrodes. Results were combined with published intracellular Na^+, K^+ and Cl^- activities to calculate the electrochemical gradient for each ion. Preliminary results show a large lumen to cell directed H^+ electrochemical potential. Both K^+ and Na^+ electrochemical potentials favour movement from lumen to cell, consistent with active Na^+ and K^+ transport by exchange for lumenal H^+. In contrast, Cl^- electrochemical potentials indicate passive transport from cell to lumen. Supported by NSERC (Canada) grants to MJO.

7.8

The effects of amino acids on ion transport and fluid secretion in the Malpighian tubules of *Rhodnius prolixus*.

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One striking feature of insect haemolymph is that it contains a total concentration of amino acids (a.a.) 50-100 times that which is normal for mammalian plasma. We studied the role of a.a. role in fluid secretion and ion transport in Malpighian tubules (MT). *Rhodnius* MTs stimulated with serotonin or cAMP in a.a. –free saline secrete at very high rates for the first 30 mins, then decrease to a stable plateau value (~30% of peak rate) approximately 90 min after stimulation. Addition of specific a.a., in particular glutamine (gln) to the bathing saline, restores secretion rates to within ~5% of their maximal rate. Moreover, pre-incubating MTs in gln and then subsequently running them in a.a.-free saline produces similar effects, suggesting long-lasting effects of exposure to gln. The mechanism of a.a stimulation is unknown. MTs secrete at marginal rates in presence of gln and absence of glucose. Dramatic changes in Na^+/K^+ ratio and pH of the secreted fluid in response to gln suggests that a.a. may modulate specific ion transporters rather than exert a general effect on metabolism. Stimulation by a.a. in presence of cycloheximde or colchicine suggests that protein synthesis or microtubule polymerization, respectively, are not required We are currently examining the roles of intracellular second messengers and kinases (e.g. osmosignaling pathways involving mitogen-activated protein kinases) in mediating gln effects. Supported by NSERC (Canada) grants to MJO.

7.9

Reduced GFR During Cold Acclimation of Freeze-Tolerant Cope's Gray Treefrog Helps to Conserve Circulating Cryoprotectant Glycerol

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Freeze-tolerant Cope's grey treefrog, Hyla chrysoscelis, accumulates high plasma concentrations of cryoprotective glycerol during weeks of cold acclimation. We hypothesized that H. chrysoscelis must possess mechanisms to prevent urinary loss of this small solute. Moreover, we hypothesized that similar mechanisms would be induced by dehydration, from which freeze tolerance may have evolved. To test this, we measured glomerular filtration rate (GFR, inulin clearance), urine flow rate (UFR), and plasma and urine concentrations of glycerol (Pgl and Ugl). Hydrated frogs at 23C had GFR = 215 µl/h, UFR = 85 µl/h, and low levels of glycerol in plasma (0.18 mM) and urine (0.3 mM). Frogs dehydrated by 15-20% over about 2 d at the same temperature reduced GFR (by 84%) and UFR (by 97.9%), but Pgl and Ugl remained not significantly changed from the hydrated condition (0.4 and 0.5 mM, respectively). Like dehydration, cold (3 months at 2-5C) induced a marked reduction in GFR (to 27 µl/h) and UFR (to 3.4 µl/h). However, hydrated, cold-acclimated frogs also had markedly elevated Pgl (50 mM) and Ugl (27 mM). The glycerol measured in the urine of cold-acclimated frogs represented ~7% of the filtered load, suggesting that the animals reabsorbed this solute from the renal tubules or the urinary bladder. The combination of reduced filtration and solute reabsorption allow the frogs to retain glycerol during the weeks during which it circulates at high concentration.

7.10

Osmoregulation in Avian Nectarivores - an Integrative Approach

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Nectar-feeding birds must often deal with large ingested water loads in order to meet their energetic demands. We investigated the importance of the integration of digestive and renal function for maintaining water homeostasis in these animals. We compared the fractional absorption of water from the gastrointestinal tracts of Broad-tailed Hummingbirds (*Selasphorus platycercus*) and Palestine Sunbirds (*Nectarinia osea*). Hummingbirds absorbed the majority of dietary water (~ 80%) regardless of the amount ingested, while sunbirds appeared to be able to modulate absorption of water across the intestine. Fractional water absorption by sunbirds decreased from 100% to 33% as water intake increased, allowing them to dispose of significant amounts of ingested water at the supply side when feeding on dilute nectars. To our knowledge this is the first documentation of adaptive regulation of water flux from the gastrointestinal tract to the body. Glomerular filtration rate in Palestine Sunbirds was 42% of that predicted based on body mass (1.69 ± 0.90 mL h-1, mean ± SD) and was not significantly correlated with water intake. Our results suggest that renal processing of water and recovery of filtered glucose may not limit energy assimilation in sunbirds because GFR, and thus glucose filtered load, remains relatively low even when birds are faced with large dietary water loads. Reseach was funded by the US-Israel Binational Science Foundation (BSF 98-178).

7.11

Saluretic Actions of Acutely Elevated Vasopressin in Fasting Northern Elephant Seals

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In a previous study, vasopressin (VP) was suggested to possess antidiuretic and natriuretic functions as well as play a role in the elevation of glomerular filtration rate (GFR) in fasting northern elephant seal pups (*Mirounga angustirostris*). Therefore, to examine the renal actions of VP in fasting seals, pups (n = 7; 99 ± 4 kg) were infused with VP (20 ng kg^{-1} bolus, then 0.6 ng kg^{-1} mL^{-1} for 34 min). Renal and hormonal responses were quantified for 24 h prior to infusion (control) and for 24 h post-infusion. VP increased urine output and osmotic clearance by 69 ± 18 % (± SE) and 36 ± 10 %, respectively, but did not change free water clearance, GFR or excreted cAMP. Osmolal and electrolyte (Na^+, K^+, Cl^-) excretion, and fractional excretion of electrolytes were also increased when compared to control. Excreted cortisol and plasma aldosterone and cortisol were increased while plasma renin activity (PRA) was decreased. The increase in cortisol suggests that VP may possess corticotropin releasing hormone-like activity in elephant seals. If osmotic diuresis, natriuresis, and suppressed PRA are typical consequences of elevated VP, then it would behoove pups not to increase [VP] during the fast in order to avoid the potential loss of Na+ induced by elevated VP. Therefore, under natural fasting conditions, pups may be highly sensitive to low [VP] resulting in the maintenance of water balance. VP appears to be more dynamic in marine mammals than originally believed.

7.12

Elimination of plant toxins: an explanation for dietary specialization in mammalian herbivores?

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Constraints on rates of elimination of plant toxins are hypothesized to be responsible for limiting dietary specialization in mammalian herbivores. This hypothesis, suggests that most mammalian herbivores are generalists so as to avoid overdosing on toxins from a single plant species. The hypothesis also predicts that the few mammalian specialists that exist should have adaptations for rapid detoxification and elimination of plant toxins. We took a pharmacological approach to test whether a juniper specialist, Neotoma stephensi, eliminates toxins from the body faster than a generalist, N. albigula. We compared blood elimination rate, total toxin exposure and fecal elimination of alpha-pinene in specialists and generalists orally dosed with alpha-pinene, a toxin present in juniper, or fed a diet containing juniper. We found that specialists and generalists did not differ in the elimination rate of alpha-pinene from the blood. However, specialists had 5.2 X lower exposure levels of alpha-pinene than generalists due to lower initial delivery of alpha-pinene to the blood. In addition, we found that alpha-pinene concentration in the feces is 2X greater in specialists than generalists. These results demonstrate that specialists may decrease toxin exposure by reducing absorption of toxins in the gut. Lower exposure to plant toxins may allow specialists to forage on diets with high toxin concentrations thereby facilitating dietary specialization. Funded by NSF-IBN0079865.

7.13

Photoperiod-induced weight loss in lemmings is due to an increase in energy expenditure.

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Background: We have previously shown that weight gain in collared lemmings (*Dicrostonyx groenlandicus*) in response to decreasing daylength is due to a decrease in resting energy expenditure, rather than an increase in food intake.

Objective: To examine whether the weight loss induced by increasing daylength in collared lemmings was due to changes in energy expenditure (EE) or food intake.

Methods: Sixteen male lemmings were maintained in short day (SD, 8L:16D) until 11 weeks post-weaning. Total and resting energy expenditure (REE) and food intake were measured on day 0, after which 8 lemmings remained in short day (SD-SD), and 8 were transferred to long day (22L:2D) (SD-LD), with repeated measurements on day 28.

Statistics: Changes in energy expenditure, body weight, and food intake were assessed using paired t-tests within each group.

Results: Between days 0 and 28 there was a significant decrease in body weight in the SD-LD lemmings (10.8 ± 1.0 g, $P<0.001$), but not in the SD-SD lemmings ($P=0.97$). There was a borderline significant increase in REE in the SD-LD lemmings (6.2 ± 3 kJ/day, $P=0.076$), but not in the SD-SD lemmings ($P=0.77$). Food intake did not change in either the SD-SD or SD-LD lemmings ($P>0.8$).

Discussion: Collared lemmings lose weight when exposed to LD, and our results suggest that this is achieved by increasing EE, mainly REE, rather than by decreasing food intake. *Funded by NIH DK54918.*

7.14

Shunting in alligators - does it make a difference?

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Crocodilians are unique in their cardiac anatomy and physiology. Unlike other reptiles, they have a completely divided heart but have retained the ability to shunt right ventricular blood to the body via the left aorta (LAo). The left aorta continues posteriorly and supplies the digestive system with right ventricular blood during shunting. This study focuses on the functional significance of the ability to shunt right ventricular blood to the digestive system in *Alligator mississippiensis*. Two groups of alligators were used in this study. One group had their left aorta surgically tied off and cut (LAo cut; unable to shunt) and the second group had sham surgeries performed on them (LAo intact). Growth rates, food intake, metabolic rates, venous blood pH, venous blood gases and digestion rates were some of the variables measured to determine the importance of the ability to shunt right ventricular blood to the digestive system. During fasting, blood pH and PCO_2 values and metabolic rates were similar in the two groups of alligators, as were the rates of mass loss. During feeding, growth rates were similar. Respiratory exchange ratios were significantly higher in alligators with their LAo cut. Differences were also seen in blood pH and blood PCO_2 measurements as well as in digestion rates. In conclusion, the ability to shunt right ventricular blood to the gut affects acid-base regulation and digestive state in alligators. This study was supported by NSERC.

7.15

Function Of The Hammerhead Shark Cephalofoil

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The adaptive function of the unique, wing-like cephalofoil head morphology of hammerhead sharks (Chondrichthyes, Sphyrnidae) has been the subject of much speculation but little empirical testing. The scalloped hammerhead shark, *Sphyrna lewini*, and the closely related sandbar shark, *Carcharhinus plumbeus*, were used to test the "enhanced electrosensory" and the "improved hydrodynamic maneuverability" hypotheses. The electrosensory hypothesis was tested by comparing the electrosensory pore distribution of the two species as well as behavioral responses to prey-simulating electric fields. The maneuverability hypothesis was tested by quantifying the dorsal surface area of the cephalofoil and pectoral fins during straight line swimming and during execution of a sharp (90°) turn. The cephalofoil is a better detector of cryptic prey than a conventional head shape, sweeping a broader area with no loss in sensitivity. In addition, hammerheads can detect cryptic prey 1.4 times further from the swimming path than sandbar sharks. The cephalofoil is maintained in the horizontal plane during straight line swimming and during turning indicating that it is not used to bank and roll during a turn, thus falsifying the maneuverability hypothesis. Maintaining the head in a horizontal plane keeps the electrosensory system as close as possible to the substratum for localizing benthic prey even during a turn. Partial funding for this project was provided by the Raney Fund and the Lord Scholarship.

7.16

Effects of Feeding on Strong Ions and Blood Gases in *Varanus exanthematicus*

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Intermittent feeding is characterized by long fasts between large meals. This imposes a number of challenges including an increased metabolic rate and an alkaline tide associated with acid being shifted into the stomach for digestion. Previously we have reported a three-fold increase in metabolic rate following ingestion of a meal equivalent to 10% body mass in *Varanus exanthematicus*, as well as a small, but significant increase in pH peaking earlier than metabolic rate. Here we test the hypothesis that the alkaline tide in *V. exanthematicus* is countered by a respiratory acidosis prior to the peak in postprandial oxygen consumption. Following feeding, metabolic rate increased 2 fold over resting levels at 12 hrs, 3 fold at 15 hrs remaining elevated through 24 hrs (0.98±0.3, 2.0±0.7, 2.8±1 ml min^{-1} respectively). Respiratory exchange ratio, PaO_2, plasma $[K^+]$, [Lactate], $[HCO_3^-]$, and $[Cl^-]$ did not change. $PaCO_2$ increased 2 hrs postfeeding remaining elevated through 24 hrs (27.8±2.7 to 35±2 torr). Plasma $[Na^+]$ increased above fasting levels at 18 hrs postfeeding (142±1.1 to 147±3 mmol l^{-1}) and arterial pH increased above fasting levels at 15 hrs postfeeding (7.37±0.05 to 7.48±0.03). While there is a significant increase in pHa postfeeding, this is compensated by increased $PaCO_2$. We conclude that the large alkaline tide, thought to be uniform across reptiles, can be partially compensated through a respiratory acidosis. Funded by NSF IBN-9727762 to AFB and JWH.

7.17

The Effects of Pregnancy on Ventilation and Oxygen Consumption in the Lizard, *Tiliqua rugosa*.

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Tiliqua rugosa, a large viviparous skink, gives birth to 1-4 young after a gestation of 5-6 months. At birth the young are large, weighing 89.5±5.9g which represents 21.6±2.6% of maternal body mass. During development the young occupy an increasingly large proportion of the body cavity. CT scans of pregnant lizards revealed that the lungs were compressed and/or regionally collapsed by the developing fetuses, potentially compromising ventilation. To quantify the effects of lung compression, ventilation and oxygen consumption were measured in pregnant females throughout the last 50% of gestation, and in non-pregnant females and males. As gestation progressed both minute ventilation and tidal volume decreased. No compensatory changes in breathing frequency or in the duration of the non-ventilatory period were detected. Oxygen consumption did not change during gestation reflecting a relative hypoventilation and a decrease in the air convection requirement. Inhalation of hypoxic hypercapnic gas (13% O_2, 5% CO_2 in N_2) caused an increase in oxygen consumption but no changes in breathing pattern, suggesting that pregnant lizards primarily use cardiovascular rather than ventilatory adjustments in response to a respiratory stress. Compression of the lungs during pregnancy, and the resulting changes in ventilation, may reduce the aerobic scope for exercise and limit the ability to forage for food and escape predators. (Funded by the Australian Research Council).

7.18

Swimming Effects on Metabolic Recovery From Anoxia in Turtles

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Anoxic submergence in the western painted turtle causes a severe metabolic acidosis characterized by high plasma lactate and depressed arterial pH, a response similar to fishes and mammals after exhaustive exercise. We tested the hypothesis that sustained aerobic swimming after anoxia would enhance the rate of lactate disappearance from the blood, as it does in mammals and fishes after strenuous exercise. After two hours of anoxic submergence at 25°C and one hour of recovery, the pattern of plasma lactate disappearance in turtles previously trained to swim in a flume and swam aerobically for one hour did not differ significantly from that in trained and untrained non-swimming turtles. The response patterns also did not differ for arterial Po_2, Pco_2, pH, $[HCO_3^-]$ or plasma glucose between groups. These data suggest sustained activity following anoxia is not fueled by lactate, but by some other substrate such as fatty acids, which would be an abundant energy source after a winter during which ATP was generated principally through anaerobic processes utilizing carbohydrates.

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7.19

Molecular cloning of Multi-drug resistant (MDR) transporter cDNAs in the cabbage looper, *Trichoplusia ni*.

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Multi-drug resistant (MDR) transporters have been cloned from a variety of organisms from vertebrates to insects. It has been proposed that this transporter may be a potent means for elimination of toxic compounds and pesticide resistance in insects. Previous studies have shown that the Malpighian tubules (MTs) have the ability to transport various MDR substrates. In the present study we are attempting to clone cDNAs for the MDR transporter from the cabbage looper *T. ni*. Using 3 degenerate oligonucleotides derived from an alignment of highly conserved regions of hamster and fruit fly MDR genes we have isolated two 700 base pair (bp) and three 200 bp partial sequence fragments. From these partial sequences, gene specific primers were designed and used for 5 and 3 RACE. We currently have 2 partially cloned and sequenced gene fragments that correspond to our initial 700 bp sequence fragments. Both sequenced fragments have significant identity to several other MDR transporter cDNAs including 49% and 53% with MDR49 and MDR50 respectively from the fruit fly *Drosophila melanogaster*, and 49% with MDR3 from the mouse *Mus musculus*. Using gene-specific primers we have probed first strand cDNA reverse transcribed from total RNA from *T. ni*. salivary gland, hindgut, midgut, muscle, fat body, nervous tissue and MTs. These RT-PCR experiments have shown identifiable expression patterns for potential MDR genes in all tissues studied. Supported by NSERC (Canada) grants to MJO.

7.20

Comparative Effects Of The Anesthetics Brevital And Isofluorane On Cardiovascular Function In The Turtle.

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We compared the effects of mean circulatory filling pressure (MCFP) on cardiac performance in the turtle, Trachemys scripta, using two common chelonian anesthetics, Sodium Brevital (30 mg kg-1) and isofluorane (0.5-1.0% in 5% CO_2/95% O_2). MCFP was assessed as the central venous pressure during temporary occlusion of cardiac outflow tracts, and was varied by removing blood or adding albumen-containing Ringer's solution. With isofluorane, hearts exhibited a greater dynamic range with total cardiac output (CO) varying from 45-165 ml kg-1 min-1 between MCFP of 0.7-4.5 cmH2O. With Brevital, CO fell sharply at low MCFP and plateaued at only 90 ml kg-1 min-1 between MCFP of 3-9 cmH2O. Total peripheral resistance (TPR) rose steeply in the Brevital animals when blood was removed and during volume loading was some two-fold higher than in the isofluorane animals. These results suggest an adverse effect of Brevital that heightened vascular reactivity. In addition, although heart rates were not different in the two groups, ECG parameters differed in important respects. The duration of the QRS complex was higher and the duration of the T wave was lower in the isofluorane group. In addition signal-to-noise was much lower in the isofluorane animals. (Supported by NSF IBN-0078094, NSF IBN- 0110322 and the Danish Research Council).

7.21

Does Chronic Hypoxia During Postnatal Development Elicit Long-Lasting Changes in Chemosensitivity in Rats?

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The respiratory environment experienced during development can have long-lasting effects on the control of breathing. Rats exposed to neonatal hypoxia (FIO_2= 0.10, 1 week) may exhibit blunted hypoxic ventilatory responses into adulthood (Okubo & Mortola, *Am. J. Physiol.* 259: R836-R841, 1990). We asked whether this blunting results from a change in the chemical control of breathing *versus* changes in respiratory mechanics. Male rat pups were exposed to FIO_2= 0.10 for the first postnatal week and hypoxic responses were assessed at 50-60 days of age. To determine hypoxic chemoreflexes without complications attendant to changes in respiratory mechanics, respiratory motor output was recorded directly from the phrenic nerve of anesthetized, paralyzed, vagotomized and pump-ventilated rats. Hypoxic phrenic responses were not significantly different from those of age-matched control rats at three levels of isocapnic hypoxia (PaO_2= 40, 50 and 60±3 Torr; P>0.05). However, preliminary plethysmographic and metabolic data are consistent with reduced hypoxic ventilatory responses (FIO_2= 0.12 and 0.10) in awake hypoxia-treated rats. These data suggest that neonatal hypoxia alters hypoxic ventilatory responses through effects on respiratory mechanics rather than changes in chemosensitivity or CNS integration of chemoafferent inputs. This research was supported by NIH Grants HL53319 and HL07654

7.22

Metabolic Indicators in Harbor Seal Muscle Tissue

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The purpose of this study was to map the enzymatic activities of citrate synthase (CS), b-hydroxyacyl CoA dehydrogenase (HOAD) and lactate dehydrogenase (LDH), and myoglobin (Mb) concentration in the swimming muscles of harbor seals (HS). Ten freshly dead HS were sampled as part of a subsistence harvest in Prince Williams Sound, Alaska. The entire swimming (epaxial) muscle was removed and weighed, and three transverse sections (cranial, middle, caudal) were taken along the muscle bundle. Multiple samples (ca. 30 per section) were taken along points on a circular grid using a 6-mm biopsy punch. A spectrophotometric technique was used to determine CS, HOAD and LDH activities and Mb concentration. Mean values were calculated for four roughly equal quadrants (dorsal, ventral, right, left) in each transverse section. There were no significant differences among the quadrants within any of the transverse sections for the three enzymes or Mb. However, there were significant differences in the average transverse section enzyme activities and Mb concentration along the length of the muscle. The cranial section had significantly lower mean CS and LDH activities and Mb concentration than for the other two sections. Average CS and HOAD activities and Mb concentrations in the epaxial (swimming) muscles of the harbor seal were 1.1, 3.2, and 1.3-times greater than in non-swimming muscles, respectively. Funding was provided by the state of Alaska and Texas A&M University.

7.23

Fiber Type Composition in the Swimming Muscles of Harbor Seals (*Phoca vitulina*).

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In pinnipeds, the energy to power skeletal muscle contractions during subsurface swimming is derived primarily from aerobic metabolism. Initial studies in our laboratory using muscle biopsies have shown that the swimming muscles in pinnipeds are well adapted to localized hypoxia and ischemia experienced during diving. The objective of this research was to determine if the physiological indices of aerobic metabolism are consistent throughout the primary locomotory muscles of the harbor seal (*Phoca vitulina*). Data from fiber typing indicated that harbor seal swimming muscle (*Longissimus dorsi*) is comprised of 46.9% type I (slow twitch, oxidative) fibers and 53.0% IIa (fast twitch, oxidative) fibers, which are homogeneously distributed throughout the muscle. No fast twitch, glycolytic (type IIb) fibers were detected in any of the muscle sections from the seals in contrast to the published data on fiber typing of harbor seal *L. dorsi* using traditional histochemical techniques. The extreme specificity inherent to the immunohistochemical fiber typing procedure leads us to conclude that harbor seal swimming muscle is entirely comprised of oxidative fibers. This study is funded by the Exxon Valdez Oil Spill Trustee Council and the Alaska Dept. of Fish and Game.

7.24

Chemosensitivity During Sleep in the Juvenile Harbour Seal (Phoca vitulina richardsi)

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Phocid seals have remarkable breath holding abilities and can tolerate large changes in blood levels of O2 and CO2, suggesting that they have a decreased sensitivity to changes in respiratory drive. This, combined with any reduction in metabolic rate such as occurs during diving or sleep, may lead to episodic breathing. In this study, we tested the above hypothesis using harbour seals (Phoca vitulina). We analyzed the cardio-respiratory patterns of seals under hypoxic and hypercarbic conditions while awake and sleeping on land. Our results show that chemosensitivity while awake is not decreased relative to terrestrial mammals and that overall sensitivity to changes in respiratory drive is unchanged as state of arousal decreases from wake to sleep. Metabolic rate was lower in sleep than wake. The breathing pattern of harbour seals also changed as a function of state of arousal, with wake having shorter and fewer apneas than sleep. The sleep-associated apneas were similar in length to the average dive time of the harbour seal (~3 min). Tachypnea was present at all levels of increased respiratory drive, however hypoxia induced a dramatic bradycardia, while hypercarbia produced a tachycardia, regardless of state. Breathing was rarely seen in REM sleep. Given our results, it seems unlikely that the appearance of long apneas, and episodic breathing during sleep in harbour seals arises from a reduction in metabolic rate. Experiments were funded through the NSERC Canada.

7.25

A Longitudinal Study of Oxygen Store Development in Nursing Harbor Seal Pups

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Harbor seals (Phoca vitulina) are one of the most precocial phocids in that they swim and dive soon after birth. As a result, they may be more physiologically mature prior to weaning than species that delay diving activity until after weaning. As breath-hold ability is partially determined by tissue oxygen stores, characterizing the development of these stores may shed light on how physiology impacts behavior in juvenile marine mammals. This study documents oxygen store development in individual free-ranging pups from birth to weaning (n=37, 4, 3, 1 for 2, 3, 4, 5 captures respectively). Blood oxygen stores were determined by measuring HCT, Hb, and total plasma volume (Evan's blue dye methodology). The development of muscle myoglobin is currently under investigation. Overall, there was little change in the average HCT or Hb values for pups from birth through weaning (HCT 52.5±5.6, 47.3±3.7, 48.8±2.5, 52.2±3.5%; Hb 19.2±2.6, 17.8±2.4, 18.1±1.9, 19.7±2.4 g/dl for animals <5, 6-14, 15-24, >25 days, respectively), with the exception that HCT values of pups 6-14 and 15-24 days of age were significantly lower than pups >25 days old ($p<0.05$). In comparison to data from less precocial species with small oxygen stores, these results suggest that the early diving activity of harbor seal pups is supported by rapid development of tissue oxygen stores. Funding was provided by the Department of Fisheries and Oceans, Mont Joli, Quebec, and University of Waterloo, Ontario, Canada.

7.26

Does Titin Contribute To The Muscle Spring?

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During cyclic muscle use, muscles save energy by storing and recovering elastic strain energy. If titin, the large cytoskeletal protein within muscle is functioning as a locomotor spring, contributing to the storage and recovery of elastic recoil energy, it should be 'tuned' to the frequency of muscle use. Our objective is to test the hypothesis that titin is functioning as a locomotor spring by examining the expression of titin isoforms in muscles used cyclically at different frequencies. We used SDS-PAGE analysis to identify the differential expression of titin isoforms in left ventricle, diaphragm, and vastus lateralus from animals ranging in size from shrew to elephant, as heart rate, respiratory rate and stride frequency are predictable functions of body size. The results show a predictable shift in isoform from most compliant in elephant to most stiff in shrew. These results suggest that titin may be an important and 'tuned' contributor to the muscle-tendon spring. This work was funded by Arizona Proposition 301.

7.27

Biochemistry of Steller Sea Lion Muscle as it Relates to Development of Dive Physiology

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Increased muscle oxygen stores significantly enhance the amount of time pinnipeds can maintain aerobic metabolism while diving. This increase allows individuals to improve foraging efficiency by extending their immersion time. To investigate the development of foraging ability we examined the myoglobin (Mb) concentrations in muscle of 4 week old Steller sea lion pups (n=4). Muscle samples were taken from recently deceased pups found on rookeries in southeast Alaska. Myoglobin was spectrophotometrically assyed using modified methods from Reynarfarje (1963). Concentration of Mb in 4 week old pups was significantly less than values presented in Kanatous et al. (1997) for adult female Steller sea lions ($p=0.000$). In addition, pups in this study showed no difference in Mb concentration between swimming and non-swimming muscles ($p>0.05$) while the adult females in the previously listed study showed significant elevation in the Mb concentration of the swimming muscles. This result is consistent with other developmental studies done on diving species that indicate Mb stores remain at low levels until the onset of foraging (Burns et al. unpub., Dolar et al. 1999). The developmental hypothesis will be further explored with the analysis of oxidative and glycolytic muscle enzymes, and the future sampling of various age classes. Research supported through a grant provided by CIFAR (NA17RJ1224) and with a co-operative agreement through NOAA and ADF&G (NA17FX1079).

7.28

Ontogeny of Diving Bradycardia in Bottlenose Dolphins (Tursiops truncatus)

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Adult cetaceans reduce heart rate (HR) during submergence. Yet little is known regarding the dive response of immature cetaceans. The development of cardiac patterns in bottlenose dolphins was examined by recording HR during stationary respiration, voluntary breath-hold, and shallow diving. All dolphins (ages: 1.7-5.4 years and adults) showed adjustments in HR in response to submergence such that HR was significantly greater during inhalation than during breath-hold. Mean HR during surface respirations and maximum HR during surfacings were also similar across all dolphins. In contrast, the ability to reduce HR while diving changed throughout maturation; mean steady state diving HR and minimum HR during submergence differed significantly between age classes. For example, juvenile age classes, 3.7-4.3 and 4.5-5.4 year olds, had significantly lower mean steady state diving HR (45±1.6 and 43±1.8 beats min^{-1}, respectively) than calf age classes, 1.7-2.3 and 2.6-3.4 year olds (52±1.2 and 51±1.6 beats min^{-1}, respectively). As a result, juveniles and adults had greater reductions in HR while diving than calves. These results demonstrate that dolphins as young as 1.7 years old exhibit elements of cardiac control, but the capacity to reduce HR during submergence improves such that the level of bradycardia during diving increases over the first 3.5 years postpartum. Funding: American Cetacean Society, Lerner-Gray Fund for Marine Research Museum of Natural History, ONR.

7.29

Hypothalamic Thermosensitivity and Body Temperature Set-point Changes in Hypoxic Squirrels

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Hypoxia reduces the lower critical temperature and thermal conductance of golden mantled ground squirrels, and indeed many other small mammals. Hypoxia also induces temporal changes in the peripheral circulation favouring increased cutaneous heat loss, particularly from the feet and ears, the so-called 'thermoregulatory organs'. These results suggest indirectly that hypoxia lowers the set-point for body temperature (T_b) regulation, which in turn reduces metabolic rate (the hypoxic metabolic response, HMR), reducing O_2 demand under conditions of reduced O_2 supply. We tested this hypothesis further by heating and cooling only the preoptic area of the hypothalamus (a centrally thermosensitive area, which is also thought to integrate and regulate T_b set-point) using indwelling thermodes in ground squirrels during normoxia and hypoxia (7% O_2). We found that the lower critical hypothalamic temperature of 38°C in normoxia was reduced to 29°C in hypoxia and that the metabolic thermosensitivity (the change in metabolic rate for any given change in hypothalamic temperature below the lower critical temperature) was reduced 11-fold. Further, peripheral vasomotor control (i.e. heat loss and heat conservation), seems to respond appropriately to hypothalamic warming and cooling in both normoxia and hypoxia. This provides strong support for the hypothesis that the HMR results from an hypoxia-induced reduction in T_b set-point. Research support provided by NSERC.

7.30

Species and Developmental Differences in Respiratory Cold Tolerance: Hibernator Versus Non-hibernator

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Mammals that hibernate also tolerate extreme hypothermia. Adult non-hibernators die from respiratory arrest during hypothermia at high body temperatures whereas their neonates are cold tolerant and able to resist respiratory arrest. This has led to the hypothesis that the ability to hibernate is a retention of neonatal traits into adult life. To examine whether all neonates respond to hypothermia in a similar manner, or whether differences in neonatal tolerance to hypothermia exist between species, we measured the respiratory motor output of the central rhythm generator for breathing in brainstem-spinal cord preparations during hypothermia in developing (days 0-6) hamsters and rats (rodent hibernator and non-hibernator, respectively). The fictive breathing from both species was resistant to cooling, arrested at lower temperatures in older animals, and showed auto-resuscitation on rewarming, demonstrating the ability of all neonates to tolerate hypothermia. None-the-less, fictive bursts of motor activity in the hamster arrested at lower temperatures and were more "excitable" during autoresuscitation. The significance of these differences is not yet clear. Our results suggest that characteristics of cold tolerance in hibernators mirror neonatal traits, supporting the hypothesis that the ability to hibernate may be due to a retention of neonatal traits. Differences, however, exist between these species that could reflect phylogenetic or adaptive differences.

7.31

Oxygen Delivery Problems May Reduce Jumping Performance In Larger Locusts

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Due to longer tracheae, larger insects may have problems matching oxygen delivery to oxygen demand during locomotion. We tested for such a relationship between body size and respiratory function in jumping grasshoppers. In the American locust (*Schistocerca americana*), larger/older grasshoppers fatigued more rapidly during forced jumping. Larger/older grasshoppers had similar jumping muscle to body mass ratios but had reduced mechanical power outputs (calculated from ballistic equations) during jumping. Is the decline in jumping performance due to increased problems with oxygen delivery during development? Larger grasshoppers had proportionally less tracheal air in their femurs and reduced mass-specific tracheal diffusing capacities (measured morphometrically using light microscopy). In adults, leg hemolymph PO_2 (measured with electron paramagnetic resonance) decreased dramatically during jumping and recovered to resting values within 5 minutes. Lactate production rates during the first minute of jumping increased with size/age, suggesting that at least during the initial minutes of jumping, increasing size/age is associated with increasing problems of oxygen delivery. This research is supported by the NSF grants IBN-9985857 to JFH and IBN-0104959 to JFH and SDK.

7.32

Cardiovascular changes induced by voluntary and mechanical ventilation in full term emu embryos (*Dromaius novachollandiae*)

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In avian embryos, the ductus arteriosus and interatrial foramina allow for a right-to-left shunt of right atrial blood away from the lungs and to the body and chorioallantoic membrane (CAM). Changes in the degree of this right-to-left shunt were examined in full term emu (*Dromatus novachollandiae*) during hatching when the site of gas exchange moves from the CAM to the lungs. We examined the distribution of microspheres injected into a CAM vein at each stage from pre-pipped to hatch. During the transition from the pre-pipped condition to internally pipped, the blood flow pattern changed little. Two hours after external pipping, a significant ($p<0.05$) decrease in the right-to-left shunt was observed as a 3.9 fold increase in normalized per gram lung blood flow and a subsequent decrease in systemic blood flow. Upon hatching, the right-to-left shunt disappeared, suggesting complete, quick closure of the ductus arteriosus and interatrial foramina. To examine the potential stimulus for these closures, we artificially pipped and mechanically ventilated day 48 embryos on hypoxic and normoxic air for 5 min each. Mechanical ventilation resulted in a redistribution of right atrial blood flow with a 1.7 fold increase in the direction of the lungs. After 2 more hours of voluntary ventilation, this blood flow pattern remained stable. Between the initiation of external pipping and hatching the ductus arteriosus closes quickly, resulting in circulatory patterns similar to the adult.

7.33

Proteins in plastic and population variation in egg production in grasshoppers

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A major question in evolutionary physiology is how the mechanisms underlying phenotypic variation evolve. We examine simultaneous plastic and interpopulation variation in developmental profiles of hemolymph proteins during egg production in the lubber grasshopper (Romalea microptera). These grasshoppers show plasticity in response to diet of ages at maximum titers of vitellogenin (Vgmax; the egg-yolk precursor) and major hemolymph proteins (MHPmax). Grasshoppers from Florida (FL) and Georgia (GA) are more closely related to each other than to Louisiana grasshoppers (LA), based on mtDNA cytochrome-b sequences. We raised adults to first oviposition on a range of diets, collected hemolymph samples every third day, and analyzed them for Vg and MHP. There were no significant differences in the range of responses across populations, despite predictions that plasticity will prove to be evolutionarily labile. Diets affected reproduction as in previous experiments, and populations differed in their innate reproductive physiologies. Georgia grasshoppers reached Vgmax sooner than both FL ($P = 0.0019$) and LA ($P < 0.0001$) grasshoppers, which did not differ from each other ($P = 0.1886$). In contrast, age at MHPmax was not affected by population ($P = 0.2618$). These data suggest that variation in Vg timing across populations is correlated with growing season but not with phylogeny. Selection may have altered Vg timing but neither reproductive plasticity nor MHP timing. (NSF DBI-9978810)

7.34

Molecular chaperone activity in ectothermic animals: Temperature sensitivity of Hsc70 orthologues from perciform fishes

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We characterized the temperature sensitivity of Hsc70 purified from perciform fishes that inhabit different thermal environments. Hsc70 was purified from two phylogenetically related notothenioids with distinct thermal environments, *Trematomus bernacchii* and *Notothenia angustata* as well as a eurythermal goby, *Gillichthys mirabilis* that encounters temperatures at the lower end of its range which overlap with those of *N. angustata*. The thermal stability of the ATPase domain of Hsc70 was compared using an *in vitro* ^{32}P-ATP based assay. The results showed Hsc70 from *T. bernacchii*, *N. angustata*, and *G. mirabilis* lost the ability to hydrolyze ATP at 67.5, 60, and 65 °C, respectively. Our data indicate that, although the Hsc70 orthologues maintain an active ATPase domain at temperatures greatly exceeding the environmental temperature range of these fish, it is not a hyper-stable molecule. In addition, we measured the sensitivity of the substrate-binding domain by determining the ability of Hsc70 to inhibit thermal denaturation of proteins and to refold denatured proteins *in vitro*. The results of these functional assays showed Hsc70 from all species was able to extend the half life of luciferase at 38 °C, $T_{1/2}$ = 10 min, as compared to control values of $T_{1/2}$ = 4 min. These studies indicate that although Hsc70 from these species has evolved at strikingly different temperatures of –2 °C to 37 °C, they maintain similar functional properties. Supported by NSF grant IBN 0096100 to GEH.

7.35

Acclimation-induced variability in the activation of heat shock transcriptional factor HSF1 in the goby *Gillichthys mirabilis*: Implications for ecological plasticity in the heat shock response

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We investigated the effect of acclimation temperature on the activity of the transcriptional factor HSF1 in the eurythermal goby, *Gillichthys mirabilis*. HSF1 is a key regulatory molecule in the induction of heat shock genes, which code for chaperone proteins that help stabilize other thermally-damaged polypeptides during heat stress. In response to elevated temperatures HSF1 becomes activated and acquires the ability to bind the promoter of heat shock genes, initiating transcription. In this experiment, fish were acclimated to 13, 21 and 28°C for five weeks, after which, their liver tissues were exposed to a range of stressful temperatures. The level of activated HSF1 in these tissues was then determined via electrophoretic mobility assay. It was found that the temperature of HSF1 activation was strongly influenced by acclimation temperature: HSF1 activation peaked at 24°C in the 13°C-acclimated group, at 33°C in the 21°C-acclimated group, and 36°C in the 28°C-acclimated group. The concentration of HSF1 was higher in the warmer-acclimated groups, but this could not explain a shift in temperature of peak HSF1 activation. The plasticity in the activation of HSF1 in *G. mirabilis* provides new insight into the adjustable nature of the heat shock response in organisms living in variable thermal environments.

7.36

Acclimation of eurythermality: A comparative analysis of cardiac and neural thermal tolerance in porcelain crabs from different thermal habitats

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Ectotherms can experience a broad range of temperatures, and must be adapted to withstand both warm and cold thermal extremes. While many experiments have demonstrated that upper or lower thermal limits of organisms can be adjusted through thermal acclimation, we know little of the effects of acclimation on thermal ranges (i.e., the temperature span between upper and lower critical temperatures where distinct changes in physiological performance are noted). Here, I examined the effects of acclimation to warm (18°C), cold (8°C), and fluctuating (8:18°C, 12:12h) temperatures on thermal ranges of heart and nerve function in porcelain crab congeners (genus *Petrolisthes*) from different thermal habitats. Heart rate was monitored by impedance electrodes during thermal ramps (0.1°C/min) where temperature either increased or decreased from an intermediate temperature to thermal extremes. Spontaneous action potentials of leg nerves were recorded during thermal ramps (0.5°C/min) over the same range of temperatures. Arrhenius break temperatures (ABTs) for both systems were used to define upper and lower limits. The acclimation treatment that resulted in greatest thermal ranges differed among species; ABTs of heart rate indicate that one species was most eurythermal in the 18°C treatment while another was most eurythermal in the 8°C treatment. Additionally, the relative plasticity of upper and lower ABTs differed among species. This work was funded by the NSF IBN-0113184 and PISCO.

7.37

Metabolic Adjustments to Seasonal Cold Exposure in Juvenile Green Turtles

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The physiological and behavioural responses made by green turtles to cold exposure vary considerably. The rapidity and severity of the decrease in temperature, ecological factors, and inter-population differences may play roles in determining how green turtles adjust to changing environmental temperature (T_E). Acute effects of cold exposure have been studied in the past. The purpose of this study was to investigate the metabolic adjustments made by green turtles during chronic exposure to low T_E. Captive green turtles were exposed to a thermal regime that mimicked seasonal temperature changes experienced by sub-tropical populations of green turtles (T_E 17-26°C). Oxygen consumption (VO_2) was measured from turtles acclimated to 17°C and 26°C using open-circuit respirometry. Biopsy samples were obtained from the quadriceps muscle of turtles at 17°C and 26°C so that activity of aerobic and anaerobic enzymes could be measured. Mean VO_2 at 17°C was not significantly lower than the mean VO_2 at 26°C. There was no significant difference in aerobic enzyme activity at 17°C and 26°C (assay temperature 25°C), but anaerobic enzymes showed a clear and significant pattern of thermal compensation. Results show that green turtles are capable of maintaining similar rates of metabolism over the range of temperatures experienced at sub-tropical latitudes. Thermal independence of acrobic enzymes and thermal compensation of anaerobic enzymes contribute to this ability. Study funded by NSERC.

7.38

Index of biological compensation of temperature (Z-approach)

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The approach considers a new theoretical tool for evaluation of the capacity of ectothermic animals to compensate effects of seasonal temperature change. The difference between uncompensated (theoretically expected, based on Arrhenius equation) and compensated (observed after acclimation) rates of the rate-specific biological processes has been taken as the basis for a compensation index (Z). The constancy of the compensation capacity within a certain range of acclimation temperatures is considered the main feature of adequate metabolic control. The compensation capacity beyond the range of constant compensation considerably decreases and finally disappears at the critical temperature (Tc). Temperatures that limit the range of constant compensation are considered to be related to pejus temperatures, which restrict the bio-geographical distribution of the species (Tp). The Z-approach is a relative approach, because all compensation events are considered relative to the state of biological function at optimal temperature for somatic growth (Topt), therefore the individual thermal history of an animal has important implication for the Z-approach. The compensation effort is linked to the Arrhenius activation energy of a given biological process. This approach can be extended and applied to a description of effect of any abiotic factor on rate-specific biological processes of ectothermic animal.

7.39

Muscular Adaptation To Cold Exposure Increases Energetic Cost Of Locomotion In Monodelphis domestica, A Mammal Lacking Brown Adipose Tissue.

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Upon exposure to cold temperature, rodents exhibit increased shivering thermogenesis to maintain temperature homeostasis. With continued cold exposure, non-shivering thermogenesis in brown adipose tissue (BAT) replaces shivering thermogenesis. We explored muscle adaptation to prolonged cold exposure in Monodelphis domestica, a small (~100g) marsupial that lacks BAT, like all marsupials and large placental mammals. Unlike in rodents, cold exposure led to increased muscle mitochondrial volume density (by 19%). This increased aerobic capacity was utilized during locomotion as evidenced by increased maximal oxygen use while running (by 17%). However, the cost of transport (COT, mlO2/kg m) at submaximal speeds was increased (by 15% overall), suggesting that uncoupled respiration reduced the efficiency of energy conversion during locomotion. Further, as the speed of locomotion (and thus overall energetic costs) increased, the difference between thermoneutral and cold acclimated animals decreased. At 25 m/min (~70% of maximal metabolic rate), COT in cold acclimated animals was 8% greater than that of warm acclimated animals. This difference increased to 18% at 10 m/min and when extrapolated to 0 m/min via regression analysis, increased to 36%, suggesting that the contribution of uncoupling processes to metabolic control decreases with increased metabolic activity.

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7.40

Gene expression and cold adaptive phenotypes in *Caenorhabditis elegans*

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Cooling induces phenotypic adaptations both to enhance resistance to cold injury and to enhance capacity for life. Candidate genes have been proposed as mediating these effects, but in most systems they are not subject to experimental manipulation. We aim to identify mechanisms mediating resistance adaptation by ablation of cooling-induced genes in the nematode worm, *C. elegans*. We have found acclimation of worms to 10°C from the L1 stage protects the worm from lethal exposure to 0°C: thus, adult LT_{50} is 82.67h, compared to only 12.9h for 25°C acclimated worms. We have assessed genes undergoing regulation induced by acute cold using microarray technology. We found that the Δ9-desaturase gene *fat-7* is up-regulated ~20-fold following initial cold exposure, this being a long recognized adaptive response to cold that moderates membrane physical condition to changing temperatures. Furthermore, gas chromatography shows that *fat-7* up-regulation is accompanied by an increase in monounsaturated fatty acid (18:1n-7) content. RNAi-mediated partial knockdown was achieved by exposing worms to *fat-7*dsRNA from the L1 larval stage. This procedure adversely affects fecundity, alters the fatty acid profile and reduces LT_{50} following exposure to 0°C. This provides direct support for the adaptive role of lipid compositional modifications in the cold-adaptive phenotype.

This work is supported by the BBSRC

7.41

Downregulated protein synthesis during mammalian hibernation: active and passive mechanisms

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Despite the need to maintain cellular integrity, mammalian hibernators must depress biosynthetic processes in concordance with energetic demands. Energetic costs of transcription and translation must be balanced with demands for crucial gene products. Data from nuclear run-on assays indicate transcriptional initiation is reduced 2-fold in torpid golden-mantled ground squirrels as compared to euthermic animals between bouts of torpor. In addition, transcriptional elongation rates across the temperature range experienced by hibernators suggest a virtual arrest of transcription at the low body temperatures of torpor. Polysome analyses indicate a large depression of initiation of translation when the hibernators' body temperature (Tb) reaches 18° C. There is a slow run off of nascent polypeptides until, at the end of the bout, few polysomes exist. Some new initiation ocurs at Tb < 18° C but initiation and elongation are not fully recoupled until Tb > 18° C. An analysis of the factors known to regulate translational initiation reveals one major change in hibernators: eIF4EBP-1 is absent in animals sampled during summer but present in winter-sampled squirrels. eIF4EBP-1 is regulated via reversible phosphorylation during the winter as the animals cycle between torpor and euthermy. Complete reversal of the transcriptional and translational arrest during the interbout arousal allows critical gene products to be replenished while providing an energetic savings during torpor.

7.42

Sequence Mutations in Teleost Cardiac Troponin C that are Permissive of Cardiac Function at Low Temperatures

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Cold temperatures reduce the Ca^{2+} sensitivity of cardiac myofibrils of fish and mammals alike. The effects of temperature on myocyte contractility parallel the effects on Ca^{2+} sensitivity of recombinant cardiac troponin C (cTnC) from salmon (ScTnC) and mammals (McTnC). Fish avoid the cardioplegic effects of temperature, at least in part, by possessing a cardiac troponin C (cTnC) with higher inherent Ca^{2+} affinity. The amino acid sequence of ScTnC differs in 13 of 161 residues (93% identity) from McTnC. ScTnC exhibits ~2x the Ca^{2+} affinity of McTnC over a broad range of temperatures and pH values, as indicated by Ca^{2+} titrations of the recombinant proteins using a fluorescent reporter. Using site-directed mutagenesis, we established that residues at position 29 (glutamine (Q) in ScTnC, leucine (L) in McTnC) and 30 (aspartate (D) vs glycine (G)) were responsible for the differences in temperature sensitivity. Q29L and D30G mutations in ScTnC reduced Ca^{2+} affinity to that of McTnC. The extreme cold tolerance of icefish (IF) is not due to variations in cTnC as IFcTnC is identical to ScTnC at the critical residues (Q29, D30) and there is no difference in Ca^{2+} affinity. Furthermore, we showed that an increase in pH from 7.0 to 7.3 sensitizes recombinant cTnC to Ca^{2+}. As pH would be higher in an icefish heart at –1.8°C than a trout heart at 14°C, due to -stat regulation, we propose that the sensitizing effect of alkaline pH on IFcTnC allows cardiac function at –1.8°C. This work is supported by The Heart and Stroke Foundation of Canada, The Heart and Stroke Foundation of BC and Yukon, and the Natural Science and Engineering Research Council of Canada.

7.43

Snake venom: prey digestion from the inside out?

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Over the past decades, three competing hypotheses have been advanced to explain why some snakes have evolved powerful venoms, including use in prey capture, defense against potential predators, and facilitation of digestion. The first two hypotheses cannot be readily tested, however, the third hypothesis can be tested by investigating the effects of venom on reducing specific dynamic action (SDA). I investigated this hypothesis by measuring respiratory gas exchange in two species of snakes (*Elaphe obsoleta* and *Pituophis catinifer*) under two experimental treatments. Every two weeks each snake was presented with a prey item (mouse) that was intramuscularly injected with either 0.5 ml of saline or reconstituted rattlesnake venom (*Crotalus atrox*; 100 mg/ml). I then quantified the energy devoted to SDA, and the duration of SDA. Preliminary results suggest that the both the duration and energy devoted to SDA is reduced by approximately 20 % following venom treatments. Since pit-viper venom is chiefly composed of proteolytic enzymes, I conclude that venom injections facilitated the food processing by digesting from the inside out while gastric secretions digested from the outside in. Understanding the physiological relationship between venom and digestion might offer insight into the evolutionary contribution of snake venoms, and potential medicinal applications. This project was supported by NSF grant IBN 0091308 and NSF graduate research fellowship.

7.44

Strategies of digestion: Effects of age and diet quality on digestive efficiency and mean retention time in harbor seals.

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Little is known regarding mean retention time (MRT), assimilation efficiency (%AE) and feeding frequency as a function of diet quality and age in harbor seals. These digestive parameters may be important in the evolution of diverse traits such as life history attributes, activity patterns and growth rates. It is theorized that digestion time and assimilation will vary according to diet quality and intake. We hypothesized that sub-adult (SA) harbor seals (2-3y) would exhibit decreased retention times due to their relatively increased metabolic rates when compared to adults (RA, 7-14y), and non-reproductive aged adults (NA, 25+y). While absolute MRT values for SA and RA were lower than NA, when corrected for body mass SA seals exhibited higher MRT values for once per day feeding (1x/d, $p = 0.039$). On high-fat herring diets SA seals exhibited increased MRT values relative to older seals (RA, NA) regardless of feeding frequency (1x/d, $p = 0.033$; 4x/d, $p = 0.016$). While not significant with respect to age, herring always yielded higher %AE values than pollock for each group during each feeding frequency. Our data show that sub-adult harbor seals had longer retention time while on a herring diet and during once per day feedings when compared to the older age classes. We speculate that longer retention times in SA harbor seals may possibly be a function of their digestive capacity to process high-energy rich prey or a developmental advantage to retain prey if patchy in nature.

7.45

Electrophysiological properties of the L-type Ca^{2+} current in cardiomyocytes from Pacific mackerel and Bluefin tuna

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We examined the electrophysiological properties of the L-type Ca^{2+} channel current (I_{Ca}) in isolated cardiomyocytes from two closely related scombrid fish; the bluefin tuna (*Thunnus thynnus*) and the Pacific mackerel (*Scomber japonicus*). We hypothesized that Ca^{2+} influx via the L-type Ca^{2+} channel would be larger and have faster kinetics in bluefin tuna than its phylogenetic cousin the Pacific mackerel, because tuna are renowned for high maximum heart rates and high cardiac output among active fish species. In accordance with the hypothesis, atrial myocytes from bluefin tuna had a significantly greater peak current amplitude (-4.8±0.3 pA/pF) compared with mackerel (-2.7±0.5 pA/pF). However, current density in ventricular myocytes did not differ between the two species (-4.8±0.4 and -5.7±0.9 pA/pF in tuna and mackerel, respectively). The inactivation kinetics of I_{Ca} tended to be faster for both atrial and ventricular cells in bluefin tuna compared with mackerel but the differences were not statistically resolvable. Similarly, steady-state activation and inactivation parameters are not strikingly different between species. Our results suggest that the elevated maximal cardiac performance observed in tuna is probably not a result of changes in I_{Ca} and thus future studies should be directed at other Ca^{2+} flux pathways such as the sarcoplasmic reticulum. This research is supported by NSERC to APF and the Monterey Bay Aquarium Foundation to BAB.

7.46

Exercise Studies of Mudskippers

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Mudskipper fishes are amphibious air breathers that often engage in periods of intense activity on land. Mudskippers have reduced gill areas and when not in water, cannot use this medium to repay an O_2 debt or restore activity-induced imbalances in factors such as acid-base state, ion levels, and waste nitrogen. We therefore studied the role of air and water in the post-exercise (post-ex) metabolic recovery of two mudskipper genera. Mudskippers (*Periophthalmus* sp. and *Scartelaos histophorus*) were placed in respirometers containing air and a quantity of water (3x fish mass) to measure pre- and post-ex aerial and aquatic M_{O2}, aquatic CO_2 release, and changes in water pH, nitrogen content, and lactate levels for 3 h pre- and up to 6 h post-ex. After pre-ex measurements, fish were exercised for 1.5 min in a tray containing a thin film of water, and then returned for post-ex determinations. M_{O2} during the first hour post-ex was raised 1.9x in *Periophthalmus* and 1.5x in *Scartelaos*, with 99% of O_2 consumption occurring in air. O_2-debt recovery times were 130 min for *Periophthalmus* and 100 min for *Scartelaos*. Post-ex water pH underwent a greater reduction in both fish. There was more ammonia in post-ex water. No lactate release occurred in pre- or post-ex fish. Both *Periophthalmus* and *Scartelaos* recover their O_2 debts aerially but rely on water access for post-ex exchange processes. (NSF IBN 9604699 and 0111241 and UC Pacific Rim Research Program 01TPRRP060187 and 02TPRRP040065)

7.47

Sex vs. parthenogenesis: increased capacity for sustained locomotion at low temperature in parthenogenetic geckos.

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The evolution of parthenogenesis is typically associated with hybridization and polyploidy. These correlates of parthenogenesis may have important physiological consequences that must be taken into account in understanding the relative merits of sexual and parthenogenetic reproduction. We compared the thermal sensitivity of aerobically sustained locomotion in northern and southern populations of the gecko *Heteronotia binoei*. The *H. binoei* complex occurs in the Australian arid zone and includes two sexual races separated along a north/south axis, with triploid parthenogenetic hybrids occurring in near or actual sympatry with both sexual races. We focused on the impact of low temperature on locomotion in these lizards since they are nocturnal and active at low body temperature. Critical thermal minima differed significantly between localities but not between reproductive modes, with values in the northern populations being approximately 1.5°C higher than those of the southern populations. In contrast, the maximum rate of oxygen consumption and endurance times at low temperatures (10, 12.5 and 15 °C, 0.05 km/h) were significantly greater in parthenogens than in sexuals but did not differ between localities. Parthenogenetic lizards have an advantage over sexuals in being capable of greater aerobic activity at low nocturnal temperatures. Support for this project was provided by Lewis and Clark College and an Australian-American Fulbright award to M. Kearney.

7.48

Allometric Cascade: A Multiple-Causes Model of Body Mass Effects on Metabolism

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The fact that metabolic rate of organisms does not scale in proportion to body mass has intrigued biologists since the late 1800s. The well-known power function of basal metabolic rate (BMR) scaling is expressed as $BMR=aM^b$ where a corresponds to a scaling constant, M is body mass, and b is the scaling exponent. In 1932, Kleiber's analysisof body size and metabolism examined BMR in mammals, which was best described by body mass raised to the ¾ power rather than a simple surface to volume ratio. The ¾ power 'law' arose from Kleiber's curve and, since then, most workers have searched for a single-cause to explain the observed allometry. Here we present a multiple-causes model of allometry, where the global exponent of metabolic rate scaling is a consequence of the interaction of scaling exponents of the major contributor to metabolism. This concept can be described by the equation $MR = a \sum c_i M^{b_i}$, where MR is the metabolic rate in a given state, a is the constant, b_i the scaling exponent of the process i, and c_i its control contribution to overall flux, or the control coefficient of the process i. One can think of this as an allometric cascade, with the b exponent for overall energy metabolism being determined by the b_i and c_i values for key steps in the complex pathways of energy demand and energy supply. When this model is applied to maximal versus basal metabolic rate, for the first time the differential scaling of these two states can be explained.

7.49

Steady swimming muscle dynamics of the shortfin mako shark (*Isurus oxyrhincus*) and the leopard shark (*Triakis semifasciata*)

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We investigated the muscle dynamics of the aerobic red myotomal muscle during steady swimming in the shortfin mako shark (*Isurus oxyrhincus*) and the leopard shark (*Triakis semifasciata*), two species representing distinct locomotor styles. Electromyographic and sonometric techniques were used to measure red muscle activation patterns and instantaneous muscle length. The timing of muscle activation and shortening and relationships between muscle strain and local body curvature were assessed at two axial positions. In both species, onset of muscle activation occurred just before peak muscle length and offset occurred during muscle shortening; onset occurred earlier in the strain cycle in the leopard shark. Activation patterns and duty cycles were similar at both axial locations in the mako and leopard shark. Red muscle strain increased caudally and was synchronous with local body bending in the leopard shark. In contrast, muscle shortening in the mako was not in phase with local curvature but rather was in phase with bending at more posterior locations along the body, a feature similar to other thunniform swimmers such as tunas. These data contribute to a larger study whose goal is to characterize the locomotion and muscle dynamics of two shark species representing different ends of the spectrum of locomotor styles from anguilliform to thunniform and to identify similarities in the muscle dynamics of sharks to that observed in bony fishes. Funding provided by NSF.

7.50

DETERMINATION OF MECHANICAL EQUIVALENT OF HEAT AND FUNCTIONAL CAPACITY OF METABOLISM OF BODY

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Joule determined Mechanical equivalent of heat via a falling mass turned a paddle wheel immersed in water. Energy utilization of 70kg body is 100kcal/hour for rest state and 200kcal/hour for slow walking state (4022.5m/hour). Functional capacity of metabolism is 27% and the other part (73%) is released as heat into medium.

Method: Mathematical modeling and evaluations of hemodynamics of myocardium and mechanics of walking.

Results: Mechanical equivalent of heat of stroke volume is 7900 cal/day for rest state. Mechanical equivalent of heat of KE=½mv^2=37.5kcal/hour for horizontal motion and PE=mgh=53kcal/hour for 5cm up and down bobbing motions (length of lower extremities:100cm, hypotenuse of a step triangle:95cm, width of a step:62cm, height of an upward movement for a step:5cm) and functional capacity of metabolism at least 45.25% for one hour slow walking state of 70kg body. Joules' experiment is not cover whole potential energy of falling mass like as friction and collision of mass by apparatus and ground. However, two methods of this study have mass of pendulum turned water and give the whole gravitational potential energy (mgh) into water at the end of the total oscillations of falling mass (m) of pendulum from a given vertical height (h). Conclusion: Active dynamics of body is functional part of metabolism, muscles are heat source of body, and concepts of mechanical equivalent of heat, thermogenesis and functional capacity of metabolism should be revised.

7.51

Cognitive Influence on the Physiology of Diving in Harbour Seals (*Phoca vitulina*)

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In order to evaluate the cognitive influence on the mammalian diving response, eight captive harbour seals were trained to recognise visual targets representing two distinct dive durations: "short dive" (1 minute) and "long dive" (>5 minutes). Video recordings obtained during diving were analysed to quantify activity level (stationary; slight movement; increased movement; active swimming). Electrocardiogram data were collected using a purpose built data logging system and two dorsal surface electrodes. The animals were trained to surface into a respiratory dome at the completion of a dive for assessment of post-dive oxygen consumption.

When exposed to a short dive target, seals would descend more rapidly and spend significantly more time exhibiting increased movement than during long dives. Unsuccessful long dives exhibited the greatest descent times. During the first minute of a long dive, seals spent a greater proportion of time in a "stationary" position than during unsuccessful or short dives

Heart rate during short dives was significantly higher than during the first minute of a long dive, and post dive oxygen consumption (ml/kg/min) was significantly greater for short dives than for long dives. These findings suggest that seals alter their physiology in response to prior knowledge of dive duration and provide evidence for cognitive influence over the mammalian diving response. This research was supported by NSERC (Canada).

7.52

The oxidatively-stressed seal

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The present study incorporated a novel molecular technique to examine changes in the peripheral concentration of oxygen (O_2)-centered free radicals in the diving mammal. Blood samples were obtained from the extradural intravertebral vein of a fasted Grey seal (*Halichoerus Grypus*) before and immediately after a five minute voluntary dive. All procedures were conducted in accordance with the Institute for Laboratory Animal Research. Free radicals were assessed directly using the combined techniques of *ex-vivo* spin trapping with -phenyl-*tert*-butylnitrone (PBN) and electron paramagnetic resonance (EPR) spectroscopy. Samples were also assayed for biological footprints of lipid peroxidation and lipid soluble antioxidants. A clear increase in the EPR signal intensity of the PBN nitroxide adduct was observed after the dive (5268 to 10556 arbitrary units) and spectra displayed nuclear hyperfine coupling constants of $a_{Nitrogen}$: 1.36 and $a_{Hydrogen}$: 0.19 millitesla. We also observed an increase in lipid hydroperoxides (3.37 to 4.59 mmol.L^{-1}) and malondialdehyde (2.12 to 2.71 mmol.L^{-1}) despite a selective increase in -tocopherol (32.41 to 33.21 mmol.L^{-1}), the major chain-breaking antioxidant. These data provide the first direct evidence for free radical generation in the diving mammal, that we suggest may be secondary or tertiary derived O_2-centered lipid alkoxyl radicals formed as a consequence of primary O_2-centered intra/extracellular damage to membrane phospholipids.

THE POWER OF INTEGRATION

8.1

Digestive Enzyme Activity in Herbivorous and Carnivorous Prickleback Fishes (Teleostei:Stichaeidae): Ontogenetic and Phylogenetic Effects.

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We measured the activities of proteases and carbohydrases in four closely related species of pricklebacks to determine whether these fishes are genetically predisposed to digest a specific diet. Both *Cebidichthys violaceus* and *Xiphister mucosus* shift to a more herbivorous diet as they grow (>45mm SL), whereas *X. atropurpureus* and *Anoplarchus purpurescens* remain carnivores throughout life. Protease (pepsin, trypsin, aminopeptidase) and carbohydrase (amylase, maltase, isomaltase) activities of small (30-40mm SL) carnivorous fish were compared with larger (60-75mm SL) fish raised on a high-protein animal diet and with larger (60-75mm SL) wild-caught fish that had consumed a natural diet. *A. purpurescens*, a member of a carnivorous clade, appears adapted to a high-protein diet because it showed higher trypsin and aminopeptidase but lower amylase activity than the other fishes, and, uniquely, increased trypsin activity when fed the high protein diet. The three other species, members of an adjacent largely herbivorous clade, showed a significant increase in amylase activity with size, even on the high-protein diet. The increase in amylase activity in *C. violaceus* and *X. mucosus* indicates that the shift in diet and enzymatic activity may be genetically fixed, whereas in *X. atropurpureus* the increase may result from phylogenetic constraints. This study was supported by NSF grant No. OCE-9906857 (M. H. Horn, PI).

8.2

Histochemistry and Enzyme Histochemistry of the Digestive System in Herbivorous and Carnivorous Prickleback Fishes (Teleostei: Stichaeidae)

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We investigated possible diet-related specializations of the gut and liver in four related species of pricklebacks using histochemistry and enzyme histochemistry (in progress) to determine whether these fishes are genetically predisposed to digest specific diets. Both *Cebidichthys violaceus* and *Xiphister mucosus* shift to herbivory as they grow (45 mm SL), whereas *X. atropurpureus* and *Anoplarchus purpurescens* remain carnivores throughout life. Fixed, resin-embedded gastro intestinal and hepatic tissues were examined in small (30-40 mm SL) carnivorous fish, larger (60-75 mm SL) fish raised on a high-protein diet and larger (60-75 mm SL) wild-caught fish. In the stomach, mucus and intracellular proteins increased from small fish to both groups of larger fish in all four species. In the pyloric intestine, secretory goblet cells were more abundant in large wild-caught fish of all species, whereas lipid vacuoles increased in only large fed *A. purpurescens*. In the distal intestine, goblet cells also were more numerous in all wild-caught fishes, but lipid vacuoles increased in only *C. violaceus* and *X. mucosus*. In the livers of small fish, glycogen was more abundant in *C. violaceus* and *X. mucosus*. Glycogen storage increased in both large fed and large wild fish of all species, whereas lipid storage was noticeably higher in only *C. violaceus*. Results to date suggest that herbivorous pricklebacks differ from the carnivorous species in patterns of lipid assimilation and storage.

8.3

What Does It Take to be a Herbivore? Gut Structure and Function in Three Species of New World Silverside Fishes (Teleostei: Atherinopsidae) with Different Diets

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We explored the possible diet-related specializations of the gut in stomachless fishes by comparing the brush-border surface areas and enterocyte inclusions (in progress) of the gut epithelium and the digestive enzyme profiles (in progress) in three closely related atherinopsid fishes using electron microscopy and biochemical assays. For these comparisons, we examined proximal, middle and distal regions of the intestine of *Atherinops affinis*, from both kelp forest and estuarine habitats, and *Atherinopsis californiensis* and *Leuresthes tenuis*, both from open coastal habitats. *A. affinis* is generally a carnivore in kelp forests and a herbivore in at least some estuaries, whereas *A. californiensis* is mainly carnivorous and *L. tenuis* strictly carnivorous. Previous work showed that relative gut length in these species generally follows the expectation that carnivores have shorter guts than herbivores and omnivores have guts of intermediate length. The herbivore (estuarine *A. affinis*) showed greater overall microvillar surface area than the two more carnivorous species as well as the carnivorous population of *A. affinis*. Thus, a degree of congruence exists between diet, gut length and absorptive surface area, with the herbivore gut exposing a larger surface area to the ingested food material.

