

¹ Validating morphological condition indices and their relationship ² with reproductive success in great-tailed grackles

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¹⁹ ABSTRACT

Morphological and physiological variation among individuals has the potential to influence multiple life history characteristics such as dispersal, migration, reproductive success, and survival. Individuals that are in better “condition” can disperse or migrate further or more successfully, have greater reproductive success, and survive longer, particularly in years where environmental conditions are harsh. Condition is defined in various ways, but is most often measured using an individual’s energetic state. These traits are difficult to measure directly, therefore a variety of morphological proxies to quantify energetic condition are used instead, including fat score, weight, ratio of weight to tarsus length, and a scaled mass index. However, there is mixed support regarding whether these energetic condition indices relate to life history characteristics, and whether the relationship is linear. Additionally, although some investigations use multiple morphological proxies for energetic condition, rarely have there been direct comparisons among proxies to validate that they measure the same trait. In this investigation, we define condition as an energetic state and we attempt to measure it by comparing two morphological indices (fat score and the scaled mass index) to validate whether they measure the same trait and whether they correlate with measures of reproductive success in our study system, the great-tailed grackle (*Quiscalus mexicanus*). We found that the morphological proxies did not correlate with each other, indicating that they do not measure the same trait. Further, neither proxy significantly correlated with reproductive success in males, measured as whether a male held a territory containing nests

36 or not. We found that females with a high scaled mass index had a significantly lower probability that
37 their nest would survive on any given day. However, there was no relationship between female fat score and
38 nest survival. These results indicate that morphological measures of energetic condition should be validated
39 before relying on their use as a condition proxy in grackles and birds in general. Future research should
40 investigate behavioral mechanisms underlying our result that higher scaled mass index correlated with lower
41 nest survival to better understand the importance of energetic condition for reproductive success - a necessary
42 component for selection to act.

43 INTRODUCTION

44 Morphological and physiological variation among individuals has the potential to influence multiple life
45 history characteristics such as dispersal, migration, reproductive fitness, and survival (Wilder et al., 2016).
46 One trait that might be particularly likely to influence these life history characteristics is energetic condition.
47 Individuals that are in better “condition” can disperse or migrate further or more successfully, have greater
48 reproductive success, and survive longer (Heidinger et al., 2010; Liao et al., 2011; Wilder et al., 2016),
49 particularly in years where environmental conditions are harsh (Milenkaya et al., 2015). For example, a
50 study conducted on vipers showed that while the level of fat reserves in males was not related to their sexual
51 activity, females with low fat reserves engaged in sexual interactions less frequently than those with higher
52 fat reserves (Aubret et al., 2002). In contrast, mantids showed conflicting results regarding the relationship
53 between fat reserves and reproductive success (Barry & Wilder, 2013). Female mantids were fed either a
54 high protein, low lipid diet, or a high lipid, low protein diet. The females that received the high lipid diet
55 had higher lipid content in most parts of their body compared to that of their high protein diet counterparts.
56 However, they were not able to produce even half as many eggs as the females fed the high protein, low
57 lipid diet. This led to lower male attraction, measured by the number of copulation events, thus negatively
58 impacting further reproductive success.

59 A variety of morphological proxies have been used to quantify energetic condition [i.e., fat score, weight,
60 ratio of mass to structural size, residuals from a linear regression of mass as a function of structural body
61 size; Labocha et al. (2014); Jacobs et al. (2012)]. However, there is mixed support regarding whether and
62 how these proxies relate to life history characteristics (Labocha et al., 2014; Wilder et al., 2016). A review
63 conducted by Barnett (2015) shows that, while mass or body size measures of energetic condition are often
64 assumed to have a positive linear relationship with fitness, this is not always the case, and the relationship
65 should first be empirically validated before being used as a proxy (Barnett et al., 2015). In some instances, the
66 proxy might relate to life history characteristics, but in a non-linear way. For example, theoretical simulations
67 of small birds show that survival does not increase linearly with energy (i.e., fat) reserves (McNamara et
68 al., 2005). If the reserves are too low, the individual is at risk of starvation. However, once the reserves get
69 too high, the individual is at an increased risk of predation (McNamara et al., 2005). Thus, fat reserves can
70 relate to a life history variable (survival), but in a U-shaped relationship rather than a linear one.

71 Although some studies use multiple morphological proxies for energetic condition (e.g., Warnock & Bishop,
72 1998), rarely are these variables directly compared. Multiple proxies should correlate with each other if
73 they measure the same trait (energetic condition). Furthermore, there is still confusion about what trait
74 some proxies actually measure. For example, a study conducted on two species of crickets showed that three
75 estimates of energetic condition based on fat content or on the relationship between body mass and body
76 length (scaled mass index or ordinary least squares regression) did not correlate with each other (Kelly et
77 al., 2014). A similar lack of a relationship was found in flying animals such as birds (Jacobs et al., 2012;
78 **gosler1995cost?**) and bats (McGuire et al., 2018) thus indicating cross-taxon support that morphological
79 proxy measures do not always measure the same trait. This is an example of the jingle fallacy (Block,
80 1995; Carter et al., 2013), where a single trait label (“energetic condition”) actually encompasses more
81 than one distinct trait. In this case, two investigations using different proxies can be conducted on the same
82 research question, using the same species, but may end up with different results. This is problematic because
83 inconsistency in results among researchers can result in potentially misleading interpretations of the impact
84 of variation in morphology in relation to life history and population variables (Stevenson & Woods Jr, 2006).

85 Here we compare two indices (fat score and the scaled mass index) of an individual's energetic state to
86 validate whether they correlate with each other, which would indicate that they both measure energetic
87 condition. Fat score, as described by Kaiser (1993), is a numerical estimate of the amount of fat visible
88 under the skin (Fig. 1). The score ranges from 0 to 8 depending on the size and appearance of the fat
89 located in the individual's abdomen and interclavicular depression, with 0 indicating no visible fat and 8
90 indicating extensive fat covering the ventral surface such that no muscle tissue is visible. For example, a
91 score of 1 corresponds to sparse traces of fat visible in the interclavicular depression and abdomen. This
92 measure is frequently used in birds (Cornelius Ruhs et al., 2019; Erciyas et al., 2010; Merilä & Svensson,
93 1997), and is a straightforward, non-invasive method for estimating energetic condition. However, previous
94 research found that it does not always positively relate with life history variables. For example, Haas (1998)
95 found no difference between fat scores in individuals that had successful or failed nests in American robins
96 and brown thrashers, indicating that fat score may not explain much of the variation in nest success in
97 some species. Further research is needed to understand the relationship between fat score measures and life
98 history characteristics.

99 In contrast, the scaled mass index (SMI) is more difficult to calculate than the fat score, but it has become
100 the predominant ratio method for quantifying energetic condition within and among populations (Delciellos
101 et al., 2018; English et al., 2018; Maceda-Veiga et al., 2014). The SMI is an individual's mass scaled
102 by skeletal body size (Peig & Green, 2009). Unlike the common alternative which uses a simple ratio of
103 tarsus (lower leg) length to body mass, the SMI accounts for the tendency towards allometric scaling where
104 the relationship between body mass and structural size increases by a power law (Huxley, 1932). When
105 individuals with different structural body sizes can be standardized to the population average structural
106 body size, then energetic condition (the amount of mass not explained by structural body size) can be more
107 directly compared within and across populations. That is, the SMI calculates the energetic condition as
108 the mass of an individual relative to the population by first computing the mass that the individual would
109 have at the population average of a specific body measurement (e.g., tarsus length). Next, structural body
110 size of the individual is standardized by scaling the individual's structural body length by the population
111 average of that body measurement, which accounts for population differences. The SMI is calculated as:

112 $Mass_i \left[\frac{AvgLength_p}{Length_i} \right]^{slope_p}$ where $Mass_i$ is each individual's weight in grams, $Length_i$ is the value of the
113 chosen measure of structural body length for each bird, $AvgLength_p$ is the average structural body length in
114 the population, and $slope_p$ is calculated from the standard major axis regression (which is used to compare
115 variables that were both directly measured and thus have residual error) of a structural body size measure,
116 like tarsus length on mass (Peig & Green, 2009), and is interpreted as the expected change in structural
117 length for a one unit increase in mass. Therefore, individuals with superior energetic condition (larger weight
118 for their structural body size) will have a higher SMI compared to individuals that with inferior energetic
119 condition. Studies across taxa found that the SMI relates positively to reproductive success and survival. For
120 example, mallards with a lower SMI had lower rates of survival compared to their higher SMI counterparts
121 (Champagnon et al., 2012), while in crimson finches SMI was positively related to the number of young that
122 survived to independence (Milenkaya et al., 2015).

123 Our research will determine whether these two indices of energetic condition measure the same trait, and
124 whether this trait relates to an important life history characteristic: reproductive success. Measuring repro-
125 ductive success in birds involves finding and monitoring nests (Mayfield, 1961). However, nests are usually
126 built in cryptic locations and parents behave secretly (Gill, 1995), thus making it difficult to quantify the
127 number of eggs and nestlings inside the nest over time. Additionally, it is difficult and time-consuming to
128 track the survival of offspring once they leave the nest. Therefore, we will use the predominant method in
129 this field for quantifying reproductive success: whether a nest fledged offspring (Mayfield, 1961).

130 Our study system is a population of great-tailed grackles (*Quiscalus mexicanus*), hereafter "grackles," in
131 Tempe, Arizona. This system is ideal for this investigation because grackles are native to the tropical
132 climates of Central America (Johnson & Peer, 2001), but have rapidly expanded their geographic range into
133 new areas (Wehtje, 2003). Because grackles are a water-associated species, the desert habitat of Tempe
134 presents physiological challenges that could lead to an increased likelihood of a tradeoff between survival
135 and reproductive attempts (Henderson et al., 2017). Deserts are characterized by a scarcity of water and
136 extreme temperature fluctuations, which require behavioral and physiological adaptations (Costa, 2012).

