

1 Impact of radio-tags on Ruby-throated Hummingbirds

2 **The impact of radio-tags on Ruby-throated Hummingbirds (*Archilochus colubris*)**

3 Theodore J. Zenzal Jr.,<sup>1\*</sup> Robert H. Diehl,<sup>2</sup> and Frank R. Moore<sup>1</sup>

4 <sup>1</sup>Department of Biological Sciences, University of Southern Mississippi, Hattiesburg,

5 Mississippi, USA

6 <sup>2</sup>U.S. Geological Survey, Northern Rocky Mountain Science Center, Bozeman, Montana, USA

7 \*Corresponding author: [tjzenzal@gmail.com](mailto:tjzenzal@gmail.com)

8 **ABSTRACT**

9 Radio telemetry has advanced the field of wildlife biology, especially with the miniaturization of  
10 radio-tags. However, the major limitation faced with radio-tagging birds is the size of the animal  
11 to which a radio-tag can be attached. We tested how miniature radio-tags affected flight  
12 performance and behavior of Ruby-throated Hummingbirds (*Archilochus colubris*), possibly the  
13 smallest bird species to be fitted with radio-tags. Using eyelash adhesive, we fitted hatch year  
14 individuals (n=20 males and 15 females) with faux radio-tags of three sizes varying in mass and  
15 antenna length (220mg-12.7cm, 240mg-12.7cm, and 220mg-6.35cm), then filmed the birds in a  
16 field aviary to quantify activity budgets. We also estimated flight range using flight simulation  
17 models. When the three radio-tag packages were pooled for analysis, the presence of a radio-tag  
18 significantly decreased both flight time (-8%) and modeled flight range (-23%) when compared  
19 to control birds. However, a multiple comparison analysis between the different packages  
20 revealed that there was a significant difference in flight time when the larger radio-tag package  
21 (240mg) was attached and no significant difference in flight time when the lighter radio-tag  
22 packages (220mg) were attached. Our results are similar to other studies which analyzed the

“This draft manuscript is distributed solely for purposes of scientific peer review. Its content is deliberative and predecisional, so it must not be disclosed or released by reviewers. Because the manuscript has not yet been approved for publication by the U.S. Geological Survey (USGS), it does not represent any official USGS finding or policy.”

23 flight time or flight range of birds wearing radio-tags. Therefore, currently available light weight  
24 radio-tags ( $\leq 220\text{mg}$ ) may be a new option to aid in the study of hummingbird biology. Future  
25 study should focus upon the additional drag created by the radio-tag and the effects of the  
26 lightest radio-tag packages on free ranging birds. These studies would provide additional  
27 information to determine the feasibility on the use of radio-tags to study hummingbird biology.  
28 *Keywords:* radio transmitters, radio telemetry, Ruby-throated Hummingbirds, *Archilochus*  
29 *colubris*, radio-tagging, behavior, flight simulations, migration

30

## 31 **INTRODUCTION**

32 Radio telemetry has advanced our understanding of wildlife biology in lock step with advances  
33 in technology. Using radio telemetry as a way to study birds started in the early 1960s (e.g., Lord  
34 et al. 1962; Southern 1964; Graber and Wunderle 1966) and is now widely used to remotely  
35 collect movement data of free-ranging birds. Advances in technology have allowed for  
36 miniaturization of radio-tags, which has enabled researchers to radio-tag smaller and smaller  
37 animals, including hummingbirds (e.g. Hadley and Betts 2009) and arthropods (e.g. Wikelski et  
38 al. 2006, 2010). The usual limitation in the use of radio-tags in avian biology is the weight of the  
39 radio-tag in relation to total body mass, which is recommended to remain  $< 3\text{-}5\%$  (Cochran 1980;  
40 Gustafson et al. 1997; Fair et al. 2010). The added weight of a radio-tag may decrease the  
41 probability of nesting and increase energetic expenditure (Barron et al. 2010), yet field studies  
42 have found that tags up to 5% of the bird's total body weight do not meaningfully affect survival  
43 or movements of small ( $< 20\text{ g}$ ) birds (Naef-Daenzer et al. 2001; Hadley and Betts 2009).

44 Some research on passerines has shown that radio-tags negatively affect survival (Dougill  
45 et al. 2000; Mattsson et al. 2006), while other studies on large, mostly flightless birds suggest

46 negative impacts based on increased energy expenditure (Osborne et al. 1997; Godfrey et al.  
47 2002; Guthery and Lusk 2004). However, most studies investigating direct impacts of radio-tags  
48 on survival rates have found negligible effects, if any (Powell et al. 1998; Naef-Daenzer et al.  
49 2001; Hernández et al. 2004; Terhune et al. 2007; Anich et al. 2009; Townsend et al. 2012). The  
50 detrimental effects of radio-tags described by Dougill et al. (2000) were due to tag design, while  
51 Mattsson et al. (2006) found decreased survival when outfitting nestlings with radio-tags. Long-  
52 term survival of radio-tagged birds does not seem to be impeded by tags, as long as tags are well  
53 designed and attached after fledging. Additionally, temporary radio-tags, in which the tag  
54 eventually falls off, would likely affect survivorship the least (e.g. Raim 1978, Sykes et al. 1990;  
55 Naef-Daenzer 1993; Naef-Daenzer et al. 2001; Anich et al. 2009; Hadley and Betts 2009;  
56 Smolinsky et al. 2013).

57       Even if there is no increased likelihood of mortality or reduced reproductive success  
58 while wearing a radio-tag, other influences might handicap organisms during particular times of  
59 their annual cycle. While Barron et al.'s (2010) meta-analysis on the effects of radio telemetry  
60 found no significant effect on flight ability, a radio-tag externally mounted to the back of a bird  
61 will necessarily increase body drag (Obrecht et al. 1988; Pennycuick et al. 2012). The main  
62 variable in telemetry effect studies is the ratio of the equipment weight to body; arguably the  
63 additional drag created by the radio tag has a larger impact on flying animals than the increase in  
64 weight. An increase in body drag has been estimated (all things being equal) to reduce long-  
65 distance flight ranges, such as during migration (Obrecht et al. 1988; Powell et al. 1998;  
66 Pennycuick 2008; Pennycuick et al. 2012). Additionally, the extra weight of a radio-tag may  
67 exacerbate energy expenditure of flight, an especially difficult problem for migrating birds that

68 are already carrying increased fat loads. Nonetheless, most investigators make no attempt to  
69 determine any impacts of the device before implementing a radio tracking study.

