

Technical Document 2934

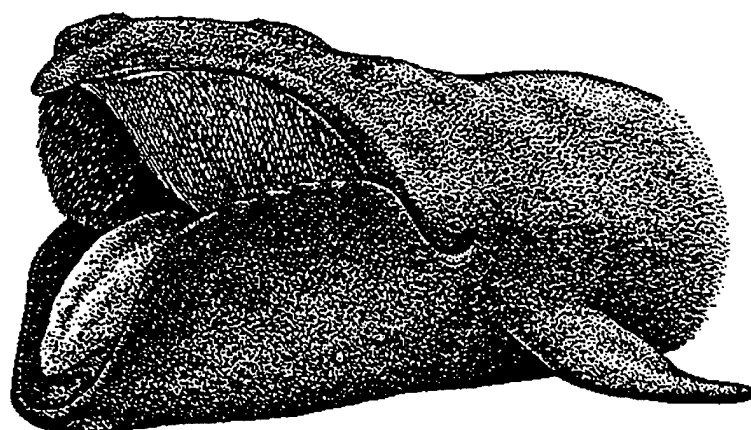
October 1996

Final Report from the Right Whale Necropsy Assessment Team

Results, Analysis, and
Recommendations

Edited by:
Sam H. Ridgway, D.V.M., Ph.D.

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Final Report from the Right Whale Necropsy Assessment Team

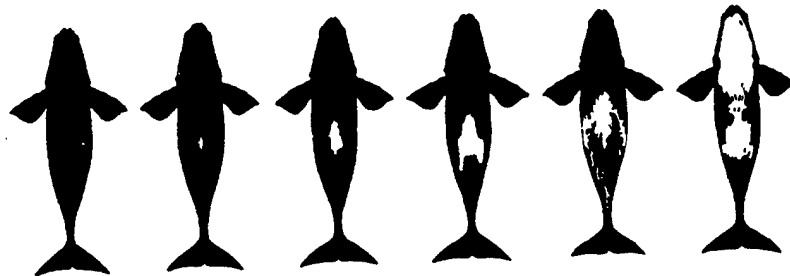
**Results, Analysis, and
Recommendations**

Prepared for:

Mr. Thomas Peeling
Chief of Naval Operations (N45)
Special Assistant for
Environmental Planning,
Environmental Protection,
Safety and Occupational Health Division
Office of the Chief of Naval Operations

Edited by:

Sam H. Ridgway, D.V.M., Ph.D.
NCCOSC RDT&E Division



**NAVAL COMMAND, CONTROL AND
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Commanding Officer

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Executive Director

ADMINISTRATIVE INFORMATION

This report was prepared for Mr. Thomas Peeling, Chief of Naval Operations (N45) Special Assistant for Environmental Planning, Environmental Protection, Safety and Occupational Health Division, Office of the Chief of Naval Operations, 2000 Navy Pentagon, Washington, DC 20350-2000. The report was edited by Sam H. Ridgway, D.V.M., Ph.D., NCCOSC RDT&E Division, Code D3503, 49620 Beluga Road, Room 200, San Diego, CA 92152-6266.

Released by
S. H. Ridgway, D.V.M. Ph.D
Senior Scientist for Animal Care

Under authority of
J. E. Haun, Head
Bioscience Division

EXECUTIVE SUMMARY

As many as six endangered right whales, *Eubalaena glacialis*, out of a western north Atlantic population estimated to number only about 300, were reported and/or found dead in their southern breeding range in the Atlantic Ocean along Georgia and Florida coasts during January and February 1996. There was suspicion by the press, public, and whale biologists studying the species that the deaths were linked to U.S. Navy operations. In view of concern within and outside the Government, the Chief of Naval Operations (N45) Special Assistant for Environmental Planning, Mr. Thomas Peeling, requested an evaluation of the four available necropsy reports.

On 19 April 1996, a panel of scientific experts (see appendix A) was convened in Washington, DC, at the Armed Forces Institute of Pathology (AFIP). The goals of the panel were to:

- review necropsy results and discuss the possible cause(s) of the recent mortalities of northern right whales along the U.S. East Coast,
- explore the possible involvement of barotrauma and ship impact in the mortalities, and
- begin to develop a necropsy protocol for right whales to be used in future assessments.

After analyzing the four available reports, viewing videotapes, and listening to the descriptions of the necropsy surgeons, the panel concluded that the cause of death could not be determined in three of the four cases. One animal, RKB 1429, had suffered massive, acute trauma, probably inflicted by a large, moving, unidentified vessel.

Although there was no evidence available that would allow for the determination of the type of vessel striking RKB 1429, the possibilities include U.S. Coast Guard, U.S. Navy, and commercial vessels. The investigation into the possible involvement of U.S. Navy exercises focused on determining whether any of the whales had suffered barotrauma or blast injury from an exploding bomb or shell and recommending tissues be taken to investigate such incidents.

The panel recommended that right whale tissue taken at necropsy be reviewed at the AFIP and made available for evaluation by other pathologists as well. The AFIP received tissues from three of the whales and rendered its report on 8 July 1996 (enclosure 1). The report concluded that injuries suggesting ship collision were seen in one animal (RKB 1429) but the pathologic findings did not support barotrauma or blast injury in any of the whales. It was suggested by Dr. Rommel (enclosure 2) that "there is an inadequate knowledge base from which to competently evaluate all the possible effects of primary blast injury on marine mammals." Much of the literature on the subject was published in the 1950s and 1960s. Many of the assumptions in this literature are based on injury from air blasts on terrestrial mammals and may not apply the same to marine mammals. Dr. Rommel also suggested further investigations be conducted to understand the injuries that underwater blasts could cause to marine mammals.

Recommendations for developing a protocol to facilitate detailed evaluations of similar incidents in the future were discussed. Panelists made suggestions about facilitating access to dead whales and improving necropsy results by other means. **Panelists recommended that Navy commands operating in right whale habitat be asked to report dead whales sighted and assist in the rapid recovery of carcasses.**

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INTRODUCTION

Reports of large and small cetaceans observed dead at sea or stranded along the coasts of the U.S. seem to have increased in recent years. The cause(s) of many of these events remain undetermined; however, anthropogenic factors, including pollution, ship strikes, and entanglement in fishing gear, have been implicated. Response time to these events has been inconsistent and investigations frequently do not reveal the cause of death. The lack of useful scientific data collected during these events stems from the fact that there is an overall lack of knowledge of pathologies associated with large whale strandings. This situation must be remedied if future efforts are to improve. Strong scientific data will annul false accusations and support solutions.

BACKGROUND OF MORTALITIES

During January and February 1996, as many as six endangered right whales, *Eubalaena glacialis*, were reported and/or found dead in their southern breeding range in the Atlantic Ocean along the Georgia and Florida coasts. Table 1 presents sighting and recovery information. Another was found further north at Near Wellfleet, Massachusetts. Four of the whales were recovered for necropsy; as part of an ongoing Office of Naval Research project, ears from three of the them were sent to Dr. Darlene R. Ketten, a noted cetacean ear anatomist at Harvard Medical School who has extensive experience from examining humpback whales, *Megaptera novaengliae*, known to be exposed to explosive events.^{1,2} Lung tissues from three of the whales (RKB 1429, RKB 1430, and GA96112201) were made available to the Armed Forces Institute of Pathology (AFIP) for histopathological analysis by marine mammal and blast trauma experts. In addition to reviewing the histologic sections of lung, they also viewed video tapes of the necropsies, met with the individuals involved in the necropsies, and reviewed the findings of Dr. Ketten.

Concurrent with the deaths were several military and commercial shipping activities including passage of U.S. Navy ships and naval training operations involving gunnery and explosions at sea. Because of the cluster of mortalities both spatially (30°16.2'N 80°47.6'W to 31°30.04'N 80°58.26'W) and temporally (1/2/96 to 2/22/96), there was public concern about the deaths of this highly endangered species and suspicion by the press, public, and whale biologists studying the species that the deaths were somehow linked to U.S. Navy activities in the area; the question was raised whether blast injury could have been the cause of any of the mortalities.

In view of concern within and outside the Government, the Chief of Naval Operations (N45) Special Assistant for Environmental Planning, Mr. Thomas Peeling, requested an evaluation of the four available necropsy reports and any other available results to determine if naval exercises, especially those that involved explosives or blasts, had precipitated any of the mortalities.

RIGHT WHALE ASSESSMENT TEAM

To review available necropsy results and discuss the possible cause(s) of recent mortalities of northern right whales, along the U.S. East Coast, a panel of scientific experts from the U.S. Navy, National Marine Fisheries Service (NMFS), Marine Mammal Commission, U.S. Fish and Wildlife

1. Todd, S., S. Stevic, J. Lien, F. Marques, and D. Ketten. 1996. "Behavioural effects of exposure to underwater explosions in humpback whales (*Megaptera novaeangliae*)." *Canadian Journal of Zoology*, 74(9), pp. 1661-1672.
2. Ketten, D. R. 1995. "Estimates of blast injury and acoustic trauma zones for marine mammals from underwater explosions." In: *Sensory Systems of Aquatic Mammals*. R. Kastelein, J. Thomas, and P. Nachtigall (eds.). De Spil Publishers, pp. 391-408.

Service, and stranding response teams (see appendix A) was convened in Washington, DC, at the AFIP on 19 April 1996. Their goals were to:

- evaluate necropsy reports for four recent right whale mortalities to reveal the cause(s) of death,
- explore the possible involvement of barotrauma and ship impact in the mortalities, and
- begin to develop a response plan and necropsy protocol for right whales to be used in future assessments.

Dr. Ketten furnished the panel written reports of her evaluation of the ears but could not attend the meeting.

Table 1. Right whale mortalities January–February 1996: sighting and recovery information.

Event #	Field #	Dates	Location	Notes	Condition/Code of Carcass
1	EGNE9601WBB	1/02/96 (0700) 1/5/96 1/11/96	Atlantic Beach, FL 30° 20.1'N 81° 23.7'W	Stranded dead right whale calf reported, carcass placed in freezer Carcass transported for necropsy, arrived on 1/08/96 Necropsy conducted	Dead 1 week; relatively good condition
2	GA96013001 RKB 1429 "Lindsey" #1623	1/30/96 (1100) 1/30/96 (1200) 1/30/96 (1830) 1/31/96 (0903) 1/31/96 (0951) 1/31/96 (1645) 2/01/96 (early am)	10 mi East of Sapelo Is, GA 31° 28.43'N 80° 59.57'W 31° 28.19'N 80° 59.91'W 31° 28.19'N 80° 59.91'W 31° 30.04'N 80° 58.26'W	First reported as dead by USCG Confirmed by GDNR from air GDNR's R/V "Bagby" unable to tow Located by UGA's "Georgia Bulldog" Towing underway Arrive at Harris Neck Natl. Wildlife Refuge Necropsy conducted	Moderately bloated, less than 10% of skin sloughed Dead probably 4–5 days prior to necropsy
3	EGNE9603WBB	2/5/96 ? 2/7/96 (1206) 2/08/96 (1200) 2/08/96 (1300)	30° 29'N 80° 37'W 30° 18.7'N 80° 57.2'W 30° 16.2'N 80° 48.3'W 30° 16.2'N 80° 47.6'W	Carcass observed by Navy but not reported to FDEP until 2/9/96 Reported by Navy to FDEP Reported by fisherman to FDEP FDEP confirmed carcass from air Carcass not recovered	Not recovered
4	GA96021901 RKB 1430	2/19/96 (0946) 2/19/96 (1800) 2/20/96 (0010)	Off Cumberland Is., GA 30° 44' N, 80° 59'W 30° 43.5'N, 80° 59.0'W	GDNR observes carcass from air GDNR's "Bagby" starts towing carcass Carcass transported to Gainesville, FL Necropsy conducted	Floating low in water; fresh No skin sloughing Internally, marked signs of advanced deterioration Dead probably 2–3 days prior to necropsy
5	GA96022201	2/22/96 (1430) 2/22/96 (1615) 2/23/96 (1745) 2/24/96 (1100) 2/24/96 (1930)	30° 56.53'N 80° 47.70'W 30° 56.00'N 80° 47.30'W 30° 59.7'N 80° 41.4'W 31° 04'N 80° 38'W	Right whale carcass reported to GDNR Right whale carcass confirmed by GDNR Right whale carcass relocated by GDNR Chartered fishing vessel starts towing carcass Necropsy conducted	Carcass scavenged by sharks Carcass badly decomposed

CASE HISTORIES AND NECROPSY FINDINGS

1. EGNE9601WBB

On 2 January 1996, a 4.8-m female right whale calf (est. 2 to 4 weeks old) was found dead on shore near St. Johns River (Jacksonville, Florida). On 11 January 1996, it was necropsied by a team headed by Dr. S. Rommel. Tissues were in poor condition and no histopathological studies were conducted. Cause of death was listed as unknown. (See appendix B.)

2. GA96013001, AKA RKB 1429, AKA #1623, AKA "Lindsey"

On 30 January 1996, the carcass of a large adult male was sighted off Sapelo Island, Georgia. The carcass was recovered and examined by a necropsy team led by R. Bonde on 1 February 1996. Because of severe, massive trauma and fractures to many bones including the skull, the team determined that the whale had probably been struck by a large vessel. Cause of death was listed as "impact, vessel collision." (See appendix C.) On 7 March 1996, Dr. Ketten received two ears collected from this whale and found that the bones of both ears had been severely fractured.

3. On 5 and 7 February 1996, a floating carcass was observed at 30°29'N 80°37'W and 30°18.7'N 80°57.2'W, respectively. The carcass was not recovered. It remains undetermined whether these sightings represent one or two animals.

4. GA96021901, AKA RKB 1430

On 19 February 1996, a 5.13-m female calf was sighted floating on its side 25 miles east of the Kings Bay Naval Base near Cumberland Island, Georgia. A necropsy was performed under the direction of R. Bonde on 20 February 1996. Unfortunately, the tissues were too decomposed for histopathological study. One ear was recovered and sent to Dr. Ketten; there were no abnormalities evident. (See appendix D.)

5. GA96022201

On 22 February 1996, another dead calf was found near Brunswick, Georgia. It was recovered and necropsied on 24 February 1996 under the direction of Dr. S. Wright and Dr. S. Rommel. The thorax had been torn open by sharks, allowing the entry of seawater that helped to preserve the lungs. Lesions in the lungs, and hemorrhage in the soft tissue surrounding one eye, led the necropsy team to suspect the calf had been subjected to external trauma. Two ears were received and examined by Dr. Ketten on 5 March 1996. She found no evidence of barotrauma. (See appendix E.)

For a review of necropsy findings, see table 2.

RESULTS OF EXAMINATION OF EARS

Dr. Darlene Ketten examined ear parts from three of the dead right whales, GA96013001/RKB 1429, GA96021901/RKB 1430, and GA96112201, that stranded near the Georgia and Florida coasts. Based on gross examination and computerized tomographic (CT) scans, she determined that one animal (GA96013001/RKB 1429) was severely traumatized by impact. Whether the trauma was due to a ship strike or blast could not be determined because of tissue decomposition. Ears of the other two calves (GA96021901/RKB 1430 and GA96112201) were reasonably well preserved, and neither showed any evidence of blast injury or impact trauma. (See appendix F.)

Table 2. Right whale mortalities January–February 1996:
gross necropsy findings and supporting diagnostics.

Event #	Field #s	Appendix (Necropsy Report)	Results of Ear Examination by Dr. Ketten	Samples Collected, Significant Findings, and General Evaluation (See Necropsy Report for Specifics)	
1	EGNE9601WBB	Appendix B		Carcass Cyamids Blubber Skin Ears Larynx Two ribs Two vertebra Skull	There were no signs of starvation or vessel collision. In the absence of other signs, a congenital defect is the most likely cause of death. Cause of death: unknown
2	GA96013001 RKB 1429 "Lindsey" #1623	Appendix C	Appendix F "...severely traumatized by impact, but whether from a ship strike or blast could not be determined because of decomposition."	Carcass Skin Blubber Cyamids Skeleton Rostral baleen Muscle Liver Colon contents Lung Intraoral rete sample Tympanic-periotic bones Ears Left eye Intestine	–Severe, massive, acute trauma and fractures to cranial bones, vertebrae, left ribs, and left scapula with associated tissue and muscle damage –Displacement of abdominal organs, possibly the result of impact trauma –Stomach empty of ingesta contents with some watery green contents throughout the descending colon and rectum Cause of death: Impact, vessel collision
3	EGNE9603WBB	Not recovered		Not recovered	Not recovered
4	GA96021901 RKB 1430	Appendix D	Appendix F Only one ear examined; no evident abnormalities.	Carcass Baleen Both eyes Cyamids Skin Muscle Blubber Larynx Intraorbital ant. palate Stomach contents Colon contents Liver Heart Skull Ears Lung intraoral rete sample Tympanic-periotic bones	–Very heavy, saturated, congested lungs –Advanced postmortem decomposition rate, associated with postmortem change and restricted to suture and growth plate bed sites –Reddened serosal surface of gastrointestinal tract –Stomach likely contained milk, and colon contained digested milk –Good overall physical condition –No cachexia or malnutrition Cause of death: unknown
5	GA96022201	Appendix E	Appendix F No evidence of trauma; possible respiratory and middle ear dysfunction.	Head Stomach Duodenum 2-m colon Heart Lungs Eyes Ears	–Lung, edema, alveolar, multifocal, moderate to severe –Lung, hemorrhage, multifocal, moderate with patchy atelectasis –Left retrobulbar periocular tissue, hemorrhage, focally extensive –skull, evidence of antemortem hemorrhage at margins of shark bite injury through nasal bones –Skin and heart, not remarkable –Spleen, autolysis precluded in-depth histological eval. Cause of death: cannot be determined from tissues examined; however, retrobulbar periocular tissue hemorrhage suggests a unilateral traumatic event, possibly a vessel collision and/or impact from a shark or other large animal.

CONCLUSIONS OF THE DEPARTMENT OF VETERINARY PATHOLOGY AND THE OFFICE OF THE ARMED FORCES MEDICAL EXAMINER (ARMED FORCES INSTITUTE OF PATHOLOGY [AFIP] BY DR. THOMAS LIPSCOMB)

1. RKB 1429 was very likely killed by massive blunt trauma affecting the head and neck. This was probably the result of a collision with a ship. No evidence implicating a specific ship is available. The pathological findings do not support blast injury as the cause of death.
2. The causes of death in the cases of GA96112201 and RKB 1430 are undetermined; however, the pathological findings do not support blast injury as the cause of death for either whale.

RECOMMENDATIONS

- Identify carcasses by marking and photography as soon as possible after being found. One economically feasible suggestion is to attach some form of radio tag to facilitate rapid retrieval.
- Develop and implement a system that will ensure speedy carcass recovery; make this a high priority. Rapid retrieval of carcasses is probably the **MOST IMPORTANT FACTOR** to improve. Only with the early retrieval of carcasses can we determine causes of death in large whales. Tissues deteriorate so quickly in large whales that diagnosis is inevitably hampered by poor carcass condition. Quicker response time will allow for the collection of fresher tissues that will produce higher quality necropsy results. For calves, a necropsy could be conducted at sea aboard a naval vessel. **In all cases, help in the immediate reporting of observed carcasses and assistance in carcass retrieval by the U.S. Navy is essential.**
- Develop and implement a right whale necropsy protocol using existing large whale protocols (one such guide is being developed by the South Eastern U.S. Large Whale Necropsy Team) with addenda for critical research interests for the right whale. (See appendix G.)
- Include methods in the protocol to extract multiple critical tissues (e.g., the lungs, spleen, ears, and brain) to optimize results from future specimens.
- Use the team approach for field examinations. Develop a telephone list of necropsy personnel and alternates that can be contacted to respond to a mortality. The team should include a veterinary and/or forensic pathologist, an experienced life history whale biologist, and a cetacean anatomist.
- To reduce variation between laboratories while still providing quality control, send samples to two different predetermined laboratories chosen for their expertise in the particular analysis. These labs must be specified and agreed upon by experts examining and evaluating the deaths. It is suggested that samples for histopathology should be sent to Dr. Greg Bossart at the University of Miami and to the AFIP. This may be critical in determining the cause of death.
- Improve relations with other federal agencies (e.g., National Park Service) and maintain good communication during all stages of a mortality event with federal, state, and local governments; this is critical and may best be coordinated by an on-site NMFS staffer or local Department of Natural Resources biologist. Because of its seagoing and port assets U.S. Navy-supported coordination at the federal level is critical.
- Encourage researchers to work together to publish findings in formal peer-reviewed reputable scientific journals.

- Obtain more data regarding air and water blast effects on large whales. A panel of prepared and knowledgeable experts should be assembled to discuss an extrapolation of demonstrable blast effects on terrestrial mammals to indicators of blast injury in marine mammals.
- Train individuals responsible for necropsy examinations in forensic pathology.
- Experts should convene annually to discuss necropsy findings, reevaluate the procedural protocol, and make recommendations for improving procedures.

CONCLUSIONS

There is a lack of useful scientific data collected during large whale mortalities that results in the failure to determine the cause(s) of death. This is often a result of delays in retrieving carcasses and the overall lack of knowledge of pathologies associated with large whale mortalities. This situation must be remedied if future efforts are to improve. Strong scientific data will annul false accusations and support solutions. In regards to the mortalities of the right whales, injuries suggesting ship collision were seen in one animal; however, the type of ship could not be determined. Dr. Greg Bossart's necropsy findings from right whale GA 96022201 include a peribulbar hemorrhage, "changes that are not inconsistent with a concussion event (appendix E, enclosure 2)." Other pathologic findings do not support barotrauma or blast injury in any of the whales. It was generally agreed that the protocol followed for such mortalities in the future is critical to ensure that better material will be available for evaluation. To that end, Dr. Thomas Lipscomb, Dr. Cindy Driscoll, Dr. Darlene Ketten, and others are drafting appropriate protocols for tissue preservation, especially for sensitive tissue such as ear, lung, and brain. (See appendix G.)

ENCLOSURES

1. Final report from the Department of Veterinary Pathology
 - a. Consultation from the Office of the Armed Forces Medical Examiner on GA96112201, RBK 1429, and RKB 1430
 - b. Consultation from the Office of the Armed Forces Medical Examiner on histologic sections of lung from RKB 1429 (AFIP 2539560)
 - c. Consultation from the Office of the Armed Forces Medical Examiner on histologic sections of lung from RKB 1430 (AFIP 2539561)
 - d. Consultation from the Office of the Armed Forces Medical Examiner on histologic sections of lung from RKB 1425 (AFIP 2539563)
2. Letter from Dr. Sentiel Rommel



DEPARTMENT OF DEFENSE
ARMED FORCES INSTITUTE OF PATHOLOGY
WASHINGTON, DC 20306-6000



8 July 1996

REPLY TO
ATTENTION OF

Department of Veterinary Pathology

Dr. Sam H. Ridgway
NCCOSC RDTE DIV 5107
49620 Beluga Road Rm 200
San Diego, CA 92152-6330

Dear Dr. Ridgway:

The Department of Veterinary Pathology and the Office of the Armed Forces Medical Examiner have completed our review of the findings relating to the deaths of three right whales (*Eubalaena glacialis*) in the Atlantic Ocean along the Georgia and Florida coasts. The identification numbers for the three whales are GA96II2201, RKB 1429 and RKB 1430. The possibility that blast injury may have been the cause of death in one or more of these mortalities has been raised by others.

After reviewing the necropsy reports, viewing video tapes of the necropsies, meeting with individuals who performed or participated in the necropsies, reviewing histologic sections of lung (RKB 1429 and RKB 1430), and reviewing the findings of Dr. Darlene R. Ketten of the Department of Otology and Laryngology of Harvard Medical School relating to the ears of the whales, we have reached the following conclusions:

1. RKB 1429 was very likely killed by massive blunt trauma affecting the head and neck. This was probably the result of a collision with a ship. No evidence implicating a specific ship is available. The pathological findings do not support blast injury as the cause of death.
2. The causes of death in the cases of GA96II2201 and RKB 1430 are undetermined; however, the pathological findings do not support blast injury as the cause of death for either whale.

Thank you for the opportunity to study these interesting cases.

Sincerely,

A handwritten signature in cursive script, appearing to read 'T P Lipscomb'.

Thomas P. Lipscomb, DVM
Diplomate, ACVP
LTC, VC, USA
Chief, Division of Veterinary Pathology

- Enclosures:
1. Consultation from the Office of the Armed Forces Medical Examiner on GA96II2201, RKB 1429 and RKB 1430.
 2. Consultation from the Office of the Armed Forces Medical Examiner on histologic sections of lung from RKB 1429 (AFIP 2539560).
 3. Consultation from the Office of the Armed Forces Medical Examiner on histologic sections of lung from RKB 1430 (AFIP 2539561)

We have reviewed this most interesting consult concerning the whale deaths. We believe each case must be examined separately to assess the possibility of blast injury.

Eubalaena glacialis Necropsy date 2-24-96
ID GA96II2201
Florida Marine Research
Institute/University of Miami

Drs. Wright and Bossart describe a badly decomposed calf which had sustained numerous shark bites to the abdomen. Additionally, the head was removed at the recovery site at sea. The pathologic findings included pulmonary edema and left retrobulbar hemorrhage.

The pulmonary edema is a nonspecific finding seen in a spectrum of pathologic and physiologic conditions. While blast injuries may cause pulmonary edema, many studies have found that it is not part of the primary blast injury sequence. The most obvious and consistent finding is that of pulmonary hemorrhage which is not described in this case.