¹³⁷ Wide variation in energetic condition and reproductive success is possible if grackle physiology requires more
¹³⁸ water than is present in the environment, and some individuals may cope with physiological stress, or find
¹³⁹ hidden sources of water, better than others (Henderson et al., 2017).

¹⁴⁰ **HYPOTHESES** We measured two morphological proxy variables of energetic condition and observed
¹⁴¹ reproductive success in grackles to test two hypotheses. The first examined the relationship between two
¹⁴² morphological proxies of energetic condition to validate that they measure the same inherent trait. Secondly,
¹⁴³ we hypothesized that energetic condition, as measured by either or both of the morphological proxy variables,
¹⁴⁴ would relate to reproductive success in male and female grackles.

145 **METHODS**

146 The methods below are based on the preregistration, with small changes summarized in the Deviations from
147 the planned methods section and further explained in the preregistration (in italics).

148 **Preregistration details** The preregistration used secondary data that were collected as part of other
149 ongoing investigations (tarsus length in http://corinalogan.com/Preregistrations/g_flexgenes.html; tarsus
150 length, body weight, number of fledglings, and whether a male holds a territory in http://corinalogan.com/Preregistrations/g_withinpop.html; and tarsus length in http://corinalogan.com/Preregistrations/g_expansion.html). The preregistration, containing the hypotheses, methods, and analysis plan, was written
151 (July 2019) and submitted to Peer Community In Ecology for pre-study peer review (August 2019) before any
152 analyses were conducted. We revised according to reviewer comments and received in principle acceptance
153 by PCI Ecology of the version on 8 Nov 2019. After that, we conducted the analyses in the preregistration.
154 Our final methods, results, and discussion, including all data and code, are listed below.



157 **Summary of methods**

158 Figure 1: A male grackle showing the yellow/orange tint of fat under the skin in the intraclavicular depression
159 (left); and a female grackle showing *no* fat under the skin of the intraclavicular region, but significant fat
160 deposits under the skin of the abdomen (right).

161 Great-tailed grackles are caught year-round in the wild in Tempe, Arizona using a variety of methods
162 (e.g., walk-in trap, bownet, mist net). After capture we immediately processed birds by attaching colored
163 leg bands in unique combinations for individual identification, conducted morphological measurements of
164 weight, tarsus length, flattened wing length, tail length, skull length, bill length and fat score (Fig. 1, the
165 amount of visible fat under the skin in the clavicle and abdomen as in Kaiser, 1993). Most grackles were
166 released after completion of color band marking, measurements, and acquiring a blood sample. A subset
167 of grackles were held in aviaries for up to 6 months for behavioral testing, and then released back to the
168 wild at their location of capture. We tested the repeatability of our structural size measures on this subset
169 of individuals by measuring them again before release. The second measures were collected by the same
170 experimenter in 11 out of 17 females and 10 out of the 18 males that were repeatedly sampled.

171 From March - August, we monitor the behavior of all color-marked grackles to determine their nesting status.
172 We follow females carrying nesting materials to find their nest. We determine whether the male territory
173 owner is color-marked as well. Then we check each nest approximately every day to determine the status
174 based on the female's behavior (building, incubation, feeding nestlings, feeding fledglings, failed).

175 Individuals included in our sample were those for which we have measures of energetic condition when they
176 were adults. We did not include individuals whose data were collected as juveniles. We also excluded data
177 that was collected from the grackles when they were released from the aviaries to avoid any confounds due
178 to their time in the aviary (e.g., perhaps unlimited nutritious food in the aviaries affected their fat score).
179 However, to validate that our measures of structural body size (tarsus length or wing length) are precise

180 and accurate, we measured twice the subset of grackles brought into aviaries - once when they were initially
181 caught, and again up to 6 months later when we released them. We calculated the repeatability of these
182 multiple measures. All other data included in this study came from wild-caught grackles (including the data
183 from the birds that were brought into the aviaries on their first capture).

184 We first used logistic mixed-effect models to determine whether SMI and fat score are correlated. We also
185 tested whether SMI and fat score varied by season because grackles are difficult to catch such that we were
186 unable to structure our data collection to coincide with the breeding season and instead caught and measured
187 grackles as often as possible. Previous research found a non-linear relationship between reproductive success
188 and energetic condition variables (Milenkaya et al., 2015). To check whether this is occurring in our data,
189 we visually examined our raw data to determine if we need to include a non-linear energetic condition
190 independent variable into our models (i.e. FatScore²). Then we used we used two types of logistic mixed-
191 effect models to determine the relationship between energetic condition and reproductive success. Both types
192 are supported in the literature, but are slightly different in the way in which the link function is specified.
193 First, we modeled the effect of energetic condition on reproductive success using a generalized linear mixed
194 model framework with a logit link function (i.e. Milenkaya et al., 2015). We then also used a logistic exposure
195 model that has a link function which accounts for the time interval between nest checks when estimating
196 the probability of daily nest survival (Bolker, 2014; Shaffer, 2004).

197 After pre-study peer review: Deviations from the planned methods

- 198 1) We realized that the sexual dimorphism of male and female body sizes necessitates separate analyses.
199 Therefore, we calculated SMI for males and females separately, and ran separate models for each sex
200 for the repeatability analysis (P1 and P2).
- 201 2) Fat score data were distributed such that the majority of scores were 0, with some 1's and very few
202 higher numbers. Specifically, of the 21 males, 15 had fat scores at 0, 5 scored 1, and a single male had
203 a fat score of 2. Out of 47 females, 26 scored 0, 18 scored 1, 2 scored 2, and a single female scored 3.
204 This lack of variance in the response variable led to problems when we ran the models: it was difficult
205 to fit models using an ordinal regression. The function “simulateResiduals,” which we used to check
206 our data, does not work with data in the ordinal family. Consequently, we modified the model to use
207 a logistic regression where the dependent variable FatScore is categorized as individuals that showed
208 no visible fat ($y = 0$), or some fat was present ($y = 1$) where we combined all individuals that had fat
209 score values of 1 or greater. Subsequent data checking indicated that these data were not zero-inflated
210 or overdispersed.

211 Deviations when testing hypothesis 1: correlation between SMI and Fat score

- 212 3) Warning messages occurred during the repeatability analysis using the “rptR” package in R (Stoffel et
213 al., 2017) indicating that the fit was singular, likely because the variance for the Experimenter random
214 effect in the model for both female and male wing length was 0.001. We thus conducted an unregistered
215 analysis where we confirmed that our repeatability values from the repeatability models were valid,
216 despite the warning, by hand calculating repeatability following Nakagawa & Schielzeth (2010). The
217 hand-calculated repeatabilities were nearly identical (female $R = 0.5$, male $R = 0.71$) to the output
218 from the rpt function.
- 219 4) Despite the data checking which indicated our model was not overdispersed or zero inflated, we could
220 not get the fixed effects or random effect to converge using the Bayesian package in R “MCMCglmm.”
221 We found no improvement in model fit by tweaking the priors or iterations/burnin/thin options. There-
222 fore, we fit these models using the function glmer, a frequentist framework.
- 223 5) The Season variable only includes 2 males in the breeding season category, thus we do not have a
224 large enough sample to produce reliable estimates. We removed the Season variable from the model
225 for males.

226 **Deviations when testing hypothesis 2: energetic condition and reproductive success**

- 227 6) Only two females had reproductive success data from more than one year in our study (2019 and 2020).
228 Consequently, there were very few repeated measures in this sample and our random effect of bird ID
229 accounted for zero variance. This led to a warning that our model fit was singular. Therefore, we
230 removed the data for these females for 2020 so we could remove ID as a random effect from the model,
231 which resulted in the model running without warnings. We removed the 2020 data for these females
232 because their energetic condition data was collected in 2019 and these measures were more likely to
233 relate to their 2019 reproductive success data than to their reproductive success in 2020.
- 234 7) The fit of the model analyzing the relationship between energetic condition and male reproductive
235 success (ability to hold a territory containing female nests) was singular. The Year random effect
236 accounted for zero variance in the data, so we removed it. The fit was still singular, but we retained
237 the ID random effect (although it also explained zero variance) to account for repeated measures in
238 this sample.
- 239 8) The model fit was again singular in our logistic exposure model because the Year random effect ex-
240 plained zero variance in the data. We removed this random effect from the analysis.

Table 1: Table 1. Sample sizes for P1 and P2. The *Breeding* and *Non-breeding season* categories refer to the number of individuals measured in each season. The *Reprod. success* category represents the total number of individuals in each year observed engaging in breeding behaviors. Note that the 2019 and 2020 reproductive success sample sizes include some of the same individuals that were observed in both years. Whereas, the *Prop. successful* category represents the proportion of the total individuals observed engaging in breeding behaviors in each year that held a territory containing nests (males) or fledged young (females).

Category	Males	Females
Breeding Season Fat	2	12
Non-breeding fat	20	35
Breeding season SMI	6	24
Non-breeding SMI	18	38
Aviaries	16	9
Repro. success 2019	8	9
Repro. success 2020	17	13
Prop. successful 2019	0.63	0.22
Prop. successful 2020	0.47	0.54

RESULTS

Prediction 1: correlation between SMI and Fat Score

We were able to calculate SMI for 24 males and 62 females, and fat score values were available for 22 males and 47 females (Table 1).

We found that wing length was more tightly correlated with body mass than tarsus length in both sexes, therefore we used wing length in our SMI calculations (female $n = 62$, $r = 0.26$, $p = 0.03$; male $n = 24$, $r = 0.35$, $p = 0.08$). This allows us to account for as much variation in body mass as possible that is associated with skeletal body size because leftover variation in body mass is more likely to relate to energetic condition. Consequently, we used wing length in our calculation of SMI.