70 The impact of radio-tags on birds is usually not tested prior to application on free-flying  
71 birds, especially for drag. If flight performance is affected by the weight of a radio-tag, then we  
72 would expect birds with the heaviest radio-tags to experience the largest effect. Drag, however,  
73 varies with transmitter and antenna size, not with mass (Pennycuick et al. 2012). Differing  
74 antenna lengths could have a disproportionate impact on the transmitter center of gravity  
75 imposing increased energetic costs per unit flight time on individuals outfitted with a longer  
76 antenna than individuals outfitted with a shorter antenna. Individuals with the longer antenna will  
77 either compensate energetically to the increased flight costs or fly less.

78 We analyzed the impact of radio-tags on the flight performance and behavior of Ruby-  
79 throated Hummingbirds (*Archilochus colubris*), a long-distance, migrant (likely both trans-Gulf  
80 and Circum-Gulf) traveling between breeding and wintering destinations (Weidensaul et al.  
81 2013), and the smallest bird to our knowledge to be outfitted with a radio-tag. We used a  
82 pairwise study design on individuals in a controlled setting to examine three different radio-tag  
83 packages varying in weight and antenna length during fall migration, a time when individuals are  
84 accumulating additional mass via fat stores in order to fuel migratory flights. Our two main  
85 objectives *a priori* were: 1) quantify the flight time of birds with and without a radio-tag, and 2)  
86 estimate the flight range of birds with and without a radio tag from a mechanical model of  
87 weight and drag (Pennycuick 2008). A secondary objective *a postori* was to determine if  
88 preening behavior differed between treatments.

## 89 **METHODS**

### 90 **Study Site and Field Methods**

91 We captured Ruby-throated Hummingbirds using nylon mist nets at the Bon Secour National  
92 Wildlife Refuge, Fort Morgan, Alabama (30°10'N, 88°00'W), between sunrise and noon from  
93 September 3 – 16, 2010. Netting effort was both active, baiting 10 nets with artificial feeders,  
94 and passive. We banded hummingbirds with a USGS aluminum band, aged and sexed them (Pyle  
95 1997), estimated fat (Helms and Drury 1960), measured wing chord and mass, and took a wing  
96 photo to determine wing span and wing area for flight range estimates.

### 97 **Aviary Routine and Radio-Tag Attachment**

98 We randomly selected a sub-sample of hatch year birds (n=35; mass= 3.80±0.73g for males  
99 (n=20) and 3.76±0.46g for females (n=15) [these and following results are reported as mean ±  
100 SD]). We individually placed birds selected for experimentation into a field aviary (2.43m X  
101 1.31m X 1.94m) with a perch and a feeder without a perch. We used a pairwise study design in  
102 which all individuals received, in random order, the control treatment (no radio-tag) and one of  
103 three experimental treatments (with faux radio-tag, Figure 1). In each experimental treatment the  
104 faux radio-tag varied by antenna length and/or mass. The first experimental group (n=15)  
105 received a heavy radio-tag (240mg; total body mass: 6.00% females, 6.32% males) with a long  
106 antenna (length: 12.7cm; diameter: 0.229mm). The second experimental group (n=10) received a  
107 light radio-tag (220mg; total body mass: 5.50% females, 5.79% males) with a long antenna  
108 (length: 12.7cm; diameter: 0.152mm). The third experimental group (n=10) received a light  
109 radio-tag (220mg; total body mass: 5.50% females, 5.79% males) with a short antenna (length:  
110 6.35cm; diameter: 0.152mm). Radio-tag design and two faux transmitters were provided  
111 courtesy of Sparrow Systems.

112 We attached radio-tags using a modified version of Raim's (1978) method. Radio-tags  
113 were first sewn to a piece of cloth the size of the radio-tag, then a second piece of cloth similar in

114 size to the one sewn to the radio-tag was glued to the back of the bird using Revlon® Fantasy  
115 Lengths® eyelash adhesive. The cloth sewn to the radio-tag was glued to the cloth on the bird  
116 (Figure 1). Cloth, thread, and glue were not included in the radio-tag weight. We removed radio-  
117 tags by clipping feathers under the cloth. Treatment order was randomized between individuals  
118 to eliminate any effect of order on subsequent analysis. Each individual tested only one radio-  
119 tag. After being prepared for the appropriate treatment (with or without radio-tag), individuals  
120 were placed in the aviary, allowed to acclimate for 10 minutes before the treatment was recorded,  
121 and then videotaped (Panasonic PV-GS65) at 1/4000 frames per second for 7 minutes to score  
122 behaviors and time spent in various activities. We then prepped the same individual for the next  
123 treatment (attachment or removal of radio-tag), allowed another 10 minutes for acclimation, and  
124 then videotaped for another 7 minutes. After a bird completed both treatments, we released it  
125 without a radio-tag.

## 126 **Flight time**

127 Flight time was quantified from the total 14 minutes (7 minutes for control treatment and 7  
128 minutes for experimental treatment) of video recording. We defined flight as any period an  
129 individual was not perched, not distinguishing hovering flight (including feeding) from forward  
130 flight. Body condition was determined for two reasons: 1) birds were randomly selected during  
131 migration, differing in the amount of fat carried, and 2) this species exhibits reverse sexual-size  
132 dimorphism (Weidensaul et al. 2013). We calculated body condition (fat) based on mass and  
133 wing length of Ruby-throated Hummingbirds captured on the Bon Secour NWR following  
134 Ellegren (1989, 1992) and Owen and Moore (2006). For both sexes, we determined fat free  
135 masses by regressing body mass on fat score for individuals in the same wing chord class (1mm  
136 increments). The intercept of the regression provided an approximation of fat free mass for a sex-

137 specific wing chord class. After performing regressions on all wing chord classes of individuals  
138 included in the study, we executed a second linear regression by regressing the intercepts on each  
139 related wing chord class for each sex. The resulting equation from the second regression allowed  
140 an estimation of size specific fat free masses for each hummingbird.

