

Hematological stress indices reveal no effect of radio-transmitters on wintering Hermit Thrushes

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ABSTRACT. Radio-telemetry is often used to track birds, and several investigators have examined the possible effects of radio-transmitters on birds. One approach to this question is to determine if transmitters induce physiological stress. Using hematological indicators of stress (heterophil-lymphocyte [H/L] ratios), studies of captive birds have revealed no evidence that radio-transmitters cause stress. However, studies in captivity may not reflect conditions faced by birds in the wild, especially during energetically demanding times, such as the overwintering period. We examined the possible effect of backpack-style transmitters on overwintering Hermit Thrushes (*Catharus guttatus*) in South Carolina by examining H/L ratios from blood smears made before and after transmitter attachment. We captured and recaptured eight thrushes in an early winter period and 11 in a late winter period, and in both cases found that H/L ratios did not change after 1 mo of carrying transmitters. H/L ratios also did not differ significantly (in either trial) between thrushes with and without transmitters. Furthermore, thrushes that carried transmitters during both of our trials did not have higher final H/L ratios than those that carried transmitters during just one trial (the late winter). These results indicate that carrying backpack-style transmitters for 1 mo did not induce stress in overwintering Hermit Thrushes. These results are consistent with studies of captive birds, and provide support for the continued use of backpack radio-transmitters in this species, as long as weight recommendations are met.

SINOPSIS. Los índices de estrés hematológico revelan ningún efecto de las radiotransmisores sobre *Catharus guttatus* durante el invierno

La radio telemetría es comúnmente usada para el seguimiento de las aves y algunos investigadores han examinado los posibles efectos de los radiotransmisores sobre las aves. Una manera de investigar esta pregunta es determinar si los transmisores causan estrés fisiológico. Cuando se usan los indicadores hematológicos del estrés (tasas de heterofilos-linfocitos [H/L]), los estudios sobre las aves en cautiverio han revelado que no existe evidencia que los radiotransmisores causan el estrés. Sin embargo, los estudios realizados en cautiverio posiblemente no reflejan las condiciones en las cuales se encuentran las aves silvestres, especialmente durante tiempos energéticamente costosos como es la época de invierno. Examinamos el posible efecto de los transmisores de tipo mochila sobre los individuos de *Catharus guttatus* en Carolina del Sur mediante un examen de la tasa H/L basado en muestras de sangre tomadas antes y después de la colocación de radiotransmisores. Capturamos y recapturamos ocho individuos a comienzos del invierno y 11 individuos a finales del invierno. En los dos casos encontramos que las tasas de H/L no cambiaron después de un mes durante la cual las aves llevaron radiotransmisores. Las tasas de H/L tampoco variaron significativamente en las dos pruebas entre individuos con radiotransmisores e individuos sin radiotransmisores. Adicionalmente, los individuos que portaban transmisores en las dos pruebas no tuvieron tasas de H/L elevadas al final de la prueba en comparación con los individuos que portaban un transmisor durante solo una prueba (la de finales del invierno). Estos resultados indican que portar radiotransmisores de tipo mochila por un mes no causa el estrés en individuos de *Catharus guttatus* durante el invierno. Estos resultados son consistentes con los estudios hechos sobre aves en cautiverio y proveen apoyo al uso de los radiotransmisores de tipo mochila en esta especie, siempre y cuando los estándares de peso sean tenidos en cuenta.

Key words: *Catharus guttatus*, Hermit Thrush, heterophil-lymphocyte ratio, radio-telemetry, stress, transmitters

Although radio-telemetry is an important tool for studies of wild birds, an underlying assumption is that transmitters do not influence the birds' movements, behavior, or other life history traits. This assumption has been tested by com-

paring birds with and without transmitters for possible differences in survival and reproductive success (Foster et al. 1992, Reynolds et al. 2004, Sunde 2006), food consumption and energy expenditure (Hernandez et al. 2004), and behavior or body condition following transmitter attachment (Brigham 1989, Mattsson et al. 2006). The results of such studies indicate that transmitters

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may (Foster et al. 1992, Mattson et al. 2006) or may not (Hernandez et al. 2004, Sunde 2006) have a negative impact on birds. More recently, investigators have examined possible adverse physiological effects of transmitters, such as increased stress levels (Schulz et al. 2001, Small et al. 2004, Schulz et al. 2005, Small et al. 2005) that could affect behavior, longevity, and reproduction, and have found little evidence of such effects. However, these studies were limited to birds in captivity and may not reflect conditions faced by wild birds, especially during potentially stressful parts of their life cycles.

