No Sex Bias in Wood Thrushes (*Hylocichla mustelina*) Captured by Using Audio Playback during the Non-breeding Season

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Published By: The Wilson Ornithological Society

DOI: [http://dx.doi.org/10.1676/13-187.1](http://dx.doi.org/10.1676/13-187.1)

No Sex Bias in Wood Thrushes (*Hylocichla mustelina*) Captured by Using Audio Playback during the Non-breeding Season

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ABSTRACT.—Conservation of migratory songbird species requires information on abundance and survival over the annual cycle, including from overwintering sites. Broadcasting recorded calls or songs (playback) often increases detections or capture rates of birds, and can improve estimates of abundance or survival. Wood
Thrushes overwintering in Belize regularly respond to broadcasted conspecific vocalizations (songs); however, it is unknown if song attracts a specific age, sex, or size class of birds. Our goal was to determine if the use of playback resulted in sex, age, or size-biases in captures of Wood Thrushes, relative to captures without playback, at a non-breeding site in Belize, Central America. We predicted that birds responding to playback would be: (1) male, (2) adults, and (3) larger than birds caught without playback, owing to social dominance of larger adult males over juveniles and females. Surprisingly, we found no significant difference in sex or age ratio, or body size of birds captured with or without playback. It may be that predicted patterns of social dominance are not apparent in non-breeding Wood Thrushes, or that song playback does not elicit responses related to local social dominance. Regardless of the mechanism, we conclude that the use of song playback during the non-breeding season does not bias captures of Wood Thrushes by sex, age, or body size, and when used with caution, can be an effective tool for studies on the species’ wintering grounds. 

Received 5 November 2013. Accepted 28 March 2014.

Key words: age bias, non-breeding season, sex bias, song playback, vocalizations, Wood Thrush.

For effective conservation, migratory birds must be studied throughout their entire life-cycle, including during the non-breeding resident and migratory periods (Faaborg et al. 2010). However, large gaps remain in our knowledge of non-breeding season behavior and ecology, which hampers our ability to effectively monitor and protect migratory birds (Faaborg et al. 2010). Researchers monitor trends and population sizes of migratory animals to collect data that are critical to successful conservation efforts; however, information for most migratory species is only available for breeding sites (Sauer et al. 2012).

Wood Thrushes (Hylocichla mustelina) exhibit seasonal site fidelity during the non-breeding season, in which they return to the same site in subsequent winters. Previous work found that birds of both sexes occupy home ranges during winter (Rappole et al. 1989), although it was not clear if birds aggressively defended these areas from conspecifics (Winker et al. 1990). Some individuals, dubbed “floaters” (Brown and Long 2007), traveled over a large area and showed no site fidelity. In Bicknell’s Thrushes (Catharus bicknelli), smaller-bodied females and first-winter birds are often subordinate to males and older birds (Townsend et al. 2010). In several Neotropical migrant songbirds, the consequences of subordinate status include lowered body condition and annual survival, as well as later arrival to their breeding grounds and reduced fecundity (Latta and Faaborg 2001, Marra and Holmes 2001, Townsend et al. 2010). Therefore, winter territoriality is an important factor in the regulation of populations of migrant species.

Conspecific playback is known to increase the captures and detections of birds at breeding sites (Yahner and Ross 1995, Atwood et al. 1996). For birds that are territorial during the non-breeding season, the addition of recorded calls or songs can also increase capture rates and provide a more realistic estimate of abundance and density of a target species (Graves 1996). However, using recorded vocalizations could bias captures towards a specific demographic group. In some species, there is a clear dominance hierarchy in winter when males are more territorial than females, as well as younger birds of both sexes (Marra et al. 1998). Therefore, males and adults might be more likely to respond to conspecific playbacks than females or juveniles. Non-singing vocalizations are thought to be the primary mechanism for territory defense for wintering Neotropical migrants. Nevertheless, we often captured Wood Thrushes responding to recordings of males singing (E. A. McKinnon, pers. obs.). Since Wood Thrushes are a sexually monomorphic species, it is unclear whether male song playback results in captures of proportionally more males, or if both sexes respond equally.