141         Restricted maximum likelihood (REML) models were used to assess the influence of  
142 radio-tags on flight time data using JMP statistical software (v.10, SAS Institute 2013). We  
143 performed a preliminary analysis on a subset of individuals balanced by experimental order  
144 (n=14 for each group) to determine if the order in which treatments were applied had an effect on  
145 flight time. Flight time (square root transformed) was the response variable in a repeated  
146 measures mixed model with radio-tag type, experimental order, and presence/absence of radio-  
147 tag as fixed factors and individual as a random nested blocking factor. We removed experimental  
148 order from subsequent analysis (see Results). We then analyzed flight time data using a repeated  
149 measures mixed model with radio-tag type, sex, body condition (described above), and  
150 presence/absence of radio-tag as fixed factors and individual as a random nested blocking factor.  
151 To determine the impact of each radio-tag type, we reran the analysis for the three radio-tags  
152 separately and interpreted p-values using the Holm-Bonferroni adjustment method for multiple  
153 comparisons (Holm 1979). Body condition failed to meet the assumptions of normality (Shapiro-  
154 Wilk ( $p=0.02$ ), even after attempting all standard transformations. Therefore, we ran each  
155 analysis with and without body condition included as a covariate. To further explore the  
156 relationship between body condition and activity budget, we used a Spearman's rank correlation  
157 between flight time and body condition.

## 158 **Preening Behavior**

159 We quantified the number of preening occurrences from the video analysis of each bird. Preening  
160 is defined as each time an individual's bill made contact with its feathers. We analyzed preening  
161 occurrences using a generalized linear mixed-effects model fit by maximum likelihood with a  
162 quasi-Poisson distribution (O'Hara and Kotze 2010) in the R statistical language (R Core Team  
163 2013), package "MASS" (Venables and Ripley 2002). The fixed factors of this model included  
164 radio-tag type, experimental order, body condition, and presence/absence of radio-tag, with  
165 individual as a random nested blocking factor. We excluded sex in this model since we did not  
166 believe there is any biological significance of sex on preening. We did however include  
167 experimental order because observations suggested that birds receiving the experimental  
168 treatment first may preen more after the back feathers were clipped to remove the transmitter. To  
169 further explore interactions we performed two additional tests in the R statistical language: First,  
170 a Nemenyi post-hoc test (Hollander and Wolfe 1999) from package "coin" (Hothorn et al. 2006,  
171 2008) was performed on significant effects of the model. Second, a Spearman's rank correlation  
172 was used to determine a relationship between preening occurrences and flight time.

### 173 **Flight Range Estimates**

174 We used Program Flight 1.24 to estimated flight range of birds with and without radio-tags.  
175 Simulations were based on wing area, fat free mass, and body condition (Pennycuick 2008), for  
176 each bird (n=31) with and without a radio-tag. We obtained fat free masses and body conditions  
177 of Ruby-throated Hummingbirds using methods described above. However, three of the  
178 individuals fell below the average fat free mass and according to the conditions of the model  
179 were not able to migrate. Therefore, two females and one male were eliminated from analysis  
180 due to lean body condition; additionally, a third female was removed because she had no  
181 associated wing photograph. We quantified wing span and wing area as described in Pennycuick



182 (2008), although we photographed rather than hand traced wings. We modified Pennycuick's  
183 (2008) wing area quantification by using ImageJ (Abramoff et al. 2014) to determine the exact  
184 wing area (partial wing area plus rootbox) from a digital tracing of an individual's semi-span  
185 instead of using a grid to quantify area as performed with hand tracings. We assumed trans-Gulf  
186 flight in still-air conditions and an altitude of 500 m (air density of  $1.17 \text{ kg/m}^3$ ; Kerlinger and  
187 Moore 1989; Woodrey and Moore 1997). When an individual was simulated with a radio-tag, a  
188 payload mass was determined for the appropriate radio-tag size (220mg or 240mg) with a drag  
189 factor of 1.5 (Pennycuick et al. 2012). We determined differences between simulated flight  
190 ranges (square root transformed) for individuals with and without a radio-tag using a REML  
191 repeated measures mixed model. Radio-tag type, sex, and presence/absence of radio-tag were set  
192 as fixed factors and individual as a random nested blocking factor. This statistical analysis was  
193 performed using JMP statistical software (v.10, SAS Institute 2013).

## 194 **RESULTS**

### 195 **Flight time**

196 We found mixed evidence of experimental order impacting flight time of hummingbirds, due to  
197 a significant interaction between experimental order and presence/absence of a radio-tag  
198 ( $F_{1,55}=21.12$ ,  $p=0.0001$ ). Individuals decreased flight time during the second treatment regardless  
199 of treatment order. There was, however, much individual variation which clouds the  
200 interpretation of the results but illustrates that attachment of a radio-tag will not elicit the same  
201 response from every individual. Individuals receiving the control treatment first had an  
202  $80.00 \pm 167.59$ s decrease when the radio-tag was attached, while individuals with a radio-tag  
203 attached first had a  $5.95 \pm 148.26$ s decrease during the control treatment. A bird undergoing the  
204 control treatment second had feathers clipped which possibly explains why there was decreased

205 flight time during the control treatment. The decrease in the subsequent treatment is likely a  
206 result of preening and possibly acclimation to captivity (see below). However, the main effect of  
207 experimental order did not affect flight time ( $F_{1,55}=0.32$ ,  $p=0.58$ ). Based on the main effect test,  
208 large amount of inter-individual variation, and the *a priori* effort to randomize treatment order,  
209 we concluded that experimental order did not meaningfully impact activity budgets and excluded  
210 it as a factor from the subsequent analysis.