The eye and surrounding structures are resistant to blast injury. However, retrobulbar hemorrhage has been described in conjunction with blast injury. Such hemorrhages are found in association with orbital fractures in cases of supralethal blasts. These blasts would be expected to result in other injuries, such as pulmonary and gastrointestinal hemorrhage.

The ears were sent to Dr. Ketten at Harvard who found no fractures and doubted that the blood in one ear arose from barotrauma associated with explosions.

In humans, the ear is the most susceptible organ to damage by blast forces. Injuries include disruption of the tympanic membrane, disruption of the bones of the middle ear and damage to the organ of corti. No equivalent injuries are noted in the report.

Eubalaena glacialis RKB-1429 "Lindsey" (1623)

The necropsy examination report describes a moderately decomposed adult male whale with multiple fractures of the skull, ribs and scapula.

These findings suggest localized blunt force trauma to the head and back of the whale rather than a blast injury.

The ears were examined by Dr. Ketten at Harvard. She found badly decomposed ears with numerous fractures. Her findings are consistent with a traumatic event, either pre or postmortem. Nothing in her description suggests trauma due to an explosion.

Eubalaena glacialis #1430

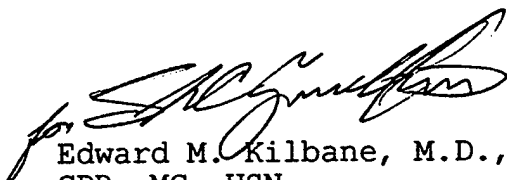
The prosector examined this immature female right whale in an advanced state of decomposition, finding pulmonary congestion and disarticulation of the cranial bones.

Similar to the first case, the pulmonary edema is a non specific finding, which as a sole finding does not support a blast injury etiology.

One ear was examined by Dr. Ketten at Harvard. She found no fracture, blood or other evidence of trauma, mechanical or blast related.

Reference: Textbook of Military Medicine: Conventional Warfare; Ballistic, Blast, and Burn Injury, Volume 5, TMM Publications, Washington, D.C., 1990, pp.271-294.


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Chief Deputy Medical Examiner

USE INK OR BALL-POINT PEN ONLY

CONSULTATIONS

AFIP ACCESSION NUMBER:

2539560

THIS IS A PERMANENT PART OF THE RECORD AND IS NOT TO BE REMOVED FROM THE FOLDER

PLEASE CIRCULATE AMONG THE FOLLOWING OFFICERS ----->
AND THEN RETURN TO THE REVIEWING OFFICER ----->~~DAFVE~~

MEMO FROM REVIEWING OFFICER:

Lung sections from a right whale.
Advanced postmortem autolysis. I do not
recognize significant lesions. Your

23 May 96 Lynwood, Vet Path
(DATE) (REVIEWING OFFICER)

Vet Path

opinion is appreciated.

28 May 96: We have reviewed the two H+E stained
slides and the necropsy report and data sheet. Although
preservation is sub-optimal, we do not recognize any
significant lesions. The stigmata of blast effects (as seen
in humans) are not observed in the sections examined.
Thank you for the opportunity to review this interesting
case.

T. R. PARSONS
MAJ, USAF, MC

STEVEN COGSWELL MD
MAJ, MC, USAF

USE INK OR BALL-POINT PEN ONLY

CONSULTATIONS

AFIP ACCESSION NUMBER:

2539561

THIS IS A PERMANENT PART OF THE RECORD AND IS NOT TO BE REMOVED FROM THE FOLDER

PLEASE CIRCULATE AMONG THE FOLLOWING OFFICERS ----->
AND THEN RETURN TO THE REVIEWING OFFICER -----v

MEMO FROM REVIEWING OFFICER:

Lung sections from a right whale.
Advanced postmortem autolysis. I
do not recognize significant lesions.

23 May 96 Lynxond, Vet Path
(DATE) (REVIEWING OFFICER)

~~DATA~~

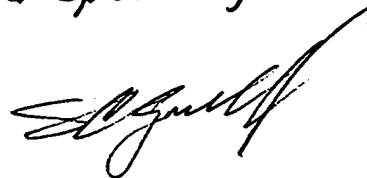
Vet Path

Your opinion is appreciated.

28 May 96: We have reviewed the autopsy reports (2) and
the data sheet and 4 H+E stained slides (2x each block).
The tissue is suboptimally preserved with architectural
collapse and loss of cytologic detail. No focal
lesions are observed in the tissue, but the autolysis
limits our review. Thank you for the opportunity to
review this interesting case.



T. R. PARSONS
MAJ, USAF, MC



STEVEN COGSWELL MD
MAJ, MC, USAF

USE INK OR BALL-POINT PEN ONLY

CONSULTATIONS

AFIP ACCESSION NUMBER:

2539563

THIS IS A PERMANENT PART OF THE RECORD AND IS NOT TO BE REMOVED FROM THE FOLDER

PLEASE CIRCULATE AMONG THE FOLLOWING OFFICERS ----->
AND THEN RETURN TO THE REVIEWING OFFICER -----v

MEMO FROM REVIEWING OFFICER:

Long sections from a stranded right whale. Advanced postmortem autolysis. I recognize no significant lesions. Your

23 May 96 Lynneil, Vet Path

(DATE)

(REVIEWING OFFICER)

~~DAFME~~

Vet Path

opinion is appreciated.

28 May 96: We have reviewed the autopsy report and data sheets and the 2418 slides (1 block). The tissue is badly autolyzed with loss of cytoplasmic detail and architectural collapse. This precludes an evaluation of anatomy in the noted neonate whale calf. No focal lesions are observed. Thank you for the opportunity to review this interesting case.

T. R. Parsons

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MAJ, USAF, MC

Steven Cogswell

STEVEN COGSWELL MD
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ECKERD COLLEGE
Collegium of Natural Sciences

Dr. Sam Ridgway
NRAD
NCCOSC RDT&E Division
San Diego, CA 92152-500

April 26, 1996

Dear Dr. Ridgway,

Thank you for inviting me to comment on the right whale mortality issue. I hope that my comments are helpful to you and your colleagues.

I would first like to add an important detail to the necropsy report for the neonate right whale examined by S. Wright and myself, in Brunswick, GA on 24 Feb 1996 (field # GA96022201). Missing from the report, but very important, is the observation that one of the shark bites, in the region surrounding the blowholes, sheared the anterior margins of both nasal bones. The remaining portions of these bones were discolored by blood - at a substantial but unmeasured depth and approximately two centimeters posteriad. This was indicative of perfusion of these bones at the time the shark bite took place - a strong indication that the animal was alive when the bones were sheared.

I came out of our meeting on 19 Apr 1996 feeling that the questions I had regarding blast injury to marine mammals were naive, since the blast injury experts at the meeting were able to dismiss most of them, presumably based on their greater knowledge of the subject. I deferred to these experts because they were introduced as knowledgeable pathologists. On 19 April I felt that my only unanswered question (actually avoided by the experts) concerned short term debilitating effects of a blast (i.e., disorientation, concussion, deafness) that might not establish lesions or any other detectable pathological change that could be determined by forensic methods.

I feel differently about the meetings now, primarily because of the information available in *The Pathology of Primary Blast Injury* by Sharpnack et al. (1991), which was distributed at the meeting. I now feel that several of the questions I raised during the meeting were dismissed inappropriately in light of the information in Sharpnack et al. After reading this material, it became clear to me that the experts were inadequately prepared to address the questions asked by my colleagues and me. Perhaps some of the experts had simply not expected questions as penetrating as those which were asked.

I strongly feel that the injuries described by Sharpnack et al. (1991) reveal an inadequate knowledge base from which to competently evaluate all the possible effects of primary blast injury on marine mammals. Please note that the "particularly noteworthy" references on blast injury are rather old ('50s and '60s). As a morphologist I recognize the value of the older literature, but surely there are more recent physiological and pathobiological studies on the effects of underwater blast injury! Additionally, the unique anatomy, physiology, behavior, and environment of diving mammals challenges many of the assumptions used in the evaluation of primary blast injury from air blasts on terrestrial mammals. Although Sharpnack et al. mention underwater blasts they do not address specifics. The physics of energy transfer, absorption, and

reflection - within and between media of different densities is complicated. I recognize that the complex geometries and compositions of biological materials make the forensic analysis of these energies in determining blast injury even more complex. However, much could be done to minimize error in analysis. Categorical statements that discount blast injury in marine mammals, based on studies of terrestrial mammals in air cannot be justified. Theoretical and experimental analyses - pertinent to the animals and their environment - should be carried out before more definitive conclusions can be made. We must recognize the potential for damage from both air blasts and underwater blasts that could occur when right or other whales swim in close proximity to Naval operations. It is very important to keep in mind the absolute size of whales. Their surface to volume ratios may profoundly influence extrapolations made from animals of more modest dimensions.

Since gas-tissue interfaces are most sensitive, and vascularized tissues most important, the unique vascular distributions of diving mammals, their air filled sinuses and lungs, and the gas in the gastro-intestinal tract all must be considered when establishing a protocol to evaluate potential blast injuries. The unique range of tissue densities (ear bones - spongy bone - foamed air sinuses - lung - muscle and unusual thickness of veins) must also be considered. One must also note the alternate circulation pathways available to marine mammals - ischemia during submergence could possibly mask the hemorrhagic appearance (indicative of blast injury) of damaged tissue. Changes in the dimensions of the alveoli with depth may also influence indicators of blast injury.

Perhaps some experiments on terrestrial mammals in hyperbaric chambers that examine blast injury at different air densities and alveolar sizes (e.g., breathing at different temperatures and pressures; or breath-holding immediately before the blast to allow compression of the alveoli) are prescribed. Studies to test changes in amount of blood in specific tissues during the ischemia of dives should be considered. Studies including fishes that have a vascularized gas bladder might be instructive. The alveolar structure in marine mammals is very different from terrestrial mammals - the density of cartilage and its distribution could affect blast injury susceptibility and appearance; therefore all evaluations must be qualified. Clearly, these are very complex problems and require further extensive thought. This list is suggestive and preliminary; it is merely offered to provoke further brainstorming. It may be profitable to assemble a panel of prepared and knowledgeable experts to discuss an extrapolation of demonstrable blast effects on terrestrial mammals to indicators of blast injury in marine mammals. Particular attention should be addressed to underwater blast injury (whether caused by in-air or under-water blast).

Please accept my comments as preliminary and suggestive. I look forward to the results of the blast expert's examination of the right whale tissues. Thank you again for involving me in the process.



Sentiel Rommel

cc.: Teri Rowles, Joseph Geraci

APPENDIX A

LIST AND ADDRESSES OF RIGHT WHALE PATHOLOGY PARTICIPANTS

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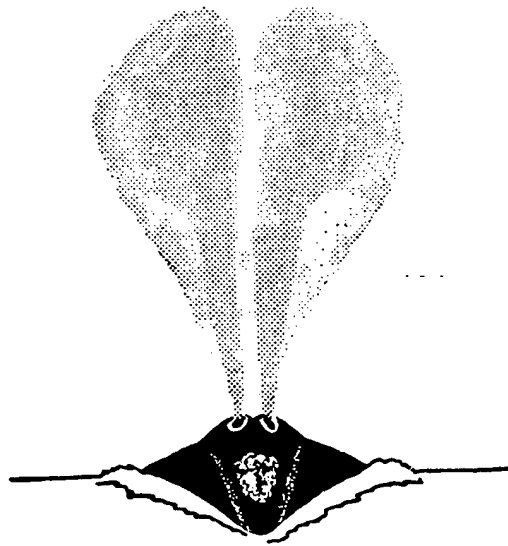
*Convener and Chairman.

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APPENDIX B

**NECROPSY
RIGHT WHALE MORTALITY
(EGNE9601WBB)**

NECROPSY

Right Whale Mortality
Atlantic Beach, Florida
January 2, 1996
NEA Mortality #

HISTORY

On January 2, 1996, a dead 4.8 m female right whale calf was reported on Atlantic Beach near Jacksonville, Fl. Bill Brooks and Marilyn Marx of the Florida Department of Environmental Protection (FLDEP) responded to the call and arrived on the beach at 9:30 am. Initial measurements and cyamids were collected and people were notified. Chris Slay and Lisa Conger from the New England Aquarium (NEA) arrived later and collected histological samples and skin for genetic analysis. After consultation with Philip Hamilton and Amy Knowlton of NEA, it was decided to have the calf frozen and shipped up to NEA for a thorough necropsy. The people on the beach arranged for the transportation and freezing of the animal which was placed in a commercial freezer by late afternoon that day.

On the afternoon of January 5, the whale was placed in a freezer truck and driven to NEA. Due to an extensive snow storm along the eastern seaboard, the whale did not arrive until the late afternoon of the 8th. Although there were ice particles throughout the body, the whale was not fully frozen. A full one-day necropsy took place on the 11th.

NECROPSY

Participants at the necropsy on January 11 were: Butch Rommel with Natalie Ward and their B.U.M.P.s class; Greg Early with Philip Hamilton, Amy Knowlton and Lucy Keith from NEA; Michael Moore with Regina Asmutis from WHOI; Tom Ford; and Darlene Ketten with Scott Kramer from Mass. Eye and Ear Infirmary.

External exam

The whale was a 478 cm female calf estimated at two to four weeks old. The carcass was gas filled and bloating. The umbilicus was prolapsed with a ragged two inch orifice with some intestines protruding out of the opening (PHOTOS). The baleen was gone and most of the skin had peeled off the back. Cyamids were present on the head and the skin was rough where callosity tissue was just beginning to erupt.

Sand had been driven between the epidermis and the dermis in several places along the length of the body indicating some rolling and surf action while on the beach. Sand was also present in the mouth, throat, esophagus and larynx, but not in the abdomen.

The whale was weighed after it was dissected at NEA and placed in buckets. All tissues were weighed, but some fluids were lost. The approximate weight was 2,345 lbs.

Internal exam

Over-all condition

B. Rommel estimated that the whale had been dead approximately one week- determined from how easily the periostium (or "bone skin"), which is normally securely attached to the bone, peeled off the bone. Considering the estimated time of decomposition, the animal was in reasonable shape. The organs were in decent enough condition to allow for an adequate assessment of their general structure. There was no obvious sign of gross trauma prior to death.

Blubber

Blubber thicknesses ranged from 2.75 cm (80 cm behind blowhole on dorsal mid-line) to 6 cm (240 cm behind blowhole on ventral mid-line). The blubber just posterior to the blowholes was noticeably redder than blubber from any other region. This condition is similar to the right whale mortality on October 20, 1995 on Long Island, Nova Scotia. Initially, we thought the coloration was a sign of trauma, but once the head of this calf was removed, it became clear that the red blubber was uniform around the body just posterior to the head (PHOTOS). The red may be an affect of bloating forcing blood into the blubber (a pooling effect which would occur on what ever side of the animal is down after death) or it may be a normal variation in blubber color for the species.

NOTE: G. Early noted that the oil appeared to have been "squeezed" from the blubber by the force of internal pressure.

Bones

There was a possible contusion forward of the right flipper (PHOTOS) and the lower growth plate of the radius on that flipper was dislocated. This may have happened post mortem. On the other side (left), both the #3 and #5 ribs were broken (PHOTOS), but there was no sign of contusion or trauma around the ribs. Broadly agreed that those breaks were post mortem.

Muscle

Muscle was pale and gas filled from decomposition. There were yellowish, waxy deposits in the muscle forward of the peduncle, along some veins, in the plural space (cavity above the lung), in the muscle of the dorsal lumbar region of the abdominal cavity near pelvis area, near auditory meatus, and some other apparently random areas (PHOTOS). First thought possibly renal failure, but then thought unlikely. B. Rommel remembered "orange marmalade" in "Popsicle" (1988 calf mortality)- may be normal or possibly an artifact caused by the heat of decomposition (although '88 calf very fresh). G. Early remembers the "marmalade" in Popsicle to be pools of proteanaceous fluid (i.e. blood serum) and believes that similar fluid would have decomposed in this animal. He also noted that there were three to five litres of bloody fluid in the abdomen which is expected in a decomposed carcass.

Organs

The lungs, kidney, heart, thymus, stomach, intestines and reproductive tract were all examined closely. The lungs were uniformly congested (PHOTOS). However, due to the lack of any pattern to this appearance, M. Moore assumed it was a post mortem change. The reproductive tract appeared normal (PHOTOS) including the uterus, cervix and bladder. The umbilical arteries were beginning to shut down as is expected. The stomach had thin coating of mucoid, yellowish substance (comparable to substance described by Bob Bonde in other southeastern right whale strandings) and the intestine had a brownish orange substance in larger (but still not full) quantities (PHOTOS).

REGARDING POSSIBLE CAUSES OF DEATH

1) It is possible that the umbilicus opening never fully closed after birth. However, M. Moore thought that, if that were the case, the resultant post natal infection would leave signs of infection tracking up the stalk and the umbilical arteries. There were no signs of an infection along the stalk nor in the abdominal mesenteries (i.e. omentum). No abscessation or other scarring or fibrosis was evident in this region. The blow out around the opening could well have been post mortem; given the "pressure effects" seen throughout the carcass, protruding bowels would be expected. However, a catastrophic prolaps due to a congenital defect in the umbilicus can't be ruled out.

2) There were no signs of starvation (from possible abandonment or mother unable to produce milk). G. Early described sea-water ingestion as a common finding in starved nursing seals and porpoises.

3) There were no signs of vessel collision.

SUMMARY

In general, all participants agreed that having a veterinary and/or forensic pathologist at each necropsy along with someone who knows cetacean anatomy well would enable us to maximize the diagnostic gross pathology observations. Michael Moore also recommends getting a portable ultrasound to do thorough mapping of blubber thicknesses. This, in time, would allow us to develop a comprehensive database of blubber thickness with which to compare future information. One question to investigate is whether these animals are getting thinner and that in turn is effecting there reproductive ability.

CAUSE OF DEATH: Unknown. G. Early believes that, given the absence of other signs, a congenital defect is the most likely cause of death.

Samples

While on the beach on January 2nd:

Florida DEP- cyamids in formalin

NEA- blubber: 1) frozen in plastic, 2) frozen in glass,
and 3) blubber/muscle in formalin.

- skin in DMSO

- cyamids in formalin

During necropsy at NEA on January 11th:

D. Ketten- both ears

J. Reidenberg- larynx (Note to Joy: one of the hyoid bones
came off and was placed in the trachea for
safe keeping)

T. Ford- rete

M. Moore- detailed blubber measurements

B. Rommel- two vertebra and two ribs

M.C.Z. will take skull eventually. Currently in NEA freezer.

OTHER

-Thymus weight: 1.04 Kg

-Ductus arteriosus was functionally patent with a diameter
of 15mm. (PHOTO)

-Mid thorax aorta was 40mm in diameter

-For Butch: "on same level, each epidermal vein is 25mm in
diameter"

-Greg remembers a vesicle with white waxy material about 2
cm in diameter in the area where the post anal sac would
have been, but the animal was flensed before it could be
investigated.

CETACEAN DATA RECORD

species Eg calf (dead) ^{Time arrived} field no.
 Date, time stranded or captured 2 January 1996 ~ 0930 date, time of data 1/2/96 ~ 1130
 observer Bill Brooks Marilyn Marx locality Atlantic Beach
 Measurements taken by Cyad: Thomas Jenner Sachs
 EXTERNAL DATA (specify units of measure used)

photographs or drawings
 tooth or baleen counts: left upper right upper l. lower r. lower
 diameter largest tooth, length longest baleen plate color of baleen
 ectoparasites

number of throat or ventral grooves weight of specimen
 MEASUREMENTS, BODY (specify units of measure used)
 2. snout to center of eye..... 105 cm 16. snout to end of ventral grooves
 3. snout to apex of melon..... 13. snout to genital slit..... ~ 310
 15. projection of lower jaw..... 14. snout to anus... ca. 1.5 m ~ 320
 4. length of gape..... 90 cm 1. total length, snout to notch... 478 cm
 5. snout to ear..... 118 cm 17. blubber thickness, mid-dorsal...
 6. center of eye to ear..... 20 cm 18. blubber thickness, mid-lateral...
 7. center of eye to angle of mouth... 20 cm 19. blubber thickness, mid-ventral...
 8. eye to blowhole (center)..... 65 cm 21. girth at axilla.....
 9. snout to center of blowhole(s)... 80 cm 21a. girth at eye..... 200
 20. length of throat grooves..... 22. maximum girth..... 260
 10. snout to flipper..... 140 cm 23. girth at anus..... 200
 11. snout to tip of dorsal fin..... 23a. girth 50 cm before notch..... 70
 12. snout to center of umbilicus....

MEASUREMENTS, APERTURES (specify units of measure used)
 24. eye:.....height 3 cm length 6 cm 27. blowhole(s): length 23 cm width 20 cm
 25. length mammary slits: r. 1 l. 28. diameter ear opening: r. 1
 26. length genital slit anal slit

MEASUREMENTS, APPENDAGES (specify units of measure used)
 29. flipper length (anterior)..... 89 cm 33. length of dorsal fin base.....
 30. flipper length (posterior)..... 50 cm 34. width of flukes..... 137 cm
 31. maximum width of flipper..... 45 cm 35. length of flukes.....
 32. height of dorsal fin..... 36. depth of fluke notch..... 52 cm

INTERNAL DATA (specify units of measure used)
 stomach contents (type and quantity)

internal parasites (see checklist)

vertebral epiphyses: open mm; closed, visible closed, invisible
 gonads: weight r. l. dimensions (LxWxD) r. l.
 pregnant? fetus length sex lactating? sperm in epididymus?
 thickness of mammary gland diameter corpus luteum diameter uterine horn

SPECIMEN COLLECTION CHECKLIST

teeth or baleen.....	bulla.....	liver sample.....
stomach contents.....	ectoparasites.....	kidney sample.....
gonads.....	endoparasites.....	skull.....
mammary gland.....	blubber sample.....	skeleton.....
ear plugs.....	muscle sample.....	fetus.....
		other.....

Remarks

APPENDIX C

**NECROPSY EXAMINATION REPORT
(GA96013001/RKB 1429)**

NECROPSY EXAMINATION REPORT

RKB-1429

Eubalaena glacialis

HISTORY

This animal was first reported by the vessel "Lady Francis" to the USCG Brunswick Station on 30 January as a floating dead right whale. The "Lady Francis" observed the carcass floating about 10 miles east of Sapelo Island, Georgia at 31°28.43' N, 80°59.57' W. The USCG forwarded the report to the Georgia Department of Natural Resources (GDNR) at approximately 1100h. The GDNR dispatched an aerial survey crew to confirm the report. The floating dead whale was observed at 1200h at 31°28.19' N, 80°59.91' W. The whale was estimated at 30-35' in total length. A GDNR 54' boat, the "Bagby", was dispatched at approximately 1500h to tow the carcass to shore. At 1700h the "Bagby" located and secured the carcass at 31°28.27' N, 80°59.91' W and commenced towing it to shore. At 1830 it became apparent that the "Bagby" was not large enough to tow the carcass, and at 1836h at 31°28.19' N, 80°59.91' W the "Bagby" cast off. The "Georgia Bulldog", a University of Georgia Marine Extension Service 75' shrimp trawler, was dispatched at approximately 2400h. The "Georgia Bulldog" travelled to the last known location of the dead whale and anchored for the night. At first light, the crew of the "Georgia Bulldog" began searching for the carcass. The carcass was located on 31 January at 0903h. Its position was 31°30.04' N, 80°58.26' W. The whale was under tow by 0951h and arrived at Harris Neck National Wildlife Refuge by approximately 1645h. Two crawler tractors and one tractor hoe were used in tandem to pull the right whale onto the shore. When the carcass was pulled up onto dry land, preliminary photographs and morphometric measurements were taken. In the early morning hours of 1 February 1996 morphometric measurements were completed and a detailed necropsy was conducted. This animal was recognized as an adult male right whale "Lindsey" (#1623) known since 1986 from the North Atlantic Right Whale Identification Catalog.

B. Zoodsma, Georgia Department of Natural Resources was the on-site coordinator. R. Bonde, National Biological Service was the necropsy team leader. Also in attendance at the necropsy were M. Harris, B. Winn, J. Couch, and S. Keating, GDNR; B. Brooks and J. Macracken, Florida Department of Environmental Protection; T. Murphy, South Carolina Department of Natural Resources; L. Conger, New England Aquarium; M. Moore, Woods Hole Institution; C. Marshall, I. Masamitsu, M. Rudin, K. Prunier, S. Malacin, J. Borrero, K. Cronin, A. Garcia, and

NECROPSY EXAMINATION REPORT
RKB-1429

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G. Pimentil from the University of Florida; J. Crawford, H. Sage, D. Maroney, K. Davis, N. Johnson, A. Kerr, M. Timmons, P. Schlein, B. Williams, and R. Edgecomb, University of Georgia Marine Extension Service at Skidaway Island; X. Prudencio, Wilderness Southeast; S. Mitchell, NOAA; and C. Sakas, C. Glock, several other employees of the U.S. Fish and Wildlife Service at the Harris Neck National Wildlife Refuge. Several 35 mm slide photographs were taken and most of the procedure was recorded on videotape. Additional video was taken by the GDNR film crew and the Skidaway Institute of Oceanography.

