

FINAL REPORT

Physiological Response and Habituation of Endangered Species to Military Training Activities

SERDP Project SI-1396

November 2009

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Report Documentation Page

REPORT DOCUMENTATION PAGE				<i>Form Approved</i> OMB No. 0704-0188	
The 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 the burden, to the Department of Defense, Executive Services and Communications Directorate (0704-0188). Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.					
1. REPORT DATE (DD-MM-YYYY) 01-12-2009		2. REPORT TYPE SERDP Final Report		3. DATES COVERED (From - To) 01-01-2006 to 30-05-2009	
4. TITLE AND SUBTITLE Physiological Response and Habituation of Endangered Species to Military Training Activities				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dr. Timothy J. Hayden, Dr. Luke Butler, Dr. L. Michael Romero, Dr. Isabelle Bisson, Dr. Martin Wikelski, Mr. Douglas Barron				5d. PROJECT NUMBER SERDP SI-1396	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) ERDC/CERL PO Box 9005 Champaign IL 61826				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Strategic Environmental Research Development Program 901 North Stuart Street, Suite 303 Arlington, VA 22203				10. SPONSOR/MONITOR'S ACRONYM(S) SERDP	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Organisms must respond to unpredictable, novel, and/or dangerous conditions in their environment to maintain homeostasis and optimize fitness. We evaluated physiological response in free-living endangered and common passerine species to human disturbance indicated by endocrine stress response (plasma corticosterone) and energy expenditure as measured by telemetered heart-rate. We conducted a series of experiments on endangered black-capped vireos and golden-cheeked warblers and non-endangered white-eyed vireos on Fort Hood, Texas to evaluate chronic and acute response to human disturbance trials. Overall, we found only weak and mixed evidence that physiological response measures in songbird species in this study are acutely or chronically sensitive to human activity at exposure levels in this study, although adults exhibited strong behavioral responses. Overall, we found minimal differences in physiological response measures to direct human disturbance among these species.					
15. SUBJECT TERMS endangered species, black-capped vireo, golden-cheeked warbler, human disturbance, stress physiology, corticosterone, energy expenditure					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Timothy J. Hayden
a. REPORT U	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code) 217 373 5859

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Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

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Acronyms

ACTH	Adrenocorticotrophic hormone
CBG	Corticosterone binding globulin
CDT	Central daylight time
CORT	Corticosterone
CPU	Central processing unit
DoD	Department of Defense
ESA	Endangered Species Act
FDB	First-difference bias
FFT	Fast Fourier Transform
HPA	Hypothalamic-pituitary-adrenal
HR	Heart rate
RF	Radio frequency
SERDP	Strategic Environmental Research and Development Program

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Acknowledgements

We would like especially to thank the staff of the Fort Hood Natural Resources Branch for site access and facilitating our field research activities during this research. Without their support, this project would not be possible. John Cornelius was Branch Chief of the Natural Resources Branch. Charles Pekins is a Wildlife Biologist and our primary installation point of contact for coordinating field activities. We would also like to thank the staff of the Fort Hood office of The Nature Conservancy, in particular David Cimprich and Rebecca Peak, for project coordination and data sharing. J. Barry, J. Brown, B. Cook, L. Duda, E. Fergus, C. Gallagher, E. Gill, J. Grainger, S. Lovell, and M. Vinson assisted with field experiments. M. Dickens and N. Cyr assisted with hormone assays. J.M. Reed, Tufts University, assisted with corticosterone statistics. This project was funded by the Department of Defense, Strategic Environmental Research and Development Program (SERDP) under project number SI-1396. Dr. Jeffrey Marqusee and Mr. Bradley Smith were, respectively, the present and former SERDP Executive Directors during this project. Dr. John Hall and Dr. Robert Holst were, respectively, the current and former Sustainable Infrastructure Program Managers during this project. The SERDP Executive Directors, Program Managers and SERDP support staff Johnathan Thigpen, Carrie Wood and Kristen Lau, and the members of the Sustainable Infrastructure Technical Committee have provided much helpful input on study objectives and approach. This project could not have been successfully completed without their assistance.

Abstract

Organisms must respond to unpredictable, novel, and/or dangerous conditions in their environment to maintain homeostasis and optimize fitness. We evaluated physiological response in free-living endangered and common passerine species to human disturbance indicated by endocrine stress response as measured by plasma corticosterone and energy expenditure as measured by telemetered heart-rate. We conducted a series of experiments on endangered black-capped vireos and golden-cheeked warblers and non-endangered white-eyed vireos on Fort Hood, Texas to evaluate chronic and acute response to human disturbance trials. Overall, we found only weak and mixed evidence that physiological response measures in songbird species in this study are acutely or chronically sensitive to human activity at exposure levels in this study, although adults exhibited strong behavioral responses. We did find a decline in baseline corticosterone in the habitat specialist golden-cheeked warblers in habitats with high road densities that we did not observe in habitat generalist white-eyed vireos. Comparison of our results with studies for other non-passerine avian taxa suggests potential differences in disturbance response in species with significantly different life-history characteristics. This is the first study integrating these two physiological measures of response to human disturbance in free-flying passerine species and addresses key knowledge gaps in how wild animals respond and adapt to potential disturbance from human activities. This research also provided the rare opportunity to directly measure these stress responses in two federally-listed endangered avian species.

Objective

This research project was performed in response to requirements of the Department of Defense (DoD), Strategic Environmental Research and Development Program (SERDP) Statement of Need CSSON-04-04 “Quantifying Physiological Stress in Threatened and Endangered Species Due to Military Activities.” By framing the research design in the context of military training activities, our research results will assist the DoD in meeting the challenges of balancing the training mission with requirements to promote conservation of endangered avian species populations on military lands.

Physiological response measures evaluated in this study were endocrine response as measured by corticosterone and energy expenditure as measured by heart-rate. This is the first study integrating these two physiological measures of response to human disturbance in free-flying passerine species. Our use of multiple measures of stress and adaptation of newly available technologies provided a unique opportunity to address key knowledge gaps in how wild animals respond and adapt to potential disturbance from human activities. This research also provided the rare opportunity to directly measure these stress responses in two federally-listed endangered avian species.

Specific research objectives were to determine: (1) chronic and acute stress response in endangered and non-endangered passerines in response to non-lethal human disturbance as measured by adrenocortical response and energy expenditure in free-flying individuals (2) whether individuals modulate their stress response to multiple exposure to human disturbance, and (3) whether species differ in stress response as a function of life history traits. Secondary tasks in support of these objectives included studies to evaluate other factors affecting endocrine

response and energy expenditure including habitat disturbance, site characteristics, and transmitter effects. Analysis programs were developed to improve data reduction and analysis capabilities for heart-rate telemetry data.

During 2006-2008 we conducted a series of studies to meet project objectives. Objectives of these individual studies are described below and methods, results and discussion sections of this report are organized by study topic.

In Study 1, we evaluated parental adrenocortical response of black-capped vireos (*Vireo atricapilla*, Fig. 1) and white-eyed vireos (*Vireo griseus*, Fig. 2) to acute and chronic threats at nests by performing a series of human or predator presentations at nests over periods of 7-12 days (Project objectives 1 and 2).



Figure 1. Male black-capped vireo on Fort Hood, Texas.



Figure 2. Male white-eyed vireo on Fort Hood, Texas.

We evaluated effects for direct human harassment on adrenocortical response in three species, white-eyed vireo, black-capped vireo and golden-cheeked warbler (*Dendroica chrysoparia*, Fig. 3) by a series of “chasing” experiments in Study 2 (Project objective 2 and 3).



Figure 3. Male golden-cheeked warbler on Fort Hood, Texas.

Other environmental or human factors may directly or indirectly alter adrenocortical response in birds. To evaluate effects of other factors relative to direct disturbance we evaluated effects of road density as a measure of habitat disturbance on adrenocortical response to capture in a habitat specialist, the black-capped vireo and golden-cheeked warbler, and a habitat

generalist, white-eyed vireo in Study 3 (Project objectives 1 and 3).

In Study 4, we compared stress response in black-capped vireos, white-eyed vireos, painted buntings (*Passerina ciris*), and northern cardinals (*Cardinalis cardinalis*) between undisturbed habitats and habitats degraded by military training activity and between regions of high brown-headed cowbird (*Molothrus ater*) nest parasitism and low cowbird parasitism (Project objectives 1 and 3).

To understand the potential interaction effects of transmitters with disturbance response, we evaluated effects of transmitters themselves on avian behavior and physiology by a meta-analysis in Study 5 of published studies using wildlife telemetry and empirical field studies in Study 6 on northern cardinals.

In Study 7, we evaluated energy expenditure in response to human disturbance by acquiring real-time heart rate from transmitters attached to free-flying white-eyed vireos and black-capped vireos. The high volume of data acquired during these trials presents a significant challenge in terms of data reduction and interpretation. We significantly advanced capabilities in these areas by developing computer programs for signal processing and improving hardware capabilities.

This is the first study to obtain heart-rate data from free-flying individuals of black-capped vireos and black-capped vireos. In Study 8, we evaluated change and habituation in energy expenditure in response to human intrusion with disturbance trials (active follows) on telemetered individuals of varying duration and frequency (Project objectives 1, 2 and 3). Disturbance trials were conducted during both day and night, and this is the first study to acquire these data relative to nocturnal disturbance.

Background

Organisms must respond to unpredictable, novel, and/or dangerous conditions in their environment to maintain homeostasis and optimize fitness (survival and reproduction). Individuals can respond proximately to environmental challenges through a variety of mechanisms, including neophobic (avoidance) and neophilic (exploratory) behaviors (Greenberg 1984, 1990, Greenberg and Hoffman 2001), endocrine responses such as the adrenocortical response in vertebrate taxa (Wingfield et al. 1997, Romero et al. 2000) or production of catecholamine's in vertebrate taxa (epinephrine and norepinephrine) through the sympathetic-adrenal medullary response (Selye 1946, Henry and Stephens 1977). Activation of these behavioral and physiological response systems represents cost/benefit tradeoffs to the individual. For example, the well-documented adrenocortical response to an environmental challenge has the benefit of mobilizing energy resources to meet the perceived threat (Wingfield et al. 1997); however, this response may have the cost of redirected behavior and deleterious physiological effects if this response persists over extended time periods (Sapolsky 1987). Proximate behaviors such as flushing from nests by parental birds in response to a perceived threat may enhance parental survival at the cost of reduced nestling survival (Steidl and Anthony 2000, Lord et al. 2001). From an ecological perspective these tradeoffs represent a "stress" when the result is reduced fitness of the individual (Hofer and East 1998).

Effects of transient human disturbance on avian species are of particular concern on DoD installations that support populations of federally listed endangered birds. Training activities on these installations are typically distributed across the landscape and not confined to roads or

trails. These activities are often conducted within habitats that support endangered bird species, thus exposing individuals of these species to “harassment” as defined under the Endangered Species Act (ESA) of 1973, as amended. If military training activities elicit a stress response in individuals of endangered species, this would constitute harassment and is considered “take” as defined under the ESA. The regulatory consequences of take are significant and can result in widespread restrictions on training activity in habitats of endangered species. Understanding avian response to transient human activity and whether individuals are capable of modulating their response to repeated disturbance is important in mitigating potential effects of military training activities and reducing potential restrictions on training activities.

Extensive research has been conducted to determine effects of human activities on wildlife, particularly species of conservation concern (e.g. Boyle and Samson 1985, Gutzwiller et al. 1998, Knight and Cole 1995, Miller et al. 1998). Much of this work has focused on birds due to their relative abundance and observability (see review by Gutzwiller and Hayden 1997). Several deleterious behavioral effects of human presence in proximity to wildlife populations have been observed including avoidance flights and abnormal vigilance induced by human activities that alter daily activity budgets and lessen the time and energy used for fitness-enhancing activities such as feeding, nest attentiveness, mate attraction, and territory defense (Burger and Gochfeld 1991, Gutzwiller et al. 1998, Lord et al. 2001). These significant behavior modifications suggested that frequent human activities near nests could adversely affect nestling survival and, therefore, reproductive success (Steidl and Anthony 2000). The energetic costs associated with behavioral response to human disturbance can have fitness consequences for the individual in terms of reduced reproduction and survival. Until recently, measures of energy expenditure were either based on non-continuous (doubly-labeled water, Nagy 1975) or non-physiological estimates (time-activity budgets, e.g., McKinney and McWilliams 2005) of energy consumption. Although comparative analyses showed that heart rate is nearly as precise as the doubly-labeled water method in estimating energy expenditure of animals in the wild (Bevan et al. 1995, Green et al. 2001), doubly labeled water provides average daily energy expenditure only over a 24-48 hour period (Nagy 1975) and cannot give instantaneous measures of energetic demands in response to specific environmental stressors or specific activities. Alternatively, heart rate allows a continuous and instantaneous measure of the energetic demands associated with specific disturbances, and is correlated with energy expenditure in fish (Lucas 1994), birds (Cochran and Wikelski 2005, Cyr et al. 2008), and mammals (Boyd et al. 2006).

Environmental and human stressors also can trigger a cascade of hormone secretions typical of stress in all vertebrates studied so far (Boersma 1987, Silverin et al. 1997, Wingfield et al. 1997, Wasser et al. 1997). The measurement of circulating levels of corticosterone, the major stress-related hormone in birds, allows monitoring of stress at the organismal level. Increases in circulating corticosterone to acute stress can be viewed as an adaptive response in that it prepares the individual for “flight or fight” (Wingfield et al. 1997). However, chronic initiation of this response has been shown to detrimentally affect an individual’s fitness by such mechanisms as reducing immune response and increased mortality (Sapolsky 1987).

Given the well-described and nearly ubiquitous correlation between acute stress and increased plasma glucocorticoid concentrations, we might predict that chronically stressed birds would have higher plasma glucocorticoids. However, recent experiments in captive (Rich and Romero 2005) and free-living (Cyr and Romero 2007) birds have demonstrated, paradoxically, that long-term or “chronic” stress lasting several days results in lower—not higher—baseline glucocorticoid concentrations. In addition to suppressing baseline glucocorticoid concentrations,

chronically-stressed birds may exhibit a damped increase in glucocorticoids in response to a threat (Rich and Romero 2005). These experimental results support observational studies of free-living animals exposed to human disturbance. Suppressed glucocorticoid concentrations have been found in spotted salamanders (*Ambystoma maculatum*) breeding near human settlement (Homan et al. 2003), and in marine iguanas (*Amblyrhynchus cristatus*, Romero and Wikelski 2002) and Magellanic penguins (*Spheniscus magellanicus*, Walker et al. 2006) exposed to tourists on foot. Furthermore, chronic stress can suppress reproduction and damage the nervous system (Sapolsky et al. 2000). In conclusion, altered glucocorticoids can reveal stress caused by a particular disturbance, but they can also be a health problem in and of themselves, and they may be correlated with other negative health effects, making them a valuable biological indicator of individual health (Romero 2004, Cockrem 2005).

Although negative effects in wildlife because of human activity have been recognized, some data indicate that wild populations may habituate in some respects to activity that may initially be perceived as threatening, but that ultimately presents no direct threat. Birds that habituate to human disturbance may devote more time to incubation, foraging, and other fitness-enhancing activities than those birds that avoid disturbance (Cooke 1980, Burger and Gochfeld 1991). These studies indicate that some species are capable of modulating their response to human activity with repeated exposure, particularly if it is perceived as not being a direct threat.

Although endocrine and energetic response measures are qualitatively different, both measures have potential costs relative to fitness of an individual. Concurrently measuring both these response systems in wild populations is important because, unlike captive experimental populations, free-flying individuals may have the option of responding to a perceived threat through activation of the adrenocortical response or behaviorally by avoiding the disturbance, thus potentially altering energy demands. Data from captive populations may not reflect this plasticity of response in wild populations.

During the 2006-2008 breeding seasons on Fort Hood, Texas (Fig. 4), we evaluated endocrine response and energy expenditure in passerine species in response to transient human disturbances characteristic of military training activities. We evaluated corticosterone response in endangered black-capped vireos and golden-cheeked warblers, and non-endangered white-eyed vireos, painted buntings and northern cardinals. We evaluated energy expenditure as measured by remotely monitored heart rate in black-capped vireos and white-eyed vireos. Our two endangered study species, the black-capped vireo and the golden-cheeked warbler have limited breeding distributions, but are locally common on Fort Hood. The white-eyed vireo and northern cardinal are common and widely-distributed in eastern North America. The painted bunting is locally common on Fort Hood.

Fort Hood encompasses 87,890 ha (217,180 ac) located in central Texas in Bell and Coryell Counties adjacent to the city of Killeen. Fort Hood lies at the northern extent of the Edward's Plateau between the cities of Waco, 64 km (40 mi) to the northeast, and Austin, 97 km (60 mi) to the south. The installation provides the infrastructure and training lands for the 1st Cavalry Division and the 4th Infantry Division (Mechanized), III Corps Headquarters and its combat aviation assets, combat support, and combat service support units. Fort Hood likely will remain the largest active U.S. installation in terms of assigned personnel with approximately 50,000 soldiers.

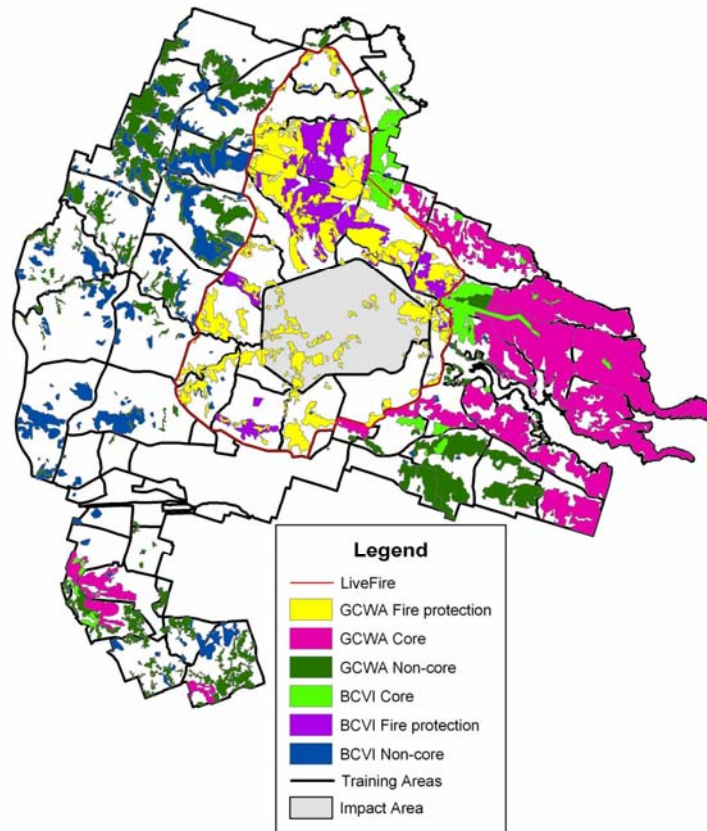


Figure 4. Endangered bird species habitats on Fort Hood, Texas. Legend represents different management prescriptions for fire management and military training restrictions. GCWA = golden-cheeked warbler. BCVI = black-capped vireo

Training events in endangered bird species habitats on Fort Hood are conducted during any hour of the day and under all climate conditions. The level of training intensity will vary according to unit size and type of unit. The characteristic of training that is the focus of this project is harassment associated with transient dismounted soldiers in endangered bird habitats including humans yelling and rapid foot traffic of soldiers. Transient human activity in endangered bird species habitats is increasing as more training areas are being opened for dismounted training for preparation of soldiers for deployment in Iraq and Afghanistan. Disturbance trials performed in this study were primarily human intrusions, presentation of predator decoys, human vocalizations and predator calls. Human intrusions represent a “worst case” disturbance associated with military training activities in that our trials presented a potential direct threat to our sample individuals and/or nests from human presence. A high level of dismounted soldier activity in endangered species habitats is a characteristic of military training activities on installations such as Fort Hood and will be the primary training activity in endangered species habitats newly opened to training. Human presence is not an actual threat, but it may be perceived as a threat by focal study birds. On the other hand, a predator presentation represents an actual threat to the individual or nest to which it is presented. Combining human intrusion or vocalizations with predator presentations or calls allows evaluation of response measures to perceived and actual threats to survival or reproduction.

Associated vehicle traffic is not emphasized in this research because it is typically limited to pre-existing roads and openings in proximity to habitats but not directly in habitat.

This is the first study integrating energy expenditure and adrenocortical response to human disturbance in free-flying passerine species. Our use of multiple measures of stress and use of newly available technologies provide a unique opportunity to address key knowledge gaps in how wild animals respond and adapt to potential disturbance from human activities. This research also provides the rare opportunity to directly measure these stress responses in two federally-listed endangered avian species. This research will assist DoD in reducing regulatory compliance requirements under the ESA that require significant administrative resources, potentially delay training activities, and may limit the scope of allowable training activities.

Materials and Methods

Study site

All project research activities were conducted at Fort Hood (Fig. 4), an 88,000-ha military training area in Texas, USA (31.2° N 97.8° W). Fort Hood supports the largest managed populations of endangered black-capped vireos and golden-cheeked warblers (Cimprich and Kostecke 2006) in these species respective ranges.

Study species

The two endangered study species evaluated in this research, the black-capped vireo and the golden-cheeked warbler have limited breeding distributions, but are locally common on Fort Hood. The white-eyed vireo and northern cardinal are common and widely-distributed in eastern North America. The painted bunting is locally common on Fort Hood.

Male golden-cheeked warblers arrive from their wintering grounds in March and prefer to defend breeding territories in mature oak-juniper woods (Ladd and Gass 1999, Fig. 5). White-eyed vireos also arrive in March and breed in a wide variety of shrubby and wooded habitats (Hopp et al. 1995). Black-capped vireos arrive in early April and prefer to breed in young secondary scrub (Grzybowski 1995, Fig. 6). All three species are common in their preferred habitats at Fort Hood (Cimprich 2006, Kostecke 2006, Peak 2006). They are nearly always observed perching in or hopping and flying through vegetation >1 m above ground, with golden-cheeked warblers frequenting upper canopy, and black-capped vireos occasionally found 1 m or less above ground in short shrubs. All three species place their nests above the ground in trees and shrubs.

Golden-cheeked warblers prefer to nest in fairly large patches (~100 ha) of mature Ashe juniper-oak (*Juniperus asheii*-*Quercus* spp.) forest, and they rely upon mature Ashe Juniper for nest building (Ladd and Gass 1999). White-eyed vireos are common in shrubby, scrubby, and forest habitats (Dickson and Segelquist 1979, Hopp et al. 1995), and have a slight preference for younger, more open habitat (Conner et al. 1983). Male white-eyed vireos will defend even very small clumps (< 0.2 ha) of habitat near our field site, including territories in suburban yards and golf courses (Luke Butler, unpublished data). Accordingly, golden-cheeked warblers are absent from a wide variety of deciduous and clumped habitat that holds large numbers of white-eyed vireos at Fort Hood (Luke Butler, Tim Hayden, Isabelle Bisson, personal observation), but white-eyed vireos are abundant in golden-cheeked warbler habitat.

Males of both endangered species are highly territorial during their breeding seasons, which begin shortly after males arrive from their wintering grounds in mid-March, and end in late May (warblers, Ladd and Gass 1999) or late June (vireos, Hopp et al. 1995). Adults of both endangered species forage primarily by gleaning insects (mainly lepidopteran larvae) from woody vegetation (Ladd and Gass 1999, Hopp et al. 1995).



Figure 5. Mature forested Ashe Juniper and mixed hardwood forests on slopes and mesa tops typical of golden-cheeked warbler habitat on Fort Hood, Texas. Northern cardinals and white-eyed vireos also occupy mature forested habitats on Fort Hood.



Figure 6. Typical black-capped vireo, white-eyed vireo, northern cardinal and painted bunting habitat on Fort Hood, Texas.

Bird capture, blood sampling and hormone analysis

Methods for bird capture were similar for all studies reported here. Blood sampling methods and hormone analyses were similar for all endocrine studies reported here. All methods were approved by the Animal Care Committees of Tufts University, Princeton University and the University of Illinois.

Focal animals were lured into mist nets using playback of conspecific songs and other

vocalizations (Figs. 7 and 8). Mist nets were monitored continuously and blood samples were acquired within 3 min of capture, so corticosterone (CORT) concentration at capture would reflect response to the preceding disturbance and not to capture (Romero and Romero 2002,



Figure 7. Setting up mist nets in black-capped vireo territory on Fort Hood, Texas.



Figure 8. Male black-capped vireo captured in mist net on Fort Hood, Texas.

Romero and Reed 2005). Blood was drawn into 60- μ l heparinized glass capillary tubes after pricking the alar vein with a 26-ga needle (Fig. 9). Bleeding was stanchied by applying cotton and pressure. After the first (baseline) sample, birds were placed in an opaque cotton bag for 30 min, and then sampled for stress-induced concentrations before release. Blood was stored on ice in the field and centrifuged within 24 h, at which time the plasma was removed using a Hamilton syringe and frozen in 0.5-ml eppendorf tubes until it was returned to Tufts University for assaying.



Figure 9. Obtaining blood sample from alar vein of male black-capped vireo.

Total plasma CORT concentration was assayed using a standard radioimmunoassay (Wingfield et al. 1992). Due to the small size of both species (8 – 10 g), and the endangered status of black-capped vireos, legal limitations precluded sampling large numbers of individuals and making comparisons of corticosterone binding globulin (CBG), which is thought to carry CORT to target substrates or bind CORT and make it unavailable to target substrates (Breuner and Orchinik 2002, Romero 2002). Thus, we report only total plasma CORT levels. Corticosterone was extracted from the plasma into 4 ml of distilled dichloromethane, which was then evaporated under nitrogen. Next the extract was reconstituted in phosphate buffer and assayed. The percent of the sample corticosterone that was recovered by the extraction process was estimated by the percent recovery of a small amount of tritiated corticosterone added to each plasma sample before extraction. Sample corticosterone concentrations were calculated by comparing the assayed extracts to a series of standards of known corticosterone concentrations.

Study 1: Chronic and acute hormonal response of endangered and common songbirds to offspring-directed threats

Disturbance trials

From 1 May to 5 June 2006, we attempted to create chronically-stressed parent vireos following the protocols of Rich and Romero (2005) as adapted to field conditions by Cyr and Romero (2007) for European starlings. Our disturbance protocol lasted 7 – 12 days and occurred while adults were incubating eggs or brooding and feeding nestlings, tasks shared about equally between sexes in black-capped vireos (Grzybowski 1995) and white-eyed vireos (Hopp et al. 1995). We presented four, 30 – 60 min offspring-directed threats or disturbances (i.e., stressors) at each nest per day, with varying amounts of time between presentations (0.5 – 3.0 h), and in random order but with the same threat never repeated at the same nest within a day. Threats were either naturalistic decoys of predators on adults and nestlings (2-m rubber snake, plastic American crow [*Corvus brachyrhynchos*] with call playback, and plastic eastern screech owl [*Megascops asio*] with call playback), or anthropogenic disturbances (human sitting or standing, human voice playback, and novel objects, such as a starkly-colored helium balloon).

From 28 April to 2 July 2007, we sampled CORT in adults randomly assigned to one of four disturbance treatments. One group was presented only with 10 – 35 min of crow decoy or human presence (as described in Experiment 1) immediately before capture and sampling. A second group was exposed to the chronic disturbance protocol of Experiment 1 for 5 – 6 days (including the crow and human presentations), and then presented with the crow or human for 10 – 35 min immediately before capture. A third group was exposed to the chronic disturbance protocol for 5 – 6 days but received no presentation immediately before capture. A fourth group received no disturbance of any kind before blood sampling. All groups were held for 30 min before a second blood sample was taken to measure the CORT response to a self-directed threat (capture and restraint).

Predator decoys and novel objects were attached to vegetation 1 – 2 m from the focal nest at or above the height of the nest. Decoys were placed in an upright, perched position, and oriented toward the nest. During human presence, one person sat or stood and occasionally moved and spoke.

Predator calls and human voice playback were made using battery-powered, handheld digital audio players (Muvo mp3 players, Creative Labs) connected to small (9 x 8 x 5 cm) amplified speakers (Mini Audio Amplifier, RadioShack) placed on the ground below or in vegetation < 2 m from the decoy. Ten-second predator calls were played randomly among two 20-s bouts of silence, and were audible no more than 5 – 10 m from the nest to avoid attracting predators. We used 35 – 40 min of a radio talk show for human voice playback.

Rich and Romero (2005) showed that a very similar chronic stress protocol caused CORT concentrations to decrease in captive starlings after 8 – 10 d, and then remain lower than non-chronically-stressed concentrations for at least 27-29 days, when the protocol was discontinued. Likewise, Cyr and Romero (2007) showed that a very similar protocol lasting 9 days caused CORT to decrease in free-living European starlings. We targeted adult vireos for sampling after 7 – 9 d of stressors in order to reduce the high risk of losing samples because of nest loss. In Experiment 1, no stressors were presented on the day CORT was sampled.

Capture of focal animals

On the day before blood sampling, one or two closed, 6 x 2.6-m mist nets were placed 2 – 10 m from the target nest and perpendicular to flight paths used by adults to access the nest. For crow and control trials, hiding places where one or two observers could continuously monitor the mist nets were also identified. Hiding places were located on the ground below or between leafy shrubs at least 5 m from the target nest, depending on vegetation density and sight lines, with other vegetation arranged to conceal the observer(s). Small speakers were placed near the net(s) and connected to audio cables extending to the hiding place for playing conspecific songs or calls to lure the focal animals to the net(s).

On the day of a crow trial, one or two people quickly entered the territory, opened the mist net(s), turned on the capture playback speakers, placed the crow in vegetation 1 – 2 m from the nest and directed it toward the nest, placed the crow call speaker under the crow, and started the crow playback. The end of a 0.6-cm nylon rope tied to the crow was then carried to the hiding place. This process lasted 1 – 3 min, and was usually completed before adults returned from foraging. Adults were exposed to the crow for 10 – 35 min before capture, and then immediately sampled for blood. Adults almost always stopped feeding nestlings during the crow trial, so after 30 – 35 min the crow was pulled away using the rope, at which point adults usually fed the nestlings or inspected the nest before flying away and being captured in one of the mist nets.

On the day of a human presence trial, one person entered the territory, opened the mist net(s), turned on the capture playback speakers, and stood 1 – 2 m from the nest in view of the adults for 10 – 35 min before blood sampling. Adults usually stopped feeding nestlings after the person approached the nest, but occasionally, especially late in a trial, the person moved around the nest to thwart adults attempting to access it.

The control group was exposed to no experimental disturbance immediately before capture (i.e., an observer simply opened the nets, turned on the speakers, and hid), or was exposed to less than 10 min of crow or human. If no adult was captured 70 min after the start of a trial, the mist nets were closed and the crow or human removed for at least 2 h before trying the other disturbance trial.

In 2006, logistics occasionally required abandoning the above protocol and setting a net on the same day we sampled adults, but this probably had no effect on CORT (see Results). In both years, trials were performed on nests containing nestlings for all black-capped vireo samples and most white-eyed vireo samples (one crow trial and two human trials were performed on white-eyed vireo nests that contained eggs).

Statistical analysis

In both study species, males take part in building the nest, incubating the eggs, and feeding nestlings and fledglings (Graber 1961, Hopp et al. 1995), and baseline and stress-induced CORT concentrations do not differ between the sexes on our breeding site (unpublished data), so we combined males and females in our analyses. Samples did not violate the assumption of homogeneous variance (all $p > 0.05$ in Levene's test, Zar 1996). We used a mixed model ANOVA (SAS Institute 2004) to compare baseline and restraint-induced CORT levels between chronically- and non-chronically-disturbed birds (Experiment 1) and among disturbance treatments (crow, human, and control; Experiment 2). This statistical procedure incorporates individuals from which only a baseline or a 30-min sample was obtained. To verify that our nest-directed threats actually disturbed adults, we made qualitative descriptions of adult behavior

during the human and crow presentations. Samples in all groups were distributed over time, but we included day of year as a covariate in 2007 analyses because sampling spanned more than two months. Alpha was set at 0.05.

Study 2: Acute hormonal response of endangered and common songbirds to human pursuit

Disturbance protocol

The disturbance protocol was similar for all species included in this study (black-capped vireo, golden-cheeked warbler and white-eyed vireo), with exceptions for warblers noted below.

Each species is highly active during the breeding season, with males singing and making other vocalizations loudly (audible >100 m away) and regularly until several hours after dawn, typical of temperate songbirds. Thus, we preceded each disturbance by walking quietly into an area with suitable breeding habitat and listening for the closest territorial male. Once this male was identified, the pursuit started when one observer walked directly toward the bird and stopped below or next to the bird. Our closest approaches were as short as 1 m for black-capped vireos in low shrubs, and 2 m for white-eyed vireos in shrubs or golden-cheeked warblers in trees, though most birds of all species flew away when the observer was 5-10 m away. For vireo trials, when the focal bird flew away, the observer watched it until it landed or as far as possible, and then walked or ran toward the next perch, relocated the focal bird, and approached it as closely as possible. This process was repeated for the duration of the disturbance period. The observer then quickly set up a mist net near the middle of the territory (estimated during the preceding disturbance period) in order to capture and sample the focal bird (see below).

We used one observer to disturb vireos, because a typical vireo territory was comprised of approximately 3 – 10 distinct clumps of vegetation separated by bare ground, so it was relatively easy for one person to quickly resume contact with the focal bird after it flew to a new perch. In order to achieve a similar level of human exposure in warblers breeding in dense juniper woods, three observers spread out in the focal bird's territory and communicated with each other using hand-held radios. When the bird flew away from one observer, another observer would locate it and pursue it. Neighbors were never disturbed on the same day.

Disturbances were conducted from 0715 h to 1030 h from 11 to 27 April 2006 in golden-cheeked warblers, from 0650 h to 1325 h from 23 April to 31 May 2006 and 2 to 29 May 2007 in black-capped vireos, and from 0810 h to 1415 h from 24 April to 30 May 2006 and 6 to 29 May 2007 in white-eyed vireos. Disturbed birds were compared to undisturbed controls distributed similarly over times and dates to control for any temporal variation in corticosterone concentrations within each species (Romero 2002). In order to minimize the number of birds caught and bled, some undisturbed vireo samples (controls) used in this study were obtained as part of another Study 1, above.

Statistical analyses

Samples did not violate the assumption of homogeneous variance (in Levene's test, all $p > 0.05$), so corticosterone concentrations were analyzed with repeated measures ANOVA using the PROC MIXED platform of SAS (SAS Institute 2004), which does not exclude individuals for which only baseline or stress-induced samples were available (for various reasons, only a baseline or a stress-induced sample was obtained for 4 of 33 black-capped vireos, 3 of 44 white-

eyed vireos, and 2 of 19 golden-cheeked warblers). Warbler latency samples were positively skewed, so we compared latency between disturbed and control warblers using a Mann-Whitney *U* test. Alpha was set at $p = 0.05$ for all statistical tests.

Study 3: Effect of road density on stress physiology of an endangered, old-growth-dependent songbird a common habitat generalist

Sampling locations

Sample sites were selected on the east and southeast parts of the installation (Fig. 4), where military activity on the roads was either prohibited (31% of sample points), or very rare and detected only a few times, either directly (e.g., seeing vehicles or military personnel) or indirectly (e.g., finding signs of recent human activity such as vehicle tracks) in >200 h of field work near the sample points between 2 April and 4 June 2008. The roads and trails transecting our sites were almost all unimproved and unpaved (described in more detail below). Many of our sample sites were along roads obstructed by dead or growing vegetation, making them impassable to vehicles.

Defining road density groups

Our goal was to establish categories of sample points representing a range of common road densities in each of ten separate areas within Fort Hood. We used Fort Hood's existing network of 31 bird survey routes within golden-cheeked warbler habitat to locate ten separate sample areas. This allowed us to compare our physiological measures to point count data (density estimates) that are collected yearly in close proximity to each of our capture sites. To choose sampling points, we pre-selected bird survey routes with a range of road densities. Only intact forest had any roadless habitat, restricting us to the 16 survey routes located in the relatively undisturbed eastern portion of the base. We used remotely-sensed imagery (false-color digital orthophotographs with 35 cm resolution, collected in 2004) to identify 10 survey routes with each of the following road density categories: Zero (no roads or trails wider than 3m within 100m of the capture site), Low (one relatively straight trail, 3-5 m wide, that runs near the capture point), Medium (the intersection of two to three 3-5m width trails), High (the intersection of several trails and roads), and Extreme (the presence of a large road creating a wide canopy gap).

The ten survey routes were distributed so that most had at least 1 km and up to 13 km between the closest capture sites. Two routes had capture sites within 600 m of each other. We selected capture sites so that there were no other habitat edges within 250 m of the capture site (and these edges were usually greater than 500 m distant). Where possible, we identified more than one potential capture site for each density category within each route, expecting that a bird would not be caught at every site for unpredictable logistical reasons. A handheld global positioning system (Explorist II, Garmin, Inc.) was used to locate targeted sites in the field, but sites occasionally had to be moved slightly due to accessibility or to place nets for capturing the target bird. One pre-selected route was abandoned due to logistical constraints and another route was added to replace it.

A total of 75 capture sites (7 – 9 per route) were used to capture 49 golden-cheeked warblers and 49 white-eyed vireos. For logistical reasons, we caught both species at 24 sites, 25 vireos at sites where no warbler was captured, and 25 warblers at sites where no vireo was

captured. Coordinates of our actual bird capture sites were recorded, and 62 (83%) were within 50 m of the original target site and 72 (96%) were within 110 m of the target site. The remaining three capture sites were within 300 m of the original target sites.

Using a digitized map of all roads and trails over 3 m, we calculated the percent canopy gap and total m of road within 100 m of each capture point to test whether our actual sample points clustered distinctly into the five planned road categories, and that the species were sampled over a similar distribution of road densities. An ANOVA with species and road category as main and crossed factors indicated that road categories differed significantly in percent canopy gap (road treatment: $F_{1,88} = 50.2, p < 0.0001$), but in post hoc analysis the High and Extreme groups were not significantly different in percent canopy gap (in Fisher's PLSD test, $p > 0.05$) so High and Extreme sites were pooled into a new High group. In the four-group analysis, all road density groups differed significantly in percent canopy gap (road density group: $F_{3,90} = 63.0, p < 0.0001$, and all pair-wise Fisher's PLSD $p < 0.05$) and total m of road (road density group: $F_{3,90} = 62.5, p < 0.0001$, and all pair-wise Fisher's PLSD $p < 0.05$), so we used four road density categories in our subsequent analyses of physiological and demographic variables. Vireos and warblers were sampled over a similar range of canopy gaps (factor species: $F_{1,90} = 0.01, p = 0.93$) and m² of road (factor species: $F_{1,90} = 0.07, p = 0.79$), and road categories were similar between species (interaction term species*road category, percent canopy gap $F_{3,90} = 0.23, p = 0.87$, $F_{3,90} = 0.12, p = 0.95$).

Sampling of focal animals

The first blood sample was taken within 3 min of capture, so these "baseline" plasma samples likely reflect pre-capture concentrations of corticosterone (the primary avian glucocorticoid), and not a response to the stress of capture (Romero and Romero 2002, Romero and Reed 2005). A second blood sample was taken 30 min after capture in order to measure the adrenal response to acute stress, which can be altered by chronic stress (Rich and Romero 2005). Although this standard "stress series" protocol (0- and 30-min samples, Wingfield 1994) is unlikely to have any serious negative effects on small birds (Sheldon et al. 2008), and this appeared to be the case for golden-cheeked warblers (Luke Butler, unpublished data), we released warblers immediately after the 30 min sample. For white-eyed vireos, an intramuscular dose (100 IU/kg, Rich and Romero 2005) of adrenocorticotrophic hormone (ACTH, Sigma Chemicals) was administered to maximally stimulate adrenal release of corticosterone. This maximal response can be altered by prior disturbance (Hopkins et al. 1999, Rich and Romero 2005). A third blood sample was taken from vireos 15 min after ACTH injection (45 min after capture) and immediately prior to release. All procedures were approved by the Institutional Animal Care and Use Committee at Tufts University.

Before release each bird was weighed with a 30 g Pesola Micro-Line spring scale graduated to 0.25 g (accuracy $\pm 0.3\%$), and body fat was scored on a scale of 0 (no visible fat) to 5 (heavy sheets of fat) following Romero et al. (1997). In order to calculate size-corrected body mass as a measure of body condition (Stevenson and Woods 2006), we measured tarsus length using a caliper (model SPI 2000, Swiss Precision Instruments, Switzerland) following the method of Pyle (1997). Age class was determined using plumage traits that distinguish males in their first breeding season (hereafter "first-year males") from older males (Ladd and Gass 1999, Hopp et al. 1995). Five of the 49 vireos sampled were scored as females based on the lack of singing before capture (Hopp et al. 1995), the presence of a developed brood patch (Pyle 1997), and the close presence of a singing male after the capture of the first bird. The golden-cheeked

warbler sample included only males (sex was easily determined by plumage color [Pyle 1997], and female warblers rarely responded to playback).

Statistical analyses

Corticosterone concentrations did not violate the assumption of homogeneity of variance (in Levene's test, all $P > 0.05$, Zar 1999), so we compared corticosterone among road density groups in an ANOVA, and planned Fisher's PLSD tests for post-hoc determination of any significant differences between pairs of road density groups. Body mass was analyzed in ANCOVA with road density group as the independent variable and tarsus length as a covariate (García-Berthou 2001), and we graphically present the residuals of body mass regressed on tarsus length (see Results). Fat score was analyzed in ANOVA. The small number of female white-eyed vireos ($n = 5$) and their distribution among road density groups (Zero $n = 0$, Low $n = 2$, Medium $n = 1$, and High $n = 2$) precluded using sex as a factor, so we report analyses of physiological variables with females included and excluded. Frequencies of males responding to playback and young males captured in each road density group were analyzed using chi-square tests (Zar 1999).

Study 4: Effect of habitat and cowbird parasitism on stress physiology of endangered, declining, and common bird species

Work was conducted during the summers of 2006, 2007, and 2008, when vireo and cardinal adults were transitioning from breeding to molting, and buntings were transitioning from breeding to migrating. All species in this study (black-capped vireo, white-eyed vireo, northern cardinal, and painted bunting) were sampled in upland juniper woods atop the Owl Creek Mountains on east Fort Hood, and in donut and other clumped deciduous habitat on west Fort Hood. Northern cardinals were also sampled in thick deciduous brushland on north Fort Hood, which was more contiguous than donut habitat, but contained few juniper trees. Painted buntings were also sampled in thick, rank riparian woods along Cowhouse Creek (east of the live fire zone on Fort Hood, Cimprich and Kostecke 2006), which contrasted strongly with the dry upland juniper woods on east Fort Hood, and the open donut scrub on west Fort Hood. Adults of all species were common in each area. Corticosterone concentrations were analyzed with repeated measures ANOVA in StatView (SAS Institute 1998). All birds were sampled within 3 min of capture and 30 min after capture.

Study 5: A meta-analysis of transmitter effects on avian behavior and ecology

Literature search

The studies used in this meta-analysis were obtained from a literature search conducted between December 2008 and March 2009. The principal method for identifying relevant studies was searching *ISI Web of Science* and *Google Scholar* using topic words: radio transmitter, transmitter effects/impacts, radio telemetry, radio tagging, device attachment, radio attachment, instrument attachment and load attachment. Because data loggers and satellite transmitters are similar in shape and methods of attachment to radio transmitters, and therefore should have comparable effects on birds, we also included them in our study by replacing 'radio' with 'data logger' or 'satellite' in our literature search. Hereafter we refer to transmitters, data loggers, etc.

as “devices”. Finally, we included additional studies found in literature reviews (Calvo and Furness 1992, Godfrey and Bryant 2003, Murray and Fuller 2000, Phillips et al. 2003, Samuel and Fuller 1994) or in the literature cited by published studies.

We identified an initial sample of 192 studies in which devices were attached to birds. For a study to be included in our analysis, however, it had to meet two criteria. First, birds with devices had to be quantitatively compared to birds without devices. Second, we had to be able to estimate effect sizes from the provided information, requiring studies to report sample sizes, direction of the effect, and one of the following statistics: mean and standard deviation, F-statistic, t-statistic, Z-statistic, chi-squared value, or p-value. A total of 84 studies met these criteria.

Data collection

We used several approaches to obtain data from the 84 studies. If results were divided (among study sites, years, etc.) without a combined analysis, we used the first result provided so only one result for a given analysis was taken from each study. If a study used devices that differed in mass and the effects were analyzed separately we used only the largest mass. If devices with different masses were combined in the analyses, however, we used the mean mass of the devices. We used multiple entries from a given study only if separate analyses were provided for different variables of interest (e.g., attachment type, species, sex). Because the few such studies usually used separate controls for each analysis, the potential lack of independence should have little effect on the results of our meta-analyses (Gurevitch and Hedges 1993). We obtained exact values from any time results provided in a graph using *GetData Graph Digitizer 2.24*.

We recorded characteristics of the birds (sex, age, primary mode of locomotion, and body mass) and the devices (% body mass and attachment type). We also recorded whether studies compared birds with devices to uncaptured birds or to previously captured procedural controls to determine the effect of capture and restraint. If studies reported results from both procedural control and uncaptured birds, we only included comparisons to procedural controls. Finally, we categorized foraging behaviors as offspring provisioning or self-provisioning.