211 Flight time was about 8% less with a radio-tag attached ( $F_{1,69}=7.36$ ,  $p=0.01$  without body  
212 condition as a factor;  $F_{1,69}=6.00$ ,  $p=0.02$  with body condition as a factor). Flight time without a  
213 radio-tag ( $182.94 \pm 121.72s$ ) was greater than when a radio-tag was attached ( $149.6 \pm 104.39s$ ,  
214 averaged across all models). However, size of the radio-tag did not have a significant effect on  
215 flight time in either model ( $F_{2,69}=0.98$ ,  $p=0.39$  without body condition as a factor;  $F_{2,69}=1.83$ ,  
216  $p=0.18$  with body condition as a factor). Further analysis using multiple comparison testing  
217 between radio-tag types using the Holm-Bonferroni adjustment (Holm 1979) revealed that the  
218 only radio-tag to have a significant decrease (~11%) in flight time between the treatment and the  
219 control was the heavy tag ( $F_{1,29}=15.06$ ,  $p=0.002$  without body condition as a factor;  $F_{1,29}=13.27$ ,  
220  $p=0.004$  with body condition as a factor; adjusted  $\alpha=0.017$ ), while flight time in both of the light  
221 tag treatments did not differ from controls (long antenna tag: ~8% decrease,  $F_{1,19}=1.76$ ,  $p=0.22$   
222 without body condition as a factor;  $F_{1,19}=2.32$ ,  $p=0.18$  with body condition as a factor; adjusted  
223  $\alpha=0.025$ ; short antenna tag: ~6.5% increase,  $F_{1,19}=0.05$ ,  $p=0.83$  without body condition as a  
224 factor;  $F_{1,19}=0.06$ ,  $p=0.82$  with body condition as a factor). Mean flight time decreased  
225 predictably from the light weight short antenna tag having the most flight time ( $210.70 \pm 127.14s$ ),  
226 followed by the light weight long antenna tag ( $148.40 \pm 74.93s$ ), while the heavy weight long  
227 antenna tag had the least flight time ( $137.00 \pm 107.92s$ ) (Figure 2). Although wing morphology of

228 Ruby-throated Hummingbirds is sex-dependent (Stiles et al. 2005), neither model showed an  
229 effect of sex on flight activity ( $F_{1,69}=2.31$ ,  $p=0.14$  without body condition as a factor;  $F_{1,69}=1.24$ ,  
230  $p=0.28$  with body condition as a factor) nor an interaction between sex and the presence of a  
231 radio-tag ( $F_{1,69}=0.21$ ,  $p=0.65$  without body condition as a factor;  $F_{1,69}=1.49$ ,  $p=0.24$  with body  
232 condition as a factor). Finally, body condition did not impact flight time ( $F_{1,69}=1.35$ ,  $p=0.26$ ), and  
233 a Spearman's rank correlation showed no relationship between flight time and body condition  
234 (with radio-tag Spearman's  $\rho=-0.01$ ,  $p=0.94$ ,  $n=35$ ; without radio-tag: Spearman's  $\rho=-0.18$ ,  
235  $p=0.30$ ,  $n=35$ ).

### 236 **Preening Behavior**

237 As expected, initial analysis revealed that preening occurrences had a significant negative  
238 correlation with flight time (with radio-tag: Spearman's  $\rho=-0.39$ ,  $p=0.02$ ,  $n=35$ ; without radio-  
239 tag: Spearman's  $\rho=-0.38$ ,  $p=0.02$ ,  $n=35$ ), which is not surprising given preening and flying are  
240 mutually exclusive behaviors. Preening occurrences did not differ between the presence/absence  
241 of a radio-tag ( $t=0.18$ ,  $df=23$ ,  $p=0.86$ ,  $n=35$ ). However, experimental order did have a significant  
242 effect on the number of preening occurrences ( $t=2.43$ ,  $df=23$ ,  $p=0.02$ ,  $n=35$ ). Birds receiving the  
243 control treatment first ( $n=16$ ) had a mean of  $25.31\pm 42.53$  preening occurrences which increased  
244 to  $37.63\pm 55.15$  preening occurrences when the radio-tag was attached. Birds that first received  
245 the experimental treatment ( $n=19$ ) increased preening occurrences from  $27.84\pm 35.06$  to  
246  $30.26\pm 40.71$  after the radio-tag was removed. However, given the close means and significant  
247 effect of preening on experimental order, a Nemenyi post-hoc test (Hollander and Wolfe 1999)  
248 was performed on the number of preening occurrences by order which yielded a non-significant  
249 effect ( $Z=0.31$ ,  $p=0.76$ ,  $n=35$ ). Although the number of preening occurrences does not differ  
250 significantly while an individual has a radio-tag attached, the number of preening occurrences

251 are greater when a radio-tag is attached ( $32.31 \pm 35.38$ ) compared to when no radio-tag is attached  
252 ( $28.00 \pm 41.01$ ) averaged across treatments. There is much individual variation between subjects  
253 regardless of treatment. When a radio-tag was attached preening occurrences ranged from 0 to  
254 184 instances over 7 minutes, individuals without a radio-tag attached showed a similar range  
255 from 0 to 157 instances over the same time frame. This analysis further explains the significant  
256 interaction between presence/absence of a radio-tag and experimental order for flight time.

### 257 **Flight Range Estimates**

258 Flight modeling provided an estimate of how a radio-tag affects flight range. The presence of a  
259 radio-tag significantly affected simulated flight ranges ( $F_{1,62}=135.26$ ,  $p<0.0001$ ), reducing an  
260 individual's flight range by ~340km on average (without radio-tag:  $1512.26 \pm 1188.65$ km; with  
261 radio-tag:  $1172.00 \pm 916.86$ km). There was no effect of sex on flight range ( $F_{1,62}=0.48$ ,  $p=0.49$ ),  
262 nor was there an effect of radio-tag mass ( $F_{2,62}=1.08$ ,  $p=0.36$ ).

### 263 **DISCUSSION**

264 Hummingbirds are challenging to study because their size and speed of movement makes  
265 detection of birds difficult by visual means. The ability to continually track and record the  
266 behavior of radio-tagged hummingbirds would measurably enhance our understanding of  
267 migratory movement, dispersal, resource use, home range activity, and habitat use. For example,  
268 the first published application of a radio-tag on a hummingbird determined the movement  
269 patterns of Green Hermits (*Phaethornis guy*) in Costa Rica (Hadley and Betts 2009). To our  
270 knowledge, the Green Hermit is the smallest bird ( $5.8 \pm 0.09$ g; Hadley and Betts 2009) that has  
271 been radio-tagged prior to our study of Ruby-throated Hummingbirds. The miniaturization of  
272 transmitters has allowed others to track flying arthropods, much smaller than most

273 hummingbirds, which provided insight to questions that would be difficult to answer using other  
274 means (e.g., Wikelski et al. 2006, 2010; Pasquet et al. 2008; Hagen et al. 2011).