8.4

Simulation of the 6000-km migration run of European eel shows remarkably low energy costs.

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The spawning site of European eel is likely located in the Sargasso Sea. Eels leaving the European coast in the fall are assumed to swim continuously at great depths during 6 months without eating. We estimated the energy costs over 6000-km in the laboratory by swimming the eels in swimtunnels. The energy consumption was calculated from the oxygen consumption data, which were based on daily measurements. The energy-balance studies were performed with adult female eels (914-g) in Blazka-type swim-tunnels of 127 liter. The water flow pattern and velocity in the tunnels was evaluated with a Laser-Doppler method. Eels (N=9) swam 117 days at 0.5 BL/sec without feeding or resting day and night, covering a distance of 5533-km. The loss of weight for swimming animals over this period was 180-g while this was for resting animals 103-g. Oxygen consumption at rest was two times lower than in the swim group (24.2 ± 2.9 vs. 57.9 ± 5.4 ml O2 kg/fish/h, respectively). The COT (Cost Of Transportation) values for adult fish species are in the range of 0.15-0.67. We found for eel a value of 0.07. This shows clearly that European Eel swims more than 2 times as efficient as other fish species, which confirms our earlier observations, based on a 1 month swimming period, suggesting very low energy costs for swimming (Nature 403, 156, 2000). Therefore, it is likely that European eels can reach their spawning grounds with sufficient reserves for reproduction. STW-project no. LBI66.4199

8.5

Lactate Processing in Endothermic Fishes: Gluconeogenic Enzyme Activities in Fast Glycolytic Myotomal Muscle and Liver of Tunas and the Short-fin Mako Shark

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When compared with related ectothermic species, endothermic tunas and lamnid sharks have higher activities of lactate dehydrogenase (LDH) in the fast, glycolytic myotomal muscle (white muscle, WM) used to power high-speed bursts. These species produce large amounts of lactate from WM glycogen which is subsequently oxidized or used to resynthesize glycogen. It is believed that gluconeogenesis occurs within the WM, yet the gluconeogenic potential of tuna and lamnid shark WM has not been reported. To assess this, we measured the activity of four enzymes required for gluconeogenesis (pyruvate carboxylase, malic enzyme, phosphoenolpyruvate carboxykinase, and fructose-1,6-bisphosphatase) in the WM and liver of five tuna species and the short-fin mako shark. Pyruvate carboxylase was not detected in any tissue samples. Malic enzyme activities in WM were less than or equal to those in liver. The other two enzymes were present in higher activities in the liver than in the WM. Because WM constitutes a larger proportion of body mass than does the liver, WM may contribute more to gluconeogenesis despite lower enzyme activities. However, without pyruvate carboxylase, gluconeogenesis may not be possible unless the reaction catalyzed by pyruvate kinase (PK) can be reversed, as has been suggested by others. Although the PK activity of WM is much greater than that of the liver in tunas, there is no direct evidence that PK is involved in gluconeogenesis in fish WM. Funded by NIH 08258S06GM.

8.6

Decrease in the degree of hyperkalemia caused by an acute lactic acid infusion

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The plasma potassium (K) concentration (P_K) rises to ~ 6 mM early in a sprint—hyperkalemia provides a stimulus for ventilation during its alkalemic phase (largely due to phosphocreatine hydrolysis), but too high a P_K poses a threat of inducing a cardiac arrhythmia. Our objective was to evaluate whether an L-lactic acid infusion would actually diminish the rise in P_K, thereby lessen the danger of this hyperkalemia. Hyperkalemia was induced by either infusing HCl (6 mmol/kg) or KCl (4 mmol/kg) for 60 min into anaesthetized rats. A post infusion period of 60 min was allowed to ensure that the P_K remained high and did not vary appreciably. The P_K at the 120 and 160 min times in the HCl and the KCl groups rose by 0.3 ± 0.1 mM (time control values). Each of the experimental groups of 6 rats received L-lactic acid (210 µmol/kg/min for 10 min) beginning at the 120 min time. The P_K fell by 1.0 ± 0.2 mM, $p < 0.01$ in the HCl group and by 0.7 ± 0.1 mM, $p < 0.01$ in the KCl group 17 min after beginning the infusion of L-lactic acid. In contrast, 7 min after stopping the infusion of L-lactic acid, the arterial P_K rose abruptly in each rat to P_K values exceeding the pre L-lactic acid infusion P_K. Plasma glucose prior to L-lactic acid infusion was 6.8 + 0.1 mM—it rose by 1.0 ± 0.1 mM after L-lactic acid infusion ($p < 0.01$). We conclude that L-lactic acid caused an acute shift of K^+ into the ICF compartment. We speculate that activation of the Na/H^+ exchanger by both a rise in insulin and a fall in ICF pH led to H^+ export and thereby Na^+ entry into cells. Because the resultant Na^+ export by the Na-K-ATPase is electrogenic, a more negative ICF voltage would be created to favor K^+ uptake into cells.

8.7

Temperature and the chemical composition of poikilotherms

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Nitrogen [N] and phosphorus [P] are constituents of key cellular structures and also play important roles as nutrients in ecological processes. N- and P-based currencies thus provide a natural way to trace temperature effects across levels of biological organization. We surveyed the literature to examine whether acclimation temperature influences organismal contents of N and P, or of N-rich (protein) and P-rich (RNA) biochemicals, in poikilotherms. Organisms in the survey—including bacteria, yeast, algae, animals, and terrestrial plants—were exposed to a mean temperature difference of about 15 °C. On average, those exposed to cold showed ~27% higher protein or N content and ~48% higher RNA or P content than did conspecifics exposed to warm temperatures. The shift occurred in all broad taxonomic groupings, except possibly bacteria. Although shifts in composition may stem from multiple causes, the simplest general explanation is that organisms in the cold increased the concentrations of enzymes and ribosomes to offset or exploit shifts in relative rates of diffusion and reaction of biochemicals. Ultimately, temperature-driven changes in the composition of individual organisms may affect ecological patterns and processes, including species interactions and patterns of C:N:P stoichiometry across latitudinal and altitudinal temperature gradients. These findings provide the kernel for an integrated framework tracing temperature's effects from biochemistry to ecology.

8.8

May We Translate Physiological Data of Rat Mud Therapy Studies to Human?

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It is well known that therapeutic mud (TM) applications (TMAs) are usually accompanied in humans with increases in heart rate (HR), blood pressure (BP), respiration rate (RR), and oxygen consumption (VO2). The aim of this study was to ascertain if such changes take place in Wistar rats used so widely in experimental therapy. Methods included measurements of HR, systolic BP (SBP), and RR by means of rheographic nonbloody approach in restrained rats lightly anesthetized with ether. VO2 was measured in restrained but unanesthetized animals. TM in Plexiglas boxes had been applied to the sheared back skin. Temperature (T) of TM was 42 °C; the duration (D) of TMA was 20 min – the most frequent T and D used in application mud therapy (AMT). For all experiments, a mean value obtained immediately before TMA had been compared with that during the impact period. Results showed a tendency to an increase in SBP (97.0±1.5 mmHg vs. 94.7±1.5 of the initial level) as well as to a decrease in HR (411±6 vs. 421±6 bpm). RR and VO2 were changed statistically significantly [122±3 vs. 104±3 breaths/min and an average 6.5 % decrease, respectively ($P < 0.05$ in both the cases)]. It is concluded that, of the four used physiological indices, RR is the only one, which demonstrates in rats a significant shift of the same direction as in humans. Thus, we should rather not use the rat as a model to study the chosen physiological effects of AMT for further extrapolation them to the clinical situation.

8.9

Measuring lean, fat and total body masses of migrant birds with dual-energy x-ray absorptiometry.

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We used dual-energy X-ray absorptiometry (DXA) to estimate body composition in birds. This method has previously been used only on mammals. We measured, fat, lean and total body masses in 10 freshly killed birds, 3 *Phylloscopus collybita* (mean mass = 7 g), 3 *Luscinia svecica* (13 g) and 4 *Calidris minuta* (22 g) with a Lunar PIXImus DXA. We also plucked and rescanned each and measured the same variables on the carcasses using gravimetry and chemical analysis. DXA values in feathered and plucked birds were significantly different for body mass ($t = 5.28$; $p<0.001$), fat mass ($t = 4.78$; $p<0.001$), and lean mass ($t = 3.35$; $p<0.01$). DXA values of feathered and plucked birds were all significantly correlated with measured values of the chemical analyses: for body mass $r^2 = 0.99$, $r^2 = 0.98$; for fat mass $r^2 = 0.84$, $r^2 = 0.89$; and for lean mass $r^2 = 0.99$, $r^2 = 0.99$, respectively, all $p < 0.01$. The slope and intercept of the regression of DXA values on chemically obtained values were always higher for the plucked than the feathered birds, indicating that feathers need to be taken into account. The results show that DXA is an accurate method to determine non-invasively fat and lean masses of dead or anesthetized birds, and will prove a useful tool to measure body condition in small endothermic animals.

POLAR MOLECULAR BIOLOGY: PROTEINS AND ENZYMES AT THEIR LOWER TEMPERATURE EXTREMES

9.1

Changes in gill basolateral membrane composition and Na^+K^+-ATPase activity in Arctic char (*Salvelinus alpinus*) exposed to seawater

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The upregulation of gill Na+K+-ATPase activity is pivotal for successful acclimation of teleost fish to seawater. Since Na+K+-ATPase activity has been shown to be sensitive to changes in the composition of the membrane environment that surrounds it changes in phospholipid, individual fatty acid and cholesterol levels were determined in isolated gill basolateral membranes from Arctic char exposed to seawater for 39 days. Analysis of plasma ion (Na+, Cl-) and osmolality revealed that seawater challenged Arctic char were able to regulate thir plasma ions at the same level as freshwater char suggesting they were fully acclimated to seawater. Basolateral membrane phospholipid and individual fatty acid levels were quite similar between freshwater and seawater acclimated Arctic char. Cholesterol levels of gill basolateral membranes were significantly lower in seawater acclimated char. A relationship between reduced basolateral membrane cholesterol content and higher gill Na+K+-ATPase activity was evident suggesting that cholesterol levels may act to regulate the activity of this transporter. This research was funded by NSERC Canada.

9.2

Osmoregulation and Freezing Avoidance in Fertilized Eggs of the Antarctic Naked Dragon Fish (*Gymnocraco acuticeps*)

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Dragon fish lays its eggs on rocks in shallow water in McMurdo Sound, often in the vicinity of anchor ice at -1.93°C, the freezing point of seawater. Upon external fertilization, the perivitelline (PV) space accounts for approximately 25% of the total volume of the egg. Osmolality of the PV fluid is 1060 mOsm, same as seawater, indicating that the chorion is permeable to ions. However, based on melting point depression, the ooplasm is only 550-600 mOsm. Ion concentrations were determined in the whole egg and the ooplasm concentrations were calculated by subtracting the contribution of the PV ions. Ooplasm values for Na, K and Cl were 83, 108 and 243 mM respectively, which accounts for 81 % of the osmolality. ^{1}H-NMR spectroscopy of the ooplasm revealed 60 mM TMAO, a known osmolyte in the muscle of this fish. Thus the combination of the three ions and TMAO accounts for much (86%) of the ooplasm osmolality. Despite being strongly hypoosmotic, the eggs are protected from freezing by the presence of antifreeze glycoproteins (AFGPs), not only in the ooplasm but also in the isoosmotic PV fluid. AFGPs, ions and TMAO together depress the freezing point of the ooplasm to −2.3°C, a temperature well below the freezing point of the seawater. The role of AFGPs in the ooplasm is obvious, but their presence in the PV fluid is not yet clear. It may be a storage site of AFGPs that are utilized by the developing embryo before it begins its own synthesis.

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9.3

Do high rates of protein degradation partially explain low growth rates in Antarctic limpets?

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Growth in Antarctic molluscs is typically slower than temperate and tropical species. The highly seasonal food availability at high latitudes and the fact many species cease, or considerably reduce feeding for long periods during the austral winter probably contributes to reduced growth rates. However, animals essentially grow by protein accretion, the net balance of protein synthesis and degradation, a shift in the balance of these three processes will also affect growth rate. Protein synthesis, degradation and accretion were measured in the Antarctic limpet, Nacella concinna. Fractional protein synthesis rates varied seasonally between 0.27 and 0.56%.d-1 and were very similar to seasonal rates measured in Mytilus edulis at considerably higher temperatures (Hawkins, 1985). In contrast, protein degradation accounted for a much higher proportion of synthesisied proteins in N. concinna (79%) than a wide range of temperate and tropical ectotherms (48%). At low water temperatures N. concinna maintains comparatively high protein synthesis rates by maintaining elevated tissue RNA concentrations to counteract low RNA translational efficiencies. However, although protein synthesis rates are comparable with M. edulis, high rates of protein degradation result in low rates of protein accretion and probably contribute to low overall growth rates. This work was funded by the Natural Environment Research Council, UK.

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9.4

Calcium Binding of Parvalbumin is Conserved at Normal Physiological Temperatures in Antarctic and Temperate Teleost Fishes

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Parvalbumin (PV) is an abundant calcium-binding protein of fast vertebrate skeletal muscle. Its proposed function is to sequester Ca^{2+} after contraction, thus facilitating relaxation. Calcium dissociation constants (K_d) of PV from two Antarctic fishes (*Gobionotothen gibberifrons* and *Chaenocephalus aceratus*) were determined and compared to those of PV from two temperate species (*Cyprinus carpio* and *Micropterus salmoides*) to assess potential differences in protein function. PV was isolated by homogenization followed by gel filtration and ion exchange chromatography. Sample purity was checked by 2-D PAGE. K_d's were determined by a competitive binding assay between PV and the fluorescent Ca^{2+} indicator fluo-3. Experiments were conducted at 5 °C intervals from 0 to 25 °C in 20 mM Hepes buffer (pH 7.2) and 150 mM KCl. Dissociation constants of PV from all species were thermally sensitive across the range of assay temperatures, and K_d's for PV from Antarctic species were consistently greater than those from temperate species. However, K_d's were relatively constant when measured at or near normal physiological temperatures: K_d was 6.63 and 6.44 at 25 °C for carp and bass and 7.03 and 6.03 at 0 °C for *G. gibberifrons* and *C. aceratus*, respectively. This indicates adjustment of thermodynamic parameters for Ca^{2+} binding to achieve similar binding constants at vastly different body temperatures. Supported by NSF IBN 9808120 and NSF OPP 9909055.

9.5

Structure Function Studies of Lens Crystallins From Cold Adapted Antarctic Notothenioid Fishes

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The eye lens of Antarctic fishes remain transparent and functional at their freezing environmental temperature of -2°C; which is in contrast to cold-induced cataracts that temperate water fishes experience when exposed to cold temperatures. To understand the molecular basis of this adaptation we characterized the lens proteins of the Antarctic toothfish, *Dissostichus mawsoni*. We separated the toothfish lens crystallins by gel filtration into three major constituent groups, confirmed to be , and crystallins by immunoblotting. Along with , the crystallin makes up the majority of the toothfish lens crystallins and upon separation from the and it has reduced solubility at the fishes' environmental temperature. Alpha crystallins are known to act as a chaperone protein in vertebrate lenses, and the crystallin fraction from the toothfish appears to stabilise or prevent precipitation of crystallin at -2°C. cDNA sequencing of some and crystallin isoforms revealed only a few amino acid differences between the Antarctic toothfish and the related New Zealand temperate water (+12°C) black cod. Comparisons with tropical fish crystallins revealed many more amino acid differences in both the and isoforms. These amino acids differences, along with chaperone activity of the crystallin at low temperature may represent adaptive changes that permit Antarctic fish lens function at the environmental temperature of -2°C. This work is supported by NSF-OPP.

9.6

Pancreatic expression of antifreeze protein is a common mechanism in all antifreeze-producing fish to prevent intestinal freezing

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To avoid freezing, many polar and subpolar fishes have evolved antifreezes (AFs) that inhibit the growth of ice crystals that enter their body. There are 5 known types of AFs - AFGPs (antifreeze glycoprotein) from Antarctic notothenioid fish and northern cods, type I AFP (antifreeze peptide) of flat fish and short-horn sculpins, type II AFP of herring, sea raven and smelt, type III AFP of zoarcid fish, and type IVAFP of long-horn sculpin. AFs are synthesized in the liver and secreted into the blood where they prevent extracellular freezing. The intestinal lumen is not in direct reach of circulatory AF, but environmental ice frequently enters in through ingestion of ice-laden food and seawater that would freeze the hypoosmotic intestinal fluid. High levels of AFGPs have been found in the intestinal fluid of notothenioid fish, but their site of synthesis is unknown. Our discovery that notothenioid AFGP evolved from a pancreatic trypsin-like protease lead us to examine for pancreatic expression of AFGPs which indeed occurs at high levels across all notothenioid taxa. Also, the pancreatic fluid AFGP protein profile was similar to that of the intestinal fluid. We further examined the other AF-bearing fishes and found all species express their respective type of AF in the pancreas. Pancreatic AF is thus the source of intestinal AF, and reaches the small intestine via the pancreatic duct. Evolutionarily diverse AF-bearing fishes have converged on this common mechanism to achieve intestinal freezing avoidance. This Work was supported by NSF OPP 9909841 and 00002654 to CHCC.

9.7

The Physiological Cost of Temperature Adaptation in Marine Ectotherms

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In recent years much attention has been paid to the molecular mechanisms of temperature adaptation in ectotherms. Broad generalisations have now emerged and we have a reasonable understanding of how cells and organisms adapt to living at different temperatures. What has received less attention is the physiological cost of such adaptation. This is expressed primarily in the rate of basal (or maintenance) metabolism. Comparative studies have shown that this varies by roughly an order of magnitude between tropical and polar marine ectotherms, variation which has profound consequences for energetics and life-history. The nature of this relationship cannot be predicted from first principles, nor do we now precisely what processes contribute to a lower basal metabolism in polar marine ectotherms. Recent studies have suggested that protein metabolism is an important component. A larger proportion of basal metabolism is used for protein synthesis n the Antarctic limpet Nacella, than in many temperate species. It is also possible that costs of maintaining elevated levels of RNA, which have also been shown to be a feature of some polar organisms, may make the unit costs of protein synthesis higher than in organisms from warmer waters.

9.8

Substrate Specificity and Structure of Fatty Acyl CoA Synthetase From Notothenioid Fishes

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Oxidative muscles of Antarctic notothenioid fishes are more dependent upon lipids than carbohydrates as their main fuel for aerobic metabolism. Furthermore, these tissues oxidize mono-unsaturated fatty acids (FA) more readily than saturated FA. We hypothesized fatty acyl CoA synthetase (FACS) in notothenioids has greater specificity for activating long-chain unsaturated FA in preference to saturated FA, targeting monoenes for oxidation in mitochondria. Using an enzyme-linked continuous spectrophotometric assay, we measured maximal FACS activity in isolated mitochondria from *Notothenia coriiceps* oxidative skeletal muscle in the presence of FA differing in chain lengths and degrees of saturation. With the exception of $C_{22:6}$, maximal activities of FACS were greater in the presence of unsaturated FA $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:4}$ and $C_{20:5}$ than in the presence of $C_{16:0}$, a saturated FA. However, monoenes as a class of substrates did not yield the highest activity. To elucidate which amino acid residues are candidates for determining FA specificity, full-length FACS cDNAs from oxidative skeletal muscles of Antarctic notothenioids *Chaenocephalus aceratus*, *Gobionotothen gibberifrons* and *N. coriiceps* and sub-Antarctic notothenioids *Eleginops maclovinus* and *Notothenia angustata* were sequenced. Comparisons of FACS from notothenioids and other organisms show substitutions within the FA binding region of FACS. Supported by NSF grants OPP 94-21657 and OPP 99-09055 to BDS.

9.9

Mechanisms of LDH adaptation to seasonal temperature change in cod (*Gadus morhua*)

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The aim of this study was to determine the effects of seasonal temperature change on the properties of lactate dehydrogenase (LDH) from white muscle and liver of Norwegian coastal cod.

Two groups of fishes were acclimated to 4°C and 12°C for 1 year. Acclimation temperature (AT) has a clear effect on the growth rate, but not on the physiological condition of the cod (k-facotr, HSI). Differences between the groups in allele frequencies of the four tested allozymes were not found. LDH-phenotypes have no effect on growth performance. AT has no effect on the LDH's isoelectrofocusing profile.

Neither kinetic (Km, Ksi, Vmax) nor thermodynamic (Ea, G) differences among the LDH-suite of LDH-phenotypes in crude homogenates were found. However, there were clear kinetic differences between the tissues. Despite of kinetic differences, the thermodynamic requirement to fulfil the pyruvate reduction in crude homogenate is the same for both tissues (Ea=47kJ/mol) at AT=12°C. However, at AT=4°C the Ea becomes higher (Ea=53-59kJ/mol). AT does not have a significant effect on the G of the reaction (56-58kJ/mol).

Differences in Km values among the acclimation groups were not observed for both tissues. Two-phase linear regression analysis revealed that at temperatures above 16±1°C the Km becomes significantly temperature dependent, which has important consequences for the "reserve capacity" of LDH, i.e. anaerobic capacity of metabolism.

Thus, the main strategies of LDH adjustment to seasonal temperature variations involves changes in LDH concentration (quantitative), adjustment of thermodynamic (Ea) and kinetic (Ksi) properties of the enzymes being present (modulative), but not the expression of alternative isoforms (qualitative). The work has been supported by EU project CLICOFI (ENV4-CT97-0596).

10.1

Is the anterior, axial position of the red myotomal muscle in tunas associated with an increased locomotor performance?

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The goal of this study was to understand how the anterior, axial position of the aerobic red myotomal muscle (RM) and an elevated RM temperature influence the swimming kinematics and energetics of tunas. We studied the eastern Pacific bonito *Sarda chiliensis*, a member of the ectothermic sister group to the tunas, and compared the results to previous studies on similar-sized tunas. A swimming tunnel respirometer was used to quantify the energetics and kinematics of *S. chiliensis* (45.5-50.5 cm) swimming at sustained speeds (50-130 cm s-1) at 18°C. *S. chiliensis* swam with a lower tail-beat frequency, greater tail-beat amplitude and yaw, and displayed a greater degree of lateral displacement than did similar-sized tunas swimming at comparable speeds. When the swimming energetics of *S. chiliensis* were compared to published data for tunas, the bonito had a similar net cost of transport but a lower standard metabolic rate (SMR). Thus, when compared with tunas, *S. chiliensis* utilizes a different swimming mode, exhibits similar locomotor costs, but has a higher SMR, despite differences in the RM position and RM temperatures. The data suggest that an elevated SMR, endothermy, anterior, axial RM, and thunniform locomotion may have evolved in concert in an ancestor of the tunas. Funded by NSF IBN-9316621 and -9973916.

10.2

Thunniform swimming: muscle dynamics and mechanical power production by aerobic fibers of yellowfin tuna (*Thunnus albacares*).

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We studied muscle performance in yellowfin tuna by sonomicrometry *in vivo* and work loops in isolated fiber bundles to understand the mechanical consequences of having the bulk of aerobic red muscle located deep in the body, rather than superficially as in most other teleosts. With sonomicrometry we found that muscle strain (relative shortening) of superficial fibers was equal to and in phase with that predicted by local body curvature, whereas strain in deep red fibers was 2X that predicted from local body curvature, and in phase with curvature 20% more posterior. This shows tuna red muscle can reside close to the backbone (as is necessary for metabolic heat conservation) without limiting its strain amplitude and at the same time direct the large strains to produce lateral motion more posteriorly on the body, the hallmark of thunniform locomotion. Work loops studies at 25°C confirm that power from deep fibers is doubled at the *in vivo* strain amplitude vs. that predicted. During steady swimming at 2-4Hz, the activation phase and duration used by fish were similar to those that gave maximal power *in vitro*, suggesting that the deep red muscle functions to maximize positive power production. At frequencies above 4Hz red muscle power declines, reaching zero at 9 Hz. At maximal burst speeds requiring the white muscle (>10Hz) the red fibers, if activated, will not contribute positive power, but may be tetanized and act as a stiff tendon-like element. Supported by NSF and NSERC.

10.3

Manipulation of Center of Mass Position in Trotting Quadrupeds

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Two force platforms in series were used to record fore- and hindlimb ground reaction forces (GRFs) independently. Dogs were trotted across the force platforms under various loading conditions and also with no added load. A saddlebag pack weighing 10% body weight was used to load the shoulders, center of mass (COM), or pelvis. Vertical and fore-aft GRFs from simultaneous fore- and hindlimb footfalls were analyzed by integrating the force-time curves and by Fourier analysis to quantify their shapes. The limbs of unloaded dogs exerted mean braking (fore) and propulsive (hind) forces of equal magnitude. Yet, this was achieved with a greater hindlimb propulsive shape bias, as shown by Fourier analysis. With a load positioned over the COM, mean fore-aft force magnitude, as well as braking (fore) and propulsive (hind) shape biases increased. This is attributed to larger moments at the hip and shoulder, which tend to dorsiflex the spine. In contrast, with the same load positioned over the pelvis or shoulders, the magnitude of the mean fore-aft force was equal to or less than that of the unloaded condition. When the pelvis was loaded, forelimb braking bias increased and hindlimb propulsive bias decreased with respect to the unloaded condition. This decrease of shape bias in response to increased vertical load is interpreted as a mechanism for maintaining fore-aft force equilibrium. No shape change was observed when the shoulders were loaded, perhaps due to their proximity to the COM.

10.4

Hind Limb Joint Kinetics of the Horse During Jumping.

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To understand the mechanics of jumping in horses, net joint moments and powers of the right hind limb were determined for five horses during the jumping take off (average barrier height = 0.63 m). Kinematic measurements determined from video recordings (250 Hz) were combined with synchronously recorded ground reaction forces (1000 Hz) using inverse dynamic analysis. Net joint moments and powers were calculated for hip, femorotibial, tarsal, metatarsophalangeal (MP) and distal interphalangeal (DIP) joints. Results show that extensor moments are produced across the hip (peak: 2.64±0.52 Nm/kg) and tarsal (peak: 2.21±0.36 Nm/kg) joints, with a strong flexor moment across the femorotibial joint (peak: 1.90±0.38 Nm/kg). There are bursts of energy generation at all joints except the DIP. These bursts occur in early stance (peak at 27% of contact time) at the femorotibial joint and in late stance at the hip (peak: 69%), tarsal (peak: 69%) and MP (peak: 82%) joints. Total power (summed across all five joints) reached a peak (13.2±6.5 watts/kg) at 74% of stance. Extensor musculature at the hip, tarsal and MP joints generate the necessary power to launch the horse off of the ground. Supported by NIH S06-GM53933 to DFH & SJW.

10.5

EMG Activity In Forelimb and Hind Limb Muscles During Level And Incline Trotting In The Horse

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Peak Ground Reaction Force (GRF) increases with running speed as a result of decreased duty factor. This necessitates an increase in the volume of active muscle. Muscle strain rate increases with trotting speed in the equine vastus lateralis and lateral triceps, and the force-velocity relationship predicts an increase in the volume of active muscle. The present study tests the hypothesis that, as trotting speed increases, the volume of active muscle increases more than would be expected from the increase in GRF alone. GRF was assumed to be inversely proportional to duty factor, which was measured using an accelerometer attached to the hoof. The volume of active muscle was assumed to be proportional to the integrated electromyogram (iEMG). EMGs were sampled at 4 kHz using a low pass filter at 1 kHz. Data were obtained from four Arabian horses (mean mass 430 kg) as they trotted on a motorized treadmill. In the vastus on the level, iEMG increased more ($p<0.005$) than GRF (158% vs 13%, respectively) as speed increased from 2.5 to 4.5 m/s. On the incline this difference ($P<0.005$) was also found (65% vs 19%). In the triceps on the level, iEMG also increased more ($p<0.0005$) than GRF (126% vs 24%) but on the incline there was no difference ($p>0.15$) in the change in iEMG and GRF (31% vs 22%). However, it should be noted that GRF on an incline may not be accurately predicted by duty factor if weight distribution between the limbs changes. Supported by NIH S06-GM53933 to DFH & SJW.

10.6

Time of Contact and Muscle Strain Rates Do Not Explain the Energetics of the Walk-Trot Transition in Horses

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In horses, metabolic rate (MR) does not change across the trot-gallop (t-g) transition or the walk-trot (w-t) transition. Because decreases in time of contact (t_c) explain the variation in MR with speed, one would expect no change in t_c at transition speeds. Previous work indicated no change in t_c at the t-g transition and the present study measured t_c at the w-t transition in three horses (average weight 431 kg) on a motorized treadmill. The t_c was lower when the horses trotted at 1.8 & 1.9 m/s than when they walked ($p=0.011$). A difference in t_c without the corresponding change in MR may be explained by a difference in muscle function, so muscle shortening velocity was measured in the vastus lateralis of the same three horses and speeds using sonomicrometry. There was no difference in positive strain rate ($p=0.98$) between the two gaits. The difference in the mechanics of the two gaits may explain the difference in t_c with no change in MR or strain rate at the w-t transition. Walking is modeled by the inverted pendulum, which is very different from the spring-mass model of the trot. The trot and the gallop are both 'bouncing' gaits and are more similar than the walk and trot. Supported by NIH S06-GM53933 to DFH & SJW.

10.7

Mitochondria Are Calcium Sinks in Rodent Extraocular Muscle

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Mitochondria are Ca^{2+} sinks in systems that require fast Ca^{2+} transients. The shallow twitches and rightward shift in the force-stimulation frequency curve of rodent extraocular muscles (EOMs) may be due to faster Ca^{2+} kinetics. Our study tested the hypothesis that mitochondria are Ca^{2+}-sequestering compartments that regulate contractile function in rat EOMs.

Methods: Superior rectus (index EOM) and lateral omohyoid (LO, relatively mitochondria-poor) muscles from male Sprague-Dawley rats were mounted between a force transducer and a micropositioner on a microscope equipped for Ca^{2+}-specific photometry. Force and myoplasmic Ca^{2+} concentration ($[Ca^{2+}]i$), and the effects of specific pharmacological interventions were measured.

Results: Calcimycin (1 M) and caffeine (5 mM) increased resting $[Ca^{2+}]i$ > 50% in LO, but not in EOMs. Carbonyl cyanide m-chlorophenyl hydrazone (CCCP, 10 M) significantly increased maximal tetanic $[Ca^{2+}]i$ in EOMs, and had a smaller effect in LO. CCCP also increased $[Ca^{2+}]i$ and force during submaximal contractions (~50% of maximal force) to a greater extent in EOMs compared to LO.

Conclusions: The results support our hypothesis that mitochondria are physiological regulators of Ca^{2+} transients during EOM contractions. Apparently, mitochondrial Ca^{2+} influx influences the extremely fast contractile events typical of EOMs, and may determine the wide frequency-response range, and fast relaxation rate of rat EOMs.

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10.8

Fascicle Strain In An Architecturally Complex Muscle In Running Birds

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Understanding the mechanical function of large muscles with extensive origins and insertions is challenging. This study examined the hypothesis that the architecture of the iliotibialis lateralis pars postacetabular (ILPO) in running birds provides for similar mechanical function in most fascicles. The ILPO is an extensor of both the hip and the knee and is the largest hindlimb muscle in Guinea Fowl Numida meleagris. The muscle is triangular in shape with short fascicles anteriorly near the hip joint and long fascicles posteriorly. The moment arm of the ILPO at the hip joint also increases posteriorly. The insertion on the patellar tendon operates as a pulley with a similar moment arm for all fascicles. However, the shorter fascicles have a longer aponeurotic connection to the patellar tendon than do the longer fascicles. We used sonomicrometry and electromyography to measure strain and muscle activity along the anteroposterior axis of the muscle. The ILPO is lengthened substantially while active during stance, and the strain during this portion of the stride was similar at all recording locations. We conclude that during stance the combination of the variable moment arm of the hip and the variable elasticity in the aponeurotic insertion at the knee compensates for differences in fascicle length such that active strain is similar across the muscle.

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10.9

Mechanical Function of a "Hamstring" Muscle in Running Guinea Fowl

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Muscle strain and activity were measured in the flexor cruris lateralis pars pelvica (FCLP), flexor cruris lateralis pars accessoria (FCLA) and intermediate gastrocnemius (IG) muscles in running guinea fowl. The FCLP complex is in the position of a "hamstring" muscle. The FCLP, FCLA and IG form a system of linked muscles and tendons that collectively span the hip, knee and ankle joints. The complexity of this system makes simple inference of muscle length change from limb kinematics difficult. We hypothesized that the joint angles at all three joints and timing of motor activity of the muscles will determine to what extent the system functions in hip retraction, knee flexion and ankle extension.

Sonomicrometry and electromyography were used to determine muscle length changes and activation patterns. Muscle activity in the FCLP and IG started in the latter part of the swing phase and persisted through the majority of the stance phase. The FCLA was primarily active during mid to late stance.

Our data are consistent with the hypothesis that early in stance the large FCLP muscle functions largely as an ankle extensor. Because it also spans the knee it likely contributes to active knee flexion. When the FCLA becomes active at midstance, this accessory muscle links the FCLP to the femur. The activity of the FCLA and the more flexed knee convert the FCLP into primarily a femoral retractor.

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10.10

The Effects of Incline on the Three-Dimensional Hindlimb Kinematics of the Arboreal Lizard, *Chamaeleo calyptratus*

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Many animals move on inclined surfaces in their natural habitat, but the effects of incline on limb movement and function during locomotion are rarely studied. Arboreal habitats have three-dimensional networks of narrow perches with variable inclines. Thus, we examined the three-dimensional kinematics of the hindlimb of a specialized arboreal lizard, *Chamaeleo calyptratus*, moving on narrow (2.3 cm) perches with inclines of -90°, -45°, 0°, 45° and 90°. We also compared our data for *C. calyptratus* to those for a similar speed of locomotion on a flat treadmill by a terrestrial lizard (*Dipsosaurus dorsalis*) with a more generalized limb posture and anatomy. Contrary to previous suggestions that chameleons have minimal axial bending, pelvic rotation of *C. calyptratus* exceeded that of *D. dorsalis* , especially on the level surface (64° vs. 42°). The angles of the knee at footfall of *C. calyptratus* commonly exceeded 120°, whereas those of *D. dorsalis* rarely exceed 90°. On the level surface, *C. calyptratus* also had greater values of long-axis femur rotation (115° vs. 83°) and femur protraction (-79° vs. -49°) than those of *D. dorsalis* . Unlike *D. dorsalis*, the knee of *C. calyptratus* was commonly above the hip at footfall. Inclined surfaces affected many attributes of the hindlimb kinematics of both species, but the effects were taxon-specific rather than globally similar.

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10.11

***In vivo* length changes of the rat rectus femoris and vastus lateralis during treadmill locomotion**

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The vastus lateralis (VL) and rectus femoris (RF) muscles constitute ~75% of the quadriceps muscle mass in the rat. The muscles are synergistic for knee extension, but RF can also flex the hip. The VL has relatively long muscle fibers with a low angle of pennation, while the RF has a bipennate architecture with relatively short muscle fibers. Based on these differences in architectural design, it has been suggested that the VL should shorten actively during locomotion to produce mechanical work at the knee, while the RF should remain nearly isometric due to symmetric movements of the hip and knee. Sonomicrometry was used to measure fractional length changes of these two muscles in the rat (n=4) during treadmill locomotion at speeds ranging from a slow walk to a gallop. RF lengthened throughout stance (~10%) at slower speeds, and at high speeds exhibited an initial lengthening phase followed by a nearly isometric period later in stance. At slow speeds, the VL lengthened (< 6%) or remained nearly isometric during stance, but at high speeds it shortened (~3%) during the second half of the stance period. The reduced excursions at higher speeds indicate a general stiffening of the joints, consistent with a transition to a bouncing gait. It is particularly interesting that the RF functions eccentrically throughout the stance phase during walking, given that such repeated eccentric contractions are widely viewed as injurious to muscles.

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10.12

Temperature-dependent Plasticity of Aerodynamic Design in *Drosophila*: Implications for Kinematics and Free-flight Ability

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In *Drosophila* and other small dipterans, there is an inverse relationship between adult body size and sub-adult rearing temperature. However, wing area increases disproportionately at low rearing temperatures such that wingloading is actually lower in larger cold-reared flies than in smaller warm-reared flies. Previous applications of theoretical aerodynamic models to tethered *Drosophila* have shown that cold-reared flies are able to generate more aerodynamic power during flight in cold air than warm-reared flies, supporting the hypothesis that this plasticity in aerodynamic design is an adaptive response allowing flies developing in cold larval and pupal environments to enhance adult flight performance in cold air. In this study, we used *D. melanogaster* to test the effects of sub-adult rearing temperature (15 °C, 23 °C or 28 °C) on adult morphology (body mass, relative thorax mass and wing area) and on free-flight ability and wingbeat frequency in cold air (14 °C, 16 °C and 18 °C). Female body mass, wing area and wingloading was significantly greater than in males for all rearing groups. For both sexes, body mass and wing area decreased with rearing temperature, while wingloading increased with rearing temperature. Relative thorax mass was significantly higher in males than in females, but was not affected by rearing temperature. Finally, cold-reared flies were best able to fly in cold air, despite having the lowest wingbeat frequencies of all rearing groups.

10.13

Effects of Load Type and Air Temperature on the Energetics of Load Carriage in the Honeybee, *Apis mellifera*

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The relationship between loading and metabolic rate in flying insects has been controversial. Some studies have found a strong linear effect of load mass on flight metabolic rate in bees. However, others have reported that flight metabolic rates of bees are related to reward rates but not load mass. We measured the effect of load type (pollen vs. nectar) and air temperature (20°C vs. 40°C) on flight metabolic rates of honeybees carrying loads. Load type strongly influenced flight metabolic rates. Pollen forager flight metabolic rates were 10% higher than nectar foragers, regardless of load size. The effect of load mass on flight metabolic depended strongly on air temperature. At 20°C and 24°C, flight metabolic rates were nearly independent of load (maximally increasing by 6%). However, at 40°C, flight metabolic rates increased linearly with load, to values approximately twice unloaded rates. Maximally loaded bees flying at 40°C had flight metabolic rates similar to bees flying at 20°C. Our data suggest that unloaded bees flying at low ambient temperature actively maintain high metabolic rates to thermoregulate. At 24°C, loading had no effect on wing kinematics, but did increase the calculated mechanical power output by a fraction similar to the increase in body mass. As a result, load carriage at low temperatures can occur with minimal increases in energetic cost, perhaps due to the high wingbeat frequencies and profile costs of flight in honeybees. In contrast, unloaded bees flying at high temperatures exhibit reduced metabolic rates and wingbeat frequencies, and load carriage requires a significant increase in metabolic rates and power output. This research was supported by NSF IBN 0093410 to J.F.H and J.H.F.

10.14

Forms of Locomotion in the Moon Snail, *Euspira lewisii*, (Mollusca: Gastropoda)

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Earlier studies on moon snails indicated that despite their large size there is an unusual if not total dependence on ciliary crawling. We studied locomotion in the largest species, *Euspira (Polinices) lewisii*, to ascertain relative contributions of muscular pedal waves and cilia to this type of movement. We simultaneously videotaped ventral and lateral views of moon snails crawling over level and inclined surfaces angled up to 30°. From the tapes, we calculated velocity, number and rate of formation of pedal contractions, and ventral pedal surface area. A mean velocity of 7.98 cm.min^{-1} (range 4.8 to 10.8 cm.min^{-1}) was obtained from 10 animals. Velocity tended to increase as larger surface areas were applied to the substrate. However, the possibility remains that other factors help determine velocity. Direct, monotaxic pedal waves were observed only on inclined surfaces and were isolated to the anterior part of the foot, the propodium. Furthermore, the number of waves increased as the angle of inclination became steeper, but pedal wave formation ceased once the animal established itself on the incline. There was a significant increase in pedal wave formation for inclinations greater 20°. As wave formation diminished on the incline, ventral surface area was increased. The data suggest that under these experimental conditions, ciliary beating played the predominant propulsive role in crawling *Euspira lewisii*.

10.15

Metabolite diffusion in giant muscle fibers of the spiny lobster *Panulirus argus*

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The time- and orientation-dependence of metabolite diffusion in giant muscle fibers of the lobster *Panulirus argus* was examined using ^{31}P- and ^{1}H-pulsed-field gradient NMR (PFG-NMR). The ^{31}P resonance for arginine phosphate and the ^{1}H resonances for betaine, arginine/arginine phosphate and –CH/-CH_2 groups were suitable for measurement of the diffusion coefficient, D. Diffusion was measured axially, $D_{\parallel}$, and radially, $D_{\perp}$, over a time course ranging from 20 to 300 ms. Diffusion was strongly anisotropic, and $D_{\parallel}$ was higher than $D_{\perp}$ at all times. Radial diffusion decreased with time until a steady-state value was reached at a diffusion time of ≈100 ms. The time scale over which changes in $D_{\perp}$ occurred was consistent with previous measurements from fish and mammalian muscle, indicating that diffusion was hindered by the same barriers in these diverse muscle types. The time-dependence indicated that the sarcoplasmic reticulum is the principal intracellular structure that inhibits mobility in an orientation-dependent manner in skeletal muscle. The abdominal muscles from *P. argus* are used for anaerobic, burst contractions during an escape maneuver. The fact that these muscle fibers have diameters that may exceed hundreds of microns in diameter, and nearly all of the mitochondria are localized near the sarcolemmal membrane, suggests that barriers that hinder radial diffusion of ATP equivalents may ultimately limit the rate of post-contractile recovery. Funded by NIH and NSF.

10.16

Gender Difference in Running Speed: Humans Versus Horses and Dogs

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A gender difference is apparent in human running performance. For example, Olympic records for 100 to 10000 m races indicate that men are ~10% faster than women. We asked if a gender difference in running speed also occurs in two other species for which race data are available; the horse and dog. Since horse races are usually segregated by gender whereas greyhound (GH) races are not, we hypothesized that a gender difference occurs in horses but not dogs. Data were obtained from the Daily Racing Form on-line (Thoroughbreds - TB), several harness tracks (Standardbreds - STB), and Bluffs Run track (GH). In a regression analysis of TB races, gender accounted for only 3.7% of the variance in speed (distance/winning time) in races ≤ one mile long (n=156), and did not significantly predict speed in races > one mile long (n=69). Analysis of STB track records for one mile indicated that gender accounted for 10.1% of the variance in speed of pacers (n=96), but did not enter the regression for trotters (n=95). Using regression, gender was not a significant predictor of GH speed at 503 m (n=146) or 603.5 m (n=23), however one-way ANOVA revealed a significant (p=0.043) effect of gender at 503 m, accounting for 2.8% of the variance in speed. In conclusion, gender has a significant but small effect on speed of both horses and dogs, particularly at shorter distances. The horse and dog gender difference in speed was ~1%, contrasting with the ~10% difference seen in humans.

10.18

The evolution of tendon - morphology and material

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Tendinous tissue first appears in the invertebrate chordate Branchiostoma as myosepta. This two dimensional array of collagen fibers is highly organized, with fibers running along two primary axes. In hagfish the first linear tendons appear and the myosepta have developed specialized regions with unidirectional fiber orientation – a linear tendon within the flat sheet of myosepturn. Tendon reacts to compressive load by forming first a fibrocartilaginous pad, and under severe stress, sesamoid bones. Evidence for this ability to react to load first arises in the cartilaginous fishes, here documented in a tendon from the jaw of a hard-prey crushing stingray. Sesamoid bones are common in bony fishes and also in tetrapods. Tendons will also calcify under tensile loads in some groups of birds, and this reaction to load is seen in no other vertebrates. We conclude that the evolutionary history of tendon gives us insight into the use of model systems for investigating tendon biology. Using mammal and fish models may be more appropriate than avian models because of the apparent evolution of a novel reaction to tensile loads in birds.

11.1

Pharmacological Anoxia and True Anoxia Result in Two Different Whole-Cell NMDAR Current Responses in Cortical Neurons from the Western Painted Turtle

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Mammalian brain undergoes rapid cell death during anoxia that is characterized by uncontrolled Ca2+ entry via N-methyl-D-aspartate receptors (NMDARs). In contrast, the western painted turtle is very anoxia-tolerant and maintains near normal [Ca2+]i for months of anoxia. A possible mechanism of anoxic survival in turtle neurons is the regulation of NMDAR to prevent excitotoxic Ca2+ injury. However, studies using metabolic inhibitors, such as NaCN, as a convenient method to induce anoxia may not represent a true anoxic stress. This study was undertaken to determine if turtle cortical neuron whole-cell NMDAR currents respond similarly to true anoxia and NaCN induced anoxia. Whole-cell NMDAR currents were measured during 1) a normoxic-anoxic transition and 2) a normoxic-NaCN transition. During true anoxia (N2) normalized NMDAR currents decreased to 35.3 ± 10.8% of control. However, exposure to pharmacological anoxia with NaCN resulted in two different NMDAR current responses, a 172 ± 51% increase in NMDAR currents, and a 52 ± 14% decrease. These two responses where neuronal cell type specific, stellate neurons responded to NaCN treatment with a decrease in NMDAR currents and pyramidal neurons showed both increased and decreased currents. NMDAR current responses were not Mg2+ dependent under either treatment. Our results show that whole-cell NMDAR currents respond differently to NaCN induced anoxia than physiologically relevant true anoxia. This work is supported by an NSERC.

11.2

Stable Isotope Changes During Fasting in Pinnipeds

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Stable carbon (13C) and nitrogen (15N) isotope changes during fasting were examined in 4 free-ranging gray seal (*Halichoerus grypus*; GS) pups, 24 free-ranging northern elephant seal (*Mirounga angustirostris*; NES) pups and 5 captive Steller sea lion (*Eumetopias jubatus*; SSL) juveniles. Blood samples were collected at intervals during fasting with the maximum fasting periods of 21, 86 and 14 days, respectively 13C showed significant depletion of 0.35‰ for NES ($p<0.05$) whereas 15N showed significant enrichment of 0.67‰ and 0.61‰ for NES and SSL ($p<0.05$), respectively. No significant changes in 13C and 15N were noted for GS. Due to variable responses, 13C did not change significantly during the fast for SSL. The trend of enriched 13C in SSL was significantly different than the depletion seen in NES but neither SSL nor NES was significantly different from GS ($p<0.05$). SSL and NES showed significantly greater enrichment for 15N compared to trends in GS but were not significantly different from one another ($p<0.05$). The 15N and 13C enrichment for SSL suggests primary use of lean body tissue during fasting compared to the younger, fatter fast-adapted phocids. In contrast, NES and GS appear to be utilizing lipids and proteins sequentially during their fasting period. GS did not appear to have reached their limit of fasting because of low depletion and enrichment trends for 13C and 15N, respectively. Research supported by CWS, NMML/NOAA, NPUMMRC, NFWF and ADF&G.

11.3

Changes in the Apoptotic Pathway in Intestinal Epithelial Cells During Hibernation

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Mammalian hibernation involves several phenotypic changes that increase the risk of physiologic stress including marked fluctuations in body temperature, metabolism, and blood flow. Strong activation of the stress-induced transcription factor, NF- B, occurs in the intestinal mucosa of hibernating 13-lined ground squirrels compared with summer animals. Regulation of apoptotic pathways is one of the consequences of NF- B transcriptional activity in many cell types. Thus, we investigated the hypothesis that the apoptotic pathway in the intestinal mucosa of hibernating squirrels differs from that in summer animals. We used TUNEL to identify apoptotic cells in intestinal sections and Western blotting to determine the expression of apoptosis-related proteins in mucosal lysates. The number of TUNEL-positive enterocytes in hibernators was much greater than in summer squirrels, with highest numbers occurring in animals that were several (>3) months into the hibernation season. The pro-apoptotic proteins Bax and caspase-8 p20 were expressed at higher levels in mucosa of entrance and torpid squirrels compared with summer animals. These results suggest that apoptosis is increased in the gut during hibernation and, therefore, the apoptotic pathway may be one transcriptional target for NF- B activation in hibernators. Supported by Army Research Office #DAAD190110455

11.4

Evidence for a cryoprotective protein in freeze-tolerant larvae of the goldenrod gall fly, Eurosta solidaginis. Nancy L. Pruitt. Department of Biology, Colgate University, Hamilton, NY 13346

Third instar larvae of the goldenrod gall fly, Eurosta solidaginis, overwinter in a ball gall on the stem of the goldenrod plant, Solidago spp., where they are exposed to a wide range of temperature extremes. Populations of gall fly larvae from central New York (USA) undergo a transition from nfreeze-susceptible to freeze-tolerant in early October. This transition is accompanied by the production of several sugars. We found that freeze-tolerant larvae also produce a soluble, heat stable, cryoprotective protein (CRP) capable of preserving up to 80% of the activity of freeze-labile rabbit muscle lactate dehydrogenase against freeze-thaw denaturation. The cryoprotective capacity of the Eurosta CRP is 10X greater than sugars and 4X greater than bovine serum albumin. Preliminary studies indicate that both freeze-susceptible larvae (collected in August and early September) and transitional larvae (collected in early October) produce the CRP, with transitional larvae having the most protein. Partial purification of the CRP resulted in an active fraction significantly enriched in a 29 kDa protein compared with crude preparations. This molecular mass compares well with that of cryoprotective proteins reported from ice nucleating bacteria (29 kDa) and Arabidopsis (15 and 26 kDa), but is not similar to heat shock proteins previously reported from gall fly larvae (71 and 94 kDa). This indicates that the CRP may be a formerly unknown protein in Eurosta. Funding Source: Colgate University Research Council.

11.5

Partial Links Between the Seasonal Acquisition of Cold Tolerance and Desiccation Resistance in the Goldenrod Gall Fly *Eurosta solidaginis*

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Larvae of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae), enhance their cold hardiness and desiccation resistance as they prepare for winter. In mid-September, gall tissues are well hydrated (64%) but later may become extremely dry as the plant senesces. Freeze tolerance gradually increased during the autumn until Oct. 30 when all larvae survived at least -20°C for 24 h. During this period there was a concomitant increase in hemolymph osmolality, consistent with previous reports of cryoprotectant (glycerol and sorbitol) accumulation at this time. In contrast to the steady increase in cold tolerance, resistance to desiccation increased abruptly between Oct. 3 and Oct. 16, when the rate of water loss decreased by 83.6%. This increase in desiccation resistance was not linked to cold hardening because neither the level of freeze tolerance nor hemolymph osmolality increased. Instead, the abrupt increase in desiccation resistance is likely associated with drying of the gall tissue; in a separate experiment, desiccated larvae significantly increased their resistance to water loss compared to larvae held at more humid conditions. However, later in the season there was a further decrease in water loss rates that correlated with increasing hemolymph osmolality, and thus suggests a link to cold hardening.

Supported by NSF#IBN-0090204

11.6

Consequences of Starvation on Metabolic Rate and Life History Traits in the Nematode, *Caenorhabditis elegans*.

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C. elegans is a terrestrial, free-living nematode worm that feeds on soil-dwelling bacteria. *C. elegans* can complete its life cycle from a newly hatched larvae to an egg-laying adult in less than two days when food is abundant. Over this period, *C. elegans* increases its body mass approximately 100-fold relative to its initial larval mass. The maintenance of this high growth rate requires that *C. elegans* ingests several times its own body mass in bacteria daily, and maintains high mass-specific metabolic rates. This study examined the effect of complete food deprivation on the metabolic rate, survivorship and growth of *C. elegans* at different developmental stages. The metabolic rates of newly hatched larvae, or young adult *C. elegans*, were measured in both starved and fed populations. When starved, both larval and adult populations of *C. elegans* reduced their metabolic rates by 50% within 24 hours, and by a factor of 10 within 5 days. *C. elegans* survived such a metabolic depression and lack of food for several weeks. However, adults that developed from starved young larvae which were subsequently returned to food typically had reduced fecundity compared to worms that were never starved. The ability of *C. elegans* to rapidly reduce its metabolic rate is consistent with its ecology as food is likely to be ephemeral in a soil environment, necessitating that soil nematodes survive periods of reduced food availability. Research support from the National Institutes Health (AG11659).

11.7

Proteomic Analysis of Brain and Heart Proteins in a Hibernating Mammal

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Hibernating mammals undergo drastic changes in body physiology in response to decreased light exposure, long periods of cold temperatures and no food in the winter. In a state of deep hibernation, the body temperature is reduced to a few degrees above 0°C, oxygen consumption is about 2% of the aroused state and the heart rate can be 3-10 beats/minute, compared to 200-300 beats/minute when the animal is awake and active. To maintain function during these physiological extremes, hibernators undergo changes in their proteome to compensate for the reduced metabolic rate and oxygen consumption. We have chosen to study hibernation at the molecular level using the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*. Identifying brain and heart proteins whose expression changes during the switch from the active to the hibernating state are of interest because both organs play a prominent role in hibernation. The protein samples are separated by two-dimensional gel electrophoresis (2D-PAGE). These 2D gels from both active and hibernating animals are analyzed by examination of stained spots corresponding to individual proteins. A comparison of the gels using Phoretix software highlights those proteins that show a modification event. Preliminary gels have shown that certain proteins in the heart are differentially regulated throughout the year. These proteins will be analyzed further with mass spectrometry in order to determine their sequence homology.

11.8

Out Cold: Protein Expression In Liver Of Golden-Mantled Ground Squirrels

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Ground squirrel hibernation is physiologically characterized by a reduction in heart, metabolic and respiratory rates to ~1-3% of summer levels and core body temperatures as low as -2.9C. The molecular and biochemical changes that underlie these physiological extremes remain largely unelucidated. Due to the close phylogenetic relationship between hibernators and non-hibernators, we propose that the phenotype of hibernation depends on the differential expression of genes common to mammals rather that the invention of new genes; therefore, we are working to identify those gene products whose expression is altered seasonally using liver tissue, because of its central role in systemic metabolism. Using well-established approaches to liver cell fractionation for subcellular enrichment coupled with two-dimensional SDS-PAGE separation of proteins, we compare summer liver protein expression to that of winter, and use liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) to identify protein spots that are differentially expressed. Post-translational modifications alter with hibernation (e.g. cytochrome B5) as do protein levels (e.g. liver fatty acid binding protein). A global perspective on mechanisms of hibernation can be obtained by this method in a variety of tissues. This research is supported by a grant from the Army Research Office.

11.9

Neuroendocrine Control of Hibernation in Mammals: Role of the HPA Axis

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Previous studies have indicated that the hypothalamic-pituitary-adrenal (HPA) axis may play an important role in triggering physiologic changes that allow animals to hibernate. We have examined the role of the HPA axis in hibernation of 13-lined ground squirrels using microarray and RT-PCR analysis of hypothalamus and adrenals. We have found that a preliminary gene expression profile of the hypothalamus in the months leading up to hibernation (September-October) is consistent with induction of kinase signaling pathways, secretory pathways, and neurotransmission. Since signals from the hypothalamus will likely impact the activities of the adrenal cortex, we examined expression of the steroidogenesis acute regulatory (StAR) protein in the adrenals using RT-PCR. The StAR protein regulates cholesterol delivery to the inner mitochondrial membrane; the tightly regulated rate-limiting step in the synthesis of all steroid hormones. We found that steady state levels of StAR mRNA were higher in active animals than in hibernating animals. These fluctuations correlate with known profiles of cortisol secretion in hibernators. These results indicate a possible mechanism for neuroendocrine initiation of hibernation: the hypothalamus senses environmental stimuli (i.e. day length, food availability, ambient temperature) and relays these signals to the adrenal cortex, which secretes steroid hormones known to regulate metabolism.

11.10

Enhanced Antioxidant Activity in the Longest-lived Rodent Species (*Heterocephalus glaber*)

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Aging is the process by which an individual's physiological capacities decline with time after maturity. Many deleterious effects of aging are thought to result from accrual of molecular oxidative damage. Lifetime damage accumulation is due to the net difference between the generation of Reactive Oxygen Species (ROS) and their removal by antioxidants. Unequal lifetime damage accrual may potentially account for variation in maximum lifespan (MLSP) among species. Naked mole-rats (*Heterocephalus glaber*) are the longest-lived rodent species known (MLSP=28y), exceeding allometric predictions for MLSP by about 8 times. We hypothesized that mole-rat longevity may be due to enhanced antioxidant activity and compared them to shorter-lived mice (*Mus musculus*, MLSP=4y). We examined: 1) whether antioxidant activity changes with age, and 2) if mole-rats have greater antioxidant activity than mice. Liver Cu/Zn superoxide dismutase (SOD) activity in young, middle-aged and old individuals of both species was assessed using colorimetric assays (Oxis). SOD activity did not vary among ages in either species. However, mole-rats had significantly higher SOD activities than mice ($P<0.05$). These results imply that unequal SOD activity may partially account for the very different MLSP between these two species, although disparate ROS generation rates may also contribute to the observed MLSP differences. Support provided by a grant from American Federation for Aging Research to TOC.

NEUROPEPTIDES INTEGRATING PHYSIOLOGICAL PROCESSES IN INVERTEBRATES: AN EVOLUTIONARY AND COMPARATIVE APPROACH

12.1

Topical Application Of An Insect Neuropeptide On Crickets (*Gryllus bimaculatus*)

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Adipokinetic hormones (AKHs) are one of the best-studied neuropeptide families in insects. They increase lipid, carbohydrate and/or proline titers in the hemolymph by mobilizing energy stores in the fat body. In addition, AKHs inhibit fat body lipid synthesis and thereby interfere with energy-demanding events such as the formation of eggs. For the Mediterranean field cricket (*Gryllus bimaculatus*), we could show that injections of Grybi-AKH, the endogenous AKH of this species, lead to a reduced egg production. However, repeated injections were required to obtain a significant effect, possibly due to the short half-life of the AKH in the hemolymph. Since repeated injections cause increased stress in the experimental animals, we felt that a non-invasive method to apply AKH would be of advantage.

We topically applied 1 to 100 pmol Grybi-AKH, dissolved in various solvents (water, methanol, acetonitrile, DMSO, 2-propanol), onto the dorsal thorax under the wings. Depending on the concentration of Grybi-AKH and on the solvent used, we found up to 100% increase in hemolymph lipid titers and a significantly reduced lipid synthesis in the fat body. Furthermore, following application, the hemolymph lipid titers remained high for a longer period (8 h or more) compared to the transient increase obtained by AKH-injection. Thus, topical application of peptides or peptide analogs may be of use in future specific insect pest control.

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12.2

Interaction of Molluscan Cardioactive Neuropeptides

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How do neuropeptides function in the integration of physiological control of molluscan muscular tissues? Tissues were isolated from gastropod heart and buccal musculature, as well as from bivalve heart. Perfused entire gastropod hearts were also studied. Synthetic neuropeptides were applied singly, and in combination, to tissues undergoing spontaneous and driven contractions. The actions of the neuropeptides were recorded using single and double sucrose gap recording in combination with recording of force and shortening or versus cardiac pumping. The response to FMRFamide (Fa) was antagonised by PQDPFLFamide and N-acetylF-NLE-RFamide. These actions may be useful in pharmacological analysis of the physiological actions of Fa-related transmitter agents. The inhibition of Fa-effects by 5HT is mimicked by dibutryl cAMP or forskolin. IBMX enhances inhibition of Fa-effects. Metoclopramide blocks maitotoxin inhibition of Fa-effects. These actions may be useful in determining whether interaction is presynaptic or intercellular.

14.2

METABOLISM AND MICROARRAY ANALYSIS OF CARDIAC GENE EXPRESSION

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Microarrays are thousands of 150 to 250 micron spots of DNA bound to microscope slides in a precise and known pattern. Each DNA spot quantitatively hybridizes to a specific mRNA so that expression of thousands of individual genes can be measured simultaneously. Microarray analyses provide data on the patterns of mRNA expression for most genes expressed in cells. Although microarrays only measure the amount of mRNA, mRNA expression affects many physiological responses (e.g., hormonal effects on metabolism1, hypoxia 2, etc.). Thus, the use of microarrays opens the door to both discover which genes are involved in physiological processes and to test hypotheses concerning the molecular mechanisms underlying these processes.
In order to apply microarray technology to physiologically insightful species of teleost fish *Fundulus heteroclitus*, we have isolated and sequenced 6,868 heart-ventricle cDNAs 3. These heart cDNAs cluster into 4,223 different genes of which 3,469 were isolated once. Of the 4,223 genes, 211 (5%) are unidentified. The heart cDNAs were subtracted from a liver cDNA library from which 9,559 liver cDNAs were isolated and sequenced. These liver cDNAs cluster into 4,412 unique genes of which 3473 were isolated once (singletons). 3,175 of these singletons are unidentified.
These cDNAs were used to investigate the experimental variation in microarray data, the variation within and between populations and the effect of body mass on mRNA expression. With substantial replication, experimental variation is equivalent to that in most biochemical assays (northerns, enzyme assays): 95% of the genes had coefficients of variation (standard deviation/mean) of less than 10%. Importantly, 18% of loci were significantly different between individuals within a population ($p < 0.001$). Body mass was a significant covariate for 11% of the loci, with the log-log slope ranging form -0.43 to 0.32 (57% were positive).

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14.4

GENE EXPRESSION ASSOCIATED WITH DIURNAL TEMPERATURE CYCLING IN THE ANNUAL KILLIFISH *AUSTROFUNDULUS LIMNAEUS*.

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Despite many years of intense research, the biochemical and molecular mechanisms that maintain cellular homeostasis during large-scale environmental fluctuations are largely unknown. Annual killifish thrive in small ephemeral ponds that experience wide fluctuations in temperature (22-39°C), oxygen (4-256% saturation), and pH (5-9) on a diurnal basis. We are using cDNA microarray technology to examine temperature adaptation by investigating changes in gene expression associated with a cycling temperature regime. An *A. limnaeus* liver-specific cDNA microarray was prepared using full-length cDNA sequences isolated from a normalized cDNA library and a subtracted cDNA library enriched for differentially expressed cDNAs. Liver tissue was chosen because of its central role in metabolic homeostasis. Adult *A. limnaeus* were exposed to three different thermal treatments: a cycling temperature regime of 20-37°C on a daily basis, and constant exposure to 20°C, 26°C, or 37°C. Differentially expressed cDNAs were identified by probing the cDNA microarray with fluorescent-labeled probes prepared from poly-A RNA isolated from fish exposed to the various thermal regimes. These cDNAs were then sequenced and identified by homology to known genes or gene products using the Blastx database search engine. Patterns of gene expression were used to construct a more "global" view of the physiological and biochemical responses to diurnal temperature cycling as well as to identify new genes and generate new hypotheses concerning the molecular basis of eurythermality. Preliminary results indicate that the transcriptional response to temperature cycling is complex but includes an induction of several classes of molecular chaperones and a cessation of cell growth and proliferation. These strong initial transcriptional responses appear to be attenuated after the first 3 diurnal cycles. Supported by NSF IBN-0133184 to GNS.

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Establishes the utility and feasibility of using microarray technology to explore adaptive physiology in non-model organisms.

14.5

EXPRESSION PROFILING DURING THERMAL AND HYPOXIC ACCLIMATION IN COMMON CARP.

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A key factor in the survival and continued success of organisms in climatically variable environments is their ability to display a suite of adaptive responses that promote both the constancy of their constituent physiological processes and increase tolerance to damaging or lethal conditions. Relatively little is known about the molecular mechanisms underlying these responses. Deploying a high-throughput microarray-based approach, we have investigated the transcriptional component of the response of common carp (*Cyprinus carpio*) to the environmental challenges of cold and hypoxia. A microarray for carp was fabricated using 14,000 cDNAs picked from a collection of normalized and serially subtracted cDNA libraries. For cold exposure, warm-acclimated (30°C) carp were cooled either to 23, 17 or 10°C, and sampled over 3 weeks. For hypoxia exposure, carp acclimated to 30 and 17°C were subjected to 0.3 mg/L O_2 at their respective acclimation temperatures, and sampled over 7 days. Liver, brain, gill, kidney, intestinal mucosa, skeletal and cardiac muscle RNA were isolated from animals throughout the time-course. We then generated the temporal gene expression profile for each individual tissue by hybridization to >600 microarrays. This data reveals that a substantial proportion of the transcriptome is regulated by cold and hypoxia. A common set of genes that were regulated in every tissue, as well as tissue-specific profiles were identified. Expression profiles were analyzed in relation to time and intensity of environmental treatment to define the thresholds for inducing different groups of genes. The significance of the transcriptional response will be discussed in the context of the known impact of cold and hypoxia on physiological performance.

Supported by NERC.

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14.6

A COMMON GENE EXPRESSION PROGRAM IN THE RESPONSE OF YEAST CELLS TO DIVERSE ENVIRONMENTAL CHANGES

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All organisms require specific and delicately balanced internal conditions for optimal growth and function. The internal milieu of the cell is maintained to promote proper operation of the cell, however fluctuations in the external surroundings can result in a variety of cellular perturbations that can disrupt the internal environment. Thus, when external conditions change abruptly, the cell must rapidly adjust its internal milieu to that required for growth at the new conditions. Genomic expression studies using DNA microarrays have provided insights into the mechanisms that the yeast *S. cerevisiae* uses to survive diverse environmental changes. One prominent feature of their response to stressful environments is the initiation of a common gene expression program, called the Environmental Stress Response (ESR). The ESR includes nearly 1000 genes (~15% of all genes in yeast) that are stereotypically induced or repressed in response to suboptimal environmental transitions. Despite the common expression changes of these genes, the regulation of the program is controlled by many condition-specific regulatory systems. Implications for the role and regulation of this response will be discussed.