To validate that we were measuring structural body size consistently across experimenters, we analyzed the repeatability of wing length in the birds in our sample that were measured more than once. We found that average wing length was repeatable ($n = 17$ females, Repeatability \pm standard error $= 0.53 \pm 0.18$; $n = 18$ males, Repeatability \pm SE $= 0.75 \pm 0.11$). Data permutations and a likelihood ratio test both confirmed that these repeatability values were statistically significant at $p < 0.01$.

In females, we found that for every one unit increase in SMI, the bird is 1.3 times more likely to have some fat (a 30% increase in the odds of having fat), which is not a statistically significant relationship (female $p = 0.81$; Table 2). In males, a one unit increase in SMI corresponds to an odds ratio of 1.6, or a 60% increase in the odds of having some fat, which is also not a statistically significant relationship ($p = 0.50$; Table 2). Together, this indicates that SMI and fat score are not equally measuring energetic condition. There was also no relationship between season (breeding or non-breeding) and female fat score ($p = 0.71$). Only 2 males were measured during the breeding season, therefore we omitted season as an independent variable in the male model (Table 1).

Prediction 2: energetic condition and reproductive success

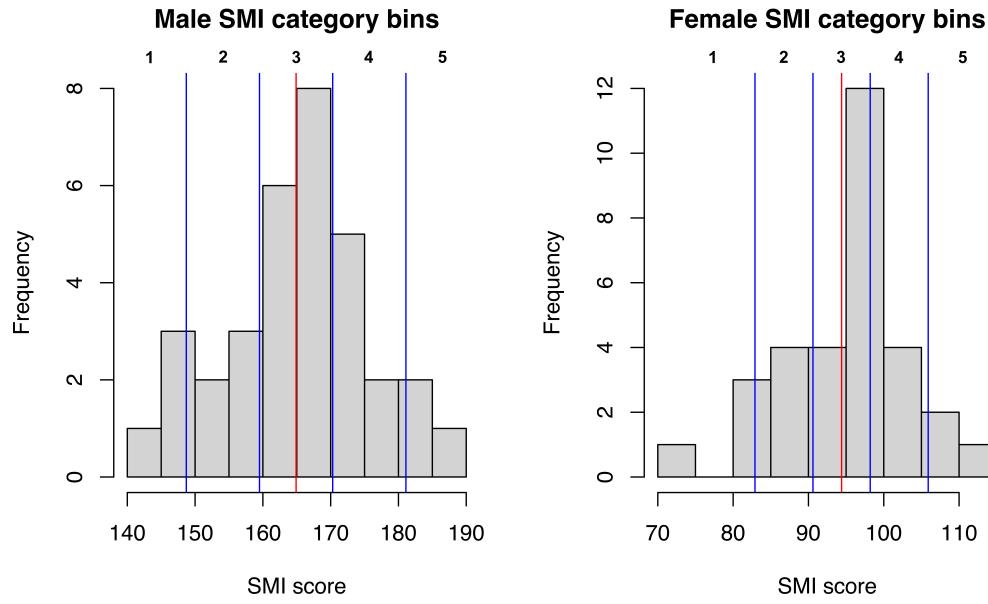
Our sample size for P2, where individuals had measures of reproductive success, SMI, and fat scores, was 20 for females and 20 for males.

To determine whether we should include any non-linear effects of SMI in our models (A. G. Gosler et al., 1995; Milenkaya et al., 2015), we visually evaluated whether individuals in any of 5 categories, ranging from low to high SMI, were more likely to be reproductively successful (Fig. 2). We found no visual evidence for

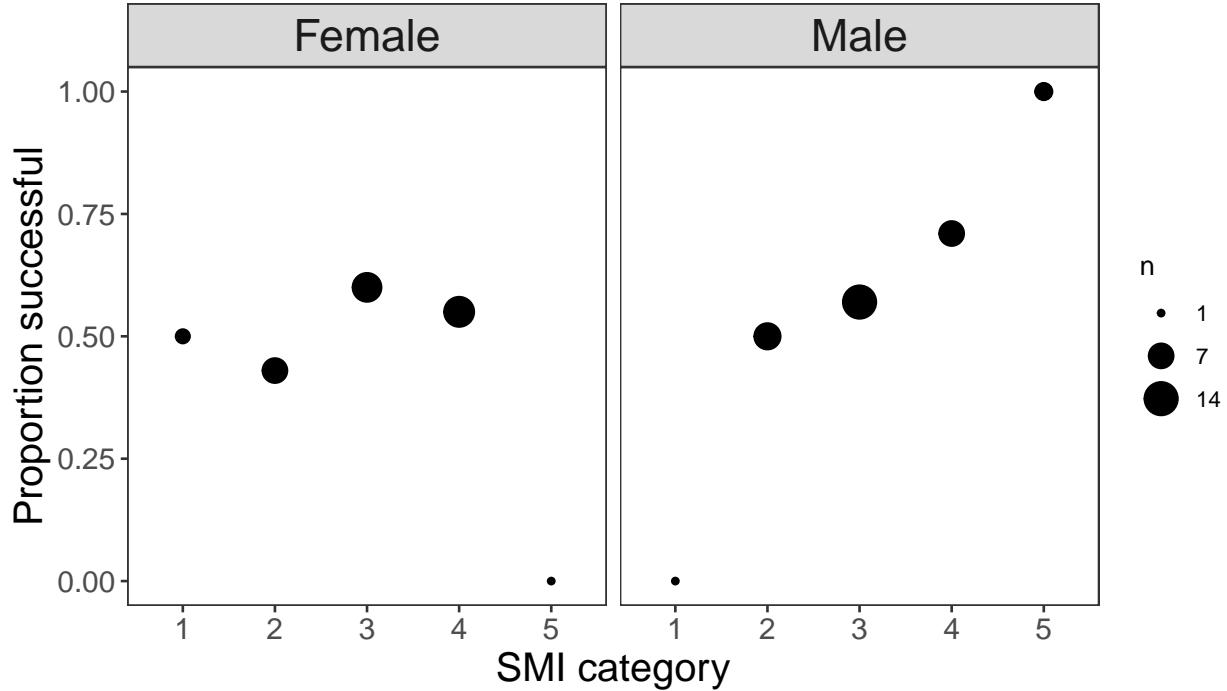
Table 2: Table 2. Results from the logistic mixed-effect regression for 47 females and fixed-effect regression for 21 males to determine whether fat score and scaled mass index (SMI) are correlated. Estimates are presented with the standard error in parentheses. Our sample size was too small to test for a season effect in males.

Parameter	Females		Males	
	Estimate (SE)	p-value	Estimate (SE)	p-value
Intercept	-0.20 (0.74)	0.79	-0.82 (0.64)	0.21
SMI	0.07 (0.30)	0.81	0.46 (0.62)	0.46
Season	0.27 (0.71)	0.70	NA	NA

269 a non-linear relationship between reproductive success and SMI for males or females (Fig. 3). Consequently,
270 we did not include non-linear terms in subsequent models.



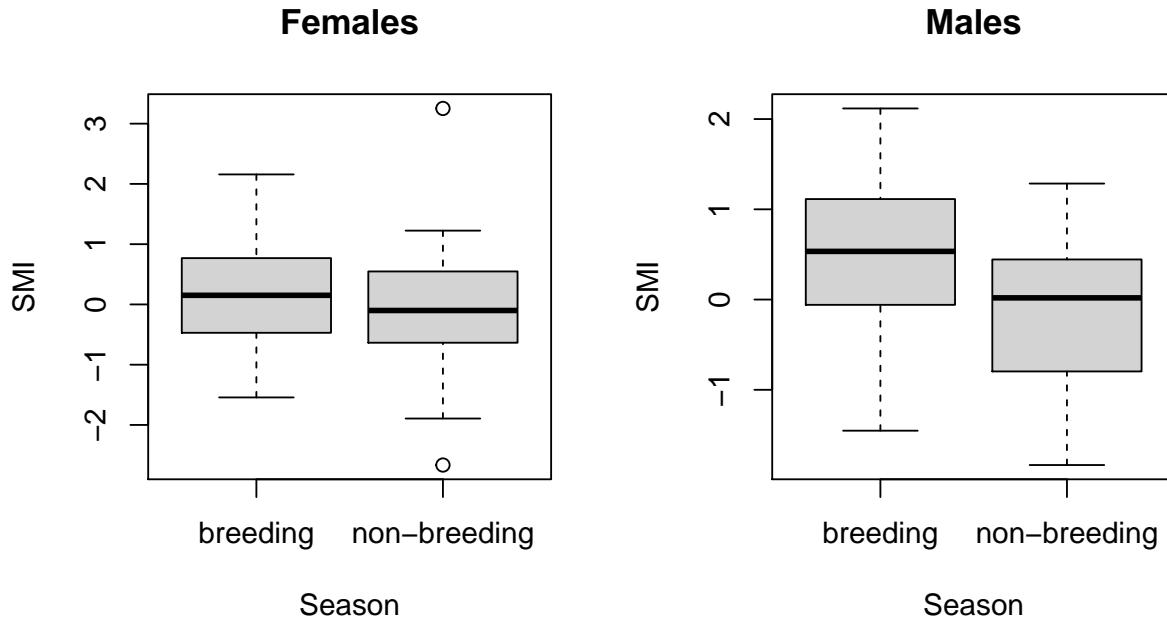
271
272 Figure 2: Frequency histogram of the SMI scores, illustrating the SMI categories, for the 33 males and 31
273 females for which we also had reproductive success data. The mean SMI value is indicated by a red vertical
274 line. We created SMI category bins (indicated with vertical blue lines) in 1 standard deviation increments,
275 centered on the mean. Category 3 indicates the SMI value is close to the population mean value. Categories
276 1 and 2 are individuals that have SMI scores that are low, and moderately low, respectively, compared to
277 the population mean value. Similarly, categories 4 and 5 contain individuals that have SMI scores that are
278 moderately high and high, respectively, compared to the population mean value.



