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*Short Communication*

# Radio transmitters do not influence physiological traits of wintering Grasshopper Sparrows (*Ammodramus savannarum*) in the Chihuahuan Desert of Mexico

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ABSTRACT. Radio transmitters are a commonly used method that allows researchers to obtain valuable biological and ecological data from avian populations. However, transmitters might negatively affect survival due to the physiological cost of carrying a device. The negative fitness effects of transmitters are likely to vary interspecifically. Therefore, understanding responses to transmitters by birds can help minimize researcher impact and refine the vital rate estimates derived from these approaches. In this study, we investigated the potential negative impact of radio transmitters on two physiological parameters (heterophil-to-lymphocyte ratio and scaled mass index) on the Grasshopper Sparrow (*Ammodramus savannarum*) during the non-breeding season in northern Mexico. Specifically, we used two sampling approaches: repeated measures and independent samples. With repeated measures, most radio-tagged individuals maintained similar values in their scaled mass index across the winter, and showed a statically non-significant increase in this variable. In addition, at the population level (independent samples), we observed no significant difference in the use of transmitters, based on the two physiological parameters. Our results for the non-breeding season are consistent with previous studies where researchers have reported no effects of radio transmitters on physiological parameters. In summary, the study provides insights into the lack of a transmitter effect on chronic stress and body mass index in this species, conducted in a challenging environmental stage of the annual cycle and within a grassland priority conservation area.

## Les émetteurs radio n'influencent pas les attributs physiologiques des Bruants sauterelles (*Ammodramus savannarum*) hivernants dans le désert de Chihuahua au Mexique

RÉSUMÉ. Le radiopistage est une méthode fréquemment utilisée pour permettre aux chercheurs d'obtenir des données biologiques et écologiques précieuses sur les populations d'oiseaux. Toutefois, les émetteurs radio pourraient avoir un effet négatif sur la survie des sujets en raison du fardeau physiologique que représente le port de l'appareil. Les effets négatifs des émetteurs sur la condition physique de l'oiseau varient probablement d'une espèce à une autre, c'est pourquoi la compréhension de la réponse des oiseaux aux émetteurs pourrait contribuer à minimiser l'impact des chercheurs et à affiner les estimations des taux de survie associés à leur utilisation. Dans cette étude, nous avons exploré l'impact négatif potentiel des émetteurs radio sur deux paramètres physiologiques (le ratio hétérophiles/lymphocytes et l'indice de masse corporelle) chez le Bruant sauterelle (*Ammodramus savannarum*) en période internuptiale dans le nord du Mexique. Plus précisément, nous avons utilisé deux stratégies d'échantillonnage : la prise de mesures répétées et d'échantillons indépendants. Les mesures répétées ont permis de constater que la plupart des individus porteurs d'un émetteur radio ont maintenu un indice de masse corporelle équivalent tout au long de l'hiver, cette variable présentant une augmentation statistiquement non significative. D'autre part, au niveau de la population (échantillons indépendants), nous n'avons pas observé de différence significative due à l'utilisation des émetteurs, sur la base des deux paramètres physiologiques mesurés. Nos résultats pour la période internuptiale corroborent des études antérieures dans lesquelles les chercheurs n'ont signalé aucun effet des émetteurs radio sur les paramètres physiologiques. En résumé, la présente étude a permis de mieux appréhender l'absence d'effet des émetteurs sur le stress chronique et l'indice de masse corporelle de cette espèce, dans une phase difficile du cycle annuel d'un point de vue environnemental et au sein d'une prairie en zone prioritaire de conservation.