NECROPSY - 1 February 1996

External exam: Adult male right whale, TL=1415 cm. The animal was examined as it lay on its right side. Less than 10% of the black skin had sloughed off. The carcass was moderately bloated due to postmortem decomposition; some of the internal gas had been released during serial measurements of blubber thickness taken the night before. Several additional morphometric measurements were taken. Template tracings of the fluke and both flippers were taken. The penis was protruding due to postmortem gaseous pressure. Two accessory mammary slits were present. Samples of skin and blubber were collected and frozen; skin samples were preserved in DMSO/hypersaline solution for genetics. Cyamid whale lice were only observed around the mouth area and in association with the bonnets or callosities. They were not observed in other crevasses usually associated with these parasites. Several cyamids were collected from the mouth area and preserved in 70% EtOH. Neither sides of the baleen rows were entire. The right and left caudal halves of baleen had washed free from the animal and were not recovered. The partial rostral right and left halves were removed intact. Both samples were collected attached to the gum line and were left at the necropsy site where they will be retained along with the complete skeleton. Disposition of the baleen will be coordinated by Georgia Dept. of Natural Resources. The color of the baleen was dark slate gray to black, with a few plates laced with whitish streaks. Both caudal halves were absent. A partial baleen plate total count was taken for the right row at 109.

No external scars were observed on the body except for some very small white scar tissue patches observed on the ventral aspect of the caudal peduncle just cranial to the junction of the fluke, and on the dorsal surfaces of both flukes. There was a small healed pie-wedge shaped scar along the posterior margin of the right fluke lobe. Photos and measurements were taken. A large line that was used for towing the carcass was still attached to the tail stock.

NECROPSY EXAMINATION REPORT
RKB-1429

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Internal exam:

Abdominal: A small amount of postmortem gas was released when the abdominal cavity was opened. Large layers of blanket blubber were peeled back with the use of a heavy tractor applying tension. A large area affecting a 2 meter by 1.5 meter area just dorsal to the left flipper insertion was infused with blood, radiating into the surrounding blubber layer and underlying musculature. This contusion was over an area of the body that corresponded with severe acute bone damage. There was no external indication of the extent of the lesion on the black skin. In general the animal appeared to be in good fat and healthy. Upon entering the caudal abdominal cavity, I was struck by the lack of loops of intestines. Upon further examination it was discovered that most of the intestines and stomach were positioned cranial to the liver. This displacement of the liver towards the mid-abdominal cavity could have been the result of severe impact causing a herniation of the area around the portal vein. There was also very little clotted blood or serous sanguinous fluid free in the abdominal cavity.

Gastrointestinal tract: The tongue was unremarkable and the mouth was empty except for a caseous pasty material that covered the mucosa. This seemed unusual to me and not characteristic of the overall decomposition. A large area within the left posterior oral cavity was markedly more advanced in decomposition. A cross section of the left anterior palate was removed and placed in 10% NBF. The esophagus was empty of contents and the mucosa was unremarkable. The laryngeal sac contained a small amount of gritty, sand-like material. The larynx/pharynx complex was autolyzed, but removed and placed aside; however it was inadvertently thrown into the burial pit and therefore not preserved. The omentum contained very heavy fat deposits. The pancreas was very badly decomposed. The forestomach and mainstomach were empty of contents and the mucosa was cream colored and unremarkable. The length of the intestines was not measured because of the advanced state of decomposition. Generally, the mucosa was light bile yellow in color in the anterior half and a dark bile green in the distal half. The intestines were primarily empty of contents. All contents were within the colon and were watery and dark green in color. The colon mucosa was light green in color. Ingesta contents were frozen and a sample of colon mucosa was collected in 10% NBF.

Liver: The liver was very large, however not very heavy. The parenchyma was dark purple in color, dry and not saturated with blood. Upon cut section a large quantity of small multifocal pale metallic particles were present in the lumen of the portal

NECROPSY EXAMINATION REPORT
RKB-1429

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veins. These flecks could be parasitic organisms or bacterial colonies. Samples were preserved frozen and in 10% NBF.

Kidneys: Both kidneys were extremely autolyzed and unremarkable. They were both too autolyzed to collect samples. The urinary bladder was empty of urine and the mucosa was cream colored and unremarkable.

Heart: The heart was badly autolyzed. The aorta was examined and unremarkable. The ventricles were unremarkable. A small chicken-fat blood clot was present in the left ventricle. The heart muscle was pale and the lumen of the heart contained very little clotted blood.

Lungs: The lungs were unremarkable. Samples of lung tissue were collected in 10% NBF. The trachea contained no aspirated foreign material.

Reproductive Tract: The testes were completely autolyzed and disintegrated. The vas deferens were empty. The penis was measured, but that measurement is not in my notes.

Brain: The brain was completely autolyzed and bone fragments were removed from the brain case. The caseous material in the oral cavity was probably decomposing brain tissue. The fractures associated with the cranium extended into the upper left palate.

Parasites: No internal parasites observed.

Skeleton: Severe, massive, comminuted fractures were observed affecting the occipital, basioccipital and parietal bones of the cranium. The lateral processes of the cervical vertebrae were also broken and the atlas was disarticulated from the occipital condyles. Left ribs #2 and #3 were also broken, as was the caudal tip of the left scapula. The fractures to the cranium extended through very massive bone elements. The fractures were deep and extended well into the brain case. The tympanic bulla and periotic bones were affected on the left side. Clotted blood was observed infused around the musculature overlying these fractures. These traumatic lesions were similar to those observed and documented in manatee mortalities caused by impact. Fractured areas were measured and photographed. Samples of surrounding tissue were collected in 10% NBF.

NECROPSY EXAMINATION REPORT
RKB-1429

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The entire skeleton was collected for accession into the University of Georgia Museum of Natural History mammal collection. Distribution of the baleen will be coordinated by GDNR. The remainder of the carcass was buried in a deep grave at the necropsy site.

Significant Findings:

- Severe, massive, acute trauma and fractures to cranial bones, vertebrae, left ribs, and left scapula with associated tissue and musculature damage
- Displacement of abdominal organs possibly the result of impact trauma
- Stomach empty of ingesta contents with some watery green contents throughout the descending colon and rectum

NECROPSY EXAMINATION REPORT
RKB-1429

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Summary:

Despite little gastrointestinal tract contents the animal appeared to be in very good body fat and was not emaciated or cachexic. The large bruised area on the left side above the left flipper insertion corresponded to massive, acute trauma and bone damage. The size of this area would indicate blunt impact trauma with a large, moving vessel. The nature and severity of these injuries would indicate that death was rapid and probably happened 4 or 5 days prior to this examination. This would be consistent with post mortem carcass condition.

CAUSE OF DEATH - IMPACT, VESSEL COLLISION

Prosecutor:



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NECROPSY EXAMINATION REPORT
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Samples Collected

Protocol for collecting materials requested in the Right Whale Samples Collection Protocol dated April 1994 were either met or exceeded for every item as determined by carcass condition, except for the larynx.

Frozen:

In Foil

Blubber

Muscle

Liver

In Plastic

Blubber

Muscle

Liver

Colon contents

Preserved in 10% N.B. Formalin:

Left eye

Right & left tympanic-periotic bones

Intraoral rete sample

Liver

Lung

Intestinal sample

Colon contents

Preserved in 70% EtOH:

Cyamid parasites

Preserved in DMSO/Hypersaline solution:

Skin

Liver

Muscle

All preserved material was retained by the National Biological Service, Gainesville Field Station.

Complete skeleton and right and partial left baleen rows were retained by the Georgia Department of Natural Resources and will be accessioned into a museum collection.

CETACEA DATA RECORD

GA-9601

Field Number: RKB-1429

Catalog Number: _____

Species: EUBALAENA GLACIALIS Sex: M Length: 14.15m Condition: 3
 Observer: B. ZOODSMA, M. HARRIS, R. BONCE Date of occurrence: 30 JAN 1996, of data 1 FEB 16
 Locality: FREE FLOATING, FIN OUT APPROX 10 MILES OFF SHORE - BUT CELIN SAP

Lat/Long: 31° 28.19' N 80° 59.91' W Reported by: B. BROOKS, R-DEPPhotographs/Drawings: VIDEO, PHOTOS & DRAWINGS

Circumstances/Cause of Death: MASSIVE, ACUTE TRAUMA TO LT. SIDE NEAR HEAD. SEVERE
CONTRUSIONS + BRUISING OF BLUBBER & MUSCLE W/ SEVERAL BROKEN & FRACTURED BONES ON CRANIUM,
CERVICAL VERTEBRAE, LT. RIBS & LT. SCAPULA.

External Description: MOST BLACK SKIN INTACT. CYAMIDS ON CALLOSITIES ON CHIN. NONE ON OP
ORACLES. POST DISTANT OF LT. BALEEN MISSING. PENIS PROTRUDING DUE TO POST MORTEM GAS. ANIN
IDENTIFIED AS #1623 LINDSEY A. ~10 YEAR OLD MALE FROM R. WHALE CATALOG

Tooth/Baleen Count: Total 109+ Up R PARTIAL Up L PARTIAL Low R _____ Low L _____

Diameter largest tooth/length longest baleen plate: _____ Baleen color: _____

MEASUREMENTS: (Specify units CM)

1 total length.....	<u>1415 cm</u>	24 number of throat grooves.....	_____
2 snout to anus.....	<u>1050</u>	25 length of throat grooves.....	_____
3 snout to genital slit.....	<u>780</u>	26 flipper length, anterior.....	<u>230</u>
4 snout to umbilicus.....	_____	27 flipper length, posterior.....	<u>230</u>
5 snout to throat grooves...	_____	28 flipper width, maximum.....	<u>132</u>
6 snout to dorsal fin tip...	_____	29 mammary slit length R <u>5</u> L <u>5</u>	
7 snout to ant. dorsal fin...	_____	30 number mammary slits.....	_____
8 snout to flipper.....	<u>383</u>	31 length genital slit _____ anal	
9 snout to ear.....	_____	32 perineal length (males).....	_____
10 snout to eye.....	<u>337</u>	33 fluke length.....	_____
11 snout to gape.....	<u>237</u>	34 fluke lobe width.....	<u>113</u>
12 snout to blowhole(s).....	_____	35 fluke notch depth.....	<u>23</u>
13 snout to melon apex.....	_____	36 dorsal fin height.....	_____
14 eye to ear.....	<u>45</u>	37 dorsal fin base length.....	_____
15 eye to gape.....	<u>25</u>	38 girth at eye.....	_____
16 eye to blowhole edge, L...	<u>~200</u>	39 girth at axilla.....	_____
17 eye to blowhole edge, R...	_____	40 girth, maximum.....	_____
18 blowhole length <u>23</u> width	_____	41 girth at anus.....	_____
19 ear opening diameter.....	<u>1</u>	42 girth midway anus to notch...	_____
20 head width at eyes.....	_____	43 height same place.....	_____
21 eye opening length.....	<u>6</u>	44 width same place.....	_____
22 rostral width, melon apex...	_____	45 blubber thickness, dorsal....	_____
23 projection up/lower jaw...	_____	46 blubber thickness, lateral... <u>15.5</u>	
		47 Blubber thickness, ventral... <u>15.7</u>	

REPRODUCTIVE SYSTEM: immature? ? mature? ?

gonads: weight R NE L NE, dimensions (LxWxD) R NE L NE
 pregnant? - fetus length - sex - LxWxD -
 lactating? - mammary gland color - LxWxD -
 sperm present in epididymis? NONE penis length _____
 remarks: _____

GASTROINTESTINAL SYSTEM:

fore stomach volume: - contents: NONE
 main stomach volume: - contents: NONE
 pyloric stomach volume: - contents: NONE
 length of intestines: - contents: DK GREEN LIQUID IN DESCENDING COLON
 remarks: _____

WEIGHTS: (specify units ND, types of scale(s) -)

SCASS.....	liver.....	kidney, R...	heart.....
all viscera..	spleen.....	kidney, L...	thymus.....
all blubber..	pancreas.....	adrenal, R..	thyroid....
all muscle...	stomach, full.	adrenal, L..	brain.....
all bone.....	stomach, empty	gonads, R...	pituitary..
all fluids...	intestines....	gonads, L...	(.....) ..
Total above..	(.....) ..	(.....) ..	(.....) ..

PARASITE/PATHOLOGY CHECKLIST: (X if present, NO if absent, NE if not examined)

barnacles.....	No	fore stomach..	No	heart.....	No	brain.....	NE
cyamids.....	X	main stomach..	No	lungs.....	No	air sinuses....	No
eyes.....	NO	pyloric.....	NO	blubber.....	No	Anasakis.....	NO
blowhole.....	No	intestines....	No	muscle.....	No	Crassicauda....	No
mouth.....	X	rectum.....	No	kidney.....	NE	Monorhagma....	No
genital slit..	No	liver.....	No ?	urethra.....	No	Nasitrema.....	No
anal slit.....	No	bile duct.....	NE	bladder.....	No	Phyllobothrium.	No
appendages....	No	pancreas.....	No	ureter.....	NO	Stenurus.....	No
(.....) ..	(.....) ..	(.....) ..	(.....) ..	(.....) ..	(.....) ..	(.....) ..	(.....) ..

SPECIMEN COLLECTION CHECKLIST:

skull.....	YES	blubber.....	Yes	GI contents..	YES	microbiology.	No
skeleton.....	YES	muscle.....	YES	gonads.....	No	toxicology...	YES
teeth/baleen..	YES	kidney.....	No	fetus.....	NONE	genetics.....	YES
toparasites..	YES	liver.....	YES	(EYE (L)) ..	YES	radiology....	No
toparasites..	NONE	lung.....	YES	(PERIOTIC) ..	YES	(.....) ..	(.....) ..
(.....) ..	(.....) ..	(.....) ..	(.....) ..	(RETE) ..	YES	(.....) ..	(.....) ..

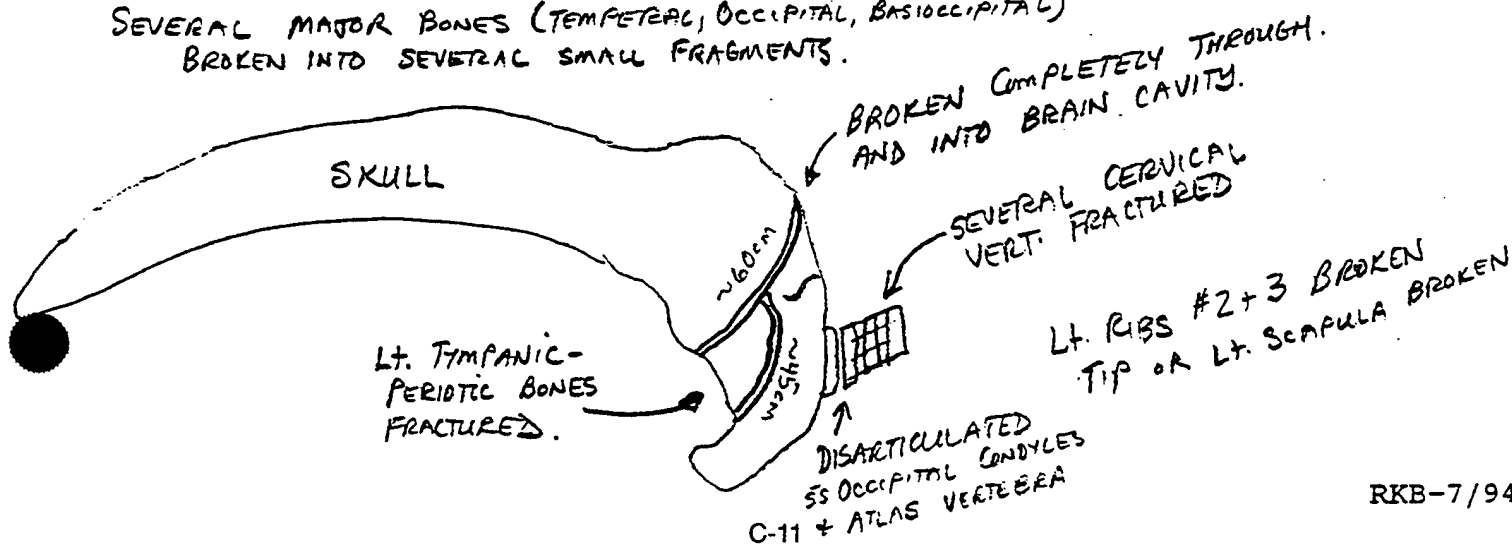
ADDITIONAL REMARKS:

SMALL TRIANGULAR NOTCH FROM POSTERIOR MARGIN OF RIGHT TAIL LOBE. SOME SLIGHT

WHITE, NON-PIGMENTED. SCAR TISSUE ON PEDUNCLE AT JUNCTION OF TAIL.

MICHAEL MOORE OF WOODSHOLE INSTITUTION CONDUCTED DETAILED STUDIES ON BLUBBER LAYER

SEVERAL MAJOR BONES (TEMPERAL, OCCIPITAL, BASIOCCIPITAL)
BROKEN INTO SEVERAL SMALL FRAGMENTS.



APPENDIX D

**NECROPSY EXAMINATION REPORT
(GA96021901/RKB 1430)**

NECROPSY EXAMINATION REPORT

RKB-1430

Eubalaena glacialis

HISTORY

On 19 February 1996, B. Zoodsma, Georgia Department of Natural Resources (GDNR), B. Mase and K. Wang, National Marine Fisheries Service (NMFS), and L. Conger, New England Aquarium (NEA), were conducting an offshore aerial survey for right whales using a NOAA twin otter aircraft. At 0946h the aircraft was requested to break track in order to investigate what appeared to be a right whale. The sighting turned out to be that of a small right whale calf carcass and was located at 30°44'N, 80°59'W, or approximately 20 nautical miles off the south end of Cumberland Island, Georgia. Based on the float characteristics (the animal was floating on it's side and was not very high in the water) the carcass was judged to be in very fresh condition. The coordinates of the calf's location were radioed to the Fernandina Airport Intercom, along with a request for them to inform C. Slay, NEA, of the carcass and request that he notify M. Harris, GDNR. M. Harris phoned the Navy at Kings Bay to request vessel support, but was unable to contact anyone due to the holiday. The U.S. Coast Guard Brunswick Station was also contacted, but they were reluctant to retrieve the carcass. Finally, arrangements were made for the GDNR's 52' research vessel "Bagby" to set sail to retrieve the animal. The R/V "Bagby" was dispatched at 1310h and arrived at the last reported location of the carcass at 1525h. Crew of the R/V "Bagby" were unable to locate the carcass and requested aircraft reconnaissance. The NEA chartered a plane and departed from the Fernandina Airport at 1615h to assist with relocating the carcass. The carcass was relocated at 1719h and at 30°43.5'N, 80°59.0'W. Crew of the R/V "Bagby" secured the carcass (GDNR field number: GA96021901) and at 1800h commenced towing it back to the GDNR docking facility where they arrived at 0010h on 20 February. A boat hoist and two straps, one wrapped around the tailstock, and another placed under the animal just posterior to pectoral flippers, were used to lift the animal out of the water and placed gently onto a trailer supplied by the Florida Department of Environmental Protection (FDEP). The carcass was covered with a cool pack and strapped to the trailer. At 0130h, B. Brooks, FDEP, departed Brunswick with the carcass and arrived in Gainesville, Florida at 0530h. The whale was taken to the University of Florida College of Veterinary Medicine for a detained examination.

NECROPSY EXAMINATION REPORT
RKB-1430

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B. Zoodsma, GDNR, was the on-site coordinator. R. Bonde, National Biological Service (NBS), was the necropsy team leader. Also in attendance at the necropsy were B. Sheppard, M. Rudin, K. Cronin, M. Pinkwasser, University of Florida, College of Veterinary Medicine; C. Beck and C. Deutsch, NBS; S. Wright and M. Sweat, FDEP Marine Mammal Pathobiology Lab; B. Brooks, J. Pennington, J. Sachs, FDEP; C. Slay, NEA; C. Marshall, M. Wesselman, A. Garcia and G. Pimentil, University of Florida. Several 35 mm slide photographs were taken and the procedure was recorded on videotape. Additional video was taken by the C. Slay, NEA.

NECROPSY - 20 February 1996

External exam: This animal is a 513 cm total length, immature, female right whale. All of the black skin remained on the body with some small blistering on the surface and a small amount of postmortem cracking at the base of the left flipper and along the lip line. No external scars, abrasions, or contusions were apparent. Large extensive colonies of live cyamid parasites were established on the head and torso areas. Several cyamid parasites were collected in 70% EtOH. Both baleen rows were intact and complete. The baleen plates were short and slate gray in color. Baleen plate counts were: right 235, left 238. Both rows of baleen were removed and will be accessioned into the mammal collection at the Florida Museum of Natural History. There was a short segment of umbilicus protruding from the umbilical region due to postmortem gas pressure. The vessels of the umbilicus had successfully clotted over and were in resorption. The right and left eyes were removed and placed in 10% NBF. Complete morphometric measurements were taken along with numerous blubber thicknesses and tracings of each appendage.

Internal exam:

Abdominal: A large amount of postmortem gas released when the abdominal cavity was opened. Several loops of reddish brown intestines were expelled along with several liters of reddish fluid. The overall appearance of the abdominal cavity showed marked signs of advanced decomposition. Internal blubber thicknesses were taken and samples of dermis and muscle were placed in DMSO/hypersaline solution for genetic analysis. Muscle and blubber samples were collected and frozen. The larynx was removed and placed in 10% NBF.

NECROPSY EXAMINATION REPORT
RKB-1430

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Gastrointestinal tract: The oral cavity was unremarkable. A cross section of the left intraorbital anterior palate was removed and placed in 10% NBF. There was a small amount of cream-colored, milky fluid in the esophagus and lumen of the forestomach. The forestomach and mainstomach mucosa was dark cream colored. The serosal surface of the intestines was dark and reddened. Loose integrity of the intestines due to advanced decomposition made total length measurement impossible, as segments came apart during handling. The colon contained soft, runny, light mustard yellow contents, which resembled digested milk. Samples of stomach and colon contents were collected and frozen.

Liver: The liver was very badly autolyzed. The parenchyma was dark purple in color. A sample was collected and frozen.

Kidneys: Both kidneys were completely autolyzed and disintegrated. No samples of kidney tissue were collected. The urinary bladder was empty of urine and the mucosa was cream colored and unremarkable.

Heart: Slight serous atrophy of the coronary fat was apparent. The surface was shiny and glistened. There were no blood clots in the atrioventricular chambers. The left ventricle wall was 5.0 cm thick, the right was 3.5 cm. Heart samples were placed in 10% NBF.

Lungs: The trachea and bronchus were open and free of mucous and foreign material, however there was a small amount of frothy fluid present. The lungs were very heavy and congested with dark red fluid which could be expressed from the alveolar sacs. Some sub-pleural clotting was present just under the lung lining, approximately 0.3 to 0.7 cm thick. This edema could have been due to postmortem change and lividity, as it was not of uniform thickness along the contour of the lung. Lung tissue sank when placed in water, then slowly started to float. Samples of lung were collected in 10% NBF and frozen.

Reproductive Tract: The ovaries were immature and autolyzed. The right measured 20x10.4x3.0 cm and the left was not measured due to loss of organ integrity. There were multiple cervical folds in the fundus of the uterus.

Brain: The entire head was removed and weighed 351 kg. The brain was completely autolyzed and liquified.

Parasites: No internal parasites observed.

NECROPSY EXAMINATION REPORT
RKB-1430

Page 4

Skeleton: Some disarticulated cranial bones were present but no signs of antemortem hemorrhage or clot was observed. Most disarticulation of bones was along fissure or growth plate sutures. The inner ear complexes were examined and removed entire and preserved in 10% NBF. A small fracture at the basioccipital bone did not have any associated tissue damage.

The entire postcranial skeleton and soft tissues were incinerated on site. The skull was retained and is in cold storage at the University of Florida College of Veterinary Medicine.

Significant Findings:

- Very heavy, saturated, congested lungs
- Advanced postmortem decomposition rate, possibly related to physiological changes at time of death
- Disarticulation of cranial bones, associated with postmortem change and restricted to suture and growth plate bed sites
- reddened serosal surface of the gastrointestinal tract
- Stomach likely contained milk and colon contained digested milk
- Good overall physical condition

NECROPSY EXAMINATION REPORT
RKB-1430

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Summary:

This animal was in good physical condition and had ample fat reserves. There was no sign of cachexia or malnutrition, which one would expect if this had been an orphaned or abandoned calf fatality. This animal had apparently been nursing prior to death. The advanced state of decomposition of certain organs was probably accelerated by physiological changes just prior to death. The excessive amount of edema in the lungs was a very suspicious finding, and has not been observed in other cetaceans I have examined. The fact that the black skin was intact at time of recovery, that there were live cyamid parasites still attached to the carcass, and the level of buoyancy at time of recovery, would indicate that time of death would have been approximately 2 to 3 days prior to this examination. Efficient insulation and blood saturated organs would have accelerated the internal decomposition rate.

CAUSE OF DEATH - UNDETERMINED

Prosecutor:



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NECROPSY EXAMINATION REPORT
RKB-1430

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Samples Collected

Protocol for collecting materials requested in the Right Whale Samples Collection Protocol dated April 1994 were either met or exceeded for every item as determined by carcass condition.