279

280 Figure 3: The proportion of individuals that successfully fledged nests (females: left) or held a territory
 281 (males: right) in low (1), moderately low (2), moderate (3), moderately high (4) and high (5) scaled mass
 282 index (SMI) categories. Dots are sized according to the number (n) of individuals in that category. There is
 283 no evidence of a non-linear relationship.

284 We used linear models to determine whether season would be important to include in our models testing
 285 whether body condition relates to reproductive success. We found that SMI did not differ by season for
 286 females (Estimate (SE): $\beta = -0.30$ (0.26), $p = 0.26$) or males ($\beta = -0.65$ (0.43), $p = 0.15$). Similarly, fat
 287 score for females ($\beta = 0.28$ (0.68), $p = 0.68$) and males ($\beta = 17.08$ (2797.4), $p = 0.99$) did not differ by
 288 season (Fig. 4). Although we note that, as stated above and indicated in the standard error value, we lack
 289 sufficient fat score data from males in the breeding season so these results should be interpreted with caution.
 290 Consequently, we did not include season as an independent variable in our subsequent models testing the
 291 relationship between our body condition proxies and reproductive success.



292

293 Figure 4: Scaled mass index (SMI) was not significantly different between the breeding and non-breeding
294 seasons for either sex.

295 Because fat score and SMI did not correlate, we included both as independent variables in our models testing
296 prediction 2. For both males and females, we found no statistically significant relationships between either
297 proxy of energetic condition and reproductive success (Table 3). Of note, the inconsistent direction of the
298 effects for the parameter estimates further supports that SMI and fat score do not measure the same trait.

299 For females, our SMI parameter estimate of -0.92 (exponentiated to get the log odds = 0.40) indicates that
300 a one unit increase in SMI corresponded to a 60% decrease in the odds a female would fledge an offspring
301 ($p = 0.13$). Whereas an increase from no visible fat to showing some fat corresponded to a 16% increase
302 in the odds a female would fledge an offspring (log odds = 1.16, $p = 0.82$). There was also no evidence of
303 a significant relationship between the ability of a female to produce fledglings and having previously spent
304 time in the aviaries (log odds = 0.25, $p = 0.22$), where the odds that a female would fledge an offspring were
305 75% lower if females spent time in the aviaries.

306 For males, there was also no statistically significant support for a relationship between whether a male
307 defended a territory and SMI (log odds = 3.25, $p = 0.13$). Nevertheless, this relationship may be biologically
308 important because a one unit increase in SMI corresponded to a more than 300% increase in the odds a
309 male will hold a territory containing nests. Fat score was also statistically unrelated to male reproductive
310 success where an increase from showing no visible fat to showing some fat corresponded to a 28% decrease
311 in territory holding (log odds = 0.72, $p = 0.76$). Lastly, we found that those males who spent time in the
312 aviaries were statistically less likely (97% decrease in the odds) to hold a territory compared with males who
313 were never in the aviaries (log odds = 0.03, $p = 0.02$). However, we stress that our sample size was relatively
314 small (20 males), and we did not have a balanced sample because there were no males that did not defend a
315 territory and were never in the aviaries. Additionally, only five males had data from more than one breeding
316 season, which resulted in our model fit being singular because the random effect for bird ID accounted for
317 essentially zero variance. However, we kept ID in the model to account for the repeated samples.

Table 3: Table 3. Results from the logistic regression for 20 females and 20 males to test whether reproductive success relates to condition. Estimates are presented with the standard error in parentheses.

Parameter	Females		Males	
	Estimate (SE)	p-value	Estimate (SE)	p-value
Intercept	-0.02 (0.73)	0.98	3.05 (1.40)	0.03
FatScore	0.15 (1.02)	0.89	-0.33 (1.10)	0.77
SMI	-0.92 (0.61)	0.13	1.18 (0.78)	0.13
Aviary	-1.38 (1.14)	0.23	-3.62 (1.56)	0.02*

318 **Prediction 2: energetic condition and probability of daily nest survival**

319 Logistic regression analyses to determine reproductive success from nests discovered in different stages will
 320 be systematically biased (Shaffer, 2004). Nests discovered at a more progressed stage (i.e., nestling stage
 321 compared to building stage) are statistically more likely to succeed and nests with frequent and prolonged
 322 adult visits (such as those that occur when nests survive longer) are more likely to be discovered. Therefore,
 323 nests that fail early are less likely to be detected (Shaffer, 2004). Consequently, we analyzed female repro-
 324 ductive success using a logistic exposure model (Bolker, 2014), which uses survival analysis to determine the
 325 factors affecting the probability of daily nest survival, while accounting for incomplete nest observations.

326 We found that the probability of daily nest survival was significantly negatively related to SMI (log odds
 327 = 0.50, $p = 0.03$; Table 4), where, for every unit increase in SMI, the odds of daily nest survival decreased
 328 by half. This indicates that a female with a larger SMI (more mass for her structural body size) was less
 329 likely to have her nest survive each day (Fig. 5). There was no statistically significant relationship between
 330 the probability of daily nest survival and fat score (log odds = 2.48, $p = 0.06$), day of the year (log odds =
 331 0.81, $p = 0.16$), or time spent in the aviaries (log odds = 0.63, $p = 0.44$, Table 4). Although not statistically
 332 significant, the effect size for the relationship between fat score and daily nest survival is large (Fig. 5) and
 333 potentially biologically meaningful. The odds of a nest surviving on a given day are almost 2.5 times greater
 334 (248%) for birds with some fat (a score of 1) compared to no fat (a score of 0).

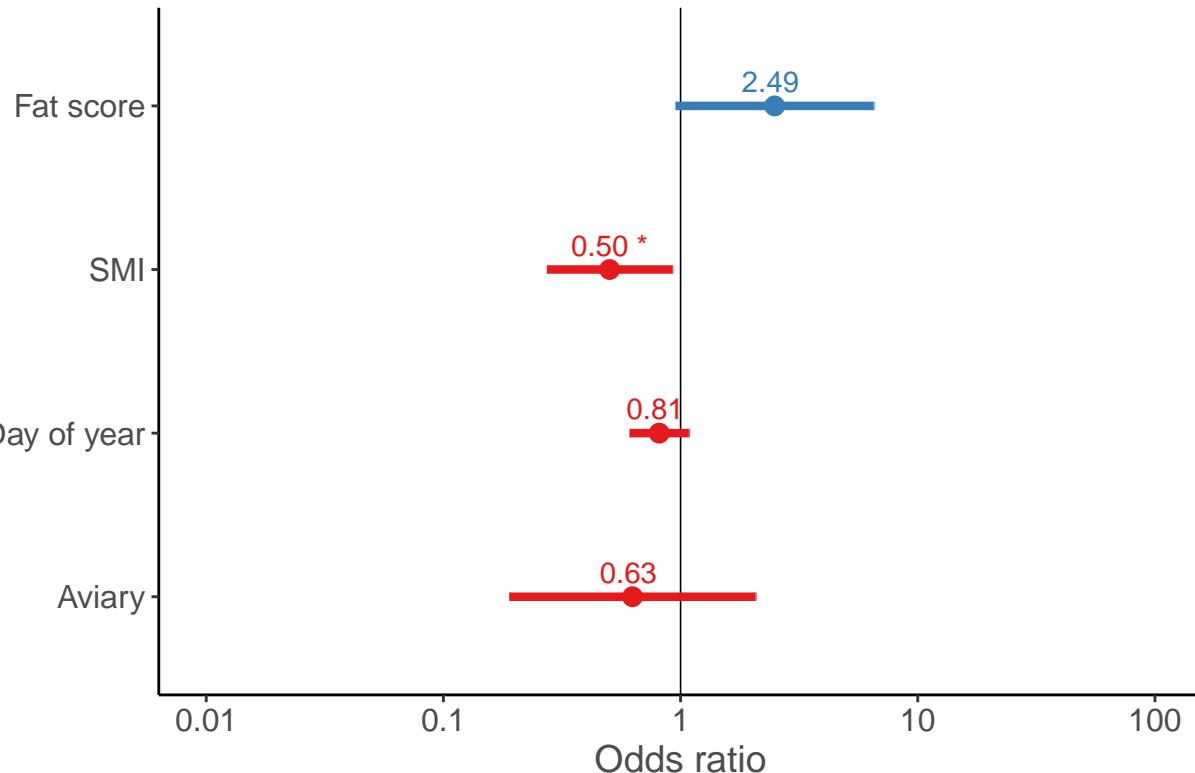
335 \begin{table}

336 \caption{Table 4. Results of the logistic exposure model showing the relationship between the probability
 337 of daily nest survival and scaled mass index (SMI), fat score, the amount of time spent in the aviaries, and
 338 the day of the year. Parameter estimates are presented with the standard error in parentheses. Odds ratios
 339 (OR) represent the exponentiated estimates, and are presented to increase interpretability with 95%
 340 confidence intervals in parentheses.}

Parameter	Estimate (SE)	OR (CI)	p-value
Intercept	1.99 (0.40)	7.32 (3.3-16.0)	<0.001
Fat score	0.91 (0.49)	0.50 (0.27-0.92)	0.06
SMI	-0.69 (0.31)	2.48 (0.95-6.49)	0.03*
Day of year	-0.21 (0.15)	0.63 (0.19-2.10)	0.16
Aviary	-0.47 (0.61)	0.81 (0.60-1.10)	0.44

342 \end{table}

Probability of daily nest survival



343
344 Figure 5: Odds ratios for independent variables affecting the probability of a nest surviving a given day.
345 The dots and corresponding values represent the odds ratio values, and lines represent the confidence
346 intervals around the odds ratio value. The vertical line at $x = 1$ delineates the odds ratio value for no
347 relationship between the estimates and the probability of daily nest survival. The asterisk indicates an
348 odds ratio value that is statistically significant.