275 Activity budgets of Ruby-throated Hummingbirds are influenced by the presence of a  
276 radio-tag, although only the heaviest radio-tag showed a significant decrease (~11%) in flight  
277 time from the control treatment. The light radio-tags had less influence in flight time with the  
278 long antenna tag decreasing flight time by ~8%, and the short antenna tag increasing flight time  
279 by ~6.5%. These radio-tags at 220 mg, just over 5% total body mass of a Ruby-throated  
280 Hummingbird, did not seem to pose a significant handicap on activity, similar to other studies  
281 using radio-tags at a comparable percent body weight (Naef-Daenzer et al. 2001; Hadley and  
282 Betts 2009). However, it is difficult to extrapolate the small differences found in flight activity  
283 that might be an artifact of a seven minute experimental period in an aviary to actual migratory  
284 flight.

285 Free ranging animals may behave differently when in captivity (see Clubb and Mason  
286 2003). The size of the aviary or simply being placed in an aviary may have limited the activity of  
287 the hummingbirds once they determined there was no way out. Although time of day was not  
288 included as a factor in analysis, most birds were tested in the late morning or early afternoon  
289 when they are typically inactive (Zenzal, personal observation) or migrating (Hall and Bell 1981;  
290 Willimont et al. 1988). The length of time allotted for birds to acclimate to the radio-tag may  
291 have been too short, affecting the outcome; most birds receiving any sort of marker (e.g. band,  
292 radio-tag) spend an unpredictable amount of time reacting to the tag (preening or attempting to  
293 remove the marker) before resuming normal behaviors.

294 Preening increased in individuals that received the experimental treatment compared to  
295 the control treatment. Increases in comfort behavior (as described by Delius 1988; i.e. preening,

296 wing flapping, head shaking) would necessarily increase the amount of time spent perching,  
297 while decreasing time spent in flight. While preening explains some of the variation found during  
298 flight activity, particularly between the different experimental orders which may be due to  
299 attaching radio-tags directly to feathers or clipping back feathers to remove the radio tag, caution  
300 is recommended when making interpretations from this analysis as handling birds seemed to  
301 increase the likelihood of birds preening. Although we found no significant effect of a radio-tag  
302 on preening, other studies have shown that preening did increase with the attachment of a  
303 transmitter (Hooge 1991; Pietz et al. 1993; Sykes et al. 1990).

304         The apparent effect of the radio-tag on activity might be influenced by attachment  
305 method, since the radio-tag was glued directly to back feathers instead of skin for easy removal  
306 after the experiment was complete. The most common adhesive attachment method requires  
307 feathers to be clipped in order to create a strong bond between the radio-tag backing and the skin  
308 of the bird or feather shaft (e.g., Raim 1978; Sykes et al. 1990; Naef-Daenzer 1993; Naef-  
309 Daenzer et al. 2001; Anich et al. 2009; Hadley and Betts 2009; Smolinsky et al. 2013). The  
310 effects of this attachment method are negligible on small birds compared to other attachment  
311 methods tested (Sykes et al. 1990). Furthermore, field studies showed no decrease in  
312 survivorship when this attachment method was used compared to non-radio-tagged birds (Naef-  
313 Daenzer et al. 2001; Anich et al. 2009).

314         The percent body mass and size of radio-tags, but not the antenna length, appeared to  
315 affect the activity budget; however much variation existed between and within treatments. Our  
316 findings are consistent with the influence of drag of the device (Barron et al. 2010; Pennycuick et  
317 al. 2012) rather than the weight of the radio-tag viz. energetic expenditure. The light radio-tag  
318 with the short antenna had the highest amount of flight time, likely due to decreased drag of the

319 antenna. However, large individual variation across all the variables explored make it difficult to  
320 suggest any hard-and-fast rules for radio-tagging hummingbirds, besides selecting a radio-tag  
321 that has the smallest weight and drag available. A valuable follow-up study to the one described  
322 would determine the amount of drag different radio-tag designs have on free-flying  
323 hummingbirds, similar to Pennycuick et al.'s (2012) study of external device drag on Rose-  
324 coloured Starlings (*Pastor roseus*), with the use of a wind tunnel.

325 Predicted flight range was affected by the presence of a radio-tag but did not vary with  
326 size of the radio-tag or sex of the individual. Individuals able to fly farther by virtue of larger fat  
327 loads experienced larger decreases in distance when radio-tagged compared to individuals with  
328 shorter flight ranges. Although flight simulations showed a decrease in flight ranges, most  
329 studies on survival and return rates of radio-tagged long-distance migrants fail to show an effect  
330 of radio-tags on survival (Powell et al. 1998; Cardinal 2005; Anich et al. 2009; Townsend et al.  
331 2012; however see Samuel and Fuller 1996). In two of these studies, a subsample of birds tagged  
332 were recaptured a year later (in one case 2 years later, Powell et al. 1998) with radios still  
333 attached (Powell et al. 1998; Townsend et al. 2012).

334 Hummingbird behavior is potentially affected by the presence of a radio-tag, so caution  
335 should be exercised when selecting individuals to tag, which will depend on season, sex, and  
336 condition. For example, a radio-tag is likely to impede nest construction in female hummingbirds  
337 (see Weidensaul et al. 2013). That said, observations of free flying Ruby-throated Hummingbirds  
338 during stopover revealed that individuals with radio-tags behave similarly to marked individuals  
339 without radio-tags in stopover duration, foraging, competitive interactions, and seasonally  
340 appropriate departure directions (Zenzal, personal observation). One of these free flying radio-  
341 tagged birds was detected, wearing its tag, at an artificial feeder in Corpus Christi, Texas (~950

342 km from Fort Morgan, Alabama) two weeks after being tagged (USGS Bird Banding Laboratory,  
343 personal communication).