When birds are stressed, glucocorticoid hormones are secreted and orchestrate the physiological stress response (Dhabhar et al. 1994, 1995, 1996). Thus, measuring the levels of stress hormones allows researchers to detect any negative reactions animals may have to specific stimuli. More recently, researchers have used a hematological approach to infer stress levels (Gross and Siegel 1983, Zulkifli et al. 1999), with increases in the relative numbers of two types of white blood cells, heterophils and lymphocytes (i.e., the H/L ratio), reflecting increases in corticosterone (Davis et al. 2008), the primary stress hormone in birds. Stress-induced increases in glucocorticoid hormones cause heterophils (or neutrophils in mammals and amphibians) to enter the circulation and, simultaneously, cause lymphocytes to egress from the circulation into tissues (Dhabhar et al. 1994, 1995, 1996), so the ratio of the two cells can be used to infer levels of stress hormones. Relative increases in H/L ratios can serve as a more reliable measure of stress in birds than plasma levels of corticosterone, especially in response to chronic, long-term stressors (McFarlane et al. 1989).

Many thrush species are secretive and difficult to observe and, therefore, have frequently been studied using telemetry (Brown et al. 2000, 2002, Lang et al. 2002, Powell et al. 2005), with considerable focus on the overwintering period (Brown et al. 2000, 2002). During late winter when food availability typically declines (Strong and Sherry 2000), birds may already be stressed and any possible effects of transmitters could be magnified. We used H/L ratios to examine the effects of backpack-style radio-transmitters on Hermit Thrushes (*Catharus guttatus*) at an overwintering site in South Carolina. Specifically, we compared H/L ratios of individuals before transmitter attachment and after carrying

transmitters for a month. We also compared the H/L ratios of thrushes with transmitters to those without transmitters. If transmitters cause stress, we expected that H/L ratios would be higher in focal birds after carrying transmitters, and that ratios would be higher in thrushes with transmitters than those without transmitters.

METHODS

Our study was conducted on a 485-ha preserve in Beaufort County, South Carolina (32.3°N, 80.8°W) from November 2006 to March 2007. Our study areas consisted primarily of mixed oak (*Quercus* spp.) species and loblolly pine (*Pinus taeda*) overstory with a moderate to dense understory of wax myrtle (*Myrica cerifera*), beautyberry (*Callicarpa americana*), and regenerating oaks.

Capturing birds and transmitter attachment. Trials were conducted during November and December 2006 and from late January to mid March 2007. During each trial, we attempted to capture as many focal birds within 1 week as possible, then recapture as many of these individuals as possible 1 mo later. All birds were captured using mist nets, banded with two color bands and one USGS aluminum leg band, and weighed to the nearest 0.01 g. We also collected a blood sample from the brachial vein using a nonheparinized capillary tube and made a standard blood smear on a clean microscope slide. Finally, we fitted each bird with a 0.75-g backpack-style radio-transmitter (Model BD-2; Holohil, Carp, Ontario, Canada) and released the bird at the site of capture. Transmitters were approximately 2.4% of the weight of most birds (mean = 30.8 g; $N = 37$) and were attached using a harness method (Rappole and Tipton 1991). Total handling time was approximately 20 min and, based on previous research, would not affect H/L ratios (Davis 2005). When birds were recaptured about 1 mo later, we weighed them, collected a blood sample for a smear, and removed the transmitter. During the same 2-week period, additional Hermit Thrushes were also captured, but not radio-tagged, for use as controls. For control birds, all procedures for capturing, measuring, and blood sampling were the same.

Leukocyte counts. Slides were stained with giemsa and leukocyte counting procedures

followed Davis et al. (2004) and Davis (2005). Briefly, slides were examined under $1000\times$ (oil) with a light microscope and all leukocytes were counted in randomly selected fields of view until at least 100 cells were counted. Only fields of view with even distributions of erythrocytes were used. Leukocytes were identified as heterophils, lymphocytes, eosinophils, basophils, and monocytes (Campbell 1995). The proportion of each cell type was then determined, and the ratio of heterophils to lymphocytes calculated.