Statistical analyses

For each study that met the criterion for inclusion we calculated the correlation coefficient r for each measured variable from one of the required statistics mentioned above along with either the sample size or degrees of freedom using the *MetaCalc Statistical Calculator* within the program *MetaWin* (Rosenberg et al. 2000). We then entered the correlation coefficients and sample sizes into *MetaWin* and used the program to calculate the effect size, Fisher’s z-transformation, and its variance for all variables from each study. We appointed positive or negative directionality to the differences between birds with and without devices in each analysis, since each aspect of behavior or ecology had a clear direction that was beneficial. Therefore, negative effect size values are in the direction considered detrimental.

For each analysis, we used a random-effects model in *MetaWin* (Rosenberg et al. 2000) because it accounts for a random component of variation in effect sizes between studies and is generally considered more appropriate for ecological data than a more restrictive fixed-effects model (Gurevitch and Hedges 1993). Categorical characteristics of the bird or device could only be analyzed if they have two or more categories, and each category contained two or more studies (required to calculate mean effect size). We calculated 95% confidence intervals of the

mean by bootstrapping with 999 iterations and considered the mean effect sizes significant if the confidence intervals did not overlap zero. We estimated probability values from randomization tests with 999 iterations and used those values to detect differences among group effect sizes (described by Q) in the categorical characteristics and to determine whether the slopes (b) of continuous characteristics plotted against effect sizes were different than zero. We report effect sizes and confidence intervals that are transformed from Fisher's z -transformation to Cohen's d (using the *MetaCalc Statistical Calculator* within the program *MetaWin* [Rosenberg et al. 2000]), because Cohen clearly defined how to interpret these values: less than 0.5 indicates a "small" effect, 0.5 to 0.8 a "medium" effect, and above 0.8 a "large" effect (Cohen 1988).

Since the tendency for studies with insignificant results to go unpublished could lead to a reporting bias known as the file-drawer effect (Sterling 1959), we addressed this problem by calculating Rosenthal's fail-safe numbers (Rosenthal 1979) with alpha equal to 0.05 in the program *MetaWin* (Rosenberg et al. 2000). This value is the number of unpublished studies with a mean effect size of zero required to reduce the combined significance to the nominated alpha value. For example, a fail-safe number of 100 would mean 100 studies with no effect must have gone unpublished to eliminate the significance of the results. If this number is large relative to the number of included studies it suggests the conclusions are relatively robust, even if some publication bias exists. We present fail-safe numbers only for marginal results where the outcome would change with a few unpublished studies showing no effect. The potential for an opposing publication bias to exist (if researchers preferentially publish results showing no impact of device attachment) cannot be investigated and will therefore not be discussed.

In our first summary analysis we determined whether devices cause an overall effect by combining all aspects of behavior and ecology into a universal meta-analysis. If studies investigated more than one aspect, we only included the one with the most robust sample size to avoid introducing nonindependence. We then ran a summary analysis for each independent aspect to determine which were affected. Next, we conducted a summary analysis for each of the categorical and continuous characteristics of the bird and device to determine their influence on device effects. Finally, we determined the role of capture and restraint by running a summary analysis for each aspect using the categorical model of whether a study compared birds with devices to uncaptured birds or procedural controls.

The potential importance of attachment type led us to also investigate its influence on the frequency of nest abandonment, physical impairment, and device-induced mortality. We could not analyze this with a meta-analytical approach because the consequences of interest had discrete (but ordinal) outcomes, thus preventing the calculation of effect sizes. Studies received a '2' if they reported at least one occurrence of a consequence, '1' if they reported no occurrence, and '0' if they did not address the topic. We included the '0' category because the frequency of non-reporting studies could be related to the frequency of a consequence if the topic is primarily addressed when it occurs or if researchers purposely omit negative findings. Data were gathered from all 192 studies because even those not meeting the criteria for inclusion in the meta-analysis frequently reported deaths, physical harm, and nest abandonment. We compiled singly ordered contingency tables for each variable and then compared the proportion of studies in each response category for each attachment type with a Kruskal-Wallis test in the program *StatXact*. The null hypothesis of no attachment effect was assessed by estimating exact p -values with a Monte Carlo procedure.

Study 6: Impact of radio transmitters on northern cardinal parental investment and productivity

Nest monitoring

We conducted this study in 2007 and 2008 on seven sites ranging from 11-150 ha at Fort Hood, in central Texas. We used parental cues to locate as many northern cardinal nests as possible on each site from mid March until late August. Nests were checked approximately every other day, with more frequent visits when the nestlings approached fledging to ensure accurate determination of nest fates.

Capture and device attachment

We restricted transmitter attachment to males because they were easier to capture using a mist-net with conspecific playback and we wanted to be consistent in which member of the pair received a transmitter. We randomly assigned each pair for which we found a nest to one of three treatments. In the “no treatment” group birds were not captured. In the “control” group, males were captured but no transmitters were attached. In the “transmitter” group, males were captured and a transmitter was attached. Males in the “control” and “transmitter” treatments were captured shortly after the nest was found, which was usually during incubation. When males were captured we collected up to 60 μ l of blood from the brachial vein for an unrelated research objective and banded each bird with a U.S Fish and Wildlife Service aluminum leg band and a unique combination of color bands before placing the birds into an opaque cloth bag. Blood collection has no major negative effects on wild birds (Sheldon et al. 2008) and should therefore not influence our results. Thirty minutes after capture we collected another blood sample and then released “control” birds. “Transmitter” birds received a mock transmitter before being released. We used mock transmitters rather than real transmitters because our goal was to assess the effects of carrying a “transmitter” and not to conduct a telemetry study. Mock transmitters were similar in size, shape, and mass to actual radio transmitters and were constructed by attaching a 0.8 mm metal wire antenna to an 8 mm cylindrical wooden body using JB Weld. The average mass of the entire package was 1.6 grams (SD = 0.2), which was approximately 4.0% of the birds’ body mass (SD = 0.3%). In 2007 we attached devices dorsally using eyelash glue (Raim 1978), but the low retention times caused us to change to a harness made of dissolvable suture (Doerr and Doerr 2002) in 2008.

Nest defense

We conducted nest defense trials up to three times during a nesting attempt: incubation, early nestling, and late nestling. A trial consisted of exposing cardinal nests sequentially to a human and a model American Crow, both of which cardinals respond to as nest threats. We randomly determined the order of the threats with a 2 h break between their presentations. The first threat was consistently presented between 2 and 2.5 h after sunrise. Following detection by either parent, we recorded the total number of vocalizations, duration of response, and closest approach to the threat by each parent. Each behavior was scored on a scale from 1 to 5, with higher values representing a stronger response. The scores of these three variables were then compiled into a composite nest defense score, ranging from 0 to 15. By recording multiple behavioral traits we were able to generate a more accurate overall estimate of nest defense (Gunness and Weatherhead 2002). After 5 min the threat was removed and the researcher left the area. Observations made during exposure to the crow were from a small, camouflaged blind

set up approximately 15-25m from the nest the previous day.

Nestling provisioning

We monitored nestling provisioning from a blind using a spotting scope (20-60x magnification) trained on the nest. Observations were made for 1 h during the interval between successive presentations in nest defense trials. We used the second hour of the interval to ensure that birds had resumed normal activity following the first nest threat presentation. In addition to recording the number of feeding visits by each parent, we also estimated food load size. Loads were given a value of 1 if they were completely contained within the parent's bill, 2 if they protruded slightly from the bill, and 3 if they protruded obviously from both sides of the bill. We calculated a feeding score for each parent in each trial by multiplying their number of visits per hour by their average prey size. If we could not determine the prey size its load value was recorded as the average of all other loads values recorded during the hour of observations.

Statistical Analyses

We analyzed daily survival rates of nests using a logistic-exposure model (Shaffer 2004). Differences in estimates of nest defense, nestling provisioning (feeding rate and feeding score), and fledging success among birds from each treatment were analyzed with a mixed model repeated measures analysis using the pair as the unit of replication. We used Bonferroni corrections for multiple comparisons between the treatments. Julian date, time after sunrise, age of young, and number of young were included as covariates in the analysis of nest defense (Montgomerie and Weatherhead 1988) and nestling provisioning. Although multiple studies have shown no impact of previous exposure on passerine nest defense (Weatherhead 1989), there has been some debate over this issue (Knight and Temple 1986, Siderius 1993). Therefore, we also included the number of times parents had been exposed to a researcher prior to the trial as a covariate of nest defense, including both regular nest visits and previous nest defense trials. Mixed model analyses were employed because treatment and nest threat were fixed models, whereas the individual birds that were monitored were randomly determined.

Study 7: Data recovery and analysis of remotely monitored heart-rate telemetry from endangered birds

We developed a number of computer programs to take continuous audio recordings of telemetry and transform them into files of heart-rate with one second time-stamps. Other programs were written to flag spurious data when the received signal is too weak or erratic. Thirteen birds were tagged in the spring of 2008 at Ft. Hood, Texas and telemetry recorded for a total of 36 days. Excluding three birds that generated essentially no usable results, the telemetry from the remaining ten birds was processed and spurious data removed.

The original program that determined heart-rate was based on an autocorrelation algorithm of filtered data within one-second analysis frames. This method is susceptible to generating spurious heart-rates that are either twice the actual heart-rate (harmonics) or one-half the actual heart-rate (sub-harmonics) (Rabiner and Schafer, 1978).

A number of significant changes were made to try to improve the autocorrelation technique used in the original program (version 24i, Appendix A). In the original program the raw WAV array was filtered using a fourth-order elliptical, 40Hz bandpass IIR filter centered on

the strongest subcarrier frequency. Autocorrelation was then performed on one millisecond envelope data within successive analysis frames of one-second duration.

Using a technique similar to the filter applied during manual processing, the new algorithm has a pair of sideband filters centered at the subcarrier frequency. Each sideband filter is 150 Hz wide centered 125 Hz from the strongest subcarrier center frequency. Similar sideband filtering is applied to the second-strongest subcarrier and that signal is added to the filtered strongest subcarrier.

A one-millisecond envelope is then calculated and a low-pass anti-alias filter applied. The filtered signal is then centered-clipped (Sondhi, 1968) at 30% of maximum signal for each half-second analysis frame. Autocorrelation of one-millisecond data is performed on five overlapping half-second analysis frames with 50% overlap. The median of the five peak frequencies is taken as the actual HR (Rabiner et al. 1975).

Improved sideband filtering, center-clipping and the use of a non-linear median filter on overlapping analysis frames greatly reduce the number of spurious heart-rates that are typically generated by the original autocorrelation method. Comparison to manually-derived heart-rates was performed on a small dataset within a limited range of heart-rates.

Study 8: Energetic cost of human disturbance on endangered and common passerine species

Heart rate transmitter placement, heart rate recording, and activity monitoring

In 2006 and 2007, we captured and transmitted 14 white-eyed vireo males (2006 and 2007) and one black-capped vireo male (for trial and testing in 2007), and 14 black-capped vireos in 2008 (12 males and two females for experimental study following successful trial in 2007). Of the total birds monitored, only three were unpaired and the remaining were nest building ($n = 2$), incubating ($n = 3$), or were feeding nestlings ($n = 3 - 2$ of which were the black-capped vireo females) or fledged young ($n = 8$). We were unable to determine the breeding status for six males. Birds were captured two to three hours before roosting (approximately 5:00 PM) by luring them into mist-nets using song playbacks. Each vireo was first fitted with a unique combination of US Fish and Wildlife Service and colour bands. We then mounted 0.5g heart rate transmitters (Sparrow Systems, Fisher, Illinois) on each male following the protocol of Cochran and Wikelski (2005). The procedure lasted an average of 15 min from the time of capture to the time of release and all males returned to territorial or nesting activities within 10-20 min after release. Heart rate transmitters emit a continuous amplitude-modulated (AM) signal by a 1,800-Hz subcarrier oscillator that is frequency modulated (FM) by heart muscle potentials. The heart muscle potential was captured with two leads placed subcutaneously on the dorsum. In 2008, we additionally transmitted three black-capped vireo males breeding in the live fire area of Fort Hood to test for physiological responses in response to specific live fire training activities. Unfortunately, due to characteristics of the terrain in these black-capped vireo territories and the time constraints imposed by working in live fire areas, we did not obtain any usable telemetry (heart rate and activity) data.

We obtained heart rate from 24 of the 29 transmitted vireos and activity data were obtained from 28 of the 29 transmitted birds. We continuously recorded heart rate for two days and three nights (average of 60 h) for each monitored vireo using a Yagi antenna connected to an AR8000 or AR8200 receiver (AOR Ltd. Tokyo, Japan), laptop computer and/or MP3

recorder (EDIROL R09, Roland Inc.). MP3 recordings that yielded the highest quality/long duration combination were obtained using a 160 kbps-sampling rate and 48 kHz-sampling frequency. Antennae and recording equipment were placed approximately 50m from the edge of the vireo's territory at a location that allowed maximal tracking range. For white-eyed vireos that were monitored in 2006 and 2007, heart rate was manually calculated using a spectrogram created in CoolEdit 2000 (sound recording analysis software, Syntrillium Software Corp., Phoenix) using a Fast Fourier Transform (FFT). Although we recorded heart rate continuously, we sampled heart rate for a 5 sec interval every 10 min during and every 30 min outside disturbance experiments for manual calculations. Heart rate was extracted using the automated program (Appendix A) described in Study 7 of this report. The program provides heart rate data as one-second time stamps.

We simultaneously recorded activity using a separate antenna connected to an automated receiver (Automatic Receiving Unit, Sparrow systems, Inc.). The automated receiver continuously records the signal strength of the radio transmitter in logarithmic units. Behavioral observations showed that an approximate doubling of signal strength, i.e., a change in signal strength by 4 dBm, corresponded to activity of the individual (Crofoot et al. 2008, Lambert unpublished data).

Experimental procedure for disturbance trials

For each bird transmitted, we used a repeated measures design to compare periods of disturbance to control periods where no experimental disturbance was conducted (Day 1 of monitoring period). The 14 white-eyed vireos monitored were separated into three groups and the eleven black-capped vireos into two groups (groups 1 and 2 only).

To evaluate the effect of long-duration continuous human disturbance, group 1 was subjected to a single four-hour continuous chase (700h -1100h local time) by three different observers on the second day of the monitoring period. The first day was used as a no-disturbance control. Chases involved one observer making loud noises while following the bird on foot at close proximity (usually <5m). Each observer tracked the individual bird with a hand-held Yagi antenna and AR800 receiver to ensure proximity to the individual being disturbed.

To evaluate the effect of short frequent disturbance and to compare natural (predator) vs. human-mediated stressors, group 2 was subjected to two types of one-hour repeated disturbances from 1100-1200h, 1300-1400h, and 1500-1600h local time on both days of the monitoring period. The type of disturbance was randomly selected as either predator presence (Screech Owl decoy with playback in vireo territory) or human disturbance. Human disturbance included chasing the bird, passively sitting in the territory by one observer, or walking through the territory without targeting the bird by one observer in random order. Group 2 was also subjected to one-hour nocturnal human disturbances on the third night. The time of the one-hour disturbance was randomly selected between 2300-0200h local time. The first two nights were used as experimental controls with no disturbance. Nocturnal disturbances included three observers simultaneously walking through the territory and playing loud music at 10m from the territory.

Group 3 (white-eyed vireos only) was subjected to a simulated conspecific territory intrusion (10 min of white-eyed vireo playback and decoy presentation in territory) at 0700h local time and three one-hour repeated human disturbances between 1100-1200h, 1300-1400h, and 1500-1600h local time on the second day. Similar to the disturbances for group 2, human disturbance in group 3 was randomly assigned as a chase, passive, or walking, applied in random

order and performed by three different observers. The first day was used as a non-disturbance control period.

Behavioral observations

Behavioral observations were conducted for four hours for two periods: during control non-disturbance periods at 50m from the territory using a blind and during disturbance trials. We specifically recorded whether males continued to perform nesting duties such as nest building, incubation and feeding young. All of our activities around and near the vireo territories were limited to the disturbance trials and non-disturbance behavioral observation periods.

Calibrating heart rate to energy expenditure

Heart rate measurements allowed us to indirectly estimate $\dot{V}O_2$ and finally energy expenditure, because the two variables are related to each other as derived by Fick's equation: $\dot{V}O_2 = f_H V_s (Ca_{O_2} - Cv_{O_2})$, where $\dot{V}O_2$ is oxygen consumption, f_H is heart rate, V_s is cardiac stroke volume, Ca_{O_2} is the oxygen content of arterial blood and Cv_{O_2} is the oxygen content of mixed venous blood (Fick 1870). If the oxygen pulse, $V_s (Ca_{O_2} - Cv_{O_2})$, remains constant, there is a linear relationship between f_H and $\dot{V}O_2$ and the former can be used to determine the latter (Green et al. 2001). However, calibration experiments to determine the exact relationship between the two variables need to be conducted for each species under study. Calibrations were performed between May 9 and 11, 2006 on five white-eyed vireos and on May 22, 2007 on three black-capped vireos that were not included in disturbance experiments. Birds fitted with heart rate transmitters were placed in 2 L plastic metabolic chambers to simultaneously measure heart rate and $\dot{V}O_2$ in 10 s intervals. On average, birds were held in the respirometry chamber for a period of 2.3 ± 0.6 h (mean $\pm$ SE). In the chamber, birds could freely move around, but when placed in a dark location, they were generally quiescent in the chambers (as determined by their low heart rate and by the lack of movement noises). Thus, we were able to obtain an estimate of over a range of heart rates. However, birds were unable to perform sustained or short bouts of $\dot{V}O_2$ true flight in the chamber as they would in the wild and therefore heart rate calibrations were not representative of such activities.

We measured oxygen consumption and carbon dioxide production $\dot{V}CO_2$ in an open-flow, push-through respirometry system. External air was dried over Drierite columns and pumped through a mass flow controller (TR-FCI, Sable Systems, Nevada, USA) and a multiplexer (V2-0, Sable Systems) into the metabolic and a reference chamber. Flow rate was 1000 ml/min and the flow controller was calibrated prior to use via a bubble meter. A previous factory calibration indicated that flow rate errors were $<1.2\%$. Air leaving the chambers was dehumidified using a Peltier-Effect Condenser (PC-1, Sable Systems) and CO_2 concentration was measured from a subsample of the outlet flow (CA-1B, Sable Systems, Henderson, USA).

Before $\dot{V}O_2$ (FC-1B, Sable Systems) was determined, Drierite was used to scrub potential remaining water from the air and CO_2 was scrubbed from the air stream. The respirometry system was tested for leaks by pressurizing it and determining that no air was lost after 10 min of observation.

We estimated instantaneous oxygen consumption using the equation of Bartholomew et al. (1981):

$$FE O_2(eq) = FE O_2(t-1) + \left[\frac{FE O_2(t) - FE O_2(t-1)}{1 - e^{\frac{-\dot{V}}{V}\Delta t}} \right]$$

where $FE O_2$ is the oxygen concentration in the excurrent air, V is the volume of the system including tubing, $\dot{V}$ is the flow rate through the system, and Δt is the interval between measurements at times t and $t-1$. We determined the denominator of this equation (the so-called Z-value) empirically using Datascan (Sable Systems). We then calculated rate of oxygen consumption by equation 4a of Withers (1977):

$$\dot{V} O_2 = \dot{V} \left[\frac{FI O_2 - FE O_2(eq)}{1 - FI O_2} \right]$$

where $FI O_2$ is the incurrent oxygen concentration. We also determined the respiratory quotient, the ratio of CO_2 produced to O_2 consumed, and used it to calculate thermal equivalents and metabolic rate (in kJ/day, following Walsberg and Hoffman 2005). Least squares regressions were used to determine the relationship between heart rate and energy expenditure (kJ/day) for each individual.

Statistical analyses

The one-second heart rate data obtained for black-capped vireos was averaged per minute for all disturbance trials. We evaluated the energetic cost in response to both human-mediated activities and natural disturbances in two ways: 1) by making repeated within-individual comparisons of the mean energy expenditure and activity between human-mediated disturbance and non-disturbance trials at the same time periods, and 2) by testing for changes in energy expenditure before (baseline), during, and after a disturbance with the exception of long duration disturbances because the four-hour baseline fell within the roosting period. We used sequential Bonferroni-corrected paired-sample t tests (Holm 1979, Rice 1989) and repeated measures analysis of variance (ANOVA) with time of day as a co-factor to control for circadian rhythms in activity and heart rate. When data could not be normalized, we used non-parametric alternatives (e.g., Wilcoxon signed rank test). Unless otherwise stated, we used one-way ANOVAs to compare mean energy expenditure and activity between different types of disturbances (three types of one-hour human disturbances, one-hour repeated vs. four-hour long duration and natural vs. human). All statistical analyses were conducted using SPSS statistical software (v. 16.0, SPSS Inc., Chicago).

Results and Discussion

Study 1: Chronic and acute hormonal response of endangered and common songbirds to offspring-directed threats

Behavioral response to crow and human

Adults at the nest flew away when the crow decoy was placed, or when the human approached and stood, at the nest. Adults then usually behaved as they do when a snake (the most common nest predators at our site, Stake and Cimprich 2003) or predatory bird is near the nest or elsewhere in the territory, by making characteristic alarm vocalizations at a high rate, and halting feeding and territorial behavior (Luke Butler and Tim Hayden, personal observation).

Chronic and acute CORT response

CORT had an interassay variation of 17.6%, an intraassay variation of 3.9%, and a detection limit of 1.34 ng/ml. CORT concentrations of white-eyed (Fig. 10a) and black-capped vireo (Fig. 10b) parents strongly increased in response to 30 min of capture and restraint (ANOVA, time after capture: $F_{1,23} = 45.5$, $p < 0.0001$ and $F_{1,28} = 33.3$, $p < 0.0001$ for white-eyed and black-capped vireos, respectively). However, contrary to prediction, chronic offspring-directed threats did not alter baseline or stress-induced plasma CORT concentrations in parents of either species (white-eyed vireos, stress treatment: $F_{1,23} = 0.01$, $p = 0.91$, stress treatment x time after capture: $F_{1,23} = 0.04$, $p = 0.85$; black-capped vireos, stress treatment: $F_{1,28} = 0.001$, $p = 0.95$; stress treatment x time after capture: $F_{1,28} = 0.02$, $p = 0.90$), compared to undisturbed (control) parents (Fig. 10).

The first exposure to the crow decoy or a human at the nest caused no significant increase in CORT compared to control (undisturbed) adults in either white-eyed vireos (ANCOVA with day of year as covariate, overall effect of threat treatment: $F_{2,13} = 1.55$, $p = 0.25$, Fig. 11a) or black-capped vireos (threat treatment: $F_{2,17} = 1.16$, $p = 0.34$, Fig. 11b). CORT increased after 30 min of capture and restraint in both species (time after capture, $F_{1,26} = 23.2$, $p < 0.0001$ and $F_{1,31} = 37.3$, $p < 0.0001$ for white-eyed and black-capped vireos, respectively), but interestingly, this response was lower in adults if they were exposed to the crow decoy immediately before capture (Fig. 11a and b). This effect was marginally non-significant in black-capped vireos (interaction term threat treatment * time after capture: $F_{2,31} = 2.90$, $p = 0.07$; white-eyed vireos: $F_{2,23} = 0.92$, $p = 0.41$), but disappeared after 5 – 6 days of offspring-directed threats ($F_{2,23} = .23$, $p = 0.80$, Fig. 11d). Although the CORT response of black-capped vireos to their first exposure to human presence (mean $\pm$ SE = 10.1 ± 5.7 ng ml⁻¹, $n = 6$) might appear higher than undisturbed controls (4.6 ± 0.9 ng ml⁻¹, $n = 8$; Fig. 11b), that difference disappears if one individual with a baseline CORT concentration (37.5 ng ml⁻¹) similar to capture stress-induced levels (35.8 ± 5.2 ng ml⁻¹, $n = 7$) is removed (first exposure to human presence excluding that sample, 4.6 ± 1.8 ng ml⁻¹, $n = 5$). The exceptionally-high CORT concentration at the time of capture in this individual might reflect inter-individual variation in sensitivity to humans, or it might indicate that the animal was exposed to an unknown stressor prior to capture.

Adults did not develop sensitivity to offspring-directed disturbances after repeated exposure (i.e., examining “baseline” concentrations before and after 5 – 6 of disturbance). Baseline CORT was unaltered by the crow disturbance and the human disturbance in both species after 5 – 6 days of offspring-directed threats (white-eyed vireos, threat treatment: $F_{2,12} = 0.60$, $p = 0.57$, Fig. 11c; black-capped vireos, threat treatment: $F_{2,12} = 2.20$, $p = 0.16$, Fig. 11d).

Finally, similar to Experiment 1, in which we found no effect of 7 – 10 days of offspring-directed threats on baseline or restraint-induced CORT levels of adults, we found no effect of 5 – 6 days of offspring-directed threats on CORT (comparing controls before and after 5 – 6 days of threats, in ANCOVA with day of year as covariate, all $p > 0.10$).

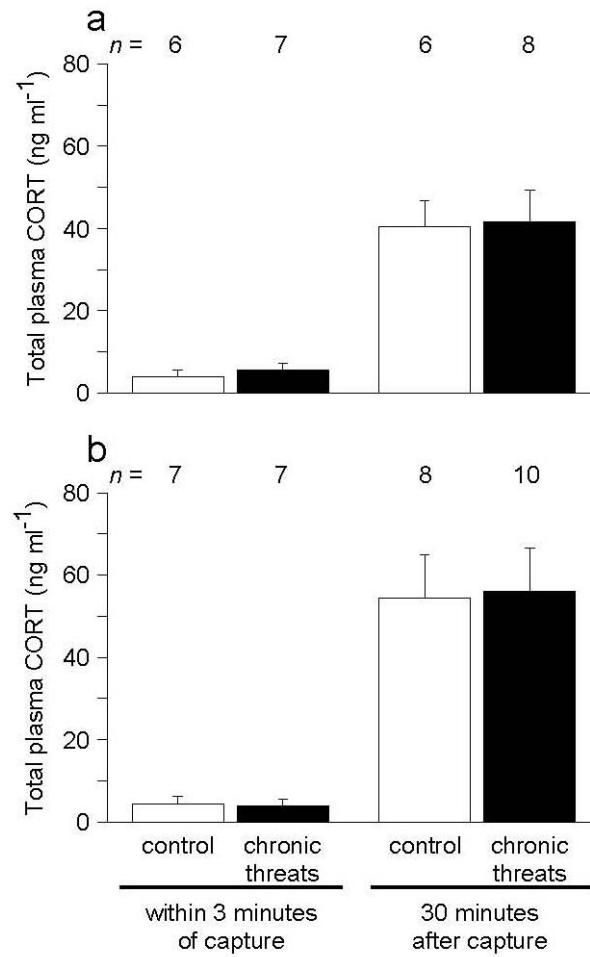


Figure 10. Corticosterone (CORT) levels (means $\pm$ SEs) in parent vireos within 3 min of capture and after 30 min of restraint.

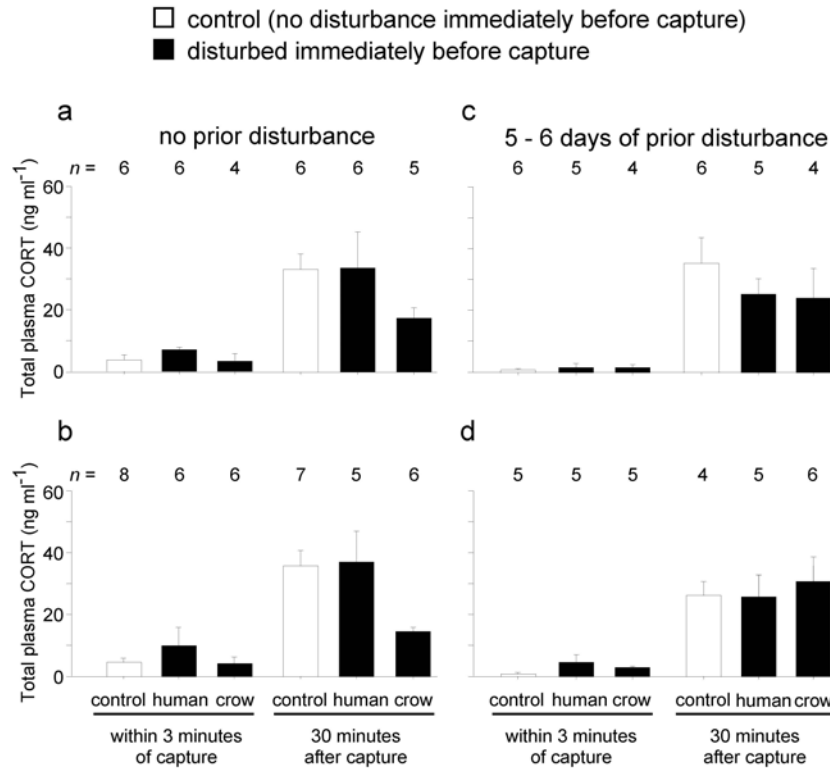


Figure 11. Corticosterone (CORT) levels (means $\pm$ SEs) in adult vireos within 3 min of capture and after 30 min of restraint following their first exposure (a, white-eyed vireos; b, black-capped vireos) to nest-directed threats (crow or human; filled bars) or no threat (open bars) and after 5 – 6 days of several nest-directed threats per day (c, white-eyed vireos; d, black-capped vireos).

Discussion

A common approach to studying how wild animals sense and respond to threats and unpredictable disturbances is to observe (Clinchy et al. 2004), introduce (Cockrem and Silverin 2002), or remove (Ahola et al. 2006) a potential threat to an animal, and measure subsequent changes in physiology, behavior, or survival. However, much less is known about how animals perceive and respond to acute threats to the survival of their offspring. Parental responses to offspring-directed threats are readily studied in nesting species with extended parental care. In particular, the adrenocortical response to stress, typified by changes in plasma concentrations of CORT (Wingfield and Romero 2001), has been a useful measure of the parental response to offspring-directed threats in birds. For example, Magellanic penguins (Fowler 1999, Walker et al. 2006) and European starlings (*Sturnus vulgaris*, Cyr and Romero 2007) show altered parental CORT concentrations following acute and chronic threats to nests containing eggs and nestlings. However, threats to offspring were not fully isolated from threats to adults in these studies because the adults could not easily escape, either because they were non-volant (Magellanic penguins, Fowler 1999, Walker et al. 2006), or because they were nesting in boxes with a single small entrance hole (European starlings, Cyr and Romero 2007). Additionally, in adult pied flycatchers (*Ficedula hypoleuca*) nesting in boxes, parental CORT increased in response to a decoy of a predator of adults and offspring (weasel, *Mustela vulgaris*), but not in response to a

predator of offspring only (woodpecker, *Dendrocopus major*, Silverin 1998). Thus, to understand the parental response to offspring-directed threats, those threats must be distinct from self-directed threats, either because parents are free to modulate their level of risk (e.g., free-living great tits exposed to a predator, Cockrem and Silverin 2002), or because the threat is offspring-specific (e.g., the offspring-only predator used by Silverin 1998).

We attempted to isolate and measure the adrenocortical response of open-nesting birds, the black-capped vireo and white-eyed vireo, to threats to their dependent young, and compare that response to threats to adults. In Experiment 1 we tested the prediction that naturalistic and anthropogenic offspring-directed threats and disturbances lasting several days would result in parents with suppressed baseline and stress-induced CORT, typical of chronic stress in European starlings nesting in boxes (Rich and Romero 2005, Cyr and Romero 2007). In Experiment 2 we tested the predictions that naturalistic and anthropogenic offspring-directed threats lasting several minutes would increase plasma CORT in parents, typical of an acute stress response, and alter CORT response of parents to a subsequent self-directed threat (capture and handling, Dallman et al. 1992). This study is distinct from all prior investigations of the parental response to offspring-directed threats because 1) the focal species were volant open-nesters, 2) responses to a naturalistic threat were compared to a human threat, 3) effects of acute offspring-directed threats were compared to chronic threats, and 4) responses of an endangered species were compared to a common species.

Adults responded to capture stress with a large increase in CORT, but acute and chronic offspring-directed threats had little effect on glucocorticoid concentrations. Our findings suggest that in our two study species, functioning of the hypothalamic-pituitary-adrenal (HPA) axis is, at most, only very weakly affected by offspring-directed threats. Nevertheless, our threats appeared to be perceived as threats by parents because normal parental care almost always ceased during presentations and alarm vocalizations and overall activity around the nest increased, very similar to the effect of live natural predators. This contrast between physiological and behavioral responses to a threat to fitness has been found in other vertebrates (e.g., Silverin 1998, Fletcher and Boonstra 2006). Thus, although a major effect of increased glucocorticoid concentrations, energy mobilization (Sapolsky et al. 2000), would seem to help sustain the observed behavioral responses of parents, or to help prepare them if the offspring-directed threat became an adult-directed threat, we found no evidence that CORT is involved in permitting or preparing for defense behaviors in parents.

Our results contrast similar studies showing that the HPA axis was altered (either stimulated or suppressed) following short- and long-term exposure to offspring-directed threats. Threats to nest boxes increased CORT in pied flycatchers after several minutes (Silverin 1998), and decreased CORT in European starlings after several days (Cyr and Romero 2007). Additionally, male East African stonechats (*Saxicola torquata axillaris*) nesting in territories shared by a fiscal shrike (*Lanius collaris*), a predator on adults and young which likely acted as a chronic stressor, had higher CORT than stonechat males in territories with no shrike (Scheuerlein et al. 2001). Parent blue tits (*Parus caeruleus*) showed no CORT response to a human 20-30 m away from a nest (Müller et al. 2006), but it is unknown whether parents perceive humans as a threat to their offspring in this species. Based on these studies, a reasonable conclusion would be that threats to nests, and thus threats to reproductive success, elicit CORT responses in parent songbirds. Our data clearly do not fit this pattern, which we suggest results from contrasts in the degree of overlap between offspring- and adult-directed threats.

We propose that nest-directed threats elicit a physiological response (i.e., CORT release)

in adults only when they threaten adults and offspring alike, which depends on the type of threat and the ability of adults to escape from it. Previous studies of the adrenocortical response to offspring-directed threats have not fully separated threats to offspring from threats to adults because the nest characteristics of the study species put adults at a higher risk of nest-associated death than the vireos in our study. Stonechats (Scheuerlein et al. 2001) build well-hidden and sometimes domed nests near or on the ground (Greig-Smith 1982, Scheuerlien and Gwinner 2006), and pied flycatchers (Silverin 1998) and starlings (Cyr and Romero 2007) were nesting in boxes with a single, small, side opening. Thus, detecting and escaping from a nest-directed threat may pose a challenge for adults of those species. Indeed, predators can be highly successful at targeting cavity-nesting adults in their nest: at least half of predation events at the natural cavity nests of marsh tits (*Parus palustris*) resulted in death of an incubating adult inside the nest cavity (Wesolowski 2002). In contrast, when we threatened active nests of black-capped and white-eyed vireos, parents could easily detect and control their perceived risk relative to the disturbance, because they build cup-shaped pendant nests attached near the tips of thin branches (Graber 1961, Hopp et al. 1995) that are often flanked by flight lanes and surrounded by a “bubble” of open space.

An alternative explanation may be that a glucocorticoid response to nest-directed threats is unfavorable when threats are common and when redirection of behavior from breeding to survival lowers overall fitness. Nest failure is much more common for black-capped and white-eyed vireos (~50-70%, Cimprich 2006, Hopp et al. 1995) than for pied flycatchers and European starlings (~10%, Huhta et al. 1998, Collins and De Vos 1966). Furthermore, vireos at our site commonly re-nest following nest failure, which is possible because of the extended breeding season in Texas (31°N). In contrast, pied flycatchers were studied at 57°N (Silverin 1998) and European starlings were studied at 42°N (Cyr and Romero 2007), where breeding seasons are considerably shorter. Therefore, a large CORT response to a nest-directed threat might reduce fitness of a Texas vireo by suppressing successful re-nesting, but in a flycatcher or starling breeding at northern latitudes, a large CORT response might increase fitness by facilitating abandonment of futile re-nesting activities in preparation for molt, migration, or winter. However, one might predict higher CORT responses in low latitude species because they can afford to abandon a breeding attempt when other attempts are possible in the same year (Wingfield and Sapolsky 2003).

However, several independent lines of evidence support our hypothesis that the ability to modulate risk exposure determines whether birds initiate a CORT response to a nest-directed threat. Incubating Magellanic penguins can see a human approaching but they cannot easily escape because they do not fly, and they increase CORT in response to a human standing nearby (Fowler 1999, Walker et al. 2006). Free-living great tits (*Parus major*) showed a much smaller CORT response to a stuffed owl (a predator on tits) than did captive tits trapped with the same owl in an aviary (Cockrem and Silverin 2002). Longnose killifish (*Fundulus majalis*) exhibited a smaller cortisol response to the sight of a predatory fish in tanks containing vegetative cover than in open tanks (Woodley and Peterson 2003). Therefore the ability to escape a threat, and not the perception of the threat alone, appears to influence whether the HPA axis is stimulated.

We cannot exclude the possibility that CBG concentrations declined in parents in response to our offspring-directed threats (Bruener et al. 2006), which would increase free CORT concentrations without increasing total CORT. In female European starlings, free CORT, and not total CORT, predicted nest desertion (Love et al. 2004). However, we never observed nest desertion by parents among the dozens of nests we exposed to our chronic disturbance

protocol (Luke Butler., unpublished data), so we believe that the types and intensities of disturbances used in this study are unlikely to cause nest abandonment in these species.

Using physiological measures to detect disturbance caused by human activity is a growing practice (Creel et al. 2002, Homan et al. 2003, Romero 2004, Gobush et al. 2008), and identifying what makes some species at risk of extinction is an important goal of conservation (Owens and Bennett 2000, Purvis et al. 2000, Reynolds et al. 2005). However, to our knowledge this is the first study to compare the endocrine responses of a free-living endangered animal and its common, sympatric relative to experimental offspring-directed disturbances. If the endangered status of black-capped vireos was due to a special sensitivity of the species to human activity during the breeding season, then we might predict that black-capped vireos would show a greater adrenocortical response than white-eyed vireos to our nest-directed threats, but this was not the case. However, the contrasting sensitivities to nest-directed threats between open-nesters (this study) and cavity-nesters (Silverin 1998, Cyr and Romero 2007) suggest that a nest style that causes a high correlation between offspring- and adult-directed threats may be a risk factor for sensitivity to human activity.

The present study illustrated a contrast in HPA axis function in two free-living birds presented with multiple threats to their offspring contained in open cup nests. Whereas glucocorticoid concentrations in parents increased dramatically following capture, two different threats to offspring caused no such increase, suggesting that adults perceived no risk to themselves from nest-directed threats. Although adults responded behaviorally to offspring-directed threats, this behavioral change was not accompanied by a change in the HPA axis, unlike the behavioral response to other stressors. Thus, a major physiological coping mechanism of vertebrates, the HPA axis, does not appear to be involved in responding to threats to reproduction as it is involved in responding to threats to survival.

Summary

Dependent young are often easy targets for predators, so for many parent vertebrates, responding to offspring-directed threats is a fundamental part of reproduction. We tested the parental adrenocortical response of the endangered black-capped vireo and the common white-eyed vireo to acute and chronic threats to their offspring. Like many open-nesting birds, our study species experience high offspring mortality. Parents responded behaviorally to a predator decoy or human 1 – 2 m from their nests, but, in contrast to similar studies of cavity-nesting birds, neither these acute threats nor chronic offspring-directed threats altered plasma corticosterone concentrations of parents. Although parents in this study showed no corticosterone response to offspring-directed threats, they always increased corticosterone concentrations in response to capture. To explain these results, we propose that parents perceive their risk of nest-associated death differently depending on nest type, with cavity-nesting adults perceiving greater risk to themselves than open-nesters that can readily detect and escape from offspring-directed threats. Our results agree with previous studies suggesting that the hypothalamic-pituitary-adrenal axis, a major physiological mechanism for coping with threats to survival, probably plays no role in coping with threats to offspring when risks to parents and offspring are not correlated. We extend that paradigm by demonstrating that nest style may influence how adults perceive the correlation between offspring-directed and self-directed threats.

Study 2: Acute hormonal response of endangered and common songbirds to human pursuit

CORT results

Intraassay variation was 2.0% for warblers and 3.9% for vireos. Interassay variation was 17.6% for vireos (all warbler samples were included in one assay).

Corticosterone concentrations increased significantly in all species 30-min after capture (Table 1), verifying that an acute threat stimulated the expected hormonal response in the adults we sampled. In contrast, corticosterone concentrations at the time of capture did not differ significantly between undisturbed birds and birds pursued by one (vireos) or three (warblers) people on foot (Table 1), suggesting that no species responded to human pursuit with an acute increase in corticosterone concentrations.

Interestingly, black-capped vireos had a significantly smaller corticosterone response to capture if they were pursued prior to capture, an effect that was consistent between years (Fig. 12), and absent in white-eyed vireos (Fig. 13). Golden-cheeked warblers had slightly lower stress-induced corticosterone concentrations following pursuit (Fig. 14), although this result did not approach statistical significance (Table 1).

Table 1. Comparisons of plasma corticosterone concentrations of three songbirds in relation to human disturbance before capture (0 and 60 min in all species, and 30 min in white-eyed vireos), time after capture (< 3 min and 30 min), and year (2006 or 2007; vireos only). Non-significant interactions were removed from the black-capped vireo analysis in step-wise fashion. The non-significant factors Year and Disturbance before capture * Time after capture, are presented for white-eyed vireos to allow direct comparison with the results for the congeneric black-capped vireo.

Species Factor	Groups <i>df</i>	Error <i>df</i>	<i>F</i>	<i>p</i>
Black-capped vireo				
Disturbance before capture	1	57	1.6	0.208
Time after capture	1	57	70.2	< 0.001
Year	1	57	6.0	0.017
Disturbance before capture * Time after capture	1	57	5.4	0.024
White-eyed vireo				
Disturbance before capture	2	72	0.5	0.608
Time after capture	1	72	32.3	< 0.001
Year	1	72	0.1	0.714
Disturbance before capture * Time after capture	2	72	0.8	0.466
Golden-cheeked warbler				
Disturbance before capture	1	32	0.8	0.391
Time after capture	1	32	49.6	< 0.001
Disturbance before capture * Time after capture	1	32	0.9	0.357

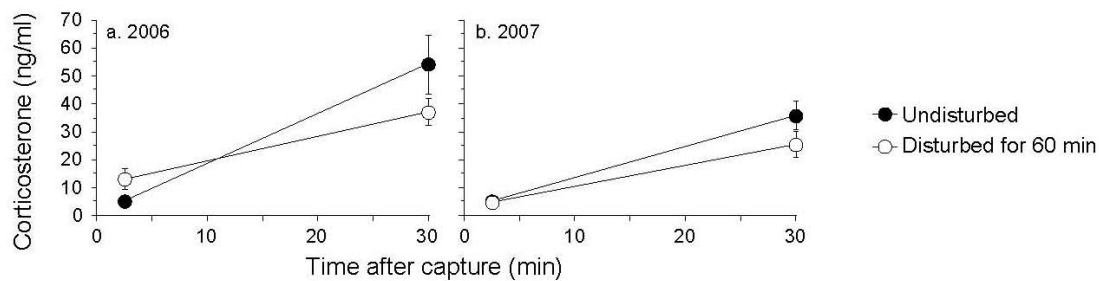


Figure 12. Corticosterone concentrations (means $\pm$ SEs) in black-capped vireos exposed to 60 min of pursuit by a person on foot (open circle), or undisturbed before capture (filled circles) in (a) 2006 and (b) 2007.

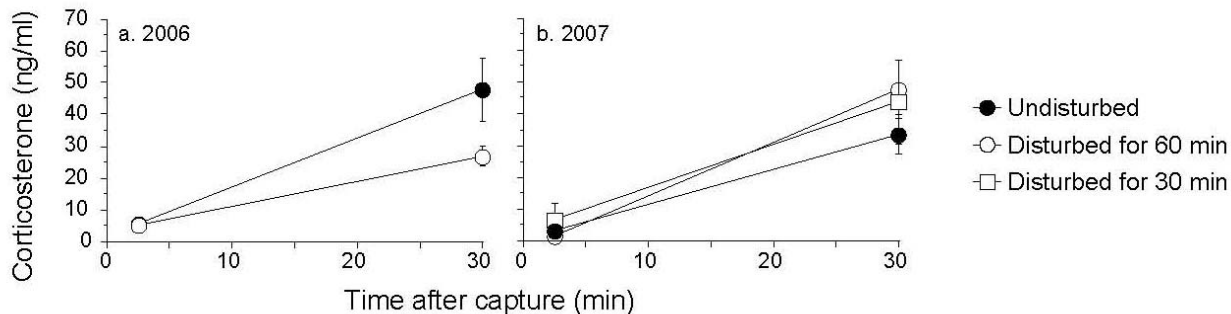


Figure 13. Corticosterone concentrations (means $\pm$ SEs) in white-eyed vireos exposed to 30 or 60 min of pursuit by a person on foot (open circle and square), or undisturbed before capture (filled circles) in (a) 2006 and (b) 2007.