Key Words: *chronic stress; Grasshopper Sparrow; grasslands; heterophil-to-lymphocyte ratio; radio-transmitter effect; physiological traits; scaled mass index; wintering*

## INTRODUCTION

Radio transmitters are a commonly used method to study movement, habitat use, and survival in bird populations (Gauthier-Clerc and Le Maho 2001, Bridge et al. 2011, Mekonnen Gutema 2015, Macías-Duarte et al. 2017, Strasser et al. 2019). For instance, this approach offers insights into how environmental variables, which change spatially, impact the presence, abundance, or survival of organisms. These factors are closely linked to both biotic and abiotic characteristics within a specific habitat,

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allowing researchers to evaluate its overall quality (Bridge et al. 2011). Methodological and technological advances in radio telemetry, e.g., battery life and size reduction, have drastically improved the quantity and quality of data provided by these methods (Bridge et al. 2011, Mekonnen Gutema 2015). The Motus Wildlife Tracking System, for instance, is a collaborative research network using coordinated automated radio-telemetry arrays (Taylor et al. 2017). However, the growing use of radio telemetry for characterizing vital rates and habitat associations (Smith et al. 2011, Macías-Duarte et al. 2017) has raised questions regarding its potential impact, which might affect parameters such as mortality, offspring size, and migration patterns (Barron et al. 2010).

Different studies have evaluated the effect of radio transmitters on birds. For example, a meta-analysis showed a negative impact of transmitters on some behavioral and ecological features (Barron et al. 2010). On the contrary, studies have also shown an absence of any effect on different physiological traits (Hernández et al. 2004, Schulz et al. 2005, Davis et al. 2008a, Rae et al. 2009, Gow et al. 2011, Townsend et al. 2012), survival (Hagen et al. 2006, Anich et al. 2009, Townsend et al. 2012), and breeding (Streby et al. 2013). The occurrence of such potential effects on birds is expected to vary interspecifically because of body size, life history, and behavior. As such, the impacts of transmitters need to be assessed and interpreted on a species-by-species basis. Exploration of the effect of transmitters and other factors on physiological and ecological aspects may be useful to ensure that the data and inference derived from these approaches, e.g., survival, habitat occupancy, and eco-physiology, are unbiased.

Various physiological measures can provide valuable insights into how individual birds respond to different stressors. These measures serve as indicators of the organism's biological perception, i.e., sympathetic nervous system, in relation to its environmental surroundings (Wikelski and Cooke 2006). The heterophil-to-lymphocyte (H:L) ratio has proven to be a valuable predictor of responses to long-term stressors in various vertebrate groups, both at the individual and population levels (Davis et al. 2008b). This method is useful for quantifying stress over a longer duration of exposure to stressors, i.e., chronic stress, compared to hormone levels (Davis and Maney 2018), which measures a more acute response to stress. The H:L ratio has been used to measure stress response to radio-tagging in Mourning Doves (*Zenaida macroura*; Schulz et al. 2005) and Hermit Thrushes (*Catharus guttatus*; Davis et al. 2008a).

Moreover, radio-tagging may induce behavioral changes in certain species, potentially altering foraging efficiency and, consequently, having an impact on overall body condition. Some studies suggest that body mass indices (BMIs) are moderately to strongly correlated with fat and protein content (Peig and Green 2010, Labocha and Hayes 2012), which represent energy reserves and muscle/organ mass, respectively (Lindström et al. 2000, Schwilch et al. 2002). However, alternatively to BMI, some studies have used only body mass i.e., weight, which showed no significant change between birds with and without radio tags (Hernández et al. 2004, Townsend et al. 2012, Peterson et al. 2015). The scaled mass index (SMI; Peig and Green 2009) is a method that has not been used to evaluate radio-tag influence on animals. This index uses the Thorpe-Lleonart allometric scaling model of mass and length and is a reliable predictor of fat reserves and body composition for evaluating the body condition of vertebrates (Peig and Green 2009, 2010). Hence, H:L ratio and SMI may be suitable tools to disentangle many aspects of the effect of transmitters and how organisms modulate their physiologic responses during a specific stage of the annual cycle (Ellis et al. 2012, Banbura et al. 2013).