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HOMEOSTASIS OF ESSENTIAL YET TOXIC METALS

15.1

HOW COPPER ENTERS CELLS: ROLES OF HIGH AFFINITY COPPER TRANSPORTERS IN PHYSIOLOGY AND DEVELOPMENT.

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Due to its ability to adopt distinct valence states, copper (Cu) serves as an essential redox-active co-factor for a wide variety of enzymatic activities including tyrosinase (pigmentation), dopamine b-hydroxylase (neurotransmitter synthesis), cytochrome oxidase (energy generation) and Cu, Zn superoxide dismutase (oxidative stress protection). The importance of Cu acquisition is underscored by the well established defects that are associated with mammalian Cu deficiency including blood vessel integrity defects, cardiac hypertrophy and other pathophysiological states. To understand how cells acquire Cu to drive important enzymatic activities, and the precise roles of Cu acquisition in cell physiology, our lab studies the structure, function and regulation of Cu transport proteins in the Ctr1 family. Our studies demonstrate that Ctr1 transports Cu with high affinity and specificity in an ATP-independent manner. Indeed, inactivation of Ctr1 genes in yeast, mice and flies generates growth and developmental defects that are consistent with biochemical defects in the acquisition and distribution of Cu to requisite biological targets.

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15.2

HEAVY METAL UPTAKE AND SEQUESTRATION IN LOBSTER HEPATOPANCREATIC EPITHELIAL CELLS AND THEIR ORGANELLES.

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The lobster (*Homarus americanus*) hepatopancreas is a site of temporary exoskeletal calcium storage during the molt cycle and of heavy metal sequestration and detoxification. This report summarizes the results of recent experiments directed at clarifying the nature of plasma membrane and organellar heavy metal transport systems in this crustacean organ and how calcium transporters may be involved in metal homeostasis. The copper-specific fluorescent dye, Phen Green, was used in experiments with suspensions of purified epithelial cell types to show that each of the four cell types transported copper across the plasma membranes by calcium-stimulated antiporter mechanisms exhibiting dissimilar kinetic constants for copper transport. Zinc was a competitive inhibitor of copper influx suggesting both metals interact at the same transporter binding sites. Phen Green was also used to define the mechanisms of copper transport into hepatopancreatic mitochondria and lysosomes. Copper uptake into mitochondria occurred by the combination of a ruthenium-red sensitive uniporter and a diltiazem-inhibited antiporter. Copper influx into lysosomes took place by vanadate- and amiloride-inhibited carrier mechanisms and by an apparent calcium-copper antiporter. This study shows that metal uptake and sequestration within hepatopancreatic epithelial cells and their organelles occurs by transport systems normally accommodating calcium as an antiport substrate or using calcium as a transport activator. Supported by NSF grant IBN99-74569 and PCCI-CONICYT.

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15.3

COPPER HOMEOSTASIS IN TELEOST FISH. Martin Grosell, Zoophysiological Laboratory, The August Krogh Institute, University of Copenhagen, Universitetsparken 13, DK-2100 Ø, Copenhagen, Denmark

At the organ level, teleost fish resemble higher vertebrates with respect to copper homeostasis. As in mammals, the liver is the major homeostatic organ, and biliary excretion is elevated in situations of elevated copper uptake. Circulating levels of copper in the plasma are under tight regulation. As in mammals, renal loss of copper is not stimulated under conditions of copper excess. Teleost fish share the dietary uptake route with mammals but recent studies demonstrate that the gills are important for whole body copper homeostasis. In some situations, copper uptake across the gill epithelium account for as much as 60 % of the whole body copper accumulation. In addition, copper uptake across the gills of freshwater fish is clearly regulated according to copper status: Copper excess is associated with reduced branchial copper uptake, while copper deficient fish exhibits elevated copper uptake across the gills. The later observations suggest involvement of copper specific carriers in copper uptake across the gill. Partial cloning of a putative Menke's type copper-ATPase from fish gills indicate that this specific copper transporter could be involved in copper uptake across the gills as it is in the intestine. Both a sodium sensitive and a sodium in-sensitive component of apical copper uptake in fish gills have been identified. Both exhibits saturation kinetics with affinity constants in the low nano-molar range. Pharmacological observations and strong interactions between copper and sodium uptake is interpreted as copper uptake via the apical epithelial sodium channel.

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15.4

PHYSIOLOGY, TOXICOLOGY, AND HOMEOSTASIS OF SILVER IN FISH AND AQUATIC INVERTEBRATES. Chris M. Wood, Dept. of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1

The toxicity of silver in aqueous environments is critically dependent on route of administration and aqueous speciation. Dietary silver exerts negligible toxicity, and for waterborne silver, only the free Ag^+ ion is acutely toxic. Complexing anions such as Cl^-, thiosulfate, sulfide, and dissolved organic matter greatly reduce silver toxicity in natural waters, but do not necessarily prevent uptake and internal accumulation. The latter occurs primarily in the liver, where silver is a powerful inducer of metallothionein. Ag^+ appears to act as a Na^+ analogue, entering the gills via the apical Na^+ channel/H^+ ATPase mechanism, and exiting the basolateral membrane via an ATP-dependent mechanism which appears to be a P-type ATPase. Acute toxicity results from an inhibition of active Na^+ (and Cl^-) uptake at the gills, ultimately attributable to a blockade of Na^+,K^+-ATPase activity at the basolateral membrane, and preceded by competitive blockade of the apical Na^+ channel and inhibition of intracellular carbonic anhydrase. An understanding of toxic mechanisms and the importance of geochemical speciation has led to the development of predictive models for assessing site-specific toxicity in a variety of natural freshwaters.

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15.5

MOLECULAR CONTROL OF ZINC TRANSPORT IN FISH. Christer Hogstrand, Sara Balesaria and Andong Qui, King's College London, School of Health and Life Sciences, Franklin Wilkins Building, London SE1 9NN, UK.

Zinc is essential to all organisms, yet it is a problematic environmental toxicant. In fish, the gill is a major uptake site for nutritional hydrominerals, including zinc, and it is also a primary site for zinc toxicity. Exposure to waterborne zinc causes hypocalcaemia through interference with branchial calcium uptake. This effect is partly due to competition with calcium for an apical uptake site. Recent evidence suggest that the nature of this apical transporter might be an orthologue to the Epithelial Calcium Channel, ECaC, present in some mammalian transporting epithelia. Although zinc and calcium seem to partly share uptake pathway, there is now evidence for a number of dedicated zinc transporters in the fish gill. These include members of the ZIP and ZnT families of zinc transport proteins. ZnT-1 from pufferfish and zebrafish show high similarities to mammalian homologues of this exporter. There are at least two putative ZIP importers in fish gill and these have strong, but mixed homologies to ZIP1 and ZIP2 from mammals. This indicates that ZIP1 and ZIP2 have evolved independently from a common ancestor in bony fish and mammals.

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15.6

UPTAKE AND REGULATION OF IRON IN TELEOST FISH. Nicolas Bury, School of Life Sciences, King's College London, SE19NN, UK.

Iron is an essential nutrient to all organisms. But, in excess it is toxic, and homeostasis is tightly controlled via absorption. In recent years the molecular basis of intestinal iron uptake in mammals has been identified. On entering the duodenum non-haem bound iron, Fe^{3+}, is reduced to Fe^{2+} via a membrane bound ferrireductase. Fe^{2+} then enters the enterocyte via a Fe^{2+}/H^+ symporter known as a divalent metal transporter (DMT). Basolateral extrusion of Fe^{2+} occurs via an iron-regulated transporter (IREG) that is linked to a membrane bound multicopper oxidase called hephaestin, which oxidises Fe^{2+} to Fe^{3+}. Fe^{3+} bound to transferrin circulates the body.

Based on molecular evidence components of the machinery for iron acquisition are present in the largest vertebrate phylum, the fishes, and cDNA homologues to DMT and ferroportin (analogous to IREG) have been identified. However, very little work has focused on how fish overcome profound geochemical obstacles to ensure functionality of iron uptake. For example, the concentrations of bioavailable iron in the water are exceptionally low. Despite this, DMT transcript is present in the gills, and non-feeding juvenile fish absorb iron. Furthermore, the intestinal fluids of marine fish possess levels of bicarbonate that results in cation precipitation, and are alkaline which would perturb the functioning of a Fe^{2+}/H^+ symporter. Even though both conditions would complicate iron uptake, the European flounder acquires iron from the diet. Establishing how fish acquire iron may help elucidate how other organisms maintain iron bioavailable.

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15.7

Bioavailability and cellular processing of zinc in fish using in vivo and in vitro approaches. Ronny Blust and Frederik Muylle,
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Essential trace metals such as copper and zinc are highly regulated within fish and other organisms. This process involves active control of both the uptake and elimination of the metals across the exchange structures. These mechanisms allow fish to keep the tissue concentrations within narrow limits and independent of changes in exposure concentrations or conditions. However, above a certain threshold concentration the regulatory capacity breaks down and net accumulation occurs. Toxic effects are observed when the cellular machinery can no longer cope with the increased influx of the metal ions by intracellular sequestration in non-toxic forms or elimination.
So far little is known concerning the relationship between metal uptake rates, intracellular processing and cellular toxicity. Using in vitro fluorescent methods in combination with laser scanning confocal microscopy we have studied the uptake and accumulation of zinc in individual cells of a trout liver cell line and primary carp hepatocytes. The results provide information concerning the characteristics of the uptake process, rates of intracellular complexation and compartmentalisation, as well as the recovery of cells from zinc loading. Changes in intracellular free zinc ion activities have been estimated as a function of exposure time and concentration. In addition, the onset of physiological disruption as a function of time and concentration was determined using different indicators of cellular functionality.

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16.2

EFFECT OF TEMPERATURE ACCLIMATION ON STRUCTURE AND THERMAL STABILITY OF MYOSIN ISOFORMS IN CARP FAST SKELETAL MUSCLE. Shugo Watabe, Laboratory of Aquatic Molecular Biology and Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo 113-8657 Japan

Carp expresses at least three myosin isoforms in association with acclimation temperature. From information on the primary structure, it is obvious that structural differences in carp myosin heavy chain isoforms are prevailed from an N-terminal myosin cross-bridge head called subfragement-1 (S1), which contains actin- and ATP-binding sites, to a C-terminal rod having coiled-coil structure of α-helices.

Taking Ca^{2+}-ATPase activity as a parameter, thermal incubation experiments demonstrated that S1 from carp acclimated to 10°C was about 3 times thermally unstable compared to that from carp acclimated to 10°C. There are only 9.1% differences in a total of 834 – 836 amino acid residues between S1 heavy chains from carp acclimated to 10 and 30°C. However, the residues responsible for such differences in thermal stability have not been identified yet

Differences in thermal stability of myosin rod in relation to structural differences have been mostly focused on L-meromyosin (LMM), a C-terminal half of rod. Three LMM isoforms including that having an intermediate structure between LMM isoforms from carp acclimated to 10 and 30°C have different thermal stability as revealed by differential scanning calorimetry (DSC). Transition temperatures in DSC for LMM isoforms indicated that LMM from carp acclimated to 10°C is clearly thermally unstable compared to that from carp acclimated to 30°C. Amino acid variations in different LMM isoforms cooperatively affect on thermal stability which was demonstrated with recombinant proteins.

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16.3

GENES REGULATING MUSCLE GROWTH IN TELEOST FISH AND THEIR RESPONSES TO TEMPERATURE CHANGE. Ian A. Johnston, Gatty Marine Laboratory, School of Biology, University of St Andrews, St Andrews, Fife, Scotland KY16 8LB, UK

Embryonic slow and fast muscles arise from adaxial cells and the lateral presomitic mesoderm respectively under the influence of distinct signalling pathways. Subsequent addition of muscle fibres involves different patterning mechanisms and populations of highly proliferative myogenic progenitor cells. Commitment and differentiation of cells to a muscle lineage involves the expression of a family of basic helix-loop-helix transcription factors belonging to the MyoD gene family (MyoD, Myf-5, myogenin, Myf-6). Temperature of embryonic development influences the expression patterns of muscle-specific genes and the relative timing of differentiation. Larval stages of herring, *Clupea harengus*, develop the adult patterns of myofibrillar isoform expression and innervation earlier with respect to body length at 12°C than 5°C, with significant consequences for escape swimming performance. In Atlantic salmon, (*Salmo salar*) each phase of myogenesis exhibits different responses to development temperature. Small differences in temperature (<2.5°C) prior to eye pigmentation affect the final number of muscle fibres in adult salmon, but not the rate of hypertrophic growth. These results are consistent with a hierarchical model of muscle stem cell commitment in which the fate of cells destined to form myotubes is determined early in development.

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16.4

MOLECULAR DETERMINANTS OF CARDIAC Na^+-Ca^{2+} EXCHANGER TEMPERATURE DEPENDENCE

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The trout cardiac Na^+-Ca^{2+} exchanger (NCX) exhibits profoundly lower temperature sensitivity in comparison to that in mammals. In order to understand the molecular mechanisms of these differences we examined the regulation and temperature dependence of cloned wild type NCX from trout (NCX-TR1) and canine (NCX1.1) hearts and chimeric constructs of these two cDNAs. NCX1.1, NCX-TR1 and chimeric cRNA were transcribed *in vitro* and injected into *Xenopus* oocytes. After 3-4 days incubation, currents were measured over a temperature range of 7-30 °C using the giant excised patch technique. With decreased temperature, both peak and steady-state currents of canine NCX1.1 decreased with Q_{10} values of 2.4 and 2.6, respectively as opposed to Q_{10} values of 1.2 and 1.1, respectively in NCX-TR1. The Q_{10} values were unaffected by chymotrypsin treatment. These and other data indicate that kinetic differences in Na^+-dependent inactivation are not responsible for the unique temperature dependence of the trout NCX-TR1. To examine further the temperature dependence of the NCX molecule, NCX1.1-NCX-TR1 chimeric proteins were constructed, expressed, and their currents measured over a temperature range of 7-30 °C. The results from these experiments place the region primarily responsible for the differential temperature dependence of the NCX isoforms within the first four transmembrane segments.

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16.5

LINKING TEMPERATURE RELATED SHIFTS IN MUSCLE GENOTYPE AND PHENOTYPE TO WHOLE ANIMAL PHYSIOLOGY AND PERFORMANCE : A CRUSTACEAN MODEL. A. J. El Haj, J. Holmes, D. Lewis* and D. Neil*, Keele University and Glasgow University* UK. During crustacean larval development, the abdominal muscle must adapt to changing functional requirements such as free swimming and tail flip as well as (water temperature) fluctuations ranging from 4-22°C. Temperature effects and phenotypic changes potentially correlate with the velocity of shortening and ATPase activity being associated with myosin heavy chain (MyHc) isoform expression. In this study, we have attempted to influence sarcometric protein expression by altering the rearing temperatures during development. Our findings correlate the functional and phenotypic requirements of the abdominal muscle and indicate that adult isoforms expressed from larval stage 2 do not alter with the changing behavioural requirements during settlement. Sequence variations in the key functional regions of the myHC molecule that are closely associated with the hydrolysis site for myofibrillar ATPase (loop 1) and the actin-binding pocket (loop 2) have been carried out in the abdominal muscles of larvae held at 10, 14 and 19±1°C. Analysis reveals developmental shifts in loop 1 as well as temperature dependant-shifts which alter the charge distribution pattern, but do not alter net charge. Loop 2 is highly conserved throughout development and is not influenced by temperature. This contrasts with our work on evolutionary adaptation to life at low temperatures amongst Antarctic species. In order to determine any long term effects of these molecular changes, we have assessed the swimming characteristics of these animals at 11 weeks of age. Our results show that although there is a clear difference between the size related swimming speed and rearing temperature there is no temperature related additional performance characteristics. In summary, we have demonstrated the developmental pattern of sarcomeric isoforms and sequence of functional loops within the myosin molecule can be altered with temperature during development, however, the long term effect of rearing temperature on swimming speed in young juveniles is not apparent despite marked changes in abdominal muscle patterns of gene expression.

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16.6

ALTERNATIVE SPLICING, MUSCLE CONTRACTION AND INTRASPECIFIC VARIATION OF DRAGONFLY FLIGHT MUSCLE. James H. Marden, Dept. of Biology, Pennsylvania State University, University Park PA 16802.

Flight muscles of *Libellula pulchella* dragonflies contain a mixture of six alternatively spliced transcripts of a single troponin T (TnT) gene. Intraspecific variation in the relative abundance of different TnT transcripts affects the calcium sensitivity of skinned muscle fibers and the performance of intact muscles during work loop contraction regimes that approximate *in vivo* conditions during flight. The relative abundance of one TnT transcript, or the pooled relative abundance of two TnT transcripts, shows a positive correlation with a ten-fold range of variation in calcium sensitivity of skinned fibers (r^2=0.77, p<0.0001), and a three-fold range in peak specific force (r^2=0.74, p<0.0001), specific work per cycle (r^2 = 0.54; p < 0.0001), and maximum specific power output (r^2=0.53, p= 0.0002) of intact muscle. Muscle power output of individual male dragonflies is positively related to their total fat content and to their mating success. Thus, it appears that alternative splicing of TnT is regulated in a manner that allows a finely tuned match between muscle energy expenditure and energy supply. Individual males able to accumulate more energy have more powerful muscle and higher fecundity.

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Relates TnT transcripts to free flight wingbeat kinematics

16.7

AN INTEGRATIVE ANALYSIS OF MYOSIN FUNCTION. Sanford I. Bernstein, Douglas M. Swank, Kimberly P. Littlefield, Aileen F. Knowles, Jennifer A. Suggs, Becky M. Sanchez and David W. Maughan*, Department of Biology and Molecular Biology Institute, San Diego State University, San Diego, CA 92182-4614 and *Department of Molecular Physiology and Biophysics, University of Vermont, Burlington, VT 05405

We are using the *Drosophila melanogaster* transgenic system to assign functions to myosin heavy chain domains. Our integrative approach analyzes biochemical and biophysical properties of myosin, structural and mechanical properties of muscle fibers, and locomotion of organisms. Typically, we replace the indirect flight muscle myosin isoform with a chimeric myosin. The chimera contains an alternative domain that is normally expressed in a different isoform. We find that the converter domain near the light chain binding region, as well as a domain near the N-terminus of myosin, modulate *in vitro* actin motility and actin-activated ATPase activity. The mechanism of converter action involves tuning kinetic properties, rather than changing the amplitude of the unitary step displacement, likely by affecting rates of conformational changes in myosin associated with product release. Both the converter and N-terminal domains regulate muscle fiber power and optimal frequency of maximal power generation (fmax), thereby affecting flight performance. Flies expressing the converter or N-terminal region of embryonic myosin in the indirect flight muscle myosin backbone are capable of flight, but exhibit reduced wing-beat frequency (wbf). The apparently voluntary reduction in wbf allows muscle oscillation frequency to more closely match fmax, enabling higher muscle power output. We conclude that the converter and N-terminal domains tune myosin kinetics to match muscle speed during locomotion.

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17.1

Origins of variation in mitochondrial content of vertebrate muscle. CD Moyes, Depts. of Biology and Physiology, Queen's University, Kingston, Canada. (http://biology.queensu.ca/~moyesc/)

Mitochondrial content is an important determinant of aerobic capacity of tissues. Muscle mitochondrial content increases dramatically during myogenesis, then diverges during development with fiber-type specialization. Adult vertebrates can remodel bioenergetics in response to physiological (e.g., exercise) and environmental (e.g., temperature) stress. Interspecies comparisons demonstrate evolutionary differences in muscle mitochondrial content associated with body size and activity levels. Our research focuses on (i) the responsiveness of individual respiratory genes (i.e., citrate synthase) to transcriptional regulation (ii) the pathways mediating the constitutive vs. inducible pathways controlling mitochondrial biogenesis in different fiber-types and (iii) the mechanisms responsible for imparting unusual mitochondrial properties in specialized tissues (e.g. billfish heater organ) and in athletic species (e.g. tunas). By integrating muscle functional parameters with signal transduction and molecular genetics, we hope to develop a unifying theory explaining the variation in mitochondrial content that exists between species, fiber-types, physiological and environmental conditions. (Supported by NSERC, Heart and Stroke Foundation of Canada, and NMFS).

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17.2

Mitochondrial Reactive Oxygen Species Production. Yulia Kushnareva, Alexander Andreyev, and Anne Murphy. MitoKor, 11494 Sorrento Valley Road, San Diego, CA 92121.

Mitochondrial dysfunction is associated with multiple diseases, including chronic forms of neurodegeneration such as Alzheimer's and Parkinson's Disease. The etiology of the reported defects in mitochondrial enzyme activities in Alzheimer's and Parkinson's Disease remains a mystery, but these alterations may relate to the enhanced levels of oxidative stress evident in these diseases. Recently, it has been reported that systemic administration of the pesticide rotenone, an inhibitor of complex I of the electron transport chain, results in pathology strikingly similar to Parkinson's Disease. Therefore, the source and mechanism of enhanced generation of reactive oxygen species (ROS) by mitochondria under these conditions has become a topic of interest. Mitochondrial ROS production can occur through autooxidation of ubisemiquinone at complex III, or within an undetermined site in complex I. Significant focus historically has been placed on the ROS producing site in complex III due to the signficantly higher rates of ROS production from this site under experimental conditions. We have found that profound loss of cytochrome c from the intermembrane space of mitochondria, an event that may occur during apoptotic signaling in multiple pathologies, significantly increases ROS production at complex I but not at complex III. Furthermore, we find that ROS production at complex I, which occurs at a site proximal to the rotenone inhibitory site, is significantly enhanced by a highly reduced state of pyridine nucleotides (NADH). These data have significant implications with regard to the potential source of oxidative stress in Parkinson's Disease.

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17.3

MITOCHONDRIAL MECHANISMS IN CELL DEATH. John J. Lemasters, T. Qian, J.-S. Kim, S.P. Elmore Sara Rodriguez-Enriquez, Y. Nishimura, D.A. Brenner and W.E. Cascio, Depts of Cell & Developmental Biology and Medicine, Univ. of North Carolina, Chapel Hill, NC 27599

During ischemia, tissue pH decreases rapidly. This naturally occurring acidosis protects profoundly against necrotic cell killing in hepatocytes, cardiac myocytes, and other cell types. However, the return to normal pH after reperfusion precipitates cell death. This 'pH paradox' is associated with the mitochondrial permeability transition (MPT). The MPT is a pore-mediated increase of mitochondrial permeability to solutes up to 1500 Da. The MPT causes mitochondrial uncoupling and cellular ATP depletion. Cell death then follows when a glycine-sensitive organic anion channel opens in the plasma membrane, initiating oncotic swelling and plasmalemmal rupture. Cyclosporin A and pH below 7 block the MPT and prevent this necrotic cell death. The MPT also causes mitochondrial swelling, outer membrane rupture and mitochondrial release of proapoptotic proteins. The MPT is implicated in necrotic and apoptotic cell death after a wide variety of stimuli, including oxidative stress, ischemia/reperfusion, tumor necrosis factor-α exposure, Fas ligation, calcium overload, Reye-related drug toxicity, ethanol hepatotoxicity and excitotoxicity. Decreased mitochondrial NAD(P)H, increased mitochondrial free calcium and increased mitochondrial oxygen radical formation promote the MPT. Necrotic or apoptotic cell death after the MPT depends, in part, on ATP. If ATP falls profoundly, necrotic killing ensues. If ATP levels are partially maintained, necrosis is prevented and apoptosis occurs instead. The MPT also signals mitochondrial autophagy, a process that removes old, damaged and superfluous organelles. Features of necrosis, apoptosis and autophagy frequently occur together after death signals and toxic stresses. The term, *necrapoptosis*, describes such death processes that begin with a common stress or death signal, progress by shared pathways, but culminate in necrosis or apoptosis depending on modifying factors like ATP.

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17.4

Role of mitochondrial reactive oxygen species in signalling in endothelial cells undergoing mechanical strain. PT Schumacker, Dept. Medicine, Univ of Chicago, Chicago, IL 60637.

Endothelial cells respond to mechanical strain or shear stress by activating signal transduction pathways, by upregulating the expression of specific genes, by altering the cell surface expression of adhesion molecules, and by increasing the generation of nitric oxide. The signaling pathways responsible for activating many of these functional responses involve reactive oxygen species (ROS), but the source of these oxidants and their relationship to the site of mechanosensing is not known. Recent studies indicate that cyclic strain stimulates mitochondrial ROS generation, and these oxidants are capable of triggering gene expression through the activation of NF-kB, and other cellular responses through the activation of Protein Kinase C, PI3-Kinase, and Akt/PKB pathways. The ROS response and subsequent signaling pathways require an intact actin cytoskeleton, but microtubules do not appear to be required. A model is proposed whereby externally applied strain is transmitted from integrins at the cell surface to the mitochondria, which respond by releasing ROS and thereby trigger functional responses. According to this model, the site of mechanosensing may be located within vicinity of the mitochondria. Supported by HL32646.

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17.5

Mitochondria: a comparative perspective on the proton leak and membrane bilayer. A.J. Hulbert, Dept. of Biol. Sci., Univ. of Wollongong, Australia

Proton leak occurs in mitochondria from all tissues and has been measured in isolated cells and whole tissues. It's magnitude varies with metabolic intensity in both mammals and birds being higher in smaller than in larger species. The acyl composition of liver mitochondrial membranes also varies allometrically in mammals and birds with the mitochondrial membranes of small species being more polyunsaturated and less monounsaturated than mitochondrial membranes from large species. Although proton leak through the mitochondrial membrane lipid bilayer itself appears not to vary with acyl composition, the more polyunsaturated the mitochondrial membrane the greater the proton leak. The most important acyl chains in this correlation appear to be the omega-3 polyunsaturates, especially docosahexaenoic acid (22:6 n-3). In ectothermic vertebrates there also appears to be a relationship between membrane polyunsaturation and mitochondrial proton leak. As polyunsaturates are prone to lipid peroxidation, the allometric changes in acyl composition of mitochondrial membranes in mammals and birds may be related to body size trends in maximum lifespan in these two groups of endothermic vertebrates. This hypothetical proposal will be discussed

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17.6

ROLE OF NITRIC OXIDE AND MITOCHONDRIA IN CONTROL OF FIREFLY FLASH

Aprille, June R.[1], Lagace, Christopher J.[2], Lewis, Sara M.[3], Michel, Thomas [3], Modica-Napolitano, Josephine S.[4], Trimmer, Barry A.[2], Zayas, Ricardo M.[2]

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In light-producing cells (photocytes) of the firefly light organ, mitochondria are clustered in the cell periphery, positioned between the tracheolar air supply and the oxygen-requiring bioluminescent reaction which is sequestered in more centrally-localized peroxisomes. This relative positioning suggests that mitochondria could control oxygen availability for the light reaction. We propose that active respiration would make the interior regions of the photocytes relatively hypoxic; the "on" signal for production of bioluminescence might depend on inhibition of respiration, which would allow delivered oxygen to pass through the peripheral mitochondrial zone to reach peroxisomes deep in the cell interior. We published recently that exogenous nitric oxide gas (NO, produced in insects in response to the neurotransmitter octopamine), induces bioluminescence in the intact firefly and in the dissected lantern, and that nitric oxide synthase is present in the light organ. New experiments showed that NO inhibited respiration in isolated photocyte mitochondria. The NO inhibition was reversed by bright light, and was reinstated when the light was turned off. Altogether, the results support the idea that NO elicited in response to neural signals triggers light production by reversible inhibition of mitochondrial respiration in photocytes. The data also suggest that the bioluminescence itself relieves NO inhibition thus contributing to rapid on/off switching.

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Evidence that NO is involved in mediating CNS control of firefly flashing; localization of nitric oxide synthase in the light organ. Hypothesis discussed for role of NO inhibition of mitochondrial respiration in regulating oxygen availablility for the luciferin-luciferase reaction as the on/off signal for flash control.

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Evidence that NO inhibits respiration in isolated photocyte mitochondria: NO inhibition is reversed by light, suggesting that *in situ* the firefly flash contributes to its own off signal.

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Excellent overview and critical review of evidence for role of NO inhibition of mitochondrial respiration in flash control, in relation to earlier hypotheses for flash control and including physiological and evolutionary contexts.

17.7

ENERGY METABOLISM AND INSECT FLIGHT

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Flying insects achieve the highest rates of aerobic metabolism known in the Animal Kingdom. We estimated mitochondrial respiration rates *in vivo* during flight in worker honeybees (*Apis mellifera*) through a combination of flow-through respirometry, ultrastructural morphometric measurements, and dual-wavelength spectroscopy. Although flight muscle mitochondrial volume and cristae surface densities are expectedly high, these cannot completely account for the high respiration rates. When expressed per unit mitochondrial volume or per unit cristae surface area, the respiration rates are significantly higher than rates estimated in mammalian locomotory muscles during exercise at VO_2max and in hummingbird (*Selasphorus rufus*) pectoral muscles during hovering flight. The numbers of respiratory chain enzyme complexes per unit cristae surface area are similar to those found in rat heart and liver. The high rates of O_2 consumption require higher rates and, possibly, an alternative mechanism for electron transfer between respiratory chain enzymes. Because energy metabolism in insect flight muscles is obligately aerobic, this analysis of the design, function, regulation and evolution of oxidative pathways in bees must necessarily include glycolysis (Funded by NSF IBN 0075817).

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Flux capacities and flux rates during flight

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Electron transfer rates during flight

17.8

MITOCHONDRIAL STRUCTURE AND FUNCTION IN RELATION TO EXERCISE.

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The malleability of the skeletal muscle tissue mitochondrial compartment as a consequence of training interventions was first noted in the late sixties when rats were subjected to strenuous running programs. The early biochemical studies indicated a massive increase of the activities of enzymes of oxidative metabolism under these conditions. Using morphometric techniques on biopsies of vastus lateralis muscle a linear relationship between whole body VO_2max and the volume density of mitochondria was found in a comparison of trained and untrained subjects. It was also noted that the proportion between mitochondrial membrane surfaces as well as compartmental spaces was constant in trained and untrained subjects. Using a comparative approach it was later suggested that all mammalian mitochondria used approx. 5mlO_2/min/ml under conditions of VO_2max. Whether mitochondria become more abundant or just bigger with endurance exercise training has remained elusive. It could be shown however, that mitochondria are separate entities and not connected into a continuous mitochondrial reticulum. Currently, the search is on for the molecular control of mitochondrial plasticity. While some of the mechanisms involved in mitochondrial biogenesis have been unraveled, the signals that induce mitochondrial growth have not yet been defined. The study of genomic regulation in humans *in vivo* will take advantage of muscle mitochondrial plasticity because mitochondrial growth can easily be manipulated and muscle tissue can repeatedly be biopsied even in humans.

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DIVING: WHERE HAVE WE BEEN AND WHERE ARE WE GOING?

18.2

DIVING BRADYCARDIA: REFLEXES, REFLEXES EVERYWHERE BUT NO TIME TO STOP AND THINK?

David R. Jones, Department of Zoology and Peter Wall Institute, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z2.

The most obvious manifestation of the cardiovascular response to forced diving is extreme bradycardia with heart rates often less than 5 beats/min. Low cardiac output is accompanied by a massive increase in peripheral resistance that serves to redirect blood, with its stored oxygen, to oxygen sensitive organs such as the heart and brain. Peripheral chemoreceptors play a crucial role in the response aided and abetted by a massive increase in circulating catecholamines. The responses are reflex, being little altered by decerebration or even brain transection at the ponto-medullary level (1). In forced dives, underwater survival times are prolonged compared with when the dive responses are inhibited.
In voluntary dives, heart rates range from no change to going up or down. Even when heart rate falls it usually increases towards the end of the dive as the animal begins to resurface. Exercising is an obvious difference between free and forced diving but empirical tests show minimal effects on heart rate (2). This heart rate variability has made it difficult to establish the part played by reflex controls. Presenting a novel stimulus to an animal in a voluntary dive often results in extreme bradycardia, arguing for a role for higher nervous centers in regulating autonomic function.
Pharmacologically inhibiting cardiac responses in free diving does not affect either dive or recovery times (3). If the free diving response lacks proof of efficacy, what is it's utility?

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18.3

Behavioral influences on diving energetics in penguins.

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Free-living Magellanic Penguins *Spheniscus magellanicus* breeding at Cabo Virgenes, Argentina were equipped with new logging technology to examine factors important in the energetics of foraging. The following parameters were recorded: swim speed, depth, flipper beat frequency and amplitude, prey ingestion and volume of air inhaled immediately prior to diving. During dive descent, flipper beat frequency and amplitude decreased with increasing depth. Bottom phases of dives featured flipper beats with extended gliding phases and ascent typically featured an increasing time spent gliding closer to the surface. Trends in these patterns were more marked in dives to greater depths, this being apparently due to steeper descent and ascent angles and larger volumes of air inhaled prior to diving. This latter attribute serves to maximize oxygen stores while negating the effect of upthrust via compressed air spaces. During prey pursuit, flipper beat frequencies and amplitudes increased dramatically leading to increased energy expenditure and correspondingly shortened dives. Knowledge of the energetics of penguin swimming underwater under varying conditions of inhaled air, hydrostatic pressure, speed etc. enables the benefits of observed strategies to be modelled out. This work was funded by ICSU under the auspices of SCAR.

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Details a new technology by which aspects of penguin foraging ecology may be determined.

18.4

THE EFFECT OF BEHAVIOR ON PHYSIOLOGICAL DIVE CAPACITY IN MARINE MAMMALS: WHAT LIES BENEATH.

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Many of the most energetically costly behaviors of marine birds and mammals occur while the animals are submerged. This not only represents a physiological challenge for air breathing vertebrates that must rely on limited oxygen stores, but also represents a logistical challenge to investigators trying to understand how these stores are used by freely diving animals. Advances in animal-borne video technology and instrumentation have provided new insights regarding the relationship between behavior and the energetic cost of diving in mammals. One strategy used by cetaceans and pinnipeds is to incorporate prolonged periods of gliding during a dive by taking advantage of changes in buoyancy at depth. For Weddell seals such "sink or swim" strategies result in a 9 - 60% savings in the energetic cost of a dive. Indeed, the cost of diving by these seals can be estimated from the total number of flipper strokes taken during submergence. By matching hunting behavior to recovery costs following a dive, we find that the energy expended to heat and assimilate prey is superimposed on other diving costs. For elephant seals and Weddell seals these additive costs represent a disadvantage in terms of dive capacity, with the animals balancing the cost of diving for distance or for digestion. For the smallest marine mammal, the sea otter, these digestive costs represent a necessary component of thermal balance, and as such provide a benefit by reducing long-term energetic costs. Supported by NSF- Polar Programs.

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18.5

PHYSIOLOGY AND BEHAVIOR OF FREE-DIVING PENGUINS.

Paul J. Ponganis, Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093-0204.

Remarkable progress in elucidation of the diving behavior and physiology of penguins has been made with the use of time depth recorders, satellite transmitters, cameras, and the continued development of sensors and microprocessor-based data loggers. A variety of foraging strategies and swim patterns have been revealed by recent research. Findings include evidence for probable benthic foraging by several species, alterations in swim speeds by different species during the foraging phases of pelagic dives, detection of prey ingestion by esophageal temperature changes, sub-ice foraging by emperor penguins, and prolonged gliding during ascents of king penguins.

Recent physiological research has focused on the respiratory system and temperature regulation. Fluctuations in air sac pressures secondary to wing beats probably contribute to air sac O_2 utilization during swimming. Calculated diving air volumes of king penguins increase with maximum depth of dive, but are reduced on a mass-specific basis in comparison to those of Adelie penguins. Remarkable anterior abdominal temperature decreases have been documented in king and emperor penguins. Although it has been suggested that this may be associated with core hypothermia and decreased O_2 consumption during dives, findings in emperor penguins are more consistent with a preservation of core temperature and cooling of peripheral tissues. Complete understanding of the mechanisms responsible for the deeper- and longer-than-expected dives of penguins awaits further investigation.

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Swim speed patterns during foraging.

18.6

The Development of Diving Ability in Pinnipeds

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Knowledge of the mechanisms by which neonatal mammals acquire the tools necessary to become competent predators is crucial to understanding how physiological processes influence behavioral strategies. While such questions can be addressed in many species, the study of how pinniped neonates become marine predators offers several unique advantages. Because oxygen stores and use rates determine foraging capacity, a suite of physiological changes must accompany the rapid transition from terrestrial neonate to diving juvenile. In phocids, blood oxygen stores develop rapidly over the lactation and postweaning fast period, such that by independence, the mass specific blood oxygen stores of juveniles are 70-90% of adult values. In contrast, muscle mass and muscle myoglobin loads are much slower to develop, and are only 30-50% of adult values at weaning. In several species, foraging is only initiated after approximately 25% of total oxygen stores are held in muscle tissue. The similar pattern of physiological development among these species is remarkable given interspecific differences in the length of the lactation and postweaning fast periods, the relative maturity of pups at birth, and the size of adult stores. These findings suggest that the rate and extent of muscle development may be a critical factor in determining when phocid pups are able to begin diving and foraging efficiently. Funded by UAA Faculty Development Grant and UCSC IMS Postdoctoral Fellowship.

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18.7

The balance between hypoxia and aerobic metabolism in seals during diving.
Davis, R.W., Kanatous, S., Polasek, L.K., Watson, R.R, Fuson, A.L. Dept. of Marine Biology, Texas A&M University, Galveston, TX

Terrestrial mammals increase ventilation and cardiac output as oxygen demand increases during exercise. For mammals adapted to hypoxic environments (e.g., high altitude), enhanced pulmonary diffusing capacity, convective oxygen transport in the blood, and cellular diffusing capacity help maintain intracellular PO_2. During submerged swimming, seals stop breathing and experience progressive hypoxic and ischemic hypoxia during diving, yet maintain aerobic metabolism at an arterial PO_2 as low as 22 torr. They accomplish this through enhancements in blood and tissue oxygen storage and intracellular diffusing capacity. Convective oxygen transport to the muscles is reduced as part of the dive response during submerged swimming. This paradoxical response to exercise under hypoxic conditions is necessary because a significant part (ca. one-third) of the seal's body oxygen is bound to myoglobin in the muscle. Because of myoglobin's high affinity for oxygen (P_{50} 2-3 torr) relative to hemoglobin (P_{50} 26-29 torr), the muscle must become hypoxic to dissociate the oxygen from myoglobin to make it available for aerobic metabolism. This is accomplished through ischemic hypoxia (peripheral vaso-constriction) and progressive hypoxic hypoxia. Modeling indicates that the degree of peripheral vasoconstriction decreases as muscular exertion increases, although long duration divers use energy-conserving modes of locomotion that routinely keep metabolism low. The dive response regulates the degree of muscle hypoxia that is necessary to access myoglobin-bound oxygen to maintain aerobic metabolism during diving.

18.8

The Energetics of Diving and the Question of Metabolic Depression.
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Underwater foraging would seem to be an energetically expensive activity. Some species of birds and mammals, however, make dives that are longer than the time in which they would consume all their O_2 stores at just the resting O_2 consumption rate. In the 1890's Bohr proposed that while aerobic metabolism was necessarily reduced during diving, it was compensated for by increased anaerobic metabolism, but Richet concluded that overall metabolism must be reduced. In the 1940's Scholander reported that although in forced submergences seals may indeed experience a reduction in total metabolism, this could "not be the case in an ordinary dive" in which seals would be actively swimming. The concept of "hypometabolism" during voluntary diving remains controversial to this day. Recent studies indicate that some seabirds make surprisingly long dives by relying on anaerobiosis, but others may experience a reduction in total metabolism due to drops in regional body temperatures. Birds may be submerged for less than 50% of the time, so it is feasible that metabolism is reduced in many organs because the concomitant reduction of function in those tissues can be compensated for by increased function when body temperature is restored. A postponement of function during diving seems unlikely in extreme divers such as elephant seals which spend 90% of time at sea underwater. For them diving is the usual, or basal state. Our symposium features innovative studies of cost-saving behaviors and physiological adjustments that may shed light on the paradox. I will focus on data relevant to the question of diving hypometabolism and how it might be resolved.

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Proposes that the cardiovascular dive response in freely diving elephant seals is a graded response that depends on the duration of the dive.

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Reports that, like heart rate, overall breathing frequency is inversely related to dive duration, supporting the suggestion that metabolic rate is reduced on longer dives, as was seen in laboratory dives.

18.9

DIVING INTO THE FUTURE

Pat Butler, School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.

Many species of marine birds and mammals when foraging under water face the potential problem of the effects of high hydrostatic pressure, and we really do not know how the deeply-diving penguins avoid these problems. All species face the problem of being restricted to the limited amount of oxygen contained within their bodies for the duration of the period of submersion. It is generally accepted now that the anaerobic production of ATP with lactic acid as an end-product, is not routinely used by aquatic birds and mammals. Thus, aerobic metabolism, with O_2 being the final electron receptor in the respiratory chain, is the major source of ATP during diving. The diving behaviour of some species of birds and mammals, e.g. ducks, fur seals, dolphins, is such that their calculated oxygen stores are sufficient for all of the dives they perform. However, for species such as the gentoo, king and emperor penguins and the elephant seals, the amount of oxygen does not appear to be sufficient for 40% or more of the dives undertaken by these animals. There are a number of possible explanations for this discrepancy. The amount of usable oxygen may be larger than we think, other sources of ATP, such as phosphocreatine, may be of greater significance than we think, the rate at which ATP, and hence oxygen, is consumed during submersion may be lower than we think. New technology is helping us to elucidate some of these unknowns, but there is still a long way to go.

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DNA MICROARRAYS: APPLICATIONS TO COMPARATIVE PHYSIOLOGY — POSTERS

20.1

Down-regulation of Metabolism in Fish Exposed to Hypoxia and Starvation

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Many fish species can tolerate periods of aquatic hypoxia and food deprivation. It is known that both low oxygen availability and food deprivation modify growth rate and metabolism in fish. Both hypoxia and starvation have been associated with a fall in oxygen uptake, lowered protein turnover rate, reduced locomotory activities and regressed or delayed sexual maturation and growth. Hypoxia is also associated with reduced food intake. These result in a general down-regulation of metabolisms in fish under both circumstances. Microarray technique has been employed to illustrate the expression profiles in fish exposed to hypoxia and starvation. Genes involved in the respiration pathways are generally down-regulated during both hypoxia and starvation

20.2

Evolution of Desiccation Resistance in Laboratory Populations of *Drosophila*: Physiological and Molecular Mechanisms

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Drosophila populations respond rapidly to selection for desiccation resistance, but contradictory physiological mechanisms have been described in previous studies. We are performing a large desiccation selection experiment in *Drosophila melanogaster*, using multiple starting populations and two types of selection control. Survival in dry air has increased by more than one hour per generation, leading to a doubling in desiccation resistance over 10 generations. The founding populations differ in lipid and carbohydrate storage, two parameters that have evolved rapidly in previous selection studies. Metabolic and biochemical measurements reveal that flies use different energy sources under different stresses. Thus, these populations differ genetically in their energy storage patterns and may differ in the molecular mechanisms for their selection responses. To test this hypothesis, we will present microarray data indicating which genes have evolved new expression patterns.

20.3

LOADING STATES MODULATE SKELETAL MUSCLE GENE PROFILE

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Using commercially available micro-array technology we analyzed the adaptation of a broad range of transcripts that accompany atrophy of rat *m. soleus* induced by 14 days of hindlimb suspension (unloading) and re-exposure to gravity for 1 day (reloading). Statistical analysis combining L1 regression with the sign test based on the conservative Bonferroni correction identified 86 of 1200 possible genes that underwent transcriptional adaptations with atrophy. Generally, the unloading-induced changes ran parallel for transcripts belonging to the same functional categories, i.e. metabolism, protein turnover, ion transport and cell regulation. They were similar to the adaptations detected previously in 35 days unloaded rat *m. soleus*. mRNA changes of 16 transcripts encoding glycolytic enzymes, voltage-dependent ion-channels and signaling molecules were reversed after 1 day of reloading. The biological role of the translation products of affected mRNAs indicates that modulation of the gene profile is an important mechanism instructing plastic adaptations of skeletal muscle fibers due to changes in its loading pattern. Changes in mRNA levels of enzymes involved in cell regulation and carbohydrate metabolism are among the most rapid expressional adaptations occurring with skeletal muscle reloading.

20.4

Proteome Analysis of Rainbow Trout Liver Proteins: Molecular Responses to Altered Diet

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The proteome is described as the expressed protein complement of a genome in a tissue or a whole organism. Protein extraction followed by high resolution 2 dimensional electrophoresis, coupled with gel image analysis allows expression of hundreds of protein to be monitored in parallel, permitting a global picture to emerge of changes in protein profile under different metabolic states. Protein spots of interest are then subjected to further analysis leading to protein identification. A proteomics approach has been used to study the protein profiles of livers of rainbow trout that have been fed two diets containing ca 30% fish meal and different levels of plant ingredients. Dietary manipulations with changes in protein sources may produce metabolic and gene expression responses in the fish. A growth trial was performed for 12 weeks, followed by protein metabolism parameters were measured. Protein growth rates were not different between diets, however protein consumption and protein synthesis rates were higher in the fish fed the diet with soybean meal (SBM). Fish fed this diet had lower efficiency of retention of synthesised protein, and greater rates of protein degradation. Ammonia excretion was increased as well as the activity of glutamate dehydrogenase. To further understand the biochemical changes that diets have caused, proteomic analysis was performed on liver proteins from fish fed the two diets. Candidate proteins have been identified that are expressed in a differential manner. During this study ~800 proteins were analysed for expression pattern, of which 20 were found to be differentially expressed. Trypsin digest fingerprinting has been used on individual proteins to allow identification of these proteins by database searching. In conclusion we demonstrate how proteomics coupled with genome characterisation could help understand dietary responses in fish and development of alternative feed sources.

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20.5

Production of a Bespoke cDNA Clone Set for Transcript Screening of Mammalian Hibernation

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Although the candidate gene approach has furnished some important information concerning transcriptional changes during hibernation our view of molecular events remains restricted and fragmentary. Contemporary microarray screening techniques offer an open-ended and systematic search for genes whose expression changes either during the circannual cycle in preparation for, or over the hibernating bout itself. Work using mouse cDNA arrays gave poor hybridization with golden-mantled ground squirrel, *Spermophilus lateralis* cDNA, suggesting that this model species is too diverged from the squirrel for this comparative approach to work. We have therefore set out to produce multi-tissue *S. lateralis* cDNA libraries to study transcript expression during hibernation. We have adopted a PCR approach incorporating suppressive subtractive hybridization (SSH) and mirror-orientated selection (MOS) to enrich cDNA populations for rare and differentially expressed genes as well as subtract abundant genes from a particular temporal state. Such an approach used in carp cDNA library construction has given libraries with 80-95% unique cDNAs. Although our array may contain some redundancy, this is tolerated in favor of the representation of potentially rare, yet important genes. Previous experience with carp cDNA libraries using cDNA fragments produced by SSH lead to highly ambiguous identification by BLASTx homology searching, due to 3' bias. We therefore developed a procedure to capture the full-length equivalents of the enriched and differentially expressed gene fragments. cDNA fragments were biotinylated and hybridized with full-length cDNA before being captured onto streptavidin. The full-length cDNAs are amplified by limited PCR, size-fractionated and cloned into a suitable vector. Each insert will be PCR amplified and the cDNA gridded onto glass slides for microarray analysis. Funded by the BBSRC.

HOMEOSTASIS OF ESSENTIAL YET TOXIC METALS

21.1

Transcriptome and Proteome Responses to Zinc in Fish

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Altered patterns of gene and protein expression provide insights into cellular mechanisms of toxicant action, means of detoxification, and potential modes of acclimation. By utilising DNA array and surface enhanced laser desorption/ionisation technology we investigated the molecular responses induced by zinc exposure in the gill of rainbow trout (*Oncorhynchus mykiss*). Zinc is both an important nutrient and contaminant to aquatic organisms yet the molecular mechanisms controlling the balance between nutrition and toxicity are unknown. Rainbow trout fingerlings were exposed to 2.3 M zinc. RNA was isolated from gill tissue. *Fugu rubripes* gill cDNA libraries were probed with mRNA from control and exposed fish. The presence of differentially expressed genes was observed. A sample of 40 of the most marked signals detected on the zinc-exposed membrane were chosen and sequenced. Control and exposed gill homogenates were loaded onto protein array chips and resulting peaks were analysed both quantitatively and qualitatively. Seven proteins were induced by zinc exposure while four proteins were suppressed. Additionally, seven proteins were upregulated and four were downregulated. Overall, the results show that genes and proteins can be grouped into categories relating to transcription and translation, structural integrity, immune response and energy mobilisation. Funding was provided by US EPA grant, R826104 and King's College London.

21.2

Investigation of Putative Transporters Responsible for Zinc Transport in the Fish Gill

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Zinc is an essential metal micronutrient. It is still unclear how zinc acquisition from external environment is carried out at the molecular level in vertebrates. In this present research, the molecular basis of zinc acquisition has been investigated in the fish gill, and several transporters have been identified from the gill of zebrafish (*Danio rerio*) and pufferfish (*Fugu rubripes*), including putative ZIP (DrZIP1 and DrKE4), ZnT (DrZnT1 and DrZnT2) and ECaC (FrECaC and DrECuC) members. DrKE4 protein displays potentially important structural differences while keeping the conserved properties of ZIP family, and is supposed to localise in the membrane of intracellular zinc storage vesicles according to its signal peptide, and translocates zinc ions into cytosol when zinc ions become limited. FrECaC and DrECaC proteins are the calcium-specific channel thought to be mainly responsible for calcium acquisition from water, and are supposed to have redundant ability to transport zinc in the fish gill due to their special structural properties. In the 5' flanking region of FrECaC gene, several steroid receptor responsive elements have been identified. Interestingly, there is a metal responsive element (MRE) in the regulatory region makes it potentially regulated by metal responsive transcription factor 1 (MTF-1).

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21.3

Long-term Kinetic Measurements of Intracellular Free Zinc Using the Fluorescent Probe FluoZin-3

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The objective of our study was to explore the cellular response to acute zinc stress with fluorescent techniques, in live fish cells acutely exposed to zinc-containing media. Moreover, we wanted to investigate heterogeneity in the distribution of the metal and estimate its activity at the subcellular level. Therefore, we loaded a monolayer of the trout R1 hepatic cell line with the zinc-specific fluorescent probe FluoZin-3 and subsequently perfused it with an iso-osmotic zinc solution. During the experiment, time series of fluorescence images of a selected cell field were collected on a Zeiss LSM510 confocal laser scanning microscope, using the 488 Ar laser line and a 63x water immersion objective. Post-hoc analysis of regions of interest was done with the LSM 5 software package. Our results clearly show that a continuous challenge with non-toxic concentrations of extracellular zinc readily induced elevated levels of free zinc, which did not recover within the experimental timeframe of one hour. Furthermore, the low fluorescence observed in the nucleus contrasted with much higher values measured in the cytoplasm, where it appeared to be localized in distinct pools. To our knowledge, continuous measurements of free intracellular zinc with a high-affinity, zinc-selective visible-wavelength probe have never been reported before. We believe that this work can be extended to the live visualization of cellular zinc management in other cell models.

21.4

Copper accumulation and metallothionein induction in three freshwater fish during sublethal copper exposure.

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This comparative study examined the relation between Cu accumulation and MT induction of *Oncorhynchus mykiss*, *Cyprinus carpio* and *Carassius gibelio* during a 1 week exposure to 1 µM Cu. Control [Cu] in the different tissues were comparable, except for kidney [Cu] which was lower in rainbow trout. Liver [Cu] in rainbow trout was almost double of carp [Cu], but variation was high.

Accumulation patterns showed remarkable differences. Gills displayed surprisingly little accumulation in rainbow trout. In common carp, an increase in gill [Cu] reached a plateau within the first 24 hours, while in prussian carp, a linear increase was observed. Liver tissue showed the highest [Cu], and a steady increase in liver [Cu] was observed in rainbow trout and common carp, with a stronger accumulation in trout. [Cu] in prussian carp livers was stable. In contrast, prussian carp kidney was the only one to show increased [Cu]. Muscle [Cu] slowly increased in the two cyprinid species.

Prussian carp had the highest capacity for MT induction which coincides with the much more resistant nature of this fish. In addition, prussian carp showed a positive significant relationship between tissue [Cu] and [MT] in gill, liver and muscle. In contrast, we found a low MT induction in rainbow trout, the most sensitive species, and no correlation at all between MT concentrations and tissue copper contents. Common carp showed an intermediate response, with significant correlations in liver and muscle tissue.

22.1

Variation in Heavy Chain Myosin Genes Between Stenothermal and Eurythermal Crustaceans: a Link Between Phenotypic Plasticity and Genotype

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To investigate the interaction between environmental temperature and structural and functional relationships in myosin heavy chain (MyHC) genes, we have compared sequence variation in two regions of the MyHC gene between stenothermal (polar) and eurythermal (temperate) isopods and amphipods. These regions (Loops 1 and 2) control ATP hydrolysis and actin binding, respectively. No difference was observed in the length, amino acid composition (100% homology) or charge distribution in Loop 1 for two species of polar and temperate isopods. Loop 2 was more variable (94% homology), though charge distribution did not differ. Primary MyHC sequences from a stenothermal amphipod, bearing 88.9% homology to both isopod species for Loop 1, differed by 89.0 and 87.6% homology for Loop 2, with greatest similarity to the polar isopod. Further, myofibrillar ATPase activities in fast muscle fibres suggest compensation for low temperature exposure in a temperate amphipod species. These data indicate adaptation of muscle function, depending on previous thermal history and current thermal acclimation, that is controlled by variation in genotype. We are currently characterising intra- and inter-specific variation in MyHC genes in amphipods along a 30° latitudinal gradient (ranging from arctic stenothermal to temperate eurythermal populations) to further increase our knowledge of the dynamic relationship between genotype and adaptive phenotype in myosin genes. This research is funded by NERC UK.

22.2

Myosin heavy chain isoform distribution and expression in lobster skeletal muscles

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Lobsters > *Homarus americanus*, like other animals, possess different skeletal muscle fiber types to serve various mechanical needs. While fibers have been differentiated by a number of characteristics including sarcomere length and myofibrillar protein assemblage, they can generally be classified as fast, slow phasic (S1) or slow tonic (S2). Fast fibers have sarcomere lengths of 2-4 μm and diagnostic myofibrillar proteins, such as P75. Both S1 and S2 fibers are composed of long sarcomeres (> 6 μm), but can be distinguished from one another by their unique proportions of troponin T and I isoforms. The objective of the current study was to further define differences among fiber types in terms of myosin heavy chain (MHC) distribution and expression in various muscles. Distinct MHCs were identified using SDS PAGE and Western blotting techniques. In complementary analyses, partial cDNAs endocing for MHC and P75 from deep abdominal flexor (fast) and MHC from crusher claw closer (slow) were used to design fiber-specific primers for RT-PCR. This technique demonstrated unique expression patterns among the different fiber types. Fibers from the crusher claw closer and from the slow fiber region of the cutter claw closer expressed slow MHC, but not fast MHC or P75. Fibers from the deep abdominal flexor expressed fast MHC and P75, but not slow MHC. In contrast, single muscle fibers from the fast fiber region of the cutter claw closer expressed both fast and slow MHC, as well as P75. These findings indicate that lobster muscles are even more heterogeneous than previously appreciated. In particular, unexpected differences exist among fast muscle fiber types that were thought to be homogeneous.

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22.3

Kinetic Differences Between *Drosophila* Muscle Types: the Fast Wild Type Myosin versus a Slow Embryonic Isoform Expressed in *Drosophila* Indirect Flight Muscle

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We are investigating which structural regions influence myosin isoform and fiber type kinetic properties using the Drosophila transgenic system. To identify key steps of the crossbridge reaction scheme that distinguish very fast and relatively slow muscles, we measured the phosphate, ADP, and ATP dependency of oscillatory work production in skinned indirect flight muscle (IFM) from wild type flies (WT). We compared these results with those from IFM obtained from a transgenic line expressing a comparatively slow, embryonic muscle myosin isoform (EMB). Expressing the slow embryonic isoform reduces the frequency of IFM maximal oscillatory work output (fmax) ten-fold. In EMB, and all other muscle types tested to date, fmax increases with increased phosphate concentration. Surprisingly, in WT IFM, fmax decreases over the same range. Preliminary studies suggest that the response to ADP and ATP are similar between the two isoforms. This remarkable qualitative difference suggests profound changes in either rates of product release, the way force-generation is coupled to product release, or perhaps even in the order of product release. As only 4 domains in the S1 MHC head vary between EMB and IFM myosin isoforms, analysis of IFM/EMB myosin chimeras should resolve which domain(s) are responsible for this unique response to phosphate.

22.4

Force Generation and Shortening Velocity in Canine Extraocular and Limb Muscle Fibers

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Contractile properties differ markedly between mammalian extraocular (EO) and limb muscles. E.g., isometric and isotonic contraction rates span much broader ranges in EO, compared to limb, muscles and EO muscles have lower max. force generating ability (Fmax). The levels of different myosin heavy chain (MHC) isoforms in EO and limb muscles of 8 species were compared. Six isoforms were detected in most EO muscles. Generally, carnivore EO muscles had higher levels of MHC isoforms not found in limb muscles, compared to non-carnivore EO muscles. An isoform, identified as EO MHC (MHC-EO), comprised most of the non-limb MHC in EO muscles. Dogs were selected to study the significance of the high level of non-limb MHC isoforms in carnivore EO muscles. Fmax and max. shortening velocity (Vmax) were measured in skinned EO and limb fibers. Vmax spanned 7- and 12-fold ranges among limb (n=23) and EO (n=37) fibers, respectively. Mean Vmax of fast limb fibers was ~3.6 fiber lengths/sec (FL/s). Mean Vmax of EO fibers expressing MHC-EO was higher, ~4.2 FL/s ($P<0.05$). Vmax in slow limb and slow EO fibers was ~1.0 FL/s. Fmax spanned 2- and 18-fold ranges in limb and EO fibers, respectively. Fmax in fast EO fibers was ~0.5 Fmax in fast limb fibers but did not differ between slow EO and slow limb fibers. Contractile properties of skinned EO fibers expressing MHC-EO differ significantly from those of limb fibers, consistent with differences between intact EO and limb muscles. Supported by NSF.

22.5

Ca^{2+}-transients activate calcineurin/NFATc1 and initiate fast-to-slow transformation. G. GROS, N. HANKE, R.J. SCHEIBE, J.D. MEISSNER and H.-P. KUBIS. Zentrum Physiologie, Medizinische Hochschule Hannover, D-30623 Hannover, Germany.

The calcineurin/NFATc1 (nuclear factor of activated thymocytes) signal transduction pathway is involved in mediating fast-to-slow transformation of skeletal muscle cells, specifically the upregulation of slow myosin heavy chain I (MHCI). In T-cells the calcineurin/NFAT pathway is activated by a small but sustained increase in intracellular resting Ca^{2+} levels, in muscle cells the type of Ca^{2+} signal necessary for the activation of calcineurin is not known. Addition of Ca^{2+}-ionophore A23187 to a primary myotube culture caused a concentration-dependent translocation of NFATc1 from the cytoplasm to the nucleus that was mediated by calcineurin. Electrostimulation of myotubes with 45 min-stimulation cycles (15 min stimulation at 1 Hz followed by a pause of 30 min) repeated continuously for 24 hrs showed: 1) an increase in MHC I and decrease in MHC IId mRNA levels, which suggested that stimulation for only 24 hrs was sufficient to start the transformation process; 2) after 24 hrs of stimulation NFATc1 was highly accumulated in the nuclei, indicating the activation of the calcineurin pathway; 3) no increase in resting $[Ca^{2+}]_i$ was found in the myotubes after 24 hrs of stimulation. We conclude that the calcineurin/NFATc1-mediated fast-to-slow transformation at the MHCI level is induced by the brief rapid calcium transients associated with excitation-contraction coupling and does not require a sustained elevation of resting $[Ca^{2+}]_i$. This work was supported by DFG Gr 489/13.

23.1

PRELIMINARY CHARACTERIZATION OF A MONOCARBOXYLATE TRANSPORTER IN ISOLATED CARDIAC MITOCHONDRIA FROM BUFO MARINUS.

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Traditionally, mitochondria have been considered a homogenous group of ATP-producing organelles. However, in the past two decades data has accumulated to suggest mitochondrial function is specially adapted to cell-type and that mitochondria participate in a variety of cellular activities (besides ATP production) such as thermogenesis and apoptosis. The mitochondrion's oxidative capacity has also been implicated in human pathologies such as post-ischemic cardiac reperfusion injury. Potential interspecific variation of tissue-specific mitochondrial function is being investigated using a comparative model. In an effort to develop such a model using anuran amphibians, mitochondria were isolated from two species of amphibian whose cardiovascular demands are adapted to either predominantly aerobic or anaerobic modes of locomotion. Cardiac muscle mitochondria was isolated from the marine toad (Bufo marinus) and the bullfrog (Rana catesbeiana). Mitochondrial oxidative capacity was compared using VO2 max. and respiratory control ratios (RCR). Bufo cardiac mitochondria demonstrated VO2 values 3-6 times greater than Rana cardiac mitochondria. Variation in VO2 max. may be explained by kinetic or other functional differences in pyruvate transport. Therefore, a radiolabeled pyruvate transport assay was developed using Bufo cardiac mitochondria. Preliminary time-course experiments suggest the presence of an alpha-cyano-hydroxycinnamate-sensitive pyruvate uptake mechanism.

23.2

Mechanisms Of Energy Conservation In The Liver Of The Overwintering Frog, *Rana temporaria*.

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The aim of this study was to investigate cellular metabolic depression during hibernation in frogs. We measured oxygen (O_2) consuming processes such as proton leak and non-mitochondrial respiration from normal and hibernating frogs. In addition, the relative contribution of ATP-consuming processes such as protein synthesis and Na^+K^+ATPase was also measured in both groups. The O_2 consumption of hepatocytes isolated from frogs prior to, and after 1, 2 and 4 months of submergence at 3°C, was measured using an 'Oxygraph' electrode system. The contribution of proton leak to the O_2 consumption was measured by inhibiting mitochondrial ATP synthesis using oligomycin. The remaining O_2 consumption is a measure of the proton leak plus any non-mitochondrial respiration. By measuring the fraction attributed to non-mitochondrial respiration, the O_2 consumption due to proton leak can be resolved. The contribution of ATP-consuming reactions was determined using ouabain and cyclohexamide to inhibit Na^+K^+ATPase and protein synthesis respectively. Results have shown that the overall O_2 consumption of these hepatocytes falls by 40% over 4 months of submergence at 3°C. The fall in O_2 consumption appears to be mostly mediated through unknown O_2 consuming processes. However, the fall in proton leak, Na^+K^+ATPase and protein synthesis suggests that these processes also contribute to bring about the metabolic depression seen in these cells.

The Natural Environmental Research Council (NERC) supported this study.

23.3

Effects of temperature, magnesium and quinine on mitochondrial proton leak in teleost fishes.

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Although mitochondrial proton leak has a thermogenic function in mammals (at least in brown adipose tissue), in poikilotherms it is an apparently wasteful process. We have investigated the factors influencing mitochondrial proton leak in several fish species under different conditions. Temperature has an important *in vitro* effect on proton leak (Q10 of ~2-3) in mitochondria isolated from diverse temperate and arctic teleosts. Winter flounder (*Pleuronectes americanus*) acclimated at ~ 9C have a reduced proton leak (at any given membrane potential) as compared to cold acclimated (0C) controls. We have also investigated the mechanism involved in mitochondrial proton leak in fish. Magnesium influences proton leak in rainbow trout (*Oncorhynchus mykiss*) heart and liver mitochondria in similar fashion to that of rat skeletal-muscle mitochondria. In these mitochondria there is a substantial reduction in proton leak associated with the presence of Mg^{2+}. In this study, we also show that quinine reduces proton leak in a similar fashion as Mg^{2+}.

Funding was provided by NSERC to JSB.

23.4

Intracellular Po_2 Is Not An Important Modulator Of Tissue Oxygen Consumption Above the P_{50} of Myoglobin in Mouse Skeletal Muscle In Vivo

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We address the role of intracellular oxygen concentration (PO_2intra) in modulating tissue oxygen consumption (MO_2) in vivo. In this study optical spectroscopy was used to follow changes in hemoglobin and myoglobin saturation throughout an ischemic period in the mouse hindlimb. Partial least squares analysis of the spectra allowed the separation of the Hb and Mb signals. Mb saturation was used to calculate the PO_2intra at each time point. From the Hb and Mb saturations and the Hb and Mb concentrations in the tissue during ischemia, the total oxygen content was also calculated and used to determine the tissue oxygen consumption. To examine the relationship between PO_2intra and MO_2 over a range of MO_2s, we treated mice with 2, 4, and 6 mg 2,4-dinitrophenol/kg body wt (DNP2, DNP4, DNP6, respectively) to partially uncouple mitochondria and raise the resting MO_2. DNP treatment resulted in a two to four-fold increase in MO_2 over the controls (control = 6.6 ± 0.9; DNP2 = 13.2 ± 4.5; DNP4 = 22.5 ± 6.8; and 23.5 ± 7.2 nmoles $O_2(g^*s)^{-1}$). In every treatment MO_2 did not fall to 90% of the maximum until PO_2intra dropped near the P_{50} of Mb (2.4 torr) (control = 2.7 ± 1.0; DNP2 = 1.8 ± 0.7; DNP4 = 2.3 ± 0.6; DNP6 = 2.6 ± 0.3 torr). These results indicate that the intracellular oxygen concentration plays little role in modulating the rate of oxygen consumption above the P_{50} of Mb. This work was supported by NIH AR45184, AR41928, and AG00057.