Frozen:

In Foil

Blubber
Muscle
Liver
Lung

In Plastic

Blubber
Muscle
Liver
Lung
Stomach and esophagus contents
Colon contents

Preserved in 10% N.B. Formalin:

Right & left eyes
Right & left tympanic-periotic bones
Intraoral rete sample
Larynx
Heart, Lung, Muscle
Colon contents

Preserved in 70% EtOH:

Cyamid parasites

Preserved in DMSO/Hypersaline solution:

Skin, Muscle, Lung, & Heart

All preserved material was retained by the National Biological Service, Gainesville Field Station.

Postcranial skeleton destroyed, cranium retained for future examination, and baleen rows were retained by the Florida Museum of Natural History for accession into the collection.

CETACEA DATA RECORD

Field Number: RKB-1430

Catalog Number: _____

Species: EUBALEANA GLACIALIS Sex: F Length: 513 cm Condition: 3-4
 Observer: R. BONDE Date of occurrence: 19 FEB 1996, of data 20 FEB 1996
 Locality: LOW FLOATING, APPROX 20 MI OFF SO. CUMBERLAND ISLAND, GA

Lat/Long: 30°44' N 80°59' W Reported by: B. ZWISMA, G.D.N.R.
 Photographs/Drawings: VIDEO, SLIDES DRAWINGS
 Circumstances/Cause of Death: UNDETERMINED - PENDING HISTOLOGICAL EXAMINATION

External Description: MOST BLACKSKIN STILL ATTACHED. CYAMIDS (LINE) ON HEAD & BODY.
BALEEN ROWS INTACT, SM PIECE OF UMBILICUS PROTRUDING FROM MID-BODY-VENTRAL.

Tooth/Baleen Count: Total _____ Up R 235 Up L 238 Low R _____ Low L _____
 Diameter largest tooth/length longest baleen plate: _____ Baleen color: _____

MEASUREMENTS: (Specify units CM)

1 total length.....	<u>513</u>	24 number of throat grooves.....	<u>-</u>
2 snout to anus.....	<u>338</u>	25 length of throat grooves.....	<u>-</u>
3 snout to genital slit.....	<u>328</u>	26 flipper length, anterior.....	<u>101</u>
4 snout to umbilicus.....	<u>240</u>	27 flipper length, posterior....	<u>85</u>
5 snout to throat grooves...	<u>-</u>	28 flipper width, maximum.....	<u>48</u>
6 snout to dorsal fin tip...	<u>-</u>	29 mammary slit length R <u>8</u> L <u>7</u>	
7 snout to ant. dorsal fin..	<u>-</u>	30 number mammary slits.....	<u>2</u>
8 snout to flipper.....	<u>109</u>	31 length genital slit <u>30</u> anal <u>1.5</u>	
9 snout to ear.....	<u>110</u>	32 perineal length (males).....	<u>-</u>
10 snout to eye.....	<u>95</u>	33 fluke length.....	<u>167</u>
11 snout to gape.....	<u>101</u>	34 fluke lobe width.....	<u>48</u>
12 snout to blowhole(s).....	<u>81</u>	35 fluke notch depth.....	<u>16</u>
13 snout to melon apex.....	<u>-</u>	36 dorsal fin height.....	<u>-</u>
14 eye to ear.....	<u>15</u>	37 dorsal fin base length.....	<u>-</u>
15 eye to gape.....	<u>12</u>	38 girth at eye..... <u>139x2</u>	<u>278</u>
16 eye to blowhole edge, L...	<u>68</u>	39 girth at axilla..... <u>166x2</u>	<u>332</u>
17 eye to blowhole edge, R...	<u>-</u>	40 girth, maximum..... <u>171x2</u>	<u>342</u>
18 blowhole length <u>11</u> width <u>2</u>		41 girth at anus..... <u>115x2</u>	<u>230</u>
19 ear opening diameter.....	<u>0.25</u>	42 girth midway anus to notch <u>67x2</u>	<u>134</u>
20 head width at eyes.....	<u>-</u>	43 height same place.....	<u>51</u>
21 eye opening length.....	<u>7</u>	44 width same place.....	<u>29</u>
22 rostral width, melon apex...	<u>-</u>	45 blubber thickness, dorsal....	<u>5.5 (2.3)</u>
23 projection up/lower jaw...	<u>-</u>	46 blubber thickness, lateral... <u>7.0 (2.2)</u>	
		47 Blubber thickness, ventral... <u>5.0 (2.1)</u>	

(SKIN)

REPRODUCTIVE SYSTEM: immature? ☒ mature? _____

gonads: weight R _____ L _____, dimensions (LxWxD) R 20x10.4x3.0 L ND
 pregnant? No fetus length _____ sex _____ LxWxD _____
 lactating? No mammary gland color _____ LxWxD _____
 sperm present in epididymis? _____ penis length _____
 remarks: _____

GASTROINTESTINAL SYSTEM:

fore stomach volume: _____ contents: WHITE MILKY SUBSTANCE
 main stomach volume: _____ contents: _____
 pyloric stomach volume: _____ contents: _____
 length of intestines: _____ contents: COLON: DIGESTED MILK CONTENTS
 remarks: _____

WEIGHTS: (specify units 3800-4000 lbs (EST), types of scale(s) _____)

(EST)

HEAD = 371 kg

CARCASS..... 3800-4000 liver..... kidney, R... heart.....
 all viscera.. spleen..... kidney, L... thymus.....
 all blubber.. pancreas..... adrenal, R... thyroid....
 all muscle... stomach, full. adrenal, L... brain.....
 all bone..... stomach, empty gonads, R... pituitary..
 all fluids... intestines.... gonads, L... (.....)
 Total above.. (.....) (.....) (.....) (.....)

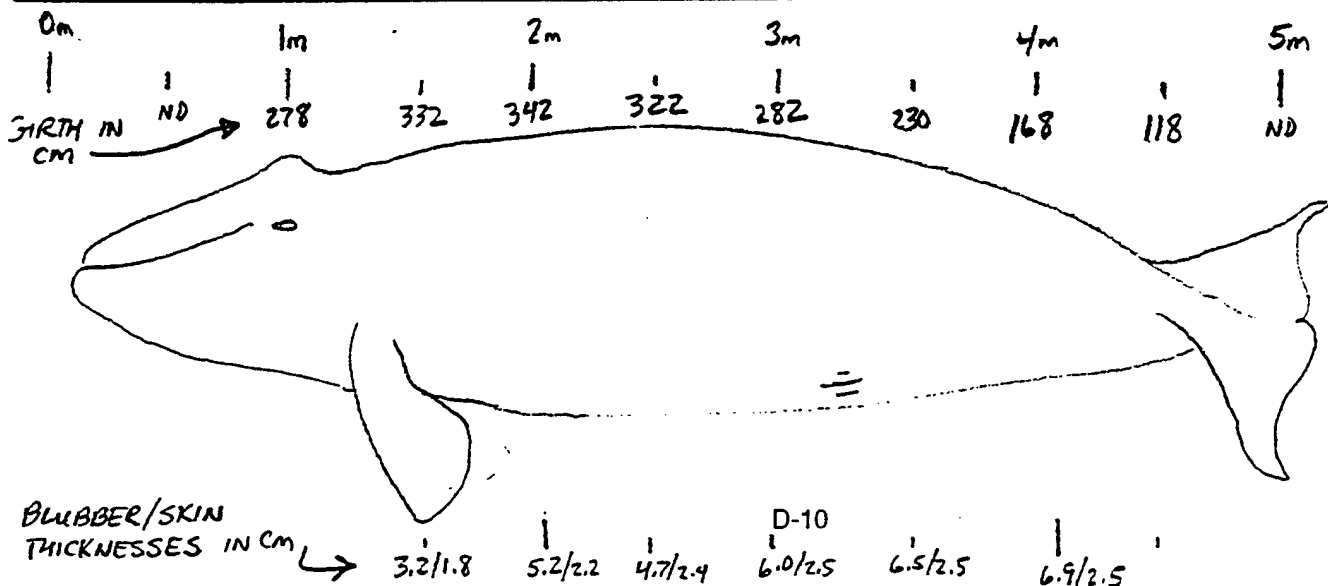
PARASITE/PATHOLOGY CHECKLIST: (X if present, NO if absent, NE if not examined)

barnacles.....	<u>No</u>	fore stomach..	<u>No</u>	heart.....	<u>No</u>	brain.....	<u>No</u>
cyamids.....	<u>X</u>	main stomach..	<u>No</u>	lungs.....	<u>No</u>	air sinuses....	<u>No</u>
eyes.....	<u>No</u>	pyloric.....	<u>No</u>	blubber.....	<u>No</u>	Anasakis.....	<u>No</u>
blowhole.....	<u>No</u>	intestines....	<u>No</u>	muscle.....	<u>No</u>	Crassicauda....	<u>No</u>
mouth.....	<u>No</u>	rectum.....	<u>No</u>	kidney.....	<u>No</u>	Monorhynchus....	<u>No</u>
genital slit..	<u>No</u>	liver.....	<u>No</u>	urethra.....	<u>No</u>	Nasitrema.....	<u>No</u>
anal slit.....	<u>No</u>	bile duct.....	<u>No</u>	bladder.....	<u>No</u>	Phyllobothrium..	<u>No</u>
appendages....	<u>No</u>	pancreas.....	<u>No</u>	ureter.....	<u>No</u>	Stenurus.....	<u>No</u>
(.....)		(.....)		(.....)		(.....)	

SPECIMEN COLLECTION CHECKLIST:

skull.....	<u>YES</u>	blubber.....	<u>YES</u>	GI contents..	<u>YES</u>	microbiology..	<u>-</u>
skeleton.....	<u>No</u>	muscle.....	<u>YES</u>	gonads.....	<u>YES</u>	toxicology...	<u>-</u>
teeth/baleen..	<u>YES</u>	kidney.....	<u>No</u>	fetus.....	<u>NA</u>	genetics.....	<u>YES</u>
ectoparasites.	<u>YES</u>	liver.....	<u>YES</u>	(.....)		radiology....	<u>-</u>
endoparasites.	<u>NONE</u>	lung.....	<u>YES</u>	(.....)		(.....)	
(.....)		(.....)		(.....)		(.....)	

ADDITIONAL REMARKS:

EXTENSIVE EDEMA IN LUNGS, REDDENED SEROSA OF GI TRACT, ADVANCED DECOMPOSITIONMILK IN GI TRACT, GOOD PHYSICAL CONDITION OTHERWISE. SOME ASSUMED FRACTURES OFCRANIUM AND EAR BONES, BUT NOT ABLE TO DETERMINE GROSSLY IF ANTE-OR POST-MORTEM

APPENDIX E

MARINE MAMMAL NECROPSY REPORT
(GA96022201)

MARINE MAMMAL NECROPSY REPORT

Florida Marine Research Institute
Marine Mammal Pathobiology Laboratory

FIELD I.D. 6A 960 22201 SPECIES Eubalaena glacialis
RECOVERY DATE 2-24-96 NECROPSY DATE 2-24-96
SEX male TL cm WT kg CONDITION Badly Decomposed

HISTORY Staff at the MMPL first received a report of the presence of a Right whale calve floating in the Atlantic on 22 February. On 23 February we packed our truck with the necessary equipment and drove to Gainesville to meet with Bob Bonde. By 1630, I was advised by Bill Brooks that the Coast Guard had aborted their search for the day and the NEA personnel that were searching for the carcass had not seen it either. We decided it was best to return to St. Petersburg. Upon our arrival to the MMPL (2030), I had a message from my wife to call Blaire Mase regarding the carcass which had been found at about 1730. After several attempts I left a message with Blaire to call me. She called the next morning (2-24) and asked me to help with the animal. I arrived at the Georgia DNR facility at approximately 1530 and the carcass arrived at approximately 1845. It was transported to an outside area for necropsy.

EXTERNAL Necropsy began at approximately 1930. The head was removed at the recovery site by Dr. Rommel. It was his decision based upon concern for damage to the soft tissues of the ear complex which could have been damaged during towing. As a result, some morphometrics were compromised by the head removal. The carcass had been scavenged by sharks. The sharks had concentrated their feeding on the lower abdomen in the vicinity of the anus and urogenital openings as well as the umbilicus. There were bites on the tail, rostrum, and most of the soft tissue of the blow hole was missing. There were no other external marks indicating trauma or propeller scars. There were no ectoparasitic aggregates.

ABDOMINAL Much of the abdominal skin and musculature was missing as a result of shark bites. Bowel was exposed outside the body through the bites.

STOMACH The stomach was autolyzed and contained a thin, brown, watery fluid. The gastric mucosa had sloughed.

DUODENUM The duodenum was only partially available and it was empty. This could have resulted from spillage during the shark feeding activity. The remaining mucosa appeared normal although autolyzed.

JEJUNUM & ILEUM Not present

CECUM Not present

DNR 33-707
approved 1/23/87)

FIELD ID _____

COLON Only the extreme distal two meters of colon remained. All of this portion of bowel contained meconium.

PANCREAS Not discernible

LIVER The liver had liquified and was barely recognizable. Much of the abdominal cavity was black stained from the decomposed liver.

GALLBLADDER Not present

REPRODUCTIVE TRACT Small cylindrical testicles were found (approx. 14 cm long and 4 cm in diameter). One testicle was torn and barely recognizable.

KIDNEYS Both kidneys were essentially gone although remnants remained in the location where the kidneys should have been.

URINARY BLADDER Not present

HEART The heart was in reasonably good condition compared to the rest of the carcass. There was little cardiac fat and there was some epicardial edema. The pericardium contained a minimum of pericardial fluid.

RESPIRATORY SYSTEM

LUNGS Both lungs were also in comparatively good condition. There was a prominent subcapsular edema that was primarily a serosanguinous exudate. Both lungs were heavy and wet on cut section. This appearance was uniform throughout the entire lung. They were a uniform pale reddish brown color. All observable airways were clear and non-inflamed. Upon applying pressure, only fluid exuded from the pulmonary parenchyma. The condition of the lungs suggest that they had not been inflated. However, a sample of lung tissue floated in fluid.

HEAD & NECK REGIONS The skull was examined thoroughly by Dr. Rommel. There did not appear to be any skull fractures. Sharks had bitten off the distal portion of the premaxillaries and the associated baleen. Both eyes were removed and the left eye exhibited hemorrhage and small blood clots in the loose connective tissue behind the orbit. The right was clean. Both orbits had collapsed presumably from decomposition. The brain was pourable. The remaining baleen was intact and removed by Dr. Rommel. The entire skull was flensed by Dr. Rommel.

SKELETON There were no indications of skeletal trauma. The skull had been removed prior to necropsy (see above) and some of the caudal vertebrae were partially separated as a result of towing the carcass.

OTHER

DNR 33-707
(approved 1/23/87)

FIELD ID _____

DIAGNOSIS The final diagnosis is open pending histopathology. Based upon gross findings three possibilities exists: the animal was still born; maturation of skeletal muscle and other elements argue against this conclusion; the animal suffered an extreme infectious process; other than diffuse extreme pulmonary edema there is little indication of infection observable grossly; acute respiratory distress (shock lung) although there are many causes of this condition, the combination of this finding and unilateral hemorrhaging behind the left eye suggest effects from external trauma.

CAUSE OF DEATH Open pending further examination

NECROPSY CONDUCTED BY: Scott D. Wright, Ph.D.
Sentiel Rommel, Ph.D.

REPORT FILED BY: Scott D. Wright

DNR 33-707
approved 1/23/87)

APPENDIX F

EXAMINATION OF EAR PARTS FROM THREE RIGHT WHALES (GA96013001/RKB 1429; GA96021901/RKB 1430; GA96112201)

HARVARD MEDICAL SCHOOL * MASSACHUSETTS EYE AND EAR INFIRMARY
DEPARTMENT OF OTOLARYNGOLOGY
THREE-DIMENSIONAL HEAD AND NECK IMAGING AND RECONSTRUCTION SERVICE
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8 March, 1996

re: *E. glacialis* ears/SE region/1996

To date, I have received ear parts from 3 right whales (*E. glacialis*) stranded near the Georgia and Florida coasts. Based my gross examination and computerized tomographic (CT) scans of these tissues, one animal (adult male) was severely traumatized by impact, but whether from ship strike or blast could not be determined because of decomposition. Ears in the other two animals (calves) were reasonably well preserved, and neither shows any evidence of blast injury or impact trauma.

All ears were examined using ultra-high resolution CT with 1 mm. contiguous scan sectioning. Two-dimensional images and three-dimensional reconstructions of the temporal bones and inner ear spaces were produced for each animal. Images will be distributed on request. Soft tissues from these ears will be processed for histology as noted below. Summaries of my findings for each animal at this point are as follows:

1. Two ears - adult male - #1429 - floating carcass near Sapelo Island, Ga. - extracted and shipped by R. Bondi, received and examined 7 March, 1996.

Dismembered ear parts were received in formalin soaked nylon bags. A third container held gauze wrapped sections of soft tissue and periotic flanges. There are two significant features to these ear components: both ears are fragmented and both are adipoceric; i.e., the ears are badly decomposed and have no residual soft tissues or fluids. Adipocere is a waxy, brownish scum made of fatty acids formed by the hydrolysis of body fat and soft tissues. The presence of adipocere is consistent with an advanced state of decomposition and with immersion. Adipocere is common, of course, in long post-mortem time marine mammal carcasses because of their high fat content and water habitat. Both ears were received as dismembered parts, but because of the lack of soft tissue and advanced decomposition, no conclusions can be made about the precise cause, nor can it be determined whether the ears were fractured pre or post-mortem. The fractures are primarily transverse, disrupting all but the densest bone. In each ear, only the periotic capsule surrounding the inner ear is intact. Both tympanics, all anterior and posterior flanges, and the ossicles are disrupted. This amount of fracturing is unprecedented in my experience. The periotic and the majority of the tympanic in mysticetes are formed of exceptionally dense compact bone. Fracturing of this type does not occur from simple decomposition. The clean edges of the breaks are consistent with healthy bone subjected to a direct, intense mechanical force. Unfortunately, because of advanced decomposition and the lack of inner and middle ear membranes, it is not possible to determine the source

of that impact. This level of damage could result from either a blast or ship strike. It is notable that both ears were impacted, which suggests multiple strikes - possibly pre and post mortem - or that the initial impact was extraordinary.

2. One ear (left) - female calf - #1430 - floating carcass near Cumberland Island, Ga. - extracted and shipped by R. Bondi; received and examined 7 March, 1996.

Only one ear, the left, has been received and examined from calf #1430. According to Dr. Bondi's report, the second ear is in formalin at Univ. of Florida College of Veterinary Medicine. One caveat: A definitive assessment requires comparison of both ears; the absence of trauma in one ear does not preclude trauma to the other ear. The ear was received as a gauze-wrapped bundle, preserved in formalin. CT scans of the intact bundle revealed that it contained the left tympanic (middle ear component), periotic (inner ear capsule), and portions of the posterior flange that connects the ear to the skull. The periotic and tympanic are separated but intact and well preserved. Three-dimensional reconstructions show that the periotic and tympanic were broken apart by snapping the lateral process. Fragments of the process are distributed between the two components. There is no blood or traumatized tissue associated with the fragments, and no blood was found in the middle or inner ear spaces. This suggests the separation occurred post-mortem and the type of break is consistent with levering or twisting of the tympanic that can occur in freeing the ear from the skull. The ossicles are intact. The malleus is attached to the tympanic and retains its connection to the incus. The stapes is attached to the oval window. The incudo-stapedial articular plates are clean and intact, consistent with a simple separation of this "soft" joint (non-ossified) when the periotic broke free. There is no blood in the inner ear. The cochlear partitions appear intact. The facial and acousto-vestibular nerves are intact and normal in size for the species. There is no evidence of fracture or disruption other than tympano-periotic separation. In summary, the ear is well-preserved with no evident abnormalities. Bone densities are consistent with an immature animal. Cochlear and neural configurations are normal. The ear will be decalcified and sectioned in order to examine residual fine structures of the inner ear.

3. Two ears - calf - extracted by S. Rommel; shipped by M. Harris, Ga. Dept. of Nat. Res., received and examined 5 March, 1996.

The ears do not show evidence of trauma but they have an unusual appearance that *may* indicate respiratory and middle ear dysfunction. The ears arrived as formalin preserved soft tissue blocks. Both blocks contained intact ears with well preserved nerves and inner ears. There is no indication of fracture or structural disruption. Mineralization of the tympanic and periotic bones was relatively low, consistent with a very young animal or with malnutrition. Tympanic membranes had been removed in both ears.

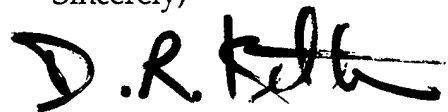
The right inner ear is normal in appearance; the left inner ear has low density fluid primarily in scala vestibuli. The exact nature of the fluid must be determined histologically, but its absorption coefficients are consistent with a mixture of cerebral spinal fluid (CSF) and blood. The inner ear presence and distribution of the fluid is consistent with a small CSF surge that would have forced fluid from the subarachnoid space into the internal auditory canal. This can be due to brain concussion or to a sudden

increase in blood pressure, as in a blow to the skull or extreme physiologic stress (ballistic vomiting, etc). Because the inner ear membranes, round window, and oval window are intact in both ears, and because the material was found in one inner ear only, it is unlikely that its presence is related to a massive atmospheric/middle ear pressure differential as occurs in explosions or barotrauma. In those events, inner ear blood is generally found bilaterally. It is also unlikely that this is a postmortem deposition because of the condition of the inner ear membranes. Principal issues to resolve histologically are whether the material is blood and whether it is a fresh bleed or does the ear contain granulomatous material indicating an active response to infection in a live animal. It would be useful to know also whether there were other signs of metabolic stress or related injury; e.g., petechial hemorrhages in the head tissues or unilateral bruises consistent with a left CSF surge or bleed.

The most important feature in this calf's ears is the abnormal middle ear anatomy. Both middle ears contained mesenchymal webs with associated amorphous soft tissue. These webs are common in fetal mammal ears. They may mean this was a recent birth and the inner ear material may be a reflection of that state. If this is not a newborn or stillborn, the webs could indicate an unusual inner ear development that may be related to a weakened condition. In a small percentage of humans (<5%), middle ear mesenchymal webs remain after birth. Their presence is often associated with respiratory, middle ear, and mastoid disease. In a recent study of infants who died within their first year from sudden respiratory failure, it was found that the majority had retained mesenchymal webs and that the lungs, trachea, throat, and middle ear were compromised by meconium (intestinal debris, mucus, bile, and epithelial cells swallowed *in utero*) that had not cleared at birth. It is not clear what exact mechanisms caused the death of these children, but they were subject to chronic infections and breathing difficulties. One possibility is that retaining mesenchymal webs is a secondary effect that accompanies abnormal mucosal and ciliary development that compromises lung function; alternatively, the webs may have a direct effect, acting as harbors for disease organisms. At the moment, only a statistical correlation between webbing and chronic, debilitating infection has been shown in humans. Based on fetal data from Solntseva and my examinations of whale calves (including sperm whale, right whale, and grey whale neonates), whales, like other mammals, commonly have fetal webs but resorb them in the last quarter before birth. My observations are certainly not a clear indication of disease or debilitation as the cause of death, but it is worth noting that in this calf, the webs remained. If the lungs and trachea are available, I believe it would be worthwhile to have them examined with this observation in mind.

I would very much appreciate any information from other researchers on these animals, and I will provide you with any additional observations I make on these tissues as they become available. Please let me know if I can be of further assistance.

Sincerely,

A handwritten signature in black ink, appearing to read "D. R. Ketten", with a stylized flourish at the end.

Darlene R. Ketten, Ph. D.

APPENDIX G

**FINAL REPORT ON THE WORKSHOP
TO COORDINATE LARGE WHALE
STRANDING RESPONSE IN THE SOUTHEAST U.S.**

DRAFT

**FINAL REPORT ON THE
WORKSHOP TO COORDINATE LARGE WHALE
STRANDING RESPONSE IN THE SOUTHEAST U.S.**

**National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southeast Fisheries Science Center
Charleston Laboratory
Charleston, South Carolina
September 27-28, 1995**

**Edited by Robert A. Blaylock and Blair G. Mase
Southeast Fisheries Science Center
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SEFSC Contribution MIA-96/97-XX

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WORKSHOP BACKGROUND

by William McLellan and Robert A. Blaylock

Introduction

Strandings of large whales generally constitute a small percentage of the total number of marine mammal strandings in any given year and the rate of large whale strandings remained level during the first two decades that regional stranding networks were in place. In 1990, however, a four-fold increase in the number of humpback whale strandings in the U.S. mid-Atlantic states occurred and humpback whale strandings in that area have continued at an elevated level to the present. The cause of the increase in humpback whale strandings is unclear, but anthropogenic factors, including ship strikes and entanglements in fishing gear, have been found to have had significant roles in recent humpback whale strandings where thorough necropsies were performed.

The northern right whale is one of the most endangered species of whale in the world and the U.S. Atlantic stock is estimated to number approximately 300 individuals. This stock utilizes an area for calving and nursing near shore in Georgia and Florida during the winter which has been designated a right whale critical habitat and includes four commercial shipping ports and two heavily trafficked naval bases. Ship collision has been the most frequently documented cause of death among right whales in this area, but because of the lack of an adequate system to provide a timely and appropriate response, determination of the cause of death is relatively rare.

The following recommendations in the inset below refer to data, in addition to cause of death, which can be collected from stranded whales and are taken from the Humpback Whale Recovery Plan. Similar recommendations are also in the Recovery Plan for the Northern Right Whale.