DISCUSSION

349
350 Energetic condition is not directly observable, but variation can affect life history characteristics (Barnett
351 et al., 2015; labocha2014which?). Consequently, a large corpus of research attempts to measure
352 energetic condition using various proxy measures (labocha2014which?) and largely assumes that the
353 chosen proxy accurately reflects energetic condition as a singular trait. Although it is often implicitly
354 assumed that all proxy measures for energetic condition reflect the same inherent trait, it is rare for one
355 study to compare multiple proxies. However, if all proxy measures are affected similarly by a singular
356 energetic condition phenotype, then multiple proxy measures should produce correlated results. The aim of
357 the current study was therefore to test the idea that multiple commonly used morphological proxies equally
358 measure energetic condition (by correlating with each other), and that these measures can explain variation
359 in reproductive success.

360 Here we found that two morphological proxies of energetic condition, fat score and SMI, did not correlate
361 with each other in the great-tailed grackle, regardless of whether it was the breeding or non-breeding
362 season. While both proxies are well supported in previous research as measures of energetic condition, our
363 results indicate that they may not be measuring the same trait. This has also been found in studies on
364 bats (McGuire et al., 2018), which are species that similarly experience distinct demands on body structure
365 to facilitate flight. There are several potential reasons why grackle fat score and SMI did not correlate.

366 First, it is possible that we were unable to accurately measure the amount of fat the birds actually stored.
367 In addition to storing fat under their skin, birds may also store fat intraperitoneally (Musacchia, 1953),
368 which would not have been detected with our fat score measure. Second, SMI and fat score may measure
369 different components of energetic condition because variation in mass among grackles could be attributable
370 to muscle or body water content, whereas fat score only accounts for subcutaneous fat (Labocha & Hayes,
371 2012). Research shows that stored fat is the primary source of energy in many taxa (Walsberg, 1988),
372 especially in birds (Blem, 1990; **pond1981physiological?**) because the energy per ounce from fat is much
373 higher than from proteins or carbohydrates (Gessaman, 1999). However, because desert birds, such as the
374 grackles in our investigation, have inconsistent access to water sources, variation in body water content
375 may obscure variation in lipid content. Measuring muscle content often requires destructive methods
376 [i.e. sacrificing the birds; Zhang et al. (2015)] or less objective assessments such as keel prominence or
377 breast muscle shape (Abolins-Abols & Ketterson, 2017; A. Gosler, 1991), which was beyond the scope of
378 the current research program. Third, it is possible that fat score and SMI did not correlate due to
379 temporal variation at a fine scale that we were unable to capture. Although we found no evidence that SMI
380 or fat score varied by season, there is evidence from other studies that avian mass changes with time of day
381 (**nip2018seasonal?**) and stage of breeding (**milenkaya2013variation?**). It was logistically impossible in
382 our project (and in many avian research programs) to capture birds multiple times within a season or at
383 several times per day, therefore temporal variation in data collection could obscure the correlation between
384 these two proxies, if such a correlation exists. However, the stage of breeding is unlikely to introduce
385 additional variance to our study because we did not catch any females that were actively engaged in any
386 stage of the breeding process. Finally, our sample sizes might have been too small to detect an effect, but
387 the effect size for the relationship between fat score and SMI was essentially zero (0.001), therefore it is
388 unlikely that a larger sample size would find a biologically informative relationship between these two
389 proxies.

390 Energetic condition can have a large impact on reproductive success in birds (Drent & Daan, 1980;
391 Montreuil-Spencer, 2017) and in flying mammals (Welbergen, 2011). For example, female chickadees with
392 higher winter fat scores are more likely to lay eggs earlier in the subsequent breeding season, as well as go
393 on to feed those offspring more frequently (Montreuil-Spencer, 2017). Energetic condition is likely a factor
394 in reproductive success in our system because previous research in great-tailed grackles found that larger
395 and heavier males were more likely to hold territories, have more social mates, and sire more offspring
396 (Johnson et al., 2000). Our study additionally considered female morphology and reproductive success,
397 subcutaneous fat, and controlled for the impact of structural body size on mass. However, we found
398 reproductive success, measured as the ability to produce fledglings (females) or to hold a territory
399 containing nests (males), did not significantly correlate with fat score or SMI. Although our results were
400 not statistically significant, in some cases the parameter estimates revealed log-odds that may be large
401 enough to be biologically significant. Notably, a one unit increase in SMI corresponded to a more than
402 300% increase in the odds a male will hold a territory containing nests, but a 60% decrease in the odds a
403 female would fledge an offspring.

404 We additionally used logistic exposure models to determine whether the energetic condition of females
405 related to the probability of daily nest survival. We only included females in this analysis because males
406 were never observed contributing to nest building, incubation, or feeding nestlings in our population and so
407 will not have a direct effect on daily nest survival. We found a negative relationship between female SMI
408 and the likelihood of daily nest survival. This could be due to larger females actually carrying
409 proportionally smaller energetic reserves than their smaller female counterparts (Jacobs et al., 2012), as
410 seen in red-winged blackbirds (Langston et al., 1990). In some species, females with smaller body sizes are
411 able to initiate breeding earlier because they can allocate more resources to reproduction compared to
412 larger individuals that have higher bodily energy demands and therefore fewer excess energetic resources
413 (Barbraud et al., 2000; Langston et al., 1990; Murphy, 1986). This indirectly affects reproductive success
414 because nesting earlier increases the probability of nesting success and multiple nesting attempts (Johnson
415 & Peer, 2001; Perrins, 1970). Yet, in our study we found no relationship between the probability of daily
416 nest survival and day of the year, therefore this is unlikely to explain the negative relationship between
417 SMI and nest survival. Alternatively, it is possible that larger females are unable to build a more concealed
418 nest in the most dense vegetation, or that larger females are unable to build nests in delicate vegetation

419 structure that is more likely to be inaccessible to predators. Moreover, the parameter estimate for the
420 relationship between fat score and the daily probability of nest survival indicates that females with some
421 visible fat are more than twice as likely to have a nest survive a given day. Because the direction of this
422 effect is opposite to the relationship between SMI and nest survival, this is further evidence that these two
423 proxies represent different traits.

424 Great-tailed grackles are an interesting system to study energetic condition and reproductive success
425 because they recently expanded their range into Arizona, where the climate and habitat are distinct from
426 that in Central America where the species originally evolved (Wehtje, 2003). The increase in temperature
427 variation and decrease in available water at our desert study site are both environmental stressors that
428 have previously been found to negatively affect energetic condition (Pendlebury et al., 2004). Although our
429 study spanned only two years, our data are likely representative of reproductive success in this
430 environment because the temperatures during our study were in line with those from the previous three
431 years (Center, 2020). Reproductive success is vital to species persistence and abundance in novel
432 environments (**masponsbehaviour2019?**). Therefore, an understanding of energetic condition and its
433 relationship with reproductive success in grackles outside of their original range could broadly inform
434 conservation research in invasive and non-native species. While reproductive success of certain avian
435 species may be easier to monitor at a more fine scale (i.e. cavity nesters), the predominant measure of
436 reproductive success currently used by avian ecologists is the ability of adults to fledge offspring (since
437 foundational work by Mayfield, 1961) because it is financially and logistically accessible to more
438 researchers. Therefore, we believe our measure of reproductive success in grackles is informative, and that
439 research that spans taxa with diverse reproductive strategies is important for understanding general trends
440 in energetic condition and the appropriate proxies.

441 The results of this study highlight the need to better understand proxy measures of energetic condition,
442 not only in grackles, but for birds in general. Most studies on avian energetic condition only use one proxy
443 variable, but because energetic condition is difficult to measure directly, it is important to compare
444 multiple proxy variables to determine whether the proxy is measuring the intended trait (Block, 1995;
445 Carter et al., 2013). If financially and logically feasible, future research could measure total body
446 composition and relative mass of fat using the relatively new and promising method of quantitative
447 magnetic resonance (Guglielmo et al., 2011), or researchers could incorporate additional physiological
448 methods to measure energetic condition, for example, blood hematocrit levels (Dawson & Bortolotti, 1997;
449 but see Fair et al., 2007). Additionally, studying traits that could relate to variation in energy stores, such
450 as dispersal (Ellers et al., 1998), migratory endurance (Deppe et al., 2015), or survival (Liao et al., 2011)
451 would allow us to disentangle whether morphological proxies like fat score and SMI are poor proxy
452 measures for energetic condition, or whether fat score and SMI do not affect reproductive success but may
453 be associated with other life history characteristics. Because SMI can perform poorly in birds with low
454 lipid mass, future research should also compare several mass by structural body size equations to determine
455 the most appropriate proxy for a specific study system (Jacobs et al., 2012). Lastly, future research would
456 benefit from using logistic exposure models to examine the relationship between energetic condition and
457 reproductive success because these models control for the bias that arises when early nest failures are not
458 detected, which is not possible in logistic regression models, and it is more sensitive to changes in a bird's
459 nest status (Shaffer, 2004).

460 **DETAILED HYPOTHESES AND METHODS FROM THE**
461 **PREREGISTRATION**

462 **HYPOTHESES**

463 We measured two morphological proxy variables of energetic condition and observed reproductive success
464 in grackles to test two hypotheses:

465 **H1 - There is a relationship between two different morphological indices of energetic**
466 **condition: fat score and the scaled mass index.**

467 **Prediction 1:** Fat score and the scaled mass index will be positively correlated. This would indicate that
468 these two indices measure the same trait, and it is likely they both are proxies for fat content.

469 **Prediction 1 alternative 1:** There is a negative correlation between fat score and the scaled mass index.
470 This would indicate that there may be a tradeoff between the two indices where a larger value of the scaled
471 mass index may measure muscle content rather than fat, and individuals with more muscle have less visible
472 fat.

473 **Prediction 1 alternative 2:** There is no correlation between fat score and the scaled mass index. This
474 indicates that these two variables do not measure the same trait. Fat score may not adequately capture a
475 bird's energetic condition because birds may be selected to only store the minimal fat necessary to prevent
476 starvation, while also minimizing the weight gain that would make them easier targets for predators
477 (Barnett et al., 2015). Similarly, the scaled mass index could be heavily influenced by body size, therefore
478 reflecting structural size rather than fat storage (Labocha & Hayes, 2012).