23.5

Changes in Mitochondrial Oxidative Phosphorylation During Insect Metamorphosis

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Early in the final larval instar of the tobacco hornworm (*Manduca sexta*) there is a small rise in hemolymph ecdysteroids, which does not induce molting, but does commit the larva to metamorphosis. Mitochondria isolated from the midguts of Day 5 larvae (after commitment) oxidize succinate at lower rates compared to those isolated from pre-commitment larvae (Day 2 larvae). In order to determine what aspects of midgut mitochondrial metabolism are responsible for this difference is succinate oxidation, a steady state kinetic analysis of oxidative phosphorylation was performed. Mitochondria were isolated from the midguts of Day 2 or Day 5 larvae and the kinetic responses (oxygen consumption) of the subsystems of oxidative phosphorylation to their common intermediate, the protonmotive force (Δp), were measured. The difference is succinate oxidation does not appear to be due to changes in the "phosphorylation system" because its kinetic response to Δp was the same in mitochondria isolated from Day 2 and Day 5 larvae. In contrast, the kinetic response of the "proton leak" to Δp is greater in Day 5 mitochondria. Compared to Day 2 mitochondria, however, the kinetic response of the "substrate oxidation system" to Δp is lower in Day 5 larvae. This indicates that processes such as the citric acid cycle, the electron transport chain, or substrate transport may be depressed after commitment. This work is supported by the National Science Foundation (IBN- 0131523).

23.6

Partial Compensation of Proton Permeability in Mitochondria and Inner Membrane Liposomes from Thermally Acclimated Trout

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Mitochondrial proton leak is dependent on membrane lipid composition, but the underlying basis for this remains unclear. Membrane lipid composition of trout liver mitochondria is restructured extensively with thermal acclimation, but the effect on proton leak is unknown. We measured the proton permeability of liver mitochondria and liposomes prepared from either inner or outer membrane lipids from trout acclimated to 20°C or 5°C. Mitochondrial proton leak was acutely dependent on measurement temperature and exhibited a pattern of partial compensation with acclimation. Maximal proton leak of mitochondria from 20°C-acclimated trout at ~185 mV was 78 ± 5 and 13 ± 1 nmoles protons per min per mg protein when measured at 20°C and 5°C, respectively. Corresponding estimates for 5°C-acclimated trout were 124 ± 4 and 30 ± 2, respectively. Bilayer surface area of the inner membrane did not change with acclimation and cannot explain the cold-induced increase in mitochondrial proton leak. Proton permeability of inner membrane liposomes exhibited a pattern of partial compensation that was qualitatively similar to that of intact mitochondria. In contrast, thermal compensation was not evident in outer membrane liposomes. Taken together, these data suggest that lipid composition can directly and significantly influence bilayer-mediated proton permeability of the inner mitochondrial membrane. Thermal compensation of the mitochondrial proton leak suggests both homeostatic regulation and a physiological imperative for doing so. Investigating the mitochondrial proton leak within a thermal acclimatory context promises to open new avenues for resolving its mechanistic and physiological significance. (JRH NSF IBN-9816438, MFG NSF DIG IBN-9902101

23.7

Bioenergetics of Diapause in Encysted Embryos of the Brine Shrimp *Artemia franciscana*

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Diapause is an endogenously controlled state of dormancy that precedes environmental insult. In *Artemia* it is characterized by developmental arrest, and depression of aerobic metabolism by >90% under normoxic conditions. Diapause is distinguished from quiescence, an environmentally controlled state of dormancy. Published NMR and biochemical data indicate that, while intracellular acidosis has a role in establishing anoxia induced quiescence, it does not appear to play a significant role in diapause. In light of the link between intracellular pH and ATP levels, our HPLC measurements support this finding by showing wide variation in ATP:ADP ratio and adenylate energy charge within diapause embryos from the Great Salt Lake. We have also shown that while respiration rate of diapause embryos is greatly inhibited, mitochondria isolated from these embryos have respiratory control ratios comparable to mitochondria of post-diapause, actively-developing embryos; yields of mitochondria per gram tissue are also similar. Thus, there appears to be an active inhibition of respiration *in vivo* that is not present when mitochondria are isolated. Comparing the activity of cytochrome c oxidase in homogenates of diapause and post-diapause embryos with enzyme activity of mitochondria isolated from these homogenates suggests a marked inhibition of this enzyme in diapause embryos. This inhibition appears to be relieved for the isolated organelle. (NSF IBN-9723746 and DARPA N00173-01-1-G011)

23.8

RNA Synthesis and Transcript Stability in Mitochondria from Embryos of *Artemia franciscana* Under Conditions of Anoxia-Induced Quiescence

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Mechanisms of transcriptional regulation were examined during anoxia-induced quiescence in mitochondria of gastrula-stage brine shrimp. During anoxia these encysted embryos undergo a profound reduction in metabolism characterized by severe intracellular acidosis (normoxia, pH 7.9; prolonged anoxia, pH 6.3) and reduced ATP levels. Isolated mitochondria were incubated in the presence of 32P-UTP under conditions simulating quiescence, and transcription products were measured by scintillation counting. Compared to normoxic controls at pH 7.9, overall RNA synthesis was reduced 49% by anoxia, 61% at low pH (6.3), and 77% by the combination of anoxia and low pH. We examined transcriptional initiation in vitro using a nuclease-protection assay and found that at pH 6.3, new initiation accounted for 31% of measured 32P-UTP incorporation, compared to 78% at pH 7.9. Using in organello DNA footprinting, we tested the hypothesis that decreased initiation at pH 6.3 was due to protein dissociation from promoters, but found no evidence of differential protein-binding among in organello treatments, which suggests mechanisms of control may include covalent modification. Finally, we measured the stability of four mRNAs under low pH and/or anoxia and their states of polyadenylation. Both anoxia and low pH promote mRNA stability, which is correlated with a decrease in mRNA polyadenylation. Plant organelles and bacteria have shown similar poly(A)/stability patterns. (NSF grant IBN-973746)

DIVING: WHERE HAVE WE BEEN AND WHERE ARE WE GOING?

24.1

DIVING EXPERIENCE AND THE AEROBIC DIVE CAPACITY OF MUSKRATS: DOES TRAINING PRODUCE A BETTER DIVER?

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We tested the hypothesis that the body oxygen stores, aerobic dive limit and dive performance of muskrats can be enhanced by dive-conditioning in a laboratory setting. The question was addressed by comparing several key variables in 12 muskrats trained for 9-11 weeks to swim a 16-m underwater course to a feeding station ("divers") with those of 12 animals precluded from diving but required to travel identical distances in water to feed ("surface swimmers"). The major finding was a significant gain in the hematocrit, blood hemoglobin concentration and blood O2 capacity of dive-trained muskrats. Dive-conditioning had no apparent effect on lung volume, whole blood and plasma volumes, nor on the glycogen level and buffering capacity of skeletal muscles. Cardiac and skeletal muscle myoglobin levels were also similar in both test groups following training. The mean total body oxygen store of "divers" was 13.5% higher than for "surface swimmers", an increase attributed entirely to the 26% gain in blood O2 storage capacity of the former group. However, owing to a slightly higher estimate of diving metabolic rate in dive-conditioned animals, the calculated aerobic dive limit for this group (61.3 s) was indistinguishable from that (61.8 s) of "surface swimmers". Few differences were observed in the post-training dive behaviour of "surface swimmers" and "divers", a finding consistent with the strong similarity in their calculated aerobic dive capacities. Funded by NSERC (Canada).

24.2

The Functional Significance of the Cardiovascular Dive Response to Routine Diving in the Harbor Seal *Phoca vitulina*

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While diving, harbor seals manage their oxygen stores through cardiovascular adjustments including bradycardia, reduced cardiac output and peripheral vasoconstriction. At the surface, tachycardia facilitates the rapid reloading of oxygen stores. Although harbor seals can tolerate over 20 min of submergence, their natural dives are typically only 2-6 min and are usually followed by surface intervals that are less than 1 min. so they spend about 80% of their time at sea underwater. We were interested in the functional role, if any, of the cardiovascular dive response during their short "routine" dives. During voluntary diving in an 11 m deep tank, the cardiovascular responses to submergence of five harbor seals were manipulated using specific pharmacological antagonists, and the effects on diving behavior were observed. The muscarinic blocker methoctramine blocked diving bradycardia; the -adrenergic blocker prazosin blocked diving vasoconstriction; and the -adrenergic blocker metoprolol blocked post-dive tachycardia. Heart rate was recorded using subcutaneous electrodes and a data logger while diving behavior was monitored using a video camera. None of the blockers had any effect on average dive or surface interval duration. Seals maintained a high percent dive time in all treatments including controls. Thus, harbor seals do not need the dive response during short dives in order to maintain an efficient dive strategy. This work was supported by an NSERCC Research Grant.

24.3

Oxygen, Carbon Dioxide, and Behavior: What are Divers Doing at the Surface?

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The post-dive surface recovery interval is a critical component in the foraging life of diving animals. From a behavioral, or optimal foraging perspective, divers should be selected to minimize time spent at the surface, because it is time not spent foraging or in other activities related to increasing fitness in an environment with increased risk of predation. From a physiological perspective, on the other hand, time spent at the surface serves the critical function of gas exchange after often extended periods of apnea. The adaptations of divers to maximizing time under water are well defined. What remains unclear are the adaptations that allow them to minimize time spent at the surface and still meet the demands of the unsteady-state gas exchange of oxygen and carbon dioxide. Until very recently, it was assumed that surface interval duration was driven by the oxygen reloading function. Recent evidence, however, suggests that the need to expunge accumulated carbon dioxide may be the determining factor. Existing models are confounded by species such as the northern elephant seal, grey seal, and New Zealand sea lion, which perform repetitive, extended deep dives with relatively short surface intervals. Here we examine the surface recovery characteristics of a variety of breath-hold divers, in terms of current models of surface recovery physiology.

24.4

Identifying prey ingestion based on blubber levels of 20:1 11 and 22:1 11 fatty acids in free-ranging Steller sea lions (*Eumetopias jubatus*).

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The fatty acid composition of blubber lipids was measured in 126 Steller sea lions (2 - 26 months of age) to determine when sea lions switch from nursing to prey ingestion. For 10 animals a sample of ingested milk provided the fatty acid signature of the milk diet. Two fatty acids in particular (20:1 11 and 22:1 11) which are present in marine fish tend to be underrepresented in pinniped milk. Weight % of 20:1 11 in ingested milk samples did not differ between area of capture or with age of the sea lion (2.30 ± 0.2 wt%, p=0.39). Young of the year (n=5) in both southeast Alaska (SEA) and Prince William Sound (PWS) showed 20:1 11 levels 1.8 fold higher than their ingested milk, while yearlings (14 mos) from PWS showed 4.2 fold higher levels in their blubber than in their milk diet. Based on subjective threshold level of 20:1 11 gauged from all 2-3 month old pups (7.0 wt %) remaining animals were divided into those judged to be nursing only and those with elevated 20:1 11 indicative of prey. Based on this threshold, 100% of animals captured in PWS that were 10 months or older showed evidence of fish ingestion. In contrast, only 1 of 70 animals captured in SEA (2 -19 months of age) indicated fish intake. These trends were also supported by 22:1 11 data. Given that high 20:1 11 levels were found in three nursing juveniles, additional variables will be required to distinguish fully weaned animals from those consuming both milk and prey. Funded by NOAA (NA17FX1079) to ADFG.

24.5

Can Terrestrial Models of "Body Condition" Be Applied to a Marine Mammal?

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In terrestrial mammal model systems, there are a variety of indices that attempt to relate anatomical or physiological variables to "condition" and therefore to assumptions of "health". For example in humans, the Body Mass Index (BMI) relates mass and height to obesity, using the assumption that obesity is indicative of poor condition in humans. On the other hand, an exceedingly low BMI is correlated with poor nutrition and poor reproductive capacity, etc. In wild ungulates, measurements of "rump fat depth" seem to be correlated with reproductive success. In marine mammals, there have been a host of indices suggested as indicators of condition. These include length x girth x mass relationships, blubber depths at certain points on the body, sculp to lean mass ratios, etc. Unfortunately, the ultimate link between "condition" and reproductive success have never been tested in marine mammals. However, even on a proximate scale, body mass and fat content appear not to be under the control of diet type, they are impacted by season and by the stage of breeding cycle. Moreover, it is not clear how fatness, blubber lipid content and blubber depth are related. We propose here that lean mass, fat mass and blubber content are being regulated in these marine species by factors that go beyond simple assumptions of body condition and therefore, terrestrial assumptions of body condition may not apply to these marine mammals.

24.6

Muscle Blood Flow and Heart Rate During Sleep Apnea in Elephant Seals

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Elephant seals, the most accomplished pinniped divers, exhibit natural, spontaneous breath holds (apneas) while sleeping. The management of oxygen (O_2) stores is essential to the breath hold capacity of these animals. In order to assess whether the bradycardia and vasoconstriction of the "diving response" isolate the muscle O_2 store from the blood O_2 store during the breath hold, we measured heart rate and muscle blood flow (by laser-Doppler flowmetry) in juvenile northern elephant seals (*Mirounga angustirostris*) throughout several sleep apnea-eupnea (breathing) cycles. Sleep apnea durations ranged from 3 to 12 minutes. Apneic heart rates were variable beat-to-beat, but averaged 60 beats per minute, or 85% of the mean eupneic rate. Muscle blood flow (MBF) decreased gradually during the course of apnea, but never reached zero. Mean apneic MBFs were approximately 44% of eupneic values, but declined to as low as 15% at the end of long apneas. Occasional, transient increases in MBF were observed during apnea. Therefore, although MBF declines progressively during sleep apneas of juvenile elephant seals, muscle is not completely isolated from the circulation. This implies that some O_2 delivery from blood to muscle may still occur during the breath hold. (Supported by NSF grant IBN-0078540 and UC Academic Senate and MRIF grants.)

24.7

Seasonal and Short-term Effect of Temperature on Metabolic Rate of the Loggerhead Turtle, *Caretta caretta*

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We measured metabolic rates (MR) of 9 loggerhead sea turtles, *Caretta caretta*, originating from the Mediterranean Sea. The turtles were kept in indoor tanks (Aquarium of Naples, Italy) supplied with circulating sea water from the Gulf of Naples and were therefore subject to the natural variation in water temperature (T_w). The study consisted of two experimental parts: (1) routine 24-h-measurements of MR from Sep 00 through Feb 01, and (2) MR measurements during short-term exposure (3 h) to experimentally manipulated T_w (15, 25 and 30°C) in Nov 00. MR was measured using an open-flow repirometry system and body temperatures (T_b) were taken either rectally or recorded by ingested miniature temperature loggers. MR decreased significantly from summer to winter with a Q_{10} of 5.8. T_b of the turtles was not different from T_w and underwent the same seasonal decline. Likewise a decrease of food intake and activity was observed during the winter, which probably contributed to the reduction in MR. However, MR correlated positively with T_w also during short-term exposure, although the effect was less profound (Q_{10} = 1.3). T_b during these short term transfers equilibrated with the experimental T_w within 2.5-3 h. The results demonstrate the importance of complete acclimation for the study of seasonal environmental temperature effects on sea turtle physiology. The study was supported by the University of Aberdeen, UK, and the Stazione Zoologica of Naples, Italy.

24.8

Fetal Lung Development in the Elephant Reflects the Adaptations Required for Snorkeling in Adult Life.

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The adult elephant is unique among mammals in that the pleural membranes are thickened and the pleural cavity is obliterated by connective tissue. It has been suggested that this peculiar anatomy developed because the animal can snorkel at depth, and this behavior subjects the microvessels in the parietal pleura to a very large transmural pressure. To investigate the development of the parietal pleura, the thickness of the endothoracic fascia (ET) was measured in four fetal African elephants of approximate gestational age 111 to 130 days, and the appearances were compared with those in human, rabbit, rat and mouse fetuses of approximately the same stage of lung organogenesis. The mean thicknesses of the ET in the elephant, human, rabbit, rat and mouse were 403, 53, 29, 27 and 37 m, respectively, and the ratios of ET thickness to rib cage diameter were 0.073, 0.019, 0.007, 0.012 and 0.012 respectively. This very early development of a thick parietal pleura in the elephant fetus is consistent with a long history of snorkeling in the elephant's aquatic ancestors. This abstract is funded by NIH grant RO1 HL 60698.

24.9

Aerobic Capacity in the Skeletal Muscles of Weddell Seals: Key to Longer Dive Durations?

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In contrast to terrestrial animals that function under hypoxic conditions but display the typical exercise response of increasing ventilation and cardiac output, marine mammals exercise under a different form of hypoxic stress. They function for the duration of a dive under progressive asphyxia, which is the combination of increasing hypoxia, hypercapnia and acidosis. Our previous studies on short duration, shallow divers found marked adaptations in their skeletal muscles, which culminated in enhanced aerobic capacities similar to athletic terrestrial mammals. The purpose of this study was to assess the aerobic capacity of skeletal muscles from long duration divers. Swimming and non-swimming muscles were collected from adult Weddell seals and processed for morphometric analysis, enzymology, myoglobin concentrations and fiber type distribution. The results showed that the skeletal muscles of Weddell seals do not have enhanced aerobic capacities compared to terrestrial mammals but are adapted to maintain low levels of an aerobic lipid based metabolism especially under the hypoxic conditions associated with diving. The lower aerobic capacity of Weddell seal muscle as compared to shorter duration divers to appears reflect their energy conserving modes of locomotion (via extended periods of extending gliding during descent) that enable longer, and deeper dives.

This work was supported by the National Heart, Lung and Blood Institutes grant PO1 HL 17731 and by the NSF Division of Polar Programs (grant OPP 14857).

24.10

Overcoming Buoyancy: Surface Descent in Thick-Billed Murres (*Uria lomvia*)

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Birds belonging to the family Alcidae are both aerial fliers and wing-propelled divers and, therefore, rely on their wings to move through two media with contrasting physical properties. Overcoming positive buoyancy is energetically expensive for diving birds (Stephenson, 1994). Previous studies calculated the total buoyancy for an adult thick-billed murre to be 4.5N (Lovvorn *et al.*, 1999). My study is designed to investigate how alcids descend into the water column from the surface. High-speed video was used to document the movements of the wings and feet employed by a murre to propel itself underwater in a variable-speed flume. Kinematic parameters investigated for the first two wingbeats include whole animal velocity and acceleration, wingbeat amplitude, and body dive angle. Thick-billed murres use their feet synchronously in addition to their wings to propel themselves underwater. All of the bird's forward acceleration occurs during the downstroke. Further, the amplitude of the first wingbeat is 30-40% larger than the second. During the initial downstroke, the body is oriented up to 30 degrees more vertical relative to the flow than it is over the next one and a half wingbeats. Thus, the first wingbeat of the surface descent is most likely specialized to generate the thrust required to overcome the bird's positive buoyancy. This work was funded by the Wyss Foundation.

24.11

The Reflex Control of Heart Rate During Diving in Lesser Scaup Ducks

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Species of birds that have evolved the ability to swim underwater have varying cardiac responses to submergence. Cardiac adjustments during voluntary dives are less pronounced than those seen during forced dives in which extreme bradycardia occurs. However, when ducks are prevented from surfacing at the end of a voluntary dive (trapping), heart rate drops to levels seen in forced dives. This study focuses on the mechanisms that may be involved in heart rate control, specifically, the contribution of reflexes to the heart rate responses to diving in lesser scaup ducks. We hypothesize that the trigeminal nerve and peripheral chemoreceptors are two major afferent inputs that modulate heart rate during short and long voluntary dives as well as during trapping. Heart rate was monitored using implantable ECG transmitters before and after trigeminal sectioning with the animal breathing air (control) or 50% oxygen before the dive. Heart rate profiles of ducks breathing oxygen before short dives did not differ from their control profiles. When ducks were temporarily trapped underwater, heart rate dropped to forced dive levels and breathing oxygen before the trapped dive did not alter this response. Future analysis will determine the contribution of trigeminal nerve reflexes to heart rate. Theses studies combined will give an understanding as to how reflexes contribute to the control of heart rate during diving in ducks. Funding was provided by an NSERCC Research Grant.

24.12

Heart Rate, Rate of Oxygen Consumption and Abdominal Temperature During Diving in Macaroni Penguins

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In many diving animals, observed diving behaviour appears greatly to exceed the limits predicted from physiological estimations of aerobic diving capacity. The present study used heart rate (f_H) to estimate the rate of oxygen consumption during diving, in order to investigate this paradox in macaroni penguins (*Eudyptes chrysolophus*) and determine whether they exceed their calculated aerobic diving limit (cADL). Thirteen free ranging breeding female penguins were implanted with miniature data loggers, which recorded f_H, abdominal temperature (T_{ab}) and diving depth. In common with other diving birds, macaroni penguins showed significant changes in f_H associated with diving. When rate of oxygen consumption was calculated for dives of different durations, 95.3% of dives measured were within the cADL for this species. The relatively high time constant of the device used meant that declines in abdominal temperature were detected during diving bouts (mean ± S.E.M.; 2.32 ± 0.2 °C) but not during individual dives. There was a linear relationship between bout length and the magnitude of the temperature drop but no commensurate increase in dive duration during dive bouts, suggesting that macaroni penguins are diving within their physiological limits and other factors are important in determining the duration of dives and dive bouts. This work was funded by a NERC/CASE studentship in association with the British Antarctic Survey.

24.13

Can Diving Optimality Models Predict Adjustments In The Diving Behaviour Of Tufted Ducks?

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We measured the effects of differing foraging costs on dive time and rate of oxygen uptake at the surface in tufted ducks during bouts of voluntary dives to a feeding tray at 1.1 m depth. The birds were trained to surface into a respirometer so that changes in the rate of oxygen uptake could be measured. The tray either held just food, or held closely packed stones on top of the food to make foraging energetically more costly. In contrast to predictions from the optimal foraging model of Houston and Carbone (1992), foraging time increased after energetically more costly dives. However, the rate of oxygen uptake was significantly higher during the surface period after these dives. This compensated for the increased energy consumption of the dive without the need to increase surface time. The 'optimal breathing model' by Kramer (1988) predicts the energy metabolised during foraging and surface time. This model was tested with data on time budgets and oxygen uptake curves from the present study, and estimates of power costs during diving from Lovvorn et al. (1991). The model successfully predicted surface time and oxygen consumption during foraging for the mean of all ducks. However, the model did not consistently predict surface time or oxygen consumption during foraging using data for individual birds. The precision of the model may have been affected by inaccurate values of power cost estimates. This work was supported by a NERC studentship.

24.14

Factors Influencing the Proximate Composition of Milk in a Sub-polar Otariid, *Callorhinus ursinus*

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Milk lipid is the primary energy source for pinniped offspring. Lipid [%] in milk varies considerably for the taxon and within species with important implications to reproductive energetics, life-history strategies, maternal investment, and population energy models. For otariid milk, days postpartum (DPP) and time ashore (TAS) are known to affect lipid [%]. Early cross species comparisons indicated longer trip durations may increase lipid [%] as well; intra-species correlations, however, remain equivocal. Diet has also been implicated in influencing total energy transferred to offspring. We used multiple linear regression models to test significance of DPP, TAS, preceding trip duration (PTD), maternal mass, dive type (proxy for diet), island and year in determining proximate composition (lipid, protein, water, and ash) of northern fur seal milk (n=189, from 101 telemetered seals; Jul-Oct 1995-96). Total lipid [%] ranged from 28.5-66.8 and gross energy averaged 21.3±0.21. PTD ranged from 2.5-14.1 days. Lipid and gross energy were correlated with DPP (positively) and TAS (negatively; $P<0.0005$; all relationships). PTD had no effect on lipid ($P=0.67$), energy content ($P=0.56$), protein ($P=0.33$), or water ($P=0.64$). Dive type, maternal mass, and year did not affect proximate composition of milk. Our results do not support the hypothesis that increased time at sea increases milk lipid in the Arctocephalinae otariids. Funding provided by NSF (OPP-9500072).

24.15

Voluntary Underwater Submergence In Conscious Rats Activates Pre-Sympathetic Brainstem Nuclei

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Underwater submergence produces a complex autonomic response that includes apnea, a parasympathetic bradycardia, and a sympathetically mediated increase in peripheral vascular tone. Cardiac output is reduced in proportion to the bradycardia, and total peripheral resistance (TPR) is increased so that mean arterial blood pressure remains almost unchanged. Because the increase in TPR is sympathetically mediated, the hypothesis was that brainstem pre-sympathetic nuclei are activated during voluntary underwater submergence. Twelve rats were trained to voluntarily dive 5 m through an underwater maze. On the day of the experiment the rats were randomly separated into a diving group (N=4) that repetitively dived underwater every 5 min for 2 hr (dive duration 12s; water temp = 30 °C), a swimming group (N=4) that repetitively swam on the surface of the water, and a control group (N=4) that remained in their cages. After the experiment the rats were deeply anesthetized, perfused, and their brainstems were immunohistologically processed for FOS as an indicator of neuronal activation, and for tyrosine hydroxylase (TH) as an indicator of catecholaminergic neurons. Double-labeled neurons (FOS+TH) were used to identify pre-sympathetic neurons that were activated during diving. In the diving rats there were an increased number of double-labeled FOS+TH neurons in brainstem pre-sympathetic nuclei, compared with control rats. This included the A1 (both caudal and rostral to the obex), A2, A5, sub-coeruleus, and A7 regions. In the swimming group the double-labeled pre-sympathetic neurons were significantly increased only in the A5 and caudal A1 region. The C2 group showed no increase in FOS+TH double-labeled neurons in the swimming or diving rats. These data suggest that pre-sympathetic neurons within the brainstem are activated by voluntary underwater submergence, and probably contribute to the increase in TPR during diving.

24.16

Antioxidant protection in marine birds and mammals.

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Marine mammals and birds are exposed to varying tissue oxygen levels resulting from regional ischemia and reperfusion during dives. They face the potential for increased production of reactive oxygen species (ROS). We examined possible adaptive responses to this condition. Production of superoxide radical ($O_2^{\cdot-}$) and lipid peroxidation (TBARS) were determined in swimming (S) and non-swimming (NS) muscle from elephant seal, ringed seal, California sea lion, and emperor penguin, and compared with non-locomotory muscle from domestic pig. $O_2^{\cdot-}$ production in ringed seal (S 0.2930 ± 0.0086, NS 0.1295 ± 0.0092 nmol/min.g), elephant seal (S 0.2440 ± 0.0219, NS 0.1840 ± 0.0346 nmol/min.g), California sea lion (S 0.0890 ± 0.0240, NS 0.1217 ± 0.0116 nmol/min.g) and penguin (S 0.2924 ± 0.0785, NS 0.4363 ± 0.0946 nmol/min.g) was, contrary to expectations, higher ($P<0.1$) than in pig (0.0173 ± 0.0017 nmol/min.g). In phocid seals $O_2^{\cdot-}$ was higher ($P<0.1$) in S than in NS muscle; the opposite was true for otariids and penguins. TBARS were not proportionally higher in seals, sea lions and penguins than in pigs. Antioxidant capacity was analyzed in ringed seal and pig, and was found to be higher ($P<0.1$) in ringed seal. These results suggest that tolerance of dive-associated ischemia/reperfusion in marine organisms may depend on enhanced intermediate scavenging of ROS. Differences between S and NS may be due to fiber type distribution; differences among species may be related to diving capacities.

25.0

INSIGHTS INTO RESPIRATORY MECHANICS: LESSONS FROM THE ELEPHANT. John B. West, Dept. of Medicine, University of California San Diego, CA 92093-0623.

A unique feature of the elephant lung is that the pleural cavity is obliterated by connective tissue. This has been known for over 300 years but never satisfactorily explained. The elephant is also unique in being able to snorkel at depth. In fact there is strong evidence that the elephant has an aquatic ancestry, and it may be that the trunk developed for snorkeling. During snorkeling the resulting differences of pressures just outside the lung mean that the small blood vessels of the parietal pleura are at great risk because of the enormous transmural pressures. Evolution has provided a remarkable solution to this problem by replacing the normally delicate parietal pleura by dense connective tissue which protects the microvessels. The visceral pleura is also somewhat thickened although its vessels appear to be at less risk. Since the microvessels of the parietal pleura normally provide the fluid that lubricates the pleural space, this option is not available in the elephant. Instead evolution has provided a layer of highly extensible, loose connective tissue between the two pleural membranes. The same distribution of pressures occurs during drinking through the trunk and this may be an additional evolutionary pressure in the modern elephant with its predominantly terrestrial lifestyle.

It is known than the fetal elephant has a normal pleural space which is obliterated late in gestation. We have recently found that the developing parietal pleura in the fetus is greatly thickened unlike that in other mammals such as human, rabbit, rat and mouse. The trunk also develops very early in fetal life, and the combination is consistent with a long history of snorkeling in the elephant's aquatic ancestors.

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PHYLOGENETIC APPROACHES TO UNDERSTANDING PHYSIOLOGICAL EVOLUTION

26.2

WHAT ARE PHYLOGENIES AND WHY DO THEY MATTER? Wayne Maddison, Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721

The broad-scale history of genetic descent takes the form of an evolutionary tree ("phylogeny"). Concordance among different sources of data confirms that we are able to reconstruct the tree with surprising accuracy. Thus, the imprint of history on the features of organisms is strong, no less so on those of physiology. Correspondingly, knowledge of phylogenetic history is a vital guide to interpeting similarities and differences among organisms. Phylogeny reveals what classes of events have been repeated during organismal evolution, such as a shifts in habitat or changes in physiological mechanism, leading us to general laws of evolutionary process. The change in perspective in comparative biology over the last 20 years from one in which species are viewed as independent points in a multivariate space describing their features, to one in which their traits are explained as changes along the branches of phylogenetic trees, is as fundamental as the change from Newtonian to Einsteinian cosmology. Indeed, phylogeny plays the role of gravity: it imposes a curvature on our multivariate character spaces, a curvature that must guide all our comparisons among organisms.

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26.3

PHYLOGENETICALLY BASED STATISTICAL METHODS: WHEN, WHY, AND HOW TO USE THEM. Theodore Garland, Jr., Dept. of Biology, Univ. of California, Riverside, CA 92521.

Interspecific comparisons, always a mainstay of comparative physiology, have been revitalized by infusion of new information on phylogenetic relationships and by new analytical methods that use this information. Although multi-species data sets can be analyzed by conventional statistical procedures (which may be termed "Phylogenetically Uninformed"), this implicitly assumes that the species included show no hierarchical relationships, i.e., that the true phylogeny is star-shaped. For most organisms, available data indicate that the phylogeny is probably strongly hierarchical, and this information can be used to perform "Phylogenetically Correct" analyses. Moreover, one can compare intermediate trees in terms of how well they fit the data, and transform branch lengths accordingly; this allows the possibility that a star may be the best tree to use, but such cases are rare. Phylogenetic information should guide choice of species to study and can allow new questions to be addressed, such as where and when particular physiological phenotypes first appeared, whether they may have constituted key innovations, and whether rates of evolution differ among lineages. Recent work has demonstrated the fundamental equality of independent contrasts and generalized least-squares approaches to phylogenetically structured data, as well as the utility of such computer-intensive methods as Monte Carlo simulations. In addition, technological advances have allowed functional biologists to become involved in generating molecular-phylogenetic data for their preferred study organisms.

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26.4

WHAT ARE THE APPROPRIATE TESTS OF MECHANISTIC AND HISTORICAL EXPLANATIONS FOR EVOLUTIONARY PATTERNS?

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Understanding how life works is the fundamental goal of biology. As modern biologists, we strive for a mechanistic explanation of phenomena ranging from molecules to ecosystems. Mechanistic understanding involves distinguishing reproducible and testable causal patterns from noncausal or nontestable associations. Reconstructing the evolution of mechanistic relationships among characters to test adaptive hypotheses is preferable to using correlational approaches because of the added strength of causal inferences. Exclusion of historical and mechanistic biology from adaptive explanations can lead to the substitution of deep understanding of the trait itself for a superficial and largely untestable narrative by assigning the significance of variants in the trait to fitness a priori. Understanding the historical and mechanistic foundations of phenotypes has the potential for increasing the accuracy and efficiency of research, and may be necessary in order to separate evolutionary causes from evolutionary effects. I argue that the proper context for challenging an adaptive hypothesis based on integrating mechanism and history is an experiment that rejects the hypothesized mechanism, or a phylogenetic study that suggests a more likely historical hypothesis. I present case studies in physiology, development, and behavior in which a false conclusion would have been reached without an integration of mechanism and history. In each of these examples, the dominant role of history combined with organismal integration makes ignoring mechanism or history a risky proposition.

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26.5

A PHYLOGENETIC PERSPECTIVE ON THE EVOLUTION OF VERTEBRATE SURFACTANTS. Christopher B. Daniels, Sandra Orgeig and Lucy C. Sullivan. Dept of Environmental Biology, University of Adelaide, Adelaide SA 5005, Australia

Pulmonary surfactant, a mixture of phospholipids, cholesterol and proteins, reduces surface tension of the fluid lining the inner lung. The surfactant system has been highly conserved, morphologically and biochemically throughout (and despite) the enormous radiation of the air-breathing vertebrates. The remarkable similarity of the proteins strongly suggests their homology, i.e., single evolutionary origin, and also an origin prior to the evolution of amniotes. Surfactant can act as an anti-adherent, prevent alveolar edema, aid the muco-ciliary escalator, increase lung compliance and be anti-microbial/viral. These roles differ in importance among species and hence each has a slightly different surfactant with different surface properties. Surfactant properties appear to have co-adapted with temperature, but not lung structure, and generally do not show lineage-specific effects. The development of the surfactant system in egg-laying and placental vertebrate embryos is remarkably similar, and is controlled by thyroid and glucocorticoid hormones. These hormonal influences disappear after birth when adrenaline, acetylcholine and temperature control the system. The surfactant system is highly conserved, yet spectacularly complex. The combination of conserved characters demonstrating important subtle differences in composition and function attributable to specific selective factors makes it an ideal system to explore evolutionary processes in respiratory physiology. *Funded by the Australian Research Council.*

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26.6

USING PHYLOGENIES TO UNDERSTAND THE EVOLUTION OF FUNCTION AND BEHAVIOR IN LIZARDS.

Duncan J. Irschick, Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA 70118.

Phylogenetic approaches have been instrumental in advancing our Understanding of basic biological issues, but the "phylogenetic revolution" has only recently made inroads into physiological studies. Studies of locomotor performance have played a particularly important role for understanding the costs and benefits of different physiological traits, but rarely from a phylogenetic perspective. Further, most performance studies have focused on maximum speeds in the laboratory, and have not considered the breadth of locomotor capacities that animals display in nature. Here, I discuss how a phylogenetic approach has proven useful for examining the evolution of performance in three contexts, using lizards as a model system: First, the evolution of kinematics during high-speed locomotion. Second, the evolution of ecological performance in arboreal specialists. Third, the evolution of acceleration in diverse lizard taxa. In each case, the phylogenetic approach revealed important information that would not been possible with an ahistorical approach. For example, comparative studies of ecological performance revealed that lizard species with high maximum capacities use a relatively smaller fraction of their capacities relative to species with low maximum capacities. Further, comparative studies showed intriguing evolutionary relationships between maximum speed and maximum acceleration. These three case studies show that a phylogenetic approach is essential for fully understanding the evolution of complex physiological systems. They also provide evidence that physiological biologists can study the evolution of traditionally "laboratory" traits in nature.

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26.7

THE EVOLUTION OF COMPLEX SYSTEMS: OXYGEN SECRETION IN THE EYE AND SWIM BLADDER OF FISHES. Michael Berenbrink, School of Biological Sciences, The University of Liverpool, Liverpool L69 3BX, England.

Modern fishes are unique among vertebrates in generating super-atmospheric oxygen (O_2) tensions at their retinas and swim bladders, respectively. While the mechanism is similar for both tissues, retinal O_2 concentration fuels the high metabolic rate of this brain-derived tissue whereas swim bladder O_2 concentration is used for buoyancy control. In this study, I mapped key anatomical, physiological and molecular elements of the retinal and swim bladder oxygen concentration mechanism onto a vertebrate phylogeny in order to reconstruct their ancestral character states. Ideally, this approach allows an estimate of the likely sequence of character changes that have given rise to the highly complex system of O_2 secretion in modern day fishes. The results indicate a severe increase in acid-sensitivity of hemoglobin O_2 binding and a simultaneous decrease in hemoglobin hydrogen ion buffering as the first steps towards any oxygen concentrating mechanism. The latter step was significantly correlated to a decrease in the histidine content of globin chains. Evolution of vascular counter-current exchange systems, first in the eye and then several times independently in the swim bladder, then allowed generation of super-atmospheric O_2 tensions. Thus, this approach allows identifying predispositions and constraints during evolution of complex systems.

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26.8

Use of Phylogenetic Information to Understand the Evolution of Anuran Thermal Biology. Carlos Navas.

Information about the evolutionary plasticity of thermal physiology is essential to understand how ectotherms invade new thermal environments. Amphibians are interesting from this point of view because, despite limited capacity for behavioral thermoregulation, have invaded contrasting thermal environments. The evolution of anuran thermal physiology has been investigated using comparative approaches based on available phylogenetic information. Comparisons within and among high-elevation anurans families, using a convergence approach, suggest that improved locomotor performance at low temperatures has evolved repeatedly and independently. No adaptive trade-offs have been observed, and swimming capacity at low temperatures is not associated to reduced swimming capacity at high temperatures. Indeed, at typical tropical temperatures high- and low-elevation Andean frogs exhibit similar locomotor performance. In addition to swimming and jumping, some of the species that live at high elevations call vigorously at very low temperatures. Given that calling is an aerobic activity, this observation poses questions regarding the evolutionary relationships between activity at low temperatures and intensity of aerobic activity. A comparative study, restricted to the tree-frog genera *Scinax*, has contributed to understand this problem. A study involving ten species showed independent evolution of the underlying physiological mechanisms related to intensity of vocal activity and activity at low temperatures. The vocally most active species exhibit high metabolic scopes at typical temperatures, but this trait is not necessarily associated to an increase in maintenance costs. A winter specialist that calls at low rates exhibits a high maintenance costs that is not coupled to an increase in aerobic scope. Neither evolutionary associations nor trade-offs have been detected, so that resting and activity metabolic states appear to evolve independently in the contexts of change in either activity temperature or intensity of activity. The evolutionarily plastic thermal physiology of anurans is evident at various systematic levels, and has allowed for remarkable behavioral and ecological diversity in these animals.

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26.9

EVOLUTIONARY PHYSIOLOGY OF LARKS ALONG TEMPERATURE AND MOISTURE GRADIENTS. Joe. Williams. Ohio State Univ.

The adaptive significance of physiological phenotype has been the subject of much research. Broad scale comparisons of species of birds indicate the possibility of adaptive modification of basal metabolic rate (BMR) and total evaporative water loss (TEWL) for species living in desert environments. Even when adjusted for phylogeny, these analyses are limited because of the diverse ecologies of the constituent species, and because of the unknown affect of acclimation. We test the hypotheses that BMR and TEWL are reduced along an aridity gradient within a single clade of birds, the larks (Alaudidae). Results showed that, for 12 species of larks, BMR and TEWL decreased along a gradient of increasing aridity, a finding consistent with our hypotheses.

We constructed a phylogeny for 22 species of larks based on sequences of two mitochondrial genes, and investigated whether historical affinity played a role in the correlation of phenotype by environment. A test for autocorrelation between mass-corrected TEWL and environment showed no influence of phylogeny in our findings. However, we did discover a significant autocorrelation between mass-corrected BMR and aridity. A test of the relationship between mass-corrected BMR and aridity using phylogenetic independent constrasts also showed that BMR decreased with increasing aridity. Phenotype by environment correlations can indicate genetic differences, or differences attributable to acclimation. Experiments showed that acclimation to environment could not explain the correlations that we have found.

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26.10

Evolutionary Physiology of Habitat Transitions

Carol Eunmi Lee. Zoology, University of Wisconsin, Madison

Invasive species serve as excellent models for studying rapid adaptation to new environments. In particular, invasions of fresh water by brackish and saline species typify many of the hazardous invaders in aquatic habitats [2]. Recent invasions of fresh water have occurred at least eight times independently in the copepod species complex *Eurytemora affinis* [1]. These independent invasions offer replicated tests of physiological adaptation [2]. Because lineages vary in low-salinity tolerance, this complex provides an ideal system for identifying mechanisms that confer the ability to invade [1]. Work in my lab has shown that the transition to fresh water involves strong selection and a heritable shift in salinity tolerance [3, Lee and Remfert, unpublished]. Within invading populations, there is considerable genetic variance for salinity tolerance, genotype by environment interaction, and differences in the ability to acclimate [3]. We are currently using gene expression analysis to determine (1) what physiological mechanisms account for differences in salinity tolerance within and between populations and (2) whether the invasion of fresh water has involved the same or different evolutionary and physiological pathways during replicate invasion events.

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THE COMPARATIVE PHYSIOLOGY OF CARBONIC ANHYDRASE

27.1

A COMPARATIVE APPROACH TO CARBONIC ANHYDRASE: THE WORK OF TOM MAREN. Erik R. Swenson, VA Puget Sound Health Care System, Department of Medicine, University of Washington, Seattle, WA 98108.

Thomas H. Maren studied carbonic anhydrase (CA) for almost 50 years, venturing into all aspects of this powerful enzyme from active site chemistry to clinical medicine. In this effort, he was a keen proponent of the power of comparative physiology to illuminate basic principles and applied it extensively to the chemistry and biology of CA by studying it in non-mammalian species every summer at the Mt. Desert Island Biological Laboratory (MDIBL). Following the venerable strategy of selecting the right creature amongst Nature's rich diversity to explore a question, Maren derived important insights into the role of CA in ion transport, acid-base regulation and gas exchange. Using the fact that tissue CAs are expressed differently in combination with differing temperatures, acid-base status and metabolic rates of many marine creatures, he more thoroughly defined the contributions of un-catalyzed and catalyzed CO2 reactions in various physiological processes. As an example, he verified the primary role of HCO3 as the lead ion in CSF formation in fish and extended this to mammals, a concept previously rejected by leaders in the field. Using marine fish whose kidneys have very little CA, his findings uncovered mechanisms of acid-base transfer independent of CA which help to explain the fact that CA inhibition does not lead to total bicarbonate depletion. His studies of aqueous humor in fish were fundamental to the ultimate discovery of topical CA inhibitors effective for glaucoma. He said of his many years at MDIBL, that the lab and comparative physiology were his scientific and spiritual "window into the world'.

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27.2

ENVIRONMENTALLY MEDIATED EXPRESSION OF CARBONIC ANHYDRASE IN THE GILLS OF EURYHALINE CRUSTACEANS. Raymond P. Henry, Auburn Univ., AL 36849

Euryhaline crustaceans make the transition from osmotic and ionic conformity to regulation below a salinity of 26 ppt by activating mechanisms of ion transport that maintain hemolymph concentrations above those in the medium. Carbonic anhydrase (CA) supports this transport process by providing H^+ and HCO_3^- through the catalyzed hydration of CO_2. During low salinity acclimation there is an 8-15 fold induction of CA activity in the posterior, ion transporting gills, the largest increase in the animal kingdom. Branchial CA induction occurs in the cytoplasmic fraction, and it is a result of selective gene activation. CA mRNA increases at 24 hr post-transfer to low salinity, and protein-specific CA activity increases immediately thereafter. Crabs must be exposed to low salinity for a critical period of time of between 12 and 24 hr for this induction to occur, but once that period has passed, the induction is irreversible.

CA gene expression is under the control of a repressor factor that is found in the eyestalk, the major endocrine complex of crustaceans. Removal of the repressor via eyestalk ablation (ESA) results in a 50-100% increase in CA activity in crabs acclimated to high salinity. ESA followed by transfer from 35 to 28 ppt (a salinity still above the critical point for CA induction) results in an 8 fold increase in CA activity. ESA also potentiates the normal low salinity-mediated CA induction by up to 30%.

When extracts of homogenized eyestalks are injected into crabs, they abolish the effects of ESA. Furthermore, these extracts also inhibit the normal low salinity-mediated induction of CA in intact crabs by 50% or greater. These results suggest that CA induction is regulated at the transcriptional level by a repressor, found in the eyestalk at high salinity, and removed upon exposure to low salinity.

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J. Exp. Biol.

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27.3

COMPARATIVE MOLECULAR PHYSIOLOGY AND EVOLUTION OF VERTEBRATE CARBONIC ANHYDRASES. Bruce L. Tufts, Susan Lund and Andrew Esbaugh, Department of Biology, Queen's University, Kingston, Ontario, Canada. K7L 3N6

In contrast to the situation in mammals, relatively little is known about the comparative molecular physiology and evolution of CA isozymes in early vertebrates. In recent years, our lab has therefore been using several approaches to learn more about the CA isozymes in early vertebrates such as agnathans, fish and amphibians. One of our objectives has been to examine the changes that have occurred in the properties of the erythrocyte CA isozyme during the transition from the slow turnover erythrocyte CA in agnathans to the fast erythrocyte CA in teleost fish. In this area, we have recently characterized the isozyme properties and nucleotide sequence of an ancient fish, the longnose gar. The kinetic properties and molecular structure of gar erythrocyte CA were not closely aligned with either mammalian CA I or CA II, but fit well into an emerging phylogenetic pattern for early vertebrates. Similar projects for erythrocyte CA from agnathans and more recently evolved teleost fish are also underway. Another objective in our lab has been to determine the diversity and structure of other CA isozymes in the tissues of early vertebrates. In this regard, we have obtained evidence that CA IV is present in a number of fish tissues. Our progress in determining the biochemical properties and molecular structure of the CA IV-like isozyme in early vertebrates will also be discussed.

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27.5

COMPARATIVE PHYSIOLOGY OF PULMONARY CARBONIC ANHYDRASE. Erich K. Stabenau and Thomas A. Heming, Department of Biology, Bradley University, Peoria, IL 61625 and Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX 77550.

Carbonic anhydrase (CA) IV participates in CO_2 excretion and postcapillary CO_2-HCO_3^--H^+ equilibration in a wide variety of terrestrial vertebrates, despite possessing vastly different lung morphology. Perfusion of reptilian and mammalian lungs with salines containing inhibitors of CA IV revealed striking similarities in the kinetics of CA inhibition. Acetazolamide produced K_I of 1.2, 1.9, and 0.9-2.2 μM during perfusion of snake, turtle and rat lungs, respectively. Moreover, perfusion of lungs with the impermeant CA inhibitor quaternary ammonium sulfanilamide indicated that the CA activity was localized to the intravascular, extracellular endothelial cell membrane in all species. The latter results were confirmed via perfusion of lungs with phosphatidylinositol specific-phospholipase C.

More recently, we performed a biochemical characterization of the microsomal CA activity in vertebrate gills and lungs. These studies revealed, in part, that gills from several species of freshwater fish did not possess SDS-resistant CA activity, whereas SDS-resistant CA activity was detected in the lung microsomal fraction in amphibian, reptilian, avian and mammalian species. Presumably, CA activity in the presence of SDS is a reliable indicator of CA IV.

Taken together, these results indicate that terrestrial vertebrates possess membrane-bound, intravascular CA IV. The enzyme activity seems remarkably conserved from an evolutionary perspective. The potential role(s) of pulmonary CA IV will be discussed.

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27.6

PHYSIOLOGICAL FUNCTIONS OF EXTRACELLULAR CARBONIC ANHYDRASES IN DIFFERENT LOCATIONS - THEORETICAL AND EXPERIMENTAL EVIDENCE. **Gerolf Gros.** Zentrum Physiologie, Medizinische Hochschule, 30623 Hannover, Germany

One type of extracellularly active CA is the GPI-anchored CAIV that is catalytically active immediately on the external surface of cells. We have shown that CAIV associated with the sarcolemma of skeletal muscle catalyses the buffering of H^+ by HCO_3^- and the generation of H^+ from CO_2, and thereby facilitates the cotransport of H^+ and lactate out of and into the muscle fiber. This results in a two-fold acceleration of lactic acid fluxes across the sarcolemma. Another type of extracellular CA is the CA found in the gastric and colonic mucus of several species including man. CA activity in the native mucus (= factor by which the uncatalysed CO_2 hydration reaction is accelerated in the mucus layer) is ~2000, which is several-fold greater than the CA activity within gastrointestinal epithelial cells and is about 1/10 of the CA activity in red cells. Mucus CA has a mol.wt. of 28.000 but differs from all other known CAs with respect to primary structure, antigenic and inhibitory properties. This CA is present on cell surfaces in a way that differs functionally from CAIV, since the latter acts only in the immediate vicinity of the cell membrane while the former acts across the entire mucus layer which is about tenfold thicker than the epithelial cell layer. A theoretical analysis demonstrates how the cellular environment copes with changes in H^+ and CO_2 when CA activity on the cell surface is either present in a 100-200 mμ thick layer or is confined to < 1 μm.

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28.1

INSPIRATION FROM COMPARATIVE PHYSIOLOGY IN THE DESIGN OF ARTIFICIAL MUSCLES, SKELETONS AND CONTROL SYSTEMS.

Robert J. Full. Department of Integrative Biology. University of California at Berkeley, Berkeley, CA 94720.

A revolution is occurring in engineering inspired from comparative physiology and biomechanics. The time of robots or prostheses with one large heavy motor connecting two stiff metal segments is ending. In its place are new light-weight, tunable materials with properties similar to natural technologies. With these new materials, nature is becoming a better teacher. However, an understanding of evolution dictates that we not copy nature, but instead extract general principles that can be most effectively used by engineers. Engineers can benefit from considering biological concepts of efficiency, economy, energy storage, exchange and management, tuning, feedback, self-stabilization and multifunctionality. Engineers now need to know what properties artificial muscles or skeletons should possess. How should they be tuned? How should they be controlled? How must they be integrated into the whole platform. At the same time comparative physiologists inspire engineers, questions arising from the building systems crystallize the problems confronting our understanding of actual muscle, skeletons and control. Supported by DARPA and ONR.

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Paper provides the inspiration from insects in the design of highly mobile robot.

28.2

THE COMPONENTS OF MUSCLE POWER OUTPUT. Robert K. Josephson, School of Biological Sciences, University of California, Irvine, CA 92697

Skeletal muscle is powered by molecular motors, which individually are capable of only pN forces and nm displacements. The arrangement of these molecular motors in serial and parallel arrays within muscle cells results in overall stresses up to 1000 kN m^{-2} and strain rates up to 20 s^{-1}. The molecular motors are activated by an increase in the concentration of cytoplasmic calcium level above a low, resting level. The calcium is released from and taken up by an internal tubular system, the sarcoplasmic reticulum (SR) in response to changes in the electrical potential across the surface membrane of the muscle cells. The capacity for high-frequency contraction is associated with hypertrophy of the SR, and an associated reduction in the cellular volume available for contractile material. Operating frequencies of muscles range up to 500 Hz in muscles turned on and off by changes in calcium levels, and more than 1 k Hz in some insect muscles that can contract in an oscillatory manner if the calcium level is maintained high and the muscle is connected to a resonant load. The power output from skeletal muscle during repetitive contraction is up to 400 W kg^{-1}, and is strongly dependent on operating temperature. Measured efficiencies of muscles in converting metabolic to mechanical energy range from 5 to 50%. Muscle efficiency is inversely related to operating frequency, in part because of the greater calcium cycling costs incurred when contracting at high frequency.

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28.3

Facilitating Control using Intelligent Mechanics in Animals and Machines

Reinhard Blickhan, Hartmut Geyer, Michael Günther, Andre Seyfarth, Heiko Wagner: Friedrich-Schiller-University Jena, Seidelstr. 20, Jena, Thuringia 07749 Germany

For fast locomotion animals use their muscle skeletal legs like springs. Losses due to the impact at touch down and the braking activity of muscles and tissues are actively compensated at each bounce. Dependent on the angle of attack of the leg the system either accelerates or decelerates for a given touch down velocity. Using a suitable angle of attack differences in the initial conditions are compensated automatically, i.e. the system provides self-stability. An active strategy, leg retraction, enhances the range of attractive behaviour. To maintain manoeuvrability animals use segmented legs instead of springy telescopes. Large animals prefer a Z-type arrangement of segments to reduce cost. Two springs in series introduce a new instability where the joints flip from Z to C mode. This can be prevented by nonlinear springs like tendons and a short angled foot. The legs of spiders are C-shaped. The hydraulic extension mechanism does not allow stable operation in the C-Mode. C-shaped operation is save, results in compliant legs however. In other legged animals a suitable joint-torque characteristics is generated by the muscle tendon-complex. A stabilizing property of muscle skeletal systems requires a careful adjustment in muscle-tendon properties, leverage defined by leg and joint geometry, and activation. Mechanical muscle properties are tuned to suppress undesired oscillations. Supported by the DFG INK A2/1, 2- A3, C1

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Linear springs result in instable behaviour of the linkage system. Instability can be avoided by a combination of measures such as J-shaped spring-characteristics, a short foot and suitable initial conditions.

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28.4

THE MYOSIN HEAVY CHAIN: THE DESIGN OF AN EVOLUTIONARILY CONSTRAINED MOLECULAR MOTOR. Gordon J. Lutz and Richard L. Lieber, Departments of Orthopaedics and Bioengineering. University of California and V.A. Medical Center, San Diego, CA 92161

The myosin heavy chain (MHC) is the molecular motor protein that powers skeletal muscle contraction. Several MHC isoforms have been cloned and sequenced in a variety of species and in a variety of muscle types. The precise reason for the existence of multiple isoforms of the same protein is not completely clear. Since the motor properties of myosin reside in the globular S1 or "globular head" region of the MHC subunit, we isolated, cloned and sequenced the S1 subunit of four MHC isoforms from skeletal muscle in *Rana pipiens*, that are specifically expressed in four mechanically divergent fibre types. Relatedness in amino acid composition was evaluated in regions reported to govern cross-bridge kinetics. Surface loops 1 and 2, thought to influence motor velocity and ATPase, respectively, were both highly divergent between isoforms. However, divergence in the loops was about equal to that of the amino-terminal region, a domain considered less important for motor function. We tested the hypothesis that the loops are more conserved in pairs of isoforms with more similar kinetics. Comparisons including other vertebrate species showed no tendency for loops from pairs with similar kinetics to be more conserved. These data provide insights into the design of the MHC molecule as a molecular motor and may be relevant to the design of artificial muscles whose properties mimic those of natural muscle.

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28.5

INTELLIGENT TRANSTIBIAL PROSTHESES WITH MUSCLE-LIKE ACTUATORS

Glenn K. Klute[1,2], J. C. Perry[2], M. S. Orendurff[1], J. M. Czerniecki[1,2]
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Many transtibial amputees exhibit lack of endurance, non-symmetrical gait, and are exposed to residual limb tissue injury while simply walking with the aid of a prosthetic limb. The development of a powered prosthesis is hypothesized to reduce metabolic costs, improve gait symmetry, and ameliorate tissue damage when compared to traditional, passive limbs. Specific aims for a powered prosthesis are based on human subject gait data and include shock absorption at foot ground contact, energy storage during loading response, and propulsive force generation through toe-off. The performance expectations for a muscle- and tendon-like actuator are based on known static and dynamic properties of biological muscle and tendon that were extracted from the comparative physiology literature. These properties are incorporated into a predictive mathematical model that describes the desired force, length, and velocity relationships. A flexible pneumatic actuator, in parallel with a hydraulic damper, together in series with an elastic tendon is proposed to serve as the artificial muscle-tendon actuator. Experimental results of the constructed device exhibit expected force-length-velocity performance in general: higher activation pressures yield higher output forces, faster concentric contractions result in lower force outputs, faster eccentric contractions produce higher force outputs, and output forces are higher at longer muscle lengths than shorter lengths.

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Supported by Dept. of Veterans Affairs.

28.6

From an engineering viewpoint, muscle can be considered a viscoelastic polymer that can change its state of stress or strain in response to control signals. It follows that synthetic electroactive polymers (EAPs), whose state of stress and strain can be controlled by electrical means, may be capable of producing muscle-like behavior. In fact, many such EAPs are often termed "artificial muscles." One type of EAP, known as dielectric elastomer, has been under development for a variety of applications including actuators for small biomimetic walking and flying robots as well as prosthetic and orthotic devices. Dielectric elastomer artificial muscles are based upon a functional unit consisting of a thin film of a dielectric elastomer polymer that is coated with compliant electrodes. When a voltage is applied across the film, the electrostatic charges in the electrodes induce stresses that can deform the film. Dielectric elastomer artificial muscles have been made into a variety of shapes, such as cylindrical rolls and expanding diaphragms, that mimic the gross shape and motion of natural muscle. The stress-strain response, temporal response, and inherent stiffness and viscoelastic damping of these devices have been shown to fall within the ranges measured in natural muscles. Biologists have shown the importance that actuator stiffness and damping play in biological motion. A current research focus is on developing robots that can exploit these characteristics.

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28.7

DYNAMIC LOCOMOTION AND ENERGETICS OF RHEX, A SIX-LEGGED ROBOT
Martin Buehler, Center for Intelligent Machines, Department of Mechanical Engineering, McGill University, Montreal, QC, Canada, H3A 2A7

Inspired by the agility, energy efficiency and speed of animals, engineers have long sought to build legged robots with similar qualities. In the process, we have learned that some classical paradigms of robotics, for example, traditional linear and nonlinear control theory, the planning and subsequent tracking of the robot pose, rigid body dynamics, full actuation and therefore complete controllability of all degrees of freedom, and the decoupled design of the mechanics, control and actuation had to be abandoned or adjusted. Insights and inspiration from biology have greatly helped in this revolutionary process. Our six-legged robot RHex is a product of this new thinking, and has been inspired by cockroach locomotion. Sprawled posture affords good stability, both at slow speeds, and when coupled with leg compliance, at high speeds as well. Leg compliance also endows the robot with preflexes, and when coupled with clock driven (open loop) leg trajectories, permit RHex to scramble successfully over a large variety of rugged terrains, reminiscent of cockroach locomotion. Indeed, RHex exhibits mobility superior to any previously reported autonomous legged robots. It serves as an ideal platform to examine fundamental questions about the role of feedforward and feedback in locomotion tasks, the effect of leg materials, shape and control on mobility and the cost of transport, the limits imposed by materials and actuators, and the advantages and disadvantages of electric motors with respect to what artificial muscles might provide on this particular platform, and in general.

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RELAXED HOMEOTHERMY

29.1

RELAXED HOMEOTHERMY IN HIBERNATING MAMMALS.
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Within mammals, hibernating species show the potential for the widest range of relaxed homeothermy, since they are capable of sustaining core body temperatures (Tb) from –2 to 40°C. This paper describes detailed patterns of Tb change in a small rodent hibernator, free-living arctic ground squirrels (ags), and a large carnivore hibernator, over-wintering black bears, and contrasts set point and diurnal and circadian range of Tb during phases in their annual cycle of reproductive maturation, gestation, lactation, pre-hibernatory fattening, and hibernation. Ags show the most narrow range of Tb, although with vastly differing set points, during hibernation. After ending torpor reproductively maturing males spend 2-3 weeks at relatively low but euthermic Tb and without circadian rhythms; upon emergence Tb increases 2-3°C and diurnal rhythms begin. Female ags show declining Tb during gestation and a sharp increase at parturition. Tb in ags remains relatively steady during fattening; entrained diurnal rhythms persist throughout the arctic summer. Tb in hibernating bears ranges from 29-36°C in 2-5 day cycles controlled by shivering; pregnant bears, however, maintain high, constant Tb until just before parturition. Tb in bears slowly returns to near summer levels before hibernation ends. In both species Tb only partially predicts metabolic rate.
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29.2

BODY TEMPERATURE AND METABOLIC RATE DURING NATURAL HYPOTHERMIA IN MAMMALS.
Gerhard Heldmaier and Kathrin Dausmann, Dept. of Biology, Philipps Univ., D-35032 Marburg, Germany

Daily Torpor and hibernation are the most powerful measures to reduce energy expenditure in endotherms. Metabolic rate is depressed to a fraction of normothermic metabolism, and body temperature gradually decreases towards ambient temperature. In recent years this behaviour has been detected in an increasing number of mammalian orders, now also including Primates. Lemurs living in Malagasy dry forest exhibit daily torpor as well as hibernation. The mouse lemurs enter torpor towards the end of cold nights (10 °C), but due to high ambient temperatures of >30 °C during daytime, they are passively rewarmed close to normothermia, and thus save the energy required for arousal. Hibernating fat-tailed lemurs display unique diurnal cycles of body temperature between 13 °C at night and 33 °C during daytime, which rather compares to ectothermic reptiles, instead of homeothermic regulation in endothermic mammals. Despite large variations in body temperature they maintain a depressed metabolic rate. This concludes that active metabolic depression is the key to energy savings and natural hypothermia in mammals.
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29.3

Relaxed Homeothermy in Insectivorous Bats Near the Northern Border of their Distribution. **John R. Speakman[1] and Paul A. Racey.** Department of Zoology, University of Aberdeen, Aberdeen, Scotland, UK. 1 Also at the Rowett Research Institute, Aberdeen, Scotland, UK.

Until the 1980s it was widely believed that insectivorous bats had only rudimentary thermoregulatory capabilities and that during the summer months they would spend large portions of their time in daily torpor unable to thermoregulate at euthermic levels. In the 1980s however this view changed and it was shown that bats given appropriate conditions would remain almost continuously endothermic and regulate their body temperatures at levels equivalent to other small mammals. Utilisation of daily torpor was considered to be an unusual occurrence precipitated only by severe weather conditions and was less likely to be seen in lactating females than others sub-populations. We measured the temperatures of a cluster of pipistrelle bats (pipistrellus pygmaeus) inhabiting a domestic house roof near Aberdeen in NE Scotland (57 oN) at 15 minute intervals for three summers. On two of the summers we also photographed the bats at 12h intervals to make sure they were in contact with the temperature probes and we ran an insect trap at a nearby site to assess nightly insect availability as well as weather conditions adjacent to the roost. We discovered that during pregnancy the bats never entered daily torpor and remained continuously at high regulated body temperatures despite occasionally encountering poor insect availabilities and low ambient temperatures. In lactation however the bats entered torpor to varying degrees. The extent of utilisation of torpor was well predicted by the previous nights ambient temperature and insect availability. Laboratory studies confirmed the independent effects of these two factors on thermoregulation in lactating pipistrelle bats. At the northern borders of their distribution lactating pipistrelle bats relax homeothermy as part of a sophisticated method for regulating their daily energy balance and matching their energy demands to available supplies.

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29.4

REGULATED DECREASE IN BODY TEMPERATURE (ANAPYREXIA) IN BIRDS WHEN MIGRATING AND FORAGING AT SEA.

Pat Butler, School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.

The lowering of the thermal set-point below the "normal" level in endothermic homeotherms is called anapyrexia. A well known example of a lowering of the set-point in birds and mammals is during sleep, when T_b may fall by 2-3° C below the temperature during the awake (active) phase. However, greater reductions of the set-point may occur during the resting phase of the daily cycle, without the animals becoming torpid, if the lack of food and/or the cold load are particularly severe, or before migration in some small birds, even if there is no lack of food or excessive cold load. The latter is thought to be related to the laying down of fat for the migratory flight. Although migratory barnacle geese do not show signs of anapyrexia before their autumn migration from the high arctic, there is a gradual decline in both day-time and night-time abdominal temperature (T_{ab}) over a period of 3 weeks starting at around the beginning of migration and continuing after many of them have arrived at their feeding grounds in southern Scotland. The average reduction is 4.4° C. The functional significance of this is unclear, but it may be related to the replacement of the fat used during migration. Most marine birds that have been studied show a fall in T_{ab} to, on average, between 35 and 30° C during bouts of diving. For penguins such as the gentoo and king, such decreases could help explain why they are able to remain submerged when foraging for such long periods of time.

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29.5

BEHAVIOURAL HETEROTHERMIA. Peter B. Frappell, Adaptive and Evolutionary Respiratory Physiology Laboratory, Dept. Zoology, La Trobe University, Melbourne, Victoria, 3086 Australia.