We studied Wood Thrushes at a non-breeding site in Belize, Central America, to determine if responses to playback were biased by social dominance hierarchies. The objective of this study was to determine if using playback of conspecific song biases captures of Wood Thrushes during the non-breeding season towards specific demographic groups. If so, this would have important implications for studies using playback to capture Wood Thrushes during the non-breeding season. We predicted that older and larger males would be the most likely to respond to playback, if Wood Thrushes show a similar social hierarchy to other Neotropical migrants (Townsend et al. 2010). We predicted that relatively more females and juveniles would be captured passively (i.e., with no playback), since captures without playback should reflect the general composition of the local population.

**METHODS**

**Study Area and Sampling Method.**—We conducted this study at the Belize Foundation for
We measured wing chord to the nearest mm for each bird captured and used this as indication of size. Birds were aged as juveniles (hatch year/second year (HY, SY)) or adults (after-hatch-year/after-second-year (AHY/ASY)) following feather characteristics (Pyle 1997). Tissues were also collected for genetic sexing (either 50 μl blood stored in 750 μl Queen’s Lysis Buffer, or dried on filter paper (Quintana et al. 2008) or plucked breast and tail feathers).

**Genetic Analyses.**—Because Wood Thrushes are a monomorphic species, genetic sexing was implemented to determine an individual bird’s sex. DNA was extracted from feathers using the DNEasy Qiagen Kits, and from blood in Queen’s Lysis Buffer using a standard Proteinase K ammonium acetate/isopropanol extraction. DNA was extracted from filter paper by adding a 1-cm² piece of blood-soaked paper to 600 μl of cell lysis buffer and 5 μl of Proteinase K. Samples were then digested at 60 °C for 5–6 hrs then held at 37 °C overnight. Following digestion, the filter paper was removed and a standard ammonium acetate/isopropanol extraction method was used to produce DNA for PCR.

Our genetic sexing protocol used modified P2/P8 primers developed specifically for Wood Thrushes (D. A. Shealer, unpubl. protocol), following Griffiths et al. (1998). The PCR cocktail included 3 μl extracted DNA, MgCl₂, Taq buffer, double-distilled H₂O, dNTPs, the modified P2 and P8 primer set, and Taq DNA polymerase, with a total reaction volume of 10 μl. The PCR was run using an Eppendorf Thermocycler with a 5-min denature step at 94 °C, 40 cycles of 94 °C for 60 sec, 48 °C for 60 sec, 72 °C for 60 sec, and a final extension at 72 °C for 2 min. PCR products were visualized on a 3% agarose gel stained with 15 μl of EtBr, photographed using an Alphalmager. Female bands were ~380 and 400 bp long, while a single male band was visible at 380 bp.

**Statistical Analyses.**—We used a Pearson’s Chi-squared test in program R (R Development Core Team 2011) to evaluate whether or not playback had an effect on the proportion of birds captured by age and sex. We used a binary logistic regression with capture method (playback used or not used) as the response variable to determine whether size (indicated by wing chord) was significantly different between birds captured by using playback versus passively, while controlling for age and sex effects (males and adults tend to be larger than females and juveniles). Alpha level...
RESULTS

Age and Sex Ratios.—Of 89 birds that were captured by using active netting, there were 36 juvenile males, 19 adult males, 24 juvenile females, and 10 adult females (Fig. 1). Of 190 birds captured passively, there were 51 juvenile males, 48 adult males, 56 juvenile females, and 35 adult females (Fig. 1). We found no significant difference in the ratio of males and females captured with (61.8% male) or without playback (52.1% male) and there was also no significant difference between ages (67.3% juveniles with playback versus 56.3% passively) (Table 1). Within females, the percent of juveniles relative to adults captured with playback (70.5%) compared to passive captures (61.5%) was not significantly different (Table 1). Within-males, the percent juveniles was not significantly different with either method (65.5% vs. 51.5%) (Table 1).

Size Effects.—Adult males had the longest mean wing length (mean ± SE = 107.3 ± 0.4 mm), followed by juvenile males (105.6 ± 0.3 mm), adult females (105.3 ± 0.4 mm), and juvenile females (103.1 ± 0.3 mm). Wing size (coefficient ± SE = -0.05 ± 0.09, Z = -0.60, P = 0.55) did not predict whether a bird was caught actively or passively, controlling for age (coefficient ± SE = -4.15 ± 13.59, Z = -0.31, P = 0.76), and sex (coefficient ± SE = 4.25 ± 14.87, Z = 0.29, P = 0.78) (Table 2). There were also no significant interactions between age, sex, and size (P > 0.05) (Table 2).