479 **H2 - Energetic condition (as measured by fat score and the scaled mass index) relates to**
480 **reproductive success (measured as a binary variable of whether a female had one or more**
481 **fledglings (1) or not (0), and whether a male defended a territory containing nests (1) or not**
482 **(0)).**

483 **Prediction 2:** Morphological indices of energetic condition (fat score and the scaled mass index) will
484 correlate positively with reproductive success. This would indicate that individuals with more fat, and
485 therefore higher energy reserves, are better able to acquire the resources necessary for reproduction.

486 **Prediction 2 alternative 1:** Morphological indices of energetic condition (fat score and the scaled mass
487 index) will correlate negatively with reproductive success. This indicates that individuals may make trade
488 offs, with some acquiring more food and increasing their energy reserves, and others prioritizing
489 reproductive activities over increasing energy reserves.

490 **Prediction 2 alternative 2:** Morphological indices of energetic condition (fat score and the scaled mass
491 index) do not correlate with reproductive success. This indicates that other, potentially non-morphological,
492 individual characteristics relate to reproductive success (i.e., cognition, nest site selection, breeding
493 experience, predator vigilance, etc.).

494 **METHODS**

495 The methods below are based on the preregistration, with small changes as described in the Deviations
496 from the planned methods section above.

497 **Planned Sample** Great-tailed grackles are caught in the wild in Tempe, Arizona using a variety of
498 methods (e.g., walk-in trap, bownet, mist net). After capture we immediately process birds by attaching
499 colored leg bands in unique combinations for individual identification, conducting morphological
500 measurements of weight, tarsus length, flattened wing length, tail length, skull length, bill length and fat
501 score (the amount of visible fat under the skin in the clavicle and abdomen as in Kaiser, 1993). Most
502 grackles are released after completion of color band marking, measurements, and acquiring a blood sample.

503 A subset of grackles are held in aviaries for up to 6 months for behavioral testing, and then released back
504 to the wild at their location of capture.

505 From March - August, we monitor the behavior of all color-marked grackles to determine their nesting
506 status. We follow females carrying nesting materials to find their nest. We determine whether the male
507 territory owner is color-marked as well. Then we check each nest approximately every day to determine the
508 status based on the female's behavior (building, incubation, feeding nestlings, feeding fledglings, failed).

509 Individuals included in this sample will be those for which we have measures of energetic condition when
510 they were adults. We will not include individuals whose data were collected as juveniles. As of 30 July
511 2019, we have fledgling data for 14 females that exhibited breeding behavior (5 had 1+ fledgling, 9 had no
512 fledglings) and breeding territory status for 10 males (7 territory holders, 3 non-territory holders, 2 not
513 observed so not part of this sample). Therefore, the minimum sample size for H2 will be 24. The minimum
514 sample size for H1 will be 72, because that is how many marked individuals we have biometric data for so
515 far. However, we expect to be able to add to the sample size for both H1 and H2 before the end of this
516 investigation in Tempe, Arizona. *UPDATE Oct 2020: In the second breeding season we had 20 females and*
517 *20 males with reproductive success and energetic condition data.*

518 **Sample size rationale** We will continue to color mark as many grackles as possible, and collect
519 biometric data and fat scores. Our current sample of reproductive success is small because the grackles in
520 Tempe nest in very tall palms, making it difficult to determine nest status. However, we plan to collect
521 additional reproductive success data during the breeding season in summer 2020. *UPDATE Oct 2020: In*
522 *the second breeding season we had 20 females and 20 males with reproductive success and energetic*
523 *condition data.*

524 **Data collection stopping rule** We will stop collecting data for this project in early August 2020 when
525 research at the Tempe, Arizona field site will be finished.

526 **Open materials** Biometric measurement protocol:
527 <https://gitlab.com/corinalogan/the-grackle-project/blob/master/protocolBiometrics.pdf>
528 Nest check protocol:
529 <https://gitlab.com/corinalogan/the-grackle-project/blob/master/protocolNestCheck.pdf>

530 **Open data** All data (Berens et al., 2020) are available at
531 <https://knb.ecoinformatics.org/view/doi:10.5063/F1NZ862D> and at github (the provided code will load
532 these files directly from github).

533 **Randomization and counterbalancing** There is no randomization or counterbalancing in this
534 investigation.

535 **Blinding of conditions during analysis** No blinding is involved in this investigation.

536 **Dependent Variables**

537 **P1: correlation between fat and the scaled mass index**

- 538 1) Fat score [the amount of visible fat under the skin in the clavicle and abdomen reported as a score
539 from 0 (no fat) to 8 (fat completely covers muscles and underside of the bird); Kaiser (1993)] *UPDATE*
540 *Oct 2020: Fat score was heavily 0 skewed with few scores greater than one. To increase model fit we*
541 *used a binomial response variable instead, where 0 is no fat and 1 is some fat observed under the skin.*

542 **P2: energetic condition and reproductive success**

- 543 1) Female had one or more fledglings (yes, no)
544 2) Male held a territory consisting of 1 to 3 clumped palms containing nests (yes, no)

545 **Independent Variables**

546 **P1: correlation between fat and the scaled mass index**

- 547 1) Scaled mass index using measures of body weight and tarsus length or flattened wing length (average
548 of left and right as in Bleeker et al., 2005). We will choose the measure that is most correlated with
549 body weight (Peig & Green, 2009).
550 2) Season (non-breeding [Sep-Feb], breeding [Mar-Aug]). *UPDATE Oct 2020: The Season variable only*
551 *includes 2 males in the breeding season category, thus we do not have a large enough sample to produce*
552 *reliable estimates. We removed the Season variable from the model for males.*
553 3) Random effect: Experimenter (because several different experimenters measure dependent variables on
554 multiple different birds)

555 **P2: energetic condition and reproductive success**

- 556 1) Fat score
 - 557 • Note 1: if the fat score and the scaled mass index are positively correlated, then we will use only
558 fat score in the model for P2. If they are not positively correlated, then we will add the scaled
559 mass index as an independent variable in the P2 analysis
 - 560 • Note 2: if fat score and/or the scaled mass index vary by season (breeding or non-breeding), then
561 we will only use the data from the breeding season to ensure that less time has elapsed between
562 the collection of energetic condition and reproductive success variables
- 563 2) Temporarily held in aviaries for behavioral testing at any point during this study, because this may
564 affect breeding behaviors (yes, no)
- 565 3) Random effect: Year (to determine whether conditions in a given breeding season similarly affected all
566 grackle behavior and nest success)
- 567 4) Random effect: Bird ID (because there may be multiple measures of reproductive success for each bird)

568 **ANALYSIS PLAN**

569 *UPDATE Oct 2020:*

- 570 1) *We realized that the sexual dimorphism of male and female body sizes necessitates separate analyses.*
571 *Therefore, we calculated SMI for males and females separately, ran separate models for each sex for the*
572 *repeatability analysis, P1 and P2.*
- 573 2) *Fat score data were distributed such that the majority of scores were 0, with some 1's and very few*
574 *higher numbers. This made it difficult to fit models using an ordinal regression. The function*
575 *simulateResiduals, which we used to check our data, does not work with data in the ordinal family.*
576 *Consequently, we used logistic regression where the dependent variable FatScore represents no fat (score =*
577 *0), or some fat (score = 1)*
- 578 3) *Despite the data checking which indicated our model was not overdispersed or zero inflated, we could not*
579 *get the fixed effects or random effect to converge using the Bayesian MCMCglmm. We found no*
580 *improvement in model fit by tweaking the priors or iterations/burnin/thin options. Therefore, we fit these*
581 *models using the function glmer, a frequentist framework.*

582 4) The Season variable only includes 2 males in the breeding season category, thus we do not have a large
583 enough sample to produce reliable estimates. We removed the Season variable from the model for males.

584 We will **exclude** data that was collected from the grackles when they were released from the aviaries to
585 avoid any confounds due to their time in the aviary (e.g., perhaps unlimited nutritious food in the aviaries
586 affected their fat score). However, to validate that our measures of structural body size (tarsus length or
587 wing length) are precise and accurate, we will measure twice a subset of grackles brought into aviaries -
588 once when they are initially caught, and again up to 6 months later when we release them. We will then
589 calculate the repeatability of these multiple measures. All other data included in this study will come only
590 from wild-caught grackles (including the birds that were brought into the aviaries on their first capture).
591 When **missing data** occur, the existing data for that individual will be included in the analyses for which
592 their data exist. Analyses will be conducted in R [current version 4.0.5; R Core Team (2017)].

593 **Ability to detect actual effects** To begin to understand what kinds of effect sizes we will be able to
594 detect given our sample size limitations, we used G*Power Faul et al. (2009) to conduct power analyses
595 based on confidence intervals. G*Power uses pre-set drop down menus and we chose the options that were
596 as close to our analysis methods as possible (listed in each analysis below). Note that there were no explicit
597 options for GLMMs, thus the power analyses are only an approximation of the kinds of effect sizes we can
598 detect. We realize that these power analyses are not fully aligned with our study design and that these
599 kinds of analyses are not appropriate for Bayesian statistics (e.g., our MCMCglmm below), however we are
600 unaware of better options at this time. Additionally, it is difficult to run power analyses because it is
601 unclear what kinds of effect sizes we should expect due to the lack of data on this species for these
602 particular research questions.

603 **Data checking** The data will be checked for overdispersion, underdispersion, zero-inflation, and
604 heteroscedasticity with the DHARMA R package (Hartig, 2019) following methods by Hartig.

605 *P1 analysis: correlation between fat and the scaled mass index*

606 We will calculate the scaled mass index as described by Peig & Green (2009) using either tarsus or
607 flattened wing length - whichever measure is most correlated with body weight (Peig & Green, 2009).