It is well established that many animal species show a definite thermal preference when free to do so. In addition, it is well known that a number of stresses alter the thermal preference. For example, in small mammals, hypoxia attenuates thermogenesis, results in hypothermia (Frappell et al. 1992), and also elicits a behavioural preference for a lower ambient temperature (Gordon and Fogelson, 1991). On the other hand, behavioural selection of a warmer environment occurs in old rats following injection of lipopolysaccharide (LPS); failure to select a warmer environment prevents a febrile response from developing (Florez-Duquet et al. 2001). Such shifts in thermal preference together with accompanying changes in body temperature are interpreted as a change in the thermal set-point (anapyrexia), rather than a deficit in thermoregulation. In this address behavioural selection of thermal preference is examined as it relates to anapyrexia (behavioural heterothermia), thermal load error (variation around the set-point) and the concept of homeothermy.

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29.6

The role of hyperthermia in the water economy of birds and mammals

Irene Tieleman, Zoological Laboratory, University of Groningen, 9750 AA Haren, The Netherlands. Joseph B. Williams, Department of Evolution, Ecology and Organismal Biology, Ohio State University, 1735 Neil Avenue, Columbus, OH 43210, USA

A number of authors have suggested that hyperthermia, the elevation of body temperature (T_b) 2-4°C above normal, contributes to a reduction in total evaporative water loss (TEWL) in endotherms. Information about the role of hyperthermia in the water economy of birds and mammals is scattered throughout the literature. We collated the available information on laboratory data on hyperthermia in birds, re-evaluated the benefits and costs of this process, and assessed its net effect on the water economy of birds, especially species living in deserts. We explored aspects of hyperthermia that reduce water loss, such as an improved thermal gradient and heat storage, and aspects that may augment water loss, the latter a result of increased respiratory water loss when T_b is elevated. Our analysis of the combination of these three factors, suggests that during acute exposure to high T_a (1 h), birds over a size range of 10-1000 g save about 50% of their TEWL by becoming hyperthermic. For chronic episodes of high T_a (5 h), small birds save water by hyperthermia but large birds do not. To test if desert birds, that experience chronic episodes of high T_a, conform to these predictions, we investigated the occurrence of hyperthermia in small and large desert birds from the Arabian Desert. Although hyperthermia is frequently reported in birds and mammals measured in the laboratory, documentations of hyperthermia in free-living animals are unavailable for birds, and few for mammals. We document, for the first time under natural conditions, the use of heterothermy in free-ranging Arabian oryx during summer and winter, and estimate its impact on their water economy.

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This paper reconsiders the model of heat balance at high temperatures, collates information on hyperthermia in birds, and reevaluates its costs and benefits, especially with respect to water economy.

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HOST-PARASITE INTERACTIONS: A COMPARATIVE APPROACH

30.1

NEW DEVELOPMENTS IN OUR UNDERSTANDING OF HOST-PARASITE INTERACTIONS BETWEEN THE SALMON LOUSE, *LEPEOPHTHEIRUS SALMONIS* AND ITS HOSTS.

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Infection by the salmon louse, *Lepeophtheirus salmonis* is a major disease problem in marine salmonid aquaculture. Indirect and direct losses attributed to sea lice are estimated to be greater than US$ 40 million annually. To date most sea lice research has focused on the identification of therapeutants and management strategies to alleviate disease. The ultimate goal for sea lice control is the development of a vaccine. The development of vaccines using the traditional approach (identification of protective antigens) has been unsuccessful for sea lice and for the majority of parasites. It has been suggested that a more effective approach for vaccine development would be to first identify the immunological events associated with infection and then modify those events to elicit an effective immune response.

Biochemical, proteomic, molecular and immunological techniques are now being used to examine the immunological interactions between *L. salmonis* and its salmonid hosts. To date a variety of salivary components and other factors that appear to be important in these interactions have been identified. Proteomic and molecular biological techniques are being used to identify these components. Their effects on salmonid immune function are being studied using classical immunological techniques. Real-Time PCR and cDNA mircoarrays. It is expected that such techniques will yield a wealth of information on these interactions and provide important information from which novel thereuputants or vaccines can be derived.

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30.2

AVIAN COCCIDIOSIS : A HOST-PARASITE RELATIONSHIP TO BE RESTORED.

A.N. Vermeulen, T.P.M. Schetters and T.C. Schaap, Dept. of Parasitology R&D, Intervet International BV, P.O. Box 31, 5830 AA Boxmeer, The Netherlands.

Poultry breeding and rearing of meat-type chickens has become a major industrial activity during the second part of the previous century resulting in an annual production of over 35 billion broiler chickens per year. By far the most economically important disease for chickens held under these circumstances has become coccidiosis, caused by coccidia of the genus Eimeria. These gut associated parasites utilize epithelial cells for proliferation resulting in severe disruption of the villi associated with enteritis, local lesions and sometimes death. These aspects have a large impact on the absorption of nutrients and resorption of water reducing growth and feed conversion into meat. Chickens become readily immune, but the immune mechanisms involved are not well understood. Both CD4 and CD8 expressing T-cells are important, found locally in the gut as well as in the peripheral blood. These cells produce lymphokines such as interferon-gamma, a factor known to be effective against intracellular stages of the parasite. We have detected antigens that were able to induce and stimulate those T-cell subsets and shown protective capacity. These could be major candidates for a vaccine against this disease, which could restore a better relationship between parasite and host. The immune mechanisms are discussed and where possible comparisons are made towards similar systems in mammals.

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30.3

TICK MODULATION OF HOST IMMUNITY: IMMUNOBIOLOGY, GENOMICS, AND PROTEOMICS

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Infectious diseases transmitted by ticks are emerging and resurging with vast consequences for global public health. Control of ticks and tick-borne diseases is complicated by the lack of effective vaccines and tick resistance to acaricides. Vaccines that block tick feeding and/or pathogen transmission are promising novel control strategies. To achieve these vaccines, understanding is needed of the immunology of the tick-host-pathogen interface. Hard ticks, Ixodidae, differentiated along two phyletic lines, Prostriata and Metastriata. Host immune responses to infestation and tick immunomodulatory countermeasures are compared for a Prostriata, *Ixodes scapularis*, and a Metastriata, *Dermacentor andersoni*. Infestation of mice with *D. andersoni* induces resistance to infestation, while resistance does not develop to *I. scapularis* feeding. Infestation with *D. andersoni* down-regulates host Th1 cytokines with little or no effect on Th2 cytokines. *Ixodes scapularis* decreases host Th1 cytokines and causes an intense Th2 polarization. Both tick species modulate other aspects of host innate and specific acquired immune defenses. Repeated infestation with *D. andersoni* causes a host response that decreases tick-induced immune deviation. This phenomenon does not occur during repeated infestations with *I. scapularis*. An immunomodulatory protein of *D. andersoni* has been purified, characterized, cloned, and expressed. An expressed sequence tags (ESTs) approach is being used to characterize genes expressed in salivary glands of feeding *D. andersoni* and *I. scapularis*. Combined genomic and proteomic approaches are employed to identify vaccine candidate immunogens among salivary gland molecules essential for blood feeding and pathogen transmission.

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Current review of topic.

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Current review of topic.

30.4

The Pathophysiology in Piscine and Mammalian Haemoflagellate Diseases Patrick T.K. Woo, Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Cryptobia and *Trypanosoma* multiply rapidly in the blood and cause morbidity/mortality in fish and mammals. *Cryptobia salmositica* is pathogenic to all *Oncorhynchus* on the Pacific coast of North America but does not cause disease in other teleosts. (Woo 2001). *Trypanosoma brucei* causes disease in man and domestic animals in Africa but wildlife are good reservoirs (Logan-Henfrey LL *et al.* 1992). In both diseases, the anaemia is caused by secreted toxins (metalloprotease, phospholipase), immune complexes on red cells, haemodilution, and decreased red cell production. Anorexia lowers parasitaemias but it contributes to the immunodepression as does complement depletion during acute disease. Phagocytosis, cell-mediated cytotoxicity and antibodies are important in protection. These parasites have a large mitochondrion and numerous glycosomes, and they consume glucose. *T. brucei* undergoes glycolysis while *C. salmositica* utilizes aerobic respiration. Trypanotolerance in wildlife is poorly understood while cryptobiatolerance is due to the neutralization of metalloprotease by α 2-macroglobulin. *T. brucei brucei* does not infect humans because it is lysed by high density lipoproteins while the Alternate Pathway of Complement Activation lyses *C. salmositica* in cyprinids.

Study is supported by the Natural Sciences and Engineering Research Council, Canada.

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POSTERS PHYLOGENETIC APPROACHES TO UNDERSTANDING PHYSIOLOGICAL EVOLUTION

31.1

Interpopulational Differences in Behavior and Exercise Physiology in an Anuran Species

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Physiological comparisons among populations have led to inconclusive results, e.g. unexpected differentiation or lack of differentiation along ecological gradients. The interpretation of these patterns is complicated by lack of integrative studies that encompass behavior, ecology and physiology. Our aim is to study the extent of physiological differentiation among two populations of *Scinax perpusilla*, a Brazilian tree-frog, and discuss morphological, ecological and behavioral correlates of such variation. We studied 87 males at Intervales and 113 at Boracéia, two localities 400km apart. In the field we performed 30min focal observations, measured calling rates and maximum number of calls in 1min. We also registered the number of active males on each night and the spacing among males. We measured snout-vent length, body mass, maximum jumping distance, distance jumped before exhaustion, and activity metabolic rate. Males are more aggregated at Boracéia, exhibit higher calling rates, and are smaller than those at Intervales. Despite differences in calling (an energetically demanding aerobic activity), activity metabolic rates were the same. Great differences were found in endurance; such variation, limited to one physiological trait, may be adaptive and product of rapid evolution. However, no behavioral data are related to endurance. We propose that exposure to higher density, more demanding territorial behavior and sexual selection are related to the physiological differences found among anuran populations.

Support: FAPESP

31.2

An Objective Ancestry Test for Fossil Bones

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By the look of the curve of a fossil toe bone and the slant of its joint surface, recent reports stated that it was from an ancestor of apes and humans. Ardipithecus ramidus kadabba, that walked on two legs. The question arose as to whether there might be a simple method yielding objective evidence to bridge the gap between those scant subjective determinations and that far-reaching conclusion. Accordingly, an objective, valid, reliable and calibrated correlational method of substantiating that conclusion was devised and successfully tested. For monkey (baboon), ape (chimpanzee) and human, similar were the ape and monkey, dissimilar were the human and monkey and most dissimilar were the ape and human. The monkey and ape similarities to human bone were less than for an anatomically different bone. The fossil toe bone had scant similarity to humans, less to monkeys and least to apes with the similarities to monkeys and apes less than for an anatomically different bone. The results of this objective ancestry test contradicted the speculation that Ardipithecus ramidus kadabba was an ancestor of apes and humans that walked on two legs. Instead, these objective analyses provided evidence that apes are similar to monkeys, but monkeys and apes have no similarity to humans.

31.3

A Discussion of the "Comparative Method" and the Mechanisms of Correlated Evolution

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Comparative physiology is the investigation of convergence and divergence in physiological processes among animals. This comparing and contrasting of physiological traits allows one to understand how distantly related organisms may adapt to similar environments or how closely related animals can diverge in response to different environments. Historically, these ideas are a common theme in comparative physiology. In recent years, "comparative method" has been used to refer to a set of statistical procedures to control for phylogenetic effects. Comparative studies without controls for phylogenetic effects may infer spurious relationships between adaptive traits and their environments. The limitations of comparative phylogenetic methods are summarized in Harvey and Pagel's (1991) remark, "Stripped to the bone, the evidence for adaptive evolution revealed by comparative studies is correlated evolution among characters or between characters and environments." Although many physiological studies have emphasized short-term ecological processes rather than phylogeny, we use phylogeny as a null hypothesis to account for that portion of interspecific physiological variation that is the result of identity by descent. We begin with a data set derived from interspecific comparisons of physiological traits. We analyzed this data first without controlling for phylogenetic effects. We then perform the same tests after controlling for phylogenetic effects using several different methods including Felsenstein's (1985) independent contrast method. We present the outcomes of these analyses and discuss the differences between them. We discuss both the biological and philosophical implications of these research methodologies.

31.4

Delta-9-Desaturase – A complex Evolutionary Tale?

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Delta-9-desaturase is the most studied of the desaturase enzymes due to its dramatic effects on the cell membrane in animals. It introduces the first double bond at the delta-9 position of saturated fatty acids to form the monounsaturate. Enzyme activity is controlled by diet and by various hormones. In poikilothermic animals, including fish, cooling is a regulatory factor.

Research shows that some species have multiple gene copies, but the reasons are unclear. Mouse has three genes with distinct expression patterns, and *Cuenorhabditiv elegans* has three genes with distinct substrate specificities. The common carp (*Cyprinus carpio*), a known tetraploid species, has two delta-9-desaturases expressed in liver, one regulated by dietary treatment and the other by cooling. The related diploid grass carp (*Ctenopharyngodon idella*) appears to possess only one. Using the grass carp as an out-group, we are testing Postlethwaite's hypothesis for the preservation of duplicated genes by complementary, degenerative mutations in the regulatory domains. We have isolated and sequenced fosmid clones containing the common carp and grass carp genes. Alignment of these and other vertebrate genomic sequences allow conserved non-coding elements to be identified. This, together with an analysis of promoter function, offers a view on the evolutionary pathways that this gene has taken. Hence being able to build a phylogenetic picture of this gene with respect to sequence divergence and gene copy number.

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31.5

The Phylogeny of Paenungulates: a Clue from Bile Salt Composition.

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One approach where physiology can directly aid in determining phylogeny is in the study of bile salts. These essential water-soluble, amphipathic endproducts of cholesterol metabolism are found in all vertebrates, and are unique in that they have chemically different structures that often parallel vertebrate evolution. Consider the problem of the location in the mammalian family tree of the Paenungulates, an assemblage of the mammalian orders Proboscidea (elephants), Hyracoidea (hyraxes), and Sirenia (manatees). Since Paenungulates are only distantly related to other mammals, molecular biologists have found this group to be vexingly problematic. The recent literature contains studies that give support for completely opposite conclusions - that the Paenungulates are either one of the more primitive, or one of the more evolved of mammalian lineages. We tested the hypothesis that bile salt composition might aid in resolution of this controversy. Bile was obtained at autopsy, and its composition analyzed by mass spectrometry (ESI-MS and GC-MS). Interestingly, bile *acids* were not present in any members of the three orders of Paenungulates. Instead, their bile salts consisted of mixtures of C_{27} bile *alcohols*, primitive precursors of the more evolved bile acids seen in other mammalian groups. The major bile alcohol (95%) of the Elephant was 3 , 7 ,25,26-tetrahydroxy-5cholestane. Bile alcohols from the Rock hyrax and Manatee showed a similar structure. The absence of bile acids and the shared presence of C_{27} bile alcohols suggests that the three orders of Paenungulates are indeed related, and that they are among the oldest extant mammals.

31.6

Reproductive constraints on adaptive differences in escape performance among guppy populations

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The empirical study of natural selection reveals that adaptations often involve trade-offs between competing influences. Because natural selection ultimately acts on whole organisms rather than individual traits, evolution may therefore be constrained by the interaction among traits or their functions. Yet, few attempts have been made to characterize how such constraints are manifested in real organisms during the process of adaptive evolution. Here, we examine the consequences of adaptive life history evolution on escape performance in the live-bearing guppy. In response to increased predation from piscivorus fish, guppies have been shown to rapidly evolve an increased allocation of resources towards reproduction. However, an unavoidable consequence of pregnancy in live-bearing fish is an increased mass and body profile as developing embryos cause the female's abdomen to swell. Here we show that differences in reproductive allocation constrain, but do not prevent, adaptive differences in burst swimming speed among populations. Guppies from high predation localities attained faster burst swimming speeds than their low predation counterparts, however, high predation females also paid a higher cost due to pregnancy; swimming speed decreased more rapidly over the course of pregnancy in high predation females than in low predation ones. The interaction of life histories and swimming performance reveals how seemingly unrelated traits are integrated together, and, highlight the complexity of studying adaptation at the whole organism level.

31.7

Rapid Evolutionary Changes in Endurance and Sprint Speed in *Tropidurus* Sister Species: Relationships with Morphology and Physiology

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Tropidurus lizards occupy a wide range of brazilian habitats. Their phylogeny and ecology have been focused intensively. The genus is suitable to study evolution of locomotor performance. As part of a broader study with the genus, we found that two sister species, *T. psamonastes* and *T. itambere*, exhibit extremes in capacity for locomotor performance. Tests include sprint speed and jumping stamina. *T psamonastes* is the fastest species with the lowest stamina, while *T. itambere* is the slowest species with the highest stamina. This finding suggests rapid evolutionary changes in performance. What are the mechanistic bases for such differentiation? We focused both on morphology and physiology - snout-vent length, foot, tail, hind and front limb lengths, muscle mechanics and metabolic rates. Work loops with ileofibularis were conducted using sinusoidal waves on cycle frequencies from 4 to 14Hz. We found no differences between species in either stress or power output (W/Kg muscle mass) of the muscle. There are no differences in limb and tail proportions between the sister species and their metabolic scope is similar. Resting metabolic rate of *T. itambere* is higher. The differences in locomotor performance between this species are not related to any of the physiological or morphological parameters studied. One possible explanation could be that the rapid evolution observed in performance between the two sister species was not accompanied by changes in more conservative parameters.

31.8

Aerobic Capacity of South American Stingless Bees

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Seven species of the Meliponinae subfamily were studied to propose a filogenetic hypothesis to explain allometric differences in oxygen consumption. This investigation contributed to clarify how such species manipulate their energy resources. Thirty individuals with similar age from each of the 7 species were used throughout the experiments. Oxygen uptake measurements indicated a positive linear correlation between the logarithms of body mass and metabolic rate. ANOVA showed that the increase in body mass explains 77% of the aerobic metabolic rate variation. Data from the literature gathered from the same species showed an inverse correlation between polen transport capacity and body mass. Higher metabolic rates of larger individuals might thus be related to generating lift to support their larger body weights, and also to thermoregulatory processes because of surface-to-volume ratio considerations. Although a definite filogenetic tree for Meliponinae is still incipient, an independent contrasts analysis was even so carried out. The results showed that the evolution rate for the dicotomy M. bicolor - M. marginata was very different, suggesting that the greater aerobic capacity for M. bicolor does not solely result from its larger body mass, but it might be related to other evolutive factors acting particularly upon body mass. This statement should however be taken with caution because of intrinsic limitations of the used cladogram.

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31.9

Post-hatching yolk consumption and stored energy reserves in hatchling snapping turtles, *Chelydra serpentina*.

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We investigated the rate at which residual yolk sac contents are consumed in addition to changes in triglyceride and glycogen of hatchling snapping turtles during the immediate neonatal period (0-4 weeks post-hatching), at the start of overwintering (15 weeks post-hatching), and at the end of overwintering (30 weeks post-hatching). Yolk sac contents were almost completely consumed within the first three weeks post-hatching. There was no associated increase in somatic (liver and carcass) triglyceride or glycogen accompanying the absorption of the yolk sac contents, but rather at decrease. Roughly 62% of the total caloric value of triglycerides and glycogen present at hatching was consumed by four weeks post-hatching. Despite being fed *ad lib.* at 25°C from Weeks 3-11 post-hatching hatchlings did not grow, nor did somatic stored energy reserves increase. Roughly 55% of the stored energy present at the start of overwintering (5°C) was consumed by the end of overwintering. These findings suggest that 1) most of the energy substrates present in the yolk sac at hatching is used to supply energy for metabolism during the immediate neonatal period, 2) hatchlings from populations in the northern part of the species range likely do not consume enough food to grow prior to overwintering, nor does residual yolk support growth, and 3) the low metabolism of overwintering turtles is essential for ensuring that stored energy reserves will last throughout the winter.

31.10

Metabolic Costs Of Egg Production: Evidence For Energy Reallocation?

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Several studies have suggested that egg production may be costly in terms of subsequent decreased provisioning ability, future fecundity or survival, but the actual energy cost of forming eggs is still unclear. This is because most estimates of egg production costs are based on theoretical models using the chemical energy content of egg components and reproductive organs. For passerine birds these models typically predict energy investment values of about 45-50% of basal metabolic rate (BMR). We investigated the metabolic cost of egg production in European starlings (*Sturnus vulgaris*) by directly measuring resting metabolic rates (RMR) in non-breeding, pre-laying and laying females. We report a wide range of between-year variation in RMR for all breeding stages. Measured laying RMR represented only 38 - 40% of the predicted peak value based on a theoretical investment model constructed from our own data set. We discuss conceptual problems associated with such models and suggest a better way to measure the energy cost of egg production: by measuring the increase in RMR over time from early follicular development to clutch completion. Using this method we found a maximal egg production related RMR increase of 22.4%. However, based on previous work in our lab we suggest that this measured increase in RMR may still underestimate the actual cost of egg production due to reallocation of energy among different physiological demands. This research was funded by an NSERC grant to TDW.

31.11

Effects of meal type on postprandial calorigenesis in *Python molurus*

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The postprandial metabolic increment associated with digestion, assimilation, and excretion is termed specific dynamic action (SDA). In ectotherms this energy can account for a large proportion of overall energy expenditure, and can reduce the metabolic scope available for other activities. During digestion the metabolic rate of Burmese pythons (Python molurus) may demonstrate increases twenty-fold over SMR. Several hypotheses have been advanced to explain the primary physiological mechanism that accounts for SDA. This experiment tested some of these hypotheses by quantifying the SDA profiles resulting from various meal types in P. molurus ($n = 8$) at 30 °C. Each snake was fed a series of 120 kcal meals consisting of purified homogenates including various purified proteins, amino acids, carbohydrates, lipids, and known substrates including chicken breast, beef suet, and mouse. VO_2 and VCO_2 were determined every two hours for 96 hours following feeding using flow-through respirometry. Our results revealed that monomeric and dimeric sugars caused metabolic rate to increase two-fold, however complex carbohydrates were unable to elicit a significant metabolic response, and were not assimilated by the snakes. Protein meals caused variable SDA responses that appeared to be related to the amino acid composition of the specific meals. Casein caused a 4-fold increase in metabolism, while gelatin caused no detectible changes and was not assimilated. Various lipid meals did not cause any significant change in VO_2 and were generally not assimilated. Our findings suggest that the relatively large SDA increment well known in this species is probably not triggered by a large food bolus and not attributable to the cost of gut upregulation. This research was sponsored by NSF grant IBN 0091308.

31.12

Stomach pH and the Cost of Gastric Digestion for the Burmese Python

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Pythons digest large intact meals (up to 100% of body mass) Gastric breakdown of such meals, including bone, understandable occurs at considerable costs, evident by their large postprandial metabolic response (specific dynamic action or SDA). To evaluated the pythons' gastric performance and relative contribution of gastric digestion to SDA, I measured stomach pH during the digestion of rodent meals, and the SDA of two feeding trials involving reduced stomach work load. Stomach pH of fasted pythons averaged 7.2, declined to 3.4 within 12 h, and to 1.6 within 48 h after feeding. pH remained between 1 and 2 for the next 3 - 5 d of digestion, returning to neutrality after the stomach empties. Work load of the stomach was reduced by feeding pythons homogenized rat. In response, pythons experienced a peak postprandial metabolic rate of 0.252 mL O_2/gh and an SDA of 943 kJ, respectively, 43% and 36% less than that experienced during the digestion of an intact meal. Next, homogenized rat was infused directly into the small intestine, thereby removing gastric function. Pythons responded with peak postprandial metabolic rates of 0.119 mL O_2/gh and SDA of 270 kJ, respectively, 74% and 73% less than that experienced during the digestion of intact meals. These findings demonstrate that gastric digestion is a substantial component of a python?s SDA, and suggest that differences in the structural composition (intact vs. chewed) of meals entering the stomach may explain observed differences in SDA among animals.

31.13

Evolution of Water Conservation Mechanisms in *Drosophila* Species

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Fruitflies of the genus *Drosophila* inhabit a wide range of habitats, from the tropics to deserts to boreal forests. The primary physiological mechanism allowing fruitflies and other insects to survive in arid habitats is a reduction in rates of water loss. To understand mechanisms of water retention in greater detail, we investigated the three main routes by which *Drosophila* lose water: excretion, cuticular transpiration and respiratory loss through the spiracles. Excretory losses comprised <6% of total water flux and did not differ between desert (cactophilic) and mesic species. No consistent relationship was observed between water-loss rates and the composition, physical properties or amounts of cuticular hydrocarbons. Desert *Drosophila* were less active and had lower metabolic rates than mesic species of the same size. They also were more likely to exhibit cyclic CO_2 release that may help to conserve water. We conclude that lower overall rates of water loss are achieved primarily by reduction of respiratory losses.

32.1

Comparative Analysis of Carbonic Anhydrase in the Midgut of Different Species of Mosquito Larvae: Do Different Species Regulate Their Midgut pH by The Same Mechanism?

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The midgut of mosquito larvae exhibits a pH value between 10.5-11.0 depending on the species. This pH seems to be related to a high concentration of luminal bicarbonate, which is in turn associated with carbonic anhydrase (CA). To determine the localization of CA activity in different species of mosquito, histochemistry was performed on isolated larval midguts from Aedes albopictus, Ochlerotatus taeniorynchus, Culex nigripalpus, Culex quinquefasciatus and Anopheles quadrimaculatus. We quantitated enzyme activity in the midgut using 18O isotope-exchange coupled to Mass Spectrometry. We also examined the effect of CA inhibitors (methazolamide and acetazolamide) on the pH of the midgut for each species as well as on different larval instars and compared their effects with previous results obtained for Aedes aegypti. Additionally, we tested the effect of these CA inhibitors on a species of fish (Sheepshead minnows) to determine if these compounds would be safe to use as mosquito larvicides. Our results indicate that different species of mosquitoes have similar distribution of CA in the midgut. Furthermore, the efficacy of the inhibitors seems to be dependent on the species. Finally, acetazolamide appeared to be less toxic than methazolamide to the species of fish tested.

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32.2

Oyster Carbonic Anhydrase

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Nielsen & Frieden (1972) found a high molecular weight carbonic anhydrase (OCA) in the hemolymph of the Eastern oyster, *Crassostrea virginica*. We have found that a low molecular weight esterase can be separated from the carbonic anhydrase by gel filtration chromatography (CL-6B), by salting out the OCA in 50% ammonium sulfate, or by DEAE-anion exchange chromatography where the OCA is strongly bound . The OCA does not react with pNitrophenyl acetate, but does react with CO_2 in the Wilbur assay. SDS-polyacrylamide gel electrophoretic analysis shows subunits with a molecular weight of ~30kDa (the size of vertebrate erythrocyte CA) for both enzymes after reduction of disulfide bonds. However, when analyzed without reduction the subunit molecular weight of the OCA was < 30kDa suggesting that it contains intramolecular disulfide bonds. Mass measurements of the carbonic anhydrase by scanning transmission electron microscopy (STEM) (Hamilton *et al.* FASEB J. 11: Abstract 3277, 1997) gave a mass of 1.9 MDa, but no clear picture of the arrangement of the *ca.* 30 kDa subunits. Supported by Fordham University Faculty Research grants and by a Dreyfus Senior Scientist Mentor Award (MGH).

32.3

The distribution and physiological significance of carbonic anhydrase in fish gills

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Carbonic anhydrase (CA) activity has been found in an intracellular location in the gills of all fish examined to date, where it appears to function in ionic regulation. High levels of CA activity are also present within the red blood cell (RBC) and contribute to CO_2 excretion. Although fish were thought for many years to lack plasma-accessible branchial CA activity, biochemical, physiological and immunohistochemical evidence now suggests that membrane-associated CA activity that is available to catalyse plasma CO_2 reactions is present in the gills of cartilaginous fish. In its susceptibility to phosphatidylinositol phospholipase C, the branchial membrane-bound CA activity is similar to the mammalian type IV isozyme. A role for branchial CA IV-like activity in CO_2 excretion in dogfish was demonstrated by (1) the increased arterial CO_2 tension and decreased arterial pH measured in dogfish treated with selective CA inhibitors and (2) the ability of dogfish subjected to severe experimental anaemia to maintain normal arterial CO_2 tensions until treated with selective CA inhibitors. By contrast, teleost fish in general appear to lack branchial membrane-bound CA activity and exhibit a significant increase in arterial CO_2 tension coupled with an acidosis of mixed respiratory and metabolic origin when subjected to severe experimental anaemia. This research was supported by the Natural Sciences and Engineering Research Council of Canada.

32.4

Quantitation and Expression of Larval Aedes aegypti Midgut Carbonic Anhydrase

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A 32 kDA protein encoding an á carbonic anhydrase(CA)was cloned from the larval mosquito, Aedes aegypti, midgut. Blast searches have revealed closest sequence homology to Drosophila melanogaster (CG3940 gene product) followed by Human CAXIV, Drosphila melanogaster (CG3669 gene product), and Horse CA1. Several methods revealed heterogeneous CA distribution along the larval Aedes gut. Real time PCR found the Aedes CA sequence to be most abundant in the gastric ceaca, followed by the posterior gut region. The anterior gut region and malpighian tubules were both negative for this particular CA sequence. This real time PCR data supports our earlier in situ hybridization findings, which also preferentially localized Aedes CA to the gastric ceaca and posterior gut regions of the mosquito. Histochemical analysis which is NOT specific for th cloned Aedes CA sequence also localized CA to the gastric ceaca and posterior regions while the anterior midgut and malpighian tubules displayed little or no activity. Other CA isoforms, if present, are therefore also likely to be found in the gastric ceaca and posterior gut regions. Recombinant expression of this protein for subsequent antibody production is currently being done using a His tag fusion and a nickel column. An antibody specific for this protein will allow the co-localization of CA with transporters, exchangers, and V-ATPases by scanning confocal microscopy. This localized network within the mosquito gut serves as a model for human kidney epithelial transport and homeostasis. Funding for these studies was provided by a UF Alumni Fellowship and by NIH Grant R01 Ai 45098.

32.5

Carbonic anhydrases in an autotrophic animal, the symbiotic tubeworm *Riftia pachyptila*

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This study investigates carbonic anhydrases (CA) in *Riftia pachyptila*, a hydrothermal vent annelid. In this worm, adaptation to extreme conditions led to the emergence of unique features including an unusual mode of animal nutrition relying on internal chemoautotrophic symbionts, using molecular CO_2 as their inorganic carbon source. The unique mode of life prompted us to characterize CAs, involved in CO_2 transport and conversion, in this model organism. A complete cDNA has been sequenced from the symbiotic tissue, and identified as a putative -CA based on BLAST analysis. In the plume, the putative CA sequence obtained from cDNA library screening was 90 % identical to the trophosome CA, except in the first 77 nucleotides downstream the initiation site identified on trophosome CA. A phylogenetic analysis showed that the annelidan *Riftia* CA emerged clustered with invertebrate CAs, the arthropodan CA and the cnidarian CA, and formed a sister group of the cluster comprising mitochondrial and cytosolic isoforms in vertebrates. Combined biochemical approaches revealed two cytosolic CA with different molecular weights and pIs in both tissues, but also suggested the occurrence of a membrane-bound CA isoform in addition of the cytosolic one in the trophosome. The discussion will focus on the key role these three CA play in the optimization of CO_2 transport to the bacteria.

This study was supported by grants from the DORSALES program and by the "Conseil Regional de Bretagne".

33.1

Biologically Inspired Self-Evolving Interfaces for the Warfighter Mission

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Groups of researchers are discovering the potential that natural systems afford for finding innovative solutions to business and engineering problems. This team brings together faculty in the areas of biology, operations research, human factors and biomedical engineering with a common goal of developing new biologically inspired paradigms that could advance the area of adaptive human-computational interfaces. Specifically the intention has been to develop interfaces for the Air Force aerospace systems that would provide operators with information as and when they need it. Our initial collaboration has studied immune system metaphors in the context of a combat mission that can be tested in the Air Force Synthesized Immersion Research Laboratory. Our work has involved using Unified Modeling Language to develop a common vocabulary for both complex systems. Additionally we are developing a biologically inspired adaptive interface for NASAs Multi-Attribute Task Battery. While the work to date has focused on military applications, this interdisciplinary collaboration hopes to extrapolate life science paradigms more broadly in the areas of decision-making and risk management, complexity, information technology and collaboration. Funding from Dayton Area Graduate Studies Institute.

33.2

Contribution of Cytological Studies of the Intrinsic Nerve Plexus of the Rat Heart to the Conception of Artificial Cardiac Pacemakers

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Fast modulation of the excitability and conduction in mammalian hearts requires the existence of an intrinsic device permitting to tune the rate of sinus node depolarizations and control the speed of intracardiac conduction. According to our cytological studies, the terminal nerve plexus of the intracardiac septum may provide for morphological substratum of such a system. Containing a heterogenous population of neurons with different histochemical properties, it transforms the specialized tissue in a neuromuscular self-governed dynamic system with variable transit functions. Large multipolar or pseudounipolar neurons associated with spindle-like sensory corpuscles of the av junction ensure short proprioceptive feedbak loops between the interventricular septum and sinus node area. This transforms both cardiac pacemakers into a system of coupled relaxation oscillators. In such a system, the av junction is not functionally subordinated to the sinus node any longer. Instead, the electromechanical events integrated at this level may, after a local processing, determine the timing of future sinus node depolarizations and modulate the execution mechanical systolae. In this respect, the adrenergic phenotype of intracardiac sensory neurons is of importance: the norepinephrine being able to trigger nodal cells depolarizations and inhibit cholinergic neurons of pacemaker area. Our data may provide a useful inspiration to the designers of arificial pacemakers.

33.3

Modulation of Power Output in Cockatiels

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We investigated the mechanisms by which cockatiels (*Nymphicus hollandicus* n=5, 97.4g) modulate pectoralis muscle power output over a range of flight speeds. *In vivo* pectoralis power output was measured using sonomicrometry and a strain gauge mounted on the deltopectoral crest of the humerus in cockatiels trained to fly in a wind tunnel over a wide range of speeds (1 – 13 m/s). We found that muscle power output varied from 74 W/kg (at 5 m/s) to 155 W/kg (at 13 m/s) over this speed range. Other factors relating to power output, including work done per wingbeat, wingbeat frequency, muscle force and strain all varied with speed to a lesser degree. Mechanical work per wingbeat was strongly correlated with total pectoralis power output (R^2 = 0.90), but wingbeat frequency varied with speed over a smaller range and was not closely tied to power output (R^2 = 0.11). Mechanical work per wingbeat was best predicted by peak muscle force. A multiple regression of pectoralis power *vs* two subcomponents of muscle work (strain and force) and wingbeat frequency resulted in an R^2 of 0.82 across individuals and speeds. In summary, cockatiels primarily modulate pectoralis power output by changing the magnitude of force and secondarily by changing strain and cycle frequency. The factors influencing power output within and across flight speeds were similar. Supported by NSF IBN-0090265.

33.4

Dynamic Properties of Isolated Gecko Setal Arrays

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Geckos have the extraordinary ability to run up walls, and to adhere to even molecularly smooth surfaces. Gecko toe pads form arrays of millions of ~5 micron diameter setae, each branched into hundreds of 200 nm spatular tips that form intermolecular bonds with most surfaces. In prior studies, we determined the mechanical and molecular mechanisms of adhesion of gecko setae. However, the simultaneous function of millions of setae in an array, and the dynamics of setal adhesion have yet to be investigated. Theory predicts that dry surfaces should exhibit no positive force dependence on velocity when pulled parallel to each other. We used a servomanipulation system and piezoresistive force sensor to test the effect of velocity on parallel force during adhesion of isolated gecko setal arrays. Surprisingly, we discovered that force increased dramatically with velocity. Thus, while dry adhesion occurs at the level of individual setae, the integration of thousands of setae in an array can yield complex -even fluid-like- dynamics at the macroscopic scale. The dynamic response of arrays of setae suggests that perturbation rejection, not merely static adhesion, may be the evolutionary design goal of the gecko adhesive system. The novel properties of gecko feet may enable development of dynamic artificial adhesives with applications ranging from slip-resistant safety devices to adhesive feet on robots for search and rescue and space exploration. DARPA N66001-00-C-8047 & N66001-01-C-8072

33.5

Voltage Clamping with Digital Signal Processor Based Feedback Control

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Voltage clamping is an important technique for studying excitable tissues of all animals. The objective of this study is to unify the design of voltage clamp, current clamp, and dynamic clamp in a single integrated system. A state-of-the-art digital signal processor (DSP) executes the feedback control, allowing for dynamic changes of clamping modes. The analog front-end consists of a membrane potential amplifier and a current injection circuit. The DSP chip (Analog Device AD73422) hosts the control algorithms and closes the feedback loop. The control algorithms are of the proportion-integral-derivative (PID) type. The DSP also communicates with a personal computer (PC) for data acquisition and user interface. To achieve an acceptable performance, the feedback is implemented with an assembly language program solely on the DSP chip. The PC is excluded from the feedback loop to ensure maximum throughput. To optimize the design of hardware and software, computer simulation for the voltage clamp system has been developed with a Hodgkin-Huxley neuron model implemented in Matlab Simulink. In-vitro validation of this instrument has been done in voltage clamping experiments with cerebral ganglion cells of the pond snail (Lymnaea stagnalis). This study has demonstrated the feasibility of digital voltage clamping with a fast DSP chip. The capability to switch dynamically among multiple clamping algorithms creates new possibilities for neuroscience research.

33.6

The Scaling of Damping: Importance for Control

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Scaling arguments imply that as the size of a limb decreases, relative friction and viscosity (damping ratio) increase. One potential disadvantage is significant energy loss. However, large passive, damping could be advantageous for control. Perturbations to limbs in the swing phase can be dissipated passively requiring less neural feedback. Muscle activation when needed could be used more as a simple position or velocity command rather than a force command. A self-stabilizing limb favors the use of simple feedforward control strategies for locomotion. To test this hypothesis, we measured the damping at an isolated femur-tibia joint of the cockroach (*Blaberus discoidalis*) to gauge its contribution to stability. The three experimental approaches included swinging a free leg, oscillating a fixed leg with a lever and developing a musculo-skeletal model. In each case, legs were overdamped during the "swing" (unloaded) phase. After removing muscles from both the animal and model, we found that the exoskeleton was consistently responsible for approximately 70% of the passive damping at the femur-tibia joint. We predict that damping should play an increasingly dominant role in the dynamics of small animals. Transferring the concept of using small, damped legs from comparative physiology studies has inspired the design of the simplest, yet most stable legged robots ever built. Supported by ONR N00014-98-1-0669 and DARPA/ONR N00014-98-1-0747.

33.7

COMPLIANT DAMPED LEGS OF ARTHROPODS INSPIRE THE DESIGN OF ROBOT LEGS

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Terrestrial runners from mammals to arthropods display large morphological variations ranging from leg number and shape to type of skeleton employed. Despite this diversity, legged runners produce ground reaction forces that can be modeled as a spring-mass system with the same dimensionless stiffness (Blickhan and Full, 1993). This comparative analysis points to the possibility that arthropods possess structures that may function as tendons, storing and returning energy. We tested the hypothesis that the legs of the deathhead cockroach, *Blaberus discoidalis*, function as springs. We used dynamic oscillations to determine the mechanical properties of the hind leg with a rigidly fixed or a freely rotating body-coxa joint. The leg was oscillated orthogonal to the plane of joint rotation and induced forces recorded. The resulting force-displacement relationships were non-linear and hysteretic. Limb resilience ranged from 85% for a fixed limb to 65% for a free limb and was independent of oscillation frequency. Although legs can function as springs, less than 10% of the total energy performed per stride is stored and returned. Alternatively, we propose that compliant, damped legs increase dynamic stability and perturbation rejection. Knowledge of the mechanical function of insect legs inspired the design of robot legs, allowing engineers to build artificial limbs with the exceptional performance of animal appendages (Xu et al, 2000).
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RELAXED HOMEOTHERMY

34.1

Does Natural Hypothermia Improve the Dive Performance of Muskrats?

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We tested the hypothesis that a hypothermia-induced depression in tissue metabolism enhances the diving capability of adult and juvenile muskrats. Voluntary diving behavior, telemetered heart rate, and rate of oxygen consumption (VO2) of normothermic and mildly hypothermic animals were compared during 15-min immersion trials in 10°C and 30°C water. Hypothermia comparable to that experienced by muskrats in nature was induced by pre-chilling muskrats in 6°C water so as to achieve a 0.5-3.5°C drop in abdominal temperature. VO2 was monitored during post-immersion recovery to assess costs of hypothermia associated with rewarming. We found no evidence that pre-chilling affected diving VO2, mean and maximum dive times, dive frequency or dive:surface ratios of adults. Hypothermia reduced diving heart rate by 16% only in dives <25 s ($P=0.01$) but did not affect the development of diving bradycardia. Post-immersion VO2 of pre-chilled adults was higher than for normothermic controls only during recovery from dives <2 min. For longer dives, post-immersion costs were similar for both groups, suggesting no appreciable energetic penalty associated with rewarming. In the youngest cohort tested (200-400 g), hypothermia was accompanied by reduced diving activity and a 24% rise in average VO2 in water ($P=0.01$). Our findings suggest that mild hypothermia reduced the dive capacity of juveniles but had little impact on either the diving behavior or metabolism of adults.
Funded by NSERC (Canada).

31.2

Hibernating Black Bears Retain Skeletal Muscle Protein And Strength

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Inactivity or starvation leads to a loss of skeletal muscle protein muscle and strength. The black bear *(Ursus americanus)* may be a paradox. It spends several winter months confined in its den where it does not eat, drink, urinate or defecate. When hibernating bears are disturbed they are capable of rapid arousal and sustained locomotion. The level of protein catabolism in the hibernating bear is much less than observed in other fasted, inactive mammals even when adjusted for metabolic demands. Some investigators have suggested that the hibernating bear is in a positive protein balance. Other studies show small losses of skeletal muscle protein throughout hibernation. Even a small loss of skeletal muscle protein may lead to substantial decrements in muscle function. This study was conducted to quantify the changes that occur in protein synthesis and breakdown rates, protein content, fiber integrity, and muscle strength between the summer active period, and early to late hibernation, from muscle biopsies from the vastus lateralis. Over 110 days of winter dormancy, bears exhibited no loss of strength or changes in fiber cross sectional area. Protein synthesis, catabolism and protein content were also unchanged in this muscle during the hibernation period.
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34.3

Effects of pyrogen-induced fever on peak metabolic rates in the nine-banded armadillo (*Dasypus novemcinctus*)

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Nine-banded armadillos exhibit a cold-induced rise in body temperature (T_b). This fever is energetically expensive and appears maladaptive unless it conveys a substantial advantage. I hypothesized that cold-induced fever allows the animal to achieve a higher maximal metabolic rate (MMR). To investigate this hypothesis, 3 series of experiments were conducted on 4 monozygotic siblings. Series I documented changes in T_b and rate of oxygen consumption (VO_2) during cold exposure. When ambient temperature was decreased from 30 to 10°C, T_b increased on average by 1.66±0.2°C, and VO_2 increased on average by 6-fold. Series II documented changes in T_b in response to injections of lipopolysaccharide (LPS). LPS is an exogenous pyrogen that causes fever in most animals, and therefore can be used to manipulate T_b. The average maximum difference in T_b following LPS compared to saline injections was 1.56±0.47°C. Finally, Series III documented the combined effects of cold-exposure and LPS injections to verify if the effects of each treatment are additive and therefore lead to increased MMR. Unforeseen problems prevented testing the additive effects of the treatments on T_b. However, MMRs were significantly higher ($P=0.002$) for saline (106.4ml min^{-1}) compared to LPS (83.45ml min^{-1}) treatments. These observations were opposite of expectations and may have been the result of endotoxic shock. This research was performed as part of an undergraduate Honors Thesis under the supervision of P. Boily.

34.4

Functional significance of cold-induced fever

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When exposed to cold temperatures, some mammals, notably the nine-banded armadillo (*Dasypus novemcinctus*) and the Siberian hamster (*Phodopus sungorus*), exhibit an increase in core temperature that is proportional to the intensity of the cold stimulus *i.e.* colder air temperatures result in higher core temperatures. This response is incompatible with the typical negative-feedback mechanisms of temperature regulation in mammals where an increase in core temperature inhibits heat production, resulting in a core temperature that cannot exceed the thermoneutral value during cold exposure. Furthermore, this response is apparently maladaptive because it increases the animal's rate of heat loss by increasing the thermal gradient between the animal's core and its environment. Using theoretical heat transfer calculations combined with preliminary empirical measurements of body temperatures and metabolic rates, we examined the energetic consequences of this response as well as its potential impact on the ability to survive severe cold exposure. The results indicate that cold-induced hyperthermia can be energetically costly but may allow the nine-banded armadillo and Siberian hamster to survive severe cold exposure by increasing peak metabolic rates, which leads to a decrease in their lower lethal temperature.

34.5

Fasting-induced shallow hypothermia in birds: effect of repeated fasts

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We subjected Japanese quails (*Coturnix coturnix japonica*) and pigeons (*Columba livia*) to repeated food deprivations (three 2-d fasts for quails, four 3-d fasts for pigeons, interval between fasts 3-7 d) at an ambient temperature of 22°C. For quails, we also varied the photoperiod duration from 12:12 to 16:8 and 20:4 hours L:D. Deep body temperatures were continuously measured using intraperitoneal radio transmitters. Both species reacted to fasting with shallow nocturnal hypothermia (nocturnal body temperature 0.5-3°C lower than in *ad lib.* birds). However, the degree of hypothermia did not increase with repeated fasts or changing photoperiod. Instead, quails reacted by decreasing their diurnal body temperatures during the second and third fasts. This partly compensated the increase in body mass loss induced by longer photoperiods. Pigeons responded with a linear increase in body mass between fasts, and a slightly lower diurnal body temperature during the fasts. Nocturnal hypothermia, often considered an adaptive and flexible response to food deprivation, seems to operate in a rather ballistic fashion and cannot be adjusted according variable energetic demands imposed by photoperiod or repeated food shortages. In quails, an earlier study showed that nocturnal hypothermia was not influenced by cold exposure. These findings suggest further studies to elucidate the costs and constraints associated with shallow hypothermia.

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34.6

Body Temperature Profiles Associated With Muscle Activity And Strength Retention In Hibernating Black Bears

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Little is known about body temperature profiles and activity of hibernating bears in a free living state. Five adult black bears (*Ursus americanus*) were trapped and fitted with tracking collars housing Onset StowAway temperature loggers with sensors close to the skin. Bears also received abdominal temperature loggers and human pacemakers during field implants which functioned as data loggers for respiratory and cardiac frequencies as well as electromyogram (EMG) signals. Bears were revisited in their dens during the late winter to retrieve the data loggers. Bears were found to maintain a body temperature around 34° C without periods of arousal. No diurnal changes in core temperature were identified; however there was an average of four bouts of elevated fur temperature each day, independent from diel ambient air fluctuations. The profile of neck surface temperature increase in bears was similar to that of a human exercising on a bicycle. Bouts of elevated heart rate and respiratory frequency corresponded to these periods of elevated surface temperature. In associated studies, we show overwintering black bears to have low skeletal muscle protein loss and maintenance of muscle fiber integrity resulting in strength retention. Data from this study suggests that bears engage in periodic muscle activity each day resulting in the loss of heat across the surface to prevent elevations in core body temperature but to assist in maintaining strength through exercise. Supported by NSF grant EPS 9614105.

34.7

Torpor upregulates UCP2 and UCP3 in mouse tissues.

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Torpor is a highly conserved adaptation to cold ambient temperatures and/or low food availability found in many small mammals. It is characterized by marked transient (2-6h) reductions in core body temperature and metabolic rate. In the present study, we examined the effect of torpor, induced by fasting, on the expression of uncoupling protein 1, 2 and 3 mRNA using real-time RT-PCR in the following tissues: liver, kidney, brown adipose (BAT), heart, soleus muscle, and tibialis anterior muscle of NIH-Swiss female mice. Temperature was monitored continuously by via implanted telemeters. Tissues were obtained from 3 sets of mice: 1) ad lib fed, 2) fasted, during torpor, and 3) fasted, just after the torpor bout (within 15 minutes). At the time of sacrifice, body temperature was 37.7 +/- 0.2 C in the ad lib fed animals, 24.6 +/- 1.0 C in the fasted torpor animals, and 34.2 +/- 1.3 C in the animals just out of torpor. UCP1 mRNA was unchanged in torpor in BAT, the only tissue where it was detected. UCP2 and 3 mRNAs were activated with torpor at least 2-fold in most tissues, and returned to normal just out of the bout of torpor. These data suggest that the expression levels of UCP2 and UCP3 can be rapidly modified (within 15) as mice come out of torpor.

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34.8

Thermal lability in the smallest marine mammal, the sea otter (*Enhydra lutris*)

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Small body size, fur insulation, high metabolic rate and coincident dietary demands result in rapid changes in core body temperature in the sea otter (*Enhydra lutris*). To determine the relationship between these temperature changes and behavior in the wild, we surgically implanted temperature sensitive VHF radio transmitters into the abdomen of 15 adult free-ranging sea otters in Monterey Bay, California (water temperature range = 11 - 16°C). Data collected during 24-hour observation periods included abdominal temperature (T_{ab}), activity state (resting, grooming, foraging) and water temperature. T_{ab} of the otters varied from 37 to 41°C and depended on the activity state of the animal. The greatest change in T_{ab} (0.6°C SE ±0.07) occurred during prolonged periods of rest (> 1 hour) on the water surface. Increased activity during grooming, swimming and diving did not result in an increase in T_{ab} as is seen in other exercising mammals. Rather, T_{ab} decreased by 0.2 to 0.4°C depending on duration of the activity. These results indicate that sea otters face a thermal challenge during both sedentary and active periods. Consequently, this small marine mammal may be especially vulnerable to environmental perturbations including changes in water temperature or prey availability. This study was funded by the Oiled Wildlife Care Network.

34.9

Metabolic Depression, Temperature Regulation and Pregnancy in Hibernating Black Bears.

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Hibernating bears reduce their metabolic rate to 25% of non-hibernating resting levels, and their core body temperature (Tb) varies between 30°C and 35°C in a cyclic 3-5 d pattern. Can female bears go through hibernation with metabolic depression and body temperature cycling and still carry out pregnancy?

We used telemetry and respirometry to investigate a female bear that became pregnant and a male black bear while they overwintered under undisturbed conditions in Alaska under video surveillance. Rates of O_2 consumption were recorded by indirect calorimetry continuously during hibernation and intermittently for one month after emergence. T_b and peripheral temperatures, EMG and EEG were recorded with radio telemetry and data loggers. During pregnancy the female maintained a steady Tb at a level normal (37-38°C) for a non-hibernating bear. However 1 day before parturition, Tb decreased by 2-3°C, resembling that of the male bear for the rest of the hibernation (the 243g cub died after birth from natural causes). Metabolic rate during pregnancy was reduced to 54.7% compared non-hibernating resting rates, and it was further reduced to 47.6% (Q_{10} of 1.8) during non-pregnant hibernation.

Pregnancy may constrain the adoption of low and cyclic Tb during hibernation to prevent abnormal embryonic development. However, metabolic depression of the mother can be present without decreases in Tb.

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34.10

Bigeye thresher sharks possess large orbital retia mirabilia and have a wide thermal niche. Kevin C. Weng and Barbara A. Block. Tuna Research & Conservation Center, Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950

The bigeye thresher shark (*Alopias superciliosus*, Lowe, 1841) is one of three species in the family Alopiidae. Alopid sharks occur in offshore and coastal waters throughout the tropics and subtropics and are renowned for their elongated tails. The bigeye thresher is easily distinguished from other Alopids by its extraordinarily large eyes and unique head morphology. Encounters with this species are rare and information about its physiology and biology limited. Here we describe the vascular anatomy of its large orbital *retia mirabilia* and provide the first measurements of its thermal niche. We attached a pop-up satellite archival tag to a shark captured and released in the Gulf of Mexico. Striking vertical migration was observed throughout the 60-day track, with most of the day spent in 9°C waters below the thermocline, and most of the night in 23°C mixed layer waters. The prolonged occupancy of waters below the thermocline precludes buffering by thermal inertia. Dissections and vessel injection preparations of bigeye thresher shark heads revealed a large arterial vascular plexus in the orbital sinus behind the eye. The structure is more developed than the orbital *retia mirabilia* of the endothermic lamnid sharks, which have elevated brain and eye temperatures. It is likely that this structure in the bigeye thresher shark is also conserving metabolic heat to maintain stable cranial temperatures during prolonged dives beneath the thermocline. Supported by NMFS.

35.1

Comparison of Plasma and Red Blood Cell Fatty Acids as Predictors of Diet in Captive Harbor Seals

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The fatty acid composition of the diet significantly impacts the fatty acid composition of various body tissues. However, because turnover rates of fatty acids differ among body tissues, differences occur in the time period required for the dietary fats to be completely reflected in the fatty acid pattern of the tissue. We compared the relative time periods necessary to correctly predict diet using either plasma or red blood cell (RBC) fatty acid (FA) profiles in the harbor seal. During a two-year feeding trial at the Alaska SeaLife Center, Seward, AK, captive harbor seals (n=8) were fed alternating diets of either herring or pollock for a four-month period or a continuous mixed diet. High frequency sampling (every two weeks) allowed us to monitor dietary changes of plasma and RBC FA over time. Using stepwise discriminant analysis, preliminary results suggest that seals could be reliably classified as belonging to pollock, herring or mixed diet groups according to their plasma and RBC FA profiles within one and two months, respectively. Dietary groups could be separated with as few as 7 predictor FA. We will continue to develop this model, incorporating the effects of seal age, gender, intake rates and metabolic state on FA profiles. This project was sponsored in part by the EVOS trustee council.

35.2

Effects of Early Nutritional Supplementation of Linoleic Acid on Memory

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Clinical Studies have linked hypertension to brain injury and cognitive impairment in elderly men and women. The loss mental speed, sustained attention and delayed verbal recall can be life altering. Spontaneously Hypertensive Rats, (SHRs) have been proposed as a possible model for investigating the role of hypertension development on cognitive behavior. We have previously found that aortic rings pretreated with linoleic acid (n-6 fatty acid) promote vasodilation within SHRs. This current study examines the hypothesis that linoleic acid may delay the onset of memory impairment due to untreated hypertension. 3, 6, and 9-month-old SHRs were used in this study. Spatial Reference Memory was evaluated in these rats using the Morris Water Maze. At 9:00 am for 14 days, one group of SHRs received linoleic acid and another group of SHRs received deionized water and the latency to find the platform in the Morris Water Maze was measured each day at 11:00 am. Results obtained indicate that early nutritional supplementation with linoleic acid significantly improved spatial reference memory in SHRs. Additionally; there was a significant increase in the B_{max} of the D_1 dopamine receptors in various brain regions. On the other hand, those rats that received deionized water displayed a gradual decline in both D_1 receptor binding and memory. (Supported by NIH Grant RR03020)

35.3

Numbers, Longevity and Dynamics of the Free Pulmonary Macrophages (FRMs) in the Chicken and the Rat

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Paucity of FRMs has been reported in birds, a taxon said to be highly susceptible to pulmonary infections. We questioned the validity of the assertion. FRMs of the chicken and the rat were studied and cell count, longevity and dynamics assessed. The FRMs were harvested by lavage, stained and counted. For longevity, the cells were held at the normal body temperature and every 30 minutes (over a period of 3 hours) the number of viable cells counted. The dynamics of the pulmonary macrophages was determined by counts made on 5 serial lavages taken over a period of 10 minutes at 2½ minute intervals.

In the chicken, the number of FRMs per gram body mass (1412±308SD) was significantly lower than in the rat (2932±518SD) and the cells were more robust. In the rat, the number of FRMs decreased progressively with lavage while in the chicken, rapid flux of cells onto the respiratory surface occurred.

In birds, FRMs are fewer and hardier and tissue macrophages are readily moved to the respiratory surface. Scarcity of FRMs alone doesn't justify inference that pulmonary defense in birds is poor. Husbandry practices and genetic manipulation for productivity may contribute to the presumed susceptibility.

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35.4

A Further Look into the Cheng-Prusoff Equation for Determination of Dissociation Constants

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Introduction: The Cheng-Prusoff equation is often used for the determination of dissociation constant of a competitive antagonist, K_B, when the IC_{50} of the antagonist and the dissociation constant of the agonist (or EC_{50}) are available. Method: A new power equation is derived for determination of the dissociation constant of a competitive antagonist by taking into account the cooperativity index of the agonist and the antagonist. Results: The new equation can avoid errors caused by the use of the Cheng-Prusoff equation when the cooperativity index deviates from unity. The slope function of the agonist concentration-response curves (K, or m for the binding ligand, not shown) and that of the inhibition curve (n) are critical for the determination of K_B values. Both equations are shown here where A is the concentration of an agonist (or of a ligand in binding assay), K_P (or K_D) is the dissociation constant of an agonist (or of a ligand in binding assay). Conclusion: Use of this new power equation would yield more accurate estimation of K_B values of antagonists.

Cheng-Prusoff equation	The new power equation
$K_B = \frac{IC_{50}}{1 + \frac{A}{K_D}}$	$K_B = \frac{(IC_{50})^n}{1 + \frac{A^n}{K_P}} = \frac{(IC_{50})^n}{1 + (\frac{A}{EC_{50}})^n}$

35.5

Fatty Acid Metabolism of Rainbow Trout: Different preferential Metabolism of Palmitate and Oleate

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Different fatty acids play different dominant roles in energy metabolism and in membrane function. The rates of incorporation of a saturated (palmitate, 16:0) and a monounsaturated fatty acid (oleate, 18:1) in tissue lipids of trout were compared to assess their involvement in neutral lipid and phospholipid metabolism (representing rough indices of primary function in energy reserve and membrane metabolism, respectively). 1-^{14}C-palmitate and 9, 10- ^{3}H-oleate were administered intravenously in adult rainbow trout and tissues were collected 24 h later. Neutral lipids and phospholipids were analyzed separately to measure palmitate and oleate specific activities in the 2 lipid groups. We found that the rates of fatty acid incorporation in total lipids were widely different between tissues ($p<0.001$): going from a low value of 10 ± 1.5 nmol fatty acid/g tissue in white muscle to 342 ± 24 in liver (intestine, gill, red muscle and heart having intermediate values). Results also showed that the 2 fatty acids have a different dominant metabolic fate. Within each tissue, oleate was incorporated more than 3 times faster than palmitate into neutral lipids ($p<0.02$), whereas both acids were included at the same rate into tissue phospholipids ($p>0.05$). We conclude that the monounsaturated acid, oleate, plays a dominant role in the metabolism of energy reserves, whereas both acids are equally involved in the membrane metabolism of rainbow trout tissues. This work was supported by an NSERC grant to J.-M. Weber.

35.6

Putative Convergent Evolution of A_4-Lactate Dehydrogenase in *Chromis* Species (Pomacentridae) From Across the Pacific: Evidence for Key Sites in Biochemical Adaptation to Temperature.

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Building on previous work elucidating the nature of adaptation of muscle-type lactate dehydrogenase (A_4-LDH) in cold-stenothermal Antarctic Notothenioids and eurythermal gobies, we investigated *Chromis* congeners from thermally stable tropical waters and variable temperate waters. The Dusky Chromis (*C. caudalis*) and Yellow-axil Chromis (*C. xanthochira*) have largely sympatric distributions restricted to the Tropical Western Pacific where temperatures are stable and average 28.5°C. The Blacksmith (*C. punctipinnis*) from the NE Pacific experiences temperatures of 10-22°C. Kinetic analyses of purified A_4-LDH show a lower Michaelis constant (K_m) and catalytic constant (k_{cat}) across 5-35°C for both the Dusky and Yellow-axil Chromis relative to the Blacksmith, such that the three species show comparable kinetics at their respective physiological temperatures. Analysis from *ldh-a* cDNA sequences reveals that four nonsynonymous substitutions are segregated between tropical and temperate species, and thus are consistent with the observed kinetic differences. These substitutions all occur in or near the two -helices (1G- 2G and -H) that have been previously implicated as adaptive "hot spots" of temperature adaptation. Thus, a few key sites, distinct from the active site, may be responsible for adjustments of flexibility/stability to effect enzyme adaptation to temperature in many different environmental regimes. This work was supported by NSF Grants IBN-9727721 and IBN-0133184 to GNS.

35.7

Alterations in Hepatic Metabolism of Sulfur-Amino Acids by Ethanol in Rats

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Alterations in hepatic metabolism of S-amino acids were monitored over one week in male rats treated with a single dose of ethanol (3 g/kg, ip). Methionine and S-adenosylhomocysteine concentrations were increased rapidly, but S-adenosylmethionine, cysteine, and glutathione (GSH) decreased following ethanol administration. Activities of methionine adenosyltransferase, cystathionine -lyase and cystathionine -synthase were all inhibited. -Glutamylcysteine synthetase activity was increased from t = 8 hr, but GSH level did not return to control for 24 hr. Hepatic hypotaurine and taurine levels were elevated immediately, but reduced below control in 18 hr. Changes in serum and urinary taurine levels were consistent with results observed in liver. Cysteine dioxygenase activity was increased rapidly, but declined from t = 24 hr. The results show that a single dose of ethanol induces profound changes in hepatic S-amino acid metabolism, some of which persist for several days. Ethanol not only inhibits the cysteine synthesis but suppresses the cysteine availability further by enhancing its irreversible catabolism to taurine, which would play a significant role in the depletion of hepatic GSH.

35.8

Sugar Preferences and Enzyme Activities in a Frugivorous Bird, the Yellow-Vented Bulbul

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The dietary preferences of frugivorous and nectarivorous birds are often linked to their sugar preferences, which in turn have been associated with digestive ability. Most of the frugivorous species studied have an aversion to sucrose, apparently because they lack sucrase. However, almost all of the species studied were North American species that normally eat fruit dominated by monosaccharides. The aim of our study was to determine the sugar preferences and sugar enzyme activity levels of one of Israel's most common frugivores, whose natural diet, like that of many old-world frugivores, regularly includes both mono and disaccharides. We used pair-wise comparisons to determine the sugar preferences of 9 yellow-vented bulbuls, *Pycnotus xanthopygos*, on four different sugar solutions: 20 % w:w fructose, glucose, sucrose, or fructose and glucose combined. We also measured maltase and sucrase activity on homogenized intestine sections of 7 birds. The bulbuls did not show strong sugar preferences, and we found high levels of both maltase and sucrase activity throughout the length of the bulbuls' intestines. The sugar preferences and digestive abilities of the yellow-vented bulbul are linked. The difference between the bulbuls' abilities and those of the North American birds studied reflect the differences in the sugar composition of the fruits available to them.

35.9

Purification and Characterization of Alanine Racemase from the Muscle of Black Tiger Prawn *Penaeus monodon*

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We purified alanine racemase to homogeneity from the muscle of black tiger prawn. The isolated enzyme had a molecular mass of 44 kDa on SDS-PAGE and 90 kDa on gel filtration, suggesting a dimeric nature of the enzyme. The purified enzyme remained its activity in the absence of pyridoxal phosphate as a cofactor but carbonyl reagents inhibited the activity, suggesting tightly binding of the cofactor to the enzyme protein.Several partial amino acid sequences of peptide fragments obtained from the isolated enzyme showed positive homologies from 52 to 76% with those of bacterial enzymes and a catalytic tyrosine residue of bacterial enzymes was also conserved in the prawn one, indicating alanine racemase gene is well conserved from bacteria to invertebrates.Funding source: a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science.

35.10

Cortisol Metabolism and Inter-population Variation in Glycolytic Enzyme Expression.

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Northern and southern populations of the killifish, *Fundulus heteroclitus*, live in substantially different thermal habitats. Associated with these temperature differences, there are differences between populations in glycolytic enzyme activities. We have previously shown that differences in lactate dehydrogenase-B (LDH-B) activity between populations are the result of mutations in a cortisol responsive element in the *Ldh-B* promoter. Here, we test the hypothesis that stress hormones are important in establishing differences between populations in the activities of other glycolytic enzymes. At rest, phosphofructokinase (PFK) and aldolase (ALD) activities were greater in southern populations, while LDH-B activity was greater in northern populations. Sham injection and injection with 200 $\mu g.g^{-1}$ cortisol had no effect on enzyme activity, but injection with 400 $\mu g.g^{-1}$ resulted in changes in the activities of PFK, ALD and LDH so that the populations no longer differed in the activity of any enzyme. These changes were reflected at the mRNA level for *Ldh-B* but not for *Pfk* or *Ald*, suggesting that the enzymes are regulated *via* different mechanisms. Plasma cortisol did not differ between populations at rest, but was significantly different between populations in sham and cortisol-injected fish, suggesting that differences in glycolytic enzyme activity may be related to underlying differences in cortisol metabolism between populations. Funded by an NSERC operating grant to P. Schulte.