DISCUSSION

Overall, there were no significant differences in the age, sex, and size of Wood Thrushes that were captured during the non-breeding season with conspecific vocalizations versus passively. Larger or older birds, or males were not captured at higher rates than smaller birds, juveniles, or females. In fact, we tended to catch more juvenile females and fewer adult males with playback (the opposite of our predictions), although this was not significant. We suggest that age and sex ratios of birds captured with both methods is simply a reflection of the general population demographics at our study site. The fact that we had fewer adult males captured with playback (although not significantly fewer) does not support the idea that large aggressive males are more responsive to playback.

It is possible that no bird captured was actually responding behaviorally to the playback (i.e., the playback had no effect). However, we often captured birds within 10 min of playback

<table>
<thead>
<tr>
<th>Category</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% males)</td>
<td>2.30</td>
<td>0.13</td>
</tr>
<tr>
<td>Age (% juveniles)</td>
<td>0.14</td>
<td>0.71</td>
</tr>
<tr>
<td>Age (% juveniles) within females</td>
<td>0.43</td>
<td>0.51</td>
</tr>
<tr>
<td>Age (% juveniles) within males</td>
<td>1.73</td>
<td>0.19</td>
</tr>
</tbody>
</table>
initiation (37% of the time), and on average all active netting captures were within 17 ± 0.9 min (n = 118 captures with playback; includes some for which sex was unknown). This suggests that birds are approaching the source of the playback, and not randomly flying into the net. Wood Thrushes are also often heard vocalizing agonistic ‘bup bup’ and ‘pit pit’ calls (Evans et al. 2011) at our study sites, suggesting some level of territorial aggression. Regardless of the mechanism, we captured birds more quickly and with less effort when using playback (17 min vs. a minimum 30-min passive net round).

Based on other studies (Marra 2000, Marra and Holmes 2001), we expected males to be more responsive to playback since they may be socially dominant over females and juveniles. Dominance hierarchies occur as a result of some critical resource becoming limited, leading to individuals of varying abilities to compete for access to that resource (Gauthreaux 1978). In American Redstarts (Setophaga ruticilla), food (arthropods) is most likely a limiting resource, and individuals compete over access to territories that will provide sufficient and consistent food levels for the duration of the non-breeding period (Marra 2000). Wood Thrushes, unlike American Redstarts, consume both arthropods and fruit throughout the non-breeding season (Blake and Loiselle 1992, Witmer 1998). Frugivorous tropical birds are less likely to defend territories than insectivorous birds (Stutchbury and Morton 2001), which could explain the more ‘even’ response of male and female Wood Thrushes to conspecific song in winter.

It is also possible that differences in habitat quality could result in slight differences between sex and age in response to playback. Male redstarts in high quality mangrove habitats responded more aggressively than females, but only slightly more than the males found in female-biased scrub habitats (Marra 2000). Aggression directed at playback could be attributed to more frequent interactions with other individuals in high quality habitats, or to a greater interest in resource defense in a higher quality habitat. Future comparisons of captures with playback over a gradient in habitat quality for Wood Thrushes could potentially reveal if a sex-bias by habitat exists in this species.

The winter social systems of Wood Thrushes may be similar to the model of Ovenbirds (Seiurus aurocapilla), as they are also sexually monomorphic ground-foragers. Strong (1999) found that wintering Ovenbirds maintain spatiotemporal territories, actively moving away from each other after chance encounters, only defending their immediate space. Brown and Sherry (2008) found that there were no sex-based differences in space-use patterns in Ovenbirds, suggesting that females can compete against males for resources, such as food and space. This agrees with evidence that females of monomorphic species are not confined to winter social subordination (Brown et al. 2000). Therefore, behaviors of sexually monomorphic species like the Wood Thrush and Ovenbird may differ from the wintering territoriality model of the American Redstart (Runge and Marra 2005), and this may explain why, in our study, we detected no differences by sex in response to playback.