- 1.4 Monitor parasite load, biotoxins, and anthropogenic contaminant level in tissues of whales and their prey.
- 1.41 Develop standardized protocols for sampling tissues of whales using strandings and biopsies.
- 1.42 Develop protocol to sample anthropogenic contaminant levels in tissues of prey.
- 1.43 Implement base-line study of parasite load in whale tissues and contaminant levels in tissues of whales and prey.
- 1.44 Monitor biotoxin concentration in tissues of prey species and whales.

A major emphasis of the 1994 Amendments to the Marine Mammal Protection Act was to take measures to reduce human-induced mortality of marine mammals. Fishery interactions and ship collisions have been identified as major contributors to large whale mortality in the National Marine Fisheries Service (NMFS) Southeast Region; however, an understanding of the causes of all large whale strandings has been hampered by a lack of consistent response. In an effort to rectify this situation, a small group of marine mammal researchers met in Orlando, Florida, in April, 1994, to plan the organization of the Southeast U.S. Marine Mammal Stranding Network (SEUS) Large Whale Stranding Response System to improve the quantity and quality of scientific data recovered from stranded large whales. The goal of the meeting was to identify SEUS Network members who had both the experience and ability to investigate large whale strandings and to initiate the organization of a system which would provide a timely and appropriate response in the event of the stranding of a large whale along the southeast U.S. Atlantic coast. A core group of experienced large whale necropsy personnel would gather specific data during necropsy examinations using standardized collection protocols designed to identify cause of death, would train others in large whale necropsy procedures, and would provide researchers and managers consistent data for comparative purposes. The necropsy personnel would receive logistical support from local on-site personnel which

would be organized into the SEUS Large Whale Stranding Response System.

That meeting led to the workshop which is the subject of this report and which was conducted at the NMFS Southeast Fisheries Science Center Laboratory in Charleston, South Carolina, on September 26-29, 1995. The goals of the workshop were to construct and formalize a system to provide an appropriate response to large whale strandings in a timely fashion, so as to maximize the quality and quantity of the data collected. An outline for such a system was discussed at the planning meeting and it was envisioned that the system would consist of two levels of response. The initial response to a large whale stranding would entail the mobilization of local SEUS Network volunteers and state, county, and city officials to assess the situation, stabilize the carcass, manage crowd control, arrange logistical support for the necropsy team, arrange for tissue sample transfer, and arrange for carcass disposal after the necropsy, in addition to other needs which might arise. A primary response team consisting of the SEUS network State Coordinator and/or the NMFS Area Representative, with assistance from SEUS Network volunteers, would be responsible for the initial response mobilization. Actual necropsy of the stranded whale would be conducted by a member of the Large Whale Necropsy Team Leader. This individual would be brought to the scene at government expense to lead the necropsy and ensure that proper sampling was conducted. This response system was adapted from the manual by J. R. Geraci and V. J. Lounsbury, *Marine Mammals Ashore, A Field Guide for Strandings*, published by the Texas A&M Sea Grant Program in 1993.

The workshop began with the presentation of a brief overview of the history of large whale strandings in the region since the establishment of the SEUS Network. This was followed by a review of similar data for Virginia and the northern portion of the North Carolina Outer Banks. These overviews provided an idea of the average number of strandings occurring annually in the Southeast Region and the adjoining State of Virginia and an idea of the typical condition of the carcasses and types of logistical conditions which might be encountered. These reviews are reproduced in this report. After the data presentations, the workshop divided into two groups to discuss the roles and needs of the two teams. The workshop participants then reconvened and presented and discussed the recommendations of each team. The notes from these discussions are reproduced in this report. Protocols for sample data and sample collection which were agreed to by all of the workshop participants are included. These protocols for response to large whale strandings on the Southeast Region Atlantic coast have been adopted by the NMFS Southeast Fisheries Science Center Marine Mammal Stranding Network Area Representatives, the SEUS Network Scientific Director, and SEUS Network State Coordinators.

The NMFS has provided funds to the New England Aquarium (NEA) to investigate northern right whale strandings in the U.S. Atlantic, so it was decided that the SEUS large whale response system should defer to the NEA in the case of right whale strandings in the Southeast Region unless the NEA could not be contacted or could not respond quickly. The SEUS stranding network would assist the NEA team utilizing the organizational structure set forth during this workshop and the complete data set would still be archived by the SEUS Stranding Network. The NEA right whale stranding protocol is appended to this report.

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HISTORICAL PERSPECTIVE ON LARGE WHALE STRANDINGS IN THE SOUTHEASTERN UNITED STATES

by Nelio Barros and Daniel K. Odell

Introduction

The Southeastern United States Marine Mammal Stranding Network was organized in 1977 as a result of a workshop sponsored by the U.S. Marine Mammal Commission in Athens, Georgia (Odell 1991). Marine mammal strandings have been recognized as an invaluable source of biological data (Heyning 1987, Odell 1987) for some time and, in the southeast U.S., thousands of records have been collected from stranded mysticetes, odontocetes, and a few species of pinnipeds since the late 1970's (Odell 1991). The National Marine Fisheries Service (NMFS) Office of Protected Resources has made suggestions to improve the efficiency of regional stranding networks (Wilkinson 1991) and a field guide detailing standardized methods for investigating marine mammal strandings has recently been published (Geraci and Lounsbury 1993).

Recovery and conservation plans have been produced for some endangered marine mammal species and those whose stocks have been depleted, and recommendations have been made for protective actions enabling stocks to be restored to pre-depletion levels. Such was the case for the mid-Atlantic migratory stock of bottlenose dolphins (Wang et al. 1994). All species of large whales are endangered or threatened along the U.S. Atlantic coast and some are at low population levels. Of particular concern is the slow recovery of the North Atlantic stock of northern right whales which is plagued by high human-related mortality (Kraus 1990, Read 1994), low reproductive success (Brown et al. 1994), and the combination of these with a number of other factors (Knowlton et al. 1994).

A review of large whale strandings in the southeastern United States from 1977 to 1995 (September inclusive) is presented here as an aid in planning response to large whale strandings and the recovery of stranded large whales. Strandings of all mysticetes are included in the review, as are those of sperm whales due to the large size they attain. Whale sightings, records with questionable species verification, and stranding records from Puerto Rico and the U.S. Virgin Islands were excluded. Seasonal and geographically based analyses are provided in the graphs, as well as the occurrence and distribution of strandings by size-class and carcass code. The species treated in this review are: minke whale (*Balaenoptera acutorostrata*); sei whale (*B. borealis*); Bryde's whale (*B. edeni*); fin whale (*B. physalus*); northern right whale (*Eubalaena glacialis*); humpback whale (*Megaptera novaeangliae*); and sperm whale (*Physeter macrocephalus*).

Results and Discussion

Large whale strandings, by species

A total of 137 large whale strandings were reported between 1977 and 1995 (Figs. 1a-h). Excluding 1977 and 1995 (years of incomplete records), strandings averaged approximately eight whales annually, with the highest numbers (14 whales/yr) reported in 1979 and 1992. A mass stranding of sperm whales (Fig. 1h) caused the 1979 stranding peak. Strandings of most large whale species showed little inter-annual variation; however, there was a noticeable increase in the number of humpback whale strandings in the late 1980's and early 1990's (Fig. 1g).

Geographical distribution of strandings

Strandings of large whales on the Atlantic coast of Florida and along the North Carolina coast during the study period totaled 102 whales, or approximately 75% of all of the large whale strandings recorded for the entire southeastern U.S. (Fig. 2a). The high number of strandings in Florida may be a result of a combination of several factors — its extensive coastline, easy accessibility to its beaches, and a historically better stranding coverage than in other states. Large whale strandings in the Gulf of Mexico were comparatively few (21 strandings or 15% of the total, Figs. 2a-h). Verified strandings of fin, right, and humpback whales occurred only on the Atlantic coast (Figs.

2e,f,g). Right whale strandings were concentrated along the Florida/Georgia border (Fig. 2f), an area along the Atlantic coast to where this species migrates for reproduction (Knowlton et al. 1992). Detailed accounts of humpback whale strandings along the Atlantic coast may be found in Wiley et al. (1995).

Temporal distribution of strandings

Although large whale strandings occur throughout the year in the southeastern United States, they are more numerous in the winter and spring (Fig. 3a). These two seasons combined accounted for 74% of all large whale strandings. Most large whale species exhibited this seasonal stranding pattern (Figs. 3b-h), with the possible exception of Bryde's whale (Fig. 3d), which tended to strand at approximately the same rate throughout the year.

Stranding distribution by size class

The logistics involved in the rescue of a large whale are directly related to its size. To aid in planning of large whale stranding responses, strandings were grouped across species into three size categories (< 5 m, 5-10 m, >10 m) regardless of biological significance. Figs. 4a-h are the result of this grouping. Throughout the study area, mid-sized whales (5-10 m category) were the most commonly stranded (68 of 128 records, or 53%, Fig. 4a). This pattern is true for minke (Fig. 4b), Bryde's (Fig. 4d), humpback (Fig. 4g) and, to some extent, sperm whales (Fig. 4h). Among fin whales, large specimens (> 10m long) predominated (Fig. 4e) and, among right whales, small specimens (<5m long) were more common (Fig. 4f). The large presence of right whale calves and juvenile humpback whales in the southeastern U.S. stranding record agrees well with the migratory patterns of both species (Knowlton et al. 1992, 1994; Wiley et al. 1995).

Stranding distribution by code

The quality of samples collected from a stranded whale largely depends on the freshness of the carcass upon examination during necropsy. Figs. 5a-h illustrate the condition of the carcass in large whale strandings which occurred in the study area. Condition 1 animals refer to animals which stranded alive and were released on site. All other live-stranded animals which subsequently died were categorized according to the condition which they were in when necropsied. Fig. 5a illustrates that nearly half of all stranded whales were fresh when examined, primarily due to the large number of fin and sperm whales (Figs. 5e,h) in that category. Right whales (Fig. 5f), on the other hand, are usually very decomposed (condition 4) by the time they are examined during necropsy. The majority of humpback whales (Fig. 5g) are moderately decomposed when examined. Nonetheless, judging by the large number of condition 2 carcasses, the potential exists for routine collection of high quality tissue samples from stranded large whales.

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Figure 1. Number of large whale strandings in the NMFS Southeast Region by year, 1977- 1995.

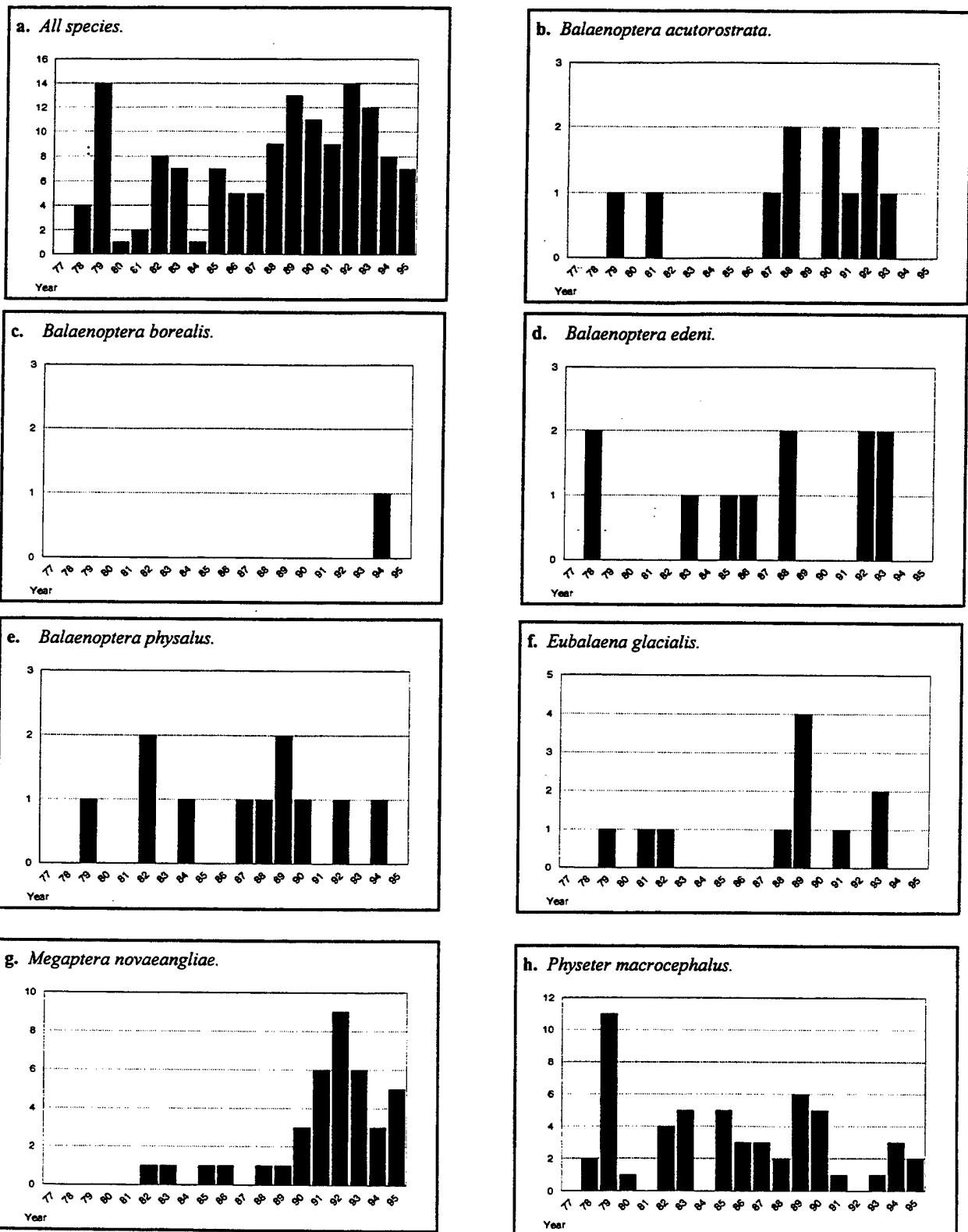


Figure 2. Distribution of large whale strandings in the NMFS Southeast Region by state, 1977-1995.

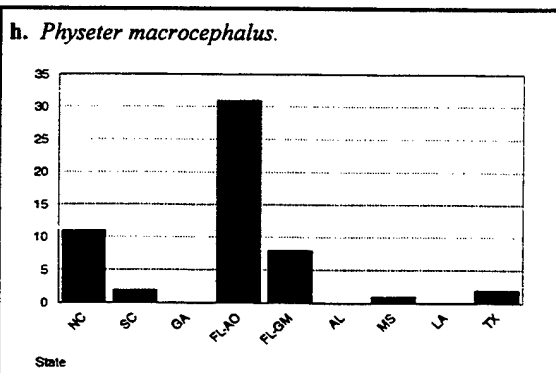
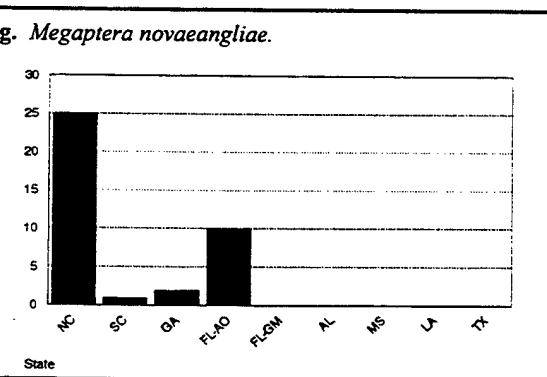
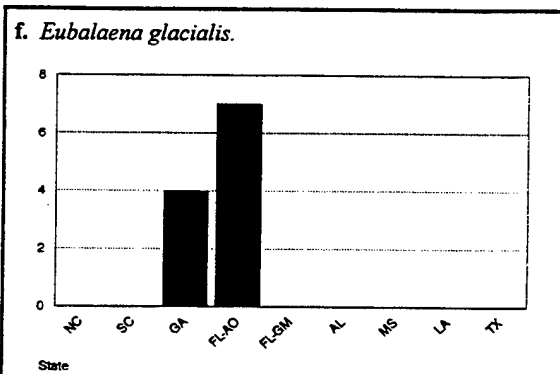
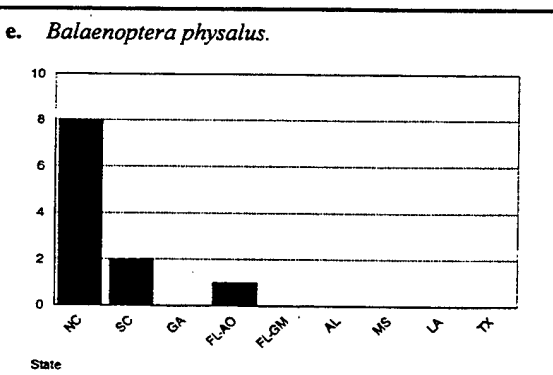
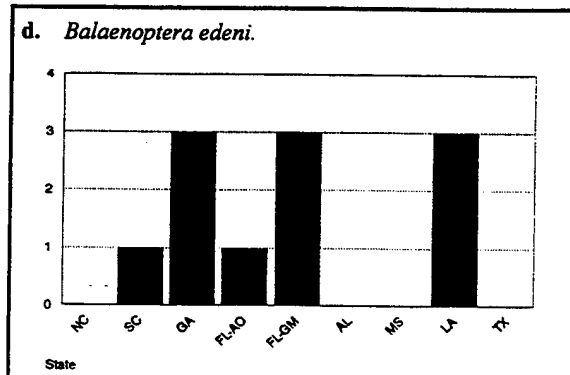
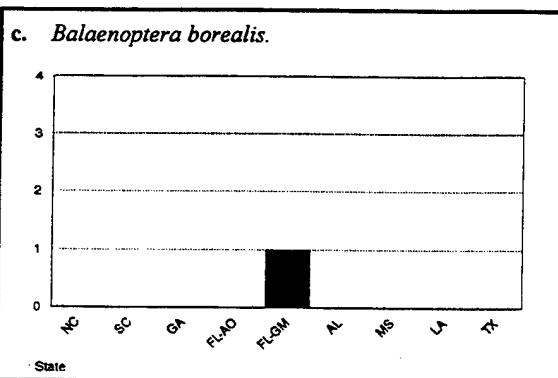
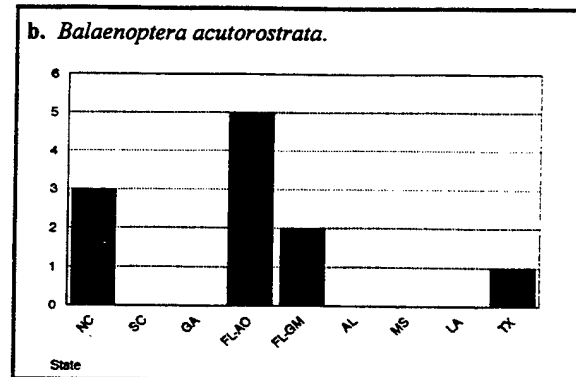
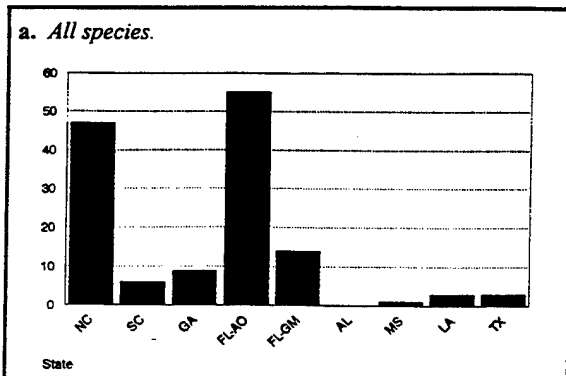


Figure 3. Distribution of large whale strandings in the NMFS Southeast Region by month, 1977-1995.

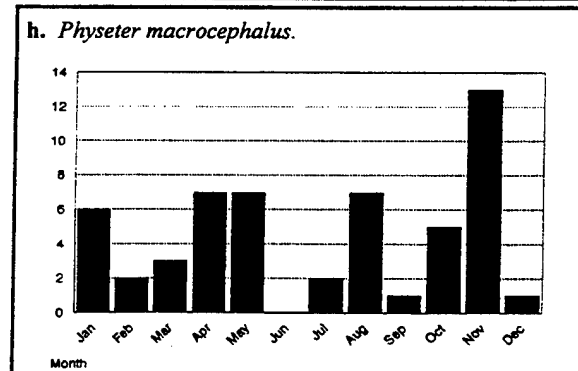
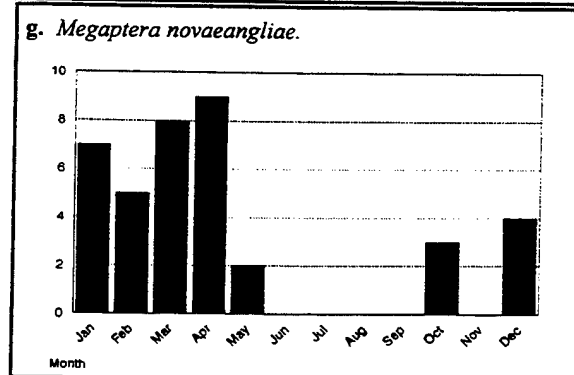
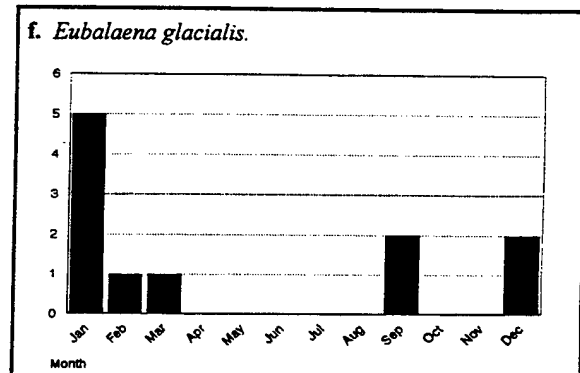
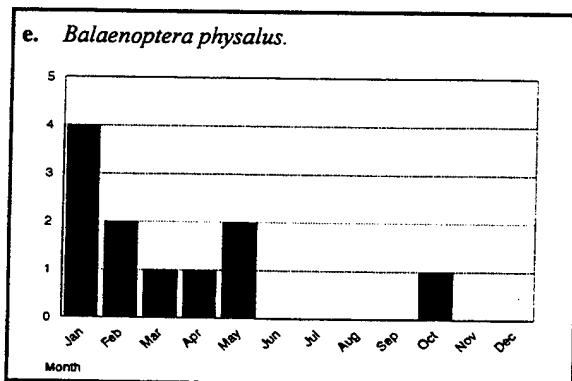
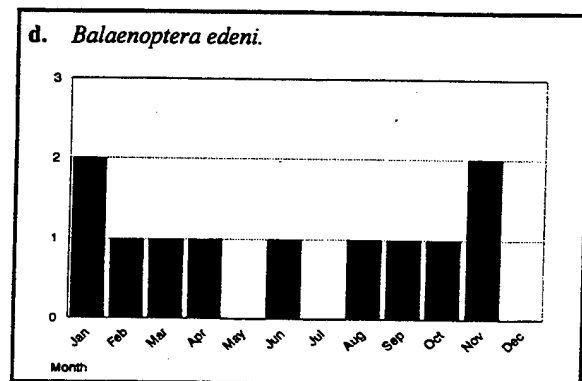
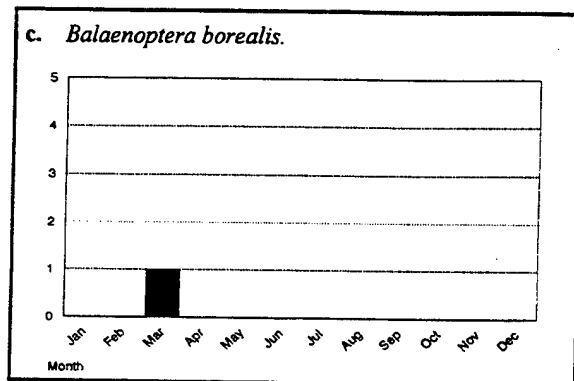
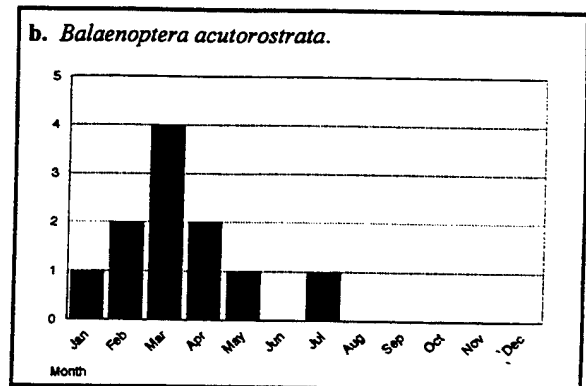
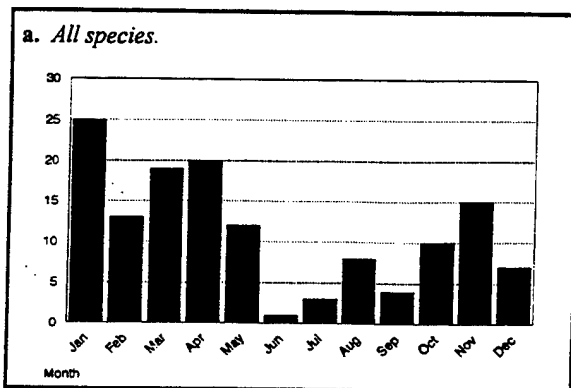


Figure 4. Distribution of large whale strandings in the NMFS Southeast Region by size class, 1977-1995.