35.11

Responses to and tolerance of temperature extremes differ among phosphoglucose isomerase genotypes in a montane leaf beetle

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Natural selection may act on loci coding for metabolic enzymes when genotypes differ in tolerance to temperature extremes. In this study, we explored mechanisms by which physiological responses to thermal extremes cause persistence of allelic variation at the enzyme locus phosphoglucose isomerase (PGI) in California populations of the willow beetle *Chrysomela aeneicollis*. PGI allele frequencies vary across a thermal gradient and shift in response to climate change. PGI allele 1 predominates in cooler localities, whereas allele 4 predominates in warm habitats. Exposure to temperature extremes resulted in physiological differences among PGI genotypes. In response to elevated temperatures, PGI 1-1 adults and larvae induced expression of a 70-kD heat shock protein (HSP70) to a greater degree and at lower temperatures than 1-4 or 4-4 individuals. Adult females expressed higher levels of HSP70 than males. However, PGI 4-4 adults had lower critical thermal maxima (CT_{max}) than 1-1 or 1-4 adults. In response to exposure to sub-zero temperatures, recovery of larval and adult PGI 1-1 homozygotes was significantly better than 1-4 or 4-4 genotypes. These data suggest that heat and cold tolerance depend on PGI genotype and may be related to HSP expression. Tradeoffs between thermal tolerance and energy allocation may maintain variation at PGI. This work was funded by a National Science Foundation grant (IBN-0078464/0078659) to EPD and NER.

35.12

Effects of temperature on locomotory performance of two species of California willow beetles

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We examined the metabolic and performance consequences of differential expression of heat shock proteins (HSPs) in the willow beetles *Chrysomela confluens* and *C. schaefferi*. *C. confluens* is more abundant at warmer, inland localities and *C. schaefferi* at cooler, coastal sites. Body temperatures vary throughout the day and are higher at inland sites. This variation in body temperature is likely to have profound impacts on locomotory activity. Differential effects of temperature on activity between species may be mediated through expression of HSPs, as individuals that express high levels of HSP may have reduced resources for movement. Using a high-speed video imaging system, we quantified walking velocities before and after exposure to a moderate (26°C) or elevated (35°C) temperature. *C. schaefferi* and *C. confluens* had similar average and maximal velocities after exposure to 26°C. However, the average velocity of *C. schaefferi* was significantly reduced after exposure to 35°C, whereas activity of *C. confluens* was not affected by heat exposure. Males had higher average and maximal velocities than females, independent of body mass and treatment temperature. Based on these data, we predict that expression of a stress-inducible isoform of HSP70 will be similar between species after exposure to 26°C and higher for *C. schaefferi* than *C. confluens* after exposure to 35°C. This work was funded by a National Science Foundation grant (IBN- 0078464/0078659) to EPD and NER.

36.1

Toxin ingestion: a behavioral adaptation of mammalian herbivores to cold?

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Two energetically demanding processes in mammalian herbivores are detoxification of plant secondary compounds and thermoregulation. The results of a recent study on mammalian herbivores suggest that detoxification under cold conditions may mitigate the costs of thermoregulation via metabolic depression. Based on these results, we hypothesized that mammalian herbivores will preferentially consume more juniper in a cold environment than when in their thermal neutral zone. We tested our hypothesis by providing Neotoma albigula, a generalist woodrat, with a choice between control and 50% juniper diet under warm (26 degrees C) and cold (20.5 degrees C) conditions. We measured dry matter intake of control and juniper diet and compared the percentage of juniper consumed between cold and warm environments. We found that N. albigula voluntarily consumed more juniper in the cold environment than the warm. We propose that N. albigula may preferentially consume more juniper under cold conditions compared to warm conditions to reduce the metabolic costs of thermoregulation. Funded by NSF-IBN0079865.

36.2

Behavioral Thermoregulation In The Amphibious Purple Shore Crab *Hemigrapsus nudus*

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The thermoregulatory behavior of the amphibious purple shore crab, *Hemigrapsus nudus*, was examined in both aquatic and aerial environments. Crabs warmed or cooled more rapidly in water than in air. Acclimation to either 16[deg]C or 10[deg]C resulted in an increased CTMax and a lower CTMin respectively. These survival regimes were not reflective of the thermal preferences of the animals. In water the thermal preference of 16[deg]C acclimated crabs was 14.6[deg]C and they avoided water warmer than 25.5[deg]C. These values were significantly lower than 10[deg]C acclimated crabs; these animals demonstrated temperature preferences for water that was 17.1[deg]C, and avoided water that was warmer than 26.9[deg]C. This temperature preference was also exhibited in air, where 10[deg]C acclimated crabs exited from under rocks at a temperature that was 3.2[deg]C higher than 16[deg]C acclimated animals. This behavioral pattern was possibly due to a decreased thermal tolerance of 16[deg]C acclimated crabs, related to the molting process. *H. nudus* were better able to survive prolonged exposure to cold temperatures than warm temperatures. Reflective of this, not all animals exited from water or air when the temperature was decreased. However, there was a trend towards lower exit temperatures with the lower acclimation (10[deg]C) temperature. By using a complex series of behaviors, *H. nudus* was able to precisely control the body temperature independent of the medium, by shuttling between air and water. The time spent in either air or water was influenced more strongly by the temperature than the medium itself. In the field, this species may experience ranges in temperatures of up to 20[deg]C, however it is able to utilize thermal microhabitats to maintain the body temperature within fairly narrow limits. The thermoregulatory behavior is discussed in relation to physiological reactions of this species in water and air. Supported by UNLV NIA grant.

36.3

Active Regulation of Brain Temperature in Yellowfin Tuna

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The objective of this study was to measure the ability of the carotid *rete* (vascular counter-current heat exchanger) to insulate the brain of yellowfin tuna *Thunnus albacares* from rapid changes in environmental temperatures, such as those experienced during "dives" through the ocean thermocline. In addition to the well-studied vascular *retia* that warm the deep red myotomal muscles in tunas, many tunas (e.g., *Thunnus* spp.) also possess a *rete* in the blood supply to the eye and brain, potentially acting as a thermal barrier to prevent heat loss from these tissues. Anesthetized tuna were implanted with a thermocouple through the pineal foramen of the skull to record brain temperature, and exposed to step changes in ambient temperature (from 25 to 15°C and then back to 25°C). Rates of brain cooling (0.154 ±0.027 °C min^{-1} $°C^{-1}$ gradient) were significantly lower than rates of heating (0.232 ±0.038 °C min^{-1} $°C^{-1}$ gradient). This difference indicates that heat exchanger efficiency was maximized to reduce heat loss during drops in ambient temperature and then minimized to increase heat gain when ambient temperature was increasing. Injection of bretylium tosylate to abolish adrenergic nervous control of the circulatory system eliminated differences in heating and cooling rates, suggesting that *retial* efficiency is actively controlled by shunting blood through alternate routes. Supported by a grant from Hawaii Pacific University's Trustee's Scholarly Endeavors Program (K.E.K.).

36.4

The relationship between body temperature, heart rate and rate of oxygen consumption in Rosenberg's goanna (*Varanus rosenbergi*) at various levels of activity.

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Rosenberg's goanna (*Varanus rosenbergi*) is found further away from the equator than any other varanid species (with the exception of *V. varius*) and has adapted to seasonal changes in the environment including large ambient temperature fluctuations. To enable the use of heart rate (f_H) for estimating field metabolic rate (FMR) in free-ranging Rosenberg's goannas, we determined the relationship between f_H and mass-specific rate of oxygen consumption (sVO_2) in 6 goannas (mean mass 1.61 ± 0.41 kg) before and during exercise on a treadmill and during the post-exercise period. The experiments were conducted between the temperature range 14 – 35°C, as these extremes represent the lowest and highest average body temperatures (Tb) of these animals in the field across the seasons. Arrhenius plots of f_H and sVO_2 revealed that both variables were related to Tb with Q_{10} values of 2.3 and 2.7 respectively. A strong positive association existed between the two traits, independent of Tb, as revealed by plotting residuals. Further, stepwise regression revealed that sVO_2 was significantly correlated with f_H (r^2 = 0.81), whereas the addition of Tb only marginally improved the correlation (r^2 = 0.82). The resultant multiple regression is reliable at predicting sVO_2 from Tb and f_H as revealed in a series of validation experiments.

36.5

Correlations Between Energy Metabolism, Thermal Environment, and Activity in Anuran Amphibians from Genus Scinax (Amphibia / Hylidae)

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Adaptation to different thermal environments involves changes in the structural and biochemical composition of the ectotherms skeletal muscles. These adjustments are related to maintenance of the energetic homeostasis and the performance in different thermal conditions. Nevertheless, the possible associations between adaptation to intense activity and activity at low temperature are less understood. We investigated the evolutionary relationships between behavioral performance and glycolytic and aerobic capacities in species of amphibians from genus Scinax that exhibits remarkable diversity in thermal and behavioral ecology. For each species we performed focal observations of field behavior and then transported frogs to the lab. Then we measured the activity of some enzymes of the glycolytic pathway and aerobic metabolism in trunk and leg muscles at different temperatures and analyzed data in an evolutionary context using a preliminary phylogenetic hypothesis. We found a correlation between intensity of interspecific calling activity and aerobic capacity in trunk muscles. The only real winter specialist species calls at low temperatures and exhibits low calling rate, and have a relative high anaerobic capacity in trunk muscles. In leg muscles, a relative high aerobic capacity characterizes species that exhibit high levels of locomotor activity. Apparently, differences in aerobic capacity are associated to the use of carbohydrates stores in addition to the contribution of lipid metabolism. Differences between species appear related to level of activity and not to activity temperature.

Financial support: FAPESP

36.6

Modification of the Physiological Stress Response in Green Sturgeon, Acipenser medirostris: The Influence of Time of Day and Temperature.

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Physiological processes in poikilothermic animals are largely regulated by their surrounding environmental conditions. The light/dark cycle and temperature are examples of environmental variables that change predictably and unpredictably respectively, with temperature becoming even more unpredictable due to the impact of man. We investigated the modification of the physiological stress response by both time of day and temperature in young-of-the-year (YOY) green sturgeon (Acipenser medirostris), which were exposed to a 1-minute air emersion stressor in a net. The stress response (plasma cortisol, lactate, and glucose) was measured at two different times of day (0800 or 2000 h), as well as after a 2-week acclimation to 11 or 19 °C. The stress response was augmented at night, reaching a peak mean of 19.09 ng/ml cortisol and 190.57 mg/L lactate compared to 4.9 ng/ml cortisol and 166.69 mg/L lactate during the day. There was no significant stress-induced change in plasma glucose levels. Temperature did not affect the peak cortisol concentrations (56.66 and 50.27 ng/ml at 11 and 19° C respectively), however the synthesis and/or clearance rate of cortisol was prolonged at 11° C. The post-stress rise in lactate was similar between temperature groups, however there was a significant increase in post-stress glucose levels at 11° C that was maintained for at least 6 h, suggesting both glycogenolysis and gluconeogenesis. This work is supported by the CALFED Bay-Delta program.

36.7

Direct Observation of Cooling in Cerebral Arterial Blood in Pigeons, *Columba livia*

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Birds maintain brain temperature lower than body temperature, preventing brain overheating during hyperthermia. Among the routes of blood supply to the brain are a paired vascular network, the rete mirabile ophthalmicum (RMO). Brain cooling is hypothesized to occur when hot arterial blood flowing to the brain is cooled in the RMO by heat transfer to cooler venous blood flowing in the opposite direction. Venous blood is cooled previously by evaporation while perfusing moist nasal and buccal surfaces. To provide the first direct evidence for heat exchange in the RMO of pigeons, arterial blood temperatures were measured using fine thermocouples at the inlet and outlet of the RMO while the birds were exposed to thermoneutrality or heat stress. Arterial blood temperature was 41.1°C at the inlet to the RMO (approximately equal to body core temperature) and decreased by about 2°C at the outlet in heat-stressed birds. To determine the correlation between temperature of blood leaving the RMO and brain temperature, we also measured hypothalamic temperature during heat stress and found that although lower than body core temperature it was higher than the RMO outflow. These data indicate that arterial blood is cooled in the RMO. They also suggest that some but not all of the blood flowing to the brain passes through the RMO. (Supported by NIH grants GM07667 and GM08136.)

36.8

Diet and the evolution of thermoregulatory energetics in the woodrats *Neotoma albigula* (a generalist) and *Neotoma stephensi* (a specialist)

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To adapt to a diet low in nutrition and high in secondary metabolites, specialist herbivores must minimize any metabolic costs and the physiological effects of secondary metabolites. To test whether dietary specialization does influence a herbivore's response to physiological stress, we compared thermoregulatory energetics of two woodrat species, *Neotoma stephensi* and *N. albigula*. Both species eat juniper (*Juniperus monosperma*), but *N. stephensi* is a juniper specialist while *N. albigula* is a generalist that feeds mostly on other plants. Because these species are sympatric, they have evolved under similar thermoregulatory regimes (i.e., differences in thermoregulatory physiology are presumably diet-related). We predicted the specialist would have lower thermoregulatory costs and exhibit fewer physiological responses to juniper secondary metabolites. Under control conditions, the specialist had a lower basal metabolic rate (BMR) than the generalist but conductance (C) and body temperature (T_b) did not differ. Comparing the effects of juniper consumption and acclimation temperature on thermoregulation found lower thermoregulatory costs for the specialist under all conditions. Acclimation temperature and juniper consumption had stronger affects on the BMR, C, and T_b of the generalist than they did on the specialist. We conclude that dietary specialization favors the evolution of reduced energetic costs and reduced physiological plasticity.

36.9

Measuring Temperatures And Heat Flux From Dolphins In The Eastern Tropical Pacific: Is Thermal Stress Associated With Chase and Capture In The Tuna Purse-seine Fishery?

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Thermal stress can be associated with chase and capture in terrestrial mammals. This study investigated whether spotted dolphins (*Stenella attenuata*) that are repeatedly chased and captured in the eastern tropical Pacific tuna purse-seine fishery suffer thermal stress. Three complementary data sets were used to investigate thermal stress: (1) deep core temperatures, (2) infrared thermographic images, and (3) dorsal fin-thermal data logger records. This study demonstrated that a dolphin increased heat flux across its dorsal fin in response to chase. Prolonged chase times (>75 min) resulted in measurably higher skin surface temperatures, suggesting relatively high rates of heat delivery to the skin's surface via blood flow. Dolphins that experienced chases less than 75 min did not appear to maintain elevated levels of heat flux and high skin surface temperatures once the chase ended and the animals were within the net corral. The animals in this study did not appear to experience any constraint to free-swimming, and, thus, to convective heat dissipation, within the net corral. Extended chase durations and capture times within the net were not statistically associated with elevated deep body temperatures in this study. These results suggest that most dolphins in this study could dissipate excess body heat generated during chase and capture. Supported by NMFS. NMFS Permit #774-1634.

36.10

Comparative Physiology of Heat Production and Its Response to Dehydration: Is It Connected to Habits and Habitats?

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Rodents adapted to xeric conditions show resting metabolic rates (RMR), which are lower than expected for their body mass. Furthermore, to compensate for such low values, they show high capacity for nonshivering thermogenesis (NST). Exogenous noradrenaline (NA) increases heat production, measured as oxygen consumption (VO_2NA). NST-capacity is calculated as the ratio between VO_2NA and RMR. Recently studies have shown that increasing salinity in drinking water of rodents kept on a high protein diet (above 40%) resulted in decreasing RMR and increasing NST. The objective of this paper is to compare between different species from different habitats, kept under the same acclimation conditions and using the same methods for collecting thermoregulatory and osmoregulatory variables. The following species are compared: golden spiny mice *Acomys russatus*, and the bushy tailed gerbil *Sekeetamys calurus* (rock dwellers), the fat jird *Meriones crassus* (burrow dweller from extreme arid environments). Tristrams' jird *Meriones tristrami* and the social vole *Microtus socialis* (mesic burrow dwellers) The mesic rodents were less tolerant to increasing salinity. The increase in salinity caused a decrease in RMR, while in such species an increase in NST-capacity was noted. The difference between *S. calurus* and *A. russatus* can be explained by their different habits. RMR values of the voles did not decrease. However, NST-capacity values increase due to an increase in VO_2NA. Oranim funded research.

HEART AND CIRCULATION

37.1

Cardiovascular Responses of the Terrestrial Hermit Crab *Coenobita clypeatus* to Changes in Body Position

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Animals living in the terrestrial environment must compensate for effects of gravity on their cardiovascular system. A number of animal models have been used to investigate gravitational effects on pressure-flow relationships including arboreal vs aquatic snakes. We have found that the terrestrial hermit crab Coenobita clypeatus also serves as an appropriate model as its closely related aquatic counterpart allows for comparative studies, investigating the adaptations to gravitational effects between the terrestrial and aquatic environments. This phenomenon has not been investigated in invertebrates. The terrestrial species spends its entire adult life on land, on the ground as well as in trees. Coenobita clypeatus are placed on a rotating platform where body position can be adjusted from horizontal to 45° and 90° with head superior and/or with head inferior. Heart rate and blood flow through the major arteries are monitored using a Pulsed Doppler flow system with probes placed adjacent to the vessels Adominal cavity volume is measured using a plethysmograph chamber to determine if hemolymph pooling occurs. Abdominal muscular activity is monitored using EMG electrodes. Limb position and activity are also monitored. Supported by NSF grant IBN 9874534 to CLR.

37.2

The Effect of Continuous and Intermittent Exercise and Temperature on Ghost Crab Heart Rate.

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Resting heart rate (HR) of land crabs is dependent upon body size and body temperature (Tb). However, the effect of exercise on HR remains controversial. We measured ghost crab (*Ocypode quadrata*, 26-74 g) HR with a pair of silver wire electrodes inserted through the carapace on either side of the heart. HR increased with Tb over 10-30C; no further increase occurred at higher temperatures (30-36C). Mean resting HR at 24C was 2.88 Hz. The resting ghost crab HR is similar to that of other land crabs with the same body mass and Tb The Q10 for 15-24C and 24-30C are 1.7 and 1.5, respectively. The Q10(15-24C) for HR is smaller than that for the ghost crab's resting metabolic rate (3.4) while the Q10(24-30C) for HR is larger than that for resting metabolic rate (1.0). Continuous exercise on a motor-driven treadmill at 5 cm/s (~45% maximum aerobic speed; MAS; Tb = 20C) increased HR by 40% above the resting value. Increasing exercise speed (up to 20 cm/s, ~180% MAS) did not further increase HR. Intermittent exercise consisted of alternating 120 s of exercise at 15 cm/s (~135% MAS) with 120 s pauses for 60 min. Mean HR during exercise intervals was 42% higher than resting HR while the mean during the pause intervals was 34% higher than the resting rate. The increase in HR due to exercise is much smaller than the ghost crab's factorial aerobic scope (~400-540%). Our data suggests that HR is a minor component of the increased metabolic rate associated with exercise.

37.3

Endothelial Cells from the Eel, Anguilla rostrata, a System to Study the Response to Environmental Changes

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Endothelial cells form the single cell tissue layer that lines, and helps to regulate, the cardiovascular system in all vertebrates. Our interest is in the role of the endothelium in pathologies resulting from exposure to aryl hydrocarbon receptor(AHR)agonists. Fish exposed during development show cardiovascular defects and many questions about this response, as well as other questions, are addressed best in cell cultures. There is little development of endothelial cell cultures from vertebrates other than from mammals. We isolated and cultured endothelial cells from the eel, Anguilla rostrata and used these to extend our studies to the cellular level. Capillary cells are isolated enzymatically from the rete mirabile and plated into fibronectin coated flasks. Capillary cells from the kidney and endocardial cells from the heart are grown as explants from which the cells are separated. The culture medium is M199 with Earle's salts plus NaCl, HEPES, NaHCO3, glutamine, pyruvate, heparin, antibiotics, endothelial cell growth supplement and 20% serum. Cultures are maintained at 25 C in humidified air. Dose response curves were determined with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and compared to those from mammalian cells and from intact eels and reflect the higher resistance in the eel to AHR agonists. These cells also were used to isolate a partial gene sequence related to endothelial NOS, thus far not identified in fish. Support:Fordham University and NIH grant P42-ES07381.

37.4

Effect of Temperature on the Sarcoplasmic Reticulum Ca^{2+}ATPase from Tuna Hearts

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Tunas have high metabolic rates, high cardiac outputs and elaborate retia mirabilia which conserve metabolic heat and elevate oxidative tissue temperatures. Archival tags indicate that Atlantic bluefin maintain elevated peritoneal temperatures (20-33°C) while swimming in a wide range of water temperatures (2.8-31°C). Although bluefin tuna maintain the brain, eyes, skeletal muscle and viscera well above ambient temperatures, the myocardium must function at ambient temperatures. Direct measurement of heart rate in our laboratory indicates that bluefin hearts function from 5 to 30°C. How bluefin hearts maintain function over large temperature changes remains unexplored. Maintenance of high heart rate may rely on increased use of intracellular Ca^{2+} stores for excitation-contraction coupling. In this study we used the fluorescent dye, fura-2, to compare Ca^{2+} uptake in ventricular sarcoplasmic reticulum (SR) vesicles of bluefin, yellowfin and albacore tuna. The rate of Ca^{2+} uptake by the SERCA 2 isoform of the Ca^{2+} ATPase in all tuna showed a decrease in pump activity at colder temperatures. The rate of SR Ca^{2+} uptake in bluefin ventricle was higher than that of yellowfin and albacore at all temperatures tested. Western blots using an antibody specific to SERCA 2 revealed that bluefin tuna ventricles contained the highest density of Ca^{2+} pump. SERCA 2 in slow-twitch muscles has a 10-fold higher activity than in ventricles, and was found to be less sensitive to temperature. Support: Pew Foundation.

37.5

Measurement of Ca^{2+} release transients in Cardiac Myocytes of Tuna and Mackerel using Confocal Microscopy

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Tunas are renowned for their endothermy and high metabolic rates. To meet these high metabolic demands, tuna hearts are large, have high maximal heart rates and produce large cardiac outputs compared with other teleosts. The cellular specializations responsible for increased cardiac performance in tunas are largely unknown. We hypothesize that maintenance of a high cardiac output may rely on an increased use of intracellular Ca^{2+} stores during myocyte excitation-contraction coupling. In this study we use confocal microscopy to compare enzymatically-isolated myocytes from yellowfin tuna, bluefin tuna and Pacific mackerel. Staining of tuna and mackerel atrial and ventricular myocytes with the membrane-selective dye, di-8-ANEPPS, revealed a lack of t-tubule membrane invaginations in these cells. Using line-scans, electrically evoked Ca^{2+} transients were recorded in Fluo-4 loaded myocytes. Preliminary results indicate Ca^{2+} transients in ventricular myocytes from yellowfin tuna and mackerel have similar kinetics with durations between 1.5 and 2.6 sec., rise times of ~250 msec. and single exponential decays (t~400 msec). The addition of 10 M ryanodine caused a 1.78 fold increase in the rise time in both species consistent with the blocking of sarcoplasmic reticululm (SR) Ca^{2+} release channels. These results indicate the presence of peripherally distributed SR in tuna and mackerel myocytes, which may contribute to increased cardiac performance.

37.6

Vascular Anatomy of SkipJack Tuna Gills

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Tunas exhibit numerous adaptations for increased aerobic capacity. In this study, the vascular anatomy of skipjack tuna, *Katsuwonus pelamis*, gills was examined by light and scanning electron microscopy of methyl methacrylate vascular replicas. Gill filaments contain three blood pathways, respiratory, interlamellar, and nutrient. Afferent and efferent filamental arteries (AFA and EFA), arterioles (ALA and ELA) and lamellae form the respiratory pathway. ALA in the basal filament are interconnected forming a vascular arcade supplying multiple lamellae. Four modifications of the lamellar circulation were evident. 1) ALA deliver blood directly to outer lamellar margin. 2) Pillar cells are closely aligned along outer boundary of inlet side and inner boundary of outlet side of lamellae forming multiple distributing and receiving blood channels. 3) Pillar cells between outer and inner boundaries are aligned forming diagonal channels to direct blood from the outer to the inner lamellar margins. 4) Lamellae are widened near their efferent end to augment oxygen saturation of blood in the inner margin. These adaptations may decrease gill vascular resistance and maximize gas-exchange capacity. Distinct interlamellar and nutrient arterio-venous pathways are only slightly modified to accommodate increased lamellar density, indicative of their vital non-respiratory homeostatic functions. NSF IBN 9723306 & Honolulu Lab, Natl. Marine Fisheries, NOAA.

37.7

Transvascular and Intravascular Fluid Transport in Rainbow Trout

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The kinetics of transvascular and intravascular fluid transport in fish capillaries are unknown. Cannulas were placed in the dorsal aorta (DA) and caudal vein (CV) of spleenectomized rainbow trout, *Oncorhynchus mykiss*, and 24 hr later a peristaltic pump completed the extra-corporeal A-V circulation. DA hematocrit (Hct) was measured prior to and at 5 min intervals for 1 hr following 1 min CV infusion of saline (SI) or plasma (PI) equal to 40% of plasma volume (PV), or hemorrhage (H) of 20 or 35% of blood volume. PV declined exponentially after infusion with a half-time of 6.6 (SI) or 15.3 (PI) min and reached a new steady state of 28.1 (SI) and 27.3 (PI) ml/kg. Whole-body transcapillary filtration rate was 1.1 (SI) and 0.9 (PI) ml/kg-mmHg-min and interstitial compliance was estimated to be 7.4 (SI) and 6.4 (PI) ml/mmHg-kg. Hct rapidly (<5 min) fell following 20 or 35% H, indicative of fluid mobilization from a low Hct microcirculation into the macrocirculation. Hct continued to slowly fall with a half-time identical to PI, indicative of transcapillary fluid reabsorption. Thus both the microcirculation and interstitium are active fluid reservoirs. Although trout interstitial compliance is similar to that of mammals, the rate and magnitude of transcapillary fluid flux and its sensitivity to hydraulic pressure is considerably greater. This suggests that plasma colloid osmotic pressure is of minimal significance in piscine fluid balance. Support: NSF IBN 9723306.

37.8

The -Adrenergic Receptor System of the Rainbow Trout.

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The -adrenoceptor (-AR) system is well characterized in mammals given its medical and pharmaceutical importance. Our understanding of this system in fish is based only on the use of mammalian-AR specific agonists and antagonists. The objective of this study was to characterize the -AR system from the rainbow trout (*Oncorhynchus mykiss*) at the molecular level and relate this information to receptor pharmacology. Partial cDNA clones of trout -ARs were amplified from total RNA using nested RT-PCR and degenerate primers. The sequence of perspective trout -AR clones were compared with sequences of -ARs reported in GenBank. Complete coding regions of the trout -ARs were obtained using 5' and 3' RACE with gene-specific primers. Standard radiolabeled binding and displacement assays (using the mixed antagonist ^{3}H-CGP) were undertaken in isolated red blood cells and semi-purified liver membranes. The three trout -AR genes identified were phylogenetically most similar to the mammalian 2-AR (RT 2-AR) and the 3-AR (RT 3a and 3b-ARs); no homologous 1-AR was found. RNase protection assays showed specific tissue distributions for each trout -AR type. Pharmacologically the RT 2-AR is similar to the mammalian 2-AR, but the RT 3-AR is 'non-typical'. These studies support a unique -AR system in this early branching vertebrate and provide some understanding of the evolution and regulation of this gene family. This study was supported by grants to TWM and GD from NSERC Canada.

37.9

Stretched Dog and Pig Femoral Arteries Relax to Acetylcholine Through Different Endothelium-dependent Mediators

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The endothelium-dependent relaxation of dog femoral arteries is mediated by endothelium-derived hyperpolarizing factor (EDHF) in stretched vascular rings and by nitric oxide (NO) and/or eicosinoids in chemically-activated rings. We hypothesized that pig femoral artery rings would utilize similar mediators. Endothelium-intact rings stretched to 15.2±0.9 g total tension or chemically-activated (10^{-7} M phenylephrine (Phe) to 12.5±0.7 g were challenged with 10^{-6} M Ach, an endothelium-dependent dilator, and sodium nitroprusside (SNP), an endothelium-independent dilator. SNP (10^{-6} M) relaxed stretched rings by 12.1±1.1 g and Phe-activated rings by 11.7±0.7 g. Ach relaxed stretched rings and Phe-activated rings to 60±8% and 83±8% of the respective SNP relaxation. To determine the nature of the endothelial-derived products involved, Ach-induced relaxation was evaluated before and after inhibition of the synthesis of eicosanoids (10^{-5} M indomethacin & 10^{-5} M nordihygroguariaretic acid) and NO (10^{-3} M Nw-nitro-L-arginine). The contribution of endothelium-derived hyperpolarizing factor was identified by blocking calcium-activated potassium channels (10^{-8} M iberiotoxin). Ach-induced relaxation of stretched rings was attenuated by inhibition of NO and/or eicosanoid synthesis but was unaffected by the presence of iberiotoxin. Ach-induced relaxation of Phe-activated rings was only attenuated by inhibition of NO synthesis. Therefore, we reject our hypothesis since Ach-induced relaxation was mediated by different factors in the pig than in the dog.

Funded in part by NSERC

37.10

The Importance Of Preload On Cardiac Performance In Bullfrogs And Turtles

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The importance of preload on cardiac performance is not well established in amphibians or reptiles. We determined the dependence of cardiac performance on filling pressures in bullfrogs, *Rana catesbeiana* and turtles. *Trachemys scripta* by manipulating vascular volumes in anesthetized animals. *Rana* was anesthetized with MS222, and *Trachemys* with Isoflurane. Mean circulatory filling pressure (MCFP) was assessed as the venous pressure during temporary occlusion of all cardiac outflow tracts. In both species, MCFP was approximately 3 cmH2O. With volume loading MCFP increased in a roughly linear fashion up to 5 cmH2O, and central venous pressures were consistently 1-2 cmH2O lower than MCFP in both species. Increased MCFP pressures increased cardiac output (CO) approximately 20% in both species, with the effect maximized early in the volume load. A lowering of MCFP by blood withdrawal had dramatic effects in both species, decreasing CO approximately 50% with a 1.5 cmH2O decrease. In both species changes in CO were due almost completely to stroke volume, heart rate remaining essentially constant. MCFP was very sensitive to volume withdrawal indicating the system is operating near the unstressed volume. Critical closing pressure was similar in both species, approximately 6-7 cmH2O (determined as the arterial pressure during outflow occlusion). Supported by NSF IBN-0078094 (SJW), NSF IBN- 0110322(DCJ) and the Danish Research Council (TW).

37.11

Delayed Depolarization of the Cog-Wheel Valve and Pulmonary-to-Systemic Shunting in Alligators

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Alligators and other Crocodilians have a cog-wheel valve located in the subpulmonary conus. Active closure of the valve during each heart beat can occlude pulmonary flow, causing a secondary rise in right ventricular (RV) pressure such that RV blood flows into the left aorta and systemic circulation rather than to the lungs (pulmonary-to-systemic shunt). To better understand control of the valve, we examined depolarization of the RV and valve muscle, and the resultant RV, pulmonary and systemic pressures in anesthetized American alligators (*A. mississippiensis*). Depolarization swept across the RV from the apex towards the base (and valve) at 91±23 cm s^{-1}; the valve ECG trailed by 248±28 ms (6-35% of a cardiac cycle) suggesting a nodal delay at the RV-valve junction. The degree of delay would limit blood flow to the lungs and thus control shunting. Left vagal stimulation reduced the delay (-20%) while direct application of acetylcholine had a variable effect (-40 to +60%). Both stimuli reduced the integrated valve ECG (vagal by 10-20%, ACh by 10-100%). When the valve muscle was killed its ECG was absent, the secondary rise in RV pressure was abolished (the RV tracked the low pulmonary pressure) and shunting did not occur. This study provides additional, direct evidence that phasic contraction of the cog-wheel valve muscle controls shunting, and that vagal and cholinergic stimulation alter the delay and strength of valve depolarization and thus the propensity to shunt. NSERC.

37.12

Regulation of systemic resistance and changes in blood flow distribution in the red-eared slider (*Trachemys scripta*) during anoxic submergence

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Cold acclimation and, to a greater degree, anoxic exposure leads to an increased systemic vascular resistance in the anoxia-tolerant turtle *Trachemys scripta*. We investigated this response in 5 and 21°C acclimated turtles through vascular injections of the -adrenergic receptor agonist and antagonist, phenylephrine and phentolamine, while monitoring arterial blood pressure and systemic blood flow ($\dot{Q}_{sys}$). We also investigated whether certain tissues were preferentially perfused during the large depressions in ($\dot{Q}_{sys}$) occurring with acute and chronic anoxic exposure through the injection of strategic coloured microspheres into the left atrium, thus allowing for determination of blood flows to the skin, muscle, bone, esophagus and stomach, intestines, spleen, ventricle, atria, brain, liver, kidney, gonads, fat, connective tissue, shell, and eyes under normoxic and anoxic conditions. The anoxia-mediated systemic vasoconstriction occurred at both temperatures, but a tonic -adrenergic tone was only present during anoxia at 21°C. Acute anoxic exposure at 21°C resulted in decreases in absolute flow to the intestines, stomach, liver, fat, bone, and muscle and decreases in the relative perfusion of the liver, stomach and intestines. In contrast, the relative perfusion to the skin, bone and shell increased. Absolute blood flow to all tissues decreased during anoxia at 5°C, but the relative perfusion of the liver and shell increased. Funded by NSERC, the Danish Research Council and a SFU Graduate Fellowship awarded to J.A.W.S.

37.13

Molecular Diagnostic in Long QT Syndrome in Mexican Patients

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Cardiac arrhytmias are a common cause of morbidity and mortality, accounting for approximately 11% of all natural deaths.

Long QT Syndrome (LQTS) is a familial disease characterized by abnormally prolonged ventricular repolarization and a high risk of malignant ventricular tachyarrhythmias. We described underwent molecular and clinical diagnostic a mexican patient with LQTS. Patient presented recurrent syncope despite with beta blockers and an implantable cardiovascular defibrillator used in high-risk patients. We identified with genetic approaches a new mutation on SCN5A gene encode an anormal Na+ channel protein. This mutation gene can produce disorder with QT prolongation, morphologic changes in the T waves and a relative high frequency of syncope and sudden death.

37.14

Kidney of Giraffes: Hypertensive Ruminants

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This is a study of aspects of renal structure of the giraffe with implications as to function. The hitherto unpublished manuscript includes 44 typewritten pages, 6 tables and 39 figures on 8 plates.

About 4,000 collecting ducts open at the truncated end of a curved crest which juts into the renal pelvis as inner medulla. Extensions of the pelvis pass between the medullary and the vascular processes almost to the corticomedullary border. The medullary processes contain inner medulla and outer medulla with its clusters of capillaries (vascular bundles) The vascular processes, on the other hand, contain the interlobar arteries and veins.

Inner medulla and almost all outer medulla, with its vascular bundles, are bathed inevitably with pelvic urine.

Cortex is 63% of the parenchyma. Outer medulla is nine times the mass of inner medulla. Inner medulla is 4% of the parenchyma. Ratio of mass of adult cortex to medulla is 1.7 to 1.0. Number of glomeruli per kidney is 6.6×10^6. Glomerular mass is 6.2 to 6.7% of renal mass in the adult and 5.2% in the 6-month calf. Dimensions of glomerular capsules are the same across thickness of cortex.

Every terminal colleging duct drains, by estimate, 1,650 nephrons.

Ratio of thickness of muscularis of main renal artery to its diameter, in adult giraffe, is 0.117 to 0.132, which is close to that in rhinoceros and ox but greater than in man. Visceral arteries (celiac, anterior mesenteric, renal) have about the same ratio of muscularis/diameter.

The inborn arterial hypertension is discussed. Atherosclerosis is absent. Serum lipid fractions are low.

This research was supported by the Kidney Foundation of Ohio.

37.15

Cardiac Hormone as a Protection Against Volume Overload

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In hypo-osmotic freshwater environment, fish face a threat of volume overload, while in hyperosmotic seawater teleosts tend to lose water. To maintain the volume and electrolyte balance, fish utilize effective regulatory mechanisms, like natriuretic peptide hormones. Salmon cardiac peptide (sCP) is a new member of the family of natriuretic peptides. The potential role of sCP as a volume-regulating hormone was studied in salmon (*Salmo salar*). Intra-arterial bolus administration of synthetic sCP resulted in volume-depleting action by increasing urine output. The sodium excretion increased in proportion to the enhanced urine flow. On the other hand, acute volume expansion of fresh water salmon with saline elevated the plasma level of sCP. Additionally, sCP secretion from isolated salmon ventricle was rapidly increased by mechanical load. In contrast to the stimulating effect of plasma volume expansion on circulating sCP, an acute transfer of salmon from fresh to sea water resulted in a increased plasma osmolality and a decreased circulating sCP level. The biological effects of sCP suggest that it has an important part in the regulation of teleost fluid balance. The volume expansion-induced elevation of circulating sCP level indicates that sCP is an homeostatic mechanism defending salmon against volume overload. This work was supported by the Academy of Finland, Sigrid Juselius Foundation, Biocenter Oulu and the Research and Science Foundation of Farmos.

RESPIRATION AND ACID-BASE

38.1

Laplace's Law and the Alveolus: A Misconception of Anatomy and a Misapplication of Physics

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Both the anatomy and the mechanics of inflation of the alveoli, as presented in most textbooks of physiology, have been misunderstood and misrepresented. The typical representation of the acinus as a "bunch of grapes" bears no resemblance to its real anatomy. The alveoli are not independent little balloons. Because of the prevalence of this misconception, Laplace's law, as it applies to spheres, has been invoked as a mechanical model for the forces of alveolar inflation and as an explanation for the necessity of pulmonary surfactant in the alveolus. The alveoli are prismatic in shape, i.e., their walls are flat, and Laplace law considerations in their inflation apply only to the curved region in the fluid where these walls intersect. Alveoli do not readily collapse into each other because they are suspended in a matrix of connective tissue "cables" and share common, often perforated walls so there can be no pressure differential across them. Surfactant has important functions along planar surfaces of the alveolar wall and in mitigating the forces that tend to close the small airways. Laplace's law as it applies to cylinders is an important feature of the mechanics of airway collapse but is not relevant to the individual alveolus.

38.2

Comparison of Oxygen Carrying Capacity of A New Perfluorocarbon (PFC) Blood Substitute in Rats Breathing Room Air or 100% Oxygen

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Oxycyte™, a new PFC-based blood substitute and therapeutic oxygen carrier, has been formulated with a PFC selected to make a stable, sub-micron emulsion, and to avoid the pulmonary toxicity and environmental ozone depletion risks associated with other PFC's. The present study was done to compare the oxygen carrying capacity of 60% w/v Oxycyte in vivo in rats breathing either room air or 100% oxygen. Male Sprague-Dawley rats weighing 320 to 360 grams were anesthetized and instrumented. Following removal of 30% of their blood volume, animals were transfused with an equal volume of a 50:50 mixture of Oxycyte and 10% pentastarch. Arterial blood gases and hemoglobin levels were measured in each animal before blood removal and 5 to 10 minutes after transfusion. Hemoglobin went from 13.4 g/dl at baseline to 8.3 g/dl post-transfusion in room air animals, and from 7.74 g/dl at baseline to 5.37 g/dl post-transfusion in 100% oxygen animals. PaO_2 in room air animals was 67.3 mmHg at baseline and 66.05 mmHg post-transfusion. In animals breathing 100% oxygen, PaO_2 increased from 321.2 mmHg at baseline to 378.5 mmHg post-transfusion. Oxycyte maintained arterial oxygen at pre-bleed levels in animals breathing room air despite a blood loss-induced 38% drop in circulating hemoglobin. In animals breathing 100% oxygen, arterial oxygen increased 18% despite a blood loss-induced drop in hemoglobin of 31%. We conclude that 60% Oxycyte is an effective oxygen carrier that warrants further study.

38.3

Avian Intrapulmonary Chemoreceptors: Role of L-type Calcium Channels in CO_2 Sensing.

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Rate and depth of breathing in birds is influenced by neural feedback from the vagal afferents of avian intrapulmonary chemoreceptors (IPC). IPC are inhibited by high CO_2 and excited by low CO_2, unlike most vertebrate respiratory CO_2 chemoreceptors. Additionally, most IPC afferents show partial spike frequency adaptation (SFA) to step CO_2 stimuli which is surprisingly increased by sK(Ca) blocker apamin and unaffected by BK(Ca) channel blocker charybdotoxin. Intravenous infusion of inorganic Ca^{++} channel blockers $CdCl_2$ or $CoCl_2$ increases IPC afferent discharge at high PCO_2 and attenuates or abolishes IPC response to CO_2 steps. To further test the role of Ca^{++} channels in IPC response to CO_2 we measured action potential rate (F_{IPC}) from single unit vagal IPC afferents in anesthetized *Anas platyrhynchos*. We inhibited L-type channels with nifedipine (i.v. 0.4-4.0 g/kg) and measured IPC afferent response to CO_2. There was no significant effect of nifedipine on SFA, but overall F_{IPC} increased ($p<0.05$, n=7). These data are most easily explained if IPC afferents are second-order neurons that synapse on a primary CO_2 sensor, somewhat like carotid or neuroepithelial bodies (Egan, Hempleman, 2001). Nifedipine may reduce Ca^{++}-induced release of an inhibitory neurotransmitter from primary CO_2 chemosensory cells that synapse with tonically active IPC afferents. Support: NSF 9723783.

38.4

Central Glutamatergic Control of Cardioventilatory Function in Catfish.

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Microinjections (36-108 nl) of kynurenic and kainic acid (10 mM) into sensory areas (primary general visceral nucleus, nGV) in the brainstem of anesthetized channel catfish (Ictalurus punctatus) were used to examine the control of cardioventilatory function and central integration of O2-sensitive afferent information. Ventilatory rate and amplitude, heart rate and blood pressure were recorded during normoxia and hypoxia (40 torr) before and after bilateral microinjections of kynurenic acid (a nonselective, NMDA/AMPA glutamate antagonist) and kainic acid (for chemical lesion of neurons) into the nGV. Kynurenic acid abolished the ventilatory response to hypoxia but did not affect normoxic ventilation. Normoxic heart rate was significantly reduced but there was still a significant hypoxic bradycardia after kynurenic acid. Blood pressure was unaffected. The lesion abolished or severely reduced normoxic and hypoxic ventilation. Resting heart rate decreased and there was no longer a hypoxic bradycardia. Blood pressure was unaffected. Control injections of mock CSF had no effect. The results show that ionotropic glutamate receptors in the nGV mediate cardiac and ventilatory hypoxic reflexes in channel catfish. The effect of nGV lesion demonstrates that this region is essential for maintaining normal ventilation.

38.5

Function of the postpulmonary septum in lung ventilation in *Varanus*.

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Monitor lizards (genus *Varanus*) are unique among the Squamates in possessing a postpulmonary septum (PPS). The PPS is a nonmuscular membrane originating from the inner body wall, which adheres closely to the posteroventral surface of the lungs. We investigated the role the PPS may play in lung ventilation by measuring gas exchange and ventilatory airflow during treadmill exercise in juvenile savannah monitors (*V. exanthematicus*, body mass range = 160-610g) before and after surgical removal of the PPS.

Maximum rate of oxygen consumption (VO_2max) was significantly lower in experimental animals (27.9 $mlO_2/kg/min$) as compared to sham-operated controls (33.3 $mlO_2/kg/min$). This was reflected in 26% reduction of locomotor endurance following PPS removal – as measured by time to exhaustion at 3 km/h. experimental animals maintained steady locomotion for 139±34 s, compared to 189±36 s of sham-operated controls. Breath-by-breath analysis of the ventilatory pneumotachograph trace showed that the tidal volume of costal inspiration (TV_t) during locomotion is reduced by PPS removal – at the maximum sustainable speed (1.5 km/h), TV_t averaged 97 ml/kg in controls, and 79 ml/kg in experimental animals.

The PPS appears to help provide structural support to the caudal region of the heterogeneously partitioned lungs of monitor lizards. Without the PPS, the poorly partitioned caudal lung regions collapse (as visualized by videoradiography) and cannot be fully inflated by costal means. This reduces ventilatory airflow, constrains respiratory gas exchange, and ultimately limits the animals' aerobic performance. Whether the PPS plays other roles in venous return or lung perfusion remains to be investigated.

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38.6

Pre-exercise Inhalation of Nedocromil sodium (an Inflammatory/mast Cell Stabilizer) Does Not Mitigate Exercise-Induced Arterial Hypoxemia (EIAH) in Thoroughbred Horses

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In view of the suggestion that pulmonary injury (capillary stress failure) induced release of histamine/other chemical mediators from airway inflammatory/mast cells contributes to EIAH, we examined the effects of pre-exercise inhalation of an airway inflammatory/mast cell stabilizer (nedocromil sodium) on EIAH and desaturation of hemoglobin in horses. Seven healthy, sound, exercise-trained Thoroughbreds were studied in the placebo and nedocromil sodium (30 puffs = 60 mg) inhalation (15 min pre-exercise) experiments carried out in random order, 7 days apart. Blood-gas data were obtained at rest and during maximal exercise, and were corrected to simultaneously measured core temperature. In both treatments, all horses experienced pulmonary hemorrhage, indicating capillary stress failure had occurred. Significant ($P<0.0001$) EIAH of a similar magnitude developed by 30s of maximal exertion in both treatments, but further significant changes in arterial O2 tension were not observed as exercise duration progressed to 120s. Thus, similar to pretreatment with IV dexamethasone (an anti-inflammatory agent; JAP in press, 2002) and with H1 receptor antagonist - tripelennamine HCl (JAP 92: 1515-1523, 2002), pre-exercise nedocromil sodium inhalation was also ineffective in mitigating EIAH. These findings argue against the pulmonary injury evoked airway inflammatory/mast cell histamine/other chemical mediator(s) release in bringing about EIAH in racehorses. Supported by HERO & USDA-Hatch funds.

38.7

The Physiology of Overwintering in the Common Snapping Turtle (*Chelydra serpentina*) and the Softshell Turtle (*Apalone spinifera*)

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Chelydra serpentina and *Apalone spinifera* were submerged in anoxic and normoxic water at 3 °C. Periodic blood samples were taken and PO_2, PCO_2, pH, $[Na^+]$, $[K^+]$, $[Cl^-]$, total Ca, total Mg, [lactate], [glucose], and osmolality measured; hematocrit and weight gain determined; and plasma $[HCO_3^-]$ calculated. PCO_2 of *C. serpentina* submerged in normoxic water fell from 10.83 to 6.91 mmHg after 125 days, buffering a slight increase in lactate and allowing the turtles to maintain a constant pH. In *A. spinifera* submerged in normoxic water, a respiratory alkalosis developed (pH 8.195, PCO_2 5.49 after 10 d) early and persisted throughout. Anoxic *C. serpentina* had a rapid increase in lactate from 1.8 to 168.1 mM after 100 d. Associated with the increased lactate was a decrease in pH from 8.057 to 7.132 and $[HCO_3^-]$ from 51.48 to 4.90 mM, and an increase in total Ca, total Mg, and $[K^+]$. Anoxic *A. spinifera* after 11 d of submergence had a fall in pH from 7.923 to 7.281 and lactate increased to 62.1 mM. Plasma $[HCO_3^-]$ fell from 34.57 to 4.53 mM. Plasma $[Cl^-]$ fell while $[K^+]$ and total Ca & Mg increased slightly. We suggest that *C. serpentina* represents a second species of turtle that is tolerant of anoxia and, therefore, is able to exploit habitats unavailable to some other species; while *A. spinifera* are an anoxia-intolerant species of turtle restricted to hibernacula that are unlikely to become hypoxic or anoxic. Funded by NSF IBN-0076592 (to GRU) and IBN-9728794 (to DCJ).

38.8

Anemia: a basis for the cost of reproduction?

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In humans, anemia is clinically defined as a decrease in hematocrit of two standard deviations from the mean for 'healthy' individuals, and can be associated with trauma (rapid blood loss), tissue disease or nutrient (iron) deficiency. By this definition birds routinely become anemic during reproduction, specifically during egg formation; the causes and consequences of this are unknown. We measured hematocrit, plasma osmolarity and yolk precursor levels during reproduction over 4 years (1998-2001) in a small passerine (*Sturnus vulgaris*). In all four years hematocrit decreased from normal non-breeding values (50-54%) to 46-49% at the 1-egg stage of laying; in two years birds were clinically 'anemic' during laying. Despite high plasma levels of yolk precursors during laying plasma osmolarity decreased from 322 mmol/kg in non-breeders to 302 mmol/kg at the 1-egg stage, remaining low through laying. This suggests that decreased hematocrit during laying was a dilution effect perhaps due to other physiological adjustments required for egg formation. However, although plasma osmolarity returned to pre-breeding levels at clutch completion (323 mmol/kg), hematocrit did not increase at this point and in some years it remained low through incubation and chick rearing. Hematocrit has been shown to be positively related to aerobic performance and we suggest that the decrease in hematocrit associated with egg formation might provide another mechanism to explain the 'cost of reproduction'.

38.9

Cutaneous CO^{18} (and thus O_2) Diffusing Capacity Decreases in Response to Dehydration in the Toad, *Bufo Woodhouseii*

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Dehydration presents toads with a dilemma - cutaneous water loss may be minimized by reducing skin blood flow, but this may also restrict cutaneous gas exchange. To determine how the xeric-adapted toad *Bufo woodhouseii* resolves this dilemma, the effect of dehydration on cutaneous gas exchange was determined. Cutaneous gas exchange (MO_2, MCO_2) and carbon monoxice (CO) diffusing capacity ($D_{Skin}CO$) were examined in unanesthetized toads. Blood gases were measured separately. In fully hydrated toads (mass 36.6±10.4 g) at 23-25°C, cutaneous gas exchange values were: MO_2=1.43±0.47 $mg.g1.h-1$, MCO_2 = 1.75±0.85 $mg\cdot{}^{-1}h^{-1}$, and R=1.36±0.56 (n = 6, x±1 s.d). $D_{Skin}CO$ was 0.218±0.013 $mg\cdot{}^{-1}h^{-1}mmHg$. Following an enforced 20-25% loss of body water, $D_{Skin}CO$ fell by 50% to 0.127±0.04 $mg\cdot{}^{-1}h^{-1}mmHg$, but cutaneous MO_2, MCO_2 and R were unchanged at 1.48±0.15 $mg\cdot{}^{-1}h^{-1}$, 1.72±0.29 $mg\cdot{}^{-1}h^{-1}$and 1.13±0.08 $mg\cdot{}^{-1}h^{-1}mmHg$, respectively. Arterial (sciatic) PO_2, about 90-100 mmHg, remained unchanged by dehydration, but arterial PCO_2 increased about 2.5 X from 7±2 up to 17±7 mmHg. The fall in $D_{Skin}CO$ during dehydration results from decreased cutaneous blood flow due to the need to reduce transcutaneous water loss (W.B., unpubl.). Yet, cutaneous MCO_2 is maintained under these conditions by a greatly increased PCO_2 diffusion gradient across the skin. Thus, *Bufo woodhouseii* appears able to restrict cutaneous blood flow to conserve water without compromising cutaneous CO_2 loss.

38.10

Effects of Chronic Cold and Submergence on Blood Oxygen Transport in Hibernating Map Turtles.

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Adult turtles (*Graptemys geographica*) at 3°C were chronically exposed (42-50 days) to one of three conditions: *air-breathing* (AB); *normoxic submergence* (NS); and *hypoxic* (PO_2 = 49 Torr) *submergence* (HS). Blood acid-base properties were determined for anaerobic samples at 3°C; isocapnic O_2 equilibrium curves (O_2EC) were generated at 3°C using thin blood film techniques. NS turtles exhibited a slight respiratory alkalosis (pH = 8.03; PCO_2 = 9.5 Torr; [Lact] = 2.2 mM/l) relative to AB animals (7.89; 13.4 Torr; 1.1 mM/l). NS turtles also revealed 47% increases in [Hb] and Hct. HS animals, on the other hand, experienced a profound metabolic acidosis (pH 7.30; PCO_2 7.9 Torr; [Lact] 81 mM/l) and no change in O_2 capacity. Half-saturation pressures (P_{50}) were: AB, 6.5 Torr; NS, 5.3; and HS, 6.5 at their respective blood pH values. CO_2-Bohr slopes ($\Delta\log PO_2/\Delta pH$) at P_{50} were -0.15, -0.16 and -0.07 for AB, NS and HS turtles, respectively. Furthermore, the Bohr coefficients were substantially depressed at the lower Hb-O_2 saturations (SO_2) for all groups. O_2EC shape was similar among the three animal groups at SO_2 below P_{50} (Hill's n = 1.7-1.9). Above P_{50}, Hill coefficients remained at 1.7-1.9 for AB and NS turtles, but declined to 1.1 for HS animals. Results suggest that increases in Hb-O_2 affinity and O_2 capacity, and reduced Bohr effect at lower SO_2 promote aerobic metabolism in normoxic water during winter hibernation. (Supported by NSF grant IBN-0076592 and DePaul Univ. LAS & URC grants.)

38.11

Modulation of Periodic Breathing by Altered Patterns of Lung Inflation in an Amphibian, Bufo marinus

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This study examined the role of pulmonary stretch receptor feedback in modifying the pattern of fictive ventilation in decerebrate and artificially-ventilated Cane toads. Ventilatory activity was recorded as motor output from the trigeminal nerve to the buccal musculature. The animals were tidally ventilated with 2.5% or 5.0% CO2. Tidal ventilation was either continuous or was triggered by the fictive breaths. Continuous ventilation produced a greater frequency of breathing than did ventilation triggered by the individual breaths. Increasing the duration of time that the lungs were inflated during triggered ventilation also increased fictive breathing frequency. Altering the pattern of phasic PSR feedback by switching from trigeminal-triggered ventilation to continuous ventilation at the onset of a breathing episode resulted in a greater number of fictive breaths per episode than did switching from continuous to triggered ventilation. Instantaneous frequency of breathing increased progressively during individual breathing episodes. The results suggest that an increased level of phasic pulmonary stretch receptor feedback enhances breathing frequency by increasing both the number of episodes per minute and the number of breaths per episode. The results also suggest that a multi-breath episode is a self-propagating phenomenon and that phasic PSR feedback exerts its effect on the breathing episode as a whole rather than on individual breaths.

OSMOTIC AND IONIC REGULATION

39.1

FLUORESCENT MEASUREMENT OF CALCIUM TRANSPORT IN CRUSTACEAN CELLS.

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Calcium (Ca) uptake in crustaceans has been studied at the whole animal level and using isolated membrane vesicles. Ca transport measurements using fluorescent markers is a promising technique for use in crustacean cells since the methodology requires minimal amounts of material. Here we measured Ca uptake by isolated cell suspensions of the stenohaline lobster (H. americanus) and the euryhaline blue crab (C. sapidus). Tissues from hepatopancreas (HP), antennal glands (AG) and ovaries (OV) were separated into cell suspensions. The dye Fluo-3 was equilibrated with the cells in a saline without Ca. After, known concentrations of Ca were added and the Ca-induced changes in fluorescence was measured. Ca influx by lobster AG was a biphasic function of [Ca], suggesting a Michaelis-Menten function combined with an apparent diffusional process. Amiloride (Al) partially reduced the carrier process, while verapamil (Vp) abolished the linear component of uptake. Similar biphasic uptake functions, sensitive to both Al and Vp, were found for cell suspensions of crab HP and OV. However, the transport rates for the crab tissues were lower than those displayed by lobster AG, probably reflecting differences in Ca ionic regulation capabilities. The results also suggest the presence of Al-sensitive and insensitive Ca carrier proteins and Vp-inhibited Ca channels in all studied tissues. Supported by CNPq (Brazil) and NSF (USA)-(FPZ-91.0114/97-8; MGW-IBN 9870374) and (GA-IBN99-74569).

39.2

Expression of PMCA3 mRNA and Protein in Crustacean during Molting

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The crustacean molting cycle is an ideal non-mammalian model system to study proteins involved in transepithelial Ca2+ transfer and the regulation of expression of the genes that encode them since the transepithelial Ca flux, which is negligible in intermolt, changes in magnitude and directionality around ecdysis. Plasma Membrane Ca ATPase (PMCA) is a Ca motive enzyme that is responsible for basolateral Ca efflux from cytoplasm into the extracellular fluid that may be upregulated during postmolt Ca net influx. Our previous work has indicated that there are 4 separate PMCA genes in crustaceans that generate multiple isoforms. Work on gill, muscle and hepatopancreas has indicated that PMCA2, PMCA3 and PMCA4 and their isoforms show distinct expression patterns with dynamic changes occurring during molting; PMCA1 meanwhile appears to be a housekeeping gene. For example, the two isoforms of PMCA3 were highly expressed during pre- and postmolt in all three tissues whereas intermolt expression was low. In the present study, we attempted to assess whether PMCA3 protein expression was correlated with mRNA levels. Western analysis using a homologous polyclonal antibody against PMCA3, clearly showed that the expression of PMCA3 protein was very similar to the expression of PMCA3 mRNA in muscle, gill and hepatopancreas. Additionally, the expression of both protein and mRNA of PMCA3 in kidney and heart during molting followed the same pattern. The molecular cloning and characterization of the PMCA3 gene will be presented. Supported by NSF grant IBN 0076035 to MGW.

39.3

Gill Na^+/H^+ Exchangers (NHE) in Marine and Freshwater Adapted Fish

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The branchial epithelium in fish is the main site of acid-base transfers. Net acid excretion is thought to be driven in part by Na^+/H^+ exchange proteins (NHE) in a fashion analogous to the mammalian renal tubule. We have cloned and sequenced full length cDNA transcripts for NHE2 isoforms from the gills of the marine long-horned sculpin (*Myoxocephalus octodecimspinosus*), mummichog (*Fundulus heteroclitus*), and spiny dogfish shark (*Squalus acanthias*). The open reading frames (2150-2250 bp) exhibited 54-68% amino acid homology to each other as well as rat NHE2. Q-PCR revealed that mRNA for sculpin NHE2 increased by 2.5x within 2 hours of an acid infusion and corresponded to an elevation of *in vivo* net H^+ transfer. Western analysis using heterologous antibodies indicated that NHE3 expression doubled in seawater (SW) adapted *F. heteroclitus* following one hour of hypercapnic acidosis. NHE2 was not consistently detected in SW fish, but expression of this isoform increased in freshwater (FW) adapted, hypercapnic animals. *In situ* hybridization studies on *S. acanthias* with an NHE2 specific RNA probe showed a population of gill epithelial cells expressing NHE2 mRNA that were also Na^+-K^+-ATPase immunoreactive. We hypothesize that during acidosis, apical gill NHE activity in mitochondrial rich cells enhances net H^+ transfer to the water in these species. Surprisingly, NHE may also play a role in acid-base regulation in the FW adapted mummichog. Supported by NSF-IBN-0111073 to JBC & AIMS.

39.4

Regulatory Volume Decrease and Increase in Northern Fur Seal Red Blood Cells

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Red blood cells (RBCs) of northern fur seal are possibly exposed to hyperosmotic pressure due to ingestion of seawater. In the RBCs, ion excretion systems may develop which contribute to maintain the appropriate ion concentration and cell volume. Mature RBCs of northern fur seal have no Na, K-pump. The cellular cation composition in their RBCs is low K and high Na, the same as in their plasma. Thus, these cations are not the driving force in other ion transport. Here, the RBCs ion transport systems for regulatory volume increase (RVI) and regulatory volume decrease (RVD) were examined. In hyperosmotic medium, amiloride-sensitive Na uptake was enhanced in the RBCs, and did not require medium K or Cl, and thus might be due to Na/H exchange. The cell volume was restored in the hyperosmotic medium in which the main cation was Na, but not in the medium in which Na was replaced with N-methyl-D-glucamine or in the medium with amiloride. In the hyposmotic medium, both the quinidine-sensitive Ca uptake and medium Ca-dependent Na efflux were enhanced in the RBCs, thereby increasing the Na/Ca exchange transport. The cell volume was restored in hyposmotic medium with Ca, but not without Ca, and the RVD was partially inhibited by quinidine. Thus, it is suggested that the RVI and RVD in northern fur seal RBCs are performed by Na/H and Na/Ca exchange transports, respectively.

39.5

Localization and Molecular Characterization of the Crayfish NCX

LaTonia M Stiner, Zhiping Zhang, Ping Gao, Michele G Wheatly: Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435

The molting cycle of the freshwater crayfish Procambarus clarkii is ideal for studying cellular/molecular mechanisms of Ca homeostasis. During intermolt transepithelial Ca flux is negligible. In premolt cuticular Ca is reabsorbed and excreted. During postmolt crayfish exhibit net Ca uptake rates of 2-10 mmol/kg/h. The transporting epithelia of the crayfish are the gills, antennal gland, hepatopancreas and cuticular hypodermis. Intermolt kinetics suggests that the NCX is primarily responsible for basolateral efflux. Previous studies have shown the increased expression of basolateral Ca pumps during postmolt. Our goal was to characterize and quantify NCX expression during premolt and postmolt compared with intermolt. Immunohistochemical studies revealed NCX Ab crossreactivity with hepatopancreas, antennal gland, and cardiac muscle. Western analysis revealed the presence of one or both NCX bands from crude homogenate and partially purified samples from antennal gland and hepatopancreas. Sporadically both bands were visualized in muscle. We cannot definitively conclude nor exclude the possibility that binding visualized via immunohistochemical analysis was due to binding of our Ab to the NCX. Northern analysis is being used to quantify NCX expression at various molting stages. Funding from NSF IBN 0076035 to MGW.

WEDNESDAY PLENARY LECTURE: RAYMOND B. HUEY

40.0

PATTERNS OF SUCCESS AND DEATH IN HIMALAYAN MOUNTAINEERING

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Mountaineers have two pragmatic goals: to reach the summit, and to return home safely. On small mountains, most mountaineers achieve these goals. On the highest Himalayan peaks, however, extremes of hypoxia and cold (plus avalanches) exact a severe toll. As a result, most Himalayan climbers fail to summit, and some die.

Repeated disasters on Mt. Everest and K2 have publicized the dangers of mountaineering in thin air, but the actual risks of high-altitude mountaineering are only beginning to be quantified and analyzed. I will summarize several descriptive analyses of statistical patterns of success and death of mountaineers on the eight-thousander meter peaks. Such patterns provide insights into the limits of human physiological performance in an extreme environment and may be useful to mountaineers themselves.

In some cases, widely held views of risks are in error. I'll also review correlations between mountain height and rates of success and of death, the "allometric scaling" of climbing permit fees, and whether use of supplemental oxygen is correlated with enhanced survival.

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Examines correlations between summit height and rates of success and of death.

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Reviews success and survival data and estimates how convective heat loss is influenced by altitude.

DEVELOPMENTAL PHYSIOLOGY: PLASTICITY AND CONSTRAINTS

41.1

DUAL PURPOSE GENES AND THE REUNIFICATION OF PHYSIOLOGY AND DEVELOPMENT. Robert E. Maxson, Dept. of Biochemistry and Molecular Biology, USC/Norris Hospital, Univ. of Southern California School of Medicine, Los Angeles, CA 90033

The central question in developmental biology is how cells become specified to form the variety of tissues and organs in an adult. Adaptational physiology seeks to determine how organisms, including embryos, function under specific environmental constraints. Although to most modern practitioners, these disciplines are distinct, earlier biologists saw them as elements of a single continuum. Recent discoveries point toward intriguing commonalities in the genetic languages of development and physiology. Master regulatory genes that are known to control cell fate during embryogenesis also control genes with physiological functions, such as transport. Even more surprisingly, such physiological genes may also have roles in cell type specification. These findings underscore the need to consider development and physiology together when formulating ideas about modes of adaptive evolution.

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41.2

PHYSIOLOGY OF MARINE INVERTEBRATE DEVELOPMENT: STARVATION SURVIVAL AND METABOLIC REGULATION. Donal T. Manahan, Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 80089-0371 USA.

Energy reserves are often utilized at different rates in similar-sized embryos and larvae of marine invertebrates developing in different environments. This results in different mass-specific metabolic rates that have important ecological implications: e.g., permitting longer life spans for feeding stages when food is scarce. Using developmental stages of sea urchin embryos and other echinoderms from temperate and 'extreme' environments (Antarctica), we have examined ontogenetic changes in physiological processes that fuel development and establish metabolic rates. Measurements of the cost of development and the biochemical processes that determine these costs will be discussed (e.g., maintenance of ion gradients; rates of protein synthesis). How these metabolic costs are fueled will be reviewed (molecular physiology of amino acid transporters; utilization of exogenous nutrients vs. endogenous reserves). In particular, studies of embryos developing in 'extreme' environments have revealed some new biological mechanisms for physiological rate regulation during development.

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41.3

TEMPORARY SUSPENSION OF DEVELOPMENTAL PROGRAMS: REQUIREMENTS AND MECHANISMS FOR SURVIVING ENVIRONMENTAL STRESS. Steven C. Hand, Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803.

Postponement of development is frequently observed among invertebrates that inhabit inconsistent environments. Interrupted development is a prerequisite that permits conservation of energy reserves through metabolic arrest. Downregulation of gene expression is observed during entry of *Artemia franciscana* embryos into anoxia-induced quiescence. Transcriptional arrest in nuclear and mitochondrial compartments is rapid, and bioenergetic constraints suggest the arrest is global. Stability of mRNA is markedly extended, and in the mitochondrion, is unexpectedly correlated with decreased polyadenylation. Polyadenylation may signal mRNA degradation, and not translation, in this organelle. Analysis of 590 genes from a full length cDNA library for *A. franciscana* embryos (prepared by reverse transcription with poly-T primers) indicates 43% of gene redundancy is explained by 16S mitochondrial rRNA (mrRNA), while no copies of nuclear rRNA were identified. Results indicate frequent polyadenylation of mrRNA but not nuclear rRNA, and considering the untranslatable nature of rRNA, suggest polyadenylation does not serve as a translation cue in mitochondria.