608 We use a Generalized Linear Mixed Model (GLMM; MCMCglmm function, MCMCglmm package;
609 (Hadfield 2010)) with an ordinal distribution (for categorical variables in MCMCglmm) and probit link
610 using 130,000 iterations with a thinning interval of 10, a burnin of 30,000, and minimal priors (V=1, nu=0)
611 (Hadfield, 2014). We will ensure the GLMM shows acceptable convergence [lag time autocorrelation values
612 <0.01; Hadfield (2010)], and adjust parameters if necessary to meet this criterion. We will determine
613 whether an independent variable had an effect or not using the Estimate in the full model.

614 Where we have multiple measures of tarsus or flattened wing length, we will check that our measurements
615 are repeatable using the rptR package (Stoffel et al., 2017).

616 To roughly estimate our ability to detect actual effects (because these power analyses are designed for
617 frequentist statistics, not Bayesian statistics), we ran a power analysis in G*Power with the following
618 settings: test family=F tests, statistical test=linear multiple regression: Fixed model (R^2 deviation from
619 zero), type of power analysis=a priori, alpha error probability=0.05. We changed the power and the effect
620 size until we reached an output that we project our sample size will be (n=90). The number of predictor
621 variables was restricted to only the fixed effects because this test was not designed for mixed models. The
622 protocol of the power analysis is here:

623 *Input:*

624 Effect size $f^2 = 0.15$

625 err prob = 0.05

626 Power (1- err prob) = 0.86

627 Number of predictors = 3

This means that, with a sample size of 89, we would have an 86% chance of detecting a medium effect (approximated at $f^2=0.15$ by Cohen, 1988).

637 *code shown in .rmd*

638 *P2 analysis: energetic condition and reproductive success*

To model the effect of energetic condition on reproductive success, we will use two types of logistic mixed-effect models. Both types are supported in the literature, but are slightly different in the way in which the link function is specified. First, we will model reproductive success using a generalized linear mixed model framework with a logit link function (i.e. Milenkaya et al., 2015). We will also use a logistic exposure model that has a link function which accounts for the time interval between nest checks when estimating the probability of daily nest survival (Bolker, 2014; Shaffer, 2004). If fat score and the scaled mass index are positively correlated in P1, then we will use only fat score as the independent variable in this GLMM. If they are not positively correlated, we will include both as independent variables.

647 Previous research found a non-linear relationship between reproductive success and energetic condition
648 variables (Milenkaya et al., 2015). To check whether this is occurring in our data, we will first plot our raw
649 data to determine if we need to include a non-linear energetic condition independent variable into our
650 model (i.e. FatScore²). Our dependent variable is binary, so to more clearly see the trends in the data, on
651 the x-axis we will bin our energetic condition scores into 5 categories based on standard deviations (sd)
652 around the mean (low = < 2 sd, moderately low = -2 sd to -1 sd, moderate = -1 sd to +1 sd, moderately
653 high = +1 sd to +2 sd, high = > 2 sd). Then on the y-axis we will use the proportion of individuals in
654 each category that had successful nests. *UPDATE Oct 2020: Because most individuals fell within the*
655 *medium category when we grouped data using 1 standard deviation around the mean, we switched to using*
656 *half standard deviation increments around the mean.*

657 A power analysis was conducted as above for P1 and the protocol reported here:

658 *Input:*

Effect size $f^2 = 0.15$

660 err prob = 0.05

661 Power (1- err prob) = 0.90

662 Number of predictors = 2

663 *Output:*

664 Noncentrality parameter = 13.2000000

665 Critical F = 3.1038387

666 Numerator df = 2

Denominator df = 85

668 Total sample size = 88

Actual power = 0.9020264

670 This means that, with a sample size of 88, we would have a 90% chance of detecting a medium effect
671 (approximated at $f^2=0.15$ by Cohen, 1988).

672 *code shown in .rmd*

673 **Do energetic condition variables vary by season?** *code shown in .rmd*

674 **Does energetic condition relate to reproductive success?** *code shown in .rmd*

675 **Does female energetic condition relate to the probability of daily nest survival?** Our measure
676 of female nest success could be systematically biased against nests that failed early (Shaffer, 2004).
677 Consequently, we also analyzed female reproductive success using a logistic exposure model. This type of
678 model determines the factors affecting daily nest survival probability.

679 *code shown in .rmd*

680 ETHICS

681 This research is carried out in accordance with permits from the:

- 682 1) US Fish and Wildlife Service (scientific collecting permit number MB76700A-0,1,2)
- 683 2) US Geological Survey Bird Banding Laboratory (federal bird banding permit number 23872)
- 684 3) Arizona Game and Fish Department (scientific collecting license number SP594338 [2017], SP606267
685 [2018], and SP639866 [2019])
- 686 4) Institutional Animal Care and Use Committee at Arizona State University (protocol number 17-1594R)

687 AUTHOR CONTRIBUTIONS

688 **Berens:** Hypothesis development, data collection, revising/editing.

689 **Logan:** Study design, write up, revising/editing, materials/funding.

690 **Folsom:** Data collection, revising/editing.

691 **Sevchik:** Data collection, revising/editing.

692 **Bergeron:** Data collection, revising/editing.

693 **McCune:** Hypothesis development, data collection, data analysis, write up, revising/editing.

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REFERENCES

- 704 Abolins-Abols, M., & Ketterson, E. D. (2017). Condition explains individual variation in mobbing
 705 behavior. *Ethology*, 123(8), 495–502.
- 706 Aubret, F., Bonnet, X., Shine, R., & Lourdais, O. (2002). Fat is sexy for females but not males: The
 707 influence of body reserves on reproduction in snakes (*vipera aspis*). *Hormones and Behavior*, 42(2),
 708 135–147.
- 709 Barbraud, C., Lormée, H., & LeNevé, A. (2000). Body size and determinants of laying date variation in the
 710 snow petrel *pagodroma nivea*. *Journal of Avian Biology*, 31(3), 295–302.
- 711 Barnett, C. A., Suzuki, T. N., Sakaluk, S. K., & Thompson, C. F. (2015). Mass-based condition measures
 712 and their relationship with fitness: In what condition is condition? *Journal of Zoology*, 296(1), 1–5.
- 713 Barry, K. L., & Wilder, S. M. (2013). Macronutrient intake affects reproduction of a predatory insect.
 714 *Oikos*, 122(7), 1058–1064.
- 715 Berens, J., Logan, C., Folsom, M., Sevchik, A., Bergeron, L., & McCune, K. (2020). Validating
 716 morphological condition indices and their relationship with reproductive success in great-tailed grackles.
 717 *Knowledge Network for Biocomplexity, Data package*. <https://doi.org/10.5063/7P8WSM>
- 718 Bleeker, M., Kingma, S. A., Szentirmai, I., Székely, T., & Komdeur, J. (2005). Body condition and clutch
 719 desertion in penduline tit *remiz pendulinus*. *Behaviour*, 142, 1465–1478.
- 720 Blem, C. (1990). Avian energy storage. *Curr Ornithol*, 7, 59–113.
- 721 Block, J. (1995). A contrarian view of the five-factor approach to personality description. *Psychological
 722 Bulletin*, 117(2), 187.
- 723 Bolker, B. (2014). Logistic regression, accounting for differences in exposure. *Version 09.30. 2014. RPubs*.
- 724 Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlishaw, G., & Heinsohn, R. (2013). Animal personality:
 725 What are behavioural ecologists measuring? *Biological Reviews*, 88(2), 465–475.
- 726 Center, N. N. C. D. (2020). *Climate data online global summary of the month*.
 727 <https://www.ncdc.noaa.gov/cdo-web/>
- 728 Champagnon, J., Guillemain, M., Elmberg, J., Massez, G., Cavallo, F., & Gauthier-Clerc, M. (2012). Low
 729 survival after release into the wild: Assessing “the burden of captivity” on mallard physiology and
 730 behaviour. *European Journal of Wildlife Research*, 58(1), 255–267.
- 731 Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* 2nd edn. Erlbaum Associates,
 732 Hillsdale.
- 733 Cornelius Ruhs, E., Vézina, F., & Karasov, W. H. (2019). Physiological and immune responses of
 734 free-living temperate birds provided a gradient of food supplementation. *Physiological and Biochemical
 735 Zoology*, 92(1), 106–114.
- 736 Costa, G. (2012). *Behavioural adaptations of desert animals*. Springer Science & Business Media.
- 737 Dawson, R. D., & Bortolotti, G. R. (1997). Are avian hematocrits indicative of condition? American
 738 kestrels as a model. *The Journal of Wildlife Management*, 1297–1306.