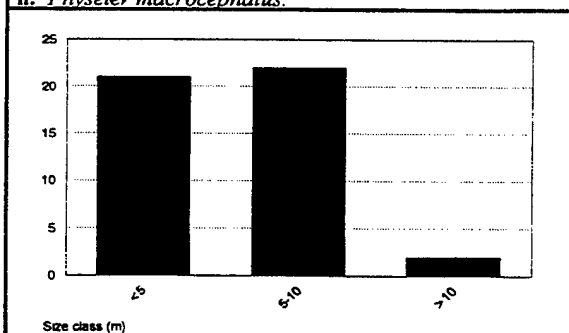
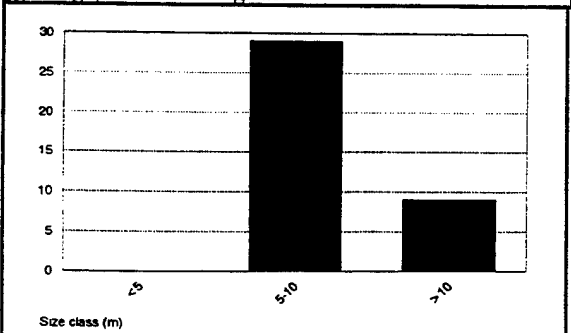
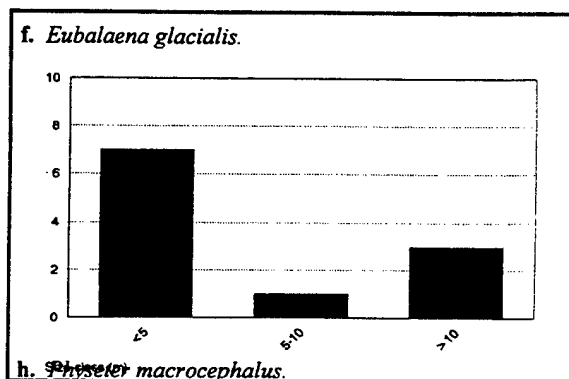
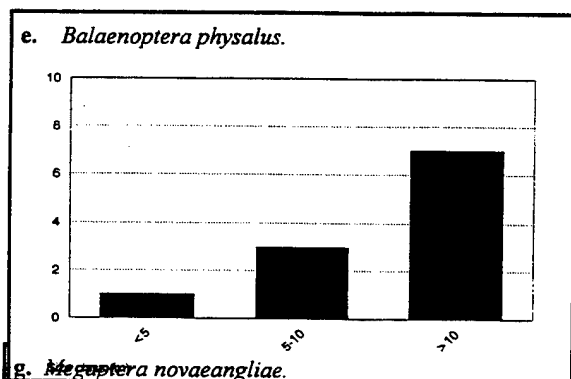
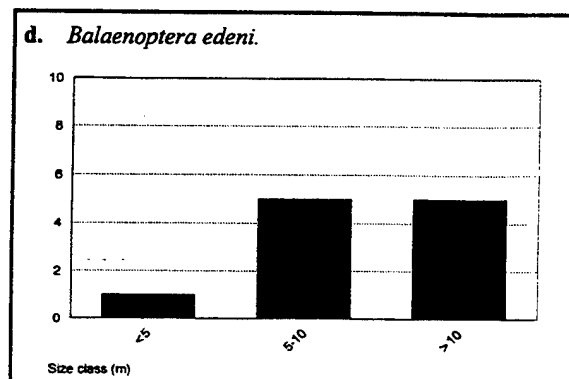
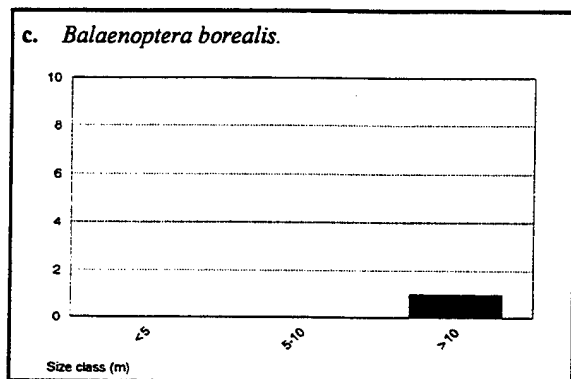
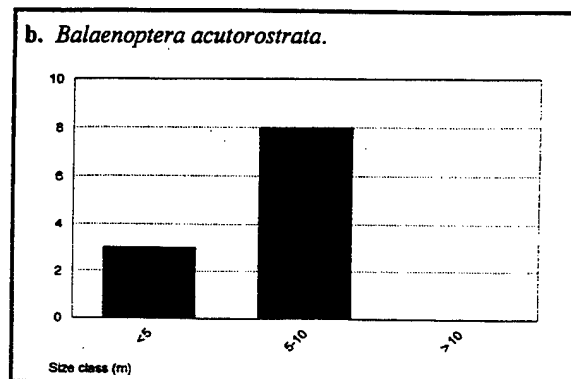
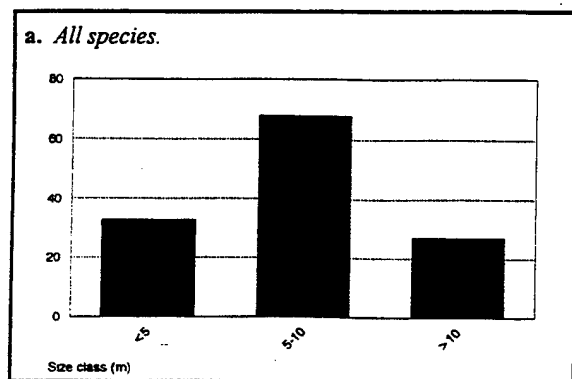
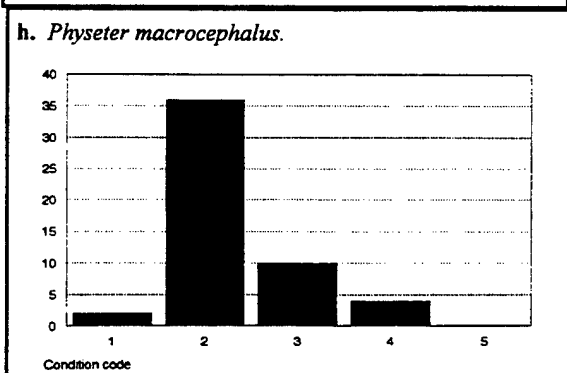
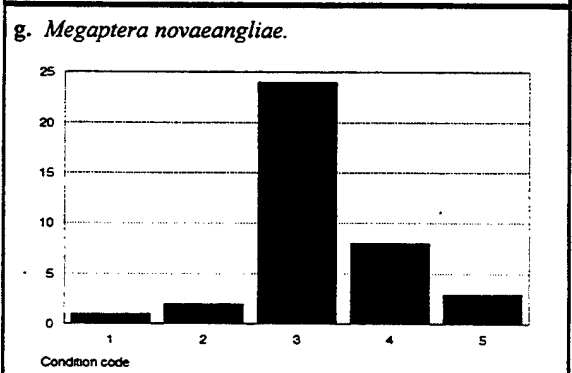
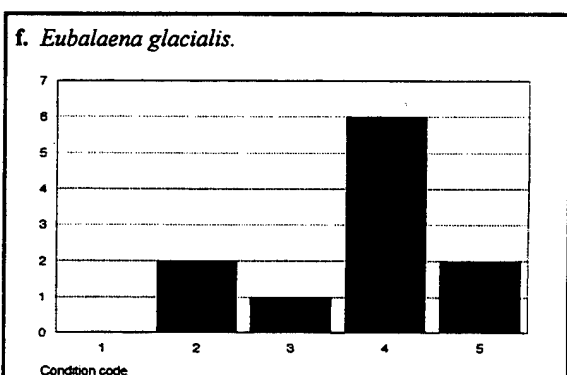
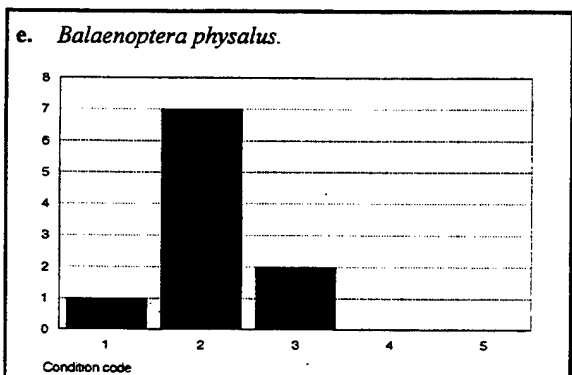
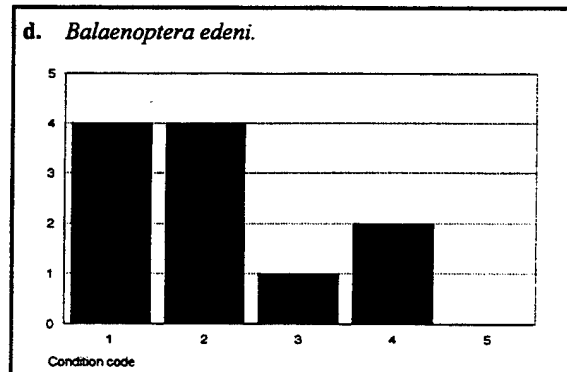
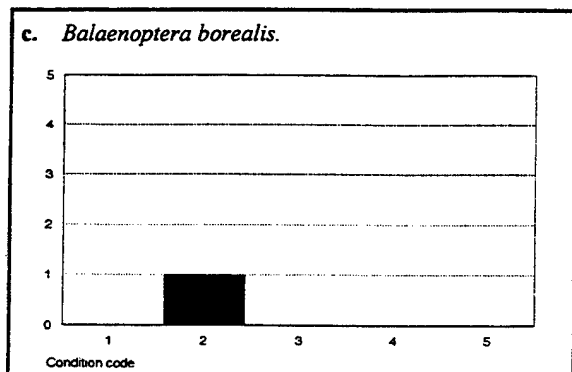
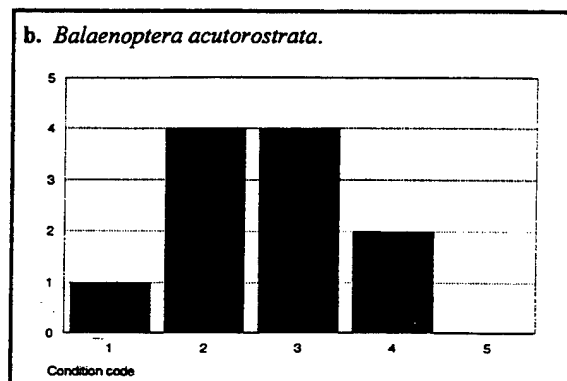
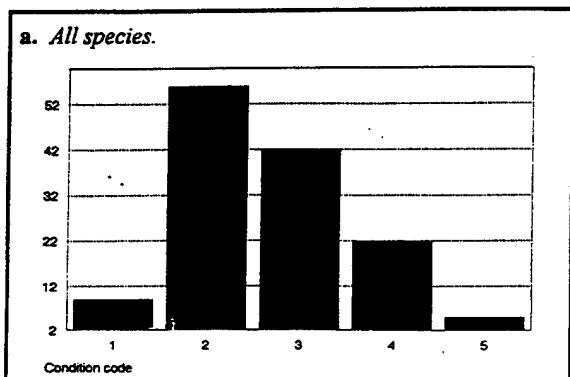


Figure 5. Distribution of carcass condition of large whales stranding in the NMFS Southeast Region, 1977-1995.



LARGE WHALE STRANDINGS IN VIRGINIA, JANUARY 1990-SEPTEMBER 1995

by Susan G. Barco and W. M. Swingle

The Virginia Marine Science Museum (VMSM) located in Virginia Beach, Virginia, has been responding to marine mammal strandings since 1987. Our response effort along the southeastern coast of Virginia has been consistent since 1990. We have been responsible for statewide stranding response since 1993. We have recorded 15 large whale strandings in Virginia from 1990 until the present (September 1995): ten humpback whales; four minke whales; and one fin whale (Table I, Fig. 1). During this time period, several floating whale carcasses, including a right whale, were reported but did not strand in Virginia. Additionally, one confirmed

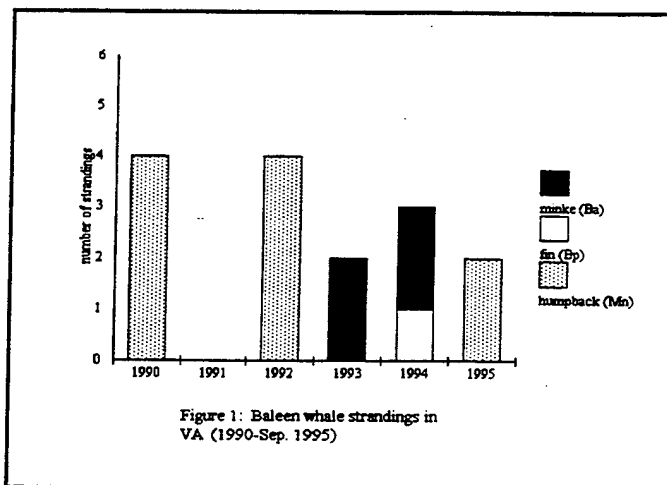
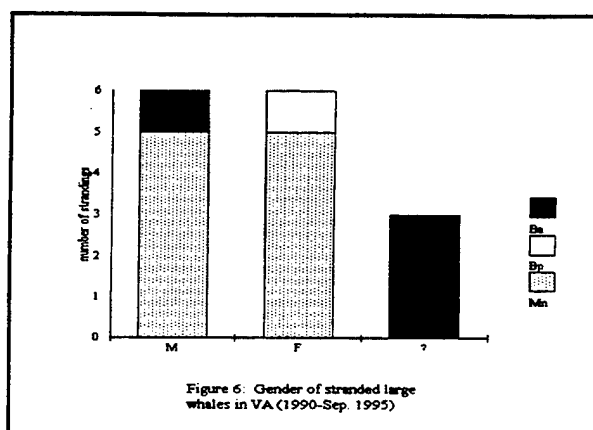
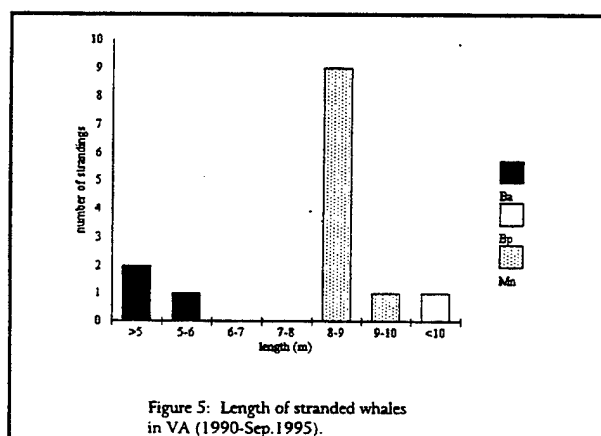
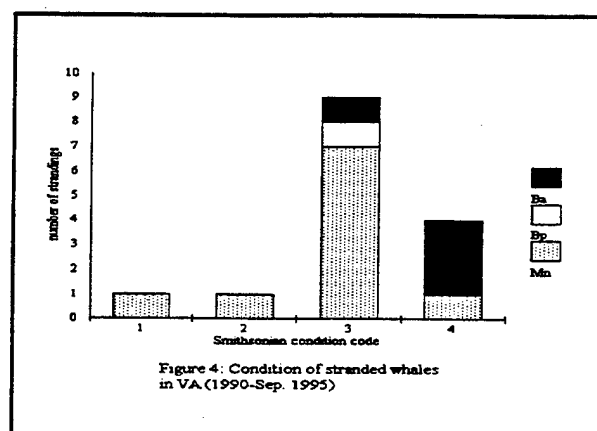
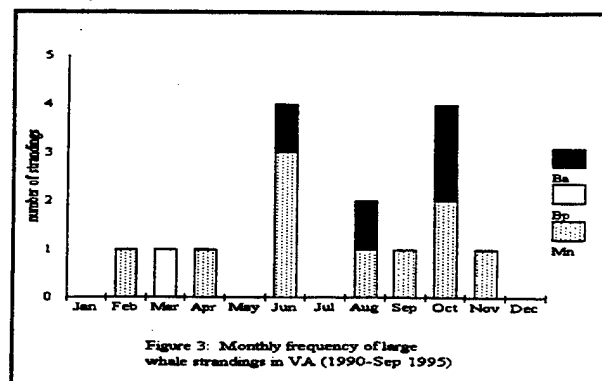
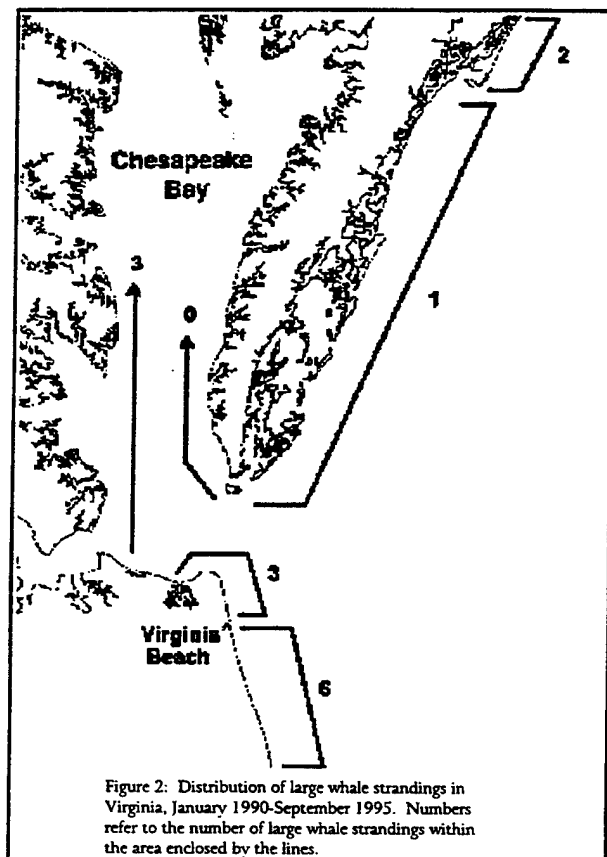


Table I. Large whale strandings in Virginia from January 1990-September 1995.

FIELD NO.	DATE	SPECIES	CITY/COUNTY	LOCALITY	HI	C	N	SEX	LENGTH
1990									
MM-01APR-90	90-04-01	<i>Megaptera novaeangliae</i>	Virginia Beach	BBNWR	Y	3	Y	F	960.1
VMSM901006	90-06-19	<i>M. novaeangliae</i>	Virginia Beach	Chesapeake Bay	N	2	Y	F	830.0
MM20JUN90	90-06-20	<i>M. novaeangliae</i>	Virginia Beach	Sandbridge	?	1	Y	F	820.0
VMSM901013	90-11-19	<i>M. novaeangliae</i>	Norfolk	Oceanview	Y	3	N	M	853.4
1991									
none									
1992									
VMSM921002	92-02-14	<i>M. novaeangliae</i>	Virginia Beach	Lynnhaven	Y	3	Y	M	857.0
92-MM-AO-MN-39	92-09-28	<i>M. novaeangliae</i>	Accomack	CNWR	?	3	Y	M	976*
92-MM-AO-MN-39	92-10-09	<i>M. novaeangliae</i>	Accomack	Metompkin Island	Y	3	Y	F	870.0
VMSM921025	92-10-22	<i>M. novaeangliae</i>	Virginia Beach	Dam Neck	Y	3	Y	M	908.0
1993									
VMSM931050	93-10-01	<i>Balaenoptera acutorostrata</i>	Virginia Beach	Sandbridge	?	3	Y	?	523.0
VMSM931051	93-10-07	<i>B. acutorostrata</i>	Virginia Beach	FCSP	?	4	N	?	337.0
1994									
VMSM 941010	94-03-12	<i>B. physalus</i>	Virginia Beach	Fort Story	Y	3	Y	F	1635*
VMSM 941078	94-06-24	<i>B. acutorostrata</i>	Accomack	CNWR	?	4	Y	M	390.0
VMSM 941084	94-08-15	<i>B. acutorostrata</i>	Hampton			4	Y	?	ND
1995									
VMSM951028	95-06-04	<i>M. novaeangliae</i>	Virginia Beach	Dam Neck	Y	3	Y	M	886.0
VMSM951043	95-08-19	<i>M. novaeangliae</i>	Mathews	Gwynns Island	?	4	Y	F	880*

KEY: HI = evidence of human interaction; LENGTH = centimeters; C = condition; N = necropsied; * = estimated; BBNWR = Back Bay National Wildlife Refuge; CNWR = Chincoteague National Wildlife Refuge; FCSP = False Cape State Park.



entanglement of a humpback whale (30 April 1990) and two vessel strikes (one humpback and one unknown large cetacean species) were reported, but these did not result in the whale stranding.

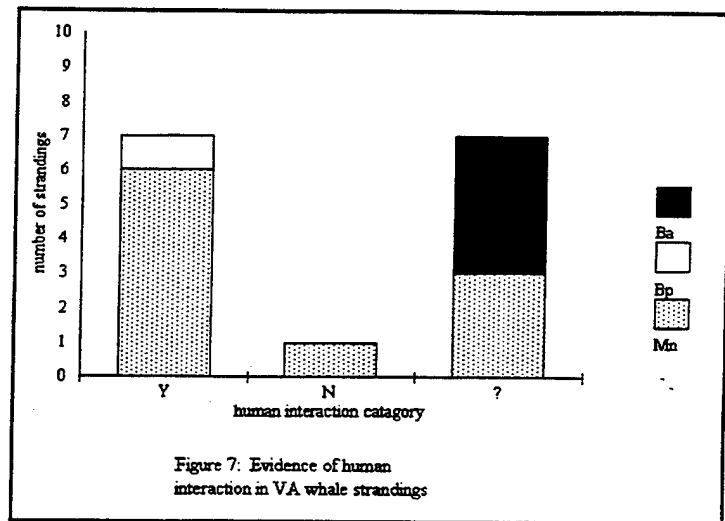
Large whale strandings in Virginia occurred primarily along the ocean coast, with a few occurring in

Chesapeake Bay, (Fig. 2) and during all seasons (Fig. 3). All carcasses were examined and a necropsy was performed on 13 of the carcasses which ranged from condition code 1 to condition code 4 upon initial examination (Fig. 4). The length of the stranded whales varied by species (Fig. 5). The total length of the carcasses indicated that sexually immature whales were primarily involved. The sex ratio, in those cases where sex was determined, was unity (Fig. 6).

Seven of the 15 carcasses (47%) showed evidence of human interaction (Fig. 7). Necropsy records indicate that three of the seven showed evidence of rope abrasion consistent with fishery interaction. Two

carcasses had terminal injuries consistent with propeller strike on the caudal peduncle and both of these had full stomachs. The remaining two carcasses possessed broken mandibles with evidence of some healing, indicating pre-mortem injuries. Carcasses placed in the "?" category were either too decomposed to determine evidence of human interaction, or had broken bones which may have occurred post-mortem.

The VMSM Stranding Team personnel examined 14 of the 15 carcasses. Cindy Driscoll (NMFS Office of Protected Resources) was present for nine of the examinations (eight humpback whales and one fin whale), and Bill McLellan and Ann Pabst (University of North Carolina - Wilmington) were present for three of the humpback necropsies. The consistency of the examinations and thoroughness of the necropsies would not have been possible without their participation. Because of equipment, time, and money constraints, most of the carcasses were examined in one day, and some carcasses were not examined completely. With the support of the National Marine Fisheries Service in the investigation of future large whale strandings, we expect to be able to conduct more comprehensive examinations to further determine cause of death, evidence of human interaction, pathology, and life history parameters during these important stranding events.



LARGE WHALE STRANDING RESPONSE PROTOCOL

by Blair G. Mase, Cindy P. Driscoll, and Robert A. Blaylock

Our goal in organizing this workshop was the development and implementation of an organized, formal response to large whale strandings utilizing the knowledge of persons in the stranding network with experience in large whale stranding response. As the SEUS Marine Mammal Stranding Network's response to large whale strandings becomes more organized, data collection and, subsequently, our understanding of the nature and causes of large whale strandings will improve. The recommendations presented here are the result of two days of collaboration among the workshop participants. Implementation of these guidelines should result in improved response to large whale strandings.

Coordination of large whale stranding response

Much of the preparation, in the form of making contacts, identifying equipment, and organizing local authorities must be made well in advance of a whale stranding so that the time required to acquire equipment and manpower during a stranding event is minimal. The timing and location of a large whale stranding cannot generally be predicted, so the location and availability of equipment and manpower for all areas along the coastline should be identified well in advance of a stranding. This advance preparation is the joint responsibility of the SEUS State Coordinator and the NMFS Area Representative.