Grassland birds populations are declining throughout North America (Brennan and Kuvlesky 2005, Ribic et al. 2009, Rosenberg et al. 2019, Vickery 2020). Therefore, it is important to consider possible adverse effects of methods used to study their ecology, even in a conservation context. One such grassland bird in decline is the Grasshopper Sparrow (*Ammodramus savannarum*), which has a wide breeding distribution in southern Canada and north-central United States, and winters in the southern United States, Mexico, Central America, and the Caribbean (Vickery 2020). On the wintering grounds, challenging weather conditions such as extreme low temperature, as well as infectious diseases and reduced food availability, can have a high physiological cost during this stage (Owen and Moore 2006, Clausen et al. 2015, Macías-Duarte et al. 2017, Montreuil-Spencer et al. 2019), and the load of the transmitter may cause this cost to increase. A study on migratory connectivity found no significant effect of high-level geolocators on the body mass of Grasshopper Sparrows between the beginning and end of their annual cycle (Hill and Renfrew 2019). Although this study provided valuable insights into the interaction between this avian species and geolocators, radio transmitters may present different challenges to the sparrows, given possible occasional snagging or entanglement of the transmitter antenna in vegetation. It is, therefore, essential to assess and evaluate the physiological status of wintering populations, including potential negative effects of radio transmitters.

The objective of this study was to evaluate the potential adverse impact of radio transmitters on physiological parameters, specifically the H:L ratio and SMI, in Grasshopper Sparrows during winter. We considered both individual, i.e., repeated measures, and population-level, i.e., transmitter versus no transmitter, comparisons. Based on previous research concerning body condition and radio-tagging (Hernández et al. 2004, Townsend et al. 2012, Hill and Renfrew 2019), we predicted that radio transmitters would not adversely affect the physiological well-being of Grasshopper Sparrows.

# METHODS

## Study area and sampling

We mist netted individual Grasshopper Sparrows in grasslands of the privately owned ranch Valle Colombia, located in the municipality of San Buenaventura, Coahuila, Mexico (28º39′ N, -102º29′W; 28º23′N, -102º29′W; 28º5′N, -102º20′W; 28º28′ W, -102º29′N; 28º18′W, - 102º15′N). This property is within the Valle Colombia grassland priority conservation area (GPCA; CEC and TNC 2005, Pool and Panjabi 2011). We captured and recaptured individuals, as described by Strasser et al. (2019), and collected blood samples during three sampling periods: (1) beginning of winter (P1), from 15 December to 20 December 2018, we deployed radio transmitters; (2) mid-winter (P2), from 29 January to 3 February 2019, we replaced radio transmitters on previously captured individuals, i.e., recaptures, and captured additional individuals to enhance our sample size; and (3) end of winter (P3), from 14 to 18 March 2019, we recaptured all individuals to remove radio tags and captured new individuals that had not been tagged during the winter period. We considered early winter to be from P1 to P2 and late winter to be from P2 to P3. The time interval between each capture-recapture period was approximately 45 days, which reflected the mean battery life of the radio transmitters (Strasser et al. 2019). We placed radio transmitters (0.5 to 0.6 g) with uncoated 13 cm long single-frequency antennas (PicoPip Ag379, Biotrack, Dorset, UK) on individuals weighing at least 15.5 g. We used a figure-eight leg-loop harness of 1-mm nylon-coated elastic to attach transmitters (Rappole and Tipton 1991). The total weight of the transmitter and harness was 3.8% of an individual's mass on average (mean ± SD = 17.01 g ± 1.22). Given that a survival study was being conducted simultaneously with the same bird population, and even though there is evidence of no negative effect of blood sampling on the survival of similar species (Smith et al. 2017), we were cautious and only blood-sampled a subset of radio-tagged birds. We collected blood samples from the brachial vein, obtaining approximately 10–20 µL of blood. The maximum blood volume collected represented 0.11% of mean body mass, which was within the recommended range according to Fair et al. (2010). We also obtained two blood smears per individual, and we placed the remaining blood sample in a cryogenic tube with Queen's lysis buffer (0.01 M Tris, 0.01 M NaCl, 0.01 M sodium EDTA, and 10% n-lauroylsarcosine) for subsequent molecular sexing. We collected standard morphometric measurements, i.e., wing chord, tail length, culmen length, tarsus length, and weight, for each bird. Blood sampling and transmitter placement of all captured individuals were carried out by a single person for each winter study period to reduce handling time of the bird, a maximum of 30 minutes, and potential associated changes in the leukocyte profiles (Davis and Maney 2018), as well as to minimize risk of injury. Fieldwork was conducted using a capture permit issued by Secretary for Environmental Management and Natural Resources of Mexico (SEMARNAT;SGPA/DGVS/12947/18).