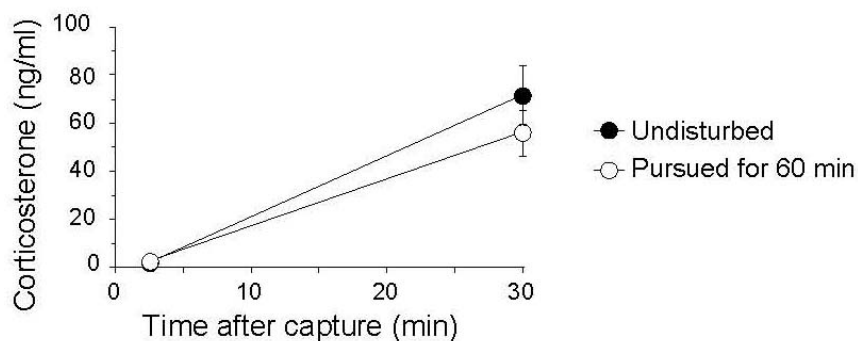


Figure 14. Corticosterone concentrations (means $\pm$ SEs) in golden-cheeked warblers exposed to 60 min of pursuit by three people on foot (open circle) in 2007, or undisturbed before capture (filled circles).

An hour of human disturbance had no significant effect on the response of golden-cheeked warblers to conspecific song playback. Latency to respond to playback was (mean $\pm$ SD) 9.9 ± 8.7 min in disturbed males ($n = 10$, including three that responded but were not caught), and 8.6 ± 10.8 min in undisturbed males ($n = 12$, including three that responded but were not caught, $U = 71.5$, $p = 0.44$).

Discussion

An especially useful approach for testing the idea that a particular transient, non-destructive human activity disturbs wildlife has been to simulate the putative disturbance and measure the responses of the target animal (Fowler 1999, Walker et al. 2005). For example, experimentally increasing the number of human visits from one to twelve per hour eliminated site use by Iberian frogs (*Rana iberica*, Rodríguez-Prieto and Fernández-Juricic 2005). Similarly, simulated tourist foot traffic elicits a physiological stress response in naïve Magellanic penguins, but not in penguins from a tourist area (Walker et al. 2005). A recent study employed experimental off-road vehicle traffic, revealing that it had no effect on the survival of flat-tailed horned lizards (*Phrynosoma mcallii*) hibernating just below the soil surface (Grant and Doherty 2009). As these studies illustrate, experimental disturbances have the advantage of exposing animals to a known “dose” of a known disturbance, rather than relying on comparing animals inhabiting sites that are thought to differ in exposure to a variety of human activities (e.g., animals in protected and unprotected habitat).

We tested the hypothesis that continuous human presence causes stress in free-living birds and therefore may contribute to population declines. Our primary goal was to expose vireos and warblers to a human on foot as continuously and as closely as possible for approximately one hour. In short, we aggressively chased the birds around their territories. After the chase period, we captured the focal bird and immediately sampled the blood for plasma concentrations of the stress-related hormone corticosterone. As in other birds (Wingfield and Romero 2001), corticosterone concentrations increase quickly in response to an acute threat in vireos (Study 1, this report) and warblers (Study 3, this report) breeding at Fort Hood. Thus, if exposure to an hour of human presence causes stress in these species, then chased birds should have higher plasma corticosterone concentrations than undisturbed conspecifics.

Our secondary goal was to compare the response of the black-capped vireo to the response of the white-eyed vireo, a common and widespread bird with similar habits and habitat preferences as the black-capped vireo (Grzybowski 1995, Hopp et al. 1995). However, unlike black-capped vireos, white-eyed vireos can be found in back yards and on golf courses near our field site (Luke Butler, personal observation), suggesting a relative insensitivity of white-eyed vireos to transient human exposure. Thus, if sensitivity to human activity has contributed to the endangered status and limited range of black-capped vireos, then black-capped vireos should exhibit a greater response to human exposure than white-eyed vireos. Finally, we predicted that golden-cheeked warblers would be as sensitive to human exposure as black-capped vireos, because golden-cheeked warblers have a very small range (Ladd and Gass 1999) and exhibit altered corticosterone concentrations in habitat disturbed by humans (Butler et al., in review), suggesting possible sensitivity to human exposure.

We conducted this study primarily because black-capped vireos and golden-cheeked warblers breed in large numbers at Fort Hood, where many individuals of these endangered species are likely to encounter people on foot during the breeding season (Hayden et al. 2001). In addition to military personnel participating in training activities, vireos and warblers are

managed and heavily studied at Fort Hood (Greene 2006). Contrary to prediction, an hour of human pursuit caused no significant increase in plasma corticosterone concentrations in black-capped vireos or golden-cheeked warblers, or in the common white-eyed vireo. All three species responded to the acute stress of capture with a large corticosterone increase, so the lack of a response to our directed disturbance was not due to a general lack of a hormonal response to perceived threats. Based on our findings, we conclude that an hour of relatively constant exposure to a person on foot causes no clear physiological stress response in these three migratory birds.

Our results agree with similar studies of human disturbance in White-eyed and black-capped vireos. We found as reported for Study 8 that human pursuit caused only a brief initial spike in heart rate (a measure of the “fight-or-flight” response) in white-eyed vireos breeding at Fort Hood, and heart rate was otherwise similar before, during, and after the pursuit. Results reported from Study 1 found that a human standing 1-2 m from active nests caused no increase in corticosterone concentrations in Black-capped or white-eyed vireos, although adults exhibited a strong behavioral response. Interestingly, the corticosterone response to 30 min of restraint was suppressed in black-capped vireos, similar to the suppressive effect of human pursuit on the response to restraint found in black-capped vireos in this study. The mechanism for this effect is unknown, although exposure to one stressor is known to alter the response to a subsequent stressor in other species (Dallman et al. 1992). Neither corticosterone concentrations nor male response to conspecific song playback were altered by human pursuit in golden-cheeked warblers, which could be because the taller habitat of warblers allowed them to achieve a greater distance from their pursuers, or because of species differences in sensitivity to human disturbance. Overall, we found only weak and mixed evidence that endangered songbirds are especially sensitive to human activity.

Our results contrast with similar studies of Magellanic penguins, in which one observer walked toward a nesting adult and stood ~ 1 m away for 5-15 min before sampling corticosterone (Fowler 1999, Walker et al. 2006). Whereas Magellanic penguins increased plasma corticosterone significantly in response to this disturbance (Fowler 1999, Walker et al. 2006), the three songbirds in our study showed no response to a very similar disturbance. This contrast likely reflects the ability of birds in our study to fly away from the disturbance, thus controlling their perceived risk. The importance of risk control for eliciting a stress response was demonstrated clearly by Cockrem and Silverin (2002): exposure to a predator decoy caused a much smaller corticosterone increase in great tits if they could control their distance from the decoy than if they were trapped with the decoy in an aviary.

All three species in this study responded to the acute stress of capture with a large increase in plasma corticosterone, so the lack of a response to our directed disturbance was not due to a general lack of a hormonal response to perceived threats. Also, it seems unlikely that our experimental disturbance elicited a corticosterone response early in the 60-min chase period, but that corticosterone concentrations returned to pre-disturbance concentrations before we sampled corticosterone from the focal birds. Breeding songbirds are able to maintain high, stress-induced corticosterone concentrations for at least an hour (Romero et al. 1998, Romero and Romero 2002), and white-eyed vireos captured after only 30 min of disturbance had corticosterone concentrations that were similar to undisturbed controls.

Summary

Wild animals are increasingly confronted with brief and unpredictable exposure to humans in their habitat, but we know little about how these exposures alter behavior and physiology in all but a few species. We investigated the adrenocortical response to human exposure in two endangered songbirds, the black-capped vireo and the golden-cheeked warbler, and one common songbird, the white-eyed vireo. Focal birds were pursued for up to an hour by one (vireos) to three (warblers) people on foot, and then captured and immediately sampled for corticosterone. Although human pursuit clearly altered the behavior of the focal birds, our disturbance caused no significant increase in plasma corticosterone concentrations in any species. Our results suggest that a relatively short, one-time disturbance by a small number of people is unlikely to cause physiological stress in songbirds breeding in shrubby and wooded habitats.

Study 3: Effect of road density on stress physiology of an endangered, old-growth-dependent songbird a common habitat generalist

Male response to playback

We chose sample sites based on habitat requirements for golden-cheeked warblers. To verify that white-eyed vireos were also common in these areas, and that we were not obviously sampling in poor vireo habitat, we examined the proportion of sites where we attracted both a golden-cheeked warbler and a white-eyed vireo to our mist net using conspecific playback (Fig. 15). We played male song of both species (sequentially) at 32 sample points that attracted at least one warbler to the net. At least one white-eyed vireo was also attracted to the net at 31 (97%) of those points, so it appeared that white-eyed vireos readily occupied golden-cheeked warbler sites for breeding.

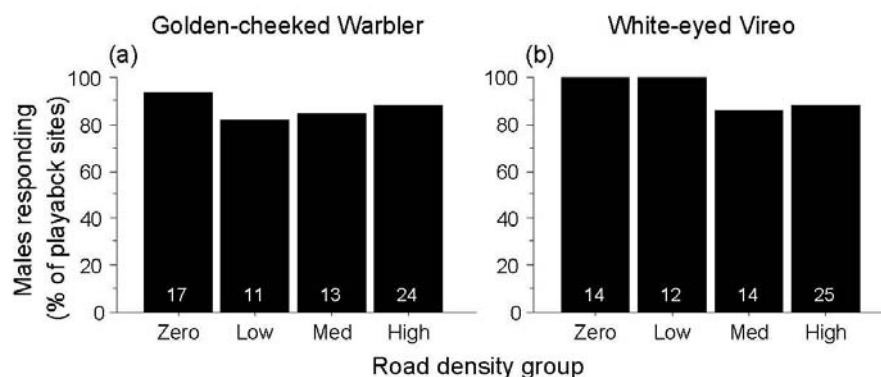


Figure 15. Percent of male (a) golden-cheeked warblers and (b) white-eyed vireos responding aggressively to conspecific song playback by road density categories. Numbers above road density labels indicate sample sizes.

We also wanted to verify that our sites with roads were not poor habitat that was strongly avoided by males of either species. To do this, we compared the frequency of males that responded to playback (i.e., were heard or seen < 50 m from the playback speakers) across road

density groups, under the assumption that very poor habitat would be less likely to hold a territorial male. Male response to playback was >80% in all road groups. Road group categories are shown in Fig. 16. There was no significant difference in the frequency of responding males among road groups in either species (Fig. 15, warblers, $\chi^2 = 1.2$, $df = 3$, $p = 0.77$; vireos, $\chi^2 = 3.7$, $df = 3$, $p = 0.30$).

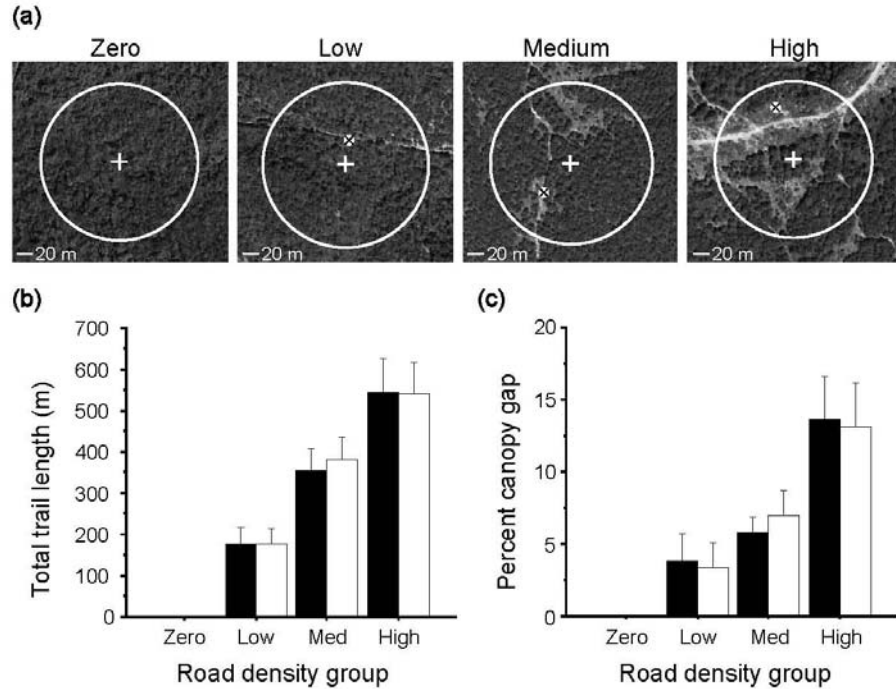


Figure 16. Aerial images (a) of four road density categories as defined by the length and width of roads within 100 m (open white circle) of the sample point (+). Dark gray areas represent juniper forest (golden-cheeked warbler habitat), medium gray areas represent grasses and shrubs, and light gray areas represent bare ground. Low, Medium, and High panels also show the location of the nearest point count (x) used to estimate warbler density. (b) Total trail and road length (mean $\pm$ 95% CI) and (c) percent canopy gap (mean $\pm$ 95% CI) among the sites where golden-cheeked warblers (filled bars) and white-eyed vireos (open bars) were sampled.

Physiological effects of roads

Baseline corticosterone concentrations differed significantly among road density groups in male golden-cheeked warblers (Fig. 17a, $F_{3,45} = 3.3$, $p = 0.029$), with Medium- and High-roads males having significantly lower corticosterone than Zero-roads males ($p = 0.027$ and $p = 0.006$, respectively). In contrast, road density had no effect on baseline corticosterone concentrations in white-eyed vireos (Fig. 17b, males only: $F_{3,39} = 1.4$, $p = 0.25$; sexes combined: $F_{3,44} = 1.4$, $p = 0.26$).

Warblers and vireos responded to the acute stress of capture with a similarly large increase in corticosterone across road density groups (Fig. 17c,d, warblers, $F_{3,43} = 0.1$, $p = 0.95$; vireos, males only: $F_{3,39} = 0.6$, $p = 0.62$; sexes combined: $F_{3,44} = 0.7$, $p = 0.54$), indicating that roads did not affect adrenal sensitivity to stress in either species. Likewise, white-eyed vireos

responded to exogenous ACTH similarly across road groups (Fig. 17d, males only: $F_{3,36} = 1.4$ $p = 0.27$; sexes combined: $F_{3,41} = 0.6$, $p = 0.60$).

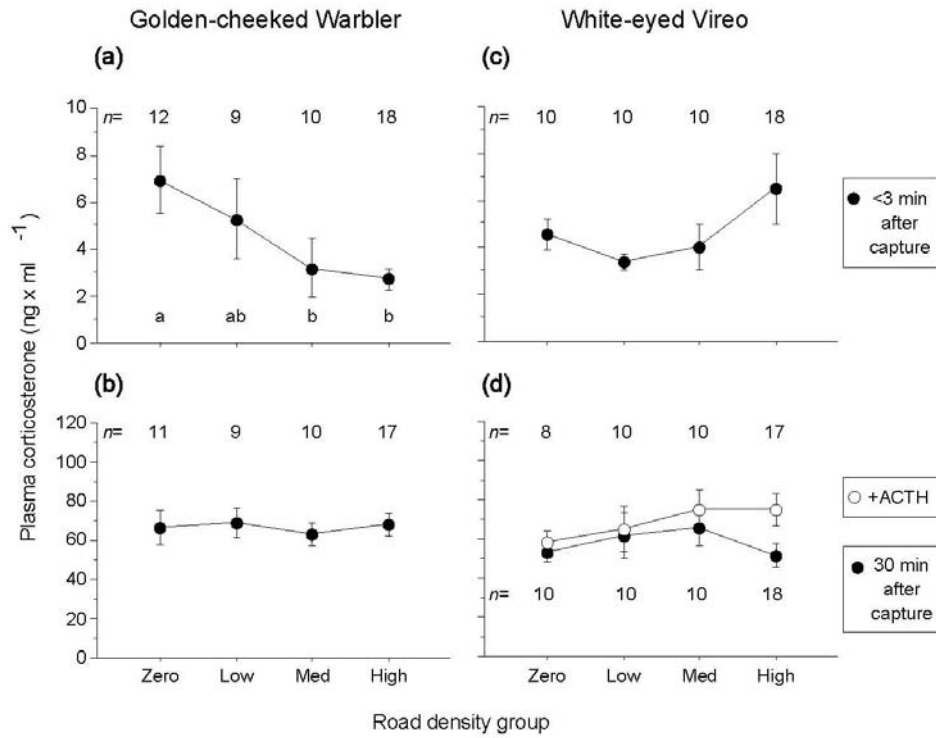


Figure 17. Plasma corticosterone concentrations (mean $\pm$ SE) in (a, b) golden-cheeked warblers and (c, d) white-eyed vireos according to road density in the surrounding habitat. Blood was sampled (a, c) within 3 min of capture, (b, d) 30 minutes after capture, and (d) 15 min after injection with adrenocorticotrophic hormone (ACTH). Road groups that share the same letter are not significantly different. Samples sizes shown (a-c) above each point or (d) below 30 min samples and above ACTH samples.

Body mass and subcutaneous fat score, were similar across road density groups in warblers (Fig. 18a,c, body mass, $F_{3,40} = 2.1$, $p = 0.11$; fat score, $F_{3,45} = 0.3$, $p = 0.83$) and vireos (body mass, males only: $F_{3,35} = 1.8$, $p = 0.16$; Fig. 18b sexes combined $F_{3,40} = 1.4$, $p = 0.25$; fat score, males only: $F_{3,40} = 1.1$, $p = 0.37$; Fig. 18d, sexes combined $F_{3,45} = 1.3$, $p = 0.30$). Residual body mass was uncorrelated with corticosterone concentrations in warblers (baseline, $r = -0.24$, $p = 0.10$; 30-min, $r = 0.01$, $p = 0.94$), and only declined significantly with 30-min corticosterone concentrations in vireos (baseline, $r = -0.07$, $p = 0.67$; 30-min, $r = -0.40$, $p = 0.009$; 45-min, $r = -0.07$, $p = 0.68$).

Demographic effects of roads

The proportion of first-year male warblers (i.e., younger subordinates) was not significantly different among the four road density categories (Fig. 19a, $\chi^2 = 4.4$, $df = 3$, $p = 0.22$), although young males were greater than three-fold more common in the Medium- and High-roads sites (32.1% of 28 males captured at Medium and High sites) compared to the Low- and Zero-roads sites (9.5% of 21 males captured at Low and Zero sites). This two-group contrast

was marginally non-significant ($\chi^2 = 3.5$, $df = 1$, $p = 0.06$). Vireos showed no significant relationship between road density and male age ratio (Fig. 19b, $\chi^2 = 1.5$, $df = 3$, $p = 0.69$).

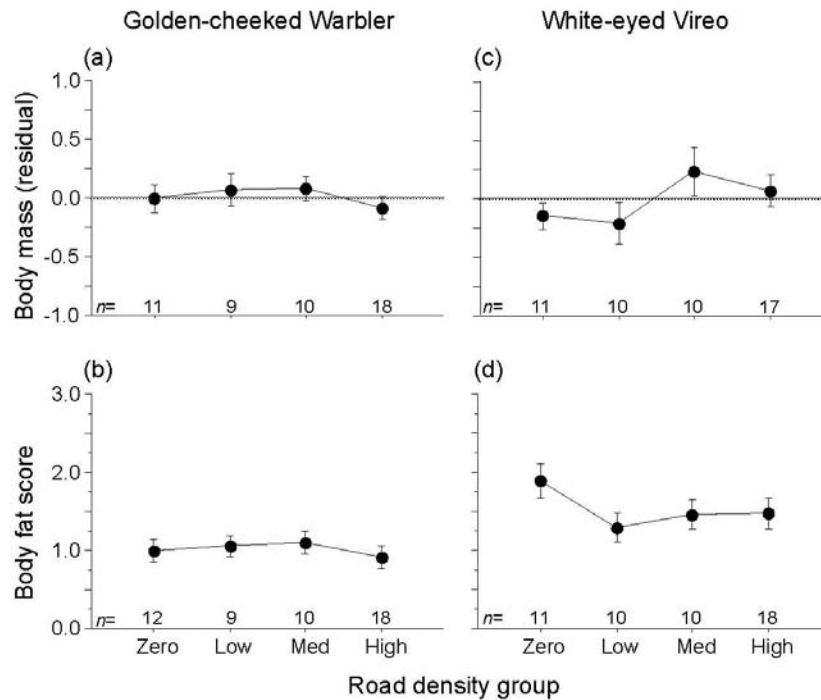


Figure 18. Residual body mass (a, b) and (c, d) body fat score in (a, c) golden-cheeked warblers and (b, d) white-eyed vireos according to road density in the surrounding habitat.

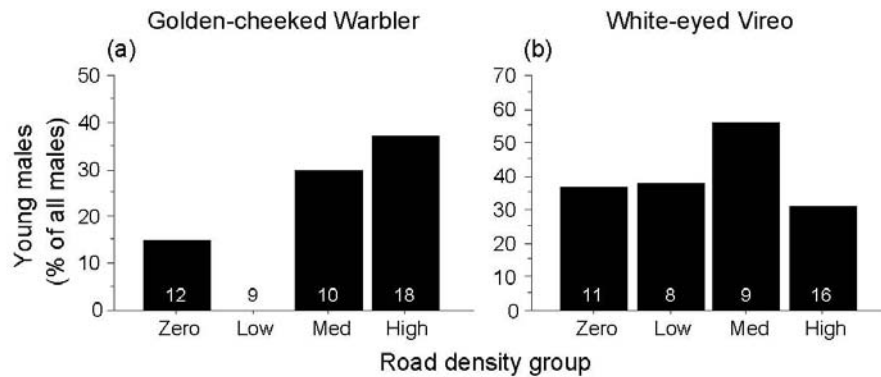


Figure 19. Frequency of young (i.e., in their first breeding season) male (a) golden-cheeked warblers and (b) white-eyed vireos according to road density group. Numbers above road density labels indicate sample sizes.

Baseline corticosterone vs. warbler density

The slightly lower proportion of first-year males in Zero-roads sites suggests the possibility that the preference of golden-cheeked warblers for large contiguous patches of forest for breeding (Ladd and Gass 1999) results in greater male-male competition for sites with fewer roads. Thus, the higher baseline corticosterone in males captured at Zero-roads sites could reflect a response to increased challenges from territorial intruders (Landys et al. 2007). To test this possibility *a posteriori*, we compared baseline corticosterone to warbler density. If male-male competition causes baseline corticosterone to increase, then male density and baseline corticosterone should be positively correlated. Density was estimated based on the mean number of detections made during two, 10-min point counts conducted yearly during the period from March – May 2002 – 2005 and 2008. The data from these five years should provide an overall estimate of the quality of the territories, with the better quality territories having a higher average warbler detection rate during point counts. Contrary to prediction, baseline corticosterone was unrelated to our density estimate in golden-cheeked warblers (Fig. 20, $r^2 = 0.001$, $p = 0.86$, $n = 49$).

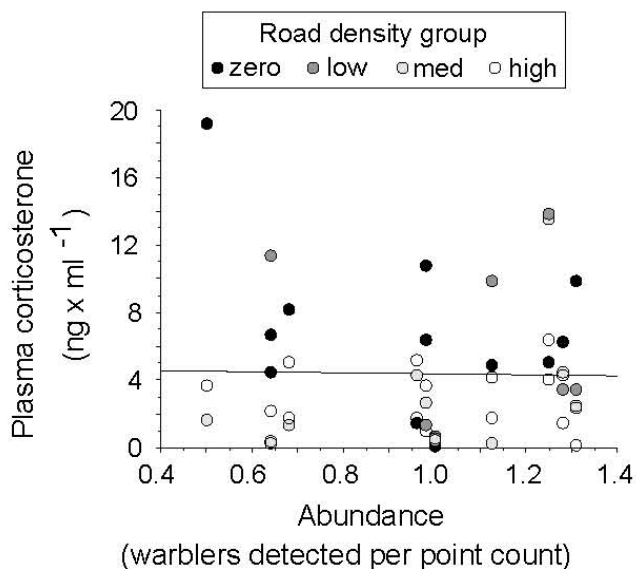


Figure 20. Baseline plasma corticosterone of male golden-cheeked warblers in relation to density of warblers as estimated by repeated point count surveys conducted yearly from 2002-2005, and 2008.

Discussion

Degradation and loss of habitat are major threats to animals worldwide (Wilcove et al. 1998, Yiming and Wilcove 2005), particularly in species that specialize on climax communities requiring many years to regenerate (Walters 1991, Gutiérrez et al. 1995). Road construction is one of the earliest, most common, and most enduring forms of human-caused habitat degradation and loss: for example, the United States is transected by more than 6×10^6 km of roads, with an average density of 0.75 km of road per km² of land (National Research Council 1997). Beyond replacing existing habitat with something much different, roads affect habitat hundreds of meters away, for example by causing erosion, introducing salts and heavy metals, creating barriers to movement, and spreading exotic plants (Forman and Alexander 1998, Forman and Deblinger

2000, Trombulak and Frissell 2000). Roads directly affect the ecology of 15-20% of land area in the USA (Forman and Alexander 1998). Thus, the effects of roads on animal populations are a major conservation concern.

Roads may negatively impact animals through direct mortality (Erritzoe et al. 2003, Mineau and Brownlee 2005), or individuals may avoid roads if they perceive that habitat quality near roads is reduced. In birds, roads have been associated with altered productivity (Kuitunen et al. 2003), altered demography (Mumme et al. 2000), decreased abundance (van der Zande et al. 1980, Forman and Deblinger 2000), decreased occupancy (Brotons and Herrando 2001), and decreased species richness (Findlay and Houlihan 1997). While useful for identifying important consequences of roads on whole populations, such measures may not reflect the mechanisms operating on individual animals that underlie altered distributions and population declines. Furthermore, measures such as abundance can be misleading indicators of habitat quality (Van Horne 1983). Instead, behavioral (Harris and Reed 2002, Laurance 2004) and physiological (Homan et al. 2003, Munshi-South et al. 2008) effects of roads have been used to illustrate effects on individuals in a variety of taxa. These measures can provide a more immediate indication of an effect of roads on species of concern (Wikelski and Cooke 2003), because behavior and physiology can change rapidly in response to environmental change. In contrast, tracking population trends required long-term data and effects may not be seen for years or decades (Brooks et al. 1999).

The major question in this study was whether roads affected the physiology and demography of the golden-cheeked warbler, an endangered songbird that prefers to breed in large tracts of old-growth forest (Ladd and Gass 1999). We also asked whether roads similarly affected the white-eyed vireo, a common songbird that with no preference for contiguous mature forest (Hopp et al. 1995). Within each species, we tested the prediction that roads alter baseline and acute stress-induced glucocorticoid concentrations. We also tested the prediction that roaded habitat is energetically-costly during breeding, by comparing body mass and fat stores to road density. Finally, we tested the prediction that each species would exhibit a preference for roadless habitat, as indicated by a higher frequency of dominant, older males holding territories in roadless areas (Holmes et al. 1996). By comparing effects of roads between species, we also tested the hypothesis that habitat sensitivity is related to differential responses to the presence of roads.

Whereas road density had no effect on vireos, baseline corticosterone concentrations were significantly lower in warbler males breeding in heavily roaded areas than in warbler males breeding in roadless areas. Lower corticosterone is often assumed to indicate lower stress because corticosterone typically increases rapidly in response to an acute threat (Sapolsky et al. 2000, Wingfield and Romero 2001). Paradoxically, long-term stress can have the opposite effect. Several recent observational studies (Romero and Wikelski 2002, Homan et al. 2003, Munshi-South et al. 2008) and controlled experiments (Rich and Romero 2005, Walker et al. 2006, Cyr and Romero 2007, Dickens et al. 2009) have shown that long-term exposure to stressful stimuli can result in suppressed, rather than elevated glucocorticoid concentrations. Males breeding at our Texas study site arrived from their wintering grounds beginning in mid-March, so they would have been exposed to any road-related stressors for several days or weeks prior to capture. Therefore, our data suggest that breeding near roads may cause chronic stress in male golden-cheeked warblers. Importantly, we found no effect of road density on white-eyed vireos breeding in the same location, suggesting a special sensitivity of golden-cheeked warblers to this form of habitat alteration.

The altered stress physiology of warblers exposed to roads suggests important fitness consequences. First, if, as we believe, their suppressed baseline corticosterone is a consequence of chronic stress (Rich and Romero 2005, Cyr and Romero 2007), then warblers breeding near roads may experience other pathologies related to chronic stress but not measured in our study, such as reproductive and immune system suppression (Sapolsky et al. 2000, Wingfield and Romero 2001). Second, regardless of the process that caused it, low baseline corticosterone may create its own physiological problems. Altered baseline corticosterone concentrations may interfere with the important roles of glucocorticoids in regulating blood glucose (Dallman and Bhatnagar 2001), feeding behavior (Dallman et al. 1993), and the immune system (Wiegers et al. 1993). Furthermore, at baseline concentrations, corticosterone binds to corticoid receptors in the brain that are involved with interpreting and responding to environmental information (de Kloet et al. 1998). Therefore, by altering baseline glucocorticoids, roads may have negative physiological effects on golden-cheeked warblers, even if they do not cause chronic stress.

Demonstrating how edge affects interior species is often easier than understanding why it affects them, and this was the case in our study. The relationship between road density and warbler corticosterone concentrations was independent of human activity, because most roads were rarely, if ever, used by people during our study. This suggests warblers were responding to ecological changes rather than to disturbance caused by road-related human activity, such as vehicle traffic or people on foot. These ecological edge effects could include micro-climatic alterations, changes in resources, or an increase in potential predators or competitors (Ries et al. 2004). Road density was unrelated to warbler body mass and body fat stores, so it seems unlikely that roads reduced warbler food availability, although warblers in roaded areas may have spent more time foraging in order to maintain their body weight. Free-living European starlings exposed to experimental chronic disturbance also had low baseline corticosterone but similar body condition compared to non-stressed starlings (Cyr and Romero 2007), so low body condition does not always reveal chronic psychological stress. White-eyed vireos, which have similar food habits to golden-cheeked warblers, also showed no body mass effect of roads, further weakening the hypothesis that warblers were affected by a road-related food shortage. An alternative hypothesis, that warblers increased corticosterone in response to higher territorial competition (Silverin 1998, van Duyse et al. 2004, Landys et al. 2007) in roadless areas, was rejected because corticosterone showed no increase over a 2.5-fold increase in male density (Fig. 20).

Edge-induced predator pressure may explain suppressed baseline corticosterone in golden-cheeked warblers. Similar to other birds (Paton 1994, Chalfoun et al. 2002), golden-cheeked warblers suffer increased nest predation as forest edge increases (Peak 2007). Texas Ratsnakes (*Elaphe obsoleta*) are the main predator on golden-cheeked warbler nests at Fort Hood (Stake et al. 2004, Reidy et al. 2008). Forest edge attracts ratsnakes at Fort Hood (Sperry et al. 2009), probably because forest adjacent to open ground provides proximate contrasting microclimates that enable efficient thermoregulation (Blouin-Demers and Weatherhead 2001, 2002, Carfagno and Weatherhead 2006). Crows and jays (corvids) are the second-most prevalent predators on warbler nests at Fort Hood (Stake et al. 2004, Reidy et al. 2008), and corvids are more common in more fragmented landscapes (Donovan et al. 1997, Ibarzabal and Desrochers 2004). In addition to threatening nests of small birds, both ratsnakes (Ernst and Ernst 2003) and corvids (Curry et al. 2002, Verbeek and Caffrey 2002) pose predatory threats to adults. Breeding birds are highly sensitive to predation risk (Ghalambor and Martin 2000, 2001, Fontaine and Martin 2006), and predatory threats to nests and adults can alter adult stress

hormones (Silverin 1998, Scheuerlein et al. 2001, Cockrem and Silverin 2002, Cyr and Romero 2007). Thus, low baseline corticosterone in warblers breeding in roaded areas may reveal chronic stress (Rich and Romero 2005, Cyr and Romero 2009) caused by more frequent and unpredictable exposures to multiple and different types of predators. It is critical to note that we are suggesting only that high risk of predation in multiple forms may be chronically-stressful, and not that predation threats, which are a routine part of life for wild animals, are chronically-stressful in general. In other words, narrow roads transecting otherwise high-quality, contiguous forest may present an ecological condition that golden-cheeked warblers are not adapted to cope with (Eggers et al. 2005). Indeed, differing effects of abnormally-high and routine predation risk would explain why white-eyed vireos, which are adapted to breeding in edge habitat, and suffer high nest failure due to predation (Hopp et al. 1995, Luke Butler, unpublished data), showed no effect of increased road density in this study.

By demonstrating an effect of roads on the stress physiology of breeding adult golden-cheeked warblers, this study broadens our knowledge of the effects of habitat fragmentation on forest songbirds. Corticosterone concentrations in particular are sensitive to a variety of forms of human disturbance across a broad diversity of taxa (e.g., Wasser et al. 1997, Fowler 1999, Creel et al. 2002, Romero and Wikelski 2002, Suorsa et al. 2003, Mullner et al. 2004, Lucas et al. 2006, Munshi-South et al. 2008, Thiel et al. 2008), arguing for their utility as a management tool for conservation biologists (Romero 2004, Cockrem 2005). Our study highlights two considerations when applying and interpreting glucocorticoid concentrations to stress-related conservation questions. First, higher corticosterone is not always found in animals exposed to presumably more stressful conditions. This has been demonstrated before in birds experimentally (Rich and Romero 2005, Cyr and Romero 2007), and it was also the case in this study, and has been shown in ground squirrels exposed to increased predation risk (Mateo 2007), elephants exposed to an industrial activity (Munshi-South et al. 2008), and salamanders breeding in disturbed habitat (Homan et al. 2003). Second, it is critical to control for pre-capture stress as much as possible when obtaining baseline samples, because baseline and stress-induced glucocorticoid concentrations do not always covary. In this study, differences between road density groups may have been missed if the birds had hung in the mist nets for more than even a few minutes before the blood sample was taken (Romero and Romero 2002).

This is the first study we are aware of that demonstrates a relationship between habitat fragmentation and plasma corticosterone concentrations in a breeding bird. Importantly, whereas roads affected an interior and old-growth dependent songbird, roads did not affect a sympatric songbird without a preference for interior habitat. Furthermore, just a 7% canopy gap, in otherwise suitable forest, and irrespective of human activity, resulted in suppressed corticosterone in the interior-dependent species, suggesting potentially long-term consequences of even narrow, unpaved road construction, and even in secluded forest patches where human activity is an unlikely occurrence (Ingelfinger and Anderson 2004). These results suggest that interior birds may be especially sensitive to seemingly minor habitat degradation, a conclusion supported by other studies demonstrating effects of minor habitat alteration on nest survival (Griesser et al. 2007) and movement patterns (Harris and Reed 2002).

Summary

Roads are a widespread and growing form of habitat alteration, making their effects on wildlife a major conservation problem. We investigated the effects of roads on the stress physiology, body condition, and demography of the golden-cheeked warbler, an endangered

songbird that depends on old-growth forest for breeding. We compared those effects in the white-eyed vireo, a common and widespread songbird that breeds in a wide variety of habitats. Warblers breeding in heavily-roaded habitat exhibited significantly lower baseline concentrations of the stress-related hormone corticosterone, typical of chronically-stressed birds. In contrast, baseline corticosterone concentrations in vireos were unrelated to road density. Stress-induced corticosterone concentrations and body condition were unrelated to road density in either species. Young, subordinate males were more common among warblers breeding in heavily-roaded habitat (although this result was marginally non-significant), suggesting a preference by male warblers for areas with fewer roads. However, baseline corticosterone concentrations were unrelated to warbler density, rejecting the hypothesis that male-male competition for territories caused increased corticosterone in warbler males breeding in roadless areas. Our results suggest that an endangered bird that depends on old-growth forest for breeding is more susceptible to chronic stress due to road-related habitat degradation than a common bird breeding in the same location.

Study 4: Effect of habitat and cowbird parasitism on stress physiology of endangered, declining, and common bird species

CORT response

Sample locations, dates, and numbers of individuals sampled by species are presented in Table 2. Intraassay variation was 3.9% for vireos, 4.5% for buntings, and 1.2% for cardinals. Interassay variation was 17.6% for vireos. Cardinal and bunting samples were assayed separately in one assay for each species. Sample site had no significant effect on baseline (within 3 min of capture) and stress-induced (30 min after capture) corticosterone concentrations in all species (Table 3, Figs. 21-24), and mean differences between east-site and west-site black-capped vireos (Fig. 21) paralleled mean differences between east-site and west-site white-eyed vireos (Fig. 22). Although the lush habitat along Cowhouse Creek differed greatly from the juniper woods and donut habitat sites, Cowhouse Creek painted buntings had intermediate baseline and stress-induced corticosterone concentrations (Fig. 23).

Table 2. Dates and locations of corticosterone sampling conducted at Fort Hood, Texas, and number of samples of each sex within each species.

Species	Location	Males	Females
Sample dates	(habitat description)	(n)	(n)
Black-capped vireo			
25 Jun – 29 Aug 2006	East Fort Hood (juniper woods atop Owl Creek Mountains)	11	2
26 Jun – 28 Aug 2006	West Fort Hood (donut habitat and oak scrub near Royalty Ridge)	8	2
White-eyed vireo			
1 Jul – 23 Aug 2006	East Fort Hood (juniper woods atop Owl Creek Mountains)	32	8
7 Jul – 28 Aug 2006	West Fort Hood (donut habitat and oak scrub near Royalty Ridge)	22	5

Painted bunting				
26 Jun – 1 Jul 2008	East Fort Hood (juniper woods atop Owl Creek Mountains)	8	0	
21 Jun – 7 Jul 2008	West Fort Hood (donut habitat and oak scrub near Royalty Ridge)	8	0	
4 Jul – 5 Jul 2008	Cowhouse Creek (lush riparian thickets on east Fort Hood)	8	0	
Northern cardinal				
23 Jun – 8 Jul 2007	East Fort Hood (juniper woods atop Owl Creek Mountains)	3	2	
20 Jun – 9 Jul 2007	West Fort Hood (donut habitat and oak scrub near Royalty Ridge)	6	1	
24 Jun – 28 Jun 2007	North Fort Hood (deciduous upland shrubs and woods)	5	4	

Table 3. Comparisons of plasma corticosterone concentrations of four songbird species breeding at Fort Hood, Texas, in relation to capture site (east side juniper woods or west side donut habitat in all species; also east side riparian woods in buntings and north side shrubland in cardinals) and time after capture (< 3 min and 30 min).

Species Factor	Groups <i>df</i>	Error <i>df</i>	<i>F</i>	<i>p</i>
Black-capped vireo				
Site at Fort Hood	1	21	0.2	0.66
Time after capture	1	21	34.8	<0.001
Site at Fort Hood * Time after capture	1	21	0.3	0.57
White-eyed vireo				
Site at Fort Hood	1	66	1.0	0.32
Time after capture	1	66	115.8	<0.001
Site at Fort Hood * Time after capture	1	66	1.7	0.20
Painted bunting				
Site at Fort Hood	2	21	0.7	0.50
Time after capture	1	19	36.3	<0.001
Site at Fort Hood * Time after capture	2	21	0.7	0.51
Northern cardinal				
Site at Fort Hood	2	18	0.9	0.42
Time after capture	1	18	78.8	<0.001
Site at Fort Hood * Time after capture	2	18	0.9	0.43

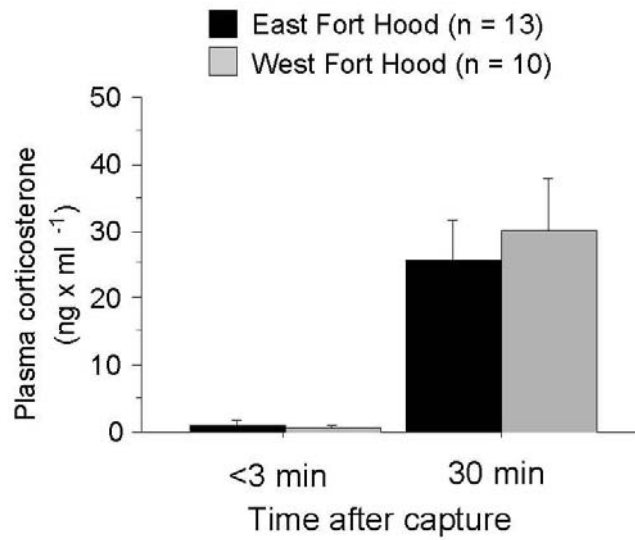


Figure 21. Plasma corticosterone concentrations (means $\pm$ SEs) of black-capped vireos captured on the Owl Creek mountains of east Fort Hood, or the donut habitat of west Fort Hood during the summer of 2006.

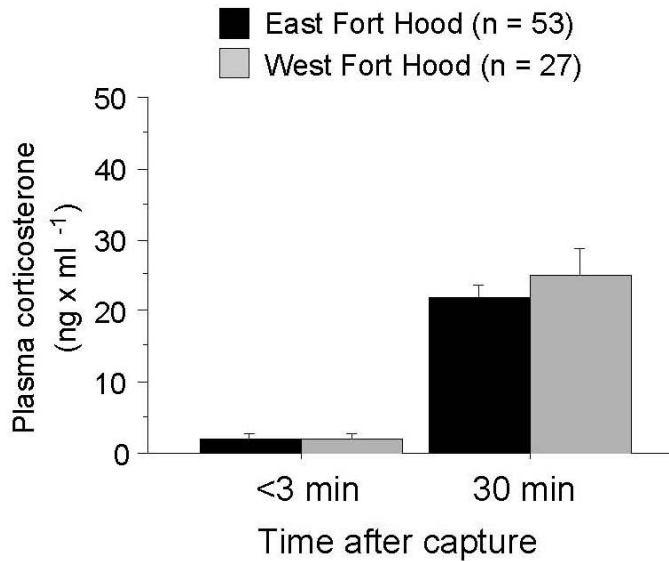


Figure 22. Plasma corticosterone concentrations (means $\pm$ SEs) of white-eyed vireos captured on the Owl Creek mountains of east Fort Hood, or the donut habitat of west Fort Hood during the summer of 2006.

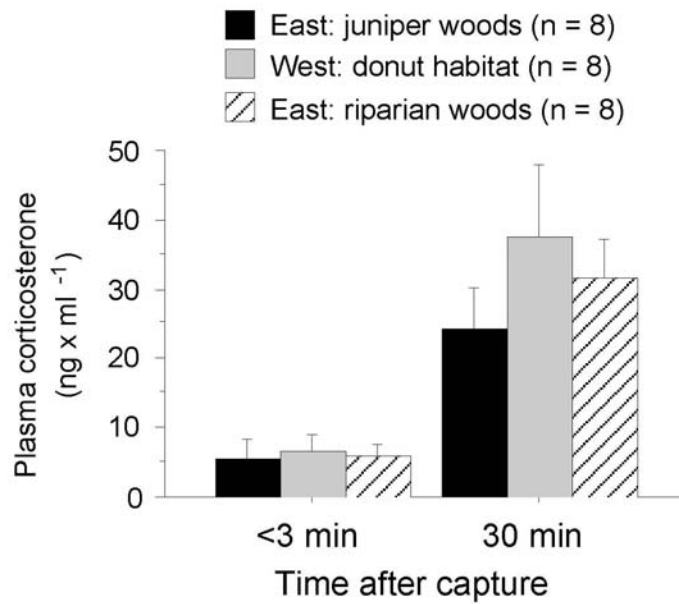


Figure 23. Plasma corticosterone concentrations (means $\pm$ SEs) of painted buntings captured on the Owl Creek mountains of east Fort Hood, or the donut habitat of west Fort Hood, or in rank riparian woods along Cowhouse Creek, during the summer of 2008.