In the event of a large whale stranding the SEUS State Coordinator or NMFS Area Representative will assign an on-site primary response team (PRT) leader and an off-site coordinator. The responsibility of the PRT leader will be to orchestrate the logistic response at the stranding site (move the carcass, if necessary; measure and photograph the carcass; control the crowd; etc.) and to assist the Necropsy Team Leader (NTL) who will perform the necropsy. The PRT leader must be familiar with the logistics required to perform a large whale necropsy and be familiar with the area where the stranding occurs (tidal range, access to site, local authorities, etc.). The off-site coordinator will contact NMFS, local authorities, heavy equipment operators, arrange for manpower, arrange for room and board for the necropsy team (if necessary), receive media inquiries, etc. The PRT leader and off-site coordinator need to maintain close communication with each other. The PRT Leader and NTL will jointly be responsible for ensuring that the complete data record is forwarded to the SEUS Scientific Coordinator for archiving.

Role of the PRT in the local stranding network

The PRT is the group of persons who initiate and carry out the local response to a large whale stranding, and assist in performing the necropsy examination. In order to maintain consistency, the local SEUS stranding network will be asked to defer to the PRT during the response; however, the PRT will usually include one or more members of the local stranding network and the PRT leader will often be the local NMFS Area Representative or the SEUS State Coordinator. It will ultimately be the decision of the PRT leader as to how involved the local network volunteers will be, but their assistance will be needed and appreciated.

Role of the NTL in the large whale stranding response

The NTL is a person with considerable experience in the dissection and examination of large whales. This person may be a State or Federal professional or an academic or museum researcher who has volunteered to assist the NMFS-SEFSC in performing the necropsy examination and sample collection from large whales stranded in the region. In the case of the northern right whale, the New England Aquarium (NEA) will field a necropsy team to respond to right whale strandings anywhere on the U.S. Atlantic coast; however, the NMFS Office of Protected Resources has made funds available through the Southeast Regional Office to provide transportation for a NTL to the scene of any large whale stranding in the region. The NEA should always be notified immediately of a right whale stranding, but this notification does not preclude the notification of the nearest NTL and the Miami Laboratory Large Whale Stranding Coordinator.

The NTL shall be responsible for performing the necropsy examination and insuring that all pertinent samples

are collected. In addition, the NTL should provide necropsy training in conjunction with the examination to enhance the Stranding Network's capabilities. The NTL will be ultimately responsible for coordinating the post-necropsy review.

On-Site Response (PRT Leader)

- I. Confirm stranding and species.
- II. Determine carcass condition.
 - A. If the carcass is floating:
 1. identify an area where it can be beached;
 2. arrange for transport to beach (USCG and others).
 - B. If the carcass is beached:
 1. arrange for heavy equipment;
 2. determine time line for arrival of heavy equipment.
 - C. Contact off-site PRT leader with time line.
- III. Examine carcass and report to NTL.
 - A. Determine carcass condition.
 - B. Determine access to carcass.
 - C. Estimate time required to complete morphometric measurements, photos, and external exam.
- IV. Initial examination and carcass manipulation.
 - A. Photograph carcass before moving.
 - B. Complete morphometric measurements.
 - C. Weigh carcass (if possible).
 - D. Manipulate carcass into position for necropsy.
 - E. Make initial disposal arrangements.
- V. Assist NTL with necropsy.
- VI. Arrange carcass disposal.

Off-Site Response (Off-Site Coordinator)

- I. Arrange communication with on-site PRT leader.
- II. Notify the following:
 - A. NTL appropriate for the species and location;
 - B. NMFS Miami;
 - C. Local authorities (police, disposal crew, crane company, etc.).
- III. Arrange for on-site manpower —
 - A. 5-15 people for 1-3 days for actual necropsy examination;
 - B. 2-5 people for crowd control and education;
 - C. media liaison- distribution of information/brochures.
- IV. Maintain contact with on-site PRT leader and provide support as necessary.
- V. Arrange for NTL to arrive on-site (arrange transportation, lodging, etc., if necessary).

Post-necropsy protocol (On-site PRT Leader, Off-site Coordinator, and NTL)

- I. On-site and off-site PRT leaders meet with NTL to arrange post-necropsy events.
 - A. Complete initial report.
 - B. Compile list of volunteers, donations, and officials associated with the event and write thank you letters.
 - C. Disseminate tissues to proper analytical facility.
 - D. Report on expenditures.
 - E. Photo and video disposition.

Reporting- Necropsy Team Leader (NTL)

- I. Written necropsy report —
 - A. Brief history to include climate, oceanographic data/life history parameters/carcass condition.*
 - B. External lesions/condition.
 - C. Internal examination.
 - D. Summary
 - E. Cause of death. List probable causes and contributing factors if they can be determined.
 - F. This report should be disseminated to the following:
 1. Large whale response group.
 2. Recovery team.
 3. Responding PRT.
 4. NMFS Regional office.
 5. NMFS headquarters for national database
 - G. Updated data will be distributed as analyses are completed.*
- II. The PRT and NTL send all data to the SEUS Scientific Director who will house the central repository. This file should include —
 - A. NMFS level A data form.*
 - B. Cetacean data form.
 - C. Human interaction assessment form.
 - D. Sample collection tracking —
 1. Check lists:
 - a. histology check list - duplicate samples (AFIP and Other), code 2 only;
 - b. contaminant check list, code 2 only;
 - c. tissue bank check list;
 2. List where of samples sent for analyses and when/where sent.*
 - E. Necropsy narrative.
 - F. Photos.*
 - G. Location data (maps, latitude and longitude).*
 - H. Ancillary information —
 1. List of people involved.*
 2. Copies of thank you letters to people and agencies who helped.*
 3. Recommendations to improve response/collection; any problems encountered that need addressing.

* Items which are primarily the responsibility of the PRT.

Necropsy Equipment

One of the primary responsibilities of the PRT is to make arrangements to have all of the necessary necropsy equipment onsite when the NTL arrives. Each NMFS Marine Mammal Stranding Network Area Representatives and State Stranding Coordinator should have at least one equipment kit available to be transported to the stranding site immediately by the PRT. In areas where there is no NMFS Area Representative, the local state official who responds to marine mammal strandings should have a kit available. The necropsy equipment checklist (Appendix I) contains the equipment recommended by the workshop participants. Each equipment kit should be labeled with a list of the items contained within. This is a suggested list. The NTL may have other items included.

Carcass Examination Protocol

The PRT and NTL will generally each be responsible for certain portions of the necropsy, depending on the circumstances of the stranding. The PRT Leader will usually see to it that all of the required external photographs are acquired, the morphometric measurements are collected and recorded, and will collect the external samples. The NTL will generally be responsible for all internal examinations; however, depending upon the skill level of the PRT members and the circumstances of the stranding, the NTL may request that some internal examination begin prior to the NTL arrival. The minimum carcass examination protocol recommended by the workshop participants is detailed below; however, additional data collection or examinations may be required for different species (See Appendices).

- I. Photos (Include animal field number and species and date at beginning of the roll and include measure bar and with ID in close-ups.) —
 - A. Whole carcass from both sides, head and fluke.
 - B. Flukes (dorsal and ventral) and right and left sides (label).
 - C. Flippers (dorsal and ventral, label).
 - D. Head/mouth.
 - E. Dorsal fin, both sides (label).
 - F. Lesions and/or wounds.
 - G. Reproductive tract.
 - H. Attached fishing gear or line.
- II. Record data/morphometrics (may be done by PRT).
- III. External examination and sampling (see Appendices for sample collection protocols): photograph lesions/parasites
 - A. skin; collect for DNA, histology
 - B. blubber; collect for tox/contaminants
 - C. external parasites; quantify mild/moderate/severe
- IV. Internal examination and sampling; photograph
 - A. Flense whole carcass.
 - B. Examine for contusion and/or scarring; note-mild/moderate/severe.
 - C. Collect samples (see Appendices for protocols).
- V. Musculature —
 - A. Examine for hemorrhaging and lesions. Note- mild/moderate/severe; photograph
 - B. Collect samples (see Appendices for protocols).
- VI. Remove pectoral fins and examine for signs of human interaction.
- VII. Remove ribs and examine for fractures and evidence of old trauma or lesions.

- VIII. Examine internal organs (see Appendices for sample collection protocols) —
- A. Internal parasites -note-mild/moderate/severe; give parasite score and collect samples for identification.
 - B. Sample internal organs as condition allows
 - C. Note reproductive organ condition and obtain measurements and photos.
 - D. Photograph any unusual findings.
- IV. Examine skeleton for evidence of recent fractures, healed injuries, and pathology.

HEMATOLOGY AND SERUM CHEMISTRY PROTOCOL

by Blair G. Mase and Ruth Ewing

Note: This protocol is intended for code 1 and/or code 1-2 animals.

MATERIALS FOR COLLECTING BLOOD:

18 gauge x 23-47 mm (1-2") needles

20 ml, syringes

2 ml, cryovials

10 ml, plastic vial

10 ml, serum separator gel Vacutainer® tubes (red/gray marble top)

10 ml, EDTA Vacutainer® tubes (purple top)

10 ml, lithium or sodium heparin Vacutainer® tubes (green top)

METHODS:

Lavender top tube with EDTA for CBC:

- Fill tube with blood via internal vacuum.
- If blood is obtained via syringe, immediately remove cap and place blood in tube.
- Gently invert tube several times to facilitate mixing of anticoagulant and prevent clotting.
- Keep specimen refrigerated during transportation and storage.

Lavender top tubes with EDTA for white blood cell separation:

- Immediately refrigerate and ship via next day service to recipient for processing.

Red and gray top tubes for serum sample:

- Fill tube.
- Allow to stand with the cap in place for 30 minutes to clot.
- Centrifuge for 10-15 minutes at 1500-2000 rpm.
- Remove serum and place in plastic vial or clean plain red top tube, labeled serum.
- Keep specimen refrigerated or frozen during transportation or storage.
- Long term storage requires a cryovial, -70 to -80 °C ultracold refrigeration, and dry ice shipment.

Green top tube with lithium or sodium heparin for plasma sample:

- Fill tube as aforementioned for the lavender top tube.
- Gently invert tube several times to facilitate mixing of anticoagulant and prevent clotting.
- Centrifuge for 10-15 minutes at 1500-2000 rpm.
- Remove plasma and place in plastic vial or clean plain red top tube, labeled plasma.
- Keep specimen refrigerated or frozen during transportation or storage.
- Long term storage requires a cryovial, -70 to -80 °C ultracold refrigeration, and dry ice shipment.

SHIP SAMPLES TO:

Freight Address:

Wayne McFee
NOAA, National Marine Fisheries Service
217 Fort Johnson Road
Charleston, SC 29424

Mailing Address:

Wayne McFee
NOAA, National Marine Fisheries Service
P.O. Box 12607
Charleston, SC 29424

Phone: 803-762-8500 (lab) or 8592 (office) - *please call to inform of incoming samples.*

MICROBIOLOGY SPECIMEN COLLECTION PROTOCOL

by Scott D. Wright

This protocol is intended for ideal conditions with the understanding that they rarely occur. Carcass condition as well as environmental factors dictate what is reasonably possible in a field necropsy situation.

General guidelines:

- Specimens should only be collected from code 1 or code 2 animals.
- Specimens must be collected carefully so as to avoid contact with the collector. This is especially true with sputum samples collected from live animals; do not get covered with sputum from the blow hole.
- Tissue samples should be collected from the edge of lesions incorporating normal tissue. This is also true of abscesses because the center of an abscess is usually sterile. If available, smaller, "newer" abscesses are more productive.
- It is important to collect the sample as aseptically as possible to avoid overgrowth of other bacteria. In the field, the site of the lesion should be decontaminated by flame. This is best accomplished with a portable propane burner; however, avoid charring the tissue in the process.
- When collecting from live animals, samples should be collected before any treatment.
- Samples should arrive at the diagnostic facility within 24 hours. When this is not possible, refrigerate the samples (wet ice), do not freeze them. The necropsy kits should contain aerobic and anaerobic transport packets. Read the directions on the vial envelope and make sure each sample is labeled with the sample site and accession number.
- Use account # 00037746 and specify the type of tests (i.e., aerobic or anaerobic).

Note: *DO NOT SEND SAMPLES ON WEEKENDS OR HOLIDAYS — HOLD THEM UNTIL THE NEXT WEEKDAY.*

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PARASITE COLLECTION PROTOCOL

by Robert K. Bonde

MATERIALS:

AFA: alcohol/formalin/acetic acid solution
10% NBF: neutral buffered formalin
70% EtOH: ethyl alcohol
70% glycerin alcohol
GAA: glacial acetic acid
0.8% saline (NaCl)
Distilled water

Disposable gloves
Scalpel and blades
Forceps
Whirlpaks®, assorted sizes
Specimen containers with screw top lids
Tape and labels

METHODS: Parasites are fragile and must be handled gently. Remove from the host by lifting from underneath using forceps, but without pinching specimens. Parasites embedded in tissue should be removed with a section of the infected tissue. If there are too many individuals of one species present, attempt to get a representative sample. Keep samples from different organs/sites in separate bags or containers. Record host species and identification number, organ or host site, density of infection and percent of total collected, preservative used, date and location of stranding. Label all bags or containers and tape closed securely. Do not freeze parasites. For each type of parasite handle as described below.

Barnacles: Fix for no more than 24 hours in 10% NBF, then transfer to 70% EtOH. If they are securely attached to the skin or baleen, cut a small piece and preserve with the parasite attached.

Copepods and amphipods: Place specimens directly into 70% EtOH.

Nematodes (roundworms): Fix in GAA for 5-10 minutes if possible. Otherwise, use 70% EtOH or 10% NBF. If formalin is used, fix for no more than a few hours. Remove larval nematodes from cysts before fixing if present. After fixing, transfer specimens to 70% glycerin alcohol.

Trematodes (flukes, flatworms): If alive, place in cool water for about an hour; this relaxes them and prevents curling. Swirl specimens in saline to clean mucus and debris from specimen. Dead or alive, fix in AFA for up to 3 days, then transfer to 70% EtOH (NOT glycerin alcohol).

Cestodes (tapeworms): Care must be taken to include the head when removing cestodes from the host. Cut host tissue if necessary. Specimens can be swirled in saline or tap water for cleaning and relaxing. Fix for 5-10 minutes by quickly adding AFA to water in at least a 4:1 ratio, then transfer to 70% EtOH. Larval cestodes must be removed from cysts before relaxing and fixing.

Acanthocephalans: If alive, place in distilled water for 5-10 minutes to extrude proboscis. Fix in AFA for up to 24 hours, then transfer to 70% glycerin alcohol.

FIXATIVES AND STORAGE MEDIA:

AFA Fixative:

ethyl alcohol, 95%	50 ml
formaldehyde, 40%	10 ml
glacial acetic acid	5 ml
distilled water	45 ml

10% neutral buffered formalin:

formaldehyde, 40%	10 ml
sodium acetate	1.0 g

	distilled water	90 ml
	OR	
	formaldehyde, 40%	10 ml
	sodium phosphate, monobasic	0.40 g
	sodium phosphate, dibasic	0.65 g
	distilled water	90 ml
70% ethyl alcohol:	ethyl alcohol, 95%	50 ml
	distilled water	15 ml
70% glycerin ethyl alcohol:	ethyl alcohol, 70%	95 ml
	glycerin	5 ml

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HISTOPATHOLOGY SPECIMEN COLLECTION PROTOCOL

By Gregory Bossart and Blair Mase

MATERIALS:

- 10% neutral buffered formalin
- Glass or plastic jars/containers
- Waterproof labels
- Scalpel handles
- Blades
- Knives
- Knife sharpeners
- Cultures
- Gloves
- Dissection board or table for cutting tissues
- Large hemostats

METHODS: Histopathologic examination of tissues collected from stranded marine mammals, including large whales, can provide researchers with the morphologic changes produced by disease caused by either anthropogenic factors or natural causes that lead to an etiologic diagnosis and cause of death. account of illnesses either caused by anthropogenic factors or natural mortality that lead us to the cause of death. Histopathologic examinations in conjunction with in conjunction with toxicology, life history, immunologic and microbiologic studies can give us an over all biological "health package" on the animal and help provide both normal and abnormal parameters for large whale species.

To retrieve as much information as possible from large whales, rapid response and tissue collection are imperative. Tissues collected from code 2 (fresh dead) are ideal, due to the rarity of large whale strandings histology specimens should be collected on up to code 3 animals. Carcasses too decomposed for histopathologic exams may be used for identifying gross lesions such as fractures, lacerations and some neoplasms.

Specimens should be obtained via standard necropsy protocol see histopathology specimen checklist in the appendix. Tissues collected should be no larger than 3 X 3 cm and ideally be 1 cm in thickness. If larger samples are collected numerous parallel cuts should be made to permit adequate fixation. Gross lesions should be described and recorded. Tissues from all representative organs should be collected. The microscopic examination of tissues frequently reveals lesions which cannot be seen grossly. Samples from gross lesions should include abnormal tissue and adjacent normal tissue and be properly labeled.

All tissues should be preserved in 10% neutral buffered formalin. A ratio of 1:10 tissue to formalin provides ideal tissue fixation. Attention to sample size and preservative to tissue proportions is imperative. All tissue samples can be placed in sealable plastic or glass jars. Samples should be doubly-labeled with a label written in indelible ink or pencil inside the jar and another on the outside of the jar.

A standardized necropsy form and histology check list should be followed to maximize data collection and for future comparative studies. Please see appendix for both.

PROTOCOL FOR COLLECTING TISSUE AND BLOOD FOR GENETIC ANALYSIS*

by Cheryl Woodley

TISSUE SOURCES: The preferred sources of tissue for genetic analysis are: 1) skin; 2) blood; 3) heart; and 4) liver. The most stable tissue for nuclear or mitochondrial DNA isolation appears to be heart muscle, although skin, blood and liver can also be used. Ideally, skin and blood can be obtained from live stranded animals. Heart and liver samples, taken from recently dead animals during the necropsy procedures, may be more degraded but are still valuable for DNA research. Genetic samples should be collected for animals including Codes 3 and 4.

NOTE: All materials needed for collecting genetic samples are provided in the large whale stranding kits.

SKIN SAMPLES

MATERIALS:

20% dimethylsulphoxide (DMSO) saturated with NaCl

Screw cap tubes (15 ml)

Scalpel/razor blades

Disposable gloves

Parafilm®

METHOD:

1. Cut 8-10 pieces of skin tissue approximately 0.25-0.5 cm³ in size (removing as much blubber layer as possible).
2. Place pieces of tissue in appropriately labeled vial containing 20% DMSO saturated with NaCl. (Note: sloughed skin may also be collected.)
3. Samples can be stored at room temperature for 1-2 years.
4. Prior to shipping, ensure that caps are on tightly and wrap upper 1/3 of tubes in Parafilm® to prevent leakage.
5. Ship samples by express mail (at room temperature).

*modified from Amos, B. and R. Hoelzel. 1991. Long-term preservation of whale skin for DNA analysis. Rep. Int. Whal. Comm. Special Issue 13:99-103.

BLOOD SAMPLES

MATERIALS:

SDS/Urea, pH 6.8: 240 mM Na₂HPO₄
 1mM ethylene-diamine tetracetic acid (EDTA)
 1% sodium dodecyl sulfate (SDS)
 8 M urea

Vacutainer® w/anticoagulant (preferably NO heparin, use either EDTA or citrate)

Screw-cap tubes

Parafilm®

Transfer pipettes

NOTE: *Optimally, blood should be collected from live animals, the minimum amount should be 10 ml; 50-100 ml represents a reasonable sample that will yield sufficient DNA to do numerous studies. It is possible to collect blood from dead animals. If the blood has not coagulated, but pooled in the body, an anticoagulant syringe may be used for collection. The heart may be a good site in obtaining blood. If the blood has coagulated, collected the clotted material and freeze; send frozen.*

METHOD:

1. Collect 4-5 ml blood in Vacutainer® with anticoagulant (eg. EDTA); do not freeze.
2. Transfer blood immediately to screw cap tubes.
3. Centrifuge at room temperature at 2,000 rpm for 3 minutes. (If centrifugation is not possible, whole blood

mixed with anticoagulant can be shipped using cold-packs).

4. Remove buffy coat (white blood cell layer between plasma and RBCs) from blood (will be about 1 ml) and add to tube containing 9 ml SDS/Urea.
5. Invert tube several times to mix.
6. Samples can be stored at room temperature.
7. Prior to shipping, ensure that tube(s) are tightly-capped and wrap in parafilm.
8. Ship samples by express mail (at room temperature).

HEART AND LIVER

MATERIALS:

SED buffer: 250mM EDTA pH 7.5; 20% DMSO saturated with NaCl

Screw-cap tubes

Razor blade or scalpel

Parafilm®

Disposable gloves

(SED buffer is nontoxic, nonflammable, and can be stored indefinitely at room temperature. Since the buffer contains saturated salt (NaCl), a white precipitate may be found. This does not affect the preservation qualities. Handling SED buffer without gloves may result in exposure to DMSO. DMSO is used to alleviate muscle aches but also produces a garlic/oyster taste and odor. Gloves are recommended when handling.)

METHOD:

1. Collect 8-10 pieces of heart or liver tissue by approximately 0.25-0.5cm³ in size using a clean razor blade to cut the tissue.
2. Add tissue to appropriately labeled tube containing SED buffer.
3. Samples can be stored at 4°C for up to a year. Avoid extended exposure to heat or sunlight.
4. Prior to shipping, ensure that tubes are tightly-capped and wrap in Parafilm®.
5. Ship samples by express mail (at room temperature).

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Phone: 803-762-8500 (lab) or 8592 (office) - *please call to inform of incoming samples.*

PROTOCOL FOR COLLECTING TISSUE AND BLOOD FOR BIOTOXIN ANALYSIS

by Fran VanDolah

TISSUE SOURCES: The preferred sources of tissue for biotoxin analysis are: 1) blood; 2) stomach; 3) liver; and 4) kidney. Blood samples should be collected for animals Code 1 and 2; tissue samples for Code 2 only.

NOTE: All materials needed for collecting biotoxin samples are provided in the large whale stranding kits.

MATERIALS:

razor blade or scalpel
disposable gloves (recommended)
Ziplock® bags or polypropylene bottles for frozen storage
heparinized syringe or vacutainer

METHODS:

Blood samples:

Collect 1 ml blood in heparinized syringe or vacutainer. Separate serum by centrifugation, if possible. Remove the serum and store the sample frozen (-20°C) for shipment. If centrifugation is not possible, send blood mixed with anticoagulant, refrigerate and send using cold-packs.

NOTE: Optimally, blood should be collected from live animals, the minimum amount should be 1 ml. It is possible to collect blood from dead animals. If the blood has not coagulated, but pooled in the body, an anticoagulant syringe may be used for collection. The heart may be a good site in obtaining blood. If the blood has coagulated, the clear (plasma) portion may be obtained via centrifugation and then frozen.

Tissues:

Collect approximately 50 g stomach contents*, liver and/or kidney tissue. Store frozen (-20°C) in zip lock bags or propylene bottles for shipment.

* After subsampling 50 g of stomach contents for biotoxin analysis, the remaining stomach contents can be sent to Nelio Barros (407)363-2664.

SHIP SAMPLES TO:

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PROTOCOL FOR COLLECTING TISSUE SAMPLES FOR CONTAMINANT ANALYSIS

by Paul Becker

The following gives procedures for collecting tissue samples for contaminant analysis (organic and heavy metal analyses) as well as specimens for archival at the National Marine Mammal Tissue Bank (NMMTB). The NMMTB provides cryogenically archived samples for various kinds of retrospective analyses. For additional information on the NMMTB refer to NOAA Technical Memorandum NMFS-OPR-94-2, Marine Mammal Health and Stranding Response Program: Program Development Plan, July 1994.

TISSUE SOURCES: Sources of tissues for contaminant analysis and specimen banking are:

- 1) blubber
- 2) liver
- 3) kidney
- 4) muscle
- 5) brain collected from Code 2 (preferably early Code 2) animals.

NOTE. All materials required for collecting contaminant samples are provided in contaminant collection kits for large whales.

MATERIALS FOR FIELD SAMPLING:

Non-talced disposable vinyl gloves
Titanium blade knives with Teflon® handles
Teflon® bags with plastic sealing tabs
Large Ziplock® bags (1 or 2 gal)
Sample Data Card
Markers for Sample Data Cards
Alcohol
High-grade distilled water
Texwipes®
Insulated cooler with ice
Garbage bags for discarded materials

METHODS:

Approximately 500 g of each tissue type is to be removed from the animal as soon as possible following death. Individuals conducting the excisions and/or handling the specimens are to wear non-talced vinyl gloves, changing gloves between specimen types. Specimens are excised using titanium knives, only. These knives are rinsed between specimen excisions with high-grade distilled water (to remove blood) followed by an ethyl alcohol rinse. Use only Texwipes® for wiping knives, bags or other materials supplied in the kits.

Using only the materials supplied in the Kit:

1. Remove the following specimens from the animal, taking the histopathology samples at the same time:
 - a. A 20 cm X 20 cm section of blubber and outer skin caudal to the dorsal fin.
 - b. A section of liver (~500 g)
 - c. A section of kidney (~500 g)
 - d. A section of muscle (~500 g)
 - e. The entire brain, including cerebrum and cerebellum
2. Immediately after removal, place each specimen in individual Teflon® bags; seal each bag with a plastic tab, attach Sample Data Card to each and place inside separate ziplock bags. This minimizes the risk of loss of Sample Data Cards and leakage from the Teflon® bags.
3. Place each bagged specimen immediately on ice in cooler and transfer to lab or protected area for subsampling and freezing.