## Scaled mass index (SMI) and heterophil-to-lymphocyte (H:L) ratio

We estimated BMI with a SMI, which integrates measurements of body mass and tarsus length, using the procedure by Peig and Green (2009). Specifically, this condition index utilizes type II regression and considers allometric scaling, ensuring that body size is standardized when determining a condition index (Peig and Green 2009).

In addition, we fixed blood smears collected in the field using 100% methanol for four minutes and subsequently stained them using the Giemsa method (Valkiūnas 2004). We used an optical microscope with 1000x magnification to carry out the leukocyte count (heterophils, lymphocytes, monocytes, eosinophils, and basophils), reaching 100 leukocytes. We calculated the H:L ratio by dividing the number of heterophils by the number of lymphocytes.

## Molecular sexing

We extracted DNA using the DNeasy Blood and Tissue kit protocol (Qiagen Inc.) and determined the sex of each Grasshopper Sparrow using a molecular protocol (Fridolfsson and Ellegren 1999). The PCR products were separated on a 1.5% agarose gel to distinguish a single band for males or two bands for females.

## Assessment of radio-transmitter effect

We analyzed the effect of radio transmitters on H:L ratio and SMI of Grasshopper Sparrows using two approaches. Initially, we employed an individual-based approach, i.e., repeated measures, to test for a change using two paired *t*-test for only SMI as the response variable in radio-tagged sparrows, monitored between P1 to P2, i.e., early winter, and between P2 to P3, i.e., late winter. Unfortunately, we were unable to recapture birds without radio tags throughout the winter, thus preventing us from evaluating changes in the condition of non-radio-tagged birds in our repeated measures analysis. Additionally, we did not consider sex, i.e., as an explanatory variable, and H:L, i.e., as the response variable, because of the small sample size, i.e., six females and 21 males and 19 individuals without a blood sample, for these analyses. Homoscedasticity and normality of the response variables were assessed using Levene's and Shapiro-Wilk tests, respectively, in each analysis. We assessed normality in repeated measures by examining the differences between periods to account for the random effects influencing SMI. We used standard deviation for error estimation, shown in parenthesis (±). All statistical analyses were conducted in R software version 4.0.3 (R Core Team 2020).

We also employed a population-based approach involving independent samples, which enables a comparison between individuals who carried the transmitter previously and those that did not. Firstly, we conducted two analyses using generalized linear models (GLM), where the explanatory variables were sex and transmitter attachment, categorized as either transmitter or no transmitter, and the response variable was SMI. In the first analysis, we compared radio-tagged birds, i.e., the same samples from repeated measures analyses, with those without radio tags, i.e., the control group, using individuals from the mid-winter period (P2). The second analysis involved the same comparison with samples from the final winter period (P3). These two analyses provided additional support for the absence of a transmitter effect, as also considered by the repeated measures analyses. Last, we performed a similar comparison using H:L as a response variable in P3. We used the Gamma distribution to account for the presence of continuous non-negative values and checked if the residuals were normally distributed using QQ-plots. Both GLM analyses were conducted using the R package lme (Bates et al. 2015).