#### 344 **ACKNOWLEDGMENTS**

345 Research funding was provided by the Department of Biological Sciences at the University  
346 of Southern Mississippi (USM) and a grant from the National Science Foundation (NSF) to FRM  
347 (IOS 844703). TJZ was also supported by a fellowship from the NSF GK-12 program  
348 “Molecules to Muscles”, Award # 0947944 through USM. We would especially like to thank the  
349 Bon Secour National Wildlife Refuge and Fort Morgan Historic Site for allowing us to work on  
350 their properties. We would also like to thank the 2010 Fort Morgan Field crew for help with data  
351 collection, C.J. Pennycuick for insightful conversations during the formulation of this  
352 manuscript, C. Qualls and J. Schaefer for statistical advice, and J. Cochran for conversations  
353 about designing radio-tags for hummingbirds and supplying faux radio-tags. We would also like  
354 to thank the Migratory Bird Research Group at USM for their support. Finally, we would like to  
355 thank Z. Németh and anonymous reviewers for helpful comments on this manuscript. This study  
356 was approved by the USM Institutional Animal Care and Use Committee as well as the USGS  
357 Bird Banding Laboratory. Any use of trade, firm, or product names is for descriptive purposes  
358 only and does not imply endorsement by the U.S. Government.

#### 359 **LITERATURE CITED**

- 360 Abramoff, M.D., P.J. Magalhaes, and S.J. Ram. 2004. Image processing with ImageJ.  
361 Biophotonics International 11: 36-42.
- 362 Anich, N.M., T.J. Benson, and J.C. Bednarz. 2009. Effect of radio transmitters on return rates of  
363 Swainson’s Warblers. Journal of Field Ornithology 80: 206-211.



- 364 Barron, D.G., J.D. Brawn, and P.J. Weatherhead. 2010. Meta-analysis of transmitter effects on  
365 avian behaviour and ecology. *Methods in Ecology and Evolution* 1: 180-187.
- 366 Cardinal, S.N. 2005. Conservation of Southwestern Willow Flycatchers: home range and habitat  
367 use by an endangered passerine. M.S. thesis, Northern Arizona University, Flagstaff, AZ,  
368 USA.
- 369 Clubb, R. and G. Mason. 2003. Captivity effects on wide-ranging carnivores. *Nature* 425: 473-  
370 474.
- 371 Cochran, W. 1980. Wildlife telemetry. In *Wildlife Management Techniques Manual*, 4<sup>th</sup> ed. (S.D.  
372 Schemnitz, Ed.). The Wildlife Society, Washington, D.C. pp. 507-520.
- 373 Delius, J.D. 1988. Preening and associated comfort behavior in birds. *Annals of the New York*  
374 *Academy of Sciences* 525: 40-55.
- 375 Dougill, S.J., L. Johnson, P.C. Banko, D.M. Goltz, and M.R. Wiley. 2000. Consequences of  
376 antenna design in telemetry studies of small passerines. *Journal of Field Ornithology* 71:  
377 385-572.
- 378 Ellegren, H. 1989. Hur kan vikt och vinglängd användas för att uppskatta mängden lagrat fett hos  
379 flyttande fåglar? en metodstudie hos höstflyttande blåhakar *Luscinia s. svecica*. *Vår*  
380 *Fågelärlid* 48: 75-85.
- 381 Ellegren, H. 1992. Estimated effects of age and sex on the fat-free body mass of autumn  
382 migrating Bluethroats. *Ardea* 80: 255-259.
- 383 Fair, J. E. Paul, and J. Jones, Eds. 2010. *Guidelines to the use of wild birds in research*.  
384 Washington, D.C.: Ornithological Council.

- 385 Godfrey, J.D., D.M. Bryant, and M.J. Williams. 2002. Radio-telemetry increases free-living  
386 energy costs in the endangered Takahe *Porphyrio mantelli*. *Biological Conservation* 114:  
387 35-38.
- 388 Graber, R.R. and S.L. Wunderle. 1966. Telemetric observations of a Robin (*Turdus migratorius*).  
389 *Auk* 83: 674-677.
- 390 Gustafson, M. E., J. Hildenbrand and L. Metras. 1997. The North American Bird Banding  
391 Manual (Electronic Version). Version 1.0.  
392 <http://www.pwrc.usgs.gov/BBL/manual/manual.cfm>. Accessed April 25, 2013.
- 393 Guthery, F.S. and J.J. Lusk. 2004. Radiotelemetry studies: are we radio-handicapping Northern  
394 Bobwhites? *Wildlife Society Bulletin* 32: 194-201.
- 395 Hadley, A.S. and M.G. Betts. 2009. Tropical deforestation alters hummingbird movement  
396 patterns. *Biology Letters* 5: 207-210.
- 397 Hagen, M., M. Wikelski, W.D. Kissling. 2011. Space use of Bumblebees (*Bombus* spp.) revealed  
398 by radio-tracking. *PloS One* 6: e19997.
- 399 Hall, G.A. and R.K. Bell. 1981. The diurnal migration of passerines along an Appalachian ridge.  
400 *American Birds* 35: 135-138.
- 401 Helms, C.W. and W.H. Drury. 1960. Winter and migratory weight and fat field study on some  
402 North American bunting. *Bird-Banding* 31: 1-40.
- 403 Hernández, F., J.A. Arredondo, F. Hernandez, D.G. Hewitt, S.J. DeMaso, and R.L. Bingham.  
404 2004. Effects of radiotransmitters on body mass, feed consumption, and energy  
405 expenditure of Northern Bobwhites. *Wildlife Society Bulletin* 32: 394-400.
- 406 Hollander, M. and D.A. Wolfe. 1999. *Nonparametric statistical methods*, 2<sup>nd</sup> edition. John Wiley  
407 and Sons, New York.