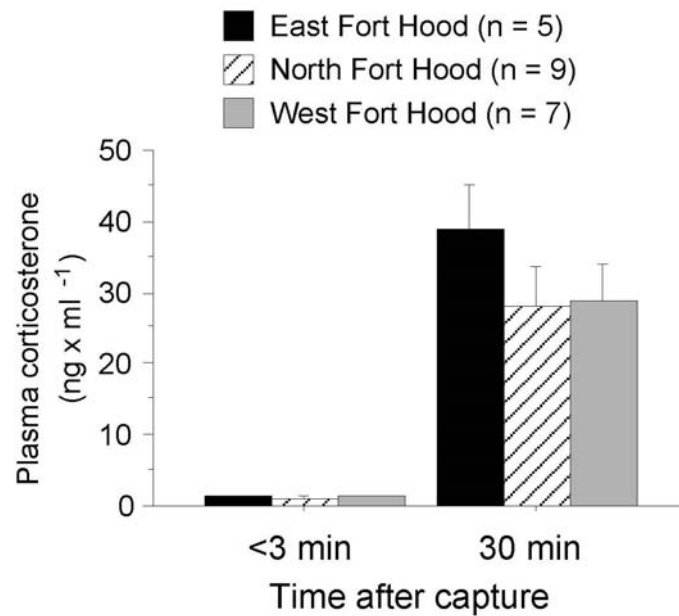


Figure 24. Plasma corticosterone concentrations (means $\pm$ SEs) of northern cardinals captured on the Owl Creek mountains of east Fort Hood, or the donut habitat of west Fort Hood, or in thick upland shrubland of north Fort Hood, during the summer of 2007

Discussion

The primary goal of this study was to compare the hormonal stress physiology of black-capped vireos and other common passerines (white-eyed vireo, northern cardinal, and painted bunting) breeding at different sites within Fort Hood, with the aim of identifying possible correlations between habitat type and physiological stress. With an area of 340 mi², Fort Hood naturally contains a wide variety of habitat types, with the east side dominated by mature juniper and mixed deciduous forest, and the west side containing large areas of anthropogenically-created scrub characterized by small scrubby clumps surrounded by patches of bare ground and grass ("donut" habitat), both of which contain substantial numbers of black-capped vireos (Cimprich and Kostecke 2006). Furthermore, the population size of the brood-parasitic brown-headed cowbird is currently controlled on the east side of Fort Hood, drastically reducing the threat of parasitism on birds breeding in the east compared to birds breeding in the west (Kostecke et al. 2007). Taking advantage of these clear ecological contrasts, we compared corticosterone concentrations in black-capped vireos breeding on east and west Fort Hood. We also included the white-eyed vireo in the study because, as a widespread congener with broad habitat preferences (Hopp et al. 1995), comparing white-eyed vireos to black-capped vireos allowed us to test the hypothesis that black-capped vireos are especially sensitive to habitat alteration.

Many other songbird species breed at Fort Hood (Kostecke 2006), including the painted bunting, a migrant that is widespread in Texas, but declining at an average rate of 3.4% per year (Sauer et al. 1997), with habitat degradation as the suspected major cause (Lowther et al. 1999). With its comprehensive wildlife management programs, Fort Hood may eventually be a refuge for this species, so we also compared corticosterone concentrations in buntings breeding on east and west Fort Hood. Finally, our study also included the northern cardinal, which allowed comparison with a very widespread and common bird that readily breeds in a very wide variety of habitats, including habitats heavily altered by human activity (Halkin and Linville 1999).

Although all species in our study responded to the acute stress of capture with a large increase in corticosterone concentrations, we found no evidence that any species in our study responded to habitat differences with an alteration of baseline or stress-induced corticosterone. We would have been surprised if corticosterone differed among sites in the common northern cardinal or the common but somewhat less human-tolerant white-eyed vireo, because those species demonstrate a wide tolerance and an ability to breed in a variety of disturbed habitats (Halkin and Linville 1999, Hopp et al. 1995). Painted buntings are declining, but they also tolerate an apparently broad variety of open wooded habitats, including habitats altered significantly by humans (Lowther et al. 1999). However, it was somewhat surprising that black-capped vireos showed no corticosterone differences between east and west Fort Hood.

Differences in the species or structure of vegetation between habitats would alone seem unlikely to alter stress physiology of breeding black-capped vireos, but east and west Fort Hood differ in important ways other than vegetation. In a demographic analysis of black-capped vireos breeding at Fort Hood, Noa et al. (2007) found that nest survival was significantly lower and cowbird parasitism was more than six times as frequent (12.3% vs. 2% of nests) in donut than in shrubland habitat. Perhaps reflecting this lower habitat quality, density of male black-capped vireo territories was lower, and the proportion of young males was higher, in donut habitat compared to shrubland (Noa et al. 2007). As a likely consequence of lower nest success, Butler et al. (2008) found that the post-breeding molt started later in west-side black-capped vireos. Thus, there is abundant evidence that east and west Fort Hood differ in important ways to the

breeding ecology of black-capped vireos, but these differences do not appear to alter the stress physiology of black-capped vireos.

The lack of an east-west difference in black-capped vireo corticosterone is also surprising because differences in ecological conditions and anthropogenic disturbance are known to alter stress physiology in a wide variety of animals in a wide variety of situations. For example, plasma corticosterone concentrations were elevated in Stonechats nesting in territories shared with a predatory shrike (Scheuerlein et al. 2001), and fecal glucocorticoid metabolites were suppressed in ground squirrels exposed to higher predation risk (Mateo 2007). Elevated fecal glucocorticoids were found in spotted owls (*Strix occidentalis*) roosting near logging activity (Wasser et al. 1997), and suppressed plasma corticosterone was found in golden-cheeked warblers breeding near roads (Butler et al. in review). Thus, a variety of animals exhibit altered stress physiology in response to the types of naturalistic and anthropogenic disturbances faced by black-capped vireos on west Fort Hood, but we found no evidence that black-capped vireos respond in a similar way.

There are at least two plausible explanations for our findings. First, although cowbird parasitism was much more common on west than east Fort Hood, cowbirds do not pose a direct survival threat to adult vireos, and threats directed solely at vireo nests (and not at adults) have little effect on adult corticosterone concentrations (Study 1). Second, we sampled vireos during an annual nadir in corticosterone concentrations, at the beginning of the prebasic molt (Luke Butler, unpublished data for vireos, also see Romero 2002). Mean corticosterone concentrations in this study were lower than samples taken on the east side of Fort Hood earlier in the breeding season (compare to results from Study 1). Thus, any effect of a suboptimal breeding site on corticosterone concentrations may have been obscured by the generally low concentrations of corticosterone during this time of year.

The demographic analysis of Noa et al. (2007) clearly demonstrates that, in terms of black-capped vireo nest survival, the donut habitat of west Fort Hood is inferior to the shrubland habitat found on other parts of the base. Butler et al. (2008) showed that west-side vireos also molt more rapidly than east-side vireos, which may reduce the feather quality, and presumably the fitness, of west-side vireos (Dawson et al. 2000). These costs were not reflected by vireo stress physiology, suggesting that changes in adrenal function do not underlie the behavioral and physiological responses to these life-history consequences of breeding in suboptimal habitat.

Summary

The adrenocortical response to stress in vertebrates is a major mechanism for coping with the environment. We compared plasma concentrations of the glucocorticoid hormone corticosterone in four species of songbirds breeding at Fort Hood: the endangered black-capped vireo and its common congener, the white-eyed vireo, the declining painted bunting, and the common and widespread northern cardinal. Within each species, we compared corticosterone concentrations between birds breeding on east Fort Hood, which is characterized by relatively contiguous juniper woodlands and relatively low abundance of the brood-parasitic brown-headed cowbird, and birds breeding on west Fort Hood, which is characterized by a heavily human-altered landscape and relatively high cowbird abundance. Breeding site had no effect on baseline or stress-induced concentrations of corticosterone in any species. This was particularly surprising for black-capped vireos, which exhibit lower density and higher nest mortality and on west than east Fort Hood. Our results suggest that the adrenocortical response to stress is not involved with coping with cowbirds or the kind of habitat alteration found on west Fort Hood.

Study 5: A meta-analysis of transmitter effects on avian behavior and ecology

Effect of device attachment

The universal analysis assessing any effect of devices showed that birds are significantly impacted by devices ($\bar{x} = -0.27$, 95% CI = -0.37 to -0.17, $n = 84$). When specific aspects of avian behavior and ecology were analyzed separately we saw a negative (detrimental) mean effect size for every aspect except flying ability, and 8 of the 12 values were significantly different from zero (Table 4). The fail-safe value for productivity (9) was lower than the number of studies, suggesting that the negative effect could be attributable to the file-drawer effect. Device attachment had a “small” effect on most aspects, except that birds with transmitters had drastically increased energetic expenditure and were much less likely to nest (Table 5). We detected no difference between foraging behaviors related to provisioning offspring and those of self-provisioning ($Q_1 = 1.23$, $p = 0.34$, $n = 38$), indicating that birds do not sacrifice self-feeding in favor of current reproduction or offspring feeding in favor of self-preservation.

Table 4. Description of each categorical characteristic of the bird or transmitter device in meta-analyses of transmitter effects. Although species in the ‘walking’ category fly during migration, studies of those species primarily used non-migrating individuals.

Characteristic	Category	Description
Attachment Type	Harness	Backpacks and harnesses
	Collar	Collars, necklaces, and pendants
	Glue	Glue and tape, whether alone or in combination with sutures, cable ties, or Velcro
	Anchor	Any method of anchoring subcutaneously
	Implant	Implanted anywhere in the body
	Breast-mounted	Attached to the breast
	Tail mount	Attached to tail
Locomotion	Walk	Shorebirds, galliforms, and rails
	Swim	Penguins
	Various	Waterfowl
	Fly	All other birds
Sex	Male	Devices on males only
	Female	Devices on females only
	Both	Devices on males and females
	Unknown	Devices on unknown sex
Age	Adult	Reproductively mature
	Juvenile	Non-reproductively mature

Table 5. The number of studies (n), estimates of Cohen's d , and 95% confidence intervals for meta-analyses of each aspect of avian ecology. Confidence intervals were obtained by bootstrapping with 999 iterations and are considered significant if they not overlapping zero.

Aspect	n	Cohen's d	95% confidence interval
Nest Success	16	-0.33	-0.59 to -0.09
Productivity	14	-0.22	-0.48 to -0.01
Clutch Size	14	-0.17	-0.31 to 0.00
Nesting Propensity	5	-0.57	-0.81 to -0.30
Nest Initiation Date	9	-0.12	-0.36 to 0.12
Offspring Quality	8	-0.42	-0.95 to 0.02
Body Condition	35	-0.38	-0.63 to -0.17
Flying Ability	7	0.27	-0.52 to 1.12
Foraging Behaviors	38	-0.26	-0.46 to -0.10
Device-induced Behaviors	18	-0.37	-0.37 to -0.12
Energetic Expenditure	13	-0.96	-1.74 to -0.32
Survival	38	-0.18	-0.28 to -0.10

Influence of bird characteristics

We found little evidence that characteristics of the bird influence its response to the device. In fact, birds were similarly affected for every aspect examined regardless of age, mode of locomotion, and body mass (Table 6). The only characteristic having any influence was sex, with birds increasing the frequency of device-induced behaviors in studies using both sexes ($\bar{x} = -0.83$, 95% CI = -1.08 to -0.38, $n = 4$), but not in studies using only females ($\bar{x} = -0.18$, 95% CI = -0.39 to 0.06, $n = 9$), only males ($\bar{x} = 0.25$, 95% CI = -0.03 to 0.55, $n = 2$), or an unknown sex ($\bar{x} = -0.62$, 95% CI = -3.86 to -0.21, $n = 3$, $p = 0.05$; Table 6). If effects on these behaviors differ between sexes, we would have expected studies using both sexes to produce an effect size between studies using only males or females. The lack of an ecological explanation for this finding, along with its marginal significance, suggests that a bird's sex has little impact on device-induced behaviors.

Influence of device characteristics

The type of attachment influenced the degree of effects for both nest success ($p = 0.05$) and device-induced behaviors ($p = 0.03$, Table 6). Birds with anchor attachment had the lowest nest success ($\bar{x} = -0.75$, 95% CI = -1.14 to -0.41, $n = 3$) followed by harness attachment ($\bar{x} = -0.33$, 95% CI = -0.99 to -0.08, $n = 5$), but glued devices caused no decrease in nest success ($\bar{x} = 0.21$, 95% CI = -0.08 to 0.55, $n = 4$). This influence on nest success, however, could be an artifact of the file-drawer effect since the fail-safe number (12) is equal to the number of studies in the analysis. Birds performed the most device-induced behaviors when wearing breast-mounted devices ($\bar{x} = -1.05$, 95% CI = -1.10 to -0.89, $n = 2$) followed by those attached with a harness ($\bar{x} = -0.51$, 95% CI = -1.16 to -0.18, $n = 7$); whereas, neither glued ($\bar{x} = -0.34$, 95% CI = -0.46 to 0.10, $n = 2$) nor implanted devices ($\bar{x} = 0.08$, 95% CI = -0.12 to 0.40, $n = 5$) caused an increase in device-induced behavior. The method of attachment had no influence on the degree of effects for the other aspects analyzed in the meta-analysis (Table 6).

Table 6. The impact of characteristics of the bird and transmitter device on aspects of behavior or ecology. The value reported for categorical characteristics is the variation in effect size explained by the model (Q), with number of categories and number of studies in parentheses. The value reported for continuous characteristics is the slope (b) $\pm$ 1 standard error, with the number of studies included in parentheses. Significant ($\alpha < 0.05$) results are designated by a single asterisk if the number of studies is similar to Rosenthal's fail-safe number and by two asterisks if Rosenthal's fail-safe number is much higher. Aspects without an entry could not be calculated because there were not two or more categories containing two or more studies.

	Nest Success	Producti vity	Clutch Size	Nestin g Propen sity	Nest Initiatio n Date	Offspri ng Qualit y	Body Condit ion	Flying Ability	Foragi ng Behavi ors	Device - induce d Behavi ors	Energeti c Expendi ture	Survival
<u>Bird</u>												
<u>Characteristics</u>												
Sex	1.62 (3, 15)	2.58 (3, 14)	2.92 (2, 14)	---	---	---	1.39 (4, 35)	---	5.24 (4, 38)	8.50** (4, 18)	0.19 (3, 12)	4.09 (4, 38)
Age	---	---	---	---	---	---	2.75 (3, 35)	---	0.02 (2, 38)	---	2.19 (2, 13)	0.62 (2, 37)
Locomotio n	3.66 (3, 15)	0.97 (2, 13)	2.93 (3, 14)	0.50 (2, 5)	---	0.19 (2, 8)	2.83 (4, 35)	0.22 (2, 7)	0.60 (4, 38)	1.37 (4, 18)	3.35 (4, 13)	0.59 (3, 38)
Body Mass	0.00 $\pm$ 0.0 0 (16)	0.00 $\pm$ 0.0 0 (14)	0.00 $\pm$ 0 .00 (14)	0.00 $\pm$ 0. 00 (5)	0.00 $\pm$ 0. 00 (9)	0.00 $\pm$ 0 .00 (8)	0.00 $\pm$ 0 .00 (35)	0.00 $\pm$ 0 .00 (7)	0.00 $\pm$ 0 .00 (38)	0.00 $\pm$ 0 .00 (18)	0.00 $\pm$ 0. 00 (13)	0.00 $\pm$ 0.0 0 (38)
<u>Device</u>												
<u>Characteristics</u>												
Attachmen t Type	8.46* (3, 12)	0.98 (3, 11)	1.19 (3, 11)	---	0.02 (2, 5)	1.01 (2, 5)	1.32 (3, 30)	0.17 (2, 5)	5.98 (5, 33)	9.9** (4, 16)	3.27 (3, 12)	2.68 (5, 34)
% Body Mass	0.07 $\pm$ 0.0 3** (16)	- 0.03 $\pm$ 0.0 6 (14)	0.01 $\pm$ 0 .05 (14)	- 0.08 $\pm$ 0. 09 (5)	- 0.14 $\pm$ 0. 09* (9)	- 0.01 $\pm$ 0 .21 (8)	0.03 $\pm$ 0 .03 (35)	0.00 $\pm$ 0 .00 (7)	0.00 $\pm$ 0 .03 (38)	- 0.07 $\pm$ 0 .07 (18)	- 0.11 $\pm$ 0. 07 (13)	0.02 $\pm$ 0.0 2** (38)

Attachment type did not influence the proportion of studies reporting physically impairment ($X^2 = 12.83$, $df = 7$, $p = 0.7$) or nest abandonment ($X^2 = 7.64$, $df = 7$, $p = 0.35$), but we did observe that the proportion of studies reporting device-induced mortality differed among attachment types ($X^2 = 29.37$, $df = 8$, $p < 0.001$). When we compared the percentage of studies reporting on mortality that had a bird die, we found that mortality was most common in studies using anchors (100%, $n = 2$), followed by implants (57%, $n = 23$), then harnesses (52%, $n = 27$), then collars (50%, $n = 6$), and finally glue (31%, $n = 13$). No mortality was reported in studies using tail mounts (0%, $n = 3$).

The effect of device attachment did not increase with increasing percent body mass for most aspects of behavior or ecology (Table 6). Birds wearing proportionally heavier devices,

however, did initiate nests later than those wearing lighter devices ($p = .03$, Table 6), but the very small fail-safe number (0) indicates little effect of proportional device mass on nest initiation date. Surprisingly, two variables showed amelioration of effects with increasing percent body mass, as birds with proportionally heavier devices had higher nest success ($p = 0.04$) and survival ($p = .05$, Table 6).

Effect of capture and restraint

Birds carrying devices showed a greater decrease in foraging behaviors when compared to uncaptured birds ($\bar{x} = -0.59$, 95% CI = -0.89 to -0.30, $n = 12$) than when compared to procedural controls ($\bar{x} = -0.02$, 95% CI = -0.26 to 0.22, $n = 24$, $Q = 10.69$, $p = 0.002$; Table 7), suggesting that birds decrease their foraging behaviors following capture and restraint. On the other hand, birds did not alter any other aspects of their behavior or ecology in response to capture and restraint (Table 7), suggesting that the negative effects described above are primarily due to device attachment.

Table 7. The effect of capture and restraint on each aspect of behavior or ecology, as calculated by contrasting studies comparing birds with devices with procedural controls and with studies using uncaptured individuals. The values reported are sample sizes (n), the variation in effect size explained by the model (Q), and randomized probability value (p). Aspects without an entry could not be calculated because there were less than two studies from one of the categories.

Aspect	n	Q	p
Nest Success	16	0.27	0.62
Productivity	14	0.20	0.72
Clutch Size	14	0.13	0.79
Nesting Propensity	---	---	---
Nest Initiation Date	9	0.16	0.76
Offspring Quality	8	0.35	0.57
Body Condition	35	2.78	0.13
Flying Ability	---	---	---
Foraging Behaviors	36	10.69	0.002
Device-induced Behaviors	18	0.03	0.89
Energetic Expenditure	13	0.42	0.56
Survival	38	0.08	0.80

Discussion

Following wild animals to monitor their activity has long been a challenge for biologists. Several decades ago, however, miniature radio transmitters were developed that allowed biologists to track free-roaming animals (Cochran and Lord 1963, LeMunyan et al. 1959). Since its initial development, this technique has become an important component of research in animal ecology. Aided by technological advancements that have produced smaller transmitters with greater battery life and signal strength, the pool of suitable species has expanded to ever-smaller animals and made the technology especially useful for studies of birds. More than 190 studies using radio telemetry on birds have been published. Growing use of telemetry in avian research makes it increasingly important that we understand how the devices affect the birds. Researchers

using radio telemetry assume that birds are unaffected by carrying the transmitters, but if this assumption is wrong, the birds could be harmed and the data collected could lead to erroneous conclusions about the populations studied and the issues addressed. Here we use a meta-analytical approach to investigate the effects of attaching devices to birds.

Clearly, researchers are aware of the potential effects of transmitter attachment. Almost 80% of the studies we reviewed addressed the topic in some fashion. Although there have been several reviews of transmitter effects on birds (e.g., Calvo and Furness 1992, Murray and Fuller 2000, Samuel and Fuller 1994), none have been comprehensive. These reviews have either evaluated transmitter effects qualitatively, or if a quantitative approach was used it relied on “vote counting” (the number of studies reporting negative effects is compared to the number reporting no effects), which ignores effect sizes (Hedges and Olkin 1980). The meta-analytical approach we use here is based upon effect sizes and allows us to explore differences quantitatively, independent of scales of measurement (Gurevitch and Hedges 1993).

Our first goal was to determine whether transmitters have any effect on birds. Secondly, we wanted to identify the specific aspects of avian behavior and ecology that were affected by transmitters. The aspects we investigated were nest success, productivity, clutch size, nesting propensity, nest initiation date, nest abandonment, offspring quality, body condition, flying ability, foraging behaviors, device-induced behaviors, energetic expenditure, survival, physical impairment, and device-induced mortality. Although not exhaustive, this list includes most important aspects that might plausibly be affected by transmitters, and for which adequate data were available. Our third objective was to identify characteristics of the birds that contributed to the transmitter effects. Characteristics considered here were sex, age, mode of locomotion, and body mass.

Our fourth goal was to identify attributes of the transmitters that contributed to their effects. We first assessed the effect of the mass of the transmitters relative to that of the birds on which they were attached. A rule of thumb is that loads weighing less than 5% of an animal's body mass have negligible effects. This “5% rule” has no empirical basis and appears to have originated from a suggestion by Brander and Cochran (1969) regarding transmitter weights. Aldridge and Brigham (1988) did assess the effect of attaching variable loads on bats and found decreased maneuverability as load mass increased. Our objective was to look for a similar effect in birds and determine if there is evidence for a threshold effect that would be consistent with the 5% rule. Lastly, we considered whether the method of transmitter attachment influences transmitter effects, because past studies that compared attachment types produced contradictory results (e.g., Garrettson and Rohwer 1998, Osborne et al. 1997, Small and Rusch 1985).

Finally, we addressed whether any negative effects were attributable only to the transmitter or whether they are partially a result of capture and restraint (Cox and Afton 1998). If birds are affected by capture and restraint, transmitters should appear to have a greater effect in studies that compare birds with transmitters to uncaptured birds than in studies that used procedural controls. Ultimately, the goal of all our analyses is to provide information that will help researchers design studies that involve attaching devices to birds in a way that will minimize negative effects on either the birds or the research.

This study provides cumulative evidence that transmitters and other devices do cause negative impacts on birds and that most aspects of avian behavior and ecology are negatively affected to some degree. The most considerable impacts were that birds with transmitters had drastically increased energetic expenditure and were much less likely to nest. Researchers may be comforted, however, that four of twelve aspects were unaffected and six were affected only to

a “small” degree, motivating little concern over generalizing estimations of these aspects from birds with devices to untagged populations.

Nest abandonment, physical impairment, and death were commonly reported in studies using devices. Our analyses suggest that although attachment type does not affect the frequency of nest abandonment or physical impairment, certain attachments are more likely to cause death. The two types with the highest percent of reported mortalities, suture and implant, require anesthesia, which highlights the risk of this procedure. Machin and Caultkett (2000) showed that anesthetizing with propofol instead of isoflurane minimizes impacts on the bird’s health and decreases the probability of nest abandonment. Harnesses and collars have the next highest mortality rates and deaths were commonly a consequence of birds becoming entangled with vegetation (36% of reported harness deaths and 33% of collar deaths). Researchers can minimize this risk by using adjustable harnesses and collars (Dwyer 1972) to custom fit each bird and by adding a weak link that allows the device to detach from the bird if entangled (Karl and Clout 1987). Unfortunately the glue and tail mount attachments, which have the lowest reported frequency of mortality, have low retention rates on many species (Woolnough et al. 2004). If long-term attachment is not required, however, they should be preferentially used.

Considering the widespread acceptance of the “5% rule” of device attachment, it is surprising that we detected little impact of proportional device mass. In fact, no aspect was negatively affected independent of the file-drawer effect and nest success and survival actually showed benefits to increasing percent body mass. This counterintuitive result cannot be explained by a life-history response where birds perceive the device as decreasing their probability of survival and therefore place extra effort into current reproduction (Trivers 1972), as that would produce the observed increase in nest success but a decrease in survival. This trend is therefore best explained by non-random sampling. Perhaps it is proportional surface area rather than proportional mass that is most important in determining a device’s effects, especially for flying and swimming birds that experience drag (Gessaman and Nagy 1988a, Obrecht et al. 1988). Researchers can minimize drag by reshaping the device, modifying its antenna, or placing it in the most caudal position (Bannasch et al. 1994, Obrecht et al. 1988, Wilson et al. 2004).

Regrettably, this meta-analysis is insufficient to properly address the “5% rule” of device attachment because its near-universal acceptance prevented studies from attaching larger devices. Indeed, only 10% of the reviewed studies exceeded this recommendation and the heaviest proportional mass used was 10%. This did not provide the variability to search for a threshold (whether 5% or a higher value) above which effects increase dramatically. Although it is possible that a device’s effect increases linearly with increasing proportional mass, our results reject this idea for relatively small devices. This topic needs further clarification by attaching a range of proportional device masses greatly exceeding 5%, although this important research may be prohibited due to permitting and animal care restrictions.

The findings of this study have strong implications for using devices on sensitive, rare, or endangered birds. Although many of the aspects investigated were minimally affected, the mean effect on almost every one was negative. The cumulative impact could be much greater than is indicated by looking at these individually. For example, the reductions in nest success, productivity, nesting propensity, and foraging behavior could combine to decrease reproductive potential. Similarly, reduced foraging behaviors, body condition, flying ability, and survival along with increased device-induced behaviors and energetic expenditure could decrease the probability that an individual endures to the following year. The combined effects on

reproduction and survival would cause a drastic decline in the fitness of device carrying birds. Device attachment could also have indirect effects on the fitness of an unmarked mate if it compensates for the decreased parental investment of the marked bird (Paredes et al. 2005), as this increase in current brood investment would decrease future reproductive potential (Trivers 1972). Our results show little effect of capture and restraint, suggesting that negative effects are solely attributable to the device. This makes it unlikely that traditional mark-recapture approaches would have similar consequences and implies that managers should carefully balance the benefits of gaining data from transmitters and other devices with the potential costs to the viability of the species.

Species from other taxa could be similarly affected by device attachment (particularly species that are small and fly) and a comparable meta-analysis would be incredibly useful in determining the universal nature of device-induced effects. Researchers using transmitters and other devices should supply the necessary information for inclusion in a meta-analysis (see methods) to ensure that this is possible.

Summary

Researchers frequently use transmitters and data loggers to collect data from free-living birds with the assumption that these devices are not harming the birds or producing biased data. Although many studies have investigated the impacts of transmitters on avian behavior and ecology, their conclusions were generally limited to a single species or type of device. To achieve a broader understanding of this topic we combined results from 84 studies into a meta-analysis that we used to explore five questions: 1) Do devices cause an overall effect on birds? 2) Which aspects of avian behavior and ecology are affected? 3) What characteristics of the birds influence effects? 4) What characteristics of the device influence effects? 5) Are effects partially a consequence of capture and restraint? We found a significant negative effect of devices on birds, both overall and for 8 of the 12 specific aspects we analyzed. The most drastic effects were that birds wearing devices expended far more energy and were much less likely to nest than birds without devices. Effects were independent of characteristics of the birds (sex, age, primary method of locomotion, body mass). We also found no evidence that the mass of the device relative to the mass of the bird contributed to effects, although researchers generally avoided using heavy devices. We did find that some methods of attachment increased device-induced behaviors such as preening at the device, whereas other attachment methods were more likely to cause mortality. The observed effects were not a consequence of capture or restraint, which suggests that traditional mark-recapture techniques would not negatively affect birds. Researchers should carefully balance the benefits of using transmitters and other devices against the potential costs to the birds and the reliability of the data obtained.

Study 6: Impact of radio transmitters on northern cardinal parental investment and productivity

Nest defense

Variable scores for nest defense trials are presented in Table 8. We found a significant effect of treatment on male nest defense ($F_{2, 271} = 10.61, p < 0.001$), but there was no difference between responses to a person vs. a model crow ($F_{1, 271} = 0.58, p = 0.45$) and the treatment effect was consistent across these threats ($F_{1, 271} = 0.32, p = 0.72$). Comparisons between treatments

confirmed that birds with transmitters defended their nests less aggressively than both no treatment ($F_{1,271} = 19.73, p < 0.001$; Fig. 25) and control birds ($F_{1,271} = 6.08, p = 0.04$; Fig. 25), but there was no difference between the control and no treatment birds ($F_{1,271} = 3.41, p = 0.20$). Females did not show a compensatory increase in nest defense when their mates had transmitters because their responses did not differ among treatments ($F_{2,271} = 0.21, p = 0.81$). The covariates age of young ($F_{1,265} = 32.09, p < 0.001$) and number of previous trials ($F_{1,265} = 10.10, p = 0.002$) significantly affected male nest defense, but not female nest defense.

Table 8. Designation of scores for each variable measured in nest defense trials. The scores of these three variables were compiled into a composite nest defense score for both the male and female parent, ranging from 0 to 15.

Variable	Value	Score
Response Duration	241-300 seconds	5
	181-240 seconds	4
	121-180 seconds	3
	61-120 seconds	2
	1-60 seconds	1
	0 seconds	0
Total Vocalizations	201+	5
	151-200	4
	101-150	3
	51-100	2
	1-50	1
	0	0
Closest Approach	0-3.9 m	5
	4-7.9 m	4
	8-11.9 m	3
	12-15.9 m	2
	16+ m	1
	Never Approached	0

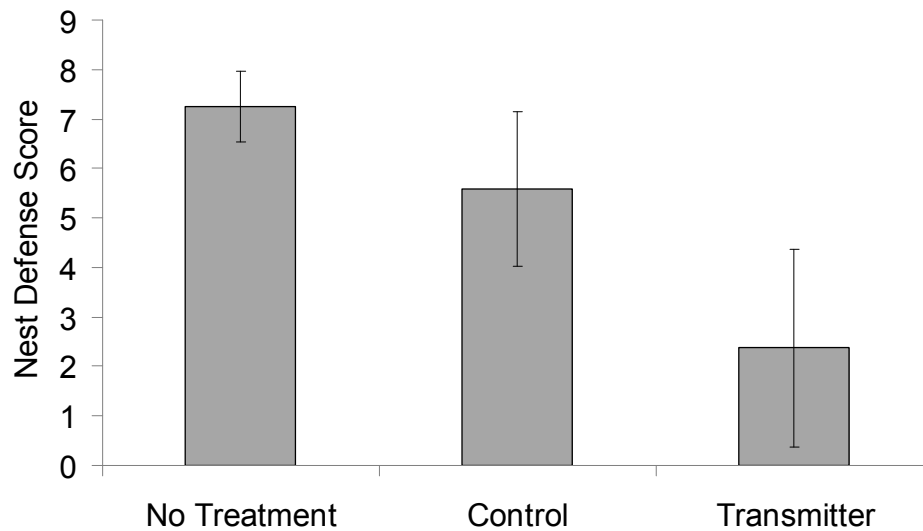
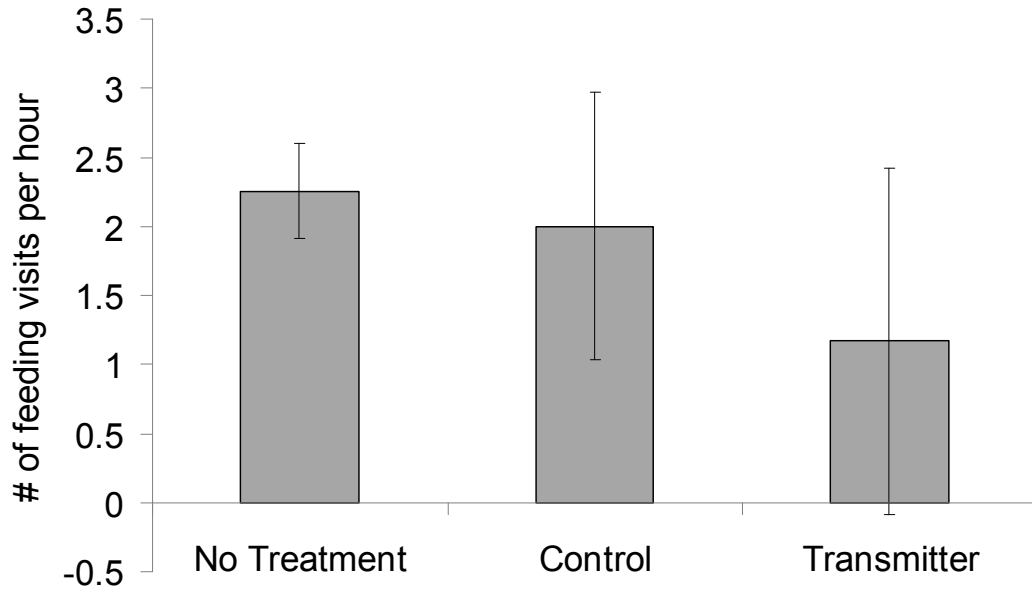


Figure 25. Mean nest defense scores ($\pm 95\%$ confidence intervals) of male cardinals from each treatment. Nest defense scores are a composite of number of vocalizations, duration of response, and closest approach. This analysis is of 276 nest defense trials (207 no treatment, 43 control, 26 transmitter) from 72 territories (56 no treatment, 8 control, 8 transmitter), with these trials divided between the crow and human nest threat.

Nestling provisioning

Males with transmitters provisioned approximately half as much as no treatment and control birds, although high predation rates during incubation limited our sample sizes and prevented us from detecting a significant effect of treatment on feeding rate ($F_{2,93} = 1.21$, $p = 0.30$; Fig. 26) or feeding score ($F_{2,91} = 1.42$, $p = 0.25$; Fig. 26). The trend of increased provisioning by mates of males with transmitters suggests that females may compensate by increasing their own feeding rate ($F_{2,93} = 1.83$, $p = 0.17$; Fig. 27) and feeding score ($F_{2,90} = 1.21$, $p = 0.06$; Fig. 27).

A.



B.

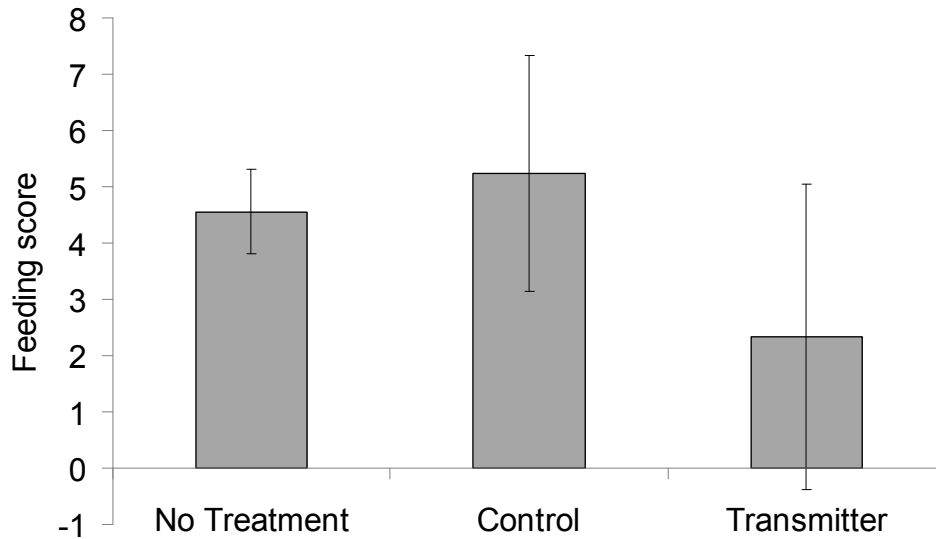
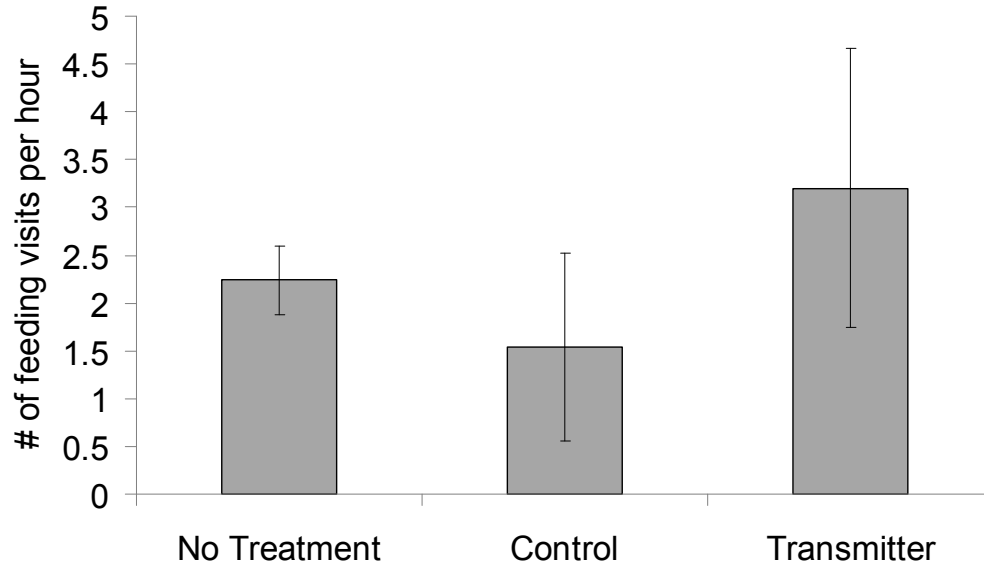


Figure 26. Mean number of feeding visits per hour (A) and feeding scores (B) of male cardinals from each treatment. Error bars are $\pm 95\%$ confidence intervals. Feeding score equals number of feeding visits multiplied by average prey size. We estimated feeding rates from 96 observations (80 no treatment, 11 control, 5 transmitter) from 57 territories (47 no treatment, 6 control, 4 transmitter). We estimated feeding scores from 94 observations (79 no treatment, 10 control, 5 transmitter) from 55 territories (46 no treatment, 5 control, 4 transmitter).

A.



B.

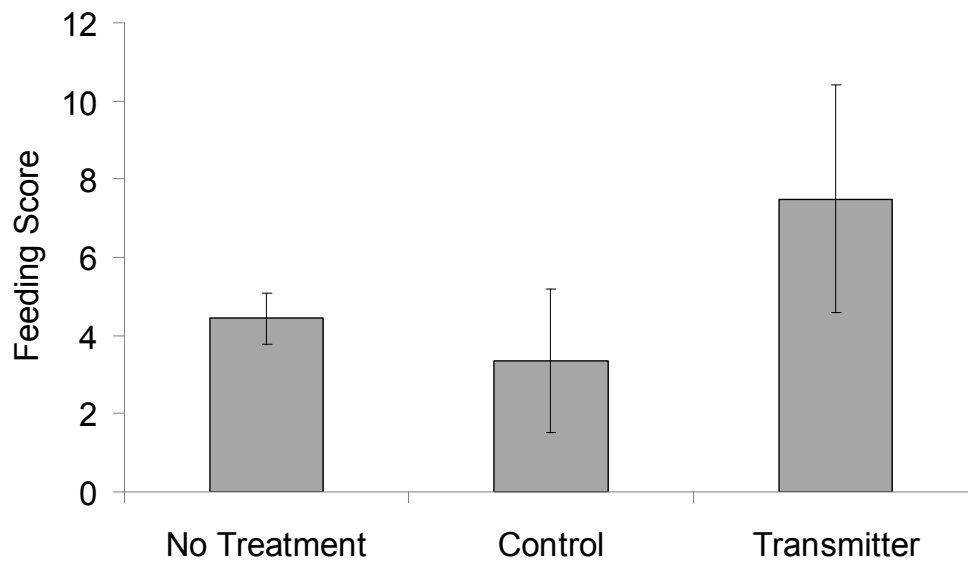


Figure 27. Mean number of feeding visits per hour (A) and feeding scores of female cardinals from each treatment (B). Error bars are $\pm 95\%$ confidence intervals. Feeding score equals number of feeding visits multiplied by average prey size. We estimated feeding rates from 96 observations (80 no treatment, 11 control, 5 transmitter) from 57 territories (47 no treatment, 6 control, 4 transmitter). We estimated feeding scores from 93 observations (79 no treatment, 10 control, 4 transmitter) from 54 territories (46 no treatment, 5 control, 3 transmitter).

Productivity

Birds with transmitters did not have altered productivity as indicated by either daily nest survival rates or fledging success (Table 9).

Table 9. Daily nest survival rate and mean number of fledglings produced per egg for birds from each treatment. Values in parentheses are the number of nests followed by the number of territories. Calculations of daily survival rates are based on 768 observation days.

Treatment	Daily Survival Rate		Number of Fledglings per Egg		<i>F</i>	<i>P</i>
	Mean	95% CI	Mean (n)	95% CI		
No Treatment	0.942	0.925-0.956	0.37 (91, 81)	0.28-0.46		
Control	0.953	0.909-0.976	0.24 (11, 9)	-0.02-0.51	0.85	0.43
Transmitter	0.943	0.900-0.969	0.24 (14, 12)	0.00-0.47		

Discussion

A major assumption of studies using radiotelemetry technologies is that transmitters do not alter the natural behavior or ecology of the birds. If this assumption is wrong, radio transmitters could harm the birds and produce unreliable data. Multiple studies have revealed negative impacts of transmitters on avian survival (e.g., Marks and Marks 1987), reproduction (e.g., Foster et al. 1992), body condition (e.g., Greenwood and Sargeant 1973), and behavior (e.g., Hooge 1991). One aspect of behavior that has received little attention in this regard, however, is parental investment, despite its importance to reproduction and survival (Trivers 1972). In this study, we investigated whether transmitters affect two components of parental investment (nest defense and offspring provisioning) by northern cardinals.

Given the importance of nest predation for most birds (Ricklefs 1969), and that nest defense can potentially deter predation (e.g., Greig-Smith 1980, Weatherhead 1990), alteration of normal nest defense could have negative implications for a bird's fitness (Montgomerie and Weatherhead 1988). There are two ways that transmitters might affect nest defense. First, transmitters could cause birds to defend their nests less aggressively simply because the cost of carrying the transmitter makes normal defense physically more difficult. Alternatively, and perhaps less intuitively, if birds with transmitters perceive their own survival value to be diminished, that could favor increased investment in their current brood (Trivers 1972), and therefore increased nest defense (Montgomerie and Weatherhead 1988). Neither of these predictions has been tested previously.

As with nest defense, offspring provisioning could either be reduced due to physical impairment associated with transmitters, or increased because transmitters cause birds to favor current reproduction over future reproduction. In the only study to investigate transmitter effects on passerine provisioning, Neudorf and Pitcher (1997) found no difference in provisioning between females with and without transmitters. A potential limitation of their study was that they considered only feeding rate, potentially missing differences associated with food load size. Here we examine both feeding rate and the size of food loads.

A potential effect of transmitters that has not been previously investigated is an indirect effect on the mate of the bird that is carrying a transmitter. If the bird with a transmitter reduces its parental investment, its unmarked mate could potentially compensate by increasing their own investment (Johnstone and Hinde 2006). To examine this possibility we determined whether transmitters caused a change in the nest defense or offspring provisioning of the mate of the bird with the transmitter.

Finally, we investigated whether transmitters affect cardinal productivity, in the form of daily nest survival rate and fledging success (fledglings produced per egg). If there is an effect the most likely outcome would be that transmitters reduce productivity, although depending on which of the predictions outlined above are supported, productivity could be unaffected by transmitters or could even increase. We predicted that transmitters would reduce productivity if they cause a decrease in nest defense, since the intensity of nest defense is positively correlated with nest success (e.g., Greig-Smith 1980, Weatherhead 1990). Because parental activity can attract predators and increase nest predation (Martin et al. 2000), we expect that if transmitters decrease offspring provisioning then birds with transmitters would have increase productivity. Although this decrease in provisioning could also decrease fledging mass, we lack sufficient data to investigate this possibility. Compensation by females would offset any changes in productivity that would have resulted from transmitter effects on male parental behaviors.

Our results indicate that transmitters cause male northern cardinals to reduce their parental investment. We found clear evidence of decreased nest defense by males with transmitters, and a non-significant trend for males with transmitters to decrease provisioning. The similarity in behavior between no treatment and control birds indicates that capturing and restraining birds was not responsible for the behavioral changes observed in birds with transmitters.

Despite the decrease in parental investment by males with transmitters we did not find evidence that transmitters caused a decline in nest productivity. Two factors may have contributed to this result. First, although past studies have found a positive correlation between nest defense intensity and nest success, the effect can be small and detectable only with relatively large samples (e.g., Weatherhead 1990). Our limited sample of nests from the transmitter treatment could have prevented us from detecting an effect of decreased nest defense. Second, although there was some evidence that males with transmitters fed their offspring less, there was also limited evidence that their mates compensated for this reduced paternal care. By compensating for their mates, females would have minimized the impact of their mates' reduced contribution. Although results indicate that adults with transmitters may provision less, the lack of statistical significance in this study of cardinals suggests why no strong effect of transmitters on nest provisioning was identified in the meta-analysis of transmitter effects reported in results for Study 5 in this report.

Our results suggesting that transmitters cause cardinals to decrease offspring feeding rate differ from those of Neudorf and Pitcher (1997), who found no comparable trend in Hooded Warblers. The different outcome is not attributable to differences in transmitter weight because their transmitters were heavier relative to the birds (7-8.5%) than were ours (4%). In both studies transmitters were attached with leg-harnesses and in both species nestlings are fed by each parent. An obvious difference between studies is that we attached transmitters to males and they attached transmitters to females. This might explain our results if greater uncertainty of parentage makes males more willing to decrease nestling provisioning when burdened with a transmitter (Sanz et al. 2000, Slagsvold and Lifjeld 1990). An appropriate study to test this

possibility would compare how transmitters affect parental care by males and females of the same species.