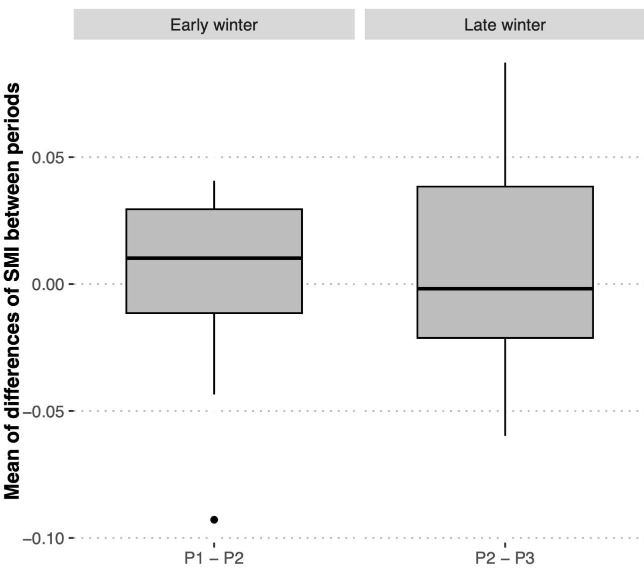
# RESULTS

## Effect of transmitter attachment

We captured a total of 52 individuals for our study. Among them, 27 radio-tagged birds were included in the analysis using repeated measures: 13 individuals were tracked from P1 to P2 (five females and eight males), and 14 individuals were monitored from P2 to P3 (one female and 13 males). These 27 individuals were used to analyze changes in SMI between the periods, based on measurements of tarsus length (mean: 19.11 ± 1.07 mm, n = 52) and body mass (mean: 17.01 ± 1.22 g, n = 52). For the wintering population in Valle Colombia, the mean H:L ratio and BMI were 0.58 (± 0.67) and 0.65 (± 0.04), respectively.

We explored whether SMI varied across winter-sampling periods for a subset of birds where all individuals were radio-tagged. These birds did not appear to change condition across the non-breeding period within early winter (paired *t*-test, $t = -0.12$, *P*-value = 0.9, $N = 13$) and within late winter (paired *t*-test, $t = -0.63$, *P*-value = 0.53, $N = 14$; Fig. 1). Whereas no significant changes in condition were observed between winter periods, we did observe a slight increase in mean condition across winter periods (SMI: P1 = 0.639, ± 0.042 and P2 = 0.640, ± 0.038; P2 = 0.655, ± 0.035, and P3 = 0.660, ± 0.039).

**Fig. 1.** Mean differences of scaled mass index (SMI) based on repeated measures of individuals of Grasshopper Sparrow (*Ammodramus savannarum*) within early (P1 versus P2) and late winter (P2 versus P3). P1 = period 1 (December 2018), P2 = period 2 (January–February 2019) and P3 = period 3 (March 2019). Solid circles represent outliers.




We found no significant difference between SMI values in P2 (transmitter = 0.66 ± 0.04, N = 11; no transmitter = 0.65 ± 0.04, N = 12, ß = 0.028, SE = 0.039, *P*-value = 0.48) and P3 (transmitter = 0.66 ± 0.04, N = 10; no transmitter = 0.65 ± 0.04, N = 20, ß = -0.012, SE = 0.04, *P*-value = 0.75; Table 1, Fig. 2). When comparing individual birds with transmitters (n = 10, two females and eight males) and without transmitters (n = 19, six females and 13 males) at the end of winter (P3), we also found no significant difference in H:L ratio (transmitter = 0.52 ± 0.38, N = 10; no transmitter = 0.60 ± 0.75, N = 20; ß= -0.41, SE = 0.69; *P*-value = 0.55; Table 1, Fig. 3).

## DISCUSSION

This study offers the opportunity to explore the potential physiological impact of radio transmitters on a new species model, the Grasshopper Sparrow. Our results expand upon earlier research that proposed radio tags might act as an additional stressor, alongside environmental stressors (Suedkamp Wells et al. 2003, Schulz et al. 2005, Mattsson et al. 2006, Davis et al. 2008a, Rae et al. 2009, Barron et al. 2010, Townsend et al. 2012, Herrod et al. 2014, Zenzal et al. 2014).

First, for individual variation, there was no significant difference between periods with individuals monitored using transmitters. Only a slight increase was observed from one period to another

**Table 1.** Parameter estimates, standard errors, and *P*-values with respect to the comparison of heterophil-to-lymphocyte (H:L) ratio and scaled mass index (SMI) for Grasshopper Sparrows (*Ammodramus savannarum*) with and without transmitters in the late winter in Valle Colombia, Coahuila, Mexico.