Data analysis. H/L ratios were arcsin-square root transformed to approximate normal distributions. We then used two approaches to evaluate the effect of transmitters. First, we compared the H/L ratios of focal individuals before and after transmitter attachment using paired t -tests. Second, we used 2-sample t -tests to compare H/L ratios of focal individuals after transmitter attachment to control birds captured during the same period. All tests were performed using the Statistica 6.0 software package (Statistica 2003).

RESULTS

Eight focal individuals were captured and followed during the early winter trial, with nine additional birds captured as controls. All radio-tracked birds were initially captured between 10 and 14 November in the early winter trial and recaptured between 6 and 15 December, with birds carrying transmitters from 26–31 d.

For the late winter trial, we followed 11 birds, with seven of these birds also used in the early winter trial. Birds were first captured between 24 January and 4 February and recaptured between 26 February and 13 March after carrying transmitters for 32–47 d. We also captured nine birds for use as controls.

Effects of transmitters. In early winter, we found a nonsignificant decline in H/L ratios of focal birds before and after transmitter attachment ($t_7 = 2.0$, $P = 0.081$; Fig. 1). Comparison of focal birds after transmitter attachment to control birds revealed that control birds had significantly higher H/L ratios ($t_{15} = -2.5$, $P = 0.023$). In the late winter trial, there was no difference in H/L ratios of focal birds before and after transmitter attachment ($t_{10} = -0.5$, $P = 0.64$; Fig. 1). We also found no difference in H/L ratios between focal and control birds ($t_{18} = 1.2$, $P = 0.26$).

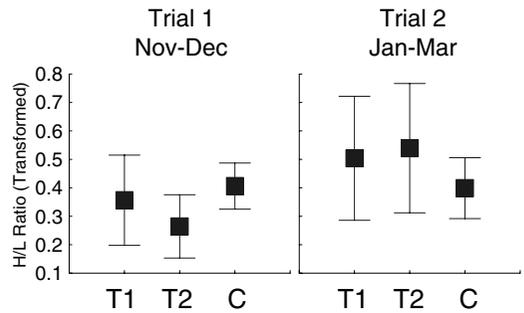


Fig. 1. Heterophil/Lymphocyte (H/L) ratios of Hermit Thrushes before (T1) and after (T2) radio-transmitter attachment ($N = 8$ for Trial 1 and 11 for Trial 2) and for control birds (C; $N = 9$ for both Trial 1 and Trial 2) with no transmitters captured at same time as T2 birds for both trials. Error bars represent 95% confidence intervals.

DISCUSSION

Based on H/L ratios that are known to reflect stress in birds (Gross and Siegel 1983, McFarlane et al. 1989, Vleck et al. 2000), we found no evidence of stress in overwintering Hermit Thrushes that carried radio-transmitters for 1 mo. H/L ratios of thrushes with transmitters did not change, and birds with transmitters did not have higher H/L ratios than birds without transmitters.

Hermit Thrushes in our study appeared to have higher H/L ratios in late winter than early winter (Fig. 1). We further assessed this by comparing the H/L ratios of focal individuals after carrying transmitters to those of control birds from the early winter trial to the same group in the late winter trial. Birds captured in December had a mean H/L ratio of 0.33 (95% CI: ± 0.08), whereas those captured in March had a mean H/L ratio of 0.48 (95% CI: ± 0.13). This increase prior to the spring migration could reflect the increase in stress experienced by migratory birds due to declining food availability during late winter (Marra and Holberton 1998, Strong and Sherry 2000, Marra and Holmes 2001, Brown and Sherry 2006).

H/L ratios are known to vary seasonally in thrushes (Owen and Moore 2006), with particularly high levels during spring migration. However, because seven of the 11 birds in the late winter trial had also carried transmitters in the earlier trial, this increase may have been caused

by the stress of carrying transmitters a second time. Comparison of the final H/L ratios (i.e., after carrying transmitters in the second trial) of the seven thrushes used in both trials to the four birds used only in the second trial revealed no significant difference (two-sample *t*-test, $t_9 = 0.5$, $P = 0.61$). Thus, thrushes that carried transmitters twice did not have higher stress levels than those that carried transmitters once, further suggesting that transmitters did not cause stress in overwintering Hermit Thrushes.

Our results, although based on a small sample size, are consistent with studies that have revealed no changes in hematological parameters of captive doves with either backpack transmitters (Small et al. 2004, 2005) or implanted transmitters (Schulz et al. 1998, 2001, 2005). Such results support the continued use of transmitters, provided they meet suggested weight limits (<3% of body mass; Bird Banding Lab, Patuxent, Maryland).

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