Johnstone and Hinde (2006) recently suggested that parents should respond to changes in their partner's cooperation with an incomplete compensatory change in the opposite direction when variation in brood need is less than variation in parental state. Cardinals may fit this pattern, as the rarity of nestling starvation and the wide range of adult body conditions (Barron, unpublished) suggests higher variation in parental state than in brood need. Furthermore, Chase (1980) predicted that any additional costs of cooperative behaviors (such as energetic costs caused by transmitters) would compel an animal to reduce such behaviors, causing a compensatory increase in the cooperative behaviors of its mate. While the female mates of cardinals with transmitters did not compensate by increasing their intensity of nest defense, they did seem to increase their offspring provisioning to compensate for their males' decreased provisioning. This insignificant trend is inconclusive, however, and further research is required to determine its accuracy. Future studies should also determine whether males compensate similarly, since males and females can respond differently to changes in partner effort (Sanz et al. 2000). Although compensatory feeding would maintain the quality of nestlings in the current brood, this life-history trade-off could limit the unmarked bird's potential investment in future broods (Trivers 1972) and therefore represent an indirect effect of transmitters.

Our findings suggest that compensatory feeding by an unmarked mate may be required to maintain offspring quality and therefore researchers should avoid placing transmitters on both parents from a single nest. Studies that only investigate transmitter effects on indices of productivity could be missing important indirect effects on the compensating mate and should therefore be cautious about declaring no effect. The potential reduction in the long-term fitness of the bird with the transmitter and its mate reveals the importance of carefully weighing the costs and benefits of using this technology on sensitive, rare, or endangered species.

Summary

Researchers commonly use radio transmitters to collect ecological data from free-living birds with the assumption that these devices do not alter their natural behavior or ecology. We tested this assumption for northern cardinal parental investment (nest defense and offspring provisioning), because such behaviors have been mostly ignored in past studies with passerines. We placed mock radio transmitters on male cardinals and compared their behaviors to uncaptured birds and similarly handled procedural controls. Birds with transmitters showed a strongly significant decrease in their nest defense and an insignificant decrease in provisioning effort. These responses were not a result of capture and restraint, as uncaptured birds and procedural controls had similar estimates for both behaviors. An insignificant increase in provisioning effort by mates of birds with transmitters hinted that compensation by unmarked females may minimize transmitter effects on current productivity. This idea is supported by our similar estimates of daily nest survival and fledging success between birds with and without transmitters. The increased energy expenditure of compensating mates could limit their future reproductive potential, representing an indirect effect of radio transmitters.

***Study 7: Data recovery and analysis of remotely monitored heart-rate telemetry from endangered birds**

Improvements in heart rate (HR) algorithm

These spurious heart-rates using the original autocorrelation method can readily be seen in Fig. 28. Fig. 29 shows the improvement using the new algorithm (version 29i, Appendix A). When the bird is close to the receiver these techniques can reduce the number of spurious points, allowing about 5-10% better recovery. There is, of course, a price to be paid by using sideband filtering and center-clipping. At low signal strength more of the original signal is lost during processing due to increased bandwidth and center-clipping. This means that when the bird is at the limit of the transmitter's range the heart-rate signal may not be recovered by the more complex algorithm. When used on the whole six receiver-days of recorded telemetry for each bird there was no gain in recovery rate using this new algorithm. For this reason the results presented in this report will be from the original algorithm.

For this dataset where the bird spends much of the daytime at or beyond the transmitter's limit, there is no advantage to using this more complex algorithm. In other datasets where the animal is close to the receiver at all times there should be an advantage.

Another disadvantage of the complex algorithm is the central processing unit (CPU) time it takes to process recorded telemetry. The original autocorrelation method (version 24) requires about six seconds of CPU time to process five minutes of telemetry, while the more complex algorithm (version 29i, Appendix A) requires about 50 seconds on the same 2GHz DDR machine.

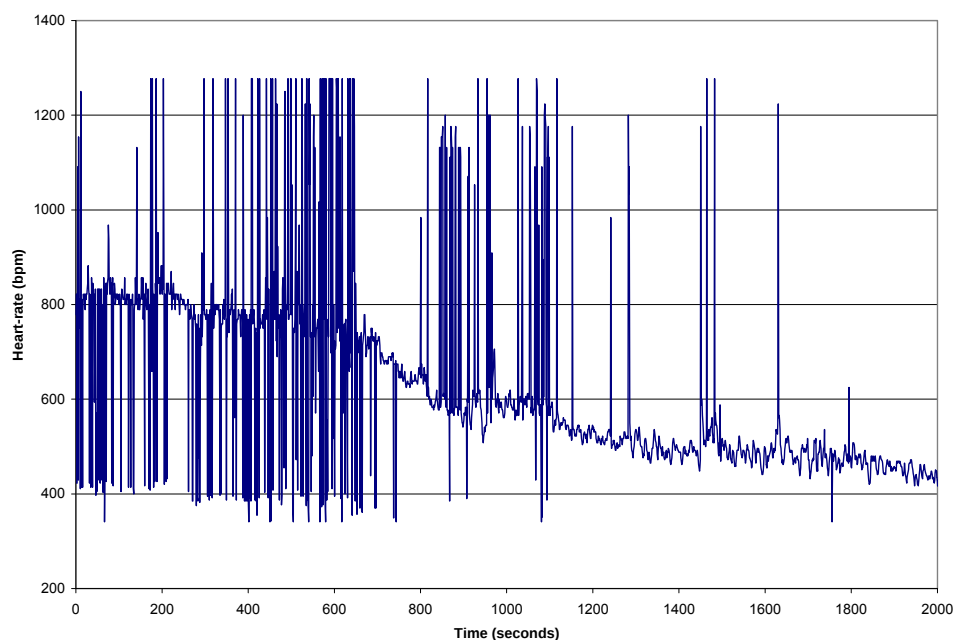


Figure 28. Derived heart-rate for 2000 seconds of recorded telemetry from bird 176_IB27 starting on 21 June 2008 at 8:24:14 AM. Processed using original autocorrelation method (version 24).

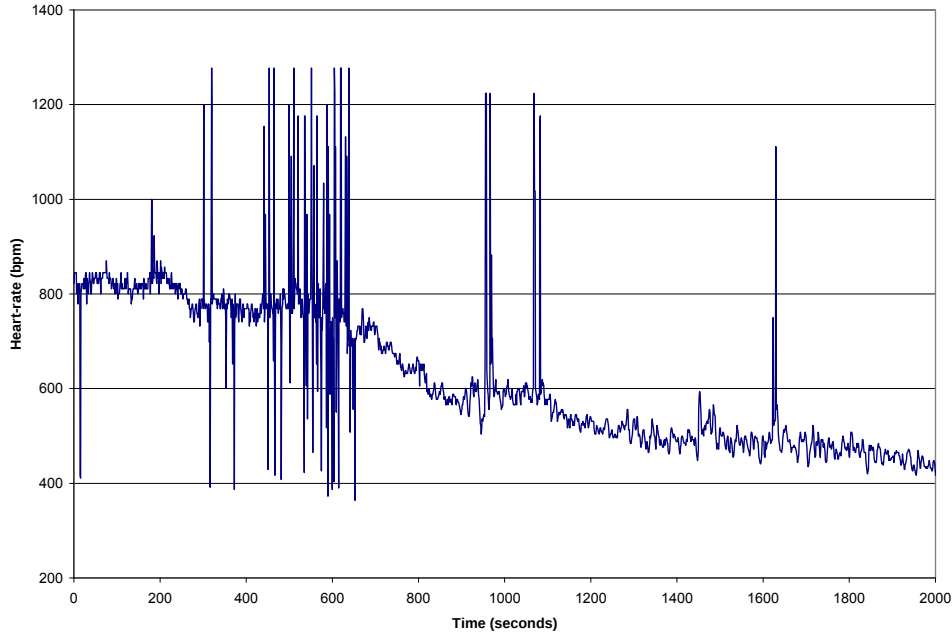


Figure 29. Derived heart-rate processed using sideband filtering, center-clipping and median filtering of five overlapping half-second analysis frames (version 29i, Appendix A). Time period is same as shown in Figure 28.

Comparison to manually-derived HR data

In previous years recorded telemetry was manually processed to obtain bird HR on a small sub-sample of all the available data. This was a very time-intensive process of filtering the recorded telemetry in sound-editing software (such as CoolEdit 2000, Syntrillium Software, Phoenix, AZ.) and manually looking at spacing of pulses on a spectrogram. In order to look for any bias of the heart-rate derived from the automated Scilab algorithm, a total of 72 seconds of recorded telemetry were processed using a manual technique. These points were spread out over 14 different recordings from three of the birds tagged in 2008.

Of the 72 manual points only 44 of them generated valid HR in both the manual and automated results, while the remaining 28 points were divided between those that did not yield valid results using the manual technique (13 points), those that did not yield valid results using the automated technique (6 points), and those that failed to yield valid results using both techniques (9 points).

Fig. 30 is a plot of manual HR vs. automated HR. The regression is quite good with $R^2 = 0.99$ ($n=44$):

$$aHR = 0.96 * mHR + 8.7 \text{ bpm} \quad (1)$$

where aHR is heart-rate (bpm) derived from automated Scilab algorithm
mHR is heart-rate (bpm) derived from manual method.

The mean difference (automatic minus manual HR) is $-18 \pm 13 \text{ bpm}$ ($\pm 1 \text{ S.D.}$, $n=44$).

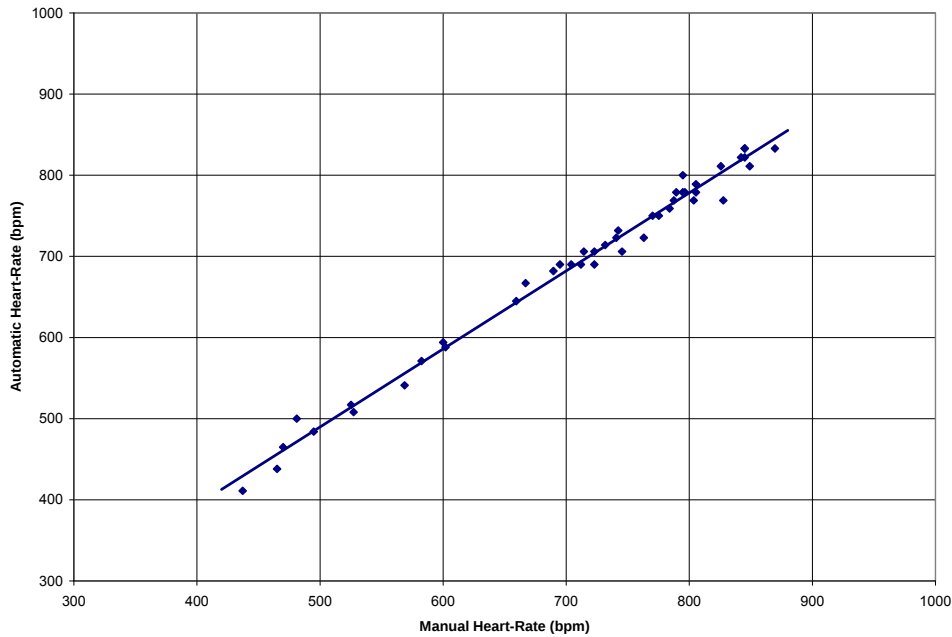


Figure 30. Correlation plot of one second HR derived from automated and manual techniques from birds 056_IB12, 998_JCB10 and 176_IB27. Blue line is the linear regression fit.

Dataset quality

Of the 13 black-capped vireos that were tagged in 2008, only ten generated meaningful amounts of data. Radio frequency (RF) transmissions from these ten birds were monitored for a total of 690 hours. After the heart-rate algorithm and filtering were applied, hourly statistics were generated and accepted only for the hours that contained a minimum of 20% valid heart-rate data. This reduced the total hours with valid heart-rate data statistics to 517 (75% of the 690 hours recorded).

Fig. 31 shows the percentage of valid heart-rate data within each hour of analyzed telemetry. The red line is the average percent valid data for all ten black-capped vireos. At night when the bird is on the nest and close to the receiving stations, the percent of valid data in each hour is typically over 90%. During the day when the bird may be out-of-range or may have its heart-rate signal mixed with wingbeat signal, the amount of valid data is reduced significantly.

We can break down the dataset into two parts: daytime (0700 to 2000 CDT) and nighttime (2200 – 0500 CDT) in order allow more direct comparisons. These time definitions purposefully omit the evening (2000 – 2200 CDT) and morning (0500 – 0700 CDT) periods to accommodate the changing sunrise and sunset times. At the beginning of field work (12 April 2008) the civil dawn was 0641 CDT and civil dusk was 2023 CDT. At the end of field work (24 June 2008) the civil dawn was 0601 CDT and civil dusk was 2107 CDT.

Fig. 32 is a plot of nighttime hourly percent valid data as a function of hourly median heart-rate for all birds. As one would expect with such high percentages of valid data, there is very little correlation ($R^2=0.006$).

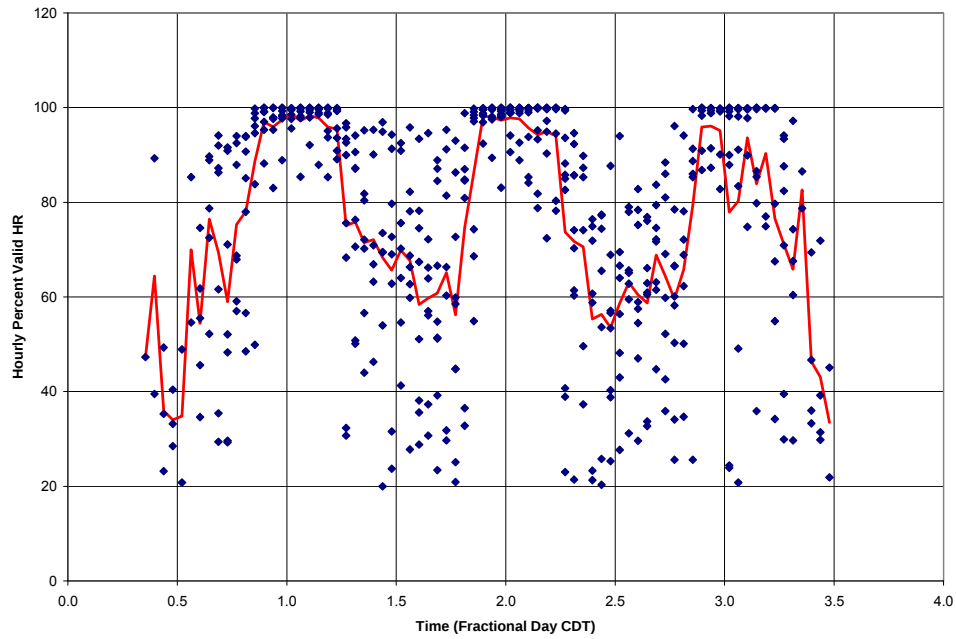


Figure 31. Plot of percent of valid data in each hour for all 10 birds as a function of time. Birds are typically tagged mid-day on day zero and monitored for until noon on the third day. Red line is average percent of valid HR data for all birds. Hours with less than 20% valid data are not included.

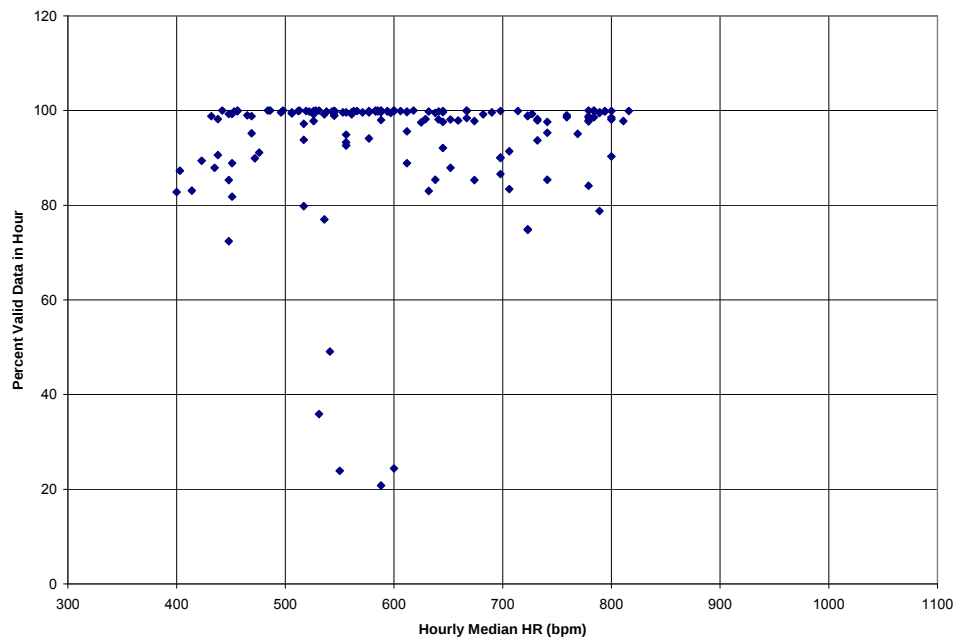


Figure 32. Plot of percent valid data in nighttime hours (2200 – 0500 CDT) for all birds as a function of hourly median HR.

Fig. 33 is the same plot for daytime data with an additional linear regression line ($R^2=0.15$). This weak correlation indicates that only small amount of hourly median HR variance is common to the variance in hourly percent valid data. This gives some confidence that the daytime hours with less valid data are still representative of the whole hour.

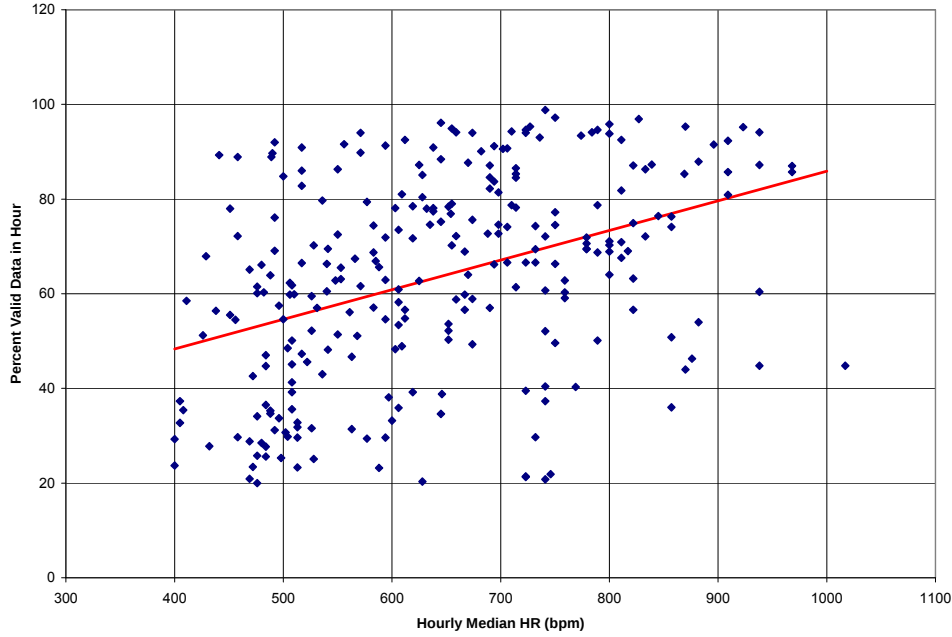


Figure 33. Plot of percent valid data in daytime hours (0700 – 2000 CDT) for all birds as a function of hourly median HR. Red line is regression fit.

Uncertainty analysis of hourly median HR

Generating an estimate of uncertainty for hourly median HR data is difficult, but lets us consider three sources: quantization noise, non-systematic sampling errors, and systematic sampling errors.

The resolution of time between one heartbeat and the next is limited to 1ms in the autocorrelation algorithm and this introduces quantization noise (Ingle and Crouch 1988):

$$\sigma_q = 0.29 * q \quad (2)$$

where σ_q is the quantization noise (bpm)
 q is the algorithm readout resolution

Algorithm readout resolution is a function of HR: at a HR of 400bpm resolution is 2.7bpm while at 1200bpm the resolution jumps up to 24.5bpm. For daytime hours the average hourly median HR is 641bpm which generates quantization noise of 2.0bpm. For nighttime hours the average hourly median HR is 605bpm which generates quantization noise of 1.8bpm.

In many hour periods we have only a sub-sample of the 3600 possible seconds of valid HR data. Excluding hours with less than 20% valid data, the daytime mean percentage of valid data is $63 \pm 21\%$ (± 1 S.D., $n=267$). For nighttime it is much better: $94 \pm 13\%$ (± 1 S.D., $n=158$).

If we assume that the sampling distribution of hourly median HR is normal with no systematic bias, then the standard error of the median (Loeve 1977) is given by

$$\sigma_{\text{Median}} = \sigma / \sqrt{N} \quad (3)$$

where σ_{Median} is the standard deviation of hourly median HR
 σ is the standard deviation of HR with an hour
 N is the number of one-second data points in hour

For daytime the mean N is 2268 (0.63×3600), and mean σ is 90bpm. This gives an hourly median sampling standard error of 1.9bpm. For nighttime the mean N is 3384 (0.94×3600), and mean σ is 35bpm. This gives a nighttime hourly median HR sampling standard error of 0.6bpm.

One test of the assumption that there is no systematic sample bias is to look at the sum of first-differences for each hour. The hourly first-difference sum is just the total of all the differences between each second of HR and the HR in the preceding second. If the preceding second of HR data is flagged as spurious for some reason, then that second of HR data makes no contribution to the sum of first-differences. First-difference bias (FDB) is defined as:

$$\text{FDB} = 100 * \sum (\text{HR}_i - \text{HR}_{i-1}) / (\text{HR}_{\text{median}} * N_{\text{valid}}) \quad (4)$$

where FDB is first-difference bias (%)
 HR_i is the valid heart-rate (bpm) at time i with valid HR at time $i-1$
 $\text{HR}_{\text{median}}$ is the median HR (bpm) for that hour
 N_{valid} is the number of valid seconds of HR data in that hour

Ideally FDB should be nearly zero for each hour of data. This would mean that each hour contained an equal amount of increasing and decreasing HR with time. If the record of valid HR was complete for an hour of data then FDB would be small as the first-difference sum would be only the net change in HR from the first to last record. A positive FDB means that there is a systematic sampling bias towards periods of rising HR. Negative FDB means that an hour of HR data is under-represented in periods of rising HR.

FDB versus percent valid data in each hour is plotted in Fig. 34. For the nighttime hours FDB is quite close to zero as one would expect with the mean percentage of valid data being so high (94%) and the variability in HR being small. The mean nighttime FDB is $-0.01 \pm 0.04\%$ (± 1 S.D., $n=158$). Fig. 35 shows that nighttime FDB is also not a function of median HR and remains close to zero for hours with median HR of 400 to 800bpm.

For the daytime we see a very different pattern in systematic sample bias as plotted in Fig. 34. As would be expected, FDB goes to zero at high percent valid data; but, as we start to look at hours with low percent valid data there is a trend towards negative FDB. Only 12 of the 267 daytime hours have positive FDB and thus there is a bias toward sampling times of decreasing HR. This may be due to better signal transmission when the bird is resting on a branch versus flying out-of-range of the receivers, or it may be caused by RF signal being modulated by both heart-muscle contraction and wing-muscle contraction during flight. The mean daytime FDB is $-0.40 \pm 0.42\%$ (± 1 S.D., $n=267$).

Fig. 35 shows there is a trend of less sample bias for daytime hours with higher median HR.

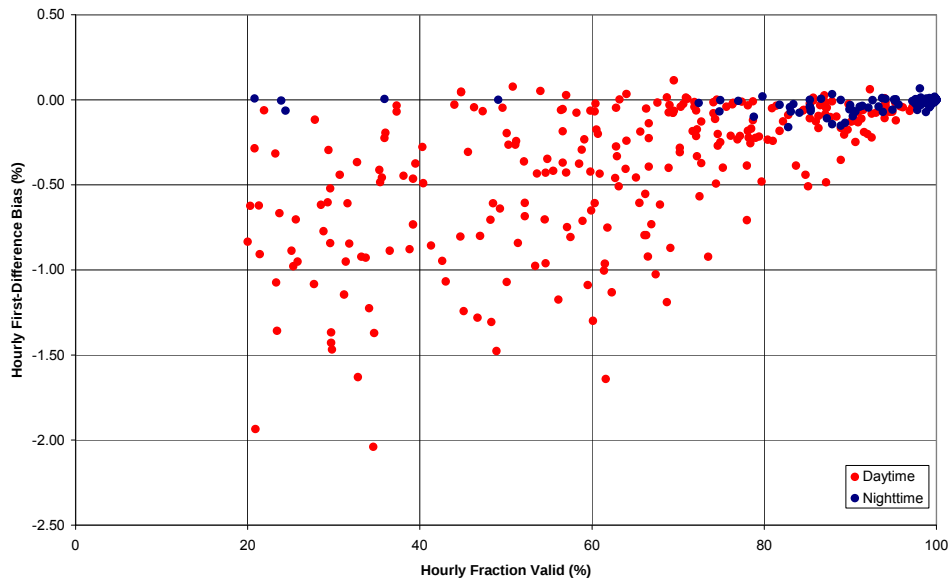


Figure 34. Plot of percent hourly first difference bias (%) as a function of hourly fraction valid data (%) for both daytime (red symbol) and nighttime (blue symbol).

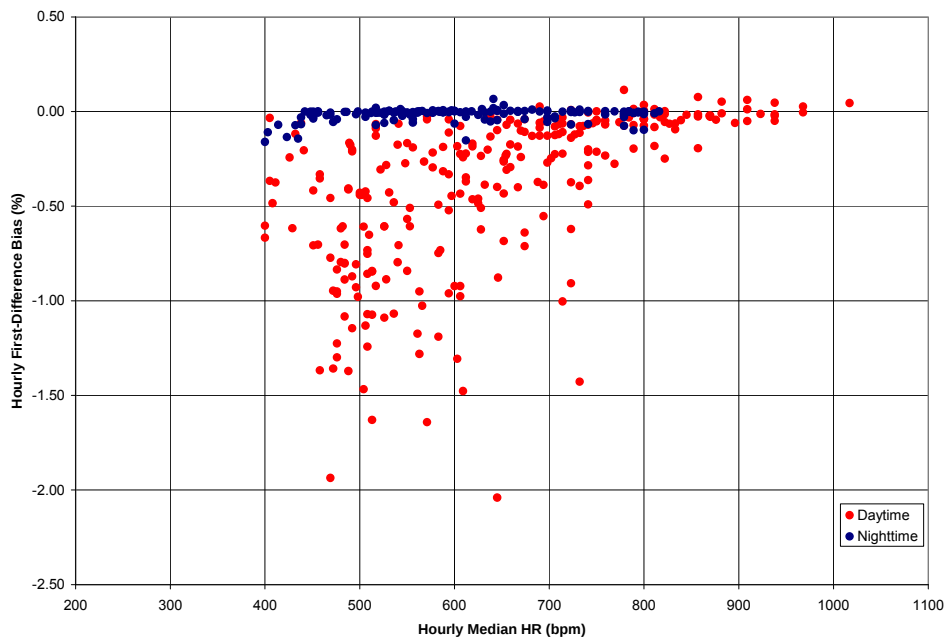


Figure 35. Plot of percent hourly first difference bias (%) as a function of hourly median HR (bpm) for both daytime (red symbol) and nighttime (blue symbol).

Estimating the uncertainty generated by this under-representation of periods of rising HR is not straightforward. One way to make this estimate is to recalculate the hourly average HR

after we have filled the gaps in one-second HR data with a linear fit between the gap starting and ending HR. In this calculation we only fill gaps that are 15seconds or shorter duration, as filling longer time gaps would not necessarily be representative of actual HR trends. For the daytime hours the mean gap duration is 6.8seconds and the 15second limit includes 93% of all gaps. The mean increase in hourly HR based on this calculation is $5.4 \pm 6.1\text{bpm}$ (± 1 S.D., $n=267$) for the daytime.

For the nighttime hours the mean gap duration is 1.4seconds and the 15second limit includes 99% of all gaps. The mean increase in hourly HR based on this calculation is $0.3 \pm 1.0\text{bpm}$ (± 1 S.D., $n=158$) for the nighttime.

We can add these three sources in quadrature to get an estimate of hourly median HR uncertainty (± 1 S.D.). For the daytime we get $\sqrt{(2.0^2 + 1.9^2 + 5.4)} = 6.1\text{bpm}$, with uncertainty generated by under-representation of periods of rising HR as the dominate source. For the nighttime we get an estimate of hourly median HR uncertainty of $\sqrt{(1.8^2 + 0.6^2 + 0.3^2)} = 1.9\text{bpm}$, with quantization noise as the dominate source.

Diurnal pattern

For hours that have less than less than 720 seconds of valid data (20% of the time within each hour) the hourly statistics are not considered representative and will not be included in any further calculations. For diurnal-averaged HR contour plots this minimum requirement was relaxed to 10% (typically 1080 seconds of valid data over the three days).

Fig. 36 is a plot of average HR versus overlapping fractional day for all birds tagged in spring 2008. The most pronounced peak is at the first hour past sunrise, with a smaller peak at the hour prior to sunset. Average temperature versus overlapping fractional day is plotted in Fig. 37 with an intra-day range of about 10 to 12 C. Large temperature changes from one hour to the next reflect the fact that only hours with valid HR are included in the average temperature.

A strong diurnal pattern of HR was found with a maximum in the hour just after dawn and another local maximum in the hour just before dusk. The lowest HR was typically found in late afternoon during peak ambient air temperatures.

Fig. 36 shows that bird HR is lower each successive night after tagging, suggesting that the bird is ‘getting used to’ the RF tag glued to its back. With the exception of the first few hours after tagging (when sedation during surgery may have caused a period of reduced HR), Fig. 36 also suggests that daytime HR generally decreases with each successive day after tag surgery.

Sleep duration

Continuous monitoring of bird heart-rate permits a measure of bird sleep duration, as on most nights there is a similar pattern of clear changes in heart-rate at the start and end of “sleep.” Fig. 38 shows a typical pattern of one-minute median heart-rate data with the start of “sleep” beginning with a sharp minimum and a large reduction in heart-rate variability as marked by the arrow. The end of “sleep” is defined by a sharp increase in heart-rate and heart-rate variability as marked by the second arrow. Whether the bird is actually asleep or just inactive during this whole period is not clear, but this pattern allows us to functionally define “sleep” onset and termination for the purposes of comparison.

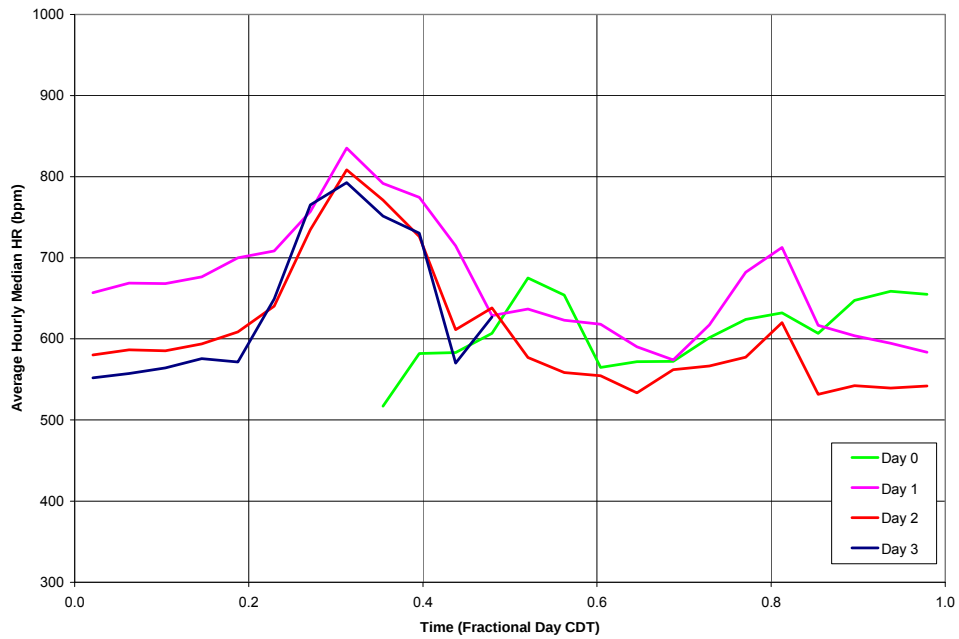


Figure 36. Plot of average hourly HR (bpm) versus fractional day for all birds tagged spring 2008.

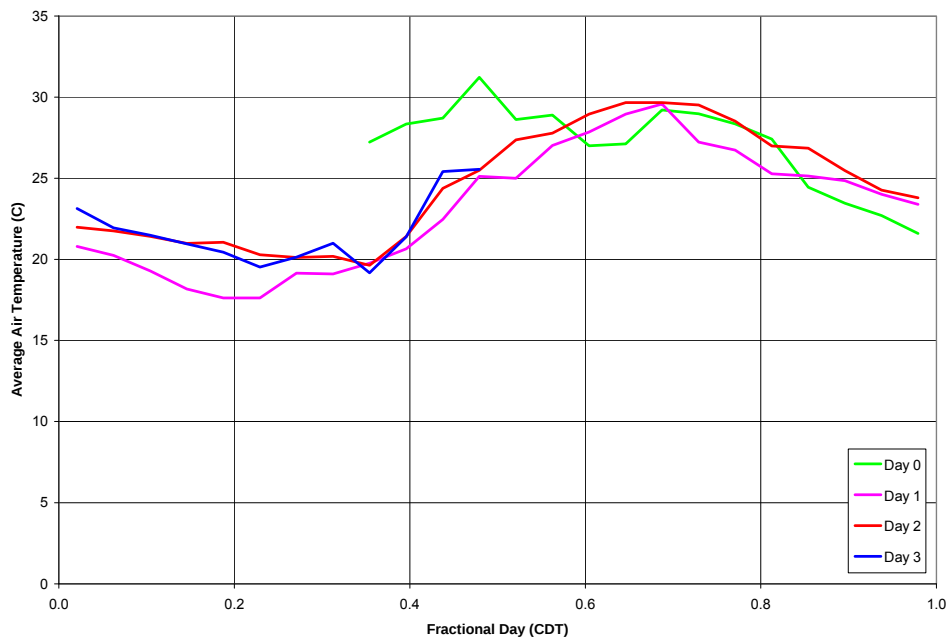


Figure 37. Plot of average ambient air temperature versus overlapping fractional day for periods of valid HR data for all birds tagged spring 2008.

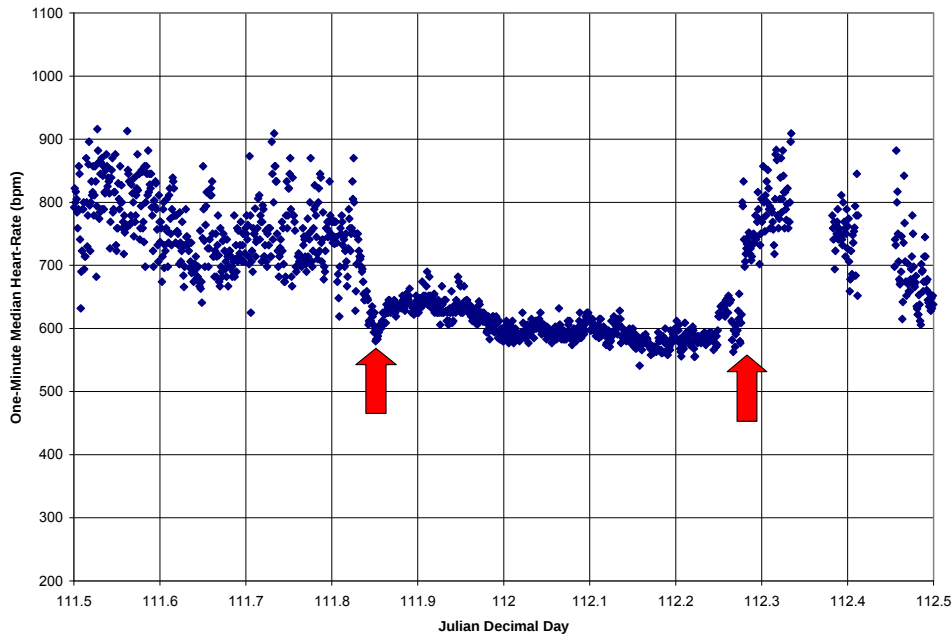


Figure 38. Plot of one-minute median heart-rate for bird 5001_IB14 for the night of April 20 / 21st, 2008. Arrows mark the start and end of defined “sleep.”

For this work we define nighttime start and end as coinciding with civil dusk and dawn as calculated by the U.S. Naval Observatory (<http://www.usno.navy.mil/USNO/astronomical-applications>) for Ft. Hood, Texas. Civil dawn and dusk are defined as the time that the Sun is six degrees below the horizon and represents the minimum limit of illumination for terrestrial objects to be clearly distinguished.

For the ten birds that yielded data in spring 2008, there were a total of 42 out of a possible 58 “sleep” onset or termination times that were identifiable in the heart-rate data. The 16 times that did not yield “sleep” onset or termination were due to poor RF signal or a HR pattern that was not typical with clear demarcation. It also must be remembered that there is a subjective element to picking the onset and termination times from the data. This may introduce errors on the order of a few minutes.

Fig. 39 shows the “sleep” onset and termination times as minutes from civil dusk and dawn. The ‘sleep-in’ time is the duration that the bird waited after civil dawn to show a marked increase in heart-rate (“sleep termination”). All the ‘sleep-in’ times are very close to zero minutes or up to 40 minutes, meaning that the birds typically ended “sleep” during the period from civil dawn to about 40 minutes after civil dawn. The average ‘sleep-in’ duration past civil dawn was 15 ± 14 minutes (± 1 S.D., $n=21$).

‘Stay-up’ time is the duration that the bird waited after civil dusk to start “sleep.” For these birds all of them had ‘stay-up’ times of near zero minutes to about -40 minutes, meaning that the birds typically started “sleep” during the period from 40 minutes before civil dusk up to civil dusk. The average ‘stay-up’ duration past civil dusk was -15 ± 16 minutes (± 1 S.D., $n=21$). There is no clear trend of increasing or decreasing ‘sleep-in’ or ‘stay-up’ duration as a function of Julian day, indicating that in this study bird “sleep” times generally were determined by sunrise and sunset times.

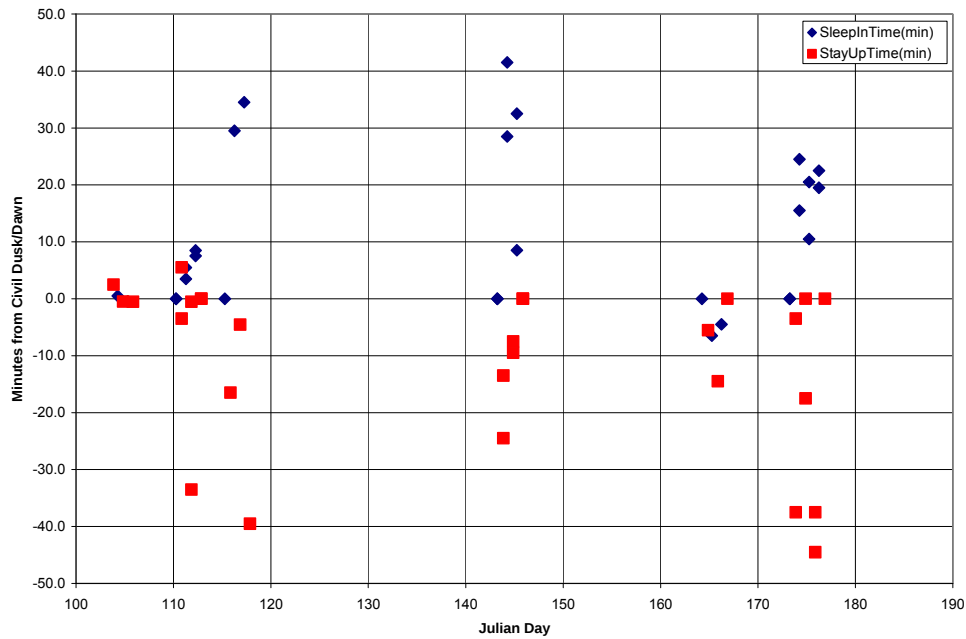


Figure 39. Plot of ‘sleep-in’ and ‘stay-up’ duration for all birds tagged in spring 2008.

“Sleep” duration is plotted in Fig. 40, with the curved line representing civil night duration. Average “sleep” duration was 29 ± 24 minutes (± 1 S.D., $n=19$) longer than civil night duration.

A one-tailed t-test was used to determine if there is a statistically significant difference in “sleep” duration between the three groups (JD 104-117, JD 144-145, and JD 165-176). At the $p = 0.05$ level there is a significant difference in “sleep” duration between groups JD 104-117 and JD 144-145. There is also a significant difference in “sleep” duration between groups JD 104-117 and JD 165-176. However, there is not a significant difference between groups JD 144-145 and JD 165-176.

Multilinear correlation

Quality-controlled weather data was provided by National Climactic Data Center (<http://www.ncdc.noaa.gov/oa/climate/rcsg/datasets.html>) for station RGAFH located on Fort Hood (Robert Gray Airport, Latitude 31.070N Longitude 97.822W Elevation 311m). Gaps in weather data shorter than three hours were filled by interpolation. Longer missing periods or periods with obviously incorrect data were filled by using data from station KILE (Skylark Field Airport, Latitude 31.083N Longitude 97.683W Elevation 257m).

Multilinear correlation was performed on hourly data for both nighttime and daytime periods. Table 10 and 11 show the correlation parameters and statistics for daytime and nighttime respectively. A two-tailed t-test was used to determine which slope coefficients were statistically significant at the 95% confidence level.

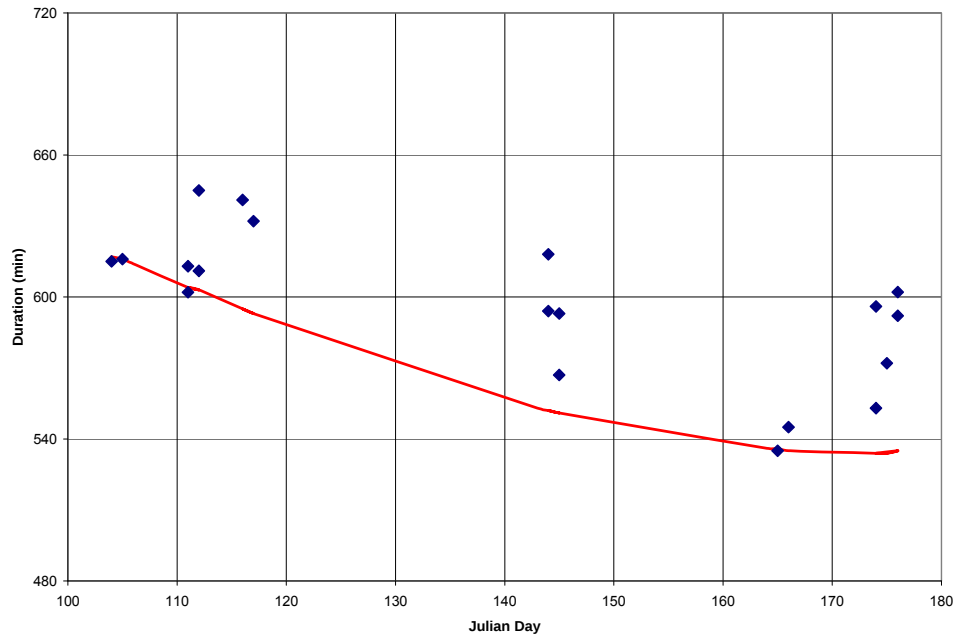


Figure 40. Plot of “sleep” duration as a function of Julian day. Curved line is the duration of civil night for Ft. Hood, TX for Julian day 103 to 176, 2008.

Table 10. Statistics for daytime multilinear regression of HR and relative humidity, air temperature, wind speed and time since bird was released from tag surgery. Slope and intercept parameters that are statistically significant ($\alpha=0.05$) are boldface.

	Fractional Day	Time Since Tag (h)	Relative Humidity (%)	Air Temperature (C)	Wind Speed (m/s)	Intercept (bpm)
Slope (bpm/X) & Intercept Parameters	71.4	-0.474	2.72	-14.9	-4.31	993
Standard Error	39.5	0.301	0.301	0.967	2.77	33.0
t-observed	1.81	1.58	9.04	15.4	1.56	27.9
t-critical ($\alpha=0.05$)	1.97					
Coefficient of Determination	0.636					
Degrees of Freedom	261					

Table 11. Statistics for nighttime multilinear regression of HR and relative humidity, air temperature, wind speed and time since bird was released from tag surgery. Slope and intercept parameters that are statistically significant ($\alpha=0.05$) are boldface.