MATERIALS FOR PROCESSING AND FREEZING SPECIMENS:

Non-talced disposable vinyl gloves
Titanium blade knives with Teflon® handles
Pre-cleaned, pre-labeled Teflon® sample jars (150 g capacity)- for tissue bank samples
Pre-cleaned, pre-labeled Teflon® sample jars (50 g capacity)- for analytical samples
Lab tape
Markers for sample labels
Teflon sheets- to provide a clean surface for subsampling specimen
Sample labels
Ethyl Alcohol
High grade distilled water
Texwipes®
Charged liquid nitrogen shipper

METHOD:

As in the case of the field collections, individuals subsampling and/or handling the specimens are to wear non-talced vinyl gloves, changing gloves between specimen types. Specimens are to be trimmed and subsampled using titanium knives, only. These knives are rinsed between specimens with high-grade distilled water (to remove blood) followed by an ethyl alcohol rinse. During trimming and subsampling, each specimen is to be placed on a Teflon® sheet and this sheet changed between specimens in order to insure a clean work surface. Use only Texwipes® for wiping knives, sample jars bags or other materials supplied in the kits.

For each specimen:

1. Remove from Teflon® bag, rinse with high-grade distilled water to remove external blood and fluids, trim away outer layer of the specimen, and divide into four samples:
 - a. Two 50g samples for immediate analysis, and
 - b. Two 150g samples for the NMMTB.In the case of the blubber specimen, the skin is separated from the blubber and either discarded or handled as an additional sample for contaminant analysis.
2. Place each of the four subsamples in the Teflon® jars provided, weigh and record sample weight, record the required information on the jar label, and wrap the label with the clear lab tape.
3. Freeze samples immediately in liquid nitrogen and express ship liquid nitrogen shipper containing samples.

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EAR EXTRACTION PROTOCOL

by Darlene Ketten/Cindy Driscoll

Materials:

- scalpels or small knife blades
- assortment of kitchen knives, including at least one butcher's knife
- 10% buffered formalin
- mallet
- chisels: 1", 2"
- screwdriver
- specimen containers including large ziplock bags or whirlpaks
- plastic or surgical gloves

CONDITION: All codes 2-5 are appropriate for collection.

ANATOMY:

Mysticete ears are removed most easily from the ventral side with lower jaw removed. There are two components, the **tympanic (middle ear bone)** and **periotic (inner ear bone)**. In some literature they are variously called bullae, temporal bones, or petrosals. The tympanic is ventral to the periotic. With the jaw gone, the tympanic is readily visible. Some soft tissue may be attached to the **tympanic**, but you should see a **large (fist-sized or bigger) ovoid bone located just in front of and lateral to the occipital condyles**. The tympanic has a hollow interior that contains the middle bones (ossicles), a spongy soft tissue (corpus cavernosum), and the **eardrum (glove finger)**. The glove finger may be visible **protruding from the lateral side of the tympanic**. Please try to **leave the glove finger and its wax cap intact**. If there is a question of blast injury, please leave as much soft tissue around the tympanic in place as possible.

Just **dorsal to the tympanic is the periotic which contains the inner ear**. On the humpback illustration (attached) one tympanic has been removed to show the periotic. **Ideally, the periotic and tympanic bones should be kept together** and removed as a unit. If the tympanic separates from the periotic during removal or is loose, please be sure to preserve the ossicles and do not forget to remove the periotic, as it is crucial for hearing analyses. **The periotic is wedged into the skull by anterior (short) and posterior (long) flanges**. The dense bulbous component at the center of the two spongy flanges is the periotic that contains the inner ear. **The solid, ball-like core of the periotic is the most important component for any hearing analysis.**

APPROACH:

To remove the whole ear, try **first to free the posterior flange by prying** it out with a chisel. If it is too tightly wedged into the skull or appears to be fused to it, it can be separated from the tympano-periotic complex by cutting through the neck of the

flange approximately 2 cm behind the tympanic. **Next use a screw driver on the flanges to lever the whole ear and remove the flanges from their troughs; pulling upward gently simultaneously on the tympanic may help.** Several nerves and vessels can be seen exiting the periotic on its medial side. The largest of these is the auditory nerve. **Cut these rather than pulling or tearing them free.** The ear should now come free from its skull bed. If the anterior flange is fused, wedge it out with a chisel or screw driver.

FIXATION:

After removal, **the ears should be placed in 10% buffered formalin for at least one week before being shipped** to the laboratory. They can be held in formalin for up to several months before shipping, but the fixative should be changed after the first week and at least twice during the first month. The best fixative is 10% buffered formalin which is available commercially through scientific chemical suppliers. Streck fixative is also acceptable. **If regular fixatives are not available, 70% ethanol may be used in extreme cases. Freezing is a last but acceptable resort. If the ears are frozen, do not thaw before shipping. If fixative is available later, place the frozen specimens in the fixative to thaw. Do not thaw in water or in air.** Formalin is used straight from the bottle and can be stored at room temperature indefinitely. If concentrated formaldehyde liquid or formaldehyde powder is used, mixing it with sea water in lieu of fresh water provides an adequate buffered solution. **Perfusion of the inner ear by injection is not necessary, but if the specimen is very fresh, and you are comfortable with the anatomy, it is an added benefit to inject formalin through the round window with a 22 or 25 gauge needle. Do not inject if only large bore needles are available.**

SHIPMENT:

Prior to shipping, the temporal bones should **be wrapped in gauze soaked with 10% buffered formalin and placed in multiple sealed bags.** Please seal containers carefully to prevent leakage during shipping. When **ready for shipment**, please contact Darlene Ketten or Scott Cramer at the numbers below before sending:

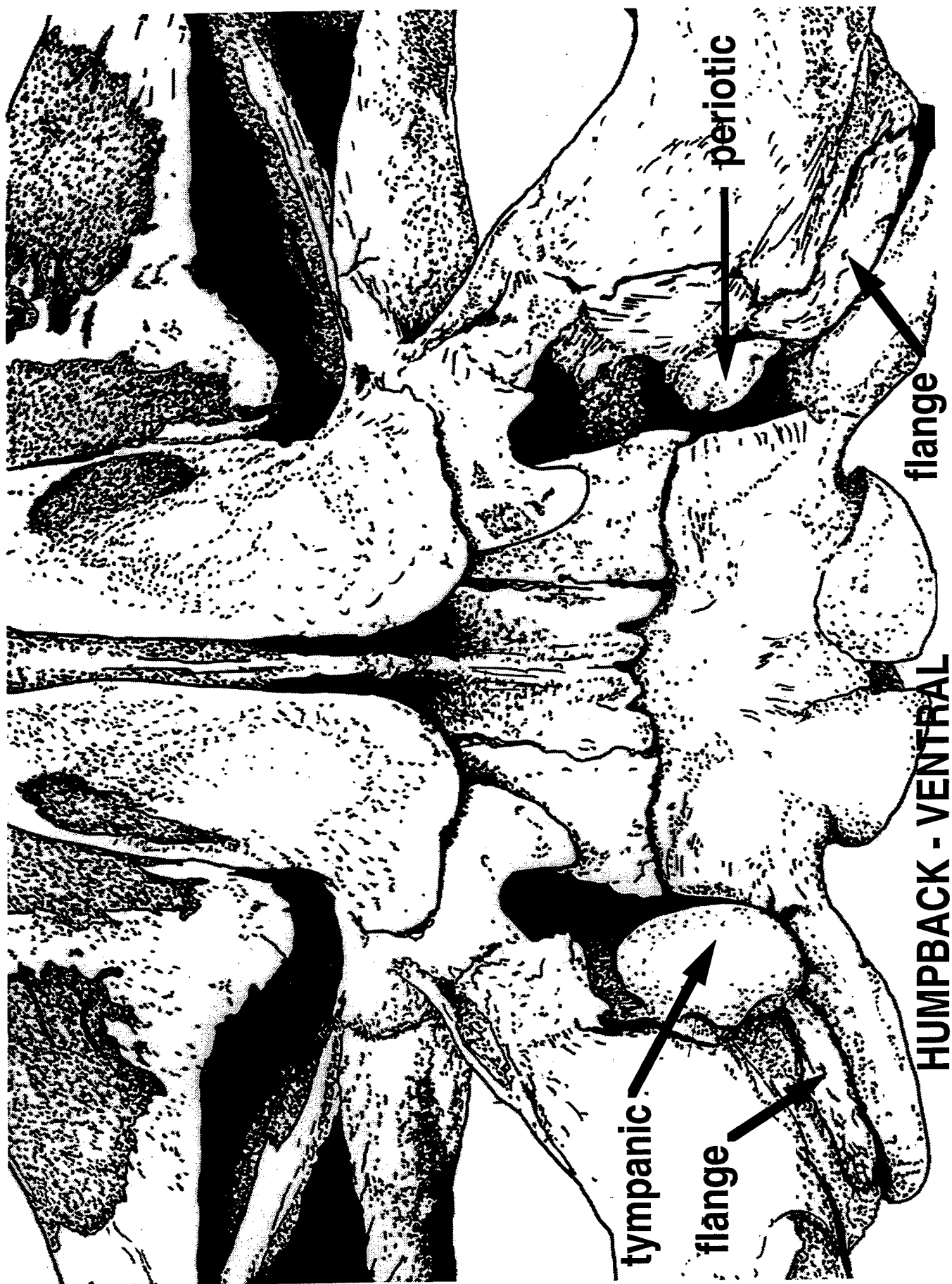
Dr. Darlene R. Ketten
Harvard Medical School
Massachusetts Eye & Ear Infirmary
243 Charles St. Rm. 705c
Boston, Massachusetts, 02114

Phone: (617) 573-4083 - please contact to inform of collected samples

FAX: (617) 573-4275

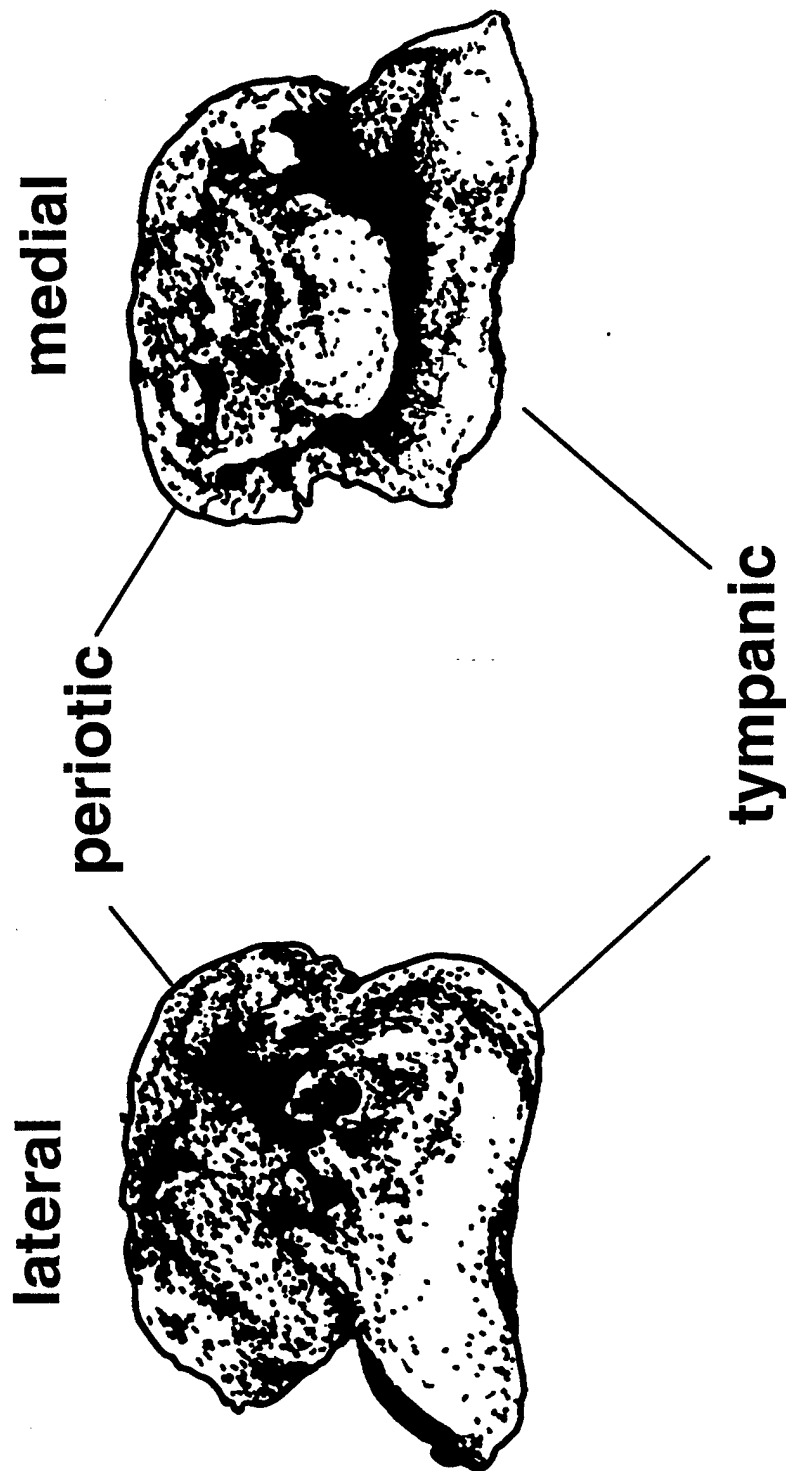
Lab: (617) 573-4273, (617)-573-4379

Email: drk@warren.med.harvard.edu



Odontocete Left Ear

Periotic has the inner ear - Required for all analyses
Tympanic is the middle ear area -Preferable to leave attached to periotic.



BRAIN EXTRACTION PROTOCOL

by Cindy Driscoll /Jim Mead/ Sam Ridgway

MATERIALS:

10% NBF: neutral buffered formalin
Mallet
Chisels: 1", 2"
Crowbar
Specimen container/plastic tub
Plastic garbage bag

CONDITION: Only *Code 2* animals should be considered for brain removal. However, *Code 3+* carcasses are good for practice if the specimen is not being saved intact as an educational/skeletal specimen.

METHODS:

Brain removal can be approached aesthetically ventrally or dorsally for preservation of skeletal specimens and museum preparation (consult your museum curator prior to the necropsy).

1. Ventral approach:

Remove lower jaw and separate head from body. Remove any soft tissue from the ventral/posterior aspect of the skull to expose the basioccipital bone and occipital condyles (see diagram). Chisel a rectangular "cap" for removal approximately 30 - 40 cm (depending on age/size of the carcass) and extend dorsally completely around occipital condyles. This may be accomplished and removed in two sections (basioccipital/occipital condyle sections) or removed as one L-shaped "cap". Since the brain tissue is fragile, extreme caution should be taken in any handling. Loosen any attachments of the brain to the skeletal cavity. To avoid tearing of brain tissue during the removal and placement in the specimen container, first place a plastic bag under the brain tissue sliding the plastic carefully. Place the container with formalin close by and lower brain into formalin gently. (*Sam has used this method and recommends it for the ventral presentation*).

2. Dorsal approach:

If the specimen is lying belly down or if you have the means to cut off the head and roll it over, you can take the brain out from a dorsal approach. Expose the occipital shield for about 50 cm dorsal to the condyles to about 30 cm wide. The bone of the occipital shield is relatively spongy and can be cut with a chisel or an axe. After having removed the calvarium with the dorsal part of the dura, run your hand around the brain and sever

the roots of the cranial nerves. The brain should then be easy to remove. *(Jim has used this method and recommends it for the dorsal approach).*

PRESERVATION:

The brain should fix intact/whole for two weeks changing formalin after one week. Contact Dr. Ridgway prior to shipping.

(Prior to shipping, a small section i.e. 1x2cm can be collected for inclusion in the general histology jar)

CONTACT DR. RIDGWAY @ VOICE (619) 553-1374 or FAX (619) 553-1346

Contact Dr. Ridgway by phone or FAX after collecting the samples. Do not send samples without prior contact. Please keep a copy of the necropsy/incident report (describing probable cause of death/circumstances surrounding death if known, i.e. entanglement, ship strike) and results of any ancillary tests with the specimen(s)..

Phone: (619) 553-1374 - please contact Dr. Ridgway to inform of collected samples

FAX: (619) 553-1346

email: ridgway@nosc.mil

APPENDICES

APPENDIX I. NECROPSY EQUIPMENT CHECKLIST

- ☐ NMFS Letter of Authorization
- ☐ necropsy, sample, and data collection protocols
- ☐ field guide "Marine Mammals Ashore"
- ☐ forms, data sheets, permits, clipboard, pencils
- ☐ two waterproof cameras
- ☐ slide/print film - Ektachrome®, 200 or 400 ASA
- ☐ video camera with tapes and extra batteries
- ☐ tape recorder
- ☐ measuring tape
- ☐ ruler
- ☐ calipers
- ☐ permanent markers
- ☐ grease markers, labels, permanent ink pens
- ☐ knives - three butcher knives, five fillet knives, two sharpening steels, Teflon®-coated knives, two long-handled flensing knives
- ☐ hooks - three short-handle, four long-handle
- ☐ bow saw
- ☐ hammer and chisels
- ☐ one pruner, rib cutters
- ☐ winch
- ☐ scalpel and blades, misc dissecting tools, scissors, forceps, probes
- ☐ tissue processing table
- ☐ ropes (10+ m length), lines, slings or lifting straps
- ☐ heavy duty aluminum foil
- ☐ heavy duty plastic bags
- ☐ heavy duty string
- ☐ bags (various size bags) - Teflon®, Whirlpaks® (various sizes), Ziplock® bags
- ☐ empty jars, vials (various sizes)
- ☐ blood collection equipment - tubes (red top), syringes, 18 gauge needles
- ☐ centrifuge tubes
- ☐ serum freezing tubes
- ☐ culture swabs, sterile urine cups
- ☐ formalin, 10% NBF, and 1 gal 37% full strength and buffer
- ☐ alcohol, EtOH, glycerin, AFA
- ☐ DMSO in vials, premixed
- ☐ gloves - heavy duty and disposable latex
- ☐ aprons, necropsy clothes, rain gear
- ☐ first aid kit, sunscreen, bug repellent
- ☐ cleaning supplies - buckets (20 L), ammonia, disinfectant, brushes
- ☐ foul weather gear and boots
- ☐ shade awning, tarpaulins, ice chests, ice, Dewar bottle with liquid nitrogen, water, 2.3 kg rock salt, dry ice
- ☐ flashlight with extra batteries

APPENDIX II. LARGE WHALE STRANDING REPORT

Date of call: _____

Time of call: _____

Reporting Source: _____

Phone number: _____

Species: _____

Length: _____

Initial report of carcass received on: _____

Location: _____

External Description: _____

Carcass condition code: 1 2 3 4 5

Bloated? y n

Skin coverage present: _____ %

Scavenger damage? y n

Baleen present? y n

Color of baleen: _____

Gear attached? y n

Describe: _____

Indication of cause of death: _____

Weather/tide conditions: _____

Personnel available: _____

Equipment available: _____

Necropsy site: mud sand marsh bulkhead/seawall mangroves car accessible? y n

Directions to site: _____

Special arrangements: _____

Comments/suggestions/additional contacts: _____

Populated or deserted area? _____

Received by: _____ Date/time _____ / _____

APPENDIX

III. HISTOPATHOLOGY SPECIMEN COLLECTION CHECKLIST

Species: _____ Date: _____ Field identification number: _____

- ☐ Adrenal
 - ☐ left
 - ☐ right
- ☐ Aorta
- ☐ Bone marrow
- ☐ Brain
- ☐ Esophagus
- ☐ Eye
- ☐ Gonad
- ☐ Heart
 - ☐ left ventricle
 - ☐ right ventricle
 - ☐ left AV valve
 - ☐ right AV valve
 - ☐ left papillary muscle
 - ☐ right papillary muscle
- ☐ Intestine
 - ☐ duodenal ampulla
 - ☐ small(20 cm from stomach)
 - ☐ large(20 cm from anus)
- ☐ Kidney
- ☐ Liver (bile ducts should be opened and examined for presence of parasites)
- ☐ Lung (collect peripheral and hilar regions)
- ☐ Lymph nodes
 - ☐ cardiac
 - ☐ lung-associated
 - ☐ intestinal
 - ☐ mesenteric
 - ☐ pancreatic
 - ☐ prescapular
- ☐ Mammary gland (ducts should be examined for presence of parasites)
- ☐ Pancreas (ducts should be examined for presence of parasites)
- ☐ Skeletal muscle
- ☐ Skin
- ☐ Spinal cord
- ☐ Spleen
- ☐ Stomach
 - ☐ first chamber
 - ☐ second chamber
 - ☐ third chamber
- ☐ Thyroid and parathyroid glands

- ☐ Tongue
- ☐ Tonsils
- ☐ Trachea
- ☐ Urinary bladder
- ☐ Prostate gland
- ☐ Thymus
- ☐ Uterus
- ☐ Other

For viral studies(if possible freeze in -70 freezer)

- ☐ 2 pieces of spleen
- ☐ 2 pieces of thymus
- ☐ 1 lung associated lymph node
- ☐ 4 pieces of lung from each lobe

Please list ant tissues collected for the National Tissue Bank

APPENDIX IV. NECROPSY REPORT FORM

Field Number:

Date and time of death (if known):

Date of carcass discovery:

Date of necropsy:

Time between death and necropsy (if known):

Examiners, contributors:

Address:

Species:

Common name:

Age (estimate):

Sex:

Length:

Weight:

Clinical Diagnosis: (state best estimate of death cause before necropsy)

Necropsy Diagnosis: (state best estimate of death cause after necropsy)

External examination: (mouth, eyes, blowhole, anus, genital slits, mammary slits, dorsal and pectoral fins, flukes, and the external surface of the carcass)

Primary incision: (blubber, subcutaneous fat, muscles, peritoneum, position of viscera, lymph nodes, mammary glands, ect. The nutritional status of the carcass should be described)

Respiratory System: (blowhole, larynx, trachea, bronchi, associated lymph nodes, lung, pleura)

Heart: (if carcass is fresh, blood should be aseptically collected from the right ventricle with a needle and a syringe for culture. Pericardium, epicardium, myocardium, endocardium, valves, coronary vessels)

Lymphatic system: (lung associated, prescapular, cardiac, pseudo pancreas, intestinal, pancreatic, mesenteric lymph nodes; spleen; thymus)

Liver: (bile ducts should be examined for the presence of trematodes)

Urinary System: (kidneys, ureters, urinary bladder, urethra. Urine should be collected for urinalysis if very fresh.)

Genital system: (testis, epididymis, spermatic cord, prostate, penis, ovaries, oviducts, uterus, cervix, vagina, vulva)

Head: (rostrum, whiskers or whisker pits, eyes, nasal sacs, sinuses, brain)

Musculoskeletal system: Note muscle mass, whether or not muscular atrophy is present. Examine skull in particular and other skeletal components for fractures. Open major joints and examine for evidence of arthritis.

Other: (peripheral nerve; spinal cord if clinical signs of spinal cord injury)

Laboratory findings: (if animal is alive, blood should be collected for a complete blood count and serum chemistry panel; additional serum should be frozen)

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE October 1996		3. REPORT TYPE AND DATES COVERED Final: October 1996	
4. TITLE AND SUBTITLE FINAL REPORT FROM THE RIGHT WHALE NECROPSY ASSESSMENT TEAM Results, Analysis, and Recommendations				5. FUNDING NUMBERS In-house Funds	
6. AUTHOR(S) Sam H. Ridgway, D.V.M., Ph.D.					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Naval Command, Control and Ocean Surveillance Center (NCCOSC) RDT&E Division San Diego, California 92152-5001				8. PERFORMING ORGANIZATION REPORT NUMBER TD 2934	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of the Chief of Naval Operations 2000 Navy Pentagon Washington, DC 20350-2000				10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.				12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) As many as six endangered right whales, <i>Eubalaena glacialis</i> , out of a western north Atlantic population estimated to number only about 300, were reported and/or found dead in their southern breeding range in the Atlantic Ocean along Georgia and Florida coasts during January and February 1996. There was suspicion by the press, public, and whale biologists studying the species that the deaths were linked to U.S. Navy operations. In view of concern within and outside the Government, the Chief of Naval Operations requested an evaluation of the four available necropsy reports. On 19 April 1996, a panel of scientific experts was convened in Washington, DC, at the Armed Forces Institute of Pathology (AFIP). This document presents results, analysis, and recommendations of the panel. After analyzing the four available reports, viewing videotapes, and listening to the descriptions of the necropsy surgeons, the panel concluded that the cause of death could not be conclusively determined in three of the four cases. One animal had suffered massive, acute trauma, probably inflicted by a large moving, unidentified vessel. It was generally agreed that the protocol followed for such mortalities in the future is critical to ensure that better material will be available for evaluation.					
14. SUBJECT TERMS Mission Area: Marine Mammals <i>Eubalaena glacialis</i> right whale necropsy assessment whale necropsy protocols				15. NUMBER OF PAGES 111	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED		18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED		19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	
				20. LIMITATION OF ABSTRACT SAME AS REPORT	

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Code D3503

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Defense Technical Information Center Fort Belvoir, VA 22060-6218	(4)	Armed Forces Institute of Pathology Washington, DC 20306-6000	(6)
NCCOSC Washington Liaison Office Washington, DC 20363-5100		University of North Carolina at Wilmington Wilmington, NC 28403	
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Navy Acquisition, Research and Development Information Center (NARDIC) Arlington, VA 22244-5114		Office of the Chief of Naval Operations Washington, DC 20350-2000	(10)
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