Entry into diapause under normoxia requires several days for maximum metabolic arrest to occur. During this transition, differential gene expression may be important in establishing the new downregulated state. For example, preliminary data suggest direct inhibition of oxidative phosphorylation under diapause. DNA microarray analysis is underway to evaluate the contribution of differential expression. (DARPA N00173-01-1-G011 and NSF IBN-9723746)

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Translocation of p26 to the nucleus is evaluated in the context of quiescent and diapause states.

41.4

FUNCTIONAL ONTOGENY OF THE CIRCULATORY SYSTEM IN FISH

Bernd Pelster, Dept. of Zoology and Limnology, University of Innsbruck, Austria

In adult vertebrates the linkage between metabolism and cardiac activity represents the main drive for adaptations of the cardiovascular system to changing environmental conditions. Although during development the circulatory system typically starts operating earlier than any other organ, blood flow apparently is not linked to metabolic requirements of tissues in early embryonic and larval stages. Nevertheless, even at this stage environmental factors like hypoxia can modify cardiac activity, blood distribution and erythropoiesis. Furthermore, exposure of early larval stages of zebrafish to a constant water current induces physiological adaptations resulting in an enhanced swimming efficiency, and in an increased tolerance towards hypoxia. In consequence, even during early development of fish larvae the performance of cardiac muscle and of skeletal muscle can be modified by environmental influences, and peripheral resistance can be adjusted. At this stage the control appears to be possible by local and hormonal components, but not by the autonomous nervous system. Thus, already at about the time of hatching the physiological performance of fish larvae is determined by the combined action of environmental influences and of genetic information.

Supported by the Austrian Science Foundation (FWF, P12571-BIO, P14976-BIO)

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41.5

PATTERNS OF GENE EXPRESSION DURING INSECT DIAPAUSE.
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Diapause (dormancy) is a developmental option for most insects, and whether an insect enters diapause commonly depends on daylength, an environmental token that reliably foretells the advent of winter. The brain is the repository of this information, and it is the brain that presides over the decision to enter and terminate this period of dormancy.

Is diapause simply a shut-down in the expression of certain genes, or does this alternative pathway result from the expression of a different set of genes? Though many genes are shut down during diapause, others are specifically expressed at this time. Classes of diapause-upregulated genes can be recognized: Some are upregulated throughout diapause (*e.g.* certain *heat shock proteins*), others are highly expressed only in early diapause (*e.g. cystatin*), while others are upregulated late in diapause (*e.g. ultraspiracle*). Of equal interest are certain genes that are shut down during diapause. One critical gene in the category is the cell cycle regulator, *proliferating cell nuclear antigen.* The upregulation of this gene is one of the first events noted when dipause is terminated.

RNA interference experiments are currently underway to probe the function of the diapause upregulated genes. Our preliminary results indicate that blockage of expression of the genes encoding the heat shock proteins results in a significant loss in protection against low temperature injury, thus suggesting a critical function for the heat shock proteins during diapause.

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41.6

DEVELOPMENTAL CONSTRAINTS ON THE EVOLUTION OF PHYSIOLOGICAL SYSTEMS. Timothy J. Bradley, Dept. of Ecol. & Evol. Biology, University of California, Irvine, CA 92697-2525 USA.

We have been using selection studies with the insect *Drosophila melanogaster* to examine patterns and mechanisms in the evolution of physiological systems. As holometabolous (metamorphosing) insects, *Drosophila* are ideal subjects for examining physiological mechanisms over a range of developmental stages, as well as the effects of selection in one stage on traits in a separate developmental stage. In conjunction with Dr. Michael Rose, we have examined the results of selection for enhanced resistance to environmental stresses (e.g. starvation or desiccation) as well as selection on demographic characters (e.g. postponed reproduction or rapid larval development). Examples will be provided in which selection in one developmental stage profoundly influences the physiological traits in a different stage; as well as other cases in which the physiological responses to selection are restricted to the stage under selection. Examination of the responses to these selection pressures is providing new insights into the physiological processes influencing and/or constraining the evolution of stress resistance and life history traits in insects.

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41.7

OXYGEN REGULATION IN CRUSTACEAN DEVELOPMENT
Nora Terwilliger Oregon Institute of Marine Biology, University of Oregon, Charleston, OR 974420 USA.

Oxygen uptake in crustaceans is affected by the availability, delivery and utilization of oxygen. Developmental stages of crustaceans show differences in oxygen uptake in response to changes in habitat temperature and salinity. The variations in physiological responses result from both a developmental trajectory and the repetitive molt cycles that overlay the developmental patterns. Oxygen delivery is affected by hemolymph oxygen affinity and oxygen carrying capacity. Hemocyanin (Hc) in *Cancer magister* undergoes stage-specific changes in expression that are reflected in functional changes in oxygen affinity. Protein, mRNA and sequencing studies indicate that Hc subunit 1 is constituitively expressed, subunit 5 appears in late megalopa , and upregulation of subunit 6 begins in 5th or 6th juvenile instar. Regulation of oxygen carrying capacity has been investigated by quantitative real-time PCR analysis of Hc cDNA throughout an entire molt cycle of a juvenile crab. Hemocyanin mRNA shows minimum fluctuation in abundance during the molt cycle compared to cryptocyanin, indicating different patterns of regulation. Preliminary studies indicate regulation of hypoxia-inducible genes in crabs by a hypoxia-inducible factor (HIF-1) which would also affect oxygen carrying capacity. Supported by NSF 9984202.

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Characterizes a new member of the Hc gene family whose synthesis is closely coordinated with the molt cycle.

42.1

ENVIRONMENTAL STRESS: A MULTIFACETED CONCEPT IN INTEGRATIVE PHYSIOLOGY. Gretchen E. Hofmann, Department of Biology, Arizona State University, Tempe, AZ 85287

Stress as an environmental concept applies to a vast array of forces that include stress resulting from physical factors such as temperature to subtler, indirect stresses such as those induced by disease and parasites. The goal of this symposium is to present an overview of research on environmental stress in the field of integrative physiology. In the Introduction to the symposium, I will discuss the topic at hand and highlight the breadth of expertise of the presenting speakers. In addition, I will use my primary study area, the function and expression of heat shock proteins in marine fishes and marine intertidal invertebrates, as a means to illustrate the common connections between the different talks and examine steps, both mechanisms and processes, that are common to pathways of response to environmental stress. These steps include: the detection of the signal (how is the thermal signal sensed?), the transduction of the stress (cell signaling used to transmit temperature cues), the response (in my case, the heat shock response), the effector molecules (the heat shock proteins), and the mechanisms that might provide plasticity in the stress response (e.g., regulation of Hsp gene expression in a manner that is sensitive to the thermal history of the organism). Supported by NSF grants IBN 0096100 and OPP 0087971.

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42.2

ADAPTATION TO STRESSFUL CONDITIONS IN *DROSOPHILA*: INSIGHTS FROM A BROAD AND MULTIFACETED APPROACH Ary A. Hoffmann CESAR, La Trobe University, Bundoora, Australia 3086

The explosion of knowledge in *Drosophila* genomics and development of technology to investigate the expression and functioning of genes provides an unparalleled opportunity to investigate the genetic and physiological basis of adaptive shifts. To this end we are working towards an adaptive genomic effort that focuses on climatic variation and links it to the genes and physiological/ biochemical processes that underlie climatic adaptation. Our starting point is clinal variation in two *Drosophila* species along the eastern coast of Australia. Through a combination of field population cages, laboratory manipulations, selection experiments, microarraying, mutagenesis and QTL mapping, we are dissecting the traits, genes and pathways that determine the ways organisms adapt to changing climatic conditions. Some early findings from this effort are discussed and the overall long-term goals are highlighted. Several genes potentially associated with adaptation to temperature extremes exhibit clinal patterns, and association studies show that much of the clinal variation in one suite of traits maps to one region of the genome. Mutants with altered levels of resistance to stressful conditions have been used to understand physiological changes. Finally shifts in traits in selected lines have identified important mechanisms underlying altered stress resistance. Ultimately, this information can be linked to physiological differences among species from different habitats.

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42.3

HEAT SHOCK PROTEINS AND THE STRESS RESPONSE: TRANSCRIPTIONAL REGULATION OF HSP GENES. Martin E. Feder, Department of Organismal Biology & Anatomy, University of Chicago, Chicago, IL 60637

Evolutionary processes have clearly adjusted stress-inducible gene expression to levels and thresholds appropriate for the specific environment in which populations and species occur, but how have they done so? We exploited the *hsp70* genes of *Drosophila* as a natural and laboratory model to address this question. Genes encoding eukaryotic heat-shock proteins such as Hsp70 are coordinately regulated in part by the binding of one or more heat-shock transcription factors (HSFs) to heat-shock elements (HSEs) in the proximal promoter. The number and spacing of HSEs (and other promoter elements) are critical for high transcription rates. We discovered that transposable elements (TEs) repeatedly and independently insert in *hsp70* promoters. Each insertion is in a strain with distinctively low Hsp70 levels or is in greater allelic frequency in populations with low Hsp70 expression. Each of the TE insertions reduces Hsp70 levels, and RNase protection assays show that such insertions reduce transcription of the *hsp70* gene. In addition, the TEs alter two measures of organismal fitness, inducible thermotolerance and female reproductive success. Thus, transposition can create quantitative genetic variation in gene expression within populations, on which natural selection can act. We hypothesize that the unusual chromatin structure of the *hsp* promoter, which poises the gene for immediate transcription, also predisposes the gene to natural mutagenesis by transposons. HSF-HSE interaction and transcription clearly are not the only ways in which nature could adjust the heat-shock response. We have surveyed the sequences of many of the co-chaperones that interact with Hsp70 proteins, and find that two (DnaJ and Hip) have distinctive signatures of nucleotide evolution. Supported by NSF IBN9972678, 9986158, and 0072944, United States-Israel Binational Science Foundation Grant 4556, and HHMI predoctoral fellowships.

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42.4

GENOMIC RESPONSE OF YEAST TO ANAEROBIOSIS. Kurt E. Kwast, Liang-Chuan Lai, and Patricia V. Burke, Department of Molecular & Integrative Physiology, University of Illinois, Urbana, IL 61801.

Recent work in our laboratory examines the physiological response of *Saccharomyces cerevisae* to anaerobiosis as revealed by O_2-dependent changes in the transcriptome. Remarkably, expression of nearly one sixth of the genome is oxygen responsive, with repression of the majority (>65%) of O_2-responsive genes during anaerobiosis. Induction of the anaerobic transcriptome appears to be regulated largely by two *trans*-acting factors, Rox1 and Upc2.

Functional analyses of anaerobically induced genes reveal predictable changes in dissimilatory pathways, including down-regulation of genes involved in respiration and the tricarboxylic acid cycle, as well as up-regulation of genes involved in glycolysis, reserve carbohydrate metabolism, and anaerobic redox balance. Extensive remodeling of the cell wall and membrane is evident from changes in the expression of genes involved in cell wall structure, protein secretion, and vesicle trafficking, as well as those involved in phospholipid, sphingolipid, and sterol metabolism. This remodeling is necessitated by anoxia-induced changes in metabolism and lack of O_2 for the synthesis of enzymes and membrane components, and likely facilitates the import of essential components for regulating membrane fluidity during anaerobiosis.

Oxygen-dependent changes will be discussed in the context of the genetic networks that control expression.

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42.5

INTRACELLULAR OSMOTIC STRESS SIGNALING IN EURYHALINE TELEOSTS: ROLE OF 14-3-3. Dietmar Kültz and Andrea Kohn. The Whitney Laboratory, University of Florida, St. Augustine FL 32080-8610.

14-3-3 proteins bind to phospho-serine or, in some cases, -threonine. Because most intracellular signal transduction pathways rely on serine/ threonine phosphorylation for information transfer, this property positions 14-3-3 proteins as putative molecular master regulators of intracellular signaling. Mammalian and plant 14-3-3 proteins interact with many components of stress-induced signaling pathways and act as a switch that induces a rapid change from one type of metabolism to another in response to environmental stress. We addressed the hypothesis that 14-3-3 is a central element of osmosensory signaling pathways that lead to the pronounced salinity-dependent changes in gill epithelium of euryhaline teleosts. When transferring fish from a plasma-hyperosmotic to –hyposmotic environment, the direction and mechanism of salt transport across gill epithelium and the activity of the underlying transporters are altered. In addition, the morphology (e.g. tight junction ultrastructure and cell morphology) and cell turnover/ differentiation patterns of gill cells change. We have cloned a novel 14-3-3 gene from *Fundulus heteroclitus* and show that it is osmoregulated. Furthermore, we show that teleost 14-3-3 protects *Xenopus laevis* oocytes from hypertonicity. One possible mechanism underlying such protective effect of 14-3-3 may be its inhibition of an endogenous chloride channel in these oocytes. Identification of 14-3-3 binding partners at different stages of salinity acclimation should provide critical insight into phospho-protein cascades that operate during salinity adaptation of osmotic stress-resistant cells from euryhaline vertebrates.

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42.6

ADAPTATIONS TO ANHYDROBIOSIS: LESSONS FROM NATURE John H. Crowe, Willem Wolkers, and Fern Tablin. Biostabilztion Program, University of California, Davis, CA 95616

Numerous organisms are capable of surviving nearly complete dehydration. Over the past several decades, extensive research on the biochemistry of such organisms has indicated the principle mechanisms by which they survive in this unique state. A major adaptation is production of large amounts of disaccharides, including sucrose or trehalose. Both sugars have the ability to preserve membranes and proteins in the dry state, and the mechanism of preservation is similar. However, because of glass transitions and the stability of the glycosidic bond, trehalose is more effective. More recent evidence suggests that the physical properties of trehalose that make the preservation possible can be mimicked by mixtures of other sugars and polymers.

Using what we have learned from anhydrobiosis, we have stabilized mammalian cells in the dry state. Blood platelets are stored in blood banks at room temperature for 3-5 days, after which they are discarded. Platelets cannot be chilled below room temperature for more then a few hours without irreversible damage, due to a lipid phase transition and subsequent lipid phase separation. We have discovered a simple mechanism for introducing trehalose into the cytosol of platelets that has permitted us to freeze-dry them, with recovery rates of about 90%. The dry platelets are stable at room temperature for at least a year (Supported by grants N66001-00-C-8048 from DARPA and PHS HL57810-04/02 and R01HL61204A from NIH.

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42.7

Corticosterone and inclement weather: mechanisms underlying adaptive behavioral responses in mountain birds. Creagh W Breuner[1] and Tom P. Hahn[2]
[1]Integrative Biology, University of Texas at Austin, Austin, TX 78712;
[2]Neurobiology, Physiology and Behavior, University of California at Davis, Davis, CA 95616

In seasonally-breeding migratory birds, the drive to reach the breeding grounds early carries with it the potential benefit of improved reproductive success, but also the risk of encountering potentially life-threatening inclement weather. This is particularly true in species breeding at high elevations or high latitudes. Storms can reduce food availability, limit foraging opportunities, and impose increased thermoregulatory costs. Individuals' reproductive success can thus depend on how effectively they assess risks, and then modify behavior. In the High Sierra of California, mountain white-crowned sparrows (*Zonotrichia leucophrys oriantha*) typically reach sub-alpine breeding areas in early May, when storms are common. Over the past 7 years, we have shown that when storm conditions become extreme, *Z. l. oriantha* typically abandon their territories and fly several kilometers to lower elevation, where conditions are milder and food is more abundant. Variation in internal reserves and/or food availability at the breeding site may modify the timing and duration of territory abandonment. We have evidence that corticosterone acts as an endocrine mediator between the environment and behavior during inclement weather. This system is proving ideal for studying the endocrine mechanisms that coordinate dramatic shifts in behavior in response to both external and internal factors. We have developed a working model of how several of these factors (weather, food availability, energy reserves, and corticosterone) interact to affect behavioral decisions.

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42.8

THE SCALE OF STRESS: TIME AND TOPOGRAPHY ON WAVE-SWEPT SHORES. Mark W. Denny, Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950.

The intertidal zone of wave-swept rocky shores is among the most physically stressful environments on earth. When the tide is in, water velocities accompanying breaking waves can exceed 25 m/s, imposing large hydrodynamic forces on the plants and animals of the shore. When the tide is out, organisms are exposed to terrestrial conditions in which their temperature and water content may fluctuate drastically. To understand the physiological and morphological responses to these stresses, it is often necessary to understand how the environment varies in both space and time. However, this variation is surprisingly complex. Spatial variation in factors such as maximum temperature and wave exposure is characterized by 1/*f* noise: the larger the scale at which the environment is measured, the larger the variability that is encountered. This pattern of variation can make it difficult to characterize the stress experienced by an organism: the mean value is scale dependent, and the standard error can increase as more measurements are made. Temporal variation is more predictable, but no less complex, and a very broad scale of variation must be taken into account. For example, short term increases in ocean temperature may actually be part of a long-term oscillation driven by the 18.6 year fluctuation in the declination of the moon's orbit.

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42.9

Evolved thermotolerance and the expression of heat inducible genes in thermally adapted *Escherichia coli*.
Richle, M. M., Bennett, A. F., Long, A. D.
Department of Ecology and Evolutionary Biology
University of California Irvine, Irvine, CA 92697-2525

Stress gene expression and stress proteins are widely known to increase in response to thermal stress, playing roles in the degradation or refolding of proteins damaged by environmental stress and in preventing the aggregation of damaged proteins. The response of individual genes and their protein products to thermal stress has been thoroughly studied; however, the application of a whole genome approach allows simultaneous investigation of all genes associated with thermal stress. We used DNA high-density array technology and data from expression profiling experiments to address the hypothesis that natural selection leads to changes in stress gene expression during the adaptation to a constant high temperature (42°C) in experimentally evolved lines of *Escherichia coli*. In comparison with their ancestor, these lines show significant and extensive changes in fitness at high temperature, growth rate at 42°C, heat-inducible gene expression, and inducible thermotolerance. These results support the hypothesis that selection has led to changes in many aspects of stress gene expression and its phenotypic consequences following evolution at high temperature.

The 8 highly conserved chaperone genes (*dnaK/hsp70, mopA hsp60* etc.) were found to have increased expression as a result of high temperature adaptation: mean expression of the 6 replicate lineages was 5.5 fold higher than the average gene vs 4.7 fold higher than the average gene in their common ancestor ($p<0.03$). In addition to being highly expressed at high temperature, the 33 stress genes examined, including 8 highly conserved chaperone genes and 25 other heat inducible genes, exhibit significant ($p<0.03$) differences in expression between the group of high temperature adapted lines and their ancestors. A number of genes appear to be targets of selection during high temperature adaptation, including genes involved in the extracytoplasmic stress response. Supported by an NSF Predoctoral Fellowship and a DDIG to MMR and NSF Grant IBN 9905980 to AFB and REL.

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42.10

Ecological Consequences of Environmental Stress and Stress Resistance: Diving into Cormorant evolution from the Cretaceous to the present. **Warren Porter**, Department of Zoology, Univ. of Wisconsin and David Gremillet, Centre d'Ecologie et Physiologie Energetiques, France.

This talk explores energetics, cold stress and its feedback on the ecology of food consumption in an aquatic medium, and behavioral constraints faced by birds that rapidly returned to an aquatic existence in the Cretaceous. The stresses they faced then and now in making this transition were very different. The dilemma these birds face today as one examines them across their South to North distribution and and across seasons exemplifies "what physiologists need to know about ecology when thinking about stress, and what physiologists can contribute to ecologists' thinking about stress". We will describe new state-of-the-art microclimate and endotherm models that have been applied and tested previously on birds and mammals in terrestrial environments. We will demonstrate how we can calculate energetic costs in different localities in Greenland that agree extremely well with field data. These calculations were done before gaining access to field data to test model accuracy. We also illustrate that seasonal changes in energetics can be used to predict timing of migration and dynamic interactions between predator and prey between at North and South Greenland sites.This talk will describe the extension of the endotherm model to aquatic environments for both steady-state and transient conditions for Cormorant sized birds. Simulations for tropical, temperate, and Arctic environments for different bird body sizes and feather properties will be explored.

ACCLIMATIZATION TO HYPOXIA: SUPPLY VS DEMAND STRATEGIES

43.1

COMPARATIVE PHYSIOLOGY OF ACCLIMATIZATION TO HYPOXIA

Frank L. Powell, Dept. of Med. and White Mt. Research Station, University of California, San Diego CA 92093-0623.

Animals respond to decreased oxygen availability by reducing oxygen demand or increasing oxygen supply. During sustained hypoxia, oxygen demand and supply acclimatize, i.e. change in a time-dependent manner. Comparative physiologists have studied acclimatization to hypoxia in a variety of vertebrates, focusing on animals that are especially tolerant of hypoxia. Experiments show that decreased oxygen demand during chronic hypoxia involves changes in behavior, energetic efficiencies, ion pumping, protein synthesis and other metabolic pathways. Acclimatization of oxygen supply also involves multiple strategies, including enhancements at every step of physiological oxygen transport. The physiological signals for increased oxygen supply during acclimatization to hypoxia are well known for mammals. In contrast, the adequate stimulus for decreased oxygen demand during hypoxia (i.e. the hypoxic metabolic response, HMR) is not known. The utility of a conceptual framework used to study the time domains of the hypoxic ventilatory response in mammals will be evaluated for application to comparative studies of the HMR. The objective of this analysis is to discover patterns of supply vs. demand strategies in acclimatization to hypoxia and better understand their evolutionary origins.
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43.2

INTERACTIONS OF THERMAL, METABOLIC AND RESPIRATORY CONTROL IN HYPOXIC HOMEOTHERMS

William K. Milsom and Glenn J. Tattersall, Department of Zoology, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

Homeothermy is characterised by the defense of a constant, elevated body temperature (T_b), achieved through regulated heat loss and heat production. In mammals and birds, this is achieved through neural regulation and integration by sites located primarily in the hypothalamus. In hypoxia (typically less than 10% O_2), however, normal homeothermy appears to be abandoned; body temperature and metabolism fall in a dose-dependent fashion, a process that has often been coined hypothermic hypometabolism. Over the past 2 decades, however, indirect evidence has accumulated to suggest that this decline is not due to non-specific mechanisms unrelated to thermoregulatory control, *i.e.* the decline in T_b is not an abandonment of homeothermy, but a regulated resetting of the set-point for T_b regulation. Recent studies examining the ability of mammalian homeotherms to elicit regulated thermoregulatory responses in hypoxia, using changes in ambient and hypothalamic temperature as stimuli, have shown that despite highly diminished shivering and non-shivering thermogenic ability, hypoxic mammals are still capable of a modest increase in heat production in the cold, and appear also to have a reduced thermal conductance in hypoxia. Part of this reduced thermal conductance is related intrinsically to a reduced hypothalamic thermosensitivity in hypoxia, since hypothalamic cooling evokes only small metabolic increments. This suggests that the lowered set-point for T_b is accompanied by an overall reduced thermosensitivity in hypoxia. This hypoxic metabolic response, although apparently adaptive, is only an immediate response to hypoxia. Other recent studies have reinforced the importance of chronic hypoxic acclimatisation in bringing about massive physiological re-adjustments that serve to restore normal T_b during chronic hypoxia. Thus, just as there are different time domains for the hypoxic ventilatory response, so too are there time domains for the hypoxic metabolic response.

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43.3

PHYSIOLOGICAL SIGNALS AND COMPARATIVE RESPONSES TO DECREASED OXYGEN SUPPLY. Dona F. Boggs, Eastern Washington University, Cheney, WA 99004

Matching oxygen supply to oxygen demand (ml O_2/min) relies upon regulation of pulmonary ventilation (V), cardiac output (C.O.) (as well as shunts in reptiles and amphibians) and O_2 capacity on the supply side, and potentially also reduction of O_2 consumption (VO_2). The signal to increase C.O. and Hb production is either reduced arterial content (e.g. fall in hematocrit) or reduced arterial PO_2, whereas the signal for increased breathing is arterial PO_2. Since pulmonary gas exchange (V/VO2) can only directly affect arterial PO_2 it seems logical that it be the regulating signal. Yet arterial oxygen content, important in the volumetric supply, depends on PO_2, hemoglobin affinity and concentration. Ventilatory responsiveness to hypoxia in terrestrial vertebrates varies interspecifically with hemoglobin affinity. A threshold PO_2 for stimulating breathing could be expected parsimoniously to vary as Hb affinity, and therefore saturation, does; hence the question arises how the control of breathing and the oxygen transport systems can become 'matched'. The answer may lie in the design of the sensors. Current models of oxygen sensing in cells responsible for signaling ventilatory or cardiovascular responses to decreased oxygen supply depend upon heme proteins. Comparative studies of oxygen sensors may reveal whether the oxygen affinities of oxygen-sensing heme proteins and oxygen-carrying heme-proteins are 'tuned' to one another.

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43.5

MOLECULAR MECHANISMS OF OXYGEN SENSING AND APOPTOSIS IN MAMMALIAN CELLS. Navdeep S. Chandel Department of Medicine, Northwestern University, Chicago, IL 60611.

Oxygen is required for the survival of all mammalian cells. Accordingly, mammalian cells have developed adaptive responses both at the organ and cellular/molecular level to counter the decreasing availability of oxygen (*hypoxia*, PO_2 < 3-35Torr). At the cellular level, the transcription factor hypoxia inducible factor 1 (HIF-1) activates a host of genes involved in glucose transport, anaerobic metabolism and angiogenesis. Induction of these genes is part of the cellular response to an adverse environment and may give cells a survival advantage. A central question in understanding the mechanism underlying the hypoxic induction of gene expression involves the identification of the cellular O_2 sensor. We have proposed a model of oxygen sensing which involves the mitochondrial electron transport chain as the site of oxygen sensing[1]. Mitochondria detect decreases in oxygen concentration by increasing the generation of reactive oxygen species (ROS). The increased levels of ROS are required for the activation of transcription factors and gene expression during hypoxia. A failure of cells to adapt to hypoxic conditions could result in oxygen concentrations close to anaerobic conditions *(anoxia)*. Anoxia causes cells to undergo apoptosis. We have recently demonstrated that the pro-apoptotic Bcl-2 family members are critical regulators of anoxia-induced apoptosis. Thus, fibroblasts from *bax*$^{-/-}$ *bak*$^{-/-}$ mice are resistant to anoxia-induced apoptosis[2]. How anoxia activates pro-apoptotic Bcl-2 family members to initiate apoptosis remains unknown?

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43.6

METABOLIC RESPONSES TO INTERMITTENT AND CHRONIC HYPOXIA IN FISHES. Nancy M. Aguilar, White Mountain Research Station, University of California, Irvine, CA 92697-2525

Dissolved oxygen can be highly variable in aquatic environments. In coastal environments, such as estuaries, aquatic hypoxia can be either intermittent or chronic. Aquatic intermittent hypoxia results from a daily pattern daytime algal photosynthesis, often producing hyperoxia (>300mmHg), and nightly algal respiration, leading to nocturnal hypoxia (<30mmHg). Chronic hypoxia typically occurs in bottom waters when an upper layer of warm, low density water prevents denser water from mixing, allowing the bottom waters to become oxygen-depleted. Although responses to acute hypoxia have been well studied, intermittent and chronic hypoxia are only beginning to be investigated in fishes.

In most fishes, chronic hypoxia causes reduced metabolic rate, activity, and growth rate, and an increase in lactate (depending on the severity of the hypoxia). Chronic moderate hypoxia can also stimulate catecholamine secretion, which may facilitate acclimation to more severe hypoxic episodes. The recent discovery that HIF-1α accumulates in trout during normoxia suggests that the patterns of hypoxic gene expression may be different in teleost and mammalian models.

Unlike mammals, in which intermittent hypoxia is generally a pathological condition, periods of low oxygen are an integral component of the life history of many fishes. However, it is not clear if routine exposure to moderate hypoxia pre-adapts fishes for more severe hypoxic events. Studies thus far with an estuarine species, *Gillichthys mirabilis*, suggest that the response to severe hypoxia is not affected by prior exposure to either chronic or intermittent hypoxia. However, physiological changes which take place during intermittent hypoxia may be more important for a rapid recovery upon return to normoxia.

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44.1

REGULATION OF RENAL BLOOD FLOW AND GLOMERULAR FILTRATION.

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For most vertebrate kidneys, the filtration of plasma in the glomerulus constitutes an essential initial step in the formation of urine by the kidney and the renal excretion of water, salts and metabolic waste products. Glomerular filtration, being a special case of transcapillary exchange, is governed by the Starling forces of transcapillary hydrostatic and osmotic pressures, and by the capillary hydraulic permeability and surface area available for filtration. Because the axial capillary profile for the net transmural force for filtration is strongly dependent on the magnitude of plasma flow, renal blood flow and glomerular filtration are often tightly coupled – and regulation of the rate of glomerular filtration occurs largely through vascular mechanisms. A summary of current concepts of regulation of RBF and GFR in vertebrate kidneys will be offered, emphasizing the extrinsic regulation of GFR. The importance of alterations in the magnitude of GFR for producing diuresis and antidiuresis in nonmammalian vertebrates will be discussed, with consideration of the role of glomerular intermittency in these responses. In addition, recent observations of the actions of vasoactive peptides and other hormones in regulating RBF and GFR especially in nonmammalian vertebrates will be reviewed. (Supported in part by grants from NSF)

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44.2

REGULATION OF PROXIMAL AND DISTAL TUBULE TRANSPORT. William H. Dantzler, Department of Physiology, Col. of Med., University of Arizona, Tucson, AZ 85724

Renal tubular transport and regulation of Na^+ (and Cl^-) and fluid and organic anions (including urate) are reviewed. Filtered Na^+ (and Cl^-) is reabsorbed along the tubules but only in mammals and birds does most of it occur in proximal tubules. Reabsorption involves active transport of Na^+ and passive reabsorption of Cl^- in all tubule regions. Active Na^+ step always involves Na-K-ATPase at basolateral membrane, but entry step at luminal membrane varies among tubule segments and among vertebrate classes (except for Na^+-$2Cl^-$-K^+ cotransporter in diluting segment). Regulation can involve intrinsic, neural, and endocrine factors. Proximal tubule fluid reabsorption is dependent on Na^+ reabsorption in all vertebrates studied, except ophidian reptiles. Fluid secretion occurs in glomerular and aglomerular fishes, reptiles, and even mammals, but its significance is not always clear. A non-specific transport system for net secretion of a wide range of organic anions (OAs) exists in proximal tubules of almost all vertebrates. Net secretion involves transport into the cells at the basolateral side against an electrochemical gradient by a tertiary active transport process, the final step in which involves OA/α-ketoglutarate exchange, and movement out of the cells across the luminal membrane down an electrochemical gradient by an unknown carrier-mediated process. Regulation may involve protein kinase C and mitogen-activated protein kinase. Urate is net secreted in proximal tubules of birds and reptiles. This process is urate-specific in reptiles but in birds it may involve both a urate-specific system and the general OA system. Regulation may relate to flow of filtrate along lumen.

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44.3

REGULATION OF WATER MOVEMENT.

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The kidney plays an essential role in body fluid homeostasis, but its water handing varies, depending on nephron structure and environment. For fish and amphibians living in a hypoosmotic environment, the kidney excretes excess water by forming a dilute urine; a diluting segment operated by a luminal Na-K-Cl cotransporter coupled with a basolateral Na-K pump plays an essential role. In contrast, in terrestrial tetrapods, water conservation by the kidney is essential for survival. In birds and mammals, because of the development of Henle's loop with which collecting ducts (CDs) run in parallel, the diluting segment provides an energy source for a counter-current urine concentration mechanism. Aquaporin (AQP) water channels, pore-forming intrinsic membrane proteins, have been identified in various transporting epithelia. In mammalian kidneys, AQP1 is expressed in proximal tubules, the descending limb, and vasa recta. AQP2 is selectively expressed in CDs and regulated by vasopressin, whereas AQP3 and 4 are constitutively expressed in the basolateral membrane. AQP1 and AQP3-homologue water channels have been cloned, respectively, from amphibian urinary bladders and teleost gastrointestinal tracts. We have cloned AQP4 (whole coding sequence) and AQP2 (partial cDNA) from medullary cones of the *Coturnix* quail (q) kidney. Water deprivation and treatment with vasotocin stimulate AQP trafficking to the apical membrane of the CDs and upregulate qAQP2 protein in the kidney. qAQP4 (335 amino acids, 77.7% identity with mammalian AQP4) mRNA is expressed in the brain, kidney, lung, and skeletal muscle. AQP water channels are evolutionarily old and play important roles in epithelial handling of water. Study of the phylogeny of AQP in vertebrates will provide insight into their functions and roles during adaptation to various environments.

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44.4

REGULATION OF NITROGEN EXCRETION. Patrick J. Walsh, M. Danielle McDonald and Chris M. Wood. NIEHS Marine and Freshwater Biomedical Sciences Center, University of Miami; Department of Biology, McMaster University.

Especially with the advent of molecular approaches, the transport of urea and other nitrogenous wastes by the vertebrate kidney (and gills of aquatic species) has re-emerged as hotbed of comparative research. A variety of transport pathways, both diffusional and active, are now known for urea, and several transporters have been cloned providing the sequence information to generate nucleotide and antibody probes for the mechanistic study of urea transport. This presentation will focus primarily on how urea is differentially handled by the gills and kidneys of marine and freshwater fish, and how these systems compare to those of higher vertebrates. In elasmobranchs, where massive gradients for urea exist, urea is retained at both the gill and kidney in part by active transporters, and at least in the gill, also in part by an unusual lipid composition which is less permeable to urea (see e.g., Fines et al., 2001). In the teleost species studied to date, where urea retention and large gradients are less of an issue, facillitative diffusion appears to predominate at the gill, whereas active secretion appears to predominate in the kidney, and these different mechanisms have been teased apart through the use of several analogs of urea (see e.g. McDonald et al., 2000). Both active and passive urea transporters are reported in the mammalian kidney, and some of the large gradients seen in elasmobranchs are also seen here. In all vertebrates studied to date, two interesting facets emerge: (1) facilitated UT transporters cannot be seen to saturate under "normal" conditions; and (2) cloning of "the" active urea transporter has remained elusive.

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44.5

Regulation of renal and lower gastrointestinal function: Role in fluid and electrolyte balance. E. J. Braun, Dept. Physiology, Univ. Arizona, Tucson, AZ 85724.

For the majority of vertebrates, the kidneys are not the sole organs that function to maintain the homeostasis of body fluid and electrolytes. Mammals are unusual in this respect, as the kidneys are the organs that fill this role. For non-mammalian vertebrates, other organs such as gills, skin, salt glands, urinary bladders, and the gastrointestinal system function in concert with the kidneys in the control of fluid and ion balance. Birds are unique and of particular interest as they do not possess urinary bladders and the renal output enters the lower gastrointestinal (GI) tract. The physiology of the interaction of avian kidneys and lower GI tract is an excellent example of integrative physiology and several aspects of it have been examined. For example, the role of the avian antidiuretic hormone (arginine vasotocin, AVT) plays in controlling renal output. AVT produces both a tubular and glomerular antidiuresis. The glomerular antidiuresis is important, as the fluid from the kidneys that enters the GI should not be highly concentrated. Another hormone, aldosterone, has been shown to play an important role in regulating the transport of sodium by the GI epithelium. In addition, the lower GI tract plays a significant role in recycling a portion of the nitrogen that leaves the kidneys as uric acid. Furthermore, the output of avian kidneys contains large amount of protein that is conserved by the lower GI tract.

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44.6

REGULATION OF SALT GLAND AND RENAL INTERACTIONS
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The high water and Na fluxes of marine birds present them with a major physiological challenge because their cells require an environment one-third as concentrated as seawater. Their kidneys do not excrete the excess NaCl, but reabsorb it for extrarenal secretion by the salt glands. During gradual acclimation to saline, osmoregulatory organs hypertrophy, usually more so in males, and extracellular fluid and Na move into the cells. Extracellular concentration is elevated following ingestion of seawater, inducing water to move out of the cells. Together increases in extracellular fluid concentration and volume stimulate secretion. The expanded intracellular fluid may facilitate rapid expansion of the extracellular fluid. Osmoregulation in birds with salt glands has been mainly studied in Charadriiform and Anseriform birds (especially the domesticated duck). Secretion concentration correlates well with the preferred habitat salinity among Charadriiform species, but not Anseriform species. Species of ducks from fresh, estuarine, or marine habitats produce secretion of similar concentration, but at different rates. Salt gland secretion rate may be constrained by rates of Na uptake in the gut and Na retrieval from the renal filtrate. These processes are integrated by a variety of physiological signals that trigger neural and/or hormonal modulators. Supported by NSERC of Canada.

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45.1

Ontogeny of the Cutaneous Permeability Barrier in Hatchling King Snakes

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Recently we investigated the importance of first postnatal ecdysis in establishing a competent barrier to transepidermal water loss (TEWL) in hatchling California king snakes (*Lampropeltis getula*). Resistance to TEWL increases two-fold following the first postnatal ecdysis and corresponds with a roughly two-fold increase in thickness and deposition of lamellar lipids in the mesos layer of the epidermis, which comprises the skin permeability barrier in snakes. Pre-shed neonatal skin contains disorganized lipid bilayers similar to those observed in mammalian fetal skin before the permeability barrier attains functional efficacy. Thus, emergence of the integument from embryonic fluids and its subsequent pan-body replacement following contact with air appear to be essential for completion of barrier competence in newborn snakes. Here we report the presence of lipid inclusions having electron-lucent morphologies within mature cells of the alpha keratin layer. Multi-lamellar bodies and different stages of "dissolution" of lamellar inclusions into electron-lucent lipids are dominant features in the immature alpha cells of pre-shed skin, similar to what is seen in avian transitional cell layers. These observations point to the possibility that alpha cells might be involved in barrier homeostasis and facultative "waterproofing" of skin, a speculation that has not been raised previously for ophidian epidermis.

45.2

The Ontogeny of Energy Consumption in Leatherback and Olive Ridley Marine Turtle Hatchlings

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Changes in activity related oxygen consumption and energy partitioning were measured in leatherback and olive ridley hatchlings over the first five weeks after emergence. In leatherbacks, resting oxygen consumption rates deceased from 0.39 to 0.17 $ml O_2 h^{-1} g^{-1}$ during this period, while ridleys declined much less, 0.23 to 0.16 $ml O_2 h^{-1} g^{-1}$. Greater differences were seen in aerobic scope. For olive ridleys the aerobic scope was 80% at emergence and 258% at week 4. By comparison, for the leatherback, the aerobic scope was only 28% at emergence and 61% at 5-weeks of age. Leatherback hatchlings gained on average 33% weight (10 g) over the first week however 70 to 80% of this increase is due to water accumulation. Leatherbacks emerge from the nest with about 75 KJ of energy in the residual yolk at their dispersal for growth and movement. By comparison the residual yolk energy reserves for the olive ridley are estimated to be much less (45 KJ). The differences in aerobic scope and energy reserves are related to differences in early life-history stages of these species. Olive ridleys swim and drift to nearby ocean gyres where they passively forage in oceanic drifts. Leatherbacks continuously swim till they reach pelagic foraging areas where they actively pursue prey for the rest of their lives. This research was supported by funds from the PADI Project AWARE Foundation, Save the Sea Turtle Foundation, EarthWatch Field Institute, FAU Graduate Student Association, and FAU Foundation.

45.3

Plasticity and Constraints of Grunion Developmental Timing

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Grunion *Leuresthes tenuis* (Osteichthyes) spawn terrestrially in beach sand following semilunar high tides. Eggs can hatch in 10 d at 20°C, when they could be washed out of the sand by the next semilunar tides. However if waves do not reach the eggs, they delay hatching and continue incubation, until inundated and environmentally triggered to hatch. During extended incubation for up to several weeks, metabolism and growth cause depletion of the yolk reserves. We examined whether this depletion could adversely affect the condition of eggs, hatchlings, and larvae. Temperature affected the time to hatching competence in grunion eggs, as well as hatching success and the duration of incubation. Warmer eggs matured quickly but had a shorter period of viability. Cooler eggs survived longer but were not ready to hatch in time for the next semilunar tides. Eggs at 20°C were more likely to survive and hatch successfully over the three subsequent semilunar tides, than eggs at 15°C or 25°C. Additional larvae at 20°C were hatched out from 10 to 35 d following fertilization, then cultured. Hatchlings from older eggs were longer initially, but all larvae grew rapidly, and there was no difference in size at 3 weeks post-hatch. Broad temperature tolerance, plasticity in incubation time, environmentally triggered hatching, and rapid larval growth enable the grunion to spawn terrestrially in a variable coastal ecosystem. This work was funded by NSF grant DBI-99-87543.

45.4

Oxygen consumption and temperature in larvae of the Antarctic starfish *Odontaster validus*

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Abstract Oxygen consumption (MO_2) of individual larvae of the Antarctic sea-star *Odontaster validus* was measured for 50 days after fertilisation. Values ranged from 0.76 pmols $O_2 h^{-1}$ for one coeloblastula to 77.6 pmols $O_2 h^{-1}$ for a bipinnaria larva. At –0.5°C mean MO_2 increased from 10.9 pmols $O_2 h^{-1}$ (SEM 0.13) for gastrulae, 13 days post-fertilisation to 25.4 pmols $O_2 h^{-1}$ (SEM 3.5) for 50 day old bipinnaria. MO_2 in gastrulae reared at –0.5°C was unchanged between days 13 and 45 post-fertilisation (mean=11.4 pmols $O_2 h^{-1}$). Individual MO_2 was highly variable, covering a >40-fold range. At 2°C gastrula MO_2 was on average 52% higher (17.35 pmols $O_2 h^{-1}$), giving a Q_{10} of 4.5. For bipinnaria mean MO_2 at 2°C (31.4 pmols $O_2 h^{-1}$) was not significantly different from larvae at –0.5°C, suggesting bipinnaria metabolism is less sensitive to temperature than gastrulae. At 2°C the bipinnaria stage was reached at 30-35 days compared with 40-45 days at 2°C (Q_{10} - 4.6). The micro-respirometry method used here is highly sensitive, inexpensive and straightforward, and was based around 35-50 1 respirometers. Larvae here have very low metabolic rates. Previous similar work required many individuals per chamber. O_2 concentration was measured using 25 1 samples injected into a couloximeter. Under optimum conditions MO_2 of a single larva of 4 pmols $O_2 h^{-1}$ was measured with 20% accuracy. This method allows MO_2 measures of field caught larvae of most marine species. Support came from the UK NERC.

45.5

CHANGES IN BLOOD CHEMISTRY DURING HYPOXIC EXPOSURE IN EMBRYOS OF THE DOMESTIC CHICKEN.

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Renewed interest has developed in using embryonic chickens as models for understanding the ontogeny of cardiovascular control. Here, we investigated the blood acid-base response of developing embryos to hypoxic exposure. Hypoxia has been used to determine not only the general cardiovascular response but also as a tool to determine the maturation of reflexive control the system. While several studies suggest that embryonic chickens regulation the cardiovascular response to hypoxia few studies have measured the blood chemistry. Therefore this study was undertaken to determine the impact 10% oxygen has on embryonic chickens. Embryonic chickens ranging from day 13 to 21 of incubation were sampled during a control and at the end of a 5-min of 10% O2 to be analyzed for lactate, glucose, ion, O2 and CO2 levels. The maximal hypoxic response occurred on day 15 with lactate increasing 7 times (2.5 to 16.6 mmol/l) while glucose levels decrease by half (136 to 63 mg/dl). Further hypoxia induced a pH (7.40 to 7.26) change that was greatest on day 15. With age (from 17 to 21 days) 10% O2 had reduced actions on all measured parameters except mixed arterial PO2 response on day 21. A recovery profile conducted on different experimental groups of embryos revealed that all blood parameters were within resting values after 1 hr. These data indicate that a 5-min exposure to 10% O2 is sufficient to induce dramatic changes in blood chemistry. This work was supported by grants from the AHA and NSF #IBN 9727762.

45.6

Comparative locomotor function in turtles: can species differences in adult motor patterns be traced to juveniles?

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Related species that prefer different habitats can exhibit marked differences in locomotor function and motor control. When in the lives of these animals do such differences first appear? To test if contrasts in motor function between adults of related species can be traced to their juveniles, we collected high-speed video and hindlimb EMGs from juveniles (8-15 weeks posthatching) and adults of two turtle species during walking and swimming: aquatic specialist spiny softshells (> *Apalone spinifera*) and habitat generalist sliders (> *Trachemys scripta*). Adults of these species show two major kinematic differences: sliders depress the femur more than softshells, and softshells hold the knee straighter than sliders (during walking). However, juveniles of these species show neither of these differences. Adult sliders and softshells also differ in limb motor patterns: in swimming, the knee extensor femorotibialis is active during recovery phase in sliders but during thrust phase in softshells. Juveniles exhibit this difference as well, but also show further differences not seen in adults of these species. For example, in juvenile sliders the puboischiofemoralis internus shows two EMG bursts during swimming, rather than the single burst typical of juvenile softshells. Thus, juveniles are not constrained to use the motor patterns of adults, or a single "juvenile" pattern related to small size, but instead may show a variety of species-specific functional specializations.

45.7

Non-skilled Motor Behavior Lateralization During The Early Postnatal Development In White Rats

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The aim of the present study was to investigate the development of lateralization in non-skilled motor reactions during the early development in the white rats. From 8 to 74 postnatal days (P8 – P74) lateralization was estimated in two motor tests: side preference in rotation with fixed tail (TR) and movement initiation in forelimb stepping test (TS). The results indicate that during early ontogenesis before sexual maturation there is lack of stable lateralizaton in non-skilled motor behavior in both

tests. Analysis shows that breaking point of lateralization development happened on P21-P23, and the final lateralization occurs both for individual rats and the whole group of tested animals in the age 6 to 8 weeks. We supposed that lateralization development in forelimb stepping test happens with the two weeks delay compare to side preference development in rotation test. Keywords: Behavioral motor asymmetry, lateralized behavior, early postnatal development, white rats.

45.8

Molt Cycle Changes in Tissue-Specific Abundance of Cryptocyanin and Hemocyanin mRNA in the Dungeness crab, *Cancer magister*.

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Hemocyanin, the copper-containing oxygen transport protein, and cryptocyanin, a protein very similar in sequence to hemocyanin but lacking some of the conserved 6 histidines necessary to bind copper, are both present in the hemolymph of *Cancer magister*, the Dungeness crab. Cryptocyanin is absent or in very low concentration in postmolt crabs, increases in intermolt and premolt to high levels, and drops precipitously at ecdysis. Quantitative, real-time PCR analysis of cryptocyanin and hemocyanin mRNA was carried out on samples obtained throughout an entire molt cycle. Results showed mRNA expression of cryptocyanin and hemocyanin in hepatopancreas tissue but not in muscle or hypodermis, consistent with previous results. The abundance of cryptocyanin mRNA in hepatopancreas tissue showed major changes similar to the hemolymph concentration of the protein, with low levels in early postmolt and late premolt and high levels during intermolt and early premolt. Hemocyanin mRNA showed minimal fluctuation in abundance during the molt cycle. Our results suggest that these two members of the same gene family are under different patterns of regulation and that cryptocyanin is closely integrated with the molt cycle. Supported by NSF 9984202 (NBT) and NSF 0100394 (DT).

45.9

Developmental expression and actions of corticotropin-releasing hormone in tadpoles of *Xenopus laevis*

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Corticotropin-releasing hormone (CRH) has been shown to stimulate both thyroid and interrenal (adrenal) hormone secretion and thereby accelerate metamorphosis in larvae of several amphibian species. We analyzed the developmental expression of CRH and its actions on the thyroid and interrenal axes of *Xenopus laevis* tadpoles. We developed a homologous radioimmunoassay (RIA) for *Xenopus* CRH (xCRH) and validated this RIA for measurement of CRH content in hypothalamus. We show that CRH-binding protein, which is expressed in tadpole brain, is removed by acid extraction and does not interfere with the RIA. Tadpole brain homogenates produced displacement curves that were parallel to the xCRH standard. Fractionation of tadpole hypothalamic extract by reverse-phase HPLC demonstrated the presence of two CRH-immunoreactive peaks, which co-eluted with either xCRH or fish urotensin I (UI) standards. Peptide content in the hypothalamus remained constant during tadpole development but increased significantly in newly metamorphosed frogs. Treating cultured pituitaries from premetamorphic tadpoles with xCRH (100 nM for 24 h) increased medium content, but decreased pituitary content of thyrotopin immunoreactivity. Injections of xCRH into premetamorphic tadpoles increased whole body thyroxine and corticosterone content. This is the first evidence for a stimulatory action of CRH on the thyroid axis in *X. laevis* (supported by NSF grant IBN9974672 to RJD and NSF predoctoral fellowship to GCB).

45.10

Effect of Photoperiod and Melatonin on Growth and Development of Neonatal Gerbils (Meriones unguiculatus)

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Animals in the wild typically respond to short winter days by channeling energy into production and retention of heat rather than reproduction and somatic growth. Several studies have shown that pregnant female rodents can communicate short photoperiod information to their unborn offspring, resulting in retarded growth and delayed sexual maturation in the young. In this study, female gerbils were exposed to 8, 12, and 16 hour daylengths during pregnancy and lactation, and the growth and development of their offspring were monitored until weaning. Pups whose mothers were exposed to 16 hr light daily grew significantly faster ($P<0.001$) than other photoperiod treatments, and had significantly larger muscle and reproductive tissue mass, but significantly smaller liver mass and brown fat mass ($P< 0.05$). Pups whose mothers were exposed to 16 hr light daily but were given melatonin in their drinking water during lactation exhibited a development pattern similar to pups whose mothers were exposed to 8 hr light: retarded growth rate, smaller muscle mass, and larger liver, kidney, and brown fat mass. Exposure to short photoperiod or melatonin (released during long nights) stimulates development of heat producing organs in gerbil pups and dramatically changes the developmental schedule of neonates prior to weaning.

PHYSIOLOGICAL AND GENETIC RESPONSES TO ENVIRONMENTAL STRESS

46.1

Phosphoserine and other unusual osmolytes in deep-sea vesicomyid bivalves: correlations with depth

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Most marine invertebrates use organic solutes such as taurine, glycine, betaine as cellular osmolytes. But recent studies show that deep-sea, hydrothermal-vent, and cold-seep animals have different solutes, perhaps adaptations to depth or unusual metabolisms. To determine which, we analyzed major osmolytes in related animals from a wide depth range: vesicomyid bivalves (Calyptogena/Vesicomya spp) were collected from cold seeps at 0.5 km (Eel River, California), 1.1 km (Okinawa Trough, Sagami Bay), 2 km (Oregon Margin), 4.4 km (Aleutian Trench), and 6.4 km (Japan Trench). Mantle tissues had very significant depth trends: contents of taurine, the major osmolyte in shallow bivalves, declined exponentially with depth; glycine peaked at 1.1 km then declined exponentially; an unidentified solute containing phosphoserine and ethanolamine, an unidentified methylamine, and myo- and scyllo- inositol increased linearly from 2 to 6.4 km. In the 6.4-km specimens, the phosphoserine solute made up over half the osmolyte pool; typical invertebrate osmolytes (taurine, betaine, glycine) were at minute levels. We propose that these unusual osmolytes are depth (pressure) adaptations. Gills had similar patterns (including dominance of the phosphoserine solute in the deepest clams), except that hypotaurine and thiotaurine were significant osmolytes (concomitant with less glycine). These had no depth correlations and probably function in sulfide metabolism of the gills' microbial symbionts.

46.2

Environmental salinity reduction leads to increased abundance of $Na^+/K^+/2Cl^-$ cotransporter mRNA in gills of the blue crab *Callinectes sapidus*.

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The mechanism by which euryhaline crustaceans transport NaCl across the gill epithelium from low salinities into the hemolymph is not well understood. Basolateral Na^++K^+-ATPase appears to provide the major driving force for Na^+ uptake as well as NH_4^+ excretion. Apical transporters may include a V-type H^+-ATPase in some species, a Na^+/H^+ exchanger, or a $Na^+/K^+/2Cl^-$ cotransporter. We have amplified and sequenced a cDNA encoding the latter protein from gills of the blue crab *Callinectes sapidus*. The 4,008-nucleotide sequence (GenBank Accession No. AF190129) contains an open reading frame coding for a 1,031-amino-acid protein that is 35-38% identical to mammalian $Na^+/K^+/2Cl^-$ cotransporter and thiazide-sensitive Na^+/Cl^- cotransporters. Quantitative real-time PCR analysis of cotransporter mRNA abundance in tissues of the blue crab showed that posterior gills and antennal gland express the highest levels, followed by anterior gills and hepatopancreas. Heart, hypodermis, and leg muscle showed very low levels of expression. Transfer of crabs from high (35 ppt) to low (5 ppt) salinity was followed by a five-fold increase in cotransporter mRNA abundance in posterior gills. Our results suggest that cotransporter gene transcription is sensitive to environmental salinity, leading to greater availability of cotransporter protein and accounting in part for enhanced NaCl uptake from dilute salinities. Supported by the National Science Foundation (IBN-9807539 and DBI-0100394).

46.3

Recovery of Water, Ion Content, and Energy Stores Following Desiccation In *Drosophila melanogaster*

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We are examining ion regulation and water balance in five, replicate populations of *Drosophila melanogaster* that have undergone laboratory selection for enhanced desiccation resistance (D populations), and in five, replicate control populations (C populations). Each D population and its paired C population are kept under identical conditions, except the D populations undergo a period of selection during which they are denied access to water. In a previous study we found that during extended bouts of desiccation, hemolymph volume is significantly diminished. As hemolymph volume is reduced, the D flies osmoregulate by excreting solutes. In this study, we investigated the importance of replenishing lost water and solutes prior to subsequent bouts of desiccation. Specifically, we examined the capacities to replace lost water, excreted sodium, and depleted carbohydrate stores following dehydration and the role of these in restoring desiccation resistance. We first dehydrated the populations and then allowed them access to either water, saline, or sucrose-saline solution. We found that the D flies are capable of returning to pre-desiccation levels of body water, dry mass, and sodium content only when provided the sucrose-saline solution during recovery. We conclude that carbohydrates are vital to the rehydration of the flies, and may play a central role in enhancing desiccation resistance in these populations.

46.4

Pelvic Skin Blood Flow And Water Uptake In Toads, *Bufo alvarius*

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Toads, Bufo alvarius, were placed in a Lucite chamber that allowed red cell velocity (RCV) in ventral pelvic skin capillaries to be measured as the voltage output of a Laser Doppler Flowcytometer, before and after the skin was allowed to contact water. Comparisons were made between hydrated and dehydrated toads and toads with or without water stored in the urinary bladder. With deionized water, hydrated toads with a full urinary bladder (13.3 % of body weight) increased RCV from 0.35 V to 0.55 V ($P<0.05$, n=20). Hydrated toads with an empty bladder increased RCV from 0.44 V to 1.63 V ($P<0.001$, n=25). In marginally dehydrated toads (3.6 %), RCV increased from 0.50 V to 1.88 V ($P<0.001$, n=30). In moderately dehydrated toads (15.4 %) RCV increased from 0.65 V to 2.60 V ($P<0.001$, n=30). An empty bladder appears to be the most important signal to increase RCV upon exposure to water. The RCV of dehydrated toads did not differ between animals placed on deionized water or 50 mM NaCl. However, water uptake across the ventral skin was significantly reduced in 50 mM NaCl compared to deionized water ($P< 0.001$, n=12). In contrast, almost full immersion in 50 mM NaCl enhanced whole body water uptake compared to immersion in deionized water: Water uptake under immersion was 9.23 g/100g/h in deionized water and 11.64 g/100g/h in 50 mM NaCl ($P<0.001$, n=15). These results suggest that the additional water uptake in toads immersed in 50 mM NaCl, is absorbed by non pelvic skin. Supported by NSF grant IBN9215023 and DNSF grant 9901768.

46.5

Paracellular Permeability And Chemosensory Function Of Toad Skin

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Dehydrated toads take up water by osmotic absorption across the skin. Chemosensory function of the skin allows toads to detect hyperosmotic solutions and then avoid dehydration. Avoidance of NaCl solutions is partially inhibited by Amiloride, suggesting that transcellular transport of Na^+ is coupled to the chemosensory process. In this study we examine the role of paracellular transport in the chemosensory process using isolated skin preparations, behavior and neural recording. Exposure of the isolated skin to 250 mM NaCl increased the electrical conductance. Inhibition of the transcellular current with Amiloride did not reduce the conductance response to 250 mM NaCl. Hyperosmotic NaCl solutions and the protein kinase C inhibitor, H-7 increased the unidirectional influx of 14C sucrose across the isolated skin. Behaviorally, the avoidance of hyperosmotic solutions of Na^+ salts decreased in the presence of larger anions or with NaCl in the presence of 5 mM $La3+$. Recordings from spinal nerves that innervate the skin showed a smaller integrated neural response and an enhanced rinse response following exposure of the skin to 250 mM sodium gluconate compared to 250 mM NaCl. Finally, $La3+$ inhibited the neural response to hyperosmotic NaCl solutions. These results are consistent with cellular regulation of occluding junctions in the toad skin and with a chemosensory function of a paracellular pathway. Supported by NSF grant IBN9215023 and DNSF grant 9901768.

46.6

The role of NaK ATPase and V type H ATPase in ion transport in euryhaline mosquito larvae

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Only 5% of the mosquito species have larvae that tolerate salt environments and two physiological strategies have been identified. Salt-tolerant osmoregulators have evolved an extra rectal segment that is the site of active NaCl excretion thereby allowing the larva to maintain constant hemolymph NaCl levels in salinities up to 300% seawater. Osmoconformers tolerate up to 70% seawater by allowing their hemolymph to conform osmotically to the environment by accumulating the organic osmolytes proline and trehalose. Osmoconformers also regulate their hemolymph NaCl levels over a range of environmental salinities but they lack the additional rectal segment and therefore differ morphologically from the salt-tolerant osmoregulators. I am examining the role of NaK ATPase and V-type H ATPase play in these two disparate strategies of salinity tolerance in mosquito larvae by examining the expression of these proteins in key ion transporting organs and determining how the population of these ATPases respond to varying salinity. Immunocytochemical analyses indicate that the V-type H ATPase is driving NaCl excretion in the extra rectal segment of salt tolerant osmoregulators when held in high salinity. Both ATPases are found in the recta and anal papillae of the freshwater and osmoconforming species however there are species and strategy specific differences in the distribution of the ATPases in the two cell types in the Malpighian tubule.

46.7

Species-specific variation in sulfide physiology between closely related Vesicomyid clams

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Symbioses involving sulfide-oxidizing bacteria and metazoan phyla dominate invertebrate assemblages at cold seeps and hydrothermal vents worldwide. The predominant species inhabiting cold seeps in Monterey Bay are the vesicomyid clams Calyptogena kilmeri and C. pacifica. The growth and survival of these clams depend directly upon the productivity of their chemoautotrophic endosymbionts, which is fueled by the oxidation of sulfide. For this reason, sulfide availability and sulfide-related physiology are thought to be the most influential factors governing the productivity of these associations. Both species inhabit sulfide-rich sediments and depend nutritionally on their symbionts, yet many aspects of their life styles differ considerably. Our results indicate that C. pacifica, which inhabits areas with lower environmental sulfide levels, is physiologically poised for the uptake and transport of sulfide, indicated by increased sulfide consumption rates, sulfide binding ability, and internal sulfide levels. C. pacifica also has a greater potential for symbiont energy turnover, supported by increased sulfide oxidation potential, enzymes involved in sulfur metabolism, and bacterial densities. C. kilmeri, on the other hand, demonstrates a less effective sulfide uptake mechanism and, therefore, a specific need for higher environmental sulfide levels. It appears that the abilities of these two species to process sulfide differ greatly and reflect not only the environments in which they are found but also the capabilities of their symbionts. This research represents the first comparative investigation of the physiological functioning of closely-related species in chemosynthetic symbioses and elucidates the constraints and advantages posed by different modes of sulfide (energy) uptake and assimilation in these, and perhaps other, symbiotic organisms.

46.8

Deleterious Effects of Mild Overwintering Temperatures on Survival and Potential Fecundity of Rose-galling *Diplolepis* Wasps (Hymenoptera: Cynipidae)

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Prepupae of the rose galling *Diplolepis spinosa*, collected from a relatively cold overwintering climate in southern Canada, and *Diplolepis variabilis*, from a milder locale in western Canada, were used to test the hypothesis that mild winter temperatures are detrimental to the survival and potential fecundity of insects. Prepupae of *D. spinosa* were either extracted from their gall or held within their gall while being exposed to simulated overwintering temperatures (-22, 0, 5, or 10°C) for approximately four months before measuring their survival, body size and potential fecundity. Similar studies were conducted using prepupae of *D. variabilis* that were removed from their gall and subjected to 0°C or 10°C treatments. *Diplolepis spinosa*, which were either extracted or held within their galls, averaged 66% less survival at 10°C than those at 0°C. Female *D. spinosa* that survived the 10°C treatment had 32% fewer eggs than those held at 0°C. In contrast, there was no difference in survival rate or numbers of eggs between *D. variabilis* held at 0°C and 10°C. Body size of adult females and size of eggs did not differ among temperature treatments for either species. We conclude that mild overwintering temperatures may be detrimental for insects by raising their metabolism, and consequently reducing energetic reserves needed for development to the adult stage and subsequent production of eggs the following spring. Supported by NSF#IBN-0090204

46.9

Cross-tolerance in Tidepool Sculpins (*Oligocottus maculosus*): A Strategy for Life in the Intertidal Zone

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In nature, a fish's thermal history is important in structuring its cellular response to stress. This study was designed to investigate the capacity of a mild heat shock to increase the tolerance of tidepool sculpins (*Oligocottus maculosus*) to a subsequent heterologous stressor, focusing on the functional role of heat shock proteins (Hsps). Survival of tidepool sculpins exposed to severe osmotic and hypoxic stressors increased from 68% to 96%, and from 47% to 76% respectively when exposed to a 22°C heat shock (fish were initially acclimated to 10°C). The magnitude of this heat shock was critical for protection. A 20°C heat shock was insufficient to confer cross-tolerance while a 25°C heat shock impaired the ability of the sculpin to tolerate a second stressor. Further experiments demonstrated that cross-tolerance was present in a defined temporal window between 12 and 48h following the 22°C heat shock. Western blot analysis revealed that hepatic Hsp70 levels were significantly elevated 12h following exposure to the mild heat shock, corresponding to the increase in stress tolerance. In their natural environment there is approximately 12 hours of high tide between low tide cycles. The time frame of this cross-tolerance may provide evidence of the tidepool sculpin's ability to invoke a protective mechanism from one low tide period for the unpredictable nature of the next. This research was supported by NSERC and the Killam Trust.

46.10

Modulation Of The Stress Response: Effects Of Breeding Stage, Season And Relationship To Nest Abandonment

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The mechanistic basis of the interaction between stress and reproduction is still poorly understood. Although previous studies have examined seasonal trends in corticosterone (B) secretion, few studies have separated specific effects of reproductive stages from season. In addition, although elevated B levels are known to increase locomotory activity and dispersal and interrupt reproduction, no studies have examined the natural variation in B in relation to nest abandonment. We examined baseline and stress-induced levels of B in wild female European starlings (*Sturnus vulgaris*) during laying, incubation and chick-rearing, and monitored nest abandonment. Stress-induced levels in chick-rearing birds were significantly lower (28 ng/ml) than incubation (37 ng/ml) and laying birds (48 ng/ml). These trends were independent of season, with laying birds exhibiting high levels throughout the season. Baseline B levels of chick-rearing birds (11 ng/ml) were significantly elevated compared with laying (8 ng/ml) and incubation (6 ng/ml) birds. Abandonment rates were correlated with stress-induced B levels through reproduction, with abandonment decreasing with each successive reproductive stage. This study illustrates that chick-rearing birds may be less sensitive to acute increases in B potentially allowing them to be more resistant to nest abandonment, while also exhibiting elevated baseline levels which may facilitate foraging and feeding of chicks. Funded by NSERC research grant to TDW.

46.11

Recent Thermal History Altered the Thermal Resistance and Hsp70 Accumulation in Tissues of the Tidepool Sculpin (*Oligocottus maculosus*) under Acute Heat Stress

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While the thermal tolerance of an animal is known to be determined genetically, at least in part, it is also known to be dependent on recent thermal history. The mechanism of this plasticity is not fully understood. Recently, we have focused on the role of 70-kDa heat shock protein (Hsp70) as one of the key factors responsible for the thermal tolerance of an animal. In the present study, we examined the effect of thermal conditions on the thermal resistance, and the cellular Hsp70 and *hsp70* mRNA response, in brain, gill, and liver tissues of an intertidal fish, the tidepool sculpin (*Oligocottus maculosus*). The laboratory-acclimatized (L) group were kept at 15 °C for 2 weeks prior to the experiment, while the field-acclimatized (F) group were collected from the intertidal zone 24 h before the experiment. During a 4 h heat shock experiment at 28 °C: 1) the F-group had no mortality, while all fish in the L-group died; and 2) Hsp70 and *hsp70* mRNA levels significantly increased in tissues of both groups, but the magnitude of increase in the Hsp70 level was significantly larger in the F-group than in the L-group. These results demonstrated that: 1) Hsp70 levels under heat stress corresponded with the thermal resistance of the fish; and 2) the F-group induced higher Hsp70 levels under heat stress faster than the L-group, implying that the translational efficiency from *hsp70* mRNA to Hsp70 may be different between these two groups. This study was funded by NSERC to GKI.