	Fractional Day	Time Since Tag (h)	Relative Humidity (%)	Air Temperature (C)	Wind Speed (m/s)	Intercept (bpm)
Slope (bpm/X) & Intercept Parameters	-105	-1.39	0.077	-14.3	-3.33	969
Standard Error	75.2	0.271	0.400	0.973	2.73	27.6
t-observed	1.39	5.12	0.192	14.7	1.22	35.1
t-critical ($\alpha=0.05$)	1.98					
Coefficient of Determination	0.687					
Degrees of Freedom	152					

The isolated multilinear regression for just the variables that were statistically correlated to HR generates the following regressions:

$$dHR = 892 - 14.7 \cdot T + 2.50 \cdot RH \quad (5)$$

$$nHR = 964 - 14.3 \cdot T - 1.42 \cdot TST \quad (6)$$

where dHR is daytime (0700 – 2000 CDT) hourly median heart-rate (bpm)
nHR is nighttime (2200 – 0500 CDT) hourly median heart-rate (bpm)
T is ambient air temperature (C)
TST is time since bird was released from tag surgery (hours)
RH is ambient relative humidity (%)

Fig. 41 shows the scatter in daytime hourly median HR versus ambient air temperature for HR that has been normalized to 50% relative humidity. For the daytime hours with valid HR the mean relative humidity was 52.8%. Fig. 42 shows the scatter in daytime hourly median HR versus relative humidity for HR that has been normalized to 25C. For daytime hours with valid HR the mean ambient air temperature was 26.1C. Fig. 43 shows the scatter in nighttime hourly median HR versus ambient air temperature for HR that has been normalized to time equal to 36hours after release from tag surgery. For the nighttime hours with valid HR the mean time since release from tag surgery was 36hours. Fig. 44 shows the scatter in nighttime hourly median HR versus time since release from tag surgery for HR that has been normalized to 25C. For nighttime hours with valid HR the mean ambient air temperature was 21.5C.

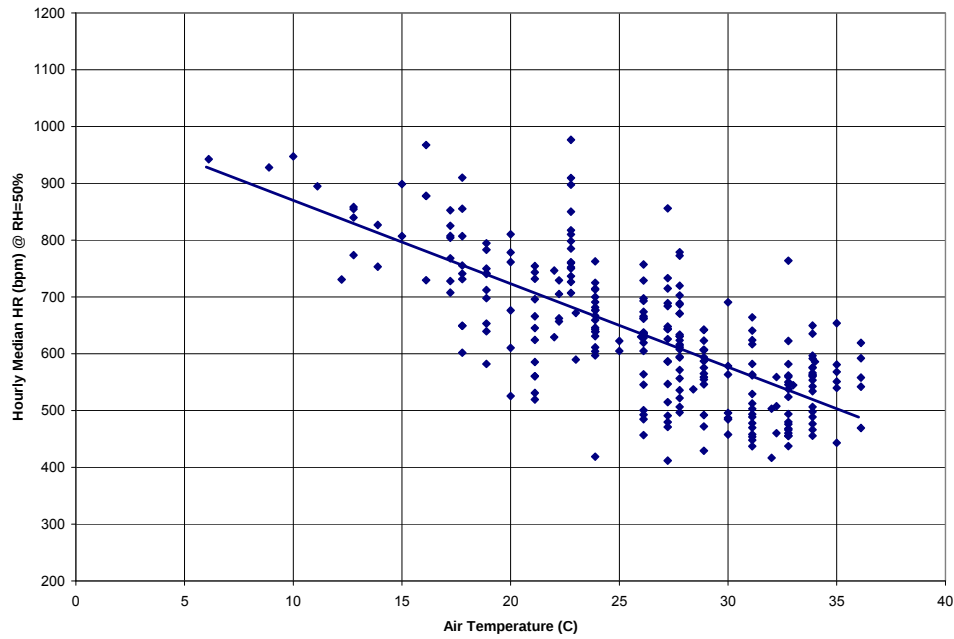


Figure 41. Plot of daytime hourly median HR versus ambient air temperature for all birds. HR data was normalized to 50% relative humidity according to Equation 5. Blue line is linear best fit.

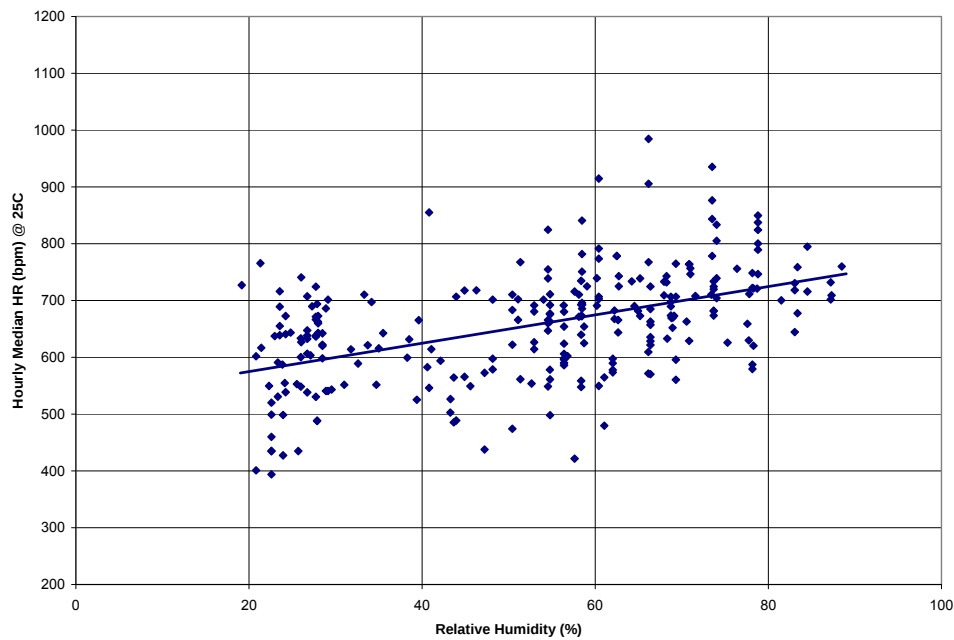


Figure 42. Plot of daytime hourly median HR versus ambient relative humidity for all birds. HR data was normalized to an ambient air temperature of 25C according to Equation 5. Blue line is linear best fit.

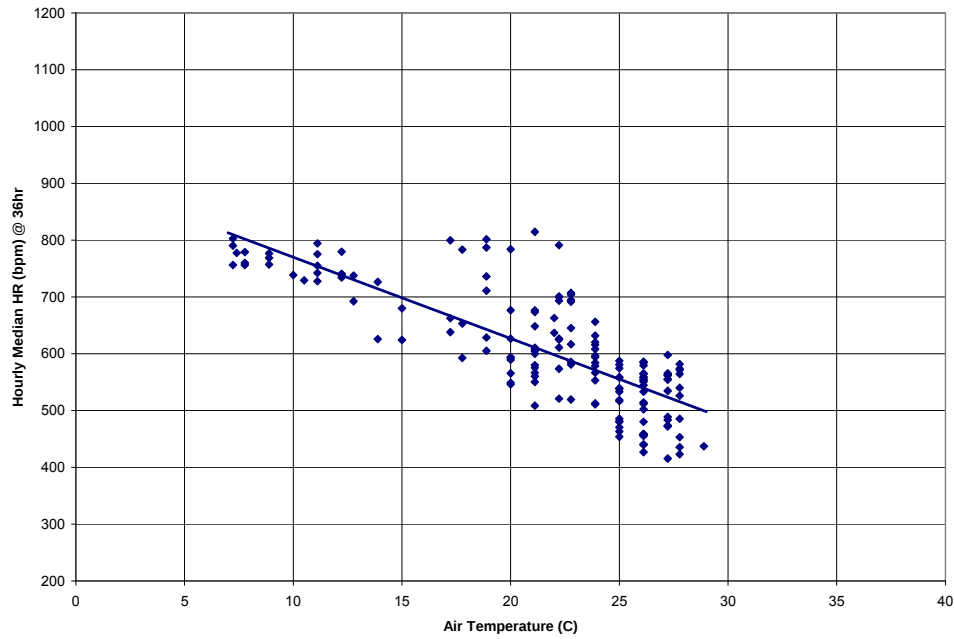


Figure 43. Plot of nighttime hourly median HR versus ambient air temperature for all birds. HR data was normalized to 36 hours after bird was released from tag surgery according to Equation 6. Blue line is linear best fit.

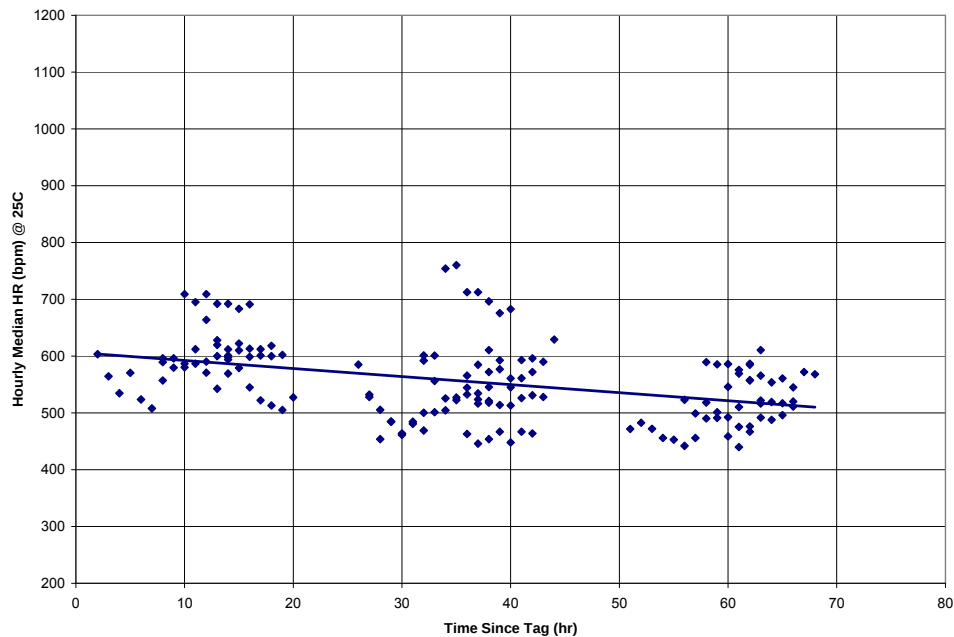


Figure 44. Plot of nighttime hourly median HR versus time since bird was released from tag surgery for all birds. HR data was normalized to an ambient air temperature of 25C according to Equation 6. Blue line is linear best fit.

Disturbance response

Nighttime disturbance was conducted for about a one hour period at about midnight CDT on the third night after tagging. Fig. 45 is a plot of median HR for periods before, during and after nighttime disturbance. If we linearly interpolate between the median HR in the hour before nighttime disturbance and the median HR for the second hour after conclusion of nighttime disturbance, we can subtract this background to get the difference or response of the bird to disturbance itself. Hopefully subtracting this linear interpolation (background-correcting) will largely remove the environmental effects that may affect the bird's HR.

Fig. 46 shows there was little change in background-corrected HR: in three birds the background-corrected HR was lower during disturbance and the hour that follows. In two other birds there was a slight increase (less than 11bpm) in median HR during nighttime disturbance and the first hour that follows. The mean background-corrected response for all five birds was slightly lower HR during nighttime disturbance and the hour that followed.

A quiet observation was typically conducted for several hours on the first full day after tag surgery. Fig. 47 shows the results, and Fig. 48 shows the background-corrected results. Bird 816_AB1 shows a 120bpm increase in background-corrected HR during the first hour of quiet observation. Birds 962_IB26 and 176_IB27 also show increases of nearly 200bpm during the first hour of quiet observation, but are not plotted in Fig. 49 because they have no valid data after quiet observation. The other five birds show little increase in HR or a marked decrease in HR during quiet observation.

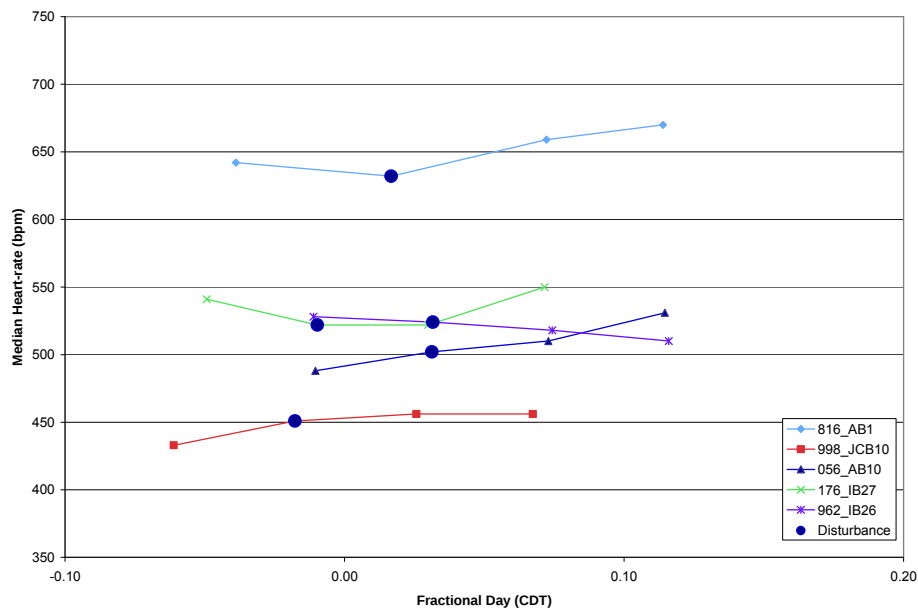


Figure 45. Plot of median HR versus fractional day (CDT) for five birds disturbed for one hour at night. Large blue symbol is the median HR for the disturbance period, while the other three symbols are the median HR for the preceding hour and two successive hours following disturbance.

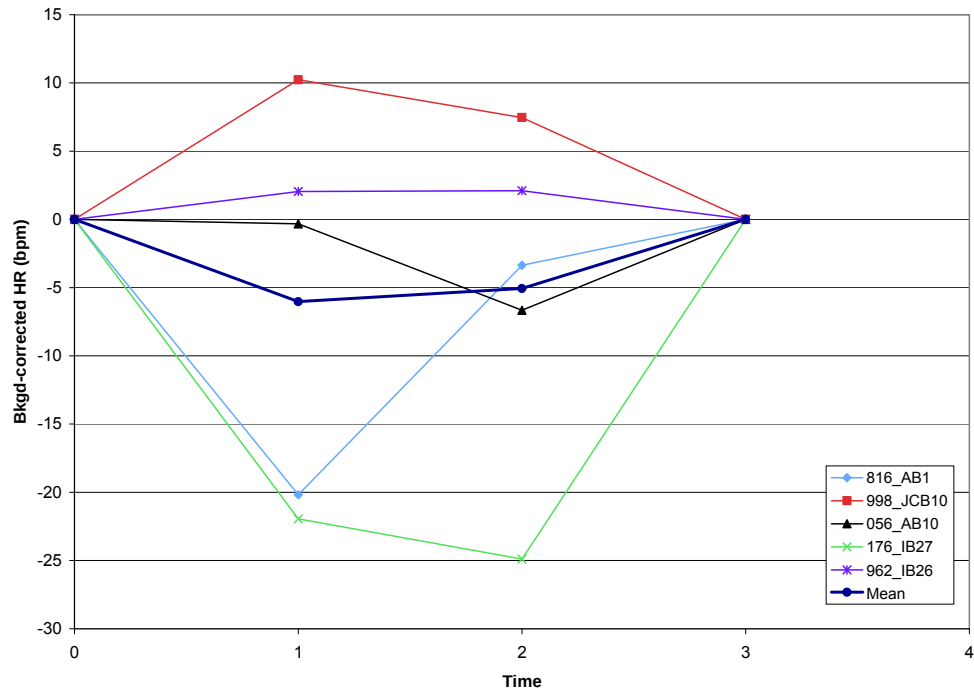


Figure 46. Linear interpolation of HR between time $t=0$ (the hour before nighttime disturbance) and time $t=3$ (the second hour after conclusion of disturbance) subtracted from HR to get background-corrected HR. At time $t=1$ the bird was disturbed for about one hour. Heavy blue line is mean of all five birds. Data analyzed are the same as in Figure 45.

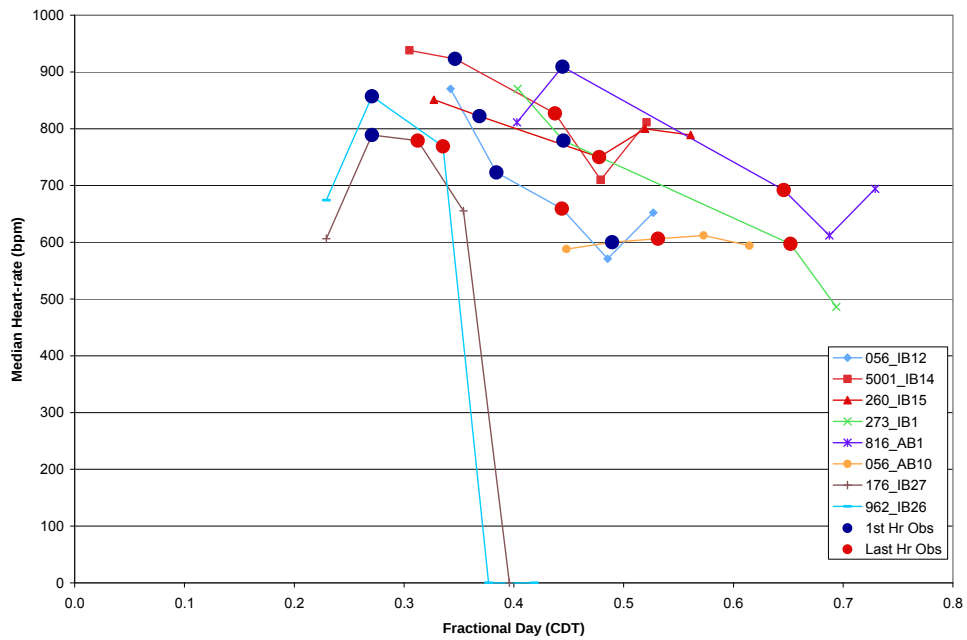


Figure 47. Plot of median HR versus fractional day (CDT) for eight birds before and after quiet observation period during the day. Large blue symbol is the median HR for

the first hour of quiet observation. Large red symbol is the median HR for the last hour of quiet observation. The other three symbols are the median HR for the preceding hour and two successive hours following quiet observation. Periods that have less than 20% valid data are marked with zero bpm HR. Data with less than 20% valid data were not included in statistical analyses reported for Study 8 in this report.

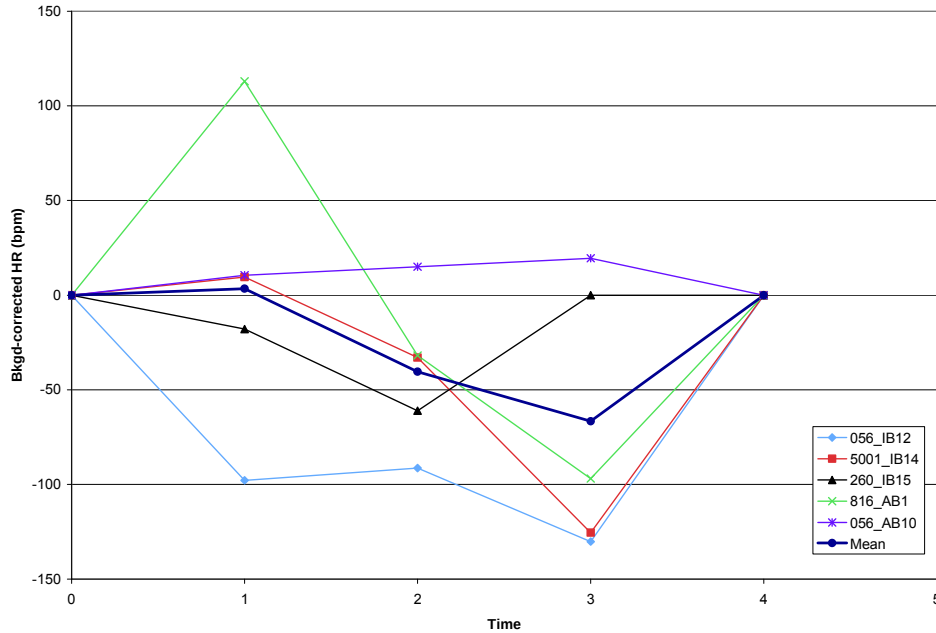


Figure 48. Linear interpolation of HR between time t=0 (the hour before quiet observation starts) and time t=4 (the second hour after conclusion of quiet observation) subtracted from HR to get background-corrected HR. Time t=1 is the first hour of quiet observation, while time t=2 is the last hour of quiet observation. Heavy blue line mean of all five birds. Data analyzed are the same as in Figure 47.

On the second full day after tag surgery the birds were disturbed three times for one hour duration, with an hour rest between each disturbance period. Each disturbance period in the sequence was a different type: nest search, nest sitting and chase. Fig. 49 shows the median HR results, while Fig. 50 is the same data once the background correction was applied.

Bird 816_AB1 showed the strongest response to this type of alternating disturbance / non-disturbance as seen in Fig. 50. This bird also showed the largest background-corrected response to quiet observation (Fig. 48), but showed a decrease in background-corrected HR during the nighttime disturbance.

The five bird background-corrected mean response to the first hour of this type of alternating disturbance was positive with an increase of $101 \pm 99\text{bpm}$ (± 1 S.D., $n=5$). For the second hour of alternating disturbance the five bird background-corrected mean response was much less with an increase of $9 \pm 110\text{bpm}$ (± 1 S.D., $n=5$). For last hour of alternating disturbance the five bird background-corrected mean response was again very small with an increase of $18 \pm 44\text{bpm}$ (± 1 S.D., $n=5$). The lower response with each additional hour of disturbance suggests that the birds are habituating quickly.

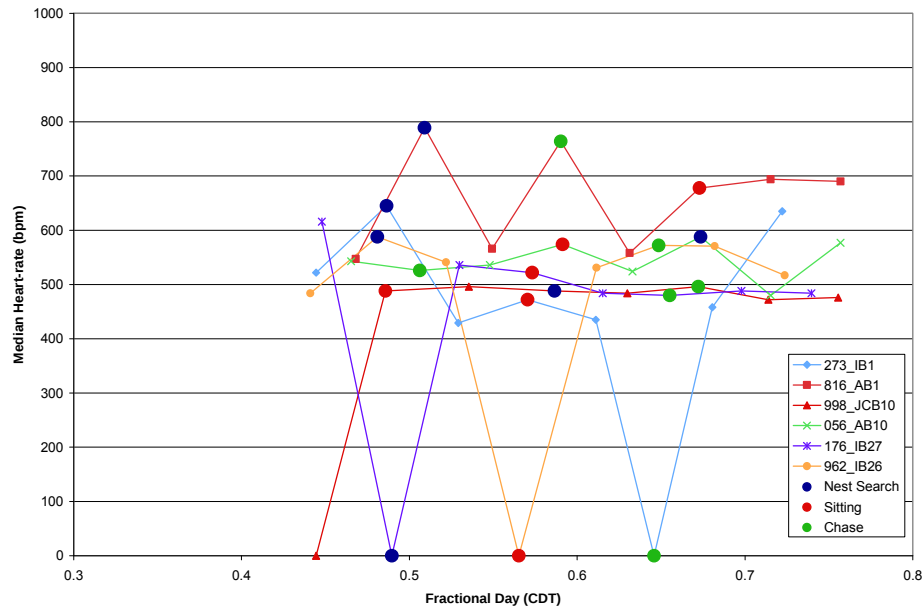


Figure 49. Plot of median HR versus fractional day (CDT) for six birds before and after alternating periods of nest searching, sitting and chase during the day. Each type of disturbance lasted approximately one hour and was separated by an hour of no disturbance. Large blue, red and green symbols are the median HR during nest search, sitting and chase respectively. The other five smaller symbols are the median HR for the preceding hour, the two hours between disturbances, and the two hours following. Periods that have less than 20% valid data are marked with zero bpm HR.

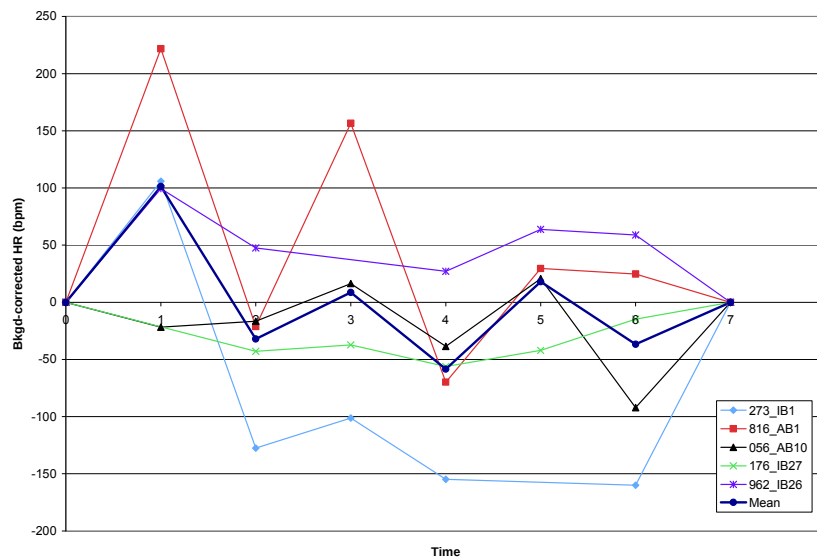


Figure 50. Linear interpolation of HR between time $t=0$ (the hour before first disturbance) and time $t=7$ (the second hour after conclusion of the third disturbance) subtracted from HR to get background-corrected HR. Time $t=1$, 3 and 5 are disturbance periods. All the others have no disturbance. Heavy blue line is mean of all five birds. Data analyzed are the same as in Figure 49.

Figs. 51 and 52 show the disturbance response during four-hour daytime chase for three birds. The four-hour chase was divided somewhat equally in time between three different individuals who pursued the birds. Similar to the alternating chase results we see that the first period of disturbance has a large increase in median HR of $78 \pm 51\text{bpm}$ (± 1 S.D., $n=3$), but successive periods have less of an increase. The second period's increase was $33 \pm 85\text{bpm}$ (± 1 S.D., $n=2$), and the third was $16 \pm 37\text{bpm}$ (± 1 S.D., $n=3$). Again this suggests that the birds are habituating quickly with each successive hour of disturbance.

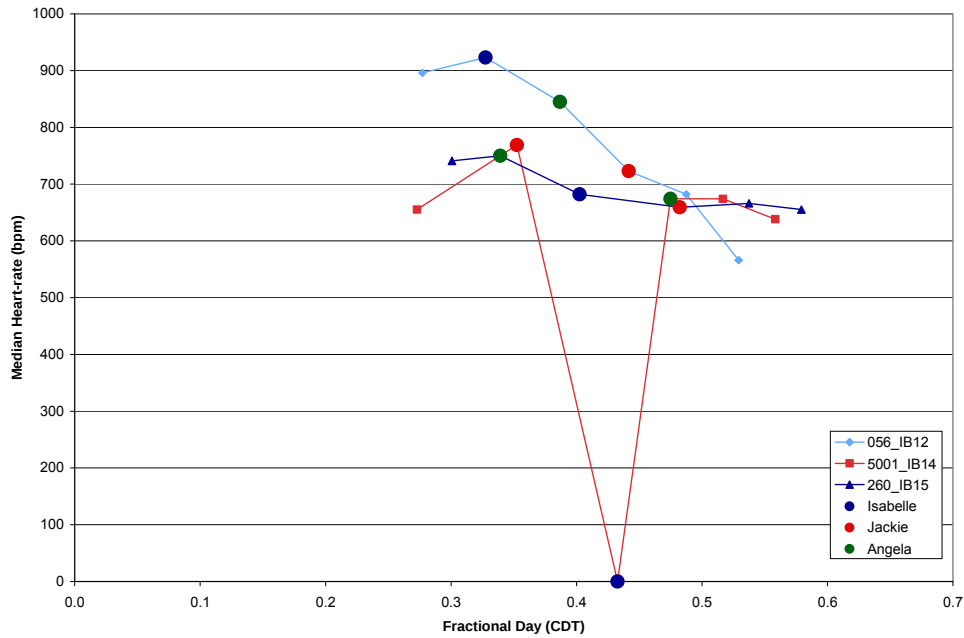


Figure 51. Plot of median HR versus fractional day (CDT) for three birds before and after a four-hour daytime chase disturbance conducted by three people: Isabelle (blue symbol), Jackie (red symbol) and Angela (green symbol). The other three smaller symbols are the median HR for the preceding hour and the two hours following the chase. Periods that have less than 20% valid data are marked with zero bpm HR.

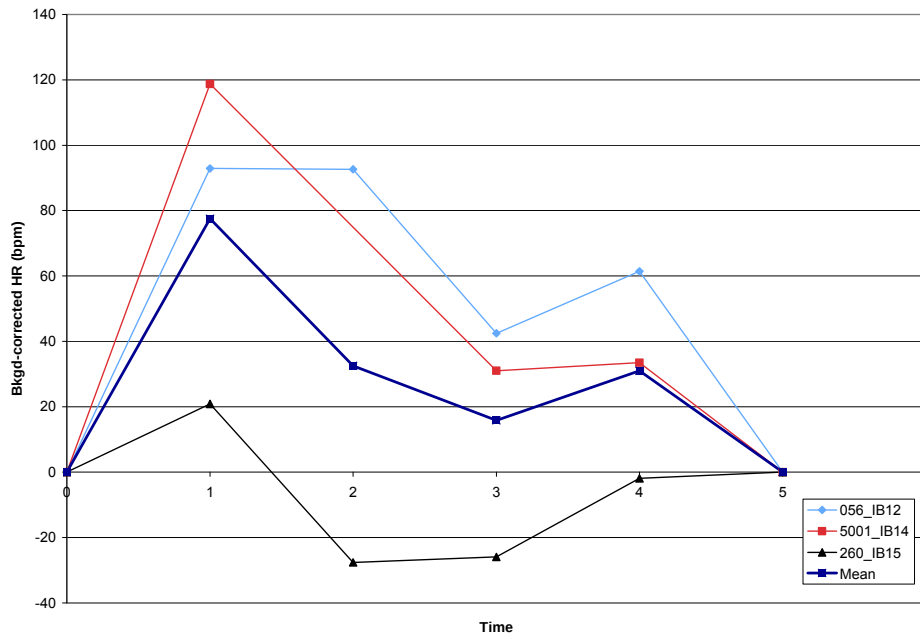


Figure 52. Linear interpolation of HR between time $t=0$ (the hour before first disturbance) and time $t=5$ (the second hour after conclusion of the four-hour disturbance) subtracted from HR to get background-corrected HR. Time $t=1, 2$ and 3 are chase periods. All the others have no disturbance. Heavy blue line is mean of three birds. Data analyzed are the same as in Figure 51.

Discussion

An improved algorithm was developed that significantly reduces spurious data points during periods of strong RF signal strength. The new algorithm does not improve recovery rates in signals that are weak or varying, as was seen in much of recorded telemetry from the spring 2008 field intensive. It should improve recovery rates for stronger telemetry signals such as those that would normally be found during monitoring of captive birds. The algorithm developed under a previous SERDP contract was used to analyze the data presented in this report.

HR derived from this automated Scilab program was compared to results obtained using a manual technique and the correlation was quite good ($R^2 = 0.99$). The regression slope was 0.96 for a range of points between 430 and 870bpm.

An analysis of uncertainty introduced solely by the automated processing technique looked at three sources: quantization noise, non-systematic sampling errors, and systematic sampling errors. The idea of first-difference bias was used to show the under-representation of periods of rising HR during the daytime. No significant sampling bias was found during nighttime hours. Adding the sources of uncertainty in quadrature gives a one-sigma uncertainty of hourly median HR equal to 6.1bpm for daytime and 1.9bpm for nighttime.

A strong diurnal pattern of HR was found with a maximum in the hour just after dawn and another local maximum in the hour just before dusk. The lowest HR was typically found in late afternoon during peak ambient air temperatures.

A common pattern of HR changes at dusk and dawn was used to calculate the start and end of “sleep.” On average “sleep” started 15 ± 16 minutes before civil dusk. “Sleep”

concluded, on average, 15 ± 14 minutes after civil dawn. Average “sleep” duration was 29 ± 24 minutes longer than civil night duration.

Multilinear regression was used to look for correlation between hourly median HR and meteorology data from nearby station RGAFF located on Ft. Hood Army Base. For daytime hours a statistically-significant correlation was found with ambient air temperature (slope = $-14.7\text{bpm}/^\circ\text{C}$) and relative humidity (slope = $+2.50\text{bpm}/\%$). For nighttime hours correlation was found with temperature (slope = $-14.3\text{bpm}/^\circ\text{C}$) and time since tag surgery (slope = $-1.42\text{bpm}/\text{hr}$).

No increase in hourly median HR was found during daytime quiet observation and nighttime disturbances. A median HR increase of $101 \pm 99\text{bpm}$ was found during the first hour of alternating daytime disturbance, but the second hour and third hours had less of an increase in median HR ($9 \pm 110\text{bpm}$ and $18 \pm 44\text{bpm}$ respectively). The same pattern was found in median HR during daytime four-hour chase. The first period had a larger increase ($78 \pm 51\text{bpm}$) than the second ($33 \pm 85\text{bpm}$) and third periods ($16 \pm 37\text{bpm}$). This suggests that the birds are habituating quickly with each successive hour of disturbance.

Summary

This research effort improved acquisition and analyses of remotely recorded heart-rate data from endangered and common passerine species on Fort Hood, Texas. We increased the quantity and quality of recorded heart-rate modulated RF transmissions through a number of hardware improvements (better wiring and cabling, new antennas, better power supplies and enclosures), operational improvements (increased antenna diversity by going to two independent receivers on opposite sides of the bird territory) and by providing diagnostic tools to quickly evaluate RF system performance (a hand-held RF spectrum analyzer and a ‘fake bird’ that transmits a known pattern of heart-rate transmissions). We developed a number of computer programs to take these continuous audio recordings of telemetry and transform them into files of heart-rate with one second time-stamps. Other programs were written to flag spurious data when the received signal is too weak or erratic. Thirteen birds were tagged in the spring of 2008 at Ft. Hood, Texas and telemetry recorded for a total of 36 days. Excluding three birds that generated essentially no results, the telemetry from the remaining ten birds was processed and spurious data removed. The average data recovery rate for these birds was nearly 60% of the time. Comparison to manually-derived heart-rates was performed on a small dataset within a limited range of heart-rates. While the agreement was within a few percent, a more careful comparison to manually-derived data using a wider range of heart-rates is needed to look for systematic biases in the computer-derived data.

Study 8: Energetic cost of human disturbance on endangered and common passerine species

Relationship between heart rate and energy expenditure

Heart rate was significantly correlated to energy expenditure in both species (Fig. 53) and the relationship across all individuals was best described by a linear fit: $\dot{V}\text{O}_2 = 0.114 f_H - 26.2$ (Fig. 53a, mean $r^2 = 0.735$) for white-eyed vireos and $\dot{V}\text{O}_2 = 0.446 f_H + 11.3$ (Fig. 53b, mean $r^2 = 0.837$) for black-capped vireos. We therefore considered heart rate a valid estimate of energy expenditure in both species and propose to use heart rate as a continuous and instantaneous estimate of energetic costs to disturbance in free-living vireos.

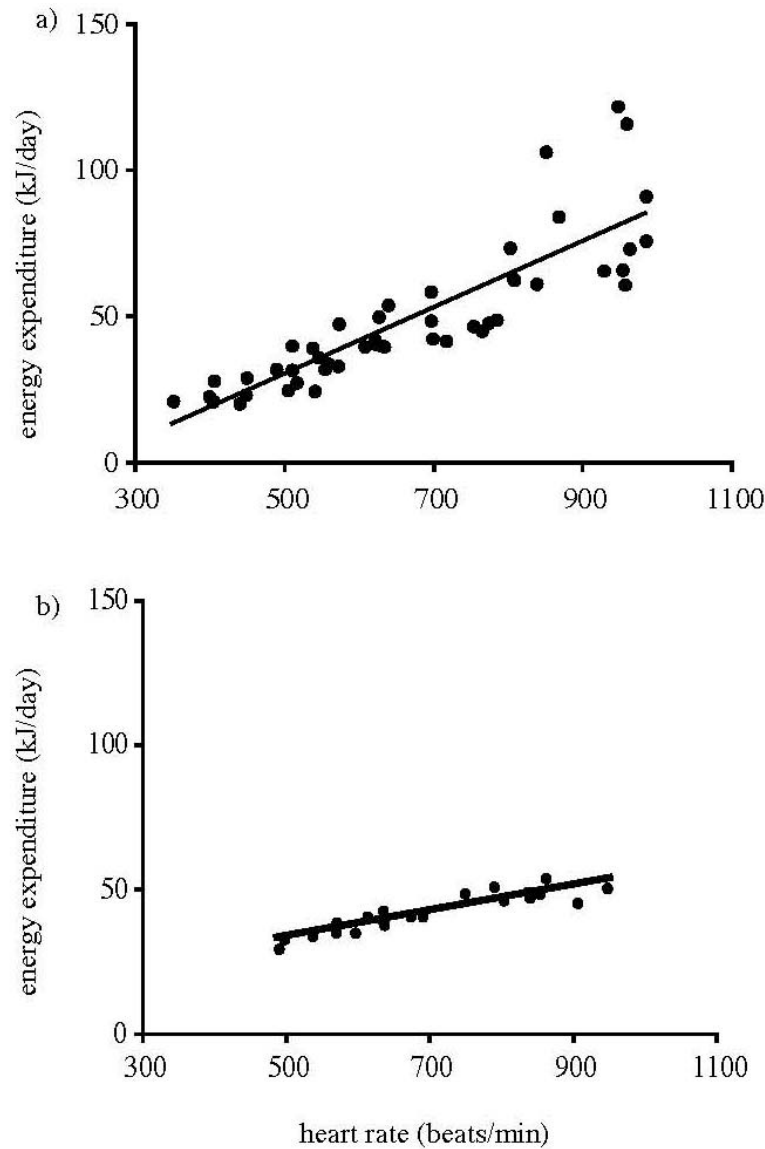


Figure 53. Relationship between heart rate (f_H bpm) and energy expenditure (kJ/day) of a) five white-eyed vireos and b) three black-capped vireos. The regression equation is: $V O_2 = 0.114 f_H - 26.2$, $r^2 = 0.735$ for white-eyed vireos (a), and $V O_2 = 0.446 f_H + 11.3$ for black-capped vireos (b). Heart rate calibrations were performed between 0700 and 2230h (local time).

Response to daytime human disturbance - white-eyed vireos

We detected a temporary (10-15 min) increase in heart rate (average 22%) shortly after the start of the long-duration (four-hour chase, Fig. 54a) human-mediated chase experiment, which was not present during non-disturbance trials on day 1 (Fig. 54a) indicating an initial alarm response to the chase. While heart rate decreased during the control period from 6h50 to

7h10, heart rate increased within the same time frame on the day of the disturbance experiment (Fig. 54a). However, individuals did not significantly differ in activity or energy expenditure

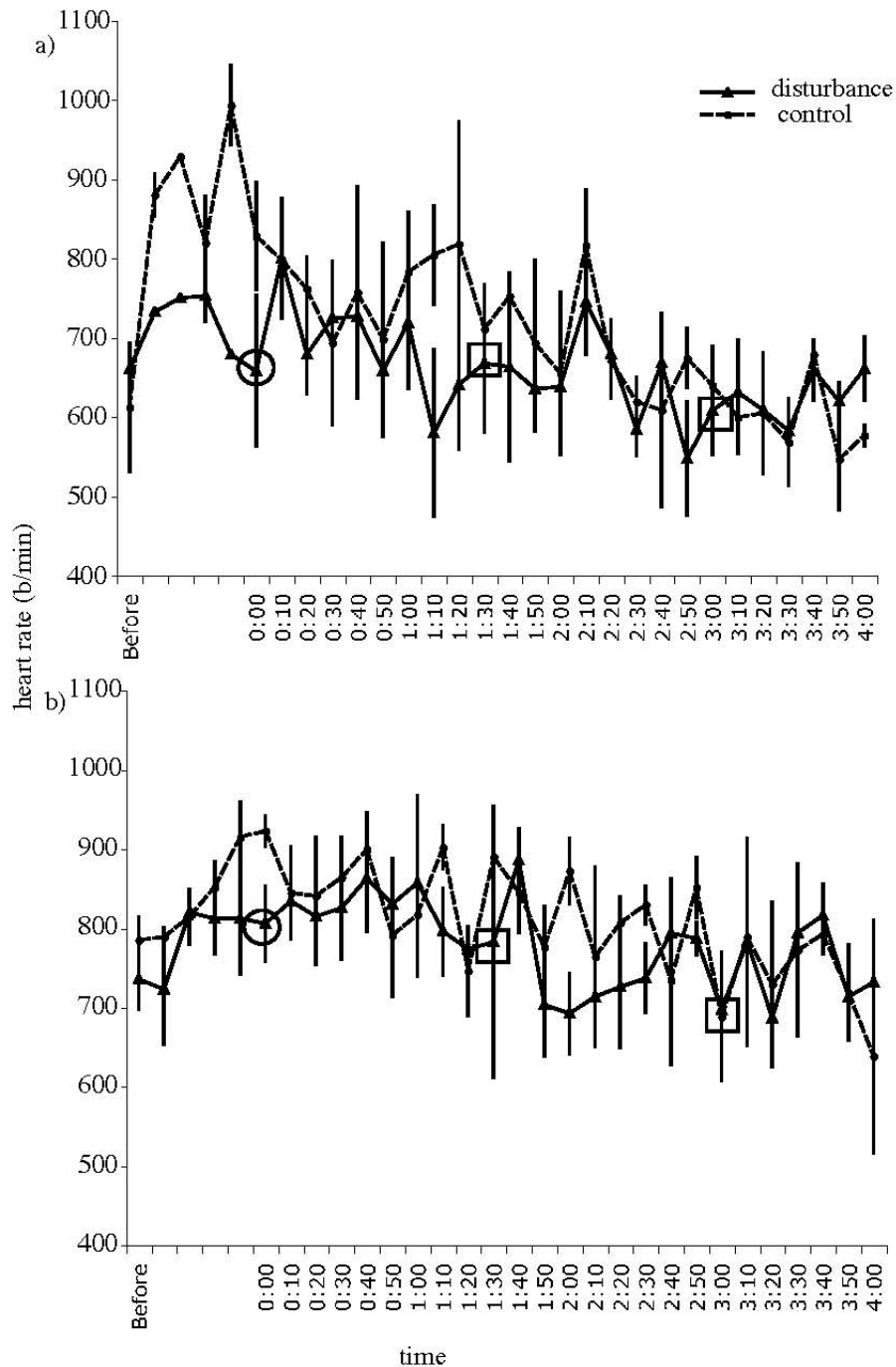


Figure 54. Mean heart rate ($\pm$ SE) for a) four male white-eyed vireos and b) for male black-capped vireos during the control no-disturbance trial (day 1), and in response to a 4-hour chase disturbance experiment (7h00-11h00 local time, day 2). Ellipses indicate start of chase and rectangles indicate change in observer for the chase experiment. Heart rate is shown from when birds became active (0600h local time).

during the four-hour chase when compared to the same time period with no disturbance (repeated measures ANOVA; energy $F_{2,6} = 1.233$, $P = 0.356$; activity $F_{2,8} = 1.424$, $P = 0.296$). Similarly, repeated one-hour disturbances (chase, passive, or walking) did not elicit a significant change in energy expenditure or activity before, during, and after each disturbance (repeated measures ANOVA; $P \geq 0.167$, Fig. 55a).

We did not detect significant differences in vireo energy expenditure and activity between different types of human disturbances, i.e., whether the one-hour disturbance was passive, walking, or a chase (repeated measures ANOVA, energy expenditure $F_{2,12} = 0.197$, $P = 0.824$; activity $F_{2,16} = 1.165$, $P = 0.337$; Fig. 56a). Birds that were subjected to four-hour disturbances did not expend more energy nor were they more active during the disturbance than birds that were exposed to one-hour repeated disturbances (one-way ANOVA, energy expenditure, $F_{1,9} = 3.678$, $P = 0.087$; activity, $F_{1,12} = 0.179$, $P = 0.680$). However, males that were subjected to one-hour disturbances on both days and night disturbances (group 2, Table 12) were significantly less active during the day (overall mean daily activity) than males that were

Table 12. Standardized disturbance trial design for breeding white-eyed vireo and black-capped vireo individuals. Heart rate and associated energy expenditure were continuously monitored for three nights and two days. Nights 1 (following capture and preceding day 1) and 2 (between days 1 and 2) are not shown because no disturbance trials were performed. Times are based on central daylight time zone.

groups		time period and disturbance type	
	day1	day2	night3
1 ($n = 4$)	no disturbance	four-hour continuous chase 0700-1100h	no disturbance
2 ($n = 4$)	one-hour repeated human or predator 1100-1200h, 1300- 1400h, and 1500-1600h	one-hour repeated human or predator 1100-1200h, 1300-1400h, and 1500-1600h	single one-hour at random time between 2300—0200h
3 ($n = 3$)	no disturbance	10min simulated territory intrusion 0700h and human 1100-1200h, 1300-1400h, and 1500-1600h	no disturbance

^aSample sizes reflect those obtained for heart rate measurements. We did not obtain heart rate for one individual in each disturbance trial. Sample sizes for activity are 5, 5, and 4, respectively.

subjected to repeated one-hour disturbances on day 2 only (group 3, Table 12: one-way ANOVA, $F_{1,7} = 14.526$, $P = 0.007$). However, we found no associated energetic costs to any such disturbances (one-way ANOVA, $F_{1,5} \leq 1.517$, $P \geq 0.273$). Heart-rate statistics for all individuals and disturbance treatments are provided in Appendix B.