- 739 Delciellos, A. C., Barros, C. dos S. de, Prevedello, J. A., Ferreira, M. S., Cerqueira, R., & Vieira, M. V.
 740 (2018). Habitat fragmentation affects individual condition: Evidence from small mammals of the
 741 brazilian atlantic forest. *Journal of Mammalogy*, 99(4), 936–945.
- 742 Deppe, J. L., Ward, M. P., Bolus, R. T., Diehl, R. H., Celis-Murillo, A., Zenzal, T. J., Moore, F. R.,
 743 Benson, T. J., Smolinsky, J. A., Schofield, L. N., & others. (2015). Fat, weather, and date affect
 744 migratory songbirds' departure decisions, routes, and time it takes to cross the gulf of mexico.
 745 *Proceedings of the National Academy of Sciences*, 112(46), E6331–E6338.
- 746 Drent, R., & Daan, S. (1980). The prudent parent: Energetic adjustments in avian breeding 1. *Ardea*,
 747 55(1–2), 225–252.
- 748 Ellers, J., Van Alphen, J. J., & Sevenster, J. G. (1998). A field study of size–fitness relationships in the
 749 parasitoid asobara tabida. *Journal of Animal Ecology*, 67(2), 318–324.
- 750 English, M. D., Robertson, G. J., Peck, L. E., Pirie-Hay, D., Roul, S., & Mallory, M. L. (2018). Body
 751 condition of american black ducks (*anas rubripes*) wintering in atlantic canada using carcass
 752 composition and a scaled mass index. *Canadian Journal of Zoology*, 96(10), 1137–1144.
- 753 Erciyas, K., Gürsoy, A., Özsemir, A., & Barış, Y. (2010). Body mass and fat score changes in recaptured
 754 birds during the autumn migration at the cernek ringing station in turkey. *The Ring*, 32(1-2), 3–15.
- 755 Fair, J., Whitaker, S., & Pearson, B. (2007). Sources of variation in haematocrit in birds. *Ibis*, 149(3),
 756 535–552.
- 757 Faul, F., Erdfelder, E., Buchner, A., & Lang, A.-G. (2009). Statistical power analyses using g* power 3.1:
 758 Tests for correlation and regression analyses. *Behavior Research Methods*, 41(4), 1149–1160.
- 759 Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G* power 3: A flexible statistical power
 760 analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, 39(2),
 761 175–191.
- 762 Gessaman, J. (1999). Evaluation of some nonlethal methods of estimating avian body fat and lean mass.
 763 *Proceedings of the 22nd International Ornithological Congress*. University of Natal Press, Durban, 2–16.
- 764 Gill, F. (1995). *Ornithology*. Freeman; Company.
- 765 Gosler, A. (1991). On the use of greater covert moult and pectoral muscle as measures of condition in
 766 passerines with data for the great tit *parus major*. *Bird Study*, 38(1), 1–9.
- 767 Gosler, A. G., Greenwood, J. J., & Perrins, C. (1995). Predation risk and the cost of being fat. *Nature*,
 768 377(6550), 621–623.
- 769 Guglielmo, C. G., McGuire, L. P., Gerson, A. R., & Seewagen, C. L. (2011). Simple, rapid, and
 770 non-invasive measurement of fat, lean, and total water masses of live birds using quantitative magnetic
 771 resonance. *Journal of Ornithology*, 152(1), 75.
- 772 Haas, C. A. (1998). Effects of prior nesting success on site fidelity and breeding dispersal: An experimental
 773 approach. *The Auk*, 115(4), 929–936.
- 774 Hadfield, J. (2010). MCMC methods for multi-response generalized linear mixed models: The
 775 MCMCglmm r package. *Journal of Statistical Software*, 33(2), 1–22.
- 776 Hadfield, J. (2014). *MCMCglmm course notes*.
 777 <http://cran.r-project.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf>
- 778 Hartig, F. (2019). *DHARMA: Residual diagnostics for hierarchical (multi-level / mixed) regression models*.
 779 <http://florianhartig.github.io/DHARMA/>
- 780 Heidinger, I. M. M., Hein, S., & Bonte, D. (2010). Patch connectivity and sand dynamics affect
 781 dispersal-related morphology of the blue-winged grasshopper *oedipoda caerulescens* in coastal grey
 782 dunes. *Insect Conservation and Diversity*, 3(3), 205–212.

- 783 Henderson, L., Evans, N., Heidinger, B., Herborn, K., & Arnold, K. (2017). Do glucocorticoids predict
784 fitness? Linking environmental conditions, corticosterone and reproductive success in the blue tit,
785 *cyanistes caeruleus*. *Royal Society Open Science*, 4(10), 170875.
- 786 Huxley, J. (1932). *Problems of relative growth*. Dover Publications.
- 787 Jacobs, S. R., Elliott, K., Guigueno, M. F., Gaston, A. J., Redman, P., Speakman, J. R., & Weber, J.-M.
788 (2012). Determining seabird body condition using nonlethal measures. *Physiological and Biochemical
789 Zoology*, 85(1), 85–95.
- 790 Johnson, K., DuVal, E., Kielt, M., & Hughes, C. (2000). Male mating strategies and the mating system of
791 great-tailed grackles. *Behavioral Ecology*, 11(2), 132–141.
- 792 Johnson, K., & Peer, B. D. (2001). *Great-tailed grackle: Quiscalus mexicanus*. Birds of North America,
793 Incorporated.
- 794 Kaiser, A. (1993). A new multi-category classification of subcutaneous fat deposits of songbirds (una nueva
795 clasificación, con multi-categorías, para los depósitos de grasa en aves canoras). *Journal of Field
796 Ornithology*, 246–255.
- 797 Kelly, C. D., Tawes, B. R., & Worthington, A. M. (2014). Evaluating indices of body condition in two
798 cricket species. *Ecology and Evolution*, 4(23), 4476–4487.
- 799 Labocha, M. K., & Hayes, J. P. (2012). Morphometric indices of body condition in birds: A review.
800 *Journal of Ornithology*, 153(1), 1–22.
- 801 Labocha, M. K., Schutz, H., & Hayes, J. P. (2014). Which body condition index is best? *Oikos*, 123(1),
802 111–119.
- 803 Langston, N. E., Freeman, S., Rohwer, S., & Gori, D. (1990). The evolution of female body size in
804 red-winged blackbirds: The effects of timing of breeding, social competition, and reproductive
805 energetics. *Evolution*, 44(7), 1764–1779.
- 806 Liao, C.-Y., Rikke, B. A., Johnson, T. E., Gelfond, J. A., Diaz, V., & Nelson, J. F. (2011). Fat maintenance
807 is a predictor of the murine lifespan response to dietary restriction. *Aging Cell*, 10(4), 629–639.
- 808 Maceda-Veiga, A., Green, A. J., & De Sostoa, A. (2014). Scaled body-mass index shows how habitat
809 quality influences the condition of four fish taxa in north-eastern spain and provides a novel indicator of
810 ecosystem health. *Freshwater Biology*, 59(6), 1145–1160.
- 811 Mayfield, H. (1961). Nesting success calculated from exposure. *The Wilson Bulletin*, 255–261.
- 812 McGuire, L. P., Kelly, L. A., Baloun, D. E., Boyle, W. A., Cheng, T. L., Clerc, J., Fuller, N. W., Gerson,
813 A. R., Jonasson, K. A., Rogers, E. J., & others. (2018). Common condition indices are no more
814 effective than body mass for estimating fat stores in insectivorous bats. *Journal of Mammalogy*, 99(5),
815 1065–1071.
- 816 McNamara, J. M., Barta, Z., Houston, A. I., & Race, P. (2005). A theoretical investigation of the effect of
817 predators on foraging behaviour and energy reserves. *Proceedings of the Royal Society B: Biological
818 Sciences*, 272(1566), 929–934.
- 819 Merilä, J., & Svensson, E. (1997). Are fat reserves in migratory birds affected by condition in early life?
820 *Journal of Avian Biology*, 279–286.
- 821 Milenkaya, O., Catlin, D. H., Legge, S., & Walters, J. R. (2015). Body condition indices predict
822 reproductive success but not survival in a sedentary, tropical bird. *PLoS One*, 10(8), e0136582.
- 823 Montreuil-Spencer, C. (2017). *Relationships between winter energetic condition and reproductive
824 investment in a wild bird* [PhD thesis].
- 825 Murphy, M. T. (1986). Body size and condition, timing of breeding, and aspects of egg production in
826 eastern kingbirds. *The Auk*, 103(3), 465–476.
- 827 Musacchia, X. (1953). A study of the lipids in arctic migratory birds. *The Condor*, 55(6), 305–312.

- 828 Nakagawa, S., & Schielzeth, H. (2010). Repeatability for gaussian and non-gaussian data: A practical
829 guide for biologists. *Biological Reviews*, 85(4), 935–956.
- 830 Peig, J., & Green, A. J. (2009). New perspectives for estimating body condition from mass/length data:
831 The scaled mass index as an alternative method. *Oikos*, 118(12), 1883–1891.
- 832 Pendlebury, C., MacLeod, M., & Bryant, D. (2004). Variation in temperature increases the cost of living in
833 birds. *Journal of Experimental Biology*, 207(12), 2065–2070.
- 834 Perrins, C. (1970). The timing of birds ‘breeding seasons. *Ibis*, 112(2), 242–255.
- 835 R Core Team. (2017). *R: A language and environment for statistical computing*. R Foundation for
836 Statistical Computing. <https://www.R-project.org>
- 837 Shaffer, T. L. (2004). A unified approach to analyzing nest success. *The Auk*, 121(2), 526–540.
- 838 Stevenson, R., & Woods Jr, W. A. (2006). Condition indices for conservation: New uses for evolving tools.
839 *Integrative and Comparative Biology*, 46(6), 1169–1190.
- 840 Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: Repeatability estimation and variance
841 decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 8(11),
842 1639–1644.
- 843 Walsberg, G. E. (1988). Evaluation of a nondestructive method for determining fat stores in small birds
844 and mammals. *Physiological Zoology*, 61(2), 153–159.
- 845 Warnock, N., & Bishop, M. A. (1998). Spring stopover ecology of migrant western sandpipers. *The
846 Condor*, 100(3), 456–467.
- 847 Wehtje, W. (2003). The range expansion of the great-tailed grackle (*Quiscalus mexicanus gmelini*) in north
848 america since 1880. *Journal of Biogeography*, 30(10), 1593–1607.
- 849 Welbergen, J. A. (2011). Fit females and fat polygynous males: Seasonal body mass changes in the
850 grey-headed flying fox. *Oecologia*, 165(3), 629–637.
- 851 Wilder, S. M., Raubenheimer, D., & Simpson, S. J. (2016). Moving beyond body condition indices as an
852 estimate of fitness in ecological and evolutionary studies. *Functional Ecology*, 30(1), 108–115.
- 853 Zhang, Y., Eyster, K., Liu, J.-S., & Swanson, D. L. (2015). Cross-training in birds: Cold and exercise
854 training produce similar changes in maximal metabolic output, muscle masses and myostatin expression
855 in house sparrows (*Passer domesticus*). *Journal of Experimental Biology*, 218(14), 2190–2200.