- 408 Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of*  
409 *Statistics* 6: 65-70.
- 410 Hooge, P.N. 1991. The effects of radio weight and harness on time budgets and movements of  
411 Acorn Woodpeckers. *Journal of Field Ornithology* 62: 230-238.
- 412 Hothorn, T., K. Hornik, M.A. Van De Wiel, and A. Zeileis. 2006. A lego system for conditional  
413 inference. *The American Statistician* 60: 257-263.
- 414 Hothorn, T., K. Hornik, M.A. Van De Wiel, and A. Zeileis. 2008. Implementing a class of  
415 permutation tests: The coin package. *Journal of Statistical Software* 28: 1-23.
- 416 Kerlinger, P and F.R. Moore. 1989. Atmospheric structure and avian migration. In: *Current*  
417 *Ornithology*, Vol. 6. (D.M. Power, ed.), pp. 109-142. Plenum Press, New York, NY.
- 418 Lord, R.D. Jr., F.C. Bellrose, and W.W. Cochran. 1962. Radiotelemetry of the respiration of a  
419 flying duck. *Science* 137: 39-40.
- 420 Mattsson, B.J., J.M. Meyers, and R.J. Cooper. 2006. Detrimental impacts of radiotransmitters on  
421 juvenile Louisiana Waterthrushes. *Journal of Field Ornithology* 77: 173-177.
- 422 Moore, F.R. and D.A. Aborn. 1996. Time of departure by Summer Tanagers (*Piranga rubra*)  
423 from a stopover site following spring trans-Gulf migration. *Auk* 113: 949-952.
- 424 Naef-Daenzer, B. 1993. A new transmitter for small animals and enhanced methods of home-  
425 range analysis. *Journal of Wildlife Management* 57: 680-689.
- 426 Naef-Daenzer, B., F. Widmer, and M. Nuber. 2001. A test for effects of radio-tagging on survival  
427 and movements of small birds. *Avian Science* 1: 15-23.

- 428 O'Hara, R.B. and D.J. Kotze. 2010. Do not log-transform count data. *Methods in Ecology and*  
429 *Evolution* 1: 118-122.
- 430 Obrecht, H.H. III, C.J. Pennycuick, and M.R. Fuller. 1988. Wind tunnel experiments to assess the  
431 effect of back-mounted radio transmitters on bird body drag. *Journal of Experimental*  
432 *Biology* 135: 265-273.
- 433 Osborne, D.A., B.J. Frawley, and H.P. Weeks Jr. 1997. Effects of radio tags on captive Northern  
434 Bobwhite (*Colinus virginianus*) body composition and survival. *American Midland*  
435 *Naturalist* 137: 213-224.
- 436 Owen, J.C. and F.R. Moore. 2006. Seasonal differences in immunological condition of three  
437 species of thrushes. *Condor* 108: 389-398.
- 438 Pasquet, R.S., A. Peltier, M.B. Hufford, E. Oudin, J. Saulnier, L. Paul, J.T. Knudsen, H.R.  
439 Herren, and P. Gepts. 2008. Long-distance pollen flow assessment through evaluation of  
440 pollinator foraging range suggests transgene escape distances. *Proceedings of the*  
441 *National Academy of Sciences* 105: 13456-13461.
- 442 Pennycuick, C.J. 2008. *Modelling the flying bird*. Academic Press (Elsevier), Boston, MA.
- 443 Pennycuick, C.J., P.L.F. Fast, N. Ballerstädt, and N. Rattenborg. 2012. The effect of an external  
444 transmitter on the drag coefficient of a bird's body, and hence on migration range, and  
445 energy reserves after migration. *Journal of Ornithology* 153: 633-644.
- 446 Pietz, P.J., G.L. Krapu, R.J. Greenwood, and J.T. Lokemoen. 1993. Effects of harness  
447 transmitters on behavior and reproduction of wild mallards. *Journal of Wildlife*  
448 *Management* 57: 696-703.
- 449 Powell, L. A., D. G. Krentz, J.D. Lang, and M.J. Conroy. 1998. Effects of Radio Transmitters  
450 on Migrating Wood Thrush. *Journal of Field Ornithology* 69: 306-315.

- 451 Pyle, P. 1997. Identification Guide to North American Birds, Part 1. Slate Creek Press, Bolinas,  
452 CA.
- 453 R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for  
454 Statistical Computing, Vienna, Austria.
- 455 Raim, A. 1978. A radio transmitter attachment for small passerine birds. *Bird-Banding* 49: 326-  
456 332.
- 457 Ridgeway, R. 1911. The birds of North and Middle America. *Bulletin of the United States*  
458 *National Museum* 50: Part V.
- 459 Samuel, M.D. and M.R. Fuller. 1996. Wildlife radiotelemetry. In *Research and Management*  
460 *Techniques for Wildlife and Habitats* (T.A. Bookhout, Ed.). The Wildlife Society,  
461 Bethesda, Maryland. pp. 370-418.
- 462 SAS Institute. 2013. JMP Statistical Analysis Software. SAS Institute, Cary, North Carolina,  
463 USA.
- 464 Smolinsky, J.A., R.H. Diehl, T.A. Radzio, D.K. Delaney, and F.R. Moore. 2013. Factors  
465 influencing the movement biology of migrant songbirds confronted with an ecological  
466 barrier. *Behavioral Ecology and Sociobiology Online First*. doi: 10.1007/s00265-013-  
467 1614-6.
- 468 Southern, W.E. 1964. Additional observations on winter Bald Eagle populations: Including  
469 remarks on biotelemetry techniques and immature plumages. *Wilson Bulletin* 76: 121-  
470 137.
- 471 Stiles, F.G., D.L. Altshuler, and R. Dudley. 2005. Wing morphology and flight behavior of some  
472 North American hummingbird species. *Auk* 122: 872-886.