Response to daytime human disturbance - black-capped vireos

We also detected a short and temporary (10 minutes) increase in heart rate after the start of the four-hour chase in black-capped vireos that was not present at the same time during the control non-disturbance period (Fig. 54b). But the response only elicited a 3% increase in heart rate and occurred 20 minutes after the start of the disturbance trial (Fig. 54b). In fact, when we compared energy expenditure between disturbance and control periods for the four-hour chase trial, we found that energy expenditure was significantly higher during the control period (mean = 46.1 ± 6.2 compared to 44.1 ± 3.1 kJ/day; repeated measures ANOVA, $F_{1,986} = 16.90$, $P \leq 0.001$). The same was found for activity levels where vireos were more active during non-disturbance control periods when compared to activity levels during the four-hour chase (mean = 0.16 ± 0.14 compared to 0.12 ± 0.14 ; Wilcoxon signed ranks test; $Z = -6.468$, $P < 0.001$).

Although one-hour repeated disturbances elicited an increase in energy expenditure during each of the three disturbances (Fig. 55b), we did not find a significant difference in energy expenditure before (baseline), during, and after each disturbance (repeated measures ANOVA; $F_{2,2,10.9} = 1.920$, $P = 0.192$). Moreover, while mean energy expenditure did increase (not significant) during each one-hour disturbance (Fig. 55b), energy expenditure during the second disturbance (13h00-14h00 local time) was lower than during the first disturbance (11h00-12h00) and comparable to energy expenditure during the last of the three disturbances (15h00-16h00; 37.0 and 37.1 kJ/day, respectively). Although not significant (repeated measures ANOVA; $F_{2,3,11.5} = 0.1448$, $P = 0.277$), black-capped vireos also increased their activity levels during the first and second 1-hour disturbance but not during the third disturbance (Fig. 55b). When we compared control and disturbance periods, we found that energy expenditure and activity were significantly higher during the control no-disturbance period (repeated measures ANOVA; $F_{1,1262} = 19.92$, $P \leq 0.001$, and $F_{1,1008} = 37.12$, $P \leq 0.001$, respectively).

Similar to white-eyed vireos, black-capped vireos did not differ in energy expenditure or activity in response to different types of human disturbances. Energy expenditure and activity levels did not significantly differ whether the 1-hour disturbance was passive, active, or a chase (repeated measures ANOVA; energy expenditure, $F_{2,10} = 2.366$, $P = 0.144$; activity, $F_{1,8,9.2} = 0.913$, $P = 0.427$; Fig. 56b). However, as in white-eyed vireos, birds that were subjected to a 4-hour chase (group 1, Table 12) expended significantly more energy (Mann-Whitney test; $Z = -23.1$, $P \leq 0.001$; Fig. 57a) and were significantly more active (Mann-Whitney test; $Z = -2.30$, $P \leq 0.001$; Fig. 57b) than birds that received the 1-hour repeated disturbance treatment (group 2, Table 12).

Response to night time human disturbance – white-eyed vireos

Heart rate increased towards the middle of the disturbance (Fig. 58a) indicating an arousal in response to nocturnal disturbance that was not associated with increased activity. However, these disturbances did not cause a significant increase in energy consumption in white-eyed vireos (Fig. 55a; $F_{2,6} = 0.632$, $P = 0.563$) when compared to energy expenditure immediately before the disturbance. Similarly, activity did not change from before to during to

after the disturbance (Fig. 58a; repeated measures ANOVA $F_{2,8} = 2.913$, $P = 0.112$). We also found no differences when comparing overall mean energy expenditure during the disturbance to the control period (first night) when no disturbance was conducted ($t_3 = -3.539$, $P = 0.038$, not significant with a sequential Bonferroni correction requiring $P \leq 0.007$). In fact, energy expenditure is higher on the night of no disturbance possibly because it was the first night following transmitter placement.

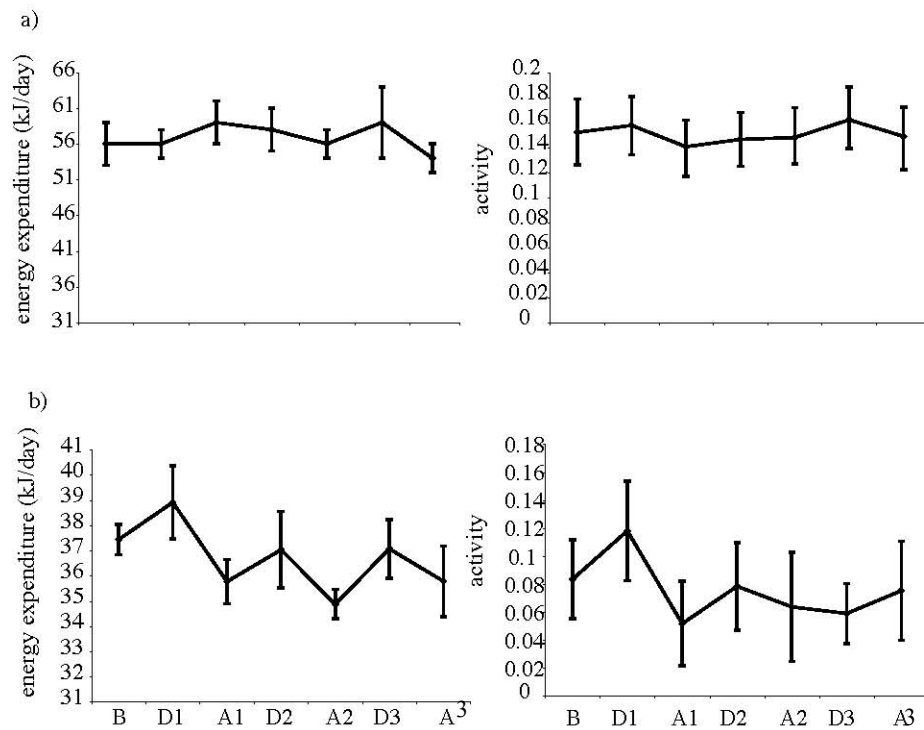


Figure 55. Mean ($\pm$ SE) energy expenditure and activity comparisons before (B), during (D), and after (A) 1-hour repeated experimental human disturbances for a) nine male white-eyed vireos and b) six black-capped vireos (two of which are females). The first disturbance was conducted between 11h00-12h00 (D1), the second (D2) between 13h00-14h00, and the third (D3) between 15h00-16h00, local time.

Response to night time human disturbance – black-capped vireos

As in white-eyed vireos, we show a slight increase in heart rate and associated energy expenditure in response to the 1-hour night disturbance (Fig. 58b) indicating awareness to the disturbance, shown also by the slight increase in activity shortly after the start of the disturbance (Fig. 58b). However, we did not find a significant difference in either energy expenditure or activity before, during, and after the disturbance at night (repeated measures ANOVA; energy expenditure, $F_{2,8} = 0.739$, $P = 0.508$; activity, $F_{1,4} = 1.02$, $P = 0.369$). When we compared control and disturbance periods, we found that energy expenditure was significantly lower on the night of the 1-hour experimental disturbance (night 3) than both other nights (nights 1 and 2) where no experimental disturbance occurred (repeated measures ANOVA; $F_{1,9,416.3} = 704.30$, $P \leq 0.001$).

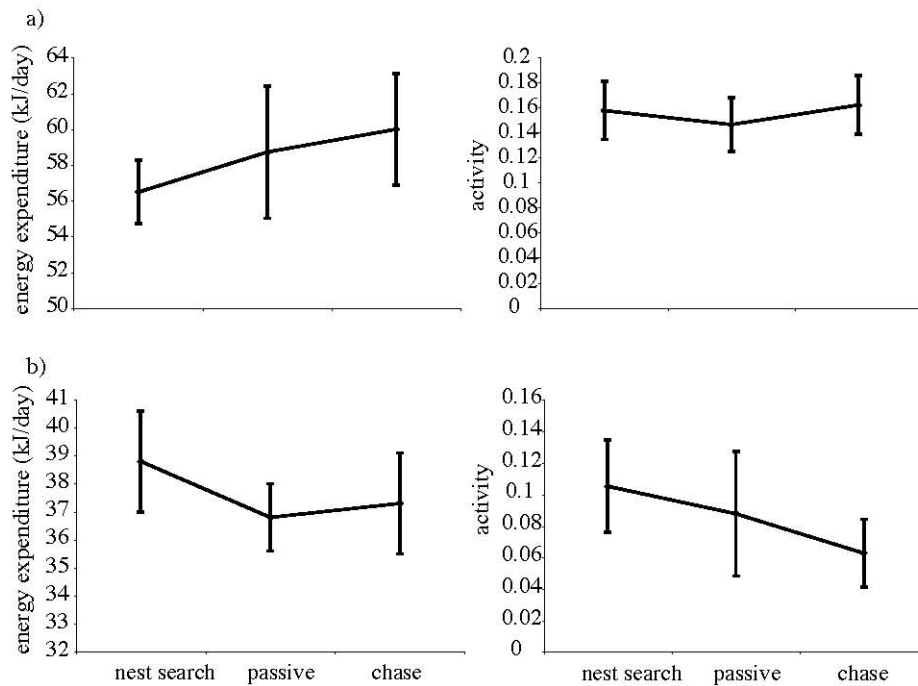


Figure 56. Mean ($\pm$ SE) energy expenditure and activity comparisons among a 1-hour nest search, passive (sitting in bird's territory), and chase disturbance for a) nine male white-eyed vireos and b) six black-capped vireos (two of which are females).

Response to natural disturbances – white-eyed vireos only

There was no significant effect of short repeated predator call and decoy presentations on energy expenditure or activity (repeated measures ANOVA, energy expenditure $F_{2,4} \leq 9.678$, $P \geq 0.029$ Bonferroni correction required $P \leq 0.007$; activity $F_{2,8} \leq 2.928$, $P > 0.1$; Fig. 55a and b) nor in relation to territorial intrusions (repeated measures ANOVA, energy expenditure $F_{2,4} = 4.177$, $P = 0.105$; activity $F_{2,6} = 1.328$, $P = 0.333$; Fig. 55b). Birds did not expend more energy and were no more active in response to human disturbances than to natural disturbances (repeated measures ANOVA, energy expenditure $F_{2,2} = 0.133$, $P = 0.882$; activity $F_{2,8} = 2.178$, $P = 0.353$).

Breeding behavior in relation to disturbances – white-eyed vireos

Whereas most males continued nesting duties (e.g., incubating, feeding young) throughout human-mediated disturbance trials, two of five white-eyed vireos that were subjected to human, predator, and night disturbances abandoned their territory some time after the 60-hour monitoring period. We surveyed both territories on five occasions four days after the monitoring period and were unable to locate either bird. Both males were transmittered early in the breeding season (April) and were in the early breeding stages (unpaired newly arrived male, and nest building).

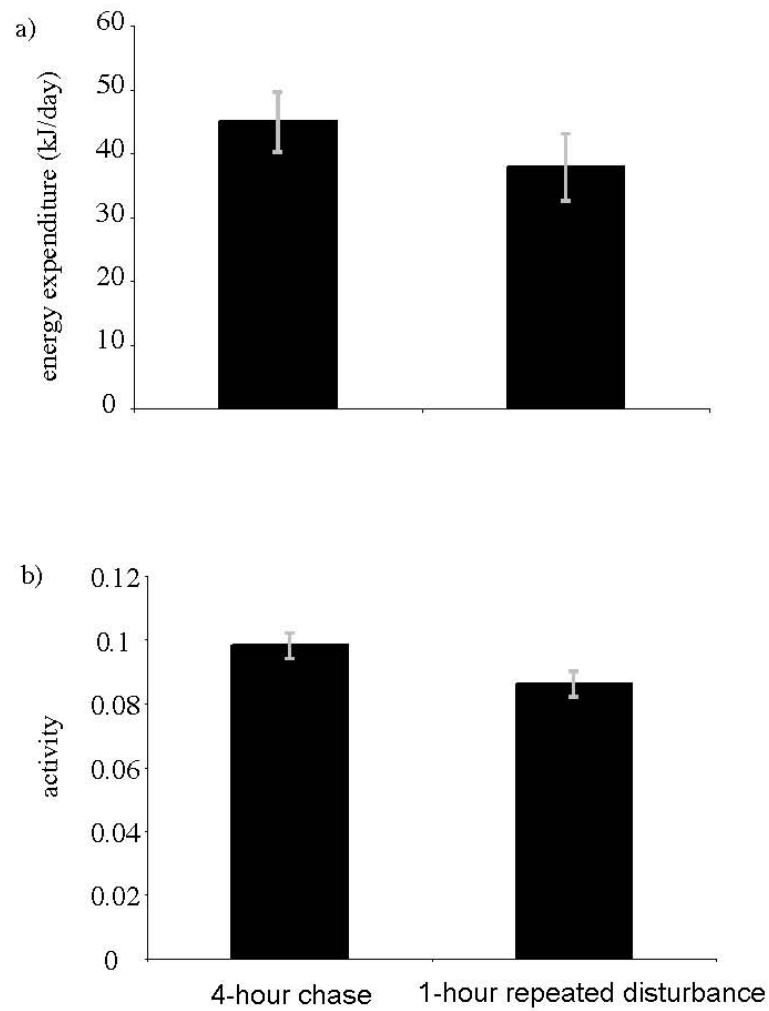


Figure 57. Mean ($\pm$ SE) energy expenditure (a) and activity (b) comparisons between black-capped vireos that were subjected to a 4-hour chase ($n=4$) and birds that were subjected to three 1-hour repeated disturbances ($n=4$ males, $n=2$ females).

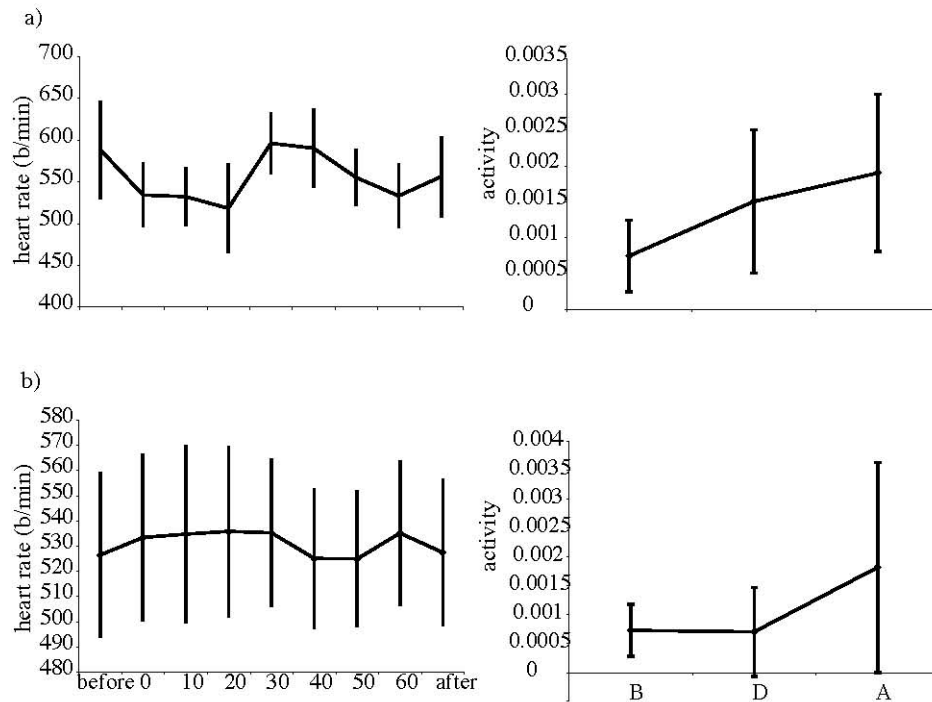


Figure 58. Mean heart rate response ($\pm$ SE) to one hour of night time human-mediated disturbance (randomly selected between 2300-0200h) and mean ($\pm$ SE) activity before (B), during (D), and after (D) the night disturbance for a) four male white-eyed vireos and b) five black-capped vireos.

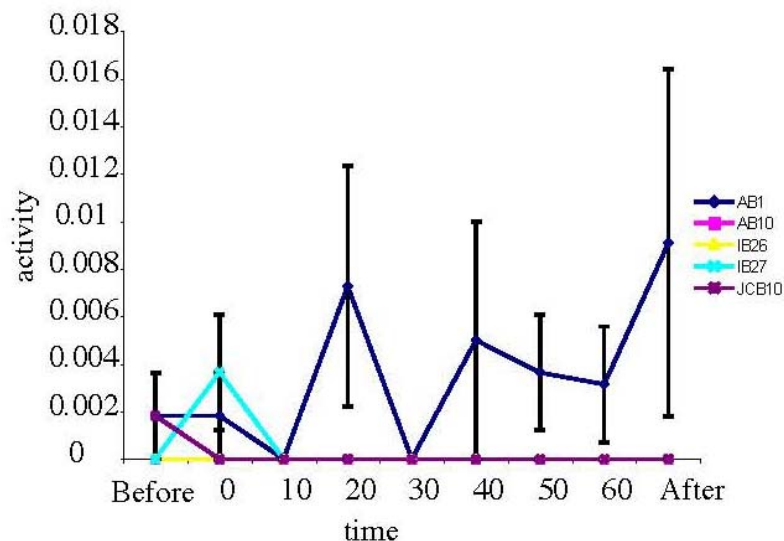


Figure 59. Mean activity per individual black-capped vireo in response to 1-hour night time disturbance (randomly selected between 2300-0200h). Individual 'AB1' was a female with three nestlings and abandoned the nest some time after night 3 of the monitoring period.

Breeding behavior in relation to disturbances – black-capped vireos

Of the 14 transmittered birds, only one brooding female (3 nestlings of 2-3 days-old) abandoned the nest some time after the third night of the monitoring period. This individual showed the highest energy expenditure and activity during the night of the disturbance trial ('AB1', Fig. 59). On the day following the disturbance, the temperature had dropped to about 38°F with high winds and rain. The nest had also been depredated.

Discussion

Birds often show negative responses to human activities, such as decreased reproductive success (Flemming et al. 1988, Piatt et al. 1990), elevated corticosterone stress responses (Wingfield et al. 1982, Nephew et al. 2003), or altered behavior (e.g., birds flee from the disturbance, Burger 1981, Henson and Grant 1991, reviewed in Gill 2007). However, we know little about energetic costs of human disturbances in free-living birds, and what we know is restricted to large, long-lived species (Nimon et al. 1996, Ackerman et al. 2004). Life history theory predicts that the costs and benefits of responding to disturbance, and diverting energy toward survival and away from reproduction, should vary across species. Slow-living animals, i.e., those with large body size, small clutch, or delayed maturity (Wikelski et al. 2003) are expected to show the strongest emergency reactions to disturbance. Under environmental challenges such long-lived animals benefit from strategies that promote long-term survival, and thus, when faced with a trade-off, they should re-direct energy allocation to activities that will enhance long-term survival over activities associated with reproduction (i.e., breeding can be postponed). On the other hand, fast-living animals such as small songbirds – organisms with small body size, large clutch size, and limited reproductive opportunities – should favor investment in current reproduction over investment in survival. Thus, short-lived organisms may appear much more tolerant towards environmental challenges, especially during breeding. Determining the energetic demands associated with unpredictable disturbances across a broad life-history spectrum can therefore provide a powerful conservation tool to understand long-term effects on survival and reproductive success across species. Most previous efforts have focused on slow-living species (e.g., Hunt et al. 2003, Walker et al. 2006, Wikelski and Cooke 2003), but here we studied two fast-lived species to extend the scope of our understanding of anthropogenic stressors on wild animals. Furthermore, with our study system, we compare the physiological response of a common generalist species, the white-eyed vireo, and an endangered species with a severely restricted breeding range, the black-capped vireo, to human-mediated disturbances.

Our study, the first to investigate energy expenditure in response to disturbance in small (<12g) free-living passerines, shows no evidence of elevated energetic costs to human or natural disturbances. Compared to control time periods, white-eyed vireos did not expend significantly more energy nor were they more active during a four-hour continuous chase by three different observers. Even repeated one-hour human-mediated activities, night disturbances and predator or conspecific presence did not elevate their average energy expenditure over a few hours. Whatever comparison of energy expenditure we conducted – either among baseline, during, and after the stressor, or between control and experimental disturbance periods – we found no differences. Similar results were found for black-capped vireos. We were not able to detect a significant difference in energy expenditure or activity before, during, and after the three 1-hour repeated disturbances, nighttime disturbance, nor whether the 1-hour disturbance passive, walking, or a chase. However, black-capped vireos expended significantly less energy and were less active during the 4-hour chase than during the control period on the previous day of the

disturbance. Similarly, energy expenditure was significantly lower during the night of the disturbance (night 3) when compared to both other nights of no disturbance (nights 1 and 2). Therefore, not only did we not find the predicted increase in energy expenditure in response to human disturbances but for black-capped vireos, the reverse was true. We found that both energy expenditure and activity were significantly lower during the human-mediated disturbances (4-hour, 1-hour repeated, and night chases) compared the control period on the previous day (day 1).

It is important to point out, however, that we did find one predicted response. Mean heart rate initially increases when birds are stressed by humans, indicating that vireos perceive the disturbance and are mounting an ‘alarm’ response. However, this was a transitory response and mean energy expenditure did not change significantly (Figs. 55 and 56). Similarly, we found evidence that mean heart rate increased in response to a one-hour night disturbance (Fig. 58) with no significant change in energy expenditure. We cannot exclude the possibility that more frequent or longer night disturbances may have elicited significant energy consumption in vireos. However, we consider this unlikely because birds did not, on average, increase energy expenditure in response to daytime disturbances of varying intensity, duration, and type. Similarly, we found no effect of heart rate transmitters on the activities of vireos because birds continued with breeding duties. We therefore propose that both white-eyed vireos and black-capped vireos did not show measurable energetic responses to either day or night stressors.

The lack of a measurable increase in energy expenditure was an unexpected result. There are several potential explanations. First, it is conceivable that vireos rapidly perceived our disturbance treatments as non-threatening. This explanation fits parallel data indicating that similar disturbances also do not elicit hormonal responses (Study 1). However, we cannot exclude that other physiological systems, such as the immune system, were affected. For example, Ots and Horak (1996) demonstrated a trade-off between health and reproductive effort. Great tits, small passerines (19g), became immune-suppressed when allocating energy towards reproduction. Such trade-offs may also occur in the face of environmental challenges including human or natural disturbances.

A second potential explanation is that because both white-eyed and black-capped vireos are a fast-living species (short-lived, breed in their first year, and have few breeding attempts), they quickly assess the severity of unpredictable changes in their environment, and when they perceive those changes as non-lethal, they minimize energy expenditure towards alleviating those disturbances in favour of maximizing energy available for reproduction. We propose that birds, and potentially other animals, with similarly fast life histories will show similar responses in energy expenditure. In contrast, when facing anthropogenic stressors, slow-living birds benefit from redirecting their physiology and behavior toward survival. For example, slow-living species such as Humboldt penguins (*Spheniscus humboldti*, Ellenberg et al. 2006) wandering albatrosses (*Diomedea exulans*, Weimerskirch et al. 2002), and Tule greater white-fronted geese (*Anser albifrons elgasi*, Ackerman et al. 2004) increased their heart rates and energetic costs to human-mediated stressors. All three species are long-lived (20 and 80 years in the penguin and albatross, respectively), lay small clutch sizes (1-2 eggs), have large body size (> 2kg), or long developmental periods, so responding to disturbances in ways that increase survival at the expense of current reproduction is consistent with our hypothesis that interspecific differences in response to unpredictable, non-life-threatening disturbance are correlated with interspecific differences in life history. Our results are also consistent with studies that showed trade-offs in slow vs. fast life-histories in relation to immune defence investment (Martin et al.

2001, Tella et al. 2002). Recently, Martin et al. (2006) showed that slow-living house sparrows (*Passer domesticus*) from tropical populations invested more energy in costly immune activities than their temperate zone fast-living conspecifics.

We further offer two potential explanations for the lower heart rate and activity levels detected in black-capped vireos during disturbance experiments (4-hour, 1-hour repeated, and night time disturbances) compared to control periods. The control no-disturbance trials were administered on day 1 of the monitoring period, the day following heart rate transmitter placement. Therefore, during this time, birds may be acclimatising to the heart rate transmitters that would elicit higher heart rate and activity during locomotion to compensate for the added weight of the transmitter (Caccamise and Hedin 1985). However, if there is an energetic cost associated with heart rate transmitters, we would expect associated behavioral changes in reproductive duties such as incubation, brooding, and feeding young as has been found in larger birds (e.g., mallards, *Anas platyrhynchos*, Pietz et al. 1993). Yet, all birds transmitted returned to normal reproductive duties upon release after transmitter placement and we did not detect any decrease in these duties during our quiet observations on the no-disturbance control day (day 1). Furthermore, this does not explain the lower values found during the 1-hour night disturbances because these were conducted on the last night of our experiments, two nights after transmitter placement. Alternatively, black-capped vireos could be suppressing heart rate and activity in response to the human-mediated disturbances conducted in our study. Such a response was observed in incubating female ptarmigans (*Lagopus mutus hyperboreus*, Gabrielsen et al. 1985) for which heart rate decreased during the presence of a human intruder at the nest (freezing behavior) and immediately increased after the intruder turned to leave (tachycardia prior to fleeing). However, if this were true in black-capped vireos, we would expect to see this pattern during the disturbance experiments. Instead, although not significant, we detected a slight increase in heart rate shortly after the start of the 4-hour chase and night time disturbance (Fig. 54b and 58b, respectively), and an increase in energy expenditure during each of the 1-hour disturbances (Fig. 55b). Conducting disturbance trials alternatively with control periods over a longer monitoring period would be necessary to understand the lower heart rate response to disturbances and tease out potential transmitter effects. Currently, heart rate telemetry monitoring periods are limited to 4 days in small passerines because of power constraints (battery life).

Finally, our results also hint at a difference at how anthropogenic stress is perceived during the reproductive season. Whereas two white-eyed vireo males and one black-capped vireo female that we disturbed early in the season abandoned their territories, all 27 birds that we disturbed later in the season continued normal breeding activities. Although this observation is somewhat anecdotal, it is consistent with the interpretation that the reproductive value of an advanced brood was higher than at the start of the breeding season. Despite the disturbance late in the breeding season, birds continued their parental investment as re-nesting opportunities would decrease as the breeding season progressed.

Understanding the effect of human activity on wildlife is a major conservation concern, and military installations in particular have become areas of special conservation attention because they often provide large expanses of rare habitat for species of concern (Duncan et al. 1995, Krausman et al. 2005). Activities associated with military training are thought to act as severe disturbances for wildlife (Maier et al. 1998, Delaney et al. 1999) because of their high intensity and episodic nature. Our study was aimed at quantifying the energy expenditure of two passerine species during the breeding season in response to non-destructive and indirect human

disturbance similar to military training on foot. While more chronic disturbances such as human-induced habitat changes could have effects, we failed to find evidence for an energetic cost to our study species in response to human intrusion on foot. The majority of the birds monitored continued to perform breeding duties such as incubating and feeding young. However, we do not yet know whether the lack of a response to disturbance is restricted to white-eyed vireos or whether other military activities such as live fire could have a significant impact on energy expenditure. This species is expected to be resistant to human disturbance because it can breed in urban environments (Hopp et al. 1995). However, we also failed to find an energetic cost to disturbance in the endangered black-capped vireo, which does not breed close to human environments and has a much more restricted breeding range (Grzybowski 1995). With only two vireo species studied to date, it is impossible to speculate whether there is a phylogenetic constraint to the physiological response to human disturbance in the Vireonidae family. However, we expect fast-living animals in general to be much less affected by human disturbances. Furthermore, if military areas provide otherwise rare optimal habitat for species, this benefit may outweigh potential disturbances associated with military training (Gill et al. 2001). We suggest that military training areas may act as preservation sites if efforts are geared toward understanding impacts on wildlife at multiple levels as has been suggested for Sonoran pronghorn (*Antilocapra americana sonoriensis*, Krausman et al. 2005) and moose (*Alces alces*, Andersen et al. 1996).

Summary

Anthropogenic or natural disturbances can have a significant impact on wild animals. Therefore, understanding when, how, and what type of human and natural events disturb animals is a central problem in wildlife conservation. However, it can be difficult to identify which particular environmental stressor affects an individual most. We use heart rate telemetry to quantify the energy expenditure associated with different types of human-mediated and natural disturbances in two breeding passerines, the common white-eyed vireo and the endangered black-capped vireo. We fitted 0.5g heart rate transmitters to 28 vireos and continuously recorded heart rate and activity for two days and three nights on the Fort Hood, Texas. We calibrated heart rate to energy expenditure for five additional males using an open flow push-through respirometry system showing that heart rate predicted 74% and 84% of energy expenditure in White-eyed and black-capped vireos, respectively. We conducted standardized disturbance trials in the field to experimentally simulate a natural stressor, predator presence (white-eyed vireos only), and two anthropogenic stressors. Although birds initially showed behavioral and heart rate reactions to some disturbances, we could not detect an overall increase in energy expenditure during one- or four-hour disturbances. Similarly, overall activity rates were unaltered between control and experimental periods and birds continued to perform parental duties despite the experimental disturbances. We suggest that vireos quickly determined that disturbances were non-threatening and thus showed no (costly) physiological response. We hypothesize that the lack of a significant response to disturbance in vireos is adaptive and may be representative of animals with fast life histories (e.g., short life span, high reproductive output) so as to maximize energy allocation to reproduction. Conversely, we predict that energetic cost of human-mediated disturbances will be significant in slow-living animals.

Conclusions and Implications for Future Research/Implementation

Project objective (1) – Acute and chronic physiological response to human disturbance

Our findings indicate that in black-capped vireos and white-eyed vireos functioning of the HPA axis is, at most, only very weakly affected by offspring-directed threats. Nevertheless, our threats appeared to be perceived as threats by parents because normal parental care almost always ceased during presentations and alarm vocalizations and overall activity around the nest increased, very similar to the effect of live natural predators. This contrast between physiological and behavioral responses to a threat to fitness has been found in other vertebrates. Thus, although a major effect of increased glucocorticoid concentrations, energy mobilization would seem to help sustain the observed behavioral responses of parents, or to help prepare them if the offspring-directed threat became an adult-directed threat, we found no evidence that corticosterone is involved in permitting or preparing for defense behaviors in parents. Repeated nest-directed threats did not elicit any indication of a chronic stress response.

In experiments with direct harassment of adult black-capped vireos, white-eyed vireos and golden-cheeked warblers, one-hour of human pursuit caused no significant increase in plasma corticosterone concentrations. Based on our findings, we conclude that an hour of relatively constant exposure to direct human harassment causes no clear acute physiological stress response in these three migratory birds.

Interestingly, the corticosterone response to 30 min of restraint was suppressed in black-capped vireos in both the nest-directed disturbance and human pursuit experiments. The mechanism for this effect is unknown, although exposure to one stressor is known to alter the response to a subsequent stressor in other species.

Results for energetic costs of disturbance are generally consistent with results from our endocrine studies. Human pursuit caused only a brief initial spike in heart rate (a measure of the “fight-or-flight” response) in black-capped vireos and white-eyed vireos breeding at Fort Hood, and heart rate was otherwise similar before, during, and after the pursuit. The finding that duration or frequency of disturbance had no significant effect on energy expenditure indicates that the levels of disturbance in this study there was no chronic elevation energy expenditure in response to disturbance.

Overall, we found only weak and mixed evidence that physiological response measures in songbird species in this study are acutely or chronically sensitive to human activity at exposure levels in this study, although adults exhibited strong behavioral responses.

Disturbance trials in this study likely exceed anything individuals might be exposed to in the context of military operations. Human presence associated with military training activities will be more transitory in relation to any individual focal bird compared with the experimental disturbance trials presented in this study. Indirect effects of training activity on habitat quality may have a more significant effect on physiological response measures as indicated by the differences in corticosterone levels in golden-cheeked warblers in habitats with high road densities.

Project objective (2) – Modulation of stress response to repeated human disturbance

Organisms' response to stressors can become increasingly sensitized with repeated exposure or alternatively reduced through habituation depending on external conditions and internal feedback mechanisms. Disturbance levels in our study did not elicit any strong acute or chronic physiological response. Thus, we did not observe significant modulation of physiological response measures with repeated disturbance trials. Transitory increase in heart rate at the start of disturbance trials as part of an "alert" phase with a subsequent decrease to comparable control levels does not indicate that there is a functional alteration of perceptual processing of feedback mechanisms. One alternative explanation for our observation of no or weak physiological response is that our study individuals were previously exposed to human disturbance from military training activities and may have been already habituated to human presence. This is possible; however, our studies were conducted for the most part in regions of Fort Hood with very limited training activity, particularly with dismounted soldiers traveling through or bivouacking in our study sites. Further evaluation of the capacity to modulate these physiological response measures would require identification of disturbance levels that would elicit a clearly recognized physiological response and then repeat these levels of disturbance over some period of time. Again, disturbance treatments in this study likely exceed any transitory human disturbance related to training activities, so that increasing experimental disturbance levels above those in this study would have limited practical application.

Project objective (3) – Stress response as a function of life-history traits

The small passerine species that were subjects in this study clearly showed behavioral responses to human disturbance but no or weak expression of physiological response in terms of total energy expenditure or activation or modulation of the adrenocortical response. This suggests that cost/benefit trade-offs are being made in the activations of response mechanisms to potential stressors in these species and differences in response mechanisms may be a function of differing life-history traits.

In this study, we were particularly interested in testing whether endangered passerine species such as the habitat specialist golden-cheeked warbler and black-capped vireo differed from more common habitat generalists such as the white-eyed vireo and northern cardinal. Overall, we found minimal differences in physiological response measures to human disturbance among these species. In terms of adrenocortical response, black-capped vireos did show a decrease in capture-induced corticosterone levels in response to human chases, whereas, golden-cheeked warblers did not. This result may be attributed to the possibility that warblers occur higher in the canopy and so may have a greater opportunity to maintain distance from the disturbance source. Neither black-capped vireo nor white-eyed vireo had significant differences in energy expenditure in response to disturbance.

The difference in baseline corticosterone in the habitat specialist golden-cheeked warblers in habitats with high road densities was not observed in habitat generalist white-eyed vireos. This is consistent with other studies on the warbler that indicate detrimental effects of habitat edge on this species. Golden-cheeked warblers are generally considered forest-interior species, which may be an adaptive response to studies on Fort Hood of increased predation risk from rat-snakes, the major predator of songbird species on Fort Hood. Warblers inhabiting

habitats with high road densities are more exposed to edge and may respond with altered stress response. Generalist white-eyed vireos have likely developed adaptive mechanisms to cope with increased predation risk associated with habitat edge. One possible adaptive mechanism is the greater reneesting potential of white-eyed vireos on Fort Hood relative to golden-cheeked warblers.

The relative lack of differences in physiological response to stress among species examined in this study may be attributed to their many similarities in life-history traits. All are small passerine species that relatively short-lived. They are neotropical or short-distance migrants and are all open nesters. Annual clutch size and reproductive potential are similar.

However, when results from our empirical studies are compared with results from studies of other species with different life-history traits, contrasts are observed. Our results contrast with similar studies showing that the HPA axis was altered following short- and long-term exposure to offspring-directed threats in cavity or ground nesting species where survival of attending adults is at greater risk. Based on these studies, a reasonable conclusion would be that threats to nests, and thus threats to reproductive success, elicit corticosterone responses in parent songbirds. Our data clearly do not fit this pattern, which we suggest results from contrasts in the degree of overlap between offspring- and adult-directed threats. We propose that nest-directed threats elicit a physiological response (i.e., corticosterone release) in adults only when they threaten adults and offspring alike, which depends on the type of threat and the ability of adults to escape from it. Previous studies of the adrenocortical response to offspring-directed threats have not fully separated threats to offspring from threats to adults because the nest characteristics of the study species put adults at a higher risk of nest-associated death than the vireos in our study. In contrast, when we threatened active nests of black-capped and white-eyed vireos, parents could easily detect and control their perceived risk relative to the disturbance, because they build cup-shaped pendant nests attached near the tips of thin branches that are often flanked by flight lanes and surrounded by a “bubble” of open space. Several independent lines of evidence support our hypothesis that the ability to modulate risk exposure determines whether birds initiate a corticosterone response to a nest-directed threat and that the ability to escape a threat, and not the perception of the threat alone, appears to influence whether the HPA axis is stimulated. Contrasting sensitivities to nest-directed threats between open-nesters (this study) and cavity-nesters suggest that a nest style that causes a high correlation between offspring- and adult-directed threats may be a risk factor for sensitivity to human activity.

The lack of a measurable increase in energy expenditure was an unexpected result. We might expect that white-eyed vireos that are ubiquitous and can breed in urban habitats would be more resilient to human disturbance than habitat specialist black-capped vireos, when in fact our results showed no difference for either species in energy expenditure in response to disturbance. It is possible that vireos rapidly perceived our disturbance treatments as non-threatening. This is consistent with parallel data indicating that similar disturbances also do not elicit hormonal responses (Study 1). However, in both cases adults did exhibit behavioral responses to human disturbance. Although total energy budgets may not have been altered, activities were redirected. A second potential explanation is that because both white-eyed and black-capped vireos are a fast-living species (short-lived, breed in their first year, and have few breeding attempts), they quickly assess the severity of unpredictable changes in their environment, and when they perceive those changes as non-lethal, they minimize energy expenditure towards alleviating those disturbances in favour of maximizing energy available for reproduction. We propose that birds, and potentially other animals, with similarly fast life histories will show

similar responses in energy expenditure. In contrast, when facing anthropogenic stressors, slow-living birds benefit from redirecting their physiology and behavior toward survival. For example, slow-living species such as Humboldt penguins, wandering albatrosses, and Tule greater white-fronted geese increased their heart rates and energetic costs to human-mediated stressors. All three species are long-lived (20 and 80 years in the penguin and albatross, respectively), lay small clutch sizes (1-2 eggs), have large body size ($> 2\text{kg}$), or long developmental periods, so responding to disturbances in ways that increase survival at the expense of current reproduction is consistent with our hypothesis that interspecific differences in response to unpredictable, non-life-threatening disturbance are correlated with interspecific differences in life history.

Implications for future research/implementation

The results of this study are generally hopeful from a conservation perspective. They suggest that mild forms of human activity for a short period of time are not likely to cause acute stress in most songbirds. However, we do not know if there are thresholds for continuous longer-term disturbances than were conducted in this study that would elicit acute or chronic stress in songbirds. Furthermore, although physiological responses were relatively small in these studies, birds nevertheless altered their behavior, including abandoning normal mating and parenting activities. This suggests that behavioral response to disturbance may provide a more direct measure of fitness consequences of human disturbance. In decisions on which response measures to evaluate relative to disturbance (physiological or behavioral) investigators will have to weigh the ability to collect relevant measures and the time and cost associated with collection of these data. Our results and results from other studies suggest that the interaction of physiological and behavioral response is quite dynamic and for any individual species is a function of life history traits. Future research will be needed to clarify these interactions and predictions of likely behavioral and physiological response as a function of life history traits.

We believe this study is ground-breaking in terms of evaluating stress response from direct measures of free-living passerines, obtaining direct measurements from two federally-listed endangered avian species, and integration of multiple response measures including the adrenocortical response and energy expenditure. To date, project collaborators have six peer-reviewed journal articles (see Appendix C) published and two in review. We will continue to publish results of this research in peer-reviewed journals as we continue to mine the wealth of data acquired under this project. One Master's thesis was also completed as part of this research effort. In addition project collaborators have published 16 technical reports and abstracts for scientific conferences and have presented and an additional 11 talks at scientific conferences nationally and internationally.

Results of this study are already being incorporated in plans for monitoring effects of Armour School transition to Fort Benning, Georgia and in a 5-year programmatic consultation for proposed range modifications at Fort Hood, Texas. Data for golden-cheeked warblers are being incorporated in a revision of the U.S. Fish and Wildlife Recovery Plan for this species.

Field methodologies from this study will set a standard and provide guidance for future research designs to evaluate stress response in wildlife species to human disturbance. Data reduction programs for heart rate telemetry data have been posted on the web as freeware and will greatly enhance the capabilities of researchers worldwide in extracting and interpreting these types of data.