| Parameter | Level | Estimate | SE | *P*-value |
|---|---|---|---|---|
| Transmitter vs. no transmitter (H:L ratio) | | | | |
| Intercept | | 2.406 | 0.721 | < 0.01 |
| Sex | Male | -0.097 | 0.795 | 0.90 |
| Transmitter | Transmitter attached | -0.419 | 0.692 | 0.55 |
| Transmitter vs. no transmitter in period 2 (SMI) | | | | |
| Intercept | | 1.516 | 0.041 | < 0.001 |
| Sex | Male | 0.009 | 0.041 | 0.82 |
| Transmitter | Transmitter attached | 0.028 | 0.039 | 0.48 |
| Transmitter vs. no transmitter in period 3 (SMI) | | | | |
| Intercept | | 1.540 | 0.037 | <0.001 |
| Sex | Male | -0.018 | 0.043 | 0.66 |
| Transmitter | Transmitter attached | -0.012 | 0.040 | 0.75 |

(0.21–1.04% increment of body mass index between sampling periods; Fig. 1). A similar tendency was observed in adult Savannah Sparrows (*Passerculus sandwichensis*) that were radio-tagged, where they exhibited an increase in body mass during the pre-migratory stage, just before the autumn migration (Rae et al. 2009). Furthermore, in a study on migratory connectivity (Hill and Renfrew 2019), researchers attached light-level geolocators weighing around 3% of each individual's body mass to individual Grasshopper Sparrows throughout the entire annual cycle, which is approximately 287 days. The birds showed only minor skin and feather abrasion, and no variation in mass between pre-deployment and post-deployment (Hill and Renfrew 2019).

Second, we performed population-level analyses using independent samples to investigate the impact of radio transmitters on SMI and on the H:L ratio. We did not find an effect of transmitters on either variable (Table 1, Figs. 2 and 3). Specifically, the absence of significant variation between non-tagged and tagged birds in P2 and P3 enables us to confirm the lack of a radio-transmitter effect on Grasshopper Sparrow individuals. This is established by utilizing birds without transmitters as a control group (Fig. 2). Similarly, the effects of radio-tagging on Savannah Sparrows, a bird of similar size, indicated no impact on body condition (Rae et al. 2009). Consequently, patterns at the population level were in line with within-individual patterns observed in repeated measures analyses related to transmitter deployment (Fig. 1). Our results are consistent with other studies, which indicated that there is no evidence of stress induced by carrying radio transmitters either in situ during winter (Davis et al. 2008a), or ex situ (Schulz et al. 2005). Viewed cumulatively, these results indicate that tagging does not have significant negative impacts on physiological condition (Peterson et al. 2015).

The results indicate a lack of adverse effects on birds in the short term, suggesting that the device itself effectively serves its purpose for biological monitoring without introducing any bias to the collected data. However, we caution against extrapolating our results to lower-quality grasslands or to years with adverse weather conditions, e.g., drought. Specifically, Grasshopper Sparrows in our study system select high quality grasslands during the winter, defined by higher summer precipitation and associated

**Fig. 2.** Box plots illustrate the variation in scaled mass index (SMI) between Grasshopper Sparrow (*Ammodramus savannarum*) individuals with and without transmitters. A) Comparison of individuals without (P2-NT) and with transmitters (P2-T) during the mid-winter sampling period (P2). B) Comparison of individuals without (P3-NT) and with transmitters (P3-T) during the final sampling period (P3). Solid circles denote outliers.