- 473 Sykes, P.W., Jr., J.W. Carpenter, S. Holzman, and P.H. Geissler. 1990. Evaluation of three  
474 miniature radio transmitter attachment methods for small passerines. *Wildlife Society*  
475 *Bulletin* 18: 41-48.
- 476 Terhune, T.M., D.C. Sisson, J.B. Grand, and H.L. Stribling. 2007. Factors influencing survival of  
477 radiotagged and banded Northern Bobwhites in Georgia. *The Journal of Wildlife*  
478 *Management* 71: 1288-1297.
- 479 Townsend, J.M., C.C. Rimmer, and K.P. McFarland. 2012. Radio-transmitters do not affect  
480 seasonal mass change or annual survival of wintering Bicknell's Thrushes. *Journal of*  
481 *Field Ornithology* 83: 295-301.
- 482 Venables, W. N. and B.D. Ripley. 2002. *Modern Applied Statistics with S*. Fourth Edition.  
483 Springer, New York.
- 484 Weidensaul, S.T., Robinson, T. R., R.R. Sargent and M.B. Sargent. 2013. Ruby-throated  
485 Hummingbird (*Archilochus colubris*). In *The Birds of North America* 204, A. Poole and  
486 F.B. Gill, Eds. Academy of Natural Sciences, Philadelphia, PA, USA, and American  
487 Ornithologists' Union, Washington DC, USA.
- 488 Wikelski, M., D. Moskowitz, J.S. Adelman, J. Cochran, D.S. Wilcove, and M.L. May. 2006.  
489 Simple rules guide dragonfly migration. *Biology Letters* 2: 325-329.
- 490 Wikelski, M., J. Moxley, A. Eaton-Mordas, M.M. López-Urbe, R. Holland, D. Moskowitz, D.W.  
491 Roubik, and R. Kays. 2010. Large-range movements of Neotropical Orchid Bees  
492 observed via radio telemetry. *PLoS One* 5: e10738.
- 493 Willimont, L.A., S.E. Senner, and L.J. Goodrich. 1988. Fall migration of Ruby-throated  
494 Hummingbirds in the northeastern United States. *The Wilson Bulletin* 100: 482-488.

495 Woodrey, M.S. and F.R. Moore. 1997. Age-related differences in the stopover of fall landbird  
496 migrants on the coast of Alabama. *Auk* 114: 695-707.

497 **Figure 1.** Ruby-throated Hummingbird with a faux radio-tag attached (photo by T.J. Zenzal).

498 **Figure 2.** Mean flight time (s) of Ruby-throated Hummingbirds per 7 min treatment with  
499 (240mg: n=15; 220mg: n=10) and without (n=35) radio-tags attached. Radio-tags are separated  
500 by mass (mg) and length of antenna (cm). Standard deviation is shown as vertical bars.  
501

502 Figure 1



503

504

505

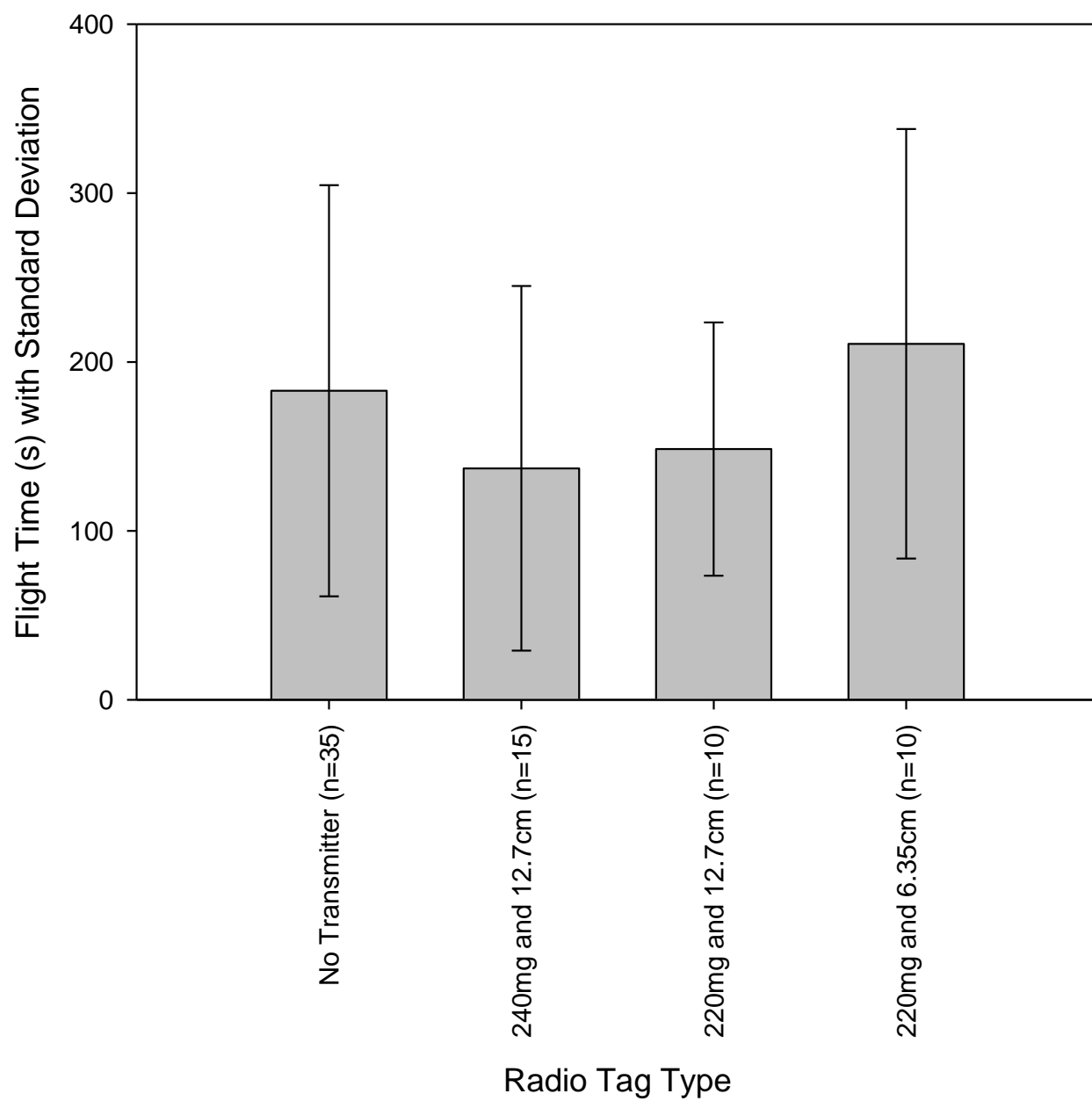
506

507

508

509

510 Figure 2



511  
512