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Appendix A. Scilab program heartrateV29i.sce

This program is referenced in Methods and Results and Discussion for Study 7 in this report. This program is an improved filtering method for analysis of heart-rate telemetry data obtained from free-flying black-capped vireos and white-eyed vireos.

```
// This program marches thru a series of 5 min .wav files and determines peak sub-carrier
// frequency and then IIR bandpass filters the file. The results for each second are not filter
// in any way, that is done in a postfilter program. Input data files must be 48000Hz
// 8bit wav format.
//
//
// v14 add confidence based on std, took out confidence test, tried to subsample
// but doesn't work well
// v15 simple peak filtering scheme works well
//
// v16 quick version
//
// v17 rips thru 40min_good just fine, but can't handle other files
// v18
// v19 lowered filter order to 4: now works on 1600Hz peaks, bandwidth=40Hz best,
// reduced MinVarianceAcc to 0.001 (works fine), tried to filter on shoulder
// 100Hz away from subCarrier (didn't work).
//
// v20 added HrConfidence=ACF*variance, improve graphs
//
// v21 lowered accept period to 200ms, removed :s from .dat file, removed
// AccBpmJump (put in post process filter), add try/catch to file open
//
// v22 add verbose, add use previous medCWfreq if none acceptable in present
// five min period
//
// v23 use median of 20 ffts to determine subCW freq.
//
// v24 use most popular of 20 ffts (this was used to process all files prior to Aug 08
//
// v26 experimental: matrix inversion during SSB filtering, Center-clipped. seems to work well, but
// doesn't chew thru more than 100s
//
// v27e put all 300s into RAM, seems to be better than v24
//
// v28e use median of 3 overlapping half-sec analysis frames, works well,
//
// v29e use median of 5 overlapping half-sec analysis frames, works well: a bout half the number of outliers,
// count up points close to median HR in each analysis frame (nClose), clean up and add save
// graphics file. Add FiltSB & FiltPB.
//
// v29f
//
// v29g start to add other inter-harmonics, doesn't line up in time
//
// v29h inversion & refilter each time: works fine, but DeltaF between interharmonics is not constant.
//
// v29i determine second highest peak, filter & add to highest peak. works well!! need to keep
// ShoulderOffset=0, fixed inversion problem, try 50ffts: no improvement. found optimum
// centerClip=0.3, about 2-5% better than v24 but slow.
//
clearglobal();
stacksize(100000000); //as large as possible
```

```

////////////////////////////////////
////////// USER SELECTABLE INPUTS //////////////////////////////////////
////////////////////////////////////
Nsec=300;           //number of seconds to examine in file in one chunk
Srate=48000;        //sample rate (Hz)
NsecEnv=0.001;      //number of seconds in envelope data
NptsEnv=Srate*NsecEnv; //number of samples in single envelope
NsecCorr=0.5;       //number of seconds in each correlation
NptsCorr=NsecCorr/NsecEnv; //number of pts in each autocorr period
FiltBW=40.0;        //bandwidth accepted by old IIR filter (Hz) 40Hz seems good, 100or20 bad
FiltPB=400.0;       //passbandwidth (Hz) 400Hz is better than 150 & 250Hz
FiltSB=100.0;       //stopbandwidth (Hz) 100Hz is best
MinHRperiod=46;     //min accepted period btw heartbeats (ms) 50=1200bpm
MaxHRperiod=175;    //max accepted periods btw heartbeats (ms) 160=375bpm, 150=400bpm, 175=342bpm
Nlags=MaxHRperiod+2; //number of lags to calc in each autocorrelation
LPfreq=100;         //cutoff period (ms) for anti-alias filter prior to ACF 22Hz=1320 Bpm
N=4096;             //number of points in FFT
FractCenterClip=0.3; //fraction of max that is center clipped
MinFreqAccFFT=1200; //minimum frequency accepted by FFT (Hz)
MaxFreqAccFFT=4000; //max freq accepted by FFT
ShoulderOffset=0;   //filter offset from subcarrier (Hz) keep =0!
MinVarianceAcc=0.001; //minimum variance in 1sec data to get ACF
MinHrConfidence=0.003; //minimum acceptable confidence in hr signal
Verbose=0;          //=1 for diagnostics, =0 for quick process
secInt=4;           //starting second after t=0 to plot
////////////////////////////////////

```

```

////////////////////////////////////
////////// ENTER FILE NAME HERE WITHOUT .WAV EXTENSION //////////////////////////////////////
////////////////////////////////////

```

```

//Fil='c:\weVireoData\Ib1214181930';
//Fil='c:\weVireoData\056IB12_12&13Apr08_1935_1';
//Fil='c:\weVireoData\056IB12_13&14Apr08_15241130_2';
//Fil='c:\weVireoData\056IB12_12Apr08_1510_1hr_2';
//Fil='c:\weVireoData\056IB12_12&13Apr08_1922_2';
//Fil='c:\weVireoData\056IB12_13&14Apr08_15421113_1';
//Fil='c:\weVireoData\056IB12_14&15Apr08_113445_2';

```

```

////////////////////////////////////

```

```

OutFile=Fil+'.dat';
fdOut=mopen(OutFile,'w');
mfprintf(fdOut,' Seconds HH MM SS  BPMuf  Confidence Variance  ACF\n');
mclose(fdOut); //close after writting headers
StatFile=Fil+'.stat';
fdStat=mopen(StatFile,'w');
mfprintf(fdStat,' File MedianFreq CWfreq(1:5) #AcceptPts #RejectPts\n');
mclose(fdStat);

```

```

TimeSec=0.5; //assign first midpoint to timestamp (sec)
medCWfreq=1700; //set highest CW freq to something reasonable to start
medCWfreq2=2500; //set second highest CW freq to something reasonable to start

```

```

fc0 = (LPfreq)*(NsecEnv);
hz9=[];
hz9=iir(6,'lp','butt',[fc0 0],[0 0]); //IIR coeff
[hzm,fr]=frmag(hz9,256);
if Verbose==3,
    scf(0);
    clf(0);

```

```

plot2d(fr',hzm');
xpause(5e6);
end;

for jFn=1:500,          //total number of 5min files to crunch
    s2=0;              //reset endpoint
    FileNum='_'+string(jFn);
    InFile=Fil+FileNum+'.wav';
    try
        fdIn=mopen(InFile,'rb');
    catch
        break;
    end;
    WavHeader=mgeti(44,'c',fdIn);    //get header
    RawWav=[];
    RawWav=mgeti(Nsec*Srate,'c',fdIn)-128;    //convert to signed int

    [nr,nc]=size(RawWav);    //determine number of rows & columns
    if modulo(nc,Srate) < 0 then,
        if modulo(nc,Srate) > 0.5*Srate then,
            RawWav(1,nc+1:nc-modulo(nc,Srate)+Srate)=0; //pad with zeros if less than half sec
        else,
            RawWav=RawWav(1:nc-modulo(nc,Srate));    //otherwise truncate to whole second
        end;
    end;
    [nr,nc]=size(RawWav);    //determine number of rows & columns
    NsecRead=nc/Srate;

    f=Srate*(0:(N/2))/N;    //associated frequency vector
    n=size(f,'*');    //size=i*j*k*...
    indexMinHz=round(MinFreqAccFFT/(Srate/N));    //calc index for minimum
    i=0;
    iAcc=0;
    CWfreq=[];
    CWacc=[];
    yAll=[];
    for j=[1:24],    //24
        i=i+1;
        q=round((j/25)*length(RawWav));    //25
        slice=q:q+N;
        y=fft(RawWav(slice));    //the fft response is symmetric
        [m,k]=max(abs(y(indexMinHz:n)));    //find max for freq greater than XX Hz
        if Verbose==10 then,
            scf(1);
            clf(1);
            plot2d(f,abs(y(1:n)),rect=[0,0,5000,1.2*m]);    //plot FFT
            legends([string(f(k+indexMinHz))],[1],opt="ur");
            xpause(5e5);
        end;
        CWfreq(i)=f(k+indexMinHz);    //peak frequency
        if (CWfreq(i)>=MinFreqAccFFT & (CWfreq(i)<=MaxFreqAccFFT)) then
            //accept only freq btw 1200Hz & 3000Hz
            iAcc=iAcc+1;
            CWacc(iAcc)=CWfreq(i);
        end;
        yAll=[yAll;y];    //concatenate all ffts
    end;
    if iAcc==0 then,
        //no acceptable CW freqs were found so use last 5min period's
    else,
        for i=[1:iAcc],
            numNeighbors(i)=0;

```

```

for j=[1:iAcc],
    if abs(CWacc(i)-CWacc(j))<=50.0 then
        //this CW frequency has a close neighbor
        numNeighbors(i)=numNeighbors(i)+1;
    end;
end;
[m,k]=max(numNeighbors(1:iAcc));
//now we know the most popular CW frequency, so lets average around it to find center
CWsum=0;
numCWsum=0;
for i=[1:iAcc],
    if abs(CWacc(k)-CWacc(i))<=50.0 then
        CWsum=CWsum+CWacc(i);
        numCWsum=numCWsum+1;
    end;
end;
medCWfreq=(CWsum/numCWsum)-ShoulderOffset;    //select avg around most popular peak as CW freq.

//now we need to find the most popular second highest peak CW
iMedCWfreq=round(medCWfreq/(Srate/N));    //calc index associated with most popular highest CW
yAll(:,iMedCWfreq-10:iMedCWfreq+10)=0;    //set all values around peak to zero
iAcc=0;
for j=[1:24],    //go thru each to determine highest remaining peak
    [m,k]=max(abs(yAll(j,indexMinHz:n)));    //find max
    CWfreq2(j)=f(k+indexMinHz);    //peak frequency
    if (CWfreq2(j)>=MinFreqAccFFT) & (CWfreq(j)<=MaxFreqAccFFT) then
        //accept only freq btw 1200Hz & 3000Hz
        iAcc=iAcc+1;
        CWacc2(iAcc)=CWfreq2(j);
    end;
end;
for i=[1:iAcc],
    numNeighbors(i)=0;
    for j=[1:iAcc],
        if abs(CWacc2(i)-CWacc2(j))<=50.0 then
            //this CW frequency has a close neighbor
            numNeighbors(i)=numNeighbors(i)+1;
        end;
    end;
end;
[m,k]=max(numNeighbors(1:iAcc));
//now we know the most popular CW frequency, so lets average around it to find center
CWsum=0;
numCWsum=0;
for i=[1:iAcc],
    if abs(CWacc2(k)-CWacc2(i))<=50.0 then
        CWsum=CWsum+CWacc2(i);
        numCWsum=numCWsum+1;
    end;
end;
medCWfreq2=(CWsum/numCWsum)-ShoulderOffset;    //select avg around most popular peak as CW freq.
end;

hz=[];
fc1 = (medCWfreq-0.5*FiltPB)/(Srate);
fc2 = (medCWfreq+0.5*FiltPB)/(Srate);
fc3 = (medCWfreq-0.5*FiltSB)/(Srate);
fc4 = (medCWfreq+0.5*FiltSB)/(Srate);

fc5 = (medCWfreq2-0.5*FiltPB)/(Srate);
fc6 = (medCWfreq2+0.5*FiltPB)/(Srate);

```

```

fc7 = (medCWfreq2-0.5*FiltSB)/(Srate);
fc8 = (medCWfreq2+0.5*FiltSB)/(Srate);

hz1=iir(5,'bp','butt',[fc1 fc2],[0 0]); //IIR coeff
hz2=iir(5,'sb','butt',[fc3 fc4],[0 0]); //IIR coeff

hz3=iir(5,'bp','butt',[fc5 fc6],[0 0]); //IIR coeff
hz4=iir(5,'sb','butt',[fc7 fc8],[0 0]); //IIR coeff

clear EnvFiltWav;
Hrate=[];
FiltRawWav=[];
if Verbose==1 then,
    scf(2);
    clf(2);
    subplot(611);
    plot2d(RawWav(secInt*Srate:(secInt+1)*Srate));
end;
FiltRawWav = flts(double(RawWav),hz1); //BP filter IIR to include sidebands around CW
FiltRawWav = FiltRawWav($:-1:1); //1st inversion
FiltRawWav = flts(FiltRawWav,hz1); //refilter
FiltRawWav = FiltRawWav($:-1:1); //2nd inversion
FiltRawWav = flts(FiltRawWav,hz2); //SB filter IIR to remove CW
FiltRawWav = FiltRawWav($:-1:1); //1st inversion;
FiltRawWav = flts(FiltRawWav,hz2); //refilter
FiltRawWav = FiltRawWav($:-1:1); //2nd inversion;
if Verbose==1 then,
    subplot(612);
    plot2d(FiltRawWav(secInt*Srate:(secInt+1)*Srate));
end;
clear q;
q=matrix(abs(FiltRawWav),NptsEnv,-1); //columnize & absolute
clear FiltRawWav;
EnvFiltWav=max(q,'r'); //find max of each column to get envelope
AAEnvFiltWav = flts(EnvFiltWav,hz9); //lp filter envelope for anti-alias
AAEnvFiltWav = AAEnvFiltWav($:-1:1); // inversion;
AAEnvFiltWav = flts(AAEnvFiltWav,hz9); //lp filter envelope for anti-alias
AAEnvFiltWav = AAEnvFiltWav($:-1:1); // inversion;
if Verbose==1 then,
    subplot(614);
    plot2d(AAEnvFiltWav(secInt*Srate/NptsEnv:(secInt+1)*Srate/NptsEnv));
end;

clear q;
FiltRawWav=[];
FiltRawWav = flts(double(RawWav),hz3); //BP filter IIR to include sidebands around CW2
FiltRawWav = FiltRawWav($:-1:1); //1st inversion;
FiltRawWav = flts(FiltRawWav,hz3); //refilter
FiltRawWav = FiltRawWav($:-1:1); //2nd inversion;
FiltRawWav = flts(FiltRawWav,hz4); //SB filter IIR to remove CW2
FiltRawWav = FiltRawWav($:-1:1); //1st inversion;
FiltRawWav = flts(FiltRawWav,hz4); //refilter
FiltRawWav = FiltRawWav($:-1:1); //2nd inversion;
if Verbose==1 then,
    subplot(613);
    plot2d(FiltRawWav(secInt*Srate:(secInt+1)*Srate));
end;
clear q;
q=matrix(abs(FiltRawWav),NptsEnv,-1); //columnize & absolute
clear FiltRawWav;
EnvFiltWav2=max(q,'r'); //find max of each column to get envelope
AAEnvFiltWav2 = flts(EnvFiltWav,hz9); //lp filter envelope for anti-alias

```

```

AAEnvFiltWav2 = AAEnvFiltWav2($:-1:1);      // inversion;
AAEnvFiltWav2 = flts(AAEnvFiltWav2,hz9); //lp filter envelope for anti-alias
AAEnvFiltWav2 = AAEnvFiltWav2($:-1:1);      // inversion;
if Verbose==1 then,
    subplot(615);
    plot2d(AAEnvFiltWav2(secInt*Srate/NptsEnv:(secInt+1)*Srate/NptsEnv));
end;

//AAEnvFiltWav=AAEnvFiltWav.*AAEnvFiltWav2; //non-linear !!!!!
AAEnvFiltWav=AAEnvFiltWav+AAEnvFiltWav2; //linear addition of two highest peak envelopes

if Verbose==1 then,
    subplot(616);
    plot2d(AAEnvFiltWav(secInt*Srate/NptsEnv:(secInt+1)*Srate/NptsEnv));
end;
FiltRawWav=[];          //clear large array
RawWav=[];              //clear large array
q=[];

if Verbose==9 then,
    subplot(614);
    plot2d(EnvFiltWav(2*Srate/NptsEnv:3*Srate/NptsEnv));
    subplot(615);
    plot2d(AAEnvFiltWav(2*Srate/NptsEnv:3*Srate/NptsEnv));
    maxH=max(AAEnvFiltWav(2*Srate/NptsEnv:3*Srate/NptsEnv));
    w=find(AAEnvFiltWav(2*Srate/NptsEnv:3*Srate/NptsEnv)<0.5*maxH);
    AAEnvFiltWav(w-1+2*Srate/NptsEnv)=0;
    w=find(AAEnvFiltWav(2*Srate/NptsEnv:3*Srate/NptsEnv)>=0.5*maxH);
    AAEnvFiltWav(w-1+2*Srate/NptsEnv)=AAEnvFiltWav(w-1+2*Srate/NptsEnv)-0.5*maxH;
    subplot(616);
    plot2d(AAEnvFiltWav(2*Srate/NptsEnv:3*Srate/NptsEnv));
    xsave(Fil+'.scg');
    xpause(8e6);
end;

slice=[MinHRperiod:MaxHRperiod]; //limit range of accept Hrates
clear q;
q=matrix(AAEnvFiltWav,NptsCorr,-1); //columnize for autocorrelation
[nr1,nc1]=size(q); //determine number of rows & columns
for l=1:nc1, //go thru envelope without offset in time
    maxCol=max(q(:,l)); //find max in each analysis frame
    VarEnvFiltWav1(l)=variance(q(:,l));
    w=find(q(:,l)<FractCenterClip*maxCol);
    q(w,l)=0; //set center-clipped pts = 0
    w=find(q(:,l)>=FractCenterClip*maxCol);
    q(w,l)=q(w,l)-FractCenterClip*maxCol; //subtract Fraction of max from peaks

    if VarEnvFiltWav1(l)>0 then,
        CorrEnvFiltWav=corr(q(:,l),Nlags)/VarEnvFiltWav1(l); //autocorr on each column normalized by variance
    else,
        CorrEnvFiltWav=zeros(1:Nlags);
    end;
    [m1(l),k]=max(CorrEnvFiltWav(slice)); //pick out peak btw min&max heartrate
    BpmUF1(l)=round(60.0*1000.0/(k+double(MinHRperiod))); //unfiltered beats per minute
    HrConfidence1(l)=VarEnvFiltWav1(l)*m1(l); //assign confidence to derived HeartRate
    if Verbose==7 then,
        scf(3);
        clf(3);
        subplot(211);
        plot2d(q(:,l));
        subplot(212);

```

```

legends([string(BpmUF1(l)),string(l/2)],[1,1],opt="ur");
plot2d(slice,CorrEnvFiltWav(slice),style=5,rect=[MinHRperiod,-0.4,MaxHRperiod,1.1]);
//if l<=5 then,
//xsave(Fil+'_'+l+'.scg');
//end;
xpause(1e5);
end;
end;

AAEnvFiltWav=AAEnvFiltWav(1+NptsCorr/2:$-NptsCorr/2); //strip off first and last half analysis frames
clear q;
q=matrix(AAEnvFiltWav,NptsCorr,-1); //columnize for autocorrelation
[nr2,nc2]=size(q); //determine number of rows & columns
for l=1:nc2, //go thru envelope with offset in time
    maxCol=max(q(:,l)); //find max in each analysis frame
    VarEnvFiltWav2(l)=variance(q(:,l));
    w=find(q(:,l)<FractCenterClip*maxCol);
    q(w,l)=0; //set center-clipped pts = 0
    w=find(q(:,l)>=FractCenterClip*maxCol);
    q(w,l)=q(w,l)-FractCenterClip*maxCol; //subtract Fraction of max from peaks
    if VarEnvFiltWav2(l)>0 then,
        CorrEnvFiltWav=corr(q(:,l),Nlags)/VarEnvFiltWav2(l); //autocorr on each column normalized by variance
    else,
        CorrEnvFiltWav=zeros(1:Nlags);
    end;
    [m2(l),k]=max(CorrEnvFiltWav(slice)); //pick out peak btw min&max heartrate
    BpmUF2(l)=round(60.0*1000.0/(k+double(MinHRperiod))); //unfiltered beats per minute
    HrConfidence2(l)=VarEnvFiltWav2(l)*m2(l); //assign confidence to derived HeartRate
    if Verbose==8 then,
        scf(4);
        clf(4);
        subplot(211);
        plot2d(q(:,l));
        subplot(212);
        legends([string(BpmUF1(l)),string(l/2)],[1,1],opt="ur");
        plot2d(slice,CorrEnvFiltWav(slice),style=5,rect=[MinHRperiod,-0.4,MaxHRperiod,1.1]);
        xpause(1e5);
    end;
end;
clear q;

Hrate=[];
//calc values for first second
nClose=0;
BpmUF=median([BpmUF1(1) BpmUF2(1) BpmUF1(2)]); //calc median heart-rate for each second
VarEnvFiltWav=mean([VarEnvFiltWav1(1) VarEnvFiltWav2(1) VarEnvFiltWav1(2)]); //calc median variance
AvgPeakAAC=mean([m1(1) m2(1) m1(2)]); //calc median peak ACF
HrConfidence=VarEnvFiltWav*AvgPeakAAC;
for k=1:2, //add up all half sec HR that are with +/-25 Bpm
    if abs(BpmUF1(k)-BpmUF)<=25 then,
        nClose=nClose+1;
    end;
end;
if abs(BpmUF2(1)-BpmUF)<=25 then,
    nClose=nClose+1;
end;
Hrate=[Hrate; [TimeSec,0,0,0,BpmUF,HrConfidence,VarEnvFiltWav,AvgPeakAAC,nClose]];
TimeSec=TimeSec+1; //assign time to midpoint

//calc values for most of matrix
for l=2:NsecRead-1,
    nClose=0;

```

```

    BpmUF=median([BpmUF2(2*I-2) BpmUF1(2*I-1) BpmUF2(2*I-1) BpmUF1(2*I) BpmUF2(2*I)]); //calc median heart-rate
    for each second
        VarEnvFiltWav=mean([VarEnvFiltWav2(2*I-2) VarEnvFiltWav1(2*I-1) VarEnvFiltWav2(2*I-1) VarEnvFiltWav1(2*I)
        VarEnvFiltWav2(2*I)]); //calc median variance
        AvgPeakAAC=mean([m2(2*I-2) m1(2*I-1) m2(2*I-1) m1(2*I) m2(2*I)]); //calc median peak ACF
        HrConfidence=VarEnvFiltWav*AvgPeakAAC;
        for k=0:2, //add up all half sec HR that are with +/-25 Bpm
            if abs(BpmUF2(2*I-k)-BpmUF)<=25 then,
                nClose=nClose+1;
            end;
        end;
        for k=0:1,
            if abs(BpmUF1(2*I-k)-BpmUF)<=25 then,
                nClose=nClose+1;
            end;
        end;
        Hh=floor(TimeSec/3600); //hours
        Mm=floor((TimeSec-3600*Hh)/60); //minutes
        Ss=TimeSec-3600*Hh-60*Mm; //seconds
        Hrate=[Hrate; [TimeSec,Hh,Mm,Ss,BpmUF,HrConfidence,VarEnvFiltWav,AvgPeakAAC,nClose]]; //append timestamp
    heartrate
    TimeSec=TimeSec+1; //assign time to midpoint
    end;

    //calc values for last second
    l=NsecRead;
    nClose=0;
    BpmUF=median([BpmUF1(2*I-1) BpmUF2(2*I-1) BpmUF1(2*I)]); //calc median heart-rate for last second
    VarEnvFiltWav=mean([VarEnvFiltWav1(2*I-1) VarEnvFiltWav2(2*I-1) VarEnvFiltWav1(2*I)]); //calc median variance
    AvgPeakAAC=mean([m1(2*I-1) m2(2*I-1) m1(2*I)]); //calc median peak ACF
    HrConfidence=VarEnvFiltWav*AvgPeakAAC;
    for k=1:0, //add up all half sec HR that are with +/-25 Bpm
        if abs(BpmUF1(2*I-k)-BpmUF)<=25 then,
            nClose=nClose+1;
        end;
    end;
    if abs(BpmUF2(2*I-1)-BpmUF)<=25 then,
        nClose=nClose+1;
    end;
    Hh=floor(TimeSec/3600); //hours
    Mm=floor((TimeSec-3600*Hh)/60); //minutes
    Ss=floor(TimeSec-3600*Hh-60*Mm); //seconds
    Hrate=[Hrate; [TimeSec,Hh,Mm,Ss,BpmUF,HrConfidence,VarEnvFiltWav,AvgPeakAAC,nClose]];
    TimeSec=TimeSec+1; //assign time to midpoint
    nCloseAvg=sum(Hrate($-NsecRead+1:$,9))/NsecRead;

    //done with one whole 5min file
    mclose(fdIn); //close 5min file

    scf(5);
    clf(5);
    subplot(311);
    plot2d([1:NsecRead],Hrate(:,5),rect=[0,-200,300,1400],style=5);
    subplot(312);
    plot2d([1:NsecRead],Hrate(:,6),style=6,logflag="nl",rect=[0,1e-3,300,1e3]);
    subplot(313);
    plot2d([1:NsecRead],Hrate(:,9),style=6,rect=[0,0,300,8]);
    legends([(Fil+FileNum)],[1],opt="lr");

    fdOut=mopen(OutFile,'a'); //re-open in append mode
    fprintf(fdOut,'%8.1f %2.2i %2.2i %2.2i %5i %9.3f %9.3f %9.3f %3i\n',Hrate);
    mclose(fdOut); //close data file out after writing

```

```

fdStat=fopen(StatFile,'a'); //re-open statsfile in append mode
fprintf(fdStat,'%50s %5.0f %5.0f %8.3f\n',InFile,medCWfreq,medCWfreq2,nCloseAvg);
fprintf('%50s %5.0f %5.0f %8.3f\n',InFile,medCWfreq,medCWfreq2,nCloseAvg);
fclose(fdStat);
end;
//done with all 5min files
fprintf('DONE WITH ALL FILES\n');
clearglobal();
abort;

```

Appendix B. Heart-rate Disturbance Statistics

The following tables provide statistics used in analyses presented in Study 8 of this report. Table headings are Bird = individual identifier of sample bird, Description = heart-rate sample relative to disturbance periods, JDD Start (CDT) = Julian start date of heart-rate sample period, JDD Finish (CDT) = Julian end date of heart-rate sample period, Duration (min) = duration in minutes of heart-rate data collection, HR Median (bpm) = median heart-rate beats per minute, HR Mean (bpm) = mean heart-rate beats per minute, HR Max (bpm) = maximum heart-rate during sample period, HR STD (bpm) = standard deviation of mean heart rate, % Valid = percentage of sample period during which valid heart-rate data were obtained. Data were not included in analyses (labeled NA) if less than 20% valid heart-rate data were obtained for the sample period. Inability to obtain valid data were likely due to the telemetered bird out of range of the receiver or low quality of recorded data precluding filtering of the heart-rate signal.

Table B-1. Heart-rate statistics for night disturbance.

Bird	Description	JDD Start (CDT)	JDD Finish (CDT)	Duration (min)	HR Median (bpm)	HR Mean (bpm)	HR Max (bpm)	HR STD (bpm)	% Valid
816_AB1	1 hr prior	117.940	117.982	60	642	651	857	42	99.9
	Night Disturbance	117.982	118.051	100	632	639	822	36	98.8
	1st hr after disturb.	118.051	118.093	60	659	665	845	51	97.7
	2nd hr after disturb.	118.093	118.135	60	670	676	851	40	97.8
	1 day earlier	116.982	117.051	100	789	790	870	34	100.0
	2 days earlier	115.982	116.051	100	736	736	876	33	98.4
998_JCB10	1 hr prior	144.918	144.960	60	433	439	1154	39	98.7
	Night Disturbance	144.960	145.005	65	451	455	1071	28	99.4
	1st hr after disturb.	145.005	145.047	60	456	459	628	23	100.0
	2nd hr after disturb.	145.047	145.088	60	456	463	714	35	99.9
	1 day earlier	143.960	144.005	65	441	448	1071	44	98.4
	2 days earlier	142.960	143.005	65	NA	NA	NA	NA	0.2
056_AB10	1 hr prior	144.969	145.010	60	488	490	659	26	100.0
	Night Disturbance	145.010	145.052	60	502	505	663	30	100.0
	1st hr after disturb.	145.052	145.094	60	510	513	638	31	100.0
	2nd hr after disturb.	145.094	145.135	60	531	532	682	30	100.0
	1 day earlier	144.010	144.052	60	594	599	759	38	99.9
	2 days earlier	143.010	143.052	60	NA	NA	NA	NA	NA

Table B-1. (continued). Statistics for night disturbance.

Bird	Description	JDD Start (CDT)	JDD Finish (CDT)	Duration (min)	HR Median (bpm)	HR Mean (bpm)	HR Max (bpm)	HR STD (bpm)	% Valid
176_IB27	1 hr prior	175.930	175.972	60	541	544	682	32	99.9
	Night Disturbance	175.972	176.009	54	522	525	706	31	99.8
	1st hr after disturb.	176.009	176.051	60	522	521	638	21	99.8
	2nd hr after disturb.	176.051	176.092	60	550	552	659	32	99.9
	1 day earlier	174.972	175.009	54	550	550	659	20	99.1
	2 days earlier	173.972	174.009	54	561	562	659	20	99.9
962_IB26	1 hr prior	175.968	176.010	60	528	546	1071	67	99.0
	Night Disturbance	176.010	176.053	63	524	539	794	64	99.5
	1st hr after disturb.	176.053	176.095	60	518	533	750	59	99.7
	2nd hr after disturb.	176.095	176.137	60	510	520	723	45	99.9
	1 day earlier	175.010	175.053	63	556	560	745	33	99.6
	2 days earlier	174.010	174.053	63	625	624	800	43	95.5
Average of five birds	1 hr prior			60	526	534	885	41	99.5
	Night Disturbance			68	526	533	811	38	99.5
	1st hr after disturb.			60	533	538	700	37	99.4
	2nd hr after disturb.			60	543	549	726	36	99.5
	1 day earlier			68	586	589	821	34	99.4
	2 days earlier			68	641	641	778	32	97.9

Table B-2. Heart-rate statistics for daytime quiet observations.

Bird	Description	JDD Start (CDT)	JDD Finish (CDT)	Duration (min)	HR Median (bpm)	HR Mean (bpm)	HR Max (bpm)	HR STD (bpm)	% Valid
056_IB12	1 hr prior	104.322	104.363	60	870	841	1091	100	82.0
	1st hr of Quiet Obs	104.363	104.405	60	723	720	1091	151	88.1
	Last hr of Quiet Obs	104.423	104.465	60	659	702	1111	137	92.4
	1st hr after Obs.	104.465	104.506	60	571	614	1071	144	92.4
	2 nd hr after Obs.	104.506	104.548	60	652	673	1111	114	87.6
5001_IB14	1 hr prior	111.284	111.326	60	938	928	1071	33	92.8
	1st hr of Quiet Obs	111.326	111.367	60	923	916	1017	36	95.2
	Last hr of Quiet Obs	111.417	111.458	60	827	813	1017	85	96.9
	1st hr after Obs.	111.458	111.500	60	710	718	953	81	94.4
	2 nd hr after Obs.	111.500	111.542	60	811	804	1053	77	92.6
260_IB15	1 hr prior	111.306	111.348	60	851	845	1091	78	51.2
	1st hr of Quiet Obs	111.348	111.390	60	822	825	1111	81	58.3
	Last hr of Quiet Obs	111.457	111.499	60	750	756	1034	62	63.9
	1st hr after Obs.	111.499	111.540	60	800	796	1053	66	63.6
	2 nd hr after Obs.	111.540	111.582	60	789	790	1111	62	68.2
273_IB1	1 hr prior	116.383	116.424	60	870	855	1154	69	52.2
	1st hr of Quiet Obs	116.424	116.466	60	779	788	1091	81	71.3
	Last hr of Quiet Obs	116.631	116.673	60	597	595	1224	149	55.8
	1st hr after Obs.	116.673	116.715	60	486	497	1250	90	84.0
	2 nd hr after Obs.	116.715	116.756	60	NA	NA	NA	NA	5

Table B-2 (continued). Statistics for daytime quiet observations.

Bird	Description	JDD Start (CDT)	JDD Finish (CDT)	Duration (min)	HR Median (bpm)	HR Mean (bpm)	HR Max (bpm)	HR STD (bpm)	% Valid
816_AB1	1 hr prior	116.382	116.424	60	811	793	1132	136	66.4
	1st hr of Quiet Obs	116.424	116.465	60	909	890	1132	109	63.4
	Last hr of Quiet Obs	116.625	116.667	60	692	724	1154	120	66.2
	1st hr after Obs.	116.667	116.708	60	612	640	1091	115	54.8
	2 nd hr after Obs.	116.708	116.750	60	694	701	1091	138	91.2
998_JCB10	1 hr prior	143.420	143.462	60	NA	NA	NA	NA	1.0
	1st hr of Quiet Obs	143.462	143.503	60	NA	NA	NA	NA	2.1
	Last hr of Quiet Obs	143.632	143.674	60	NA	NA	NA	NA	9.0
	1st hr after Obs.	143.674	143.715	60	476	491	1071	73	24.9
	2 nd hr after Obs.	143.715	143.757	60	451	468	1091	74	27.7
056_AB10	1 hr prior	143.427	143.469	60	588	614	1132	88	30.8
	1st hr of Quiet Obs	143.469	143.510	60	600	632	1154	99	30.6
	Last hr of Quiet Obs	143.510	143.552	60	606	635	1111	101	54.2
	1st hr after Obs.	143.552	143.594	60	612	639	1132	105	52.0
	2 nd hr after Obs.	143.594	143.635	60	594	622	1132	98	47.1
039_IB23	1 hr prior	164.273	164.315	60	NA	NA	NA	NA	10.9
	1st hr of Quiet Obs	164.315	164.356	60	NA	NA	NA	NA	10.5
	Last hr of Quiet Obs	164.451	164.493	60	403	462	1224	164	22.7
	1st hr after Obs.	164.493	164.535	60	NA	NA	NA	NA	7.9
	2 nd hr after Obs.	164.535	164.576	60	441	485	1250	128	29.2

Table B-2 (continued). Statistics for daytime quiet observations.

Bird	Description	JDD Start (CDT)	JDD Finish (CDT)	Duration (min)	HR Median (bpm)	HR Mean (bpm)	HR Max (bpm)	HR STD (bpm)	% Valid
176_IB27	1 hr prior	174.208	174.250	60	606	605	690	19	99.7
	1st hr of Quiet Obs	174.250	174.292	60	789	761	1111	89	90.0
	Last hr of Quiet Obs	174.292	174.333	60	779	753	1000	87	70.6
	1st hr after Obs.	174.333	174.375	60	655	664	1224	76	70.2
	2 nd hr after Obs.	174.375	174.417	60	NA	NA	NA	NA	10.1
962_IB26	1 hr prior	174.208	174.250	60	674	670	789	43	90.9
	1st hr of Quiet Obs	174.250	174.292	60	857	835	1091	70	75.6
	Last hr of Quiet Obs	174.315	174.356	60	769	757	968	82	24.0
	1st hr after Obs.	174.356	174.398	60	NA	NA	NA	NA	19.1
	2 nd hr after Obs.	174.398	174.440	60	NA	NA	NA	NA	1.0
Average of eight birds	1 hr prior			60	654	671	1171	114	70.8
	1st hr of Quiet Obs			60	609	627	1037	84	71.6
	Last hr of Quiet Obs			60	632	637	991	70	60.7
	1st hr after Obs.			60	751	740	1057	103	67.0
	2 nd hr after Obs.			60	684	684	1066	88	63.4

Table B-3. Heart-rate statistics for alternating one-hour chase.

Bird	Description	JDD Start (CDT)	JDD Finish (CDT)	Duration (min)	HR Median (bpm)	HR Mean (bpm)	HR Max (bpm)	HR STD (bpm)	% Valid
273_IB1	1 hour prior	117.424	117.465	60	522	583	1250	147	69.3
	1 hour nest search	117.465	117.508	61	645	673	1250	136	33.1
	Gap	117.508	117.551	62	429	451	1250	87	46.7
	1 hour sitting	117.551	117.590	57	472	501	1154	109	74.6
	Gap	117.590	117.632	60	435	464	1200	114	63.3
	1 hour chase	117.632	117.660	40	NA	NA	NA	NA	14.5
	1st hr after chase	117.660	117.701	60	458	503	1250	106	73.0
	2nd hr after chase	117.701	117.743	60	635	665	1250	108	28.6
816_AB1	1 hour prior	117.447	117.489	60	547	580	1132	105	45.6
	1 hour nest search	117.489	117.529	58	789	770	1132	134	63.9
	Gap	117.529	117.569	58	566	615	1071	127	73.1
	1 hour chase	117.569	117.611	60	764	782	1154	113	46.3
	Gap	117.611	117.651	58	558	577	1034	92	83.2
	1 hour sitting	117.651	117.694	62	678	715	1132	112	64.0
	1st hr after sitting	117.694	117.736	60	694	727	1111	110	80.2
	2nd hr after sitting	117.736	117.778	60	690	723	1071	141	74.3
998_JCB10	1 hour prior	144.424	144.465	60	NA	NA	NA	NA	11.8
	1 hour sitting	144.465	144.506	59	488	501	1071	82	26.7
	Gap	144.506	144.565	84	496	522	1132	108	25.4
	1 hour nest search	144.565	144.608	63	488	498	1154	71	42.2
	Gap	144.608	144.651	62	484	492	984	57	43.8
	1 hour chase	144.651	144.693	60	496	508	1091	73	38.4
	1st hr after chase	144.693	144.735	60	472	482	1034	57	42.1
	2nd hr after chase	144.735	144.776	60	476	491	1154	67	36.2

Table B-3 (continued). Statistics for alternating one-hour chase.

Bird	Description	JDD Start (CDT)	JDD Finish (CDT)	Duration (min)	HR Median (bpm)	HR Mean (bpm)	HR Max (bpm)	HR STD (bpm)	% Valid
056 AB10	1 hour prior	144.444	144.486	60	543	583	1132	110	81.9
	1 hour chase	144.486	144.526	58	526	567	1091	109	69.6
	Gap	144.526	144.569	62	536	577	1053	114	51.2
	1 hour sitting	144.569	144.613	63	574	595	1154	94	55.7
	Gap	144.613	144.653	57	524	570	1154	103	65.5
	1 hour nest search	144.653	144.694	60	588	615	1132	95	44.4
	1st hr after search	144.694	144.736	60	480	516	1154	111	75.0
	2nd hr after search	144.736	144.778	60	577	590	1034	99	36.2
176 IB27	1 hour prior	175.427	175.469	60	616	629	1176	71	27.1
	1 hour nest search	175.469	175.510	60	NA	NA	NA	NA	15.6
	Gap	175.510	175.549	56	536	555	1200	75	49.5
	1 hour sitting	175.549	175.597	69	522	530	923	51	60.2
	Gap	175.597	175.633	52	484	494	952	48	61.2
	1 hour chase	175.633	175.677	63	480	492	1154	63	63.6
	1st hr after chase	175.677	175.719	60	488	497	1154	61	66.4
	2nd hr after chase	175.719	175.760	60	484	497	1111	68	62.4
962 IB26	1 hour prior	175.420	175.462	60	484	514	1224	114	29.5
	1 hour nest search	175.462	175.500	55	588	593	1200	90	58.1
	Gap	175.500	175.544	63	541	563	1200	93	47.2
	1 hour sitting	175.544	175.587	62	NA	NA	NA	NA	14.0
	Gap	175.587	175.636	71	531	562	1224	112	35.9
	1 hour chase	175.636	175.661	36	572	594	1200	95	64.3
	1st hr after chase	175.661	175.703	60	571	584	1200	79	68.6
	2nd hr after chase	175.703	175.744	60	517	540	1224	91	48.8

Table B-3 (continued). Statistics for alternating one-hour chase.

Bird	Description	JDD Start (CDT)	JDD Finish (CDT)	Duration (min)	HR Median (bpm)	HR Mean (bpm)	HR Max (bpm)	HR STD (bpm)	% Valid
Average of six birds	1 hour prior			58	498	519	1094	84	50.7
	1 hour disturbance			66	524	547	1113	95	50.3
	Gap			63	508	531	1156	88	48.9
	1 hour disturbance			56	538	558	1105	81	55.8
	Gap			61	547	562	1148	82	58.8
	1-hour disturbance			59	507	532	1167	88	54.9
	1st hr after disturb.			61	544	557	1102	76	67.6
	2nd hr after disturb.			58	501	523	1100	84	47.8

Table B-4. Heart-rate statistics for daytime four-hour chase.

Bird	Description	JDD Start (CDT)	JDD Finish (CDT)	Duration (min)	HR Median (bpm)	HR Mean (bpm)	HR Max (bpm)	HR STD (bpm)	% Valid
056_IB12	1 hr prior	105.256	105.298	60	896	897	1132	71	87.4
	Isabelle Chase	105.298	105.357	85	923	902	1154	83	88.7
	Angela Chase	105.357	105.417	86	845	838	1132	88	75.0
	Jackie Chase	105.417	105.467	72	723	733	1132	117	80.9
	1st hr after	105.467	105.508	60	682	688	1000	124	41.6
	2nd hr after	105.508	105.550	60	566	608	1053	132	75.7
5001_IB14	1 hr prior	112.252	112.294	60	655	672	992	73	99.4
	Jackie Chase	112.294	112.411	169	769	780	1053	69	53.5
	Isabelle Chase	112.411	112.454	62	NA	NA	NA	NA	1.1
	Angela Chase	112.454	112.496	60	674	689	1000	70	82.6
	1 hr after	112.496	112.538	60	674	684	984	63	95.3
	2 hr after	112.538	112.579	60	638	651	1000	65	76.4
260_IB15	1 hr prior	112.280	112.322	60	741	755	1091	64	59.5
	Angela Chase	112.322	112.357	51	750	759	1132	80	38.3
	Isabelle Chase	112.357	112.448	131	682	698	1111	91	57.5
	Jackie Chase	112.448	112.517	99	659	680	1071	76	60.8
	1st hr after	112.517	112.558	60	666	690	1071	88	76.2
	2nd hr after	112.558	112.600	60	655	675	1034	79	80.2

Table B-4 (continued). Statistics for daytime four-hour chase.

Bird	Description	JDD Start (CDT)	JDD Finish (CDT)	Duration (min)	HR Median (bpm)	HR Mean (bpm)	HR Max (bpm)	HR STD (bpm)	% Valid
039 IB23	1 hr prior	165.257	165.299	60	NA	NA	NA	NA	17.9
	Isabelle Chase	165.299	165.361	90	NA	NA	NA	NA	13.0
	Jackie Chase	165.361	165.417	80	NA	NA	NA	NA	7.2
	Angela Chase	165.417	165.458	60	NA	NA	NA	NA	0.1
	1st hr after	165.458	165.500	60	NA	NA	NA	NA	4.0
	2nd hr after	165.500	165.542	60	NA	NA	NA	NA	1.2
Average of three birds	1 hr prior			60	764	775	1072	69	82.1
	1st Chaser			99	814	814	1113	77	60.2
	2nd Chaser			90	764	768	1122	90	66.3
	3rd Chaser			73	685	701	1068	88	74.8
	1st hr after			60	674	687	1018	92	71.0
	2nd hr after			60	620	645	1029	92	77.4

Appendix C. Technical Publications

Journal publications

- Bisson, I.-A., L. K. Butler, T. J. Hayden, L. M. Romero, and M. Wikelski. 2009. No energetic cost of anthropogenic disturbance in a songbird. *Proceedings of the Royal Society of London Series B, Biological Sciences* 276:961-969.
- Bisson, I.-A., L. Butler, M. Romero, T. Hayden, M. Wikelski. Energy expenditure in response to experimental human disturbance in a free-living endangered songbird. In prep. Potential outlet: *Conservation Biology*.
- Butler, L. K., I.-A. Bisson, T. J. Hayden, M. Wikelski, and L. M. Romero. 2009. Adrenocortical responses to offspring-directed threats in two open-nesting birds. *General and Comparative Endocrinology* 162:313-318.
- Butler, L. K., L. Reis, I.-A. Bisson, T. J. Hayden, M. Wikelski, and L. M. Romero. In review. Road density alters the stress physiology of an endangered, old-growth-dependent songbird, but not a habitat generalist. *Conservation Biology*.
- Butler, L. K., T. J. Hayden, and L. M. Romero. 2008. Prebasic molt of Black-capped and White-eyed Vireos: effects of breeding site and the El Niño-Southern Oscillation. *Condor* 110:428-440.
- Romero, L. M. and L. K. Butler. 2007. Endocrinology of stress. *International Journal of Comparative Psychology* 20:89-95.

Master's thesis

- Barron, D. G. 2009. Applied aspects of avian reproduction on Fort Hood, TX. Master of Science thesis. University of Illinois, Champaign-Urbana, IL.

Technical publications

- Hayden, T.J., I.-A Bisson, M.C. Wikelski, L. K. Butler, and L. M. Romero. 2008. Physiological response and habituation of endangered species to military training activities: SERDP 2006 Annual Report. ERDC/CERL SR-08-08.
- Hayden, T.J., I.-A Bisson, M.C. Wikelski, L. K. Butler, and L. M. Romero. In editing. Physiological response and habituation of endangered species to military training activities: SERDP 2007 Annual Report. ERDC/CERL SR-XX-XX.

Published technical abstracts

- Bisson, I.-A., L. K. Butler, T. J. Hayden, L. M. Romero, M. C. Wikelski. Heart rate response and associated energy expenditure to simulated military training in a common and an endangered songbird. Poster. Partners in Environmental Technology Technical Symposium and Workshop, Strategic Environmental Research and Development Program, Washington, D.C. (2008).

- Butler, L. K. When is corticosterone of useful measure of short- and long-term disturbance in birds? Lessons from field experiments in endangered and common birds. Invited Presentation. American Ornithologists' Union Annual Meeting, Portland, OR (2008)
- Butler, L. K. Parental responses to offspring-directed threats in birds with high nest failure rates. Invited Presentation. 9th International Symposium on Avian Endocrinology, Leuven, Belgium (2008).
- Butler, L. K. and L. M. Romero. Relationships between corticosterone concentrations and the onset, progression, intensity, and rate of molt in two free-living songbirds. Poster. Society for Integrative and Comparative Biology, Boston (2009).
- Butler, L. K., T. J. Hayden, and L. M. Romero. Effects of heavy rainfall on the physiology and life-history of migratory songbirds breeding in the south-central United States. Poster. Society for Integrative and Comparative Biology, San Antonio, Texas (2008).
- Butler, L. K., T. J. Hayden, and L. M. Romero. Population differences in the onset and rate of prebasic molt in Black-capped and White-eyed Vireos. Poster. Society for Integrative and Comparative Biology, Phoenix, Arizona (2007).
- Butler, L. K., L. Ries, I. Bisson, T. J. Hayden, M. Wikelski, and L. M. Romero. Physiological and demographic effects of roads on an endangered, old-growth specialist and a common generalist. Presentation. Society for Integrative and Comparative Biology, Boston (2009).
- Butler, L. K., I. Bisson, T. J. Hayden, M. Wikelski, and L. M. Romero. Corticosterone response to nest-directed disturbances in two migratory songbirds. Presentation. Society for Integrative and Comparative Biology, San Antonio, Texas (2008).
- Butler, L. K., T. J. Hayden, I. Bisson, M. Wikelski, and L. M. Romero. Chronic stress and corticosterone: CORT responses to seven days of rotating stressors in two free-living songbirds. Presentation. Society for Integrative and Comparative Biology, Phoenix, Arizona (2007).
- Butler, L. K., I. A. Bisson, T. J. Hayden, M. Wikelski, and L. M. Romero. Using hormonal indicators of chronic stress to study military training disturbance on avian species of concern. Poster. Partners in Environmental Technology Technical Symposium and Workshop, Strategic Environmental Research and Development Program, Washington, D.C. (2006).
- Butler, L. K., T. J. Hayden, I. Bisson, M. Wikelski, and L. M. Romero. Testing for stress caused by military personnel on foot in the breeding habitat of two avian species of concern at Fort Hood. Partners in Environmental Technology Technical Symposium and Workshop, Strategic Environmental Research and Development Program, Washington, D.C. (2007).
- Butler, L. K., L. Ries, T. J. Hayden, I. Bisson, M. Wikelski, and L. M. Romero. Physiological and demographic effects of road density on an endangered songbird breeding at Fort Hood. Poster. Partners in Environmental Technology Technical Symposium and Workshop, Strategic Environmental Research and Development Program, Washington, D.C. (2008).
- Glassman, L. W., L. K. Butler, and L. M. Romero. Behavioral responses of an endangered and a common bird to disturbances at the nest. Poster. Society for Integrative and Comparative Biology, Boston (2009).
- Kostelanetz, S. A., M. J. Dickens, L. K. Butler, and L. M. Romero. Effects of chronic stress during molt on the heart rate and heart rate variability of European starlings. Poster. Society for Integrative and Comparative Biology, Boston (2009).

Guest lectures and conference presentations

- Bisson, I.-A. 2009. Multiple facets of bird migration. Departmental seminar, University of Minnesota, St. Paul, MN.
- Bisson, I.-A. 2009. Energetic cost of human disturbance in a common and an endangered songbird. Southeastern Ecology and Evolution Conference, Gainesville, FL.
- Bisson, I.-A. 2008. Conservation physiology: energetic cost of human disturbance in a songbird. Job interview at Concordia University, Montreal, QC.
- Bisson, I.-A. 2008. Society for Integrative and Comparative Biology. San Antonio, TX. Conservation Physiology: energetic cost of human disturbance in a songbird.
- Butler, L. K. 2009. Multiple facets of bird migration. Departmental seminar, University of Minnesota, St. Paul, MN.
- Butler, L. K. 2009. Energetic cost of human disturbance in a common and an endangered songbird. Southeastern Ecology and Evolution Conference, Gainesville, FL.
- Butler, L. K. 2008. Conservation physiology: energetic cost of human disturbance in a songbird. Job interview at Concordia University, Montreal, QC.
- Butler, L. K. 2008. Society for Integrative and Comparative Biology. San Antonio, TX. Conservation Physiology: energetic cost of human disturbance in a songbird.
- Romero, L. M. 2009. From the Arctic to the Equator - Stress in Wild Animals. Presentation, University of Massachusetts, Amherst, MA
- Romero, L. M. 2008. From the Arctic to the Equator - Stress in Wild Animals. Presentation. Tufts University, Amherst, MA
- Romero, L. M. 2008. From the Arctic to the Equator - Stress in Wild Animals. Presentation. University of Washington, Aubrey Gorbman Memorial Lecture, Seattle, WA