Age can also have an effect on territoriality of avian species (Stutchbury 1994). In wintering Hooded Warblers (Setophaga citrina) (Stutchbury 1994), there were relatively more juvenile, non-territorial ‘floaters’ captured relative to territorial adults. We also captured more juvenile Wood Thrushes overall with both active and passive

<table>
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<th>Independent variable</th>
<th>Coefficient ± SE</th>
<th>Z-value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5.39 ± 6.9</td>
<td>0.56</td>
<td>0.57</td>
</tr>
<tr>
<td>Wing chord</td>
<td>−0.05 ± 0.1</td>
<td>−0.60</td>
<td>0.55</td>
</tr>
<tr>
<td>Age</td>
<td>−4.15 ± 13.6</td>
<td>−0.31</td>
<td>0.76</td>
</tr>
<tr>
<td>Sex</td>
<td>4.25 ± 14.9</td>
<td>0.29</td>
<td>0.78</td>
</tr>
<tr>
<td>Age*sex</td>
<td>16.34 ± 23.0</td>
<td>0.71</td>
<td>0.48</td>
</tr>
<tr>
<td>Age*wing chord</td>
<td>0.03 ± 0.1</td>
<td>0.27</td>
<td>0.79</td>
</tr>
<tr>
<td>Sex*wing chord</td>
<td>−0.05 ± 0.1</td>
<td>−0.33</td>
<td>0.74</td>
</tr>
<tr>
<td>Age<em>sex</em>wing chord</td>
<td>−0.15 ± 0.2</td>
<td>−0.70</td>
<td>0.48</td>
</tr>
</tbody>
</table>

TABLE 2. Size of Wood Thrush was not a significant predictor of being captured by active (using playback) versus passive (no playback) netting during the non-breeding season. Since size is affected by age and sex, we used a binary linear regression to include wing chord, age, and sex, as well as interactions between all variables (indicated by *).
netting (67.4 and 56.3%, respectively). It is possible that more juveniles were captured because they were more likely to be floaters (as opposed to adults, of which we should only catch those owning local territories). Previous work on non-breeding Wood Thrushes in Veracruz documented the presence of floaters, although the sex and age of these birds was mostly unknown (Winker et al. 1990). It is also possible that naïve birds (in our study, juveniles) were more likely to be captured in general than experienced birds, as has been shown in Willow Warblers (Phylloscopus trochilus) (Linhart et al. 2012). Comparing capture rates of marked adult birds versus unmarked adults could assess if it is naïveté or age that influences the overall capture rates of Wood Thrushes.

Despite the benefits of using audio playback concurrent with mist-netting and the lack of sex, age, or size bias in our study population of Wood Thrushes, there are drawbacks. The possible consequences of simulated territorial intrusions produced by playback are poorly understood. Birds may take time away from foraging, expend energy, or make themselves vulnerable to predation or extra-pair copulations when responding to playback. Rufous Antpittas (Grallaria rufula) and Plain-tailed Wrens (Pheugopedius euophrys) altered their singing behavior in response to playback by increasing vocalizations (Harris and Haskell 2013). Since birds may expend extra energy, become stressed, or divert time from other activities to respond to playback, this method should be used at a minimum to limit such costs, especially for vulnerable species or those for which other behavioral data are being collected (e.g., nest success).

This study determined that there were no biases in sex, age, or size when using audio playback to capture Wood Thrushes. Active netting (a single net with an mp3-player and speaker playback) is less labor intensive than erecting 10–20 nets in a passive array and waiting for a Wood Thrush to be captured, especially when working in dense tropical forest where net-lanes must be cleared by hand. We suggest that a combination of active and passive netting will provide the ‘best of both worlds’ in that playback can be used sparingly within passive net arrays to boost overall captures of Wood Thrushes. The use of active netting does not measure territoriality or aggression directly, and thus additional analyses need to be conducted to determine if males and females, and adults and young birds are truly equal competitors in winter. The use of playbacks to capture and detect wintering Wood Thrushes could save time and money for important studies of population dynamics, habitat use, and resources needs, for this rapidly declining species (Sauer et al. 2012).

ACKNOWLEDGMENTS

Field assistance was provided by William Garcia, Liberato Pop, Roni Florian, Alejandro Chollum, Marlyn Cruz, Abidas Ash, Alan Romeros, Thomas Pop, and Ross Kresnik. Jacob Marlin and Staff at BFREE facilitated fieldwork. Lab assistance was provided by Steven Yee, Jackie Day, Armita Afzalimehr, and Colleen Murchison. We thank Dave Shealer for the modified primers and PCR protocol and Calandra Stanley for help testing the protocol. Funding was provided by NSERC, the American Ornithologists’ Union, York University, the Schad Foundation, Molson Foundation, Earth Rangers, the Queen Elizabeth II Graduate Scholarship in Science and Technology, and proceeds from Silence of the Songbirds (B. J. M. Stutchbury 2007, Walker & Co.).

LITERATURE CITED