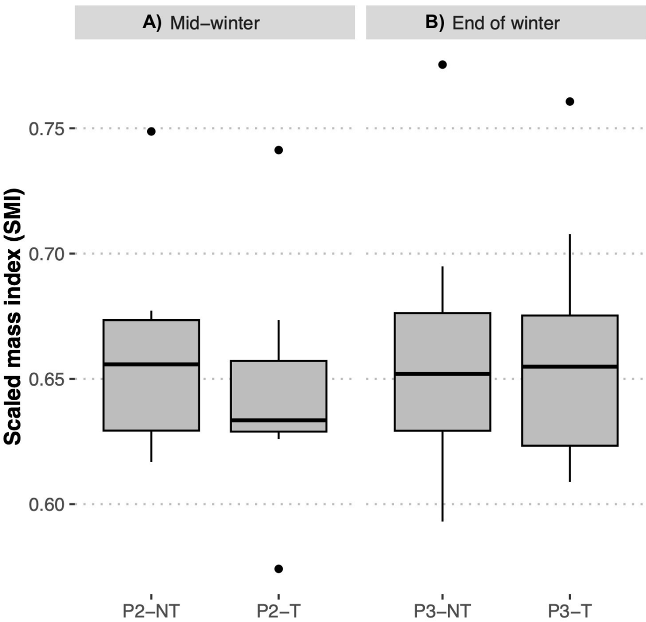
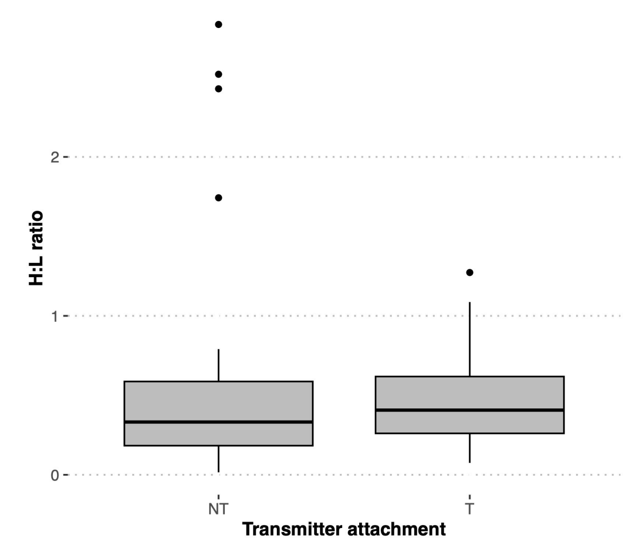



**Fig. 3.** Box plots showing heterophil-to-lymphocyte (H:L) ratio variation between Grasshopper Sparrow (*Ammodramus savannarum*) individuals with transmitter (T) versus no transmitter (NT), during the final sampling period (P3). Solid circles represent outliers.




fall vegetation (Macías-Duarte et al. 2018). Furthermore, Valle Colombia GPCA use the livestock-rotation approach, which is known to maintain healthy grassland and high plant diversity in semi-arid habitat (McDonald et al. 2019). Therefore, further research is needed to evaluate how the condition of the grasslands, i.e., sites that vary in grassland quality, may offset the potential detrimental effects of radio transmitters.

These data support previous findings that suggest radio transmitters have a negligible effect on physiology and body condition. Moreover, this research makes a unique contribution by addressing a gap in the existing literature concerning the use of specific physiological measures, i.e., H:L ratio and SMI, of radio-tagging on smaller passerine birds. However, further studies to evaluate whether there are short- or long-term effects on bird population are still warranted (Peniche et al. 2011). Although some findings have suggested an adverse effect of transmitters (e.g., Barron et al., 2010), we posit that over the past few years, advancements in technical and technological innovations have taken various factors into account. These innovations are specifically designed to mitigate the potential impact of radio-tagging on other bird species, assuming such an effect does exist. For example, device attachment, material quality, and attachment type (Rappole and Tipton 1991, Fair et al. 2010, Bridge et al. 2013, Zenzal et al. 2014, Streby et al. 2015), as well as the qualifications of researchers deploying tags (Streby et al. 2015), can all minimize the potential harmful effects of tag deployment on other bird species. Ultimately, radio-tracking provides useful applications for ornithological fieldwork, allowing researchers to attain otherwise unattainable data on fine-scale movement, habitat use, and seasonal survival for highly nomadic species. Hence, it is crucial to assess the potential impacts the device might have on the bird to prevent any direct interference with the biological or ecological data gathered by researchers. This ensures the obtained data accurately mirror the organism's interaction with its environment.

## Author Contributions:

*Conceived and designed the work: J. G. H.-D. and R. C.-D.-C. Conducted the field work: J. G. H.-D. and R. C.-D.-C. Laboratory procedures: J. G. H.-D. Analyzed the data: J. G. H.-D. and I. R.-O. Provided reagents and materials: R. C.-D.-C. and I. R.-O. Manuscript writing: J. G. H.-D., R. C.-D.-C. and I. R.-O. Revised and approved the final version of the manuscript: all authors.*

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