46.12

Extreme Resistance to Desiccation and Microclimate Related Differences in Cold-hardiness of Overwintering Gall Wasps (Hymenoptera: Cynipidae) on Roses in Southern Canada

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Four species of cynipid wasps of the genus *Diplolepis* that induce galls on roses (*Rosa* spp.) in southern Canada, and two species of inquiline cynipids associated with these galls, were studied for their cold-hardiness and resistance to water loss, as well as for possible links between these adaptations. Winter acclimated supranivean *D. spinosa* and *P. pirata* which overwinter exposed, above the snowpack had lower average supercooling points (-39°C) and higher hemolymph osmolalities (1813 mOsm) than subnivean *D. polita*, *D. gracilis*, *D. radicum*, and *Periclistus* sp. (-32°C and 1287 mOsm) which overwinter in the buffered subnivean space. During a simulated transition from fall to winter conditions, *D. spinosa*'s glycerol concentration more than tripled reaching a value of 0.98 *M*, while its supercooling point decreased by 13°C from the initial -27.4°C; however, glycerol content and supercooling point did not change for subnivean species. Cuticular permeability for all species was extremely low (0.33 to 1.00 $\mu g \cdot h^{-1} \cdot cm^{-2} \cdot Torr^{-1}$), even when compared to desert species; however, there was no difference in permeability between supranivean and subnivean prepupae. Transition temperatures ranged between 32.3 and 34.6°C; below 30°C, temperature had little effect on rates of water loss for all species (Q10 1.13 to 1.87). In conclusion, supranivean species had increased cold-hardiness, however there was no difference in water loss rates between the species. Supported by NSF#IBN-0090204.

46.13

Influence of thermal stress on rates of protein synthesis and metabolism in an intertidal crustacean

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The semi-terrestrial isopod, *Ligia oceanica*, inhabits humid micro-habitats at the high water mark of Spring tides, where it experiences elevated temperatures in the summer months due to the radiant heating effects of the sun. SDS PAGE and autoradiography of acclimatised animals demonstrated that *L. oceanica* survives periodic exposure to high temperatures (25, 27, 29 and 31°C for 3 h) by synthesising heat shock proteins (hsp) with molecular weights of 70, 68, 60 and 53 kDa. In contrast, general rates of protein synthesis decrease significantly at each heat shock temperature. For example, absolute rates of protein synthesis fell from 2.75±0.37(10) to 1.24±0.23(10) mg protein $animal^{-1}$ day^{-1} after heat shock at 25°C for 3h. Despite the increase in temperature, whole animal rates of oxygen uptake also declined during heat shock, falling from 206±26(10) to 58±6(10) mol $animal^{-1}$ day^{-1} between 15 and 25°C.

The relationship between whole animal rates of protein synthesis and metabolism was further investigated by injecting heat shocked animals with the protein synthesis inhibitor, cycloheximide. Such treatment inhibited hsp synthesis but had little effect on general rates of protein synthesis, while there was a slight decline in oxygen uptake rates. From this data it appears that the preferential synthesis of heat shock proteins in *L. oceanica*, at least within 3h of heat shock, are less metabolically costly than general rates of protein synthesis. LSF received a NERC studentship.

46.14

CO_2 Release Pattern in Female *Culex tarsalis* and Effect of Age, Flight, Egg Production and Blood-Feeding

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Using flow-through respirometry, we have measured the pattern and rate of CO_2 release from single adult female mosquitoes in the species *Culex tarsalis*. Adult females at rest released CO_2 at a mean rate of 0.65nl/min, in rhythmic bursts with a periodicity of 22± 3.5 seconds. Flight attempts in the small chambers (0.5 ml^3) resulted in CO_2 bursts reaching 25 times resting values. Individual rates of CO_2 release and dry mass were measured at rest for virgin and fertilized females at ages 4, 8, 12 and 16 days. Release rate was found to increase with dry mass with an exponent of 0.69. Fertilized females weighed significantly more than virgins and had significantly lower mass-specific CO_2 release rates. Average VCO_2 was negatively correlated with age in virgins whereas for fertilized females it was lowest at age 12 days. CO_2 release was also measured over a six-day period following a blood or sugar meal. Blood-feeding elicited a substantial specific dynamic action, while the response to sugar-feeding was much reduced. This last experiment also provided the diurnal patterns of CO_2 release.

These are the first data on CO_2 release in single adult mosquitoes using flow-through respirometry. They allowed us to estimate the effects of life history factors (flight, feeding and reproduction) on metabolic rate in a medically important mosquito, and can be used to estimate the effects of environmental factors (temperature, relative humidity) as well.

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46.15

Physiological and Behavioral Sensitivity to Environmental Stressors Measured by Changes in Fish Guild Structure in Urbanized Streams

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Indicator guilds of fishes in Georgia Sand Hills streams were used to quantify metric values for a localized index of biological integrity. Land use impacts have decreased biodiversity in streams north of Fort Benning, Georgia compared to reference assemblages. Species endemic to the region that have been characterized previously as intolerant species (e.g. blacktip shiner, Lythrurus atripiculus), occurred in numbers too low to correlate with environmental analytes associated with urban disturbance. We adapted index metrics to reflect occurrence of fishes currently inhabiting urbanized streams. The blacktail shiner (Cyprinella venusta) and silverjaw minnow (Ericymba buccata) were found to be sensitive species and the bluegill (Lepomis macrochirus) and western mosquitofish (Gambusia affinis) to be tolerant species. The adapted sensitivity metric correlated with turbidity and the tolerance metric with siltation. Other metrics correlated with factors influencing behavioral requirements of indicator species. However, physiological stressors influenced metrics including native sunfishes, native cyprinids, biomass, and reproductive specificity. Combinations of chlorine, detergents, dissolved oxygen, salinity, and water velocity influenced these metrics. Urban land use introduces physiological and behavioral stressors that influence fish guild structure. Funding for this research was provided by the Construction Engineering Research Laboratory Contract # DACA42-00-C-0047.

46.16

Urine Composition in Water Stressed Cricetid Rodents: Sodium Oxalate

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When subjected to prolonged water deprivation, several species of cricetid rodents from the Namib Desert produce allantoin that precipitates in their urine. This shift in nitrogen excretion from urea to allantoin allows them to realize considerable water savings. However, as this has not been reported in any other rodent species, it is not known whether this is a general trait of cricetids, or if it is unique to Namib Desert species. We did not find precipitated allantoin in the urine of any of four rodent species from the Negev Desert (three cricetids and one murid), when deprived of water under laboratory conditions. There was no discernable precipitate at all in the urine of Anderson's gerbil >*Gerbillus andersoni allenbyi*) or Cairo spiny mouse >*Acomys cahirinus*). Precipitate found in the urine of the fat sand rat >*Psammomys obesus*) was sodium oxalate, and the as yet unidentified precipitate that was found in the urine of Wagner's gerbil >*Gerbillus dasyurus*), was not allantoin. Apparently, rodents of the Negev Desert do not share with cricetids from the Namib the ability to switch from urea to allantoin production. Therefore, this shift in nitrogenous excretion may be unique to the cricetids of the Namib Desert.

46.17

Characterization of Very-Low Density Lipoprotein Particle Size During Avian Egg Production

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Because animals require energy to fuel all aspects of life, trade-offs arise between different activities when resources are limited. Consideration of differential allocation of energy-rich lipids during avian egg production may give insight into the physiological basis of trade-offs between current reproduction and both maternal survival and future reproduction. Egg production is dependent on dramatic changes in lipid metabolism, including a marked increase in hepatic production of yolk-targeted very-low density lipoprotein (VLDLy), which represents an alteration in plasma VLDL structure and function from larger 'generic' VLDL, which is involved in triglyceride transport, to smaller VLDLy, which supplies the yolk with energy-rich lipid. We characterized VLDL particle size distribution (PSD) in laying female Zebra Finches (*Taeniopygia guttata*) to examine the dynamics of lipid metabolism during egg production in passerines in comparison with poultry, and determine the extent of inter-individual variation and repeatability of PSD, and the relationship between PSD and egg size. Blood samples were taken on the day females laid their first egg, and PSD was measured using dynamic laser scattering. VLDLy particle diameter ranged from 20-36 nm in laying Zebra Finches, compared to 15-44 nm in laying hens. The extent of inter-individual variation in PSD in Zebra Finches was not as marked as in poultry, and was independent of egg size. Funded by an NSERC research grant to TDW.

46.18

Molecular Basis of Angiogenetic Disturbances in Baltic Salmon Early Mortality Syndrome

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Baltic salmon have been suffering from a maternal-dependent disease, M74, which results in high yolk-sac fry mortality. Symptoms of M74 include circulatory disturbances and oxidative stress. In mammals, one important factor regulating angiogenesis and embryonic survival is basic-helix-loop-helix-PAS (bHLH-PAS) protein hypoxia inducible transcription factor 1 alpha (HIF-1α). To study the possible role of HIF in the etiology of M74 we have analyzed its expression in hatchery-reared, wild healthy and M74-fry aged 50 to 190 ATUs (Accumulated Temperature Units). We found that healthy fry have an increasing expression pattern of HIF from age 50 to 190 ATUs. In M74-fry, the nuclear localization and the binding of HIF to response elements in DNA are decreased. Since Baltic salmon fry contain organochlorine contaminants that induce expression of another bHLH-PAS protein, aryl hydrocarbon receptor (AhR), disturbances in HIF transactivation could be explained by competition for common dimerization partner, ARNT. However, in M74-fry also the binding of AhR to response elements in DNA is decreased. Therefore, we propose that during yolk-sac fry development formation of hypoxic regions in the embryo activates HIF, which increases expression of genes involved in vascularization and erythropoiesis. In M74-fry, oxidative stress may explain the disturbances in HIF DNA-binding activities, as DNA-binding of both HIF-1α and AhR may be redox-regulated. The work is supported by Academy of Finland.

46.19

Neonates of the common map turtle (*Graptemys geographica*) overwinter terrestrially in northern Indiana: Does Hatchling Cold Hardiness Influence Geographic Distribution?

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Field observations and laboratory experiments were conducted on hatchlings of the common map turtle (*Graptemys geographica*), a species that reportedly overwinters within the natal nest in the northern parts of its range. The cold hardiness of *G. geographica* was assessed by measuring a suite of physiological adaptations that contribute to winter survivorship in other terrestrially hibernating hatchlings. We examined the frequency of spring versus fall emergence in natural *G. geographica* nests, the minimum winter temperatures experienced in the nest, the frequency and duration of sub-zero events, and survivorship. Laboratory experiments were conducted using lab-reared hatchlings to determine intrinsic supercooling capacity, tolerance of somatic freezing, resistance to inoculation by environmental ice, as well as desiccation resistance. Field observations confirmed that hatchling map turtles regularly overwinter within the nest chamber despite the occurrence of subfreezing (-4°C) nest temperatures. The hatchlings supercooled deeply (-14.8°C), exhibited a remarkably high resistance to inoculation by external ice nuclei (-9.7°C), and they had relatively low rates of evaporative water loss (1.99 mg g^{-1} d^{-1}). However the hatchlings were intolerant of somatic freezing at -2.5°C for 24h, which suggests that this species survives severe winter conditions by remaining unfrozen in a state of supercooling.

46.20

Cold Hardiness and Desiccation Resistance in Hatchling *Emydoidea blandingii*

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Parameters of cold hardiness and desiccation resistance of hatchling Blanding's turtles (*Emydoidea blandingii*) were examined to suggest potential overwintering sites. Terrestrially overwintering hatchlings either tolerate freezing or avoid ice nucleation behaviorally or physiologically by supercooling. The ability of hatchlings to supercool depends upon their resistance to inoculative freezing. In the absence of external ice nuclei, hatchlings supercooled to –14.3°C. Furthermore, supercooled hatchlings were able to survive 1h exposures to –8.0°C and 7d exposures to –4.0°C. However, when immersed in frozen soil, their ability to remain supercooled was compromised by their inability to resist inoculation by external ice nuclei below –1.2°C. Hatchlings survived somatic freezing to –3.5°C for 72h. This profound freeze tolerance is comparable to that of hatchling painted turtles (*Chrysemys picta*) and box turtles (*Terrapene ornata*). Evaporative water loss rates of *E. blandingii* hatchlings (4.1mg g^{-1} d^{-1}) were intermediate to both terrestrial and aquatic overwintering turtle species. Our results suggest that hatchling *E. blandingii* possess physiological adaptations that may allow them to overwinter in terrestrial habitats. However, due to the lack of inoculation resistance it is doubtful that hatchling *E. blandingii* use their pronounced ability to supercool, but rather they rely on freeze tolerance. This work was supported by National Science Foundation (IBN 98017087).

46.21

Are physical factors facilitating marine species invasions?

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The bay mussels *Mytilus trossulus* and *M. galloprovincialis* co-occur from Monterey to Cape Mendocino in a patchy hybrid zone. *M. galloprovincialis* appeared in Southern California before 1950 and currently dominates the region. We are interested in whether small-scale physical conditions and differential physiological adaptation explain the observed distribution. To address this question, we surveyed adult and recruit populations, monitored temperature and salinity and tested physiological limits of adults. Scoring individuals using multiple gene loci, we genetically identified adult and recruit populations in Monterey Bay (MBay) and San Francisco Bay (SFBay). In both systems, we found an increased abundance of adult *M. trossulus* as we move up the estuarine gradient (toward higher temperature and lower salinity). However, *M. trossulus* has a lower heat tolerance than *M. galloprovincialis*, implying that salinity -not temperature- is driving the observed distribution. We also found that recruit abundance does not match adult patterns in MBay but does in SFBay, suggesting that MBay is a more open system, with respect to recruitment. The mismatch in MBay is due to a greater abundance of *M. trossulus* recruits at sites where adults are rare or absent. Finally, our results have implications for invasive species prevention and water resource management in areas where species distributions follow estuarine gradients. Sea Grant, NSF.

46.22

Characterization of Oxidative Stress in *Saccharomyces cerevisiae* Mutants Lacking Superoxide Dismutase

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Superoxide dismutase (SOD) catalyzes the reduction of superoxide to hydrogen peroxide and water and thus protects cells against protein, lipid and DNA damage caused by reactive oxygen species (ROS). The yeast *Saccharomyces cerevisiae* expresses two forms of SOD, a CuZnSOD present in both the cytosol and mitochondria, and a MnSOD found only in mitochondria. We examined the level of oxidative stress in *S. cerevisae* mutants lacking SOD to clarify the role of each SOD in protecting cells against ROS. Respiration rates and protein carbonylation levels were measured at four points during growth, as cells shifted from fermentation to respiration. Surprisingly, protein carbonylation does not increase as respiration rate increases. Protein carbonylation differs among strains only in late-stationary phase, at which point carbonylation of mitochondrial proteins is higher in mutants lacking CuZnSOD or both CuZnSOD and MnSOD, compared to wild-type and MnSOD mutants. 2D-electrophoresis reveals that similar proteins are carbonylated in all strains until late-stationary phase. We are currently using mass spectrometry to identify these proteins. These data indicate that CuZnSOD is more important than MnSOD in protecting mitochondria from oxidative damage. Furthermore, these results suggest SOD is only critical in protecting cells against oxidative stress during late-stationary phase. Funding was provided by an NIH post-doctoral fellowship to K.M.O. and NIH grant HL63324 to R.O.P.

46.23

Fluorescein Transport in Malpighian Tubules of the Cricket, Acheta domesticus: Affinity and Specificity Characteristics

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Previous studies have used fluorescein accumulation in renal tissue as an indicator for the presence of the organic anion transport system. We characterized kinetic and specificity characteristics of this transport process in the renal tissue of the cricket, *Acheta domesticus*, using epifluorescence microscopy. Malpighian tubules showed a rapid accumulation of fluorescein, with a calculated K_m of 7.8 uM. Fluorescein (1 µM) accumulation was substantially blocked by 1 mM probenecid and 100 µM bromosulphophthalein (uptake was ~5% and 13% of control, respectively), but was largely unaffected by 250 µM glutarate or 3 mM *p*-aminohippuric acid (uptake was 89% and 80% of control). Likewise, fluorescein (0.5 µM) uptake was unaffected by the presence (1 mM) of the monocarboxylates valeric acid and caprylic acid. The lack of inhibition by classic organic anion substrates indicates substantial differences in its specificity compared to organic anion transport in mammals. The herbicide 2,4-D (1 mM) reduced uptake to 50% of control, suggesting a role for this process in handling environmental toxins. However, uptake of fluorescein was not inhibited by exposure to 100 µM of the insecticide chlorpyrifos and was stimulated 22% by its oxon metabolite. We conclude that cricket Malpighian tubules possess a vigorous transport process for organic anions that is positioned for the renal handling of xenobiotics to which insects are exposed. Supported by Jeffress Trust J-618.

46.24

Physiological Responses, Desaturase Activity and Fatty Acid Composition in Milkfish (Chanos chanos) Under Cold Acclimation

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Physiological responses of milkfish (*Chanos chanos*) under cold shock and acclimation were investigated by monitoring the parameters of plasma glucose, lactate, and lipids, as well as stearoyl-CoA desaturase activity and fatty acid compositions of hepatic membranes. The experimental milkfish, a warm-water teleost, were initially acclimated at 25 °C and then transferred directly to 15 °C; stress responses of this species were monitored for 1 week. All the monitored parameters showed significant changes in milkfish under cold acclimation. A hyperglycemic response, indicated by a notable increase in plasma glucose levels from 85 mg dl^{-1} to 458.2 mg dl^{-1} in 24 h, was followed by a rapid decline thereafter. Plasma lactate concentrations remarkably increased from 47 mg dl^{-1} on day 0 to 149.6 and 120.4 mg dl^{-1} on days 1 and 2, respectively, and then rapidly declined to the same level as the control. In contrast, plasma lipids increased gradually from 44.8 mg dl^{-1} to 191 mg dl^{-1} over the 5-day period, followed by a declining trend. Furthermore, changes in monounsaturated fatty acids were highly correlated with those of stearoyl-CoA desaturase activities in hepatic microsomes of milkfish during cold acclimation. Results indicate that in milkfish subjected to cold stress, plasma hyperglycemic and hyperlactemic responses can be used as acute stress indicators, and plasma lipids can be used as a chronic stress indicator. This work was funded by the Council of Agriculture, Taiwan.

46.25

The pathway to heat acclimation: Does HIF-1 plays a role? A lesson from C. elegans mutants

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Chronic exposure to environmental heat induces protective mechanisms leading to heat acclimation (HA). Our data on mammals indicates that reprogrammed expression of genes coding for heat shock proteins and enzymes involved in energy metabolism play major roles. Since our knowledge of the "architecture" of acclimatory signaling in mammals is limited we decided to characterize a Caenorhabditis elegans heat-acclimation genetic model, allowing identification of selective evolutionary conserved pathways involved in the acclimatory response. In wild type C. elegans HA (25oC, 24hrs) significantly increased heat endurance during exposure to heat stress at 35oC. To identify the mediating signaling pathways we examined knock out mutations in two candidate genes: the daf-16 (insulin receptor pathway, known to enhance stress tolerance) and hif-1 (hypoxia inducible factor). vhl-1 and egl-9 mutants (overexpressing HIF-1) were also examined. LD50, HIF-1 level(Western immunoblotting) were measured before and after HA . While the wild type C. elegans and daf-16 mutants showed enhanced heat endurance following acclimation, hif-1 knock-out mutant could not acclimate. Non-acclimated vhl-1 and egl-9 mutants showed markedly better endurance to heat than wild type. Our results suggest a central role for HIF-1 in heat acclimation, as HIF-1 expression is both necessary and sufficient for this process. The observation that HA elevates HIF 1a in rats suggests conservation of this pathway.

46.26

Stressor-dependent Regulation of Heat Shock Response in Zebrafish, *Danio rerio*

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Divergent environmental signals trigger heat shock transcription factor (HSF) -mediated activation of *hsp* (heat shock protein) genes. We have previously shown in zebrafish disappearance of a unique isoform zHSF1b following heat shock with concomitant induction of Hsp70, the major heat-inducible heat shock protein. This observation led to the question of the role of these isoforms in stress response. Among the reported inducers of Hsps are heavy metals, which are known to induce also another protein, metallothionein (MT), involved in metal homeostasis and detoxification. To characterize the role of zHSF1-isoforms *in vivo*, we have carried out copper (5-100µM) and cadmium (5-100µM) exposures in order to specify whether the disappearance of HSF1b is stressor-dependent.

After four-hour exposures we analysed transcription of HSF1, *hsp70*, *hsc70* (constitutive form of Hsp70) and *MT* by reverse transcriptase polymerase chain reaction (RT-PCR). Copper and cadmium induced *MT* in gills and liver but not in gonads, at concentrations ≥ 20 M. *hsp70* was only slightly activated in gonads and liver upon exposures. Neither of the metals affected HSF1a/b-ratio despite the target gene upregulation. This study indicates complexicity of the stress response, which becomes apparent when experiments are conducted at the organismic instead of cellular level.

This work is supported by the Academy of Finland, projects 40830 and 42186 and 50748.

46.27

A Comparative Study Examining the Utility of Hsp 70 mRNA and Protein in Red Blood Cells as Bio-Indicators of Acute and Chronic Temperature Stress in the Thermo-Sensitive Brook Trout (*Salvelinus fontinalis*).

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The main objectives of this study were to examine the Hsp 70 mRNA and protein response in various tissues of brook trout under both acute and chronic heat stress conditions to determine the utility of red blood cells (rbcs) as a bio-indicator tissue of sub-lethal temperature stress. Rbcs consistently produced one of the highest heat shock mRNA and protein responses of all of the tissues examined. Hsp 70 mRNA was significantly induced in brook trout rbcs at 22°C, a temperature that is commonly experienced by this species during the summer months. Recovery of Hsp 70 mRNA following an acute temperature increase required between 24 and 48 hours. In contrast, Hsp 70 protein levels did not become significantly elevated until a temperature of 25°C, but remained elevated for over 48 hours after the temperature returned to control levels. During a six day chronic (23°C) heat stress, rbc Hsp 70 mRNA returned to baseline between 24 and 72 hours of exposure while Hsp 70 protein was still significantly elevated after six days. This study provides evidence of the utility of rbcs as a valuable indicator tissue of thermal stress in the brook trout, and indicates that water temperatures presently being reached in brook trout habitats are capable of inducing a significant heat shock response in this species. While Hsp 70 mRNA proved to be a more rapid and sensitive indicator of stress in all tissues examined, protein remained elevated over a longer period. Funded by NSERC Canada and the ASF.

46.28

A Role for hsp90 in the Estrogenic Response of Juvenile Rainbow Trout (*Oncorhynchus mykiss*) to ß-Estradiol and 4-Nonlyphenol

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The ubiquitous anthropogenic pollutant, 4-nonylphenol (4-NP) exerts an estrogenic effect on fish and has been shown to influence growth in salmonids. To date, the molecular processes underpinning these physiological effects are not well understood. In this regard, stress proteins (hsps) are thought to have a critical role in endocrine signal transduction in that they complex with steroid receptors and contribute to receptor activation. The goal of this study was 1) to determine if environmentally relevant levels of 4-NP cause an estrogenic response in juvenile rainbow trout and 2) to determine if the magnitude of the estrogenic response is correlated with hsp90 levels. We found that exposure to 2 24 h pulses of 20 µg/l 4-NP did not significantly affect growth in these fish up to 6 weeks following treatment. This dose was sufficient to induce moderate endocrine disruption as indicated by an increase in liver zona radiata protein (Zrp), but these estrogenic effects were not correlated with increases in hsp90. β-estradiol (E2) also resulted in a significant increase in Zrp within 24 hours of exposure that remained elevated for 6 weeks. Although the constitutive form of hsp90 was not affected by E2 treatment, the inducible form of this hsp was significantly increased 1 week after treatment. The correlation of hsp90 and Zrp levels suggests a role for hsp90 in the estrogenic response of fish and perhaps in endocrine disruption. NSERC of Canada supported this study.

46.29

Phenostasis and patterns of growth: a framework from which to interpret adaptive capacity

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Numerous investigations have documented the variety and range of the adaptive capacities of individual organisms. Among vertebrates, endotherms generally demonstrate a greater capacity for physiological accommodation to stresses such as hypoxia or exercise than ectotherms. Despite the obvious endotherm/ectotherm distinction, this view may obscure the actual causative factor responsible for these differences. I propose that the monitoring and response necessary to maintain the functional capacity of a determinant grower's phenotype (phenostasis) facilitates its adaptive capacity. In contrast, indeterminant growers follow a simpler program of maintaining functional capacity by continually building upon the present morphology, but with little or no feedback of functional status. This explains how a determinant grower with a long evolutionary heritage at low elevations can defend the functional capacity of its phenotype if moved to a hypoxic environment; a novel environmental stress. Although many indeterminant growers have evolved programmed responses to specific environmental influences (polyphenisms), without a "sensed" maintenance mode they have limited capacity to respond to novel stresses unknown to their heritage, e.g. chemical pollutants. The observation that children demonstrate less response to exercise and strength training than adults (male or female) provides intriguing support to separate adaptive capacity along growth pattern rather than thermoregulatory behavior.

46.30

Basal Metabolic Rate may not be related to Body Composition.

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Several recent studies on birds, including some long-distance, nonstop migratory shorebirds, have asserted a relationship between body composition and basal metabolic rate (BMR). The most common linkage established in these studies is between visceral (digestive) organs and BMR, though occasionally skeletal muscle has been implicated. The tone of some papers suggests that a connection between body composition and BMR is to be expected.

We have established that in migrating Eared Grebes (*Podiceps nigricollis*), mass-specific BMR shows no change compared with staging grebes in spite of substantial changes in body composition, especially digestive organs. This reinforces the findings of Geluso and Hayes (1999) who found no significant change in BMR in European Starlings (*Sturnus vulgaris*) despite large differences in body composition (including digestive organs) attributable to diet. Constancy in BMR may be a function of compensating changes in tissue metabolic rates. Alternatively, it may be due to a higher integrative control of BMR.

ACCLIMATIZATION TO HYPOXIA: SUPPLY VS DEMAND STRATEGIES

47.1

Intracellular pH Regulation of Rainbow Trout (*Oncorhynchus Mykiss*) Hepatocytes: Hypoxia Stimulates Sodium/Proton Exchange

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We studied pH regulation of rainbow trout (*Oncorhynchus mykiss*) hepatocytes under normoxic and hypoxic conditions using microspectrofluorometry. The cells were experimentally acidified by propionate. The transport pathways involved in maintenance of steady-state intracellular pH and in recovery from acidification were identified by using ion substitutions and transport inhibitors.

In accordance with earlier data, hepatocyte pH was regulated by Na^+/H^+-exchange, Na^+-independent Cl^-/HCO_3^-exchange and Na^+-dependent HCO_3^--transport

Under hypoxia (1 % O_2), the acid extrusion rate after acidification increased by 24.8 % ($p<0.001$) compared to normoxic conditions (21% O_2). The increase was due to activation of Na^+/H^+-exchanger since methyl isobutyl amiloride fully inhibited the recovery.

To examine the role of OH^--radicals, which appear to mediate the oxygen-dependency of ion transport in rainbow trout erythrocytes, the scavenger of OH^--radicals, N-(2-mercaptopropionyl)-glycine (MPG) was used. The effect of hypoxia was not mimicked by MPG, indicating that OH^--radicals do not cause the oxygen-dependency of Na^+/H^+-exchange in hepatocytes. This may be due to absence of Fe-ions, which participate in the formation of OH^--radicals, in the vicinity of hepatocyte membrane. This study was supported by the Academy of Finland (grants no. 40830, 50748 and 51860) and the National Graduate Program on Biological Interactions.

47.2

DEPRESSION OF LIPOLYSIS IN CARP; A POSSIBLE HYPOXIA PROTECTION MECHANISM

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During exposure of carp (Cyprinus carpio) to hypoxia fatty acids levels fall rapidly together with a significant increase of norepinephrine. Infusion of norepinephrine lowers plasma fatty acid levels, while it increases glucose levels. In mammals, however, adrenergic stimulation always increases plasma fatty acids mediated via -stimulation. Accumulation of fatty acids and their metabolites under hypoxia is destructive for biomembranes. So, depression of lipolysis under hypoxia may be a protection mechanism.Carp were cannulated in the dorsal aorta and infused with different salines: 1) control, 2) noradrenaline, 3) yohimbine (2-antagonist) & norepinephrine, 4) clonidine (2-agonist), and 5) isoproterenol (non selective -agonist). Infusion with norepinephrine decreases the plasma FFA level and increases the plasma glucose level for several hours. Norepinephrine in combination with yohimbine resulted in retardation of the FFA decrease, indicating the involvement of 2-adrenoceptors. Infusion with isoproterenol caused a marked increase of glucose levels, and a decline of plasma FFA levels, indicating a direct involvement of -adrenoceptors. Combination of isoproterenol with either atenolol (1-antagonist) or ICI 118,551 (2-antagonist) showed a 1+ 2 effect on the glucose release, and a -effect on the FFA decline. It is not yet clear how -stimulation results in inhibition of lipolysis. The mechanism is currently studied with adipocytes.

47.3

Hypoxia induces gross-morphological changes in crucian carp gills

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We have found that crucian carp kept in normoxic water have gills that lack protruding lamellae, a very atypical feature of fish. The lamellae was found to be buried in a cell mass, here denoted the interlamellar cell mass (ILCM). The lamellae of the gills are the principal site of gas exchange in fish, but also a major site of costly ion-fluxes between blood and water. Matching the gill surface area to respiratory need should therefore be advantageous. The haemoglobin of Carassius has an extremely high affinity for O2, which can explain why under normoxic conditions crucian carp does not need a large respiratory surface area. But such a morphological optimisation of one particular aspect of gill function (ion loss) could limit the ability of the crucian carp to cope with falling environmental O2 levels, unless relatively rapid morphological changes can take place. By employing scanning and transmission electron microscopy together with TUNEL and BrdU labelling we found that exposing crucian carp to several days of hypoxia induced a striking morphological change in the gills. The lamellae became protruding due to a large reduction in the ILCM caused by increased apoptosis and reduced mitosis. Consequently, the ability to take up oxygen from the water increased, but at the same time we found indications of increased ion loss to the water. The morphologial change was reversed within a week and was not induced by exposure to a total lack of oxygen. To our knowledge, this is the first example of an adaptive and reversible gross-morphological change in the respiratory organ of an adult vertebrate.

47.4

Effect of hypoxia on fish - what role(s) does apoptosis play?

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When fish face prolonged hypoxia, their metabolism, swimming activity, food intake, and reproduction are reduced. Growth is also reduced and this may due to some or all of the above factors, involving changes in cell proliferation and apoptotic rates. It is possible that the up regulation of apoptosis and/or the down regulation of cell proliferation reduces growth and decreases energy expenditure during hypoxia.

TUNEL (Tdt-mediated dUTP nick-end labelling) was used to analyse apoptotic rates. From preliminary results, 24hr hypoxic liver cells showed apoptotic signals while normoxic liver cells did not. Results from prolonged hypoxia (4-8 weeks exposure) in which cell proliferation rate and apoptosis were measured, to determine the relative roles of these mechanisms in determining organ size during hypoxia, will be discussed.

47.5

Developmental Plasticity In Tadpole Shrimp: Cardiac and Respiratory Responses to Chronic Hypoxic Exposure

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Tadpole shrimp (*Triops longicaudatus*) live in ephemeral pools throughout the desert southwest of the United States. These pools routinely become very warm (40° C) and close foo anoxic during the summer months before complete evaporation. Behavioral modifications (increased surfacing activity) along with compensatory physiological responses (increased ventilation and heart rates) allow these animals to survive under acute hypoxic exposure. Extended or chronic hypoxic exposure during embryonic and larval (juvenile) developmental stages results in more profound changes in cardiac and respiratory physiology and morphology. Animals exposed to normoxia, moderate and sever hypoxia during embryonic and larval periods showed significant differences in gill surface area, ventilation rates and hemoglobin concentrations. Heart rate, ventilation rate and surface area for gas exchanges showed significant changes in response chronic hypoxic exposure. Hemoglobin concentration more than doubled with a significant increase in oxygen binding affinity which results in an almost 10 fold increase in oxygen capacitance. The hypoxia induced up-regulation of hemoglobin production maybe more complex than a simple increase in circulating Hb concentration. 2D gel electrophoresis suggest a second form of Hb maybe produced, which could result in the shift in oxygen bind affinity. Supported by NSF grant IBN 9874534 to CLR.

47.6

Effect of Reproductive State and Hypoxia on Cardiovascular Responses in the Grass Shrimp *Palaemonetes Pugio*

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Decapod crustaceans encounter hypoxic environments and have evolved compensatory mechanisms to deal with the effects of low O_2. Large crustaceans have been shown to alter their cardiovascular parameters. Specifically, they maintain cardiac output by increasing stroke volume, which compensates for the hypoxia-induced bradycardia. This allows for maintenance of cardiac output even under hypoxic conditions. However, grass shrimp (*P. pugio*), small hypoxic tolerant decapod crustaceans, utilize a different compensatory mechanism. These adult shrimp exhibit a hypoxia-induced tachycardia with a concomitant decrease in stroke volume yet maintain cardiac output. Yet gravid female grass shrimp exposed to mild hypoxia (13.3 KPa O_2) do not follow the same pattern as these non-gravid adult shrimp. Cardiac output of gravid females is not maintained, as both heart rate and stroke volume decrease in response to hypoxic conditions. Grass shrimp ovaries are paired organs that run from the anterior portion of the shrimp ventrally through the pericardial sinus. As oocytes develop within the ovaries the confined space of the pericardial sinus becomes limited. This increased mass may restrict the space available for the heart to expand, thereby limiting the ability of the gravid female to maintain stroke volume. In order to elucidate the differences in cardiovascular response of grass shrimp to hypoxic conditions, cardiac parameters (heart rate, stroke volume and cardiac output) of males, non-gravid and gravid females exposed to progressive hypoxia (Po_2s = 20, 13.33, 10, 6.7 and 2 KPa O_2) are monitored. Water Po_2 within the experimental chamber is maintained using a gas mixing flowmeter (Gas Mixing Flowmeter GF3/MP) and cardiac parameters are determined using videomicroscopic dimensional analysis. Supported by NSF grant 9874534 to CLR.

47.7

Metabolic and Thermal Acclimation to Hypoxia in Rats.

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The ability to acclimate metabolically to chronic hypoxia, (i.e. regain a normoxic level of metabolism), has been described to be present in mature rats and absent in young rats. The control and interaction of the hypometabolic and hypothermic responses to hypoxia remain poorly understood. Therefore we asked the following questions: 1) Is there a relationship between the magnitude of the acute hypoxic hypometabolic or hypothermic response (which may be greater in younger animals) and the ability to acclimate or the time course of acclimation? 2) Does the ability to acclimate metabolically develop gradually or over a narrow age range? 3) Are metabolic rate and body temperature restored over similar or differing time courses? Preliminary data from 8 rats of varying ages exposed acutely and chronically (up to two weeks) to 12% oxygen suggest that: 1) the magnitude of the acute response is not correlated with the acclimation rate (r=-0.18); 2) the ability to acclimate metabolically develops at about 34 ? 36 days post-natally; and 3) body temperature can recover more quickly than metabolic rate during acclimation to chronic hypoxia. This developmental ?window? provides a time frame in which to look for changes that may relate to mechanisms controlling hypoxic hypometabolism and metabolic acclimation to hypoxia.

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47.8

2,3-DPG Changes in Horses, Mules and Burros with Exposure to Altitude

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There are limited studies that address the physiological changes in equids in response to high altitude, and the present study had the goal of increasing our understanding of high altitude acclimation in horses, mules and burros by measuring 2,3-diphosphoglycerate [DPG]. Increases in DPG would facilitate oxygen unloading at the tissues and has been argued as an adaptation to high altitude. Two separate animal populations were sampled. Population 1: pack animals consisting of 10 mules and 15 Quarter Horse-type, were sampled following 4.5 months at 3000-3500 m. They had been moved to low altitude (1050 m) 12 hours prior to sampling. Twelve other horses (275 m) served as another low altitude comparison. Population 2: 12 burros on pasture at 1220 m for one year and 12 other burros that had been at altitude (3000-3400 m) for six weeks. The high-altitude acclimated burros were sampled approximately 4 hours after arrival at 1220 m. There were no differences between the two populations of horses measured at low altitude. Altitude acclimatization increased DPG/Hb (in mmol•g hemoglobin^{-1}) in horses (18.0 ± 1.0 to 23.8 ± 1.2) and in mules (14.8 ± 1.3 to 29.9 ± 1.3) but had no effect on burros (16.8 ± 0.8 to 15.7 ± 0.5).

47.9

Respiratory consequences of mouthbrooding and hypoxia in coral reef fish

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A mouth full of eggs can be expected to pose a considerable respiratory problem. Still, the respiratory consequences of mouthbrooding in fish have previously not been examined. Cardinalfishes (Apogonidae) are all mouthbrooders. This study was carried out at Lizard Island on the Great Barrier Reef. Using closed respirometry, we found that mouthbrooding significantly reduced the ability of cardinalfish (Apogon fragilis) to take up oxygen at low ambient oxygen levels. Hypoxia may also reduce the breeding success, since it made the males spit out the whole brood. Moreover, mouthbrooding was found to significantly reduce aerobic swimming performance in cardinalfish (Apogon leptacanthus). Finally, a surprisingly high ability to maintain oxygen uptake at relatively severe hypoxia (critical PO2= 18 - 50 mmHg) was displayed not only by cardinalfishes, but by several other fishes, including damselfishes, occurring in the same shallow coral reef habitat.

It is concluded that, while the direct energetic cost of mouthbrooding is insignificant at rest in well oxygenated water, mouthbrooding significantly reduces the ability of the fish to cope with environmental factors such as hypoxia and water currents.

It is also suggested that hypoxia tolerance is widespread among coral reef fishes, indicating that hypoxia may be a more common occurrence in this ecosystem than generally thought.

Financed by the Research Council of Norway.

47.10

Effects of Hypoxia and Epinephrine on Erythrocytes of High-Altitude Acclimated Pigeons, *Columba livia*

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We previously reported that mean corpuscular volume increases in red blood cells (RBC) of high-altitude acclimated pigeons. Others have shown that β-adrenergic receptors on avian RBC bind catecholamines, causing net water uptake and a RBC-volume increase. To examine the effects of catecholamines and hypoxia on avian RBC *in vitro*, we acclimated pigeons to 7 km simulated altitude (inspired PO_2 (PIO_2) 65 Torr) for ≥ 3 weeks. Arterial blood was sampled from the undisturbed birds while they breathed normoxic air. The heparinized whole blood (WB) was tonometered continuously at 41°C for 30-40 min with saturated gases to replicate the arterial blood gases of altitude-acclimated pigeons breathing normoxic (PIO_2=139, PaO_2=95, $PaCO_2$=30 Torr) or hypoxic (PIO_2=65, PaO_2=30, $PaCO_2$=17 Torr) air. At intervals, 20-μL WB aliquots were cold-centrifuged to determine hematocrit (Hct). Exposure to tonometer gases alone, or after saline addition to WB, had no effect. Epinephrine (epi) addition to WB increased Hct significantly over 10-15 min in both hypoxic and normoxic WB. This suggests that ion transport caused cell water uptake and increased RBC volume. In normoxic WB, addition of the ion-transport blocker furosemide (furo) abolished the epi-induced Hct increase, whereas in hypoxic, furo-treated WB, Hct still increased significantly. The results suggest that epi and hypoxia acted synergistically to increase RBC volume and Hct. (NIH grants GM61222 & GM08136; NIH NRSA GM19791 to ESQ)

47.11

Properties of skeletal muscle in mice with an inherited capacity for hypoxic exercise tolerance

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Functional and metabolic properties of muscle were examined in mice with an inherited capacity for hypoxic exercise tolerance. Following 8 weeks exposure to hypobaric hypoxia (1/2 atm), two strains of mice, BALB/cBy (C) and C57BL/6J (B6), and their F1 hybrid have dramatically different capacities for endurance exercise under hypoxia. Prior to hypoxic exposure, the mice differ little in hypoxic exercise capacity. C mice do not improve their endurance capacity following hypoxic exposure, B6 mice improve considerably, and the F1 hybrid has an endurance capacity that far exceeds that of either parent. These performance differences are associated with two loci of major effect. Fiber type and myosin heavy chain profiles were consistent among the three strains and were not influenced by hypoxia. However, the peak tension produced by predominantly fast-twitch muscle differed with strain and hypoxic treatment. Peak tension increased in the C and B6 mice following hypoxic exposure, but decreased in the F1. Capillary density did not differ with strain or hypoxic treatment. However, myoglobin (Mb) concentration changed dramatically following hypoxia in a strain and tissue-specific manner. [Mb] increased in skeletal and cardiac muscle of the B6 mice. In contrast, [Mb] decreased in skeletal muscle of the worst-performing C mice and in skeletal and cardiac muscle of the best-performing F1 mice. In cardiac muscle of C mice, however, [Mb] increased following hypoxic exposure. A reduced tension production and decreased [Mb] therefore characterizes skeletal muscle from F1 mice. These and other properties may reflect a tighter coupling of energy supply and demand in skeletal muscle that is beneficial for endurance exercise.

47.12

Amino Acid Sequences of the Embryonic Globin Chains of a Marsupial, the Tammar Wallaby (Macropus eugenii)

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We have previously reported function and sequence studies of embryonic marsupial blood.The sequences are of interest for two main reasons. Firstly they give an insight into the evolution of marsupials; and secondly the embryonic type blood present before and soon after birth has unusual functional properties. In particular these are a right-shifted oxygen equilibrium curve (OEC), and a high Hill coefficient (up to 6.5) indicating that the functional unit is larger than a tetramer. We have now the sequences of all the embryonic globin chains of the Tammar Wallaby. These were determined on the blood of neonatal animals, in which the Hb is all or nearly all of embryonic type. Sequencing was by Edman degradation or mass spectrometry with or without prior digestion of the individual chains into peptides. There are four Hbs present, named 1-4 in order of decreasing isoelectric point. They are Hb 1 ($\alpha_2\omega_2$), Hb 2 ($\alpha_2\varepsilon_2$), Hb 3 ($\zeta'_2\varepsilon_2$), Hb4 ($\zeta_2\varepsilon_2$). About 80% of the total Hb is Hb 3 or Hb 4. The ε-chain is similar to other mammalian embryonic β-type chains; the ω-chain is unusual in being more like bird β-type chains. The two embryonic α-type chains (ζ)

differed by 14 residues, showing there are two different ζ genes, the first time this has been found in any mammal. We have not been able to find any sequences that account for the low oxygen affinity or the high cooperativity of the embryonic or neonatal blood.

Supported by the Australian Research Council

47.13

Inhibition of Hypoxic Pulmonary Vasoconstriction (HPV) Reduces High Altitude Pulmonary Edema (HAPE) in Rats.

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Rapid ascent to high altitude causes HAPE in susceptible humans. The "stress failure" hypothesis proposes that HPV is uneven and capillaries leak in overperfused regions. This study was designed to determine the role of vascular pressure in a rat model of HAPE. Rats were pretreated with novel substances known to prevent HPV (60 mg/kg NiCl2 or 20 mg/kg acetazolamide, i.p.) with control rats receiving equal volume saline (1 ml, i.p.). Alveolar lavage and ventricular weight changes were then assessed. We found that saline-treated rats develop a mild protein leak during high altitude exposure (0.5 atm./24 hour) indicative of early HAPE. The leak was prevented by inhibition of HPV: protein in lavage fluid of HPV-inhibited rats, 13.2 ± 0.8 mg/dL (mean ± SEM, n=8); saline-treated rats, 22.8 ± 3.9 mg/dL, n=8; sea level-exposed rats, 13.5 ± 1.7 mg/dL, n=5 ($p<0.05$ saline vs other groups, ANOVA). The lungs of saline-treated rats were mildly hemorrhagic and lungs of HPV-inhibited rats appeared normal. Finally, the ratio of right ventricle/left ventricle+septum in saline-treated rats was greater: HPV-inhibited, 0.29 ± 0.01, n=6; saline-treated, 0.34 ± 0.01, n=7; sea level, 0.30 ± 0.01, n=4 ($p<0.05$, saline vs other groups). These results show that HPV-inhibition prevents HAPE in rats and suggest that acetazolamide, in addition to preventing acute mountain sickness, protects against HAPE. Funded by the Hawaii Community Foundation (Geist Award #HCF 20010643) and NIH NHLBI #HL24163.

47.14

Hypoxia Regulation of Gene Expression in Crustaceans: A Potential HIF-1 System.

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It is now known that mammals possess the molecular machinery, namely hypoxia-inducible factor 1 (HIF-1), to abate hypoxic situations. HIF-1 participates in acute and acclimatory responses to low oxygen in humans by upregulating genes involved in abrogating the negative physiological consequences associated with hypoxia. An example of such a system is the upregulation of erythropoietin (EPO) by HIF-1. HIF-1 mediates these responses by inducing EPO, which increases overall RBC production and thus the amount of hemoglobin and oxygen carrying capacity of the blood. The environment of many crustaceans is far from homeostatic and they undoubtedly experience hypoxia on a frequent basis, yet little is known about the mechanisms underlying hypoxia stress responses and tolerance in crustaceans. Since it has been shown that some crustaceans increase the concentration of hemocyanin in response to hypoxia, we wondered if crustaceans have a HIF-1 system that regulates gene expression to increase oxygen transport and delivery. Using hepatopancreas cDNA from *Cancer magister* as a template and degenerate primers designed from human HIF-1a, mouse HIF-1a, and *Drosophila* Similar (Sima), a HIF-1 like sequence was amplified using RT-PCR. The 647bp fragment amplified in *Cancer magister* is 82-98% identical to *Drosophila* Sima, a homologue of HIF-1a. Experiments are underway to further characterize this potential HIF-1 system in crustaceans. *Supported by NSF 9984202.*

47.15

HIF-1alpha, erythropoietin and adaptation to excessive erythrocytosis

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Hypoxia induces gene expression of specific genes such as erythropoietin (Epo) and vascular endothelial growth factor (VEGF) that allow physiological adaptation to the environmental conditions at the cellular, local and systemic levels. The hypoxia-inducible transcription factor-1 (HIF-1), a heterodimer consisting of the oxygen-regulated alpha-subunit and the constitutively expressed Beta/ARNT-subunit, serves as a master regulator of oxygen-dependent gene expression. We determined the accumulation of HIF-1alpha over a range of pathophysiological oxygen concentrations in vitro and in vivo. In HeLa cells, hypoxia-induced translocation of HIF-1alpha to the nucleus is instantaneous. In mice kept at normoxic conditions, we detected HIF-1alpha in brain, kidney, liver, heart, and skeletal muscle. Under hypoxic conditions HIF-1alpha expression levels varied depending on the tissue and duration of insult. These findings suggest that HIF-1alpha may be required for basal expression of genes that are necessary to provide the cellular energy requirements under normoxic conditions.

In another line of investigation, we followed the consequences of chronic erythrocytosis by generating a transgenic mouse line constitutively overexpressing the human Epo gene . These mice reach adulthood with a hematocrit of about 80-90%. Unexpectedly, blood pressure is not elevated and cardiac output is not decreased. We showed that increased activity of endothelial NO synthase prevents cardiovascular disease. Still, life expectancy of transgenic mice is reduced to about 8-10 months. Further adaptational mechanisms to excessive eythrocytosis as well as the cause of early death are under current investigation.

47.16

Acute And Long-term Neuroprotective Responses To Hypoxia In Snail Neurons

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Mammalian nerve cells are exquisitely sensitive to oxygen deprivation and suffer irreversible injury after 3 minutes of anoxia. However, snail neurons are able to pre-emptively reduce their requirement for oxygen as the availability of oxygen declines. This 'oxyconformation response' has not been reported previously for any other neuron. The oxygen consumption rates of acutely dissociated neurons from active snails are 60% higher than neurons obtained from estivating snails. Further experiments indicate that acutely isolated neurons from estivating snails can depress their already reduced metabolic rate by a further 40% in response to decreasing oxygen tensions. The oxyconformation response in snail neurons is reversible at oxygen tensions ranging from 0-40 mmHg and is modulated by extracellular pH. The observation that oxyconformation in snail neurons occurs at oxygen partial pressures (P_{O2}) that are in excess of the critical P_{O2} at which diffusion of oxygen to the mitochondria begins to limit oxidative phosphorylation suggests that the neuron senses declining oxygen availability, and in response, reduces its aerobic metabolic rate. This response offers great promise in identifying mechanisms that control and co-ordinate a hypometabolic strategy of neuroprotection.

47.17

Microcalorimetric evidence of an oxyconformism in tissue metabolism of mammalian neonates

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The specific metabolic rate (MR) of mammalian fetuses equals the maternal one and is thus lower than expected from body mass. The suppression of size allometry acts as an adaptation to low intrauterine pO2 and has been shown to persist even after birth in very immature neonates. A similar mechanism might contribute to neonatal hypoxia tolerance. To test this hypothesis, ischemic hearts of neonatal, juvenile, and adult mice were studied by microcalorimetry and -respirometry. Additionally, heat output of 300 µm tissue slices from neonatal and adult murine myocardium was measured at different pO2. The ischemic decline in heat output was slowest in neonatal hearts, corresponding to the highest specific O2 consumption. Thus, ischemic survival seems to partly depend on the uptake of residual O2 from the surroundings. Although this is mainly due to the small size of neonatal organs, it would be greatly favored by a low MR. Surprisingly, the specific heat output of tissue slices, at any given pO2, was higher in neonatal than in adult mice, fitting the usual size relationship. However, this results in the fact that neonatal tissue, at low (intrauterine) pO2, still has the same MR as adult tissue at high (extrauterine) pO2. Altogether, these findings suggest that, based on its fetal metabolic adaptation, neonatal tissue is able to vary in an oxyconformistic manner between a higher (size-related) and a lower (adult-

REGULATION OF VERTEBRATE RENAL FUNCTION: A COMPARATIVE APPROACH

48.1

Sipping human, gulping camel: The story behind future sweat

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The ingestion of a large electrolyte-free water (EFW) load causes a prompt water diuresis. The rise in urine flow rate (U_{FR}) is the result of a fall in the plasma sodium concentration (P_{Na}), and thereby, antidiuretic hormone (ADH). If EFW could be retained, our Paleolithic ancestors would have used it as 'future sweat' in a prolonged hunt. Our objective was to examine conditions needed to prevent the rapid excretion of ingested EFW. Five male subjects were studied while they had a positive balance of 10 ml EFW/kg in the fasting state on 2-4 occasions. If the 10 ml/kg EFW load was ingested over 30 min (gulping), the 6.6 ml/min U_{FR} was submaximal and reproducible. This U_{FR} was maintained over 1.5 h by maintaining this positive balance of EFW. In contrast, if a positive balance of 10 ml EFW/kg was induced over 3 h (sipping), the U_{FR} did not rise (0.6 ml/min) despite a similar decrease in venous P_{Na}. Hence, factors other than the steady-state P_{Na} influenced ADH release. We speculate that a fall in the portal venous, and thereby arterial P_{Na} was the tonicity signal to inhibit ADH release. The venous P_{Na} should be lower than the arterial P_{Na} because water has equilibrated across the ECF and ICF compartments when the venous P_{Na} is obtained. To gain insight into this control system, subjects drank 20 ml EFW/kg while fasting. After achieving a steady state U_{FR} of 11 ml/min, EFW intake was curtailed. The U_{FR} fell and the urine became hypertonic despite the retention of 10 ml EFW/kg. Now a rise in the renal venous, and thereby the arterial P_{Na} could stimulate ADH release before there was equilibration with total body water. Conclusion: Sipping rather than gulping EFW made ingested EFW 'invisible' to the central tonicity-stat. Retaining more EFW could have conferred a survival advantage in Paleolithic times.

48.2

ENDOTHELIN INHIBITS NaCl TRANSPORT ACROSS THE FISH GILL BY RELEASE OF NITRIC OXIDE AND PROSTAGLANDIN E.

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Recent evidence (mammalian tissues) suggests that vasoactive paracrines such as endothelin (ET), nitric oxide (NO), and prostaglandins (PGs) may have significant effects on epithelial ion transport steps, including those that control NaCl extrusion across the marine teleost fish gill. To test this hypothesis, the opercular epithelium from seawater-acclimated killifish (Fundulus heteroclitus) was clamped to zero (Ussing chamber) and the short circuit current (Isc) recorded. ET-1 inhibited the Isc in a concentration-dependent manner, as did SNP (NO donor), PGE_2, and sarafotoxin S6c (SRX; specific for the ET_B receptor) which suggests that an ET_B-like receptor mediates the ET inhibition. Inhibition of nitric oxide synthase (0.1 mM L-NAME) stimulated the baseline Isc and reduced the SRX-induced inhibition of the Isc by 15%; inhibition of PG synthesis (10 µM indomethacin: cyclooxygenase inhibitor) did not affect the baseline Isc, but it reduced the SRX-induced inhibition by 90%. These data suggest that NO, but not PG, is tonically released to partially inhibit NaCl transport across the fish gill and that ET can inhibit this transport by the release of both NO and a PG, with PG playing the dominant role. Immunohistochemical studies suggest that big ET (ET precursor), nNOS, and COX are expressed in chloride cells, cells adjacent to chloride cells, and the arterio-venous interlamellar network, respectively. Supported by NSF IBN-9604824 and IBN-0089943.

48.3

Redistribution of Body Water and Salt Tolerance in Wild Ducks

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When Pekin ducks drink saline, some extracellular (EC) water and sodium shifts into the intracellular compartments. There is a greater decrease in EC water and EC sodium among females and females are the more saline tolerant sex. This led us to compare the degree of fluid and sodium shifting in the two sexes in three species of wild ducks: Mallards, Canvasbacks, and Goldeneyes that have low, modest, and high saline tolerance, respectively. We tested two hypotheses: 1) level of saline tolerance is related to the proportion of EC water and EC sodium redistributed in response to saline intake and 2) redistribution of EC fluid and EC sodium is greater in female birds. We compared total and EC water and sodium of birds drinking freshwater or saline (300 mM NaCl). Total body water and sodium did not differ between sexes or treatments in any species. Drinking saline has no effect on EC volume and EC sodium of Mallards or Canvasbacks, but lowers both in both sexes of Goldeneyes. Redistribution of water and sodium into cells correlates with level of saline tolerance and we accept our first hypothesis. Redistribution does not differ between the sexes and we reject the second hypothesis. Expansion of the extracellular fluid volume triggers salt gland secretion. An expanded intracellular volume may provide a fluid reservoir that can rapidly expand extracellular fluid volume in response to salt loading and thereby initiate secretion. (Supported by NSERC grant to MRH)

48.4

Distribution and Possible Function of Aquaporin Water Channels in Amphibian Skin

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Amphibians are unusual among vertebrates in that they are capable of "drinking" by absorbing water through their skin. Evidence from previous studies suggest that aquaporin (AQP) water channels located in the membranes of skin epithelial cells might facilitate water transport. Our goal is to understand the role AQPs play in cutaneous water absorption, and determine if they are regulated as a means of adjusting skin permeability. For our anuran model we used *Bufo marinus*, and our caudate model was *Taricha* granulose. We used standard immunohistochemical and molecular protocols to localize and characterize AQP proteins in the skin of both anuran and caudate amphibians. In addition, we used an ELISA assay to measure plasma ADH level in hydrated, dehydrated, and rehydrated amphibians to see if ADH might be involved in regulation of skin permeability. Using a primary antibody for mammalian AQP 1, immunohistochemistry showed a high density of AQP in the outermost epithelial layer of the entire skin surface of *Taricha*. In *Bufo* AQP was only found in skin associated with the seat patch, a specialized region of skin used in water absorption. Unlike *Taricha*, the AQP layer in *Bufo* skin was located a few cell layers below the surface. The reason for this difference AQP distribution is unclear, but might be related to differences in their water absorption behavior. A partial amino acid sequence of the AQP protein in both species suggests they are an AQP 1 homolog, and thus ADH insensitive. This is supported by the fact that endogenous ADH level in *Taricha* did not change with hydration state, and thus does not appear to impact skin permeability in caudates.

48.5

Comparison of Renal and Salt Gland Function in Three Species of Wild Ducks

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Three processes central to osmoregulation of marine birds were examined and compared in three species of ducks that differ in habitat affinity, diet and saline tolerance. These processes are: filtration of Na^+ and water from the plasma by the kidneys, reabsorption of filtered Na^+ by cells along the renal tubules, and secretion of Na^+ by the salt glands. Barrow's Goldeneyes (*Bucephala islandica*), the most marine species, have the highest rates of filtration, fractional reabsorption of water and Na^+, and salt gland Na^+ excretion. Only this species can secrete all the infused salt by the salt glands. The rate of all these processes is lower in Mallards (*Anas platyrhynchos*), the most freshwater species, but, following saline acclimation, they can excrete all the infused Na^+ by a combined Na^+ excretion of the kidneys and salt glands. Canvasbacks (*Aythya valisineria*), despite being more saline tolerant than Mallards, are unable excrete all the infused Na^+. They produce a large volume of urine (like Mallards) that has a low $[Na^+]$ (like Goldeneyes). Salt gland secretion of all three species is more concentrated than the water they drink, but only Goldeneyes secrete at a rate sufficient to eliminate all infused Na^+ via the salt glands. These three processes do not account for the differences in salt tolerance of ducks, suggesting that postrenal modification of their urine plays an important role in the conserve water. (Support by NSERC grant to MRH).

48.6

Renal structure and function in Notomys alexis and Mus musculus domesticus

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Glomerular filtration rate (GFR) was measured in Notomys alexis (N.a.) and Mus musculus domesticus (M.m.d.) when animals were provided with water ad libitum, following 15 or 24 hours of water deprivation and after 10 days of gradual water restriction. GFR was calculated from the rate of disappearance from plasma of radioactive EDTA. Plasma and urine osmolality, urea and electrolytes were measured. There was no change in GFR in response to water restriction in either species. Urine flow rate decreased in N.a. following 15 hours of water deprivation and in M.m.d. after 24 hours. Urine osmolality, potassium, chloride and urea increased in N.a. after 15 hours of water deprivation. N.a. was able to rapidly form a concentrated urine and also to dilute urine rapidly once water was restored. The response of M.m.d. was slower. The vascular bundles of N.a. are numerous and simple in structure whereas those of M.m.d. are fewer but more complex. The results are not consistent with either of the models of inner medullary insulation proposed by Bankir and DeRouffingnac (1985). The absence of change in GFR in response to water deprivation in N.a. suggests that urine concentrating is due to change in tubular properties rather than altered GFR. N.a. is better able to maintain plasma homeostasis when water restricted than is M.m.d. This study received approval from the UNE Animal Ethics Committee.

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48.7

A novel, non-invasive electrophysiological technique for analysis of organic cation transport by isolated cells and tissues.

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Endogenous organic cations (OCs) include choline, catecholamines and N-methylnicotinamide. OC transport is often studied by measuring fluxes of radiolabelled OCs such as the model compound tetraethylammonium (TEA). Our novel method exploits the ~300-fold higher selectivity of Corning ion exchanger 477317 for TEA relative to K^+. The ~3 um diameter tip of a self-referencing ion-selective (SeRIS) microelectrode (ME) is moved by computer controlled stepper motors (Applicable Electronics), between two points 50 - 100 um apart in the unstirred layer near the surface of the tissue. [TEA] is proportional to SeRIS ME voltage, and TEA flux is proportional to the measured concentration difference. SeRIS MEs provide good spatial and temporal resolution of TEA fluxes across isolated tissues of the fruit fly. TEA transport is highest in the lower Malpighian (renal) tubule (LMT) and associated ureter. Fluxes are smaller in the upper Malpighian tubule and midgut, and negligible in the foregut or hindgut. TEA influx is inhibited by OC transport blockers such as cimetidine and quinidine, but also by P-glycoprotein substrates (Rhodamine 123, nicotine) and blockers (verapamil). Our working hypothesis for the LMT invokes both potential-dependent OC transport and P-glycoprotein-like transport. We are also applying the SeRIS ME technique to the study of organic anion transport, using ion exchangers such as Corning 477913. Supported by NSERC (Canada) grants to MJO.

48.8

Contribution of Cytoskeletal Elements to Rapid Fluid Transport in Insect Malpighian Tubules.

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In the Malpighian tubules (Mt) of the house cricket, *Acheta domesticus*, the immediate response to stimulation is the vesiculation of the cytoplasm. With db-cAMP, vesicle formation is extremely rapid (15 sec) and 60% of the cell volume can be occupied by vacuoles. The rapid translocation of membrane seen in histological studies strongly suggests that the cytoskeleton is involved in fluid transport.

Initially we used the microtubule-disrupting agent, oryzalin, because it is effective at low concentrations (5-50 $mol.l^{-1}$). When added to control preparations, oryzalin reduced fluid secretion by roughly 50% over a 90-min period. When oryzalin and cAMP were added simultaneously, fluid secretion increased, but not in the expected manner. Normally the effects of cAMP are evident within the first measurement interval. Oryzalin delayed the onset of the cAMP-induced increase in secretion by a full 30 min, and the magnitude of the increase was reduced by approximately 65%. When the actin-disrupting agent, cytochalasin-B (cytoB) and cAMP were added together to a stable control preparation, the expected increase in fluid secretion was completely blocked. In stimulated preparations, cytoB lowered the secretion rate back to control values during the first measurement interval, following which the rate rebounded to about 65% of the original increase.

Microtubules are clearly involved in both normal and rapid fluid transport, but other cytoskeletal elements can compensate to a significant degree for disruptions in normal microtubule function. The initiation of the cAMP-mediated increase in fluid secretion involves an obligate, actin-dependent step, although once initiated, other elements can sustain transport.

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48.9

Cell-to-Lumen Taurine Efflux During Net Secretion by Primary Monolayer Cultures of Flounder Renal Epithelium.

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Cell-to-lumen exit is the critical step in renal transepithelial taurine secretion in marine fishes. The characteristics of the luminal taurine efflux system were examined in primary monolayer cultures of renal proximal tubule cells (PTCs) of the winter flounder. The PTCs mounted in Ussing chambers exhibited net transepithelial secretion of taurine at all concentrations examined (0.1 - 0.5 mM in both hemichambers). The cell-to-lumen taurine efflux rate was measured in monolayer culture mates mounted in Ussing chambers, preloaded with [^{3}H]taurine at the same concentrations as in the transepithelial flux studies, and with the peritubular membrane covered with mineral oil. Intracellular concentrations of taurine in these PTCs were measured before and after the efflux measurements. The cell-to-lumen efflux rate of taurine correlated closely with the net secretory rate. The efflux of taurine across the luminal membrane appeared to be independent of the basolateral transport processes but varied with intracellular taurine concentrations. With increasing intracellular taurine concentrations in PTCs (from ~ 10 to 150 mM), the luminal taurine efflux exhibited saturation kinetics (Km for luminal efflux of ~ 96 mM and maximal rate for luminal taurine efflux of ~ 23 nmol/cm^2/h). These data demonstrate for the first time that the cell-to-lumen transport step in transepithelial taurine secretion is saturable and carrier-mediated.

(Supported by OUHSC and NSF)

SCHOLANDER AWARD BANQUET LECTURE: BARBARA BLOCK

49.0

THE FIRE INSIDE: SAVING BLUEFIN TUNA. B. A. Block.

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Electronic tagging and remote sensing technologies herald a new era for biological oceanography. The deployment of small microprocessor based data storage tags that are implanted or satellite-linked provides marine researchers novel avenues for examining the migrations and behaviors of pelagic vertebrates. When biological and physical data from the tags are combined with satellite derived sea surface temperature and ocean color data, the relationship between the movements and behaviors of organisms can be linked to oceanographic processes. In this talk I will report on the results obtained from the electronic tagging of Atlantic bluefin tuna with implantable archival and externally attached pop-up satellite archival tags. The natural history and migratory abilities of Atlantic bluefin tuna have fascinated mankind for millennia. These fish grow to over 300 cm and attain 680 kg in size. They range from the tropics to polar latitudes and are renowned for their endothermic physiology, which is rare among fishes. Despite a history of exploitation that spans thousands of years, little is known about the spatial dynamics of bluefin tuna movements, depth preferences or thermal biology. The tagging data are providing new insights into seasonal movements, trans-Atlantic migrations, mixing rates, depth preferences, thermoregulatory biology and breeding behaviors. These data are critical for the future management and conservation of bluefin tuna in the Atlantic. This research is primarily supported by NMFS, NSF, NFWF, and the Packard, Pew and MacArthur Foundations.

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