1    Investigating a trade-off between the quality of nest grown feathers and pace of

2    development in an altricial bird.

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18    **ABSTRACT**

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20         Life history theory provides a framework for understanding how trade-offs

21    generate negative trait associations. Among nestling birds, developmental rate, risk of

22    predation, and lifespan covary, but some associations are only found within species

23    while others are only observed between species. A recent comparative study suggests

24    that allocation trade-offs may be alleviated by disinvestment in ephemeral traits, such

25    as nest-grown feathers, that are quickly replaced. However, direct resource allocation

26    trade-offs cannot be inferred from inter-specific trait-associations without

27    complementary intra-specific studies. Here, we asked whether there is evidence for a

28    within-species allocation trade-off between feather quality and developmental speed in

29    tree swallows (*Tachycineta bicolor*). Consistent with the idea that ephemeral traits are

30    deprioritized, nest-grown feathers had lower barb density than adult feathers.

31    However, despite substantial variation in fledging age among nestlings, there was no

32    evidence for a negative association between developmental pace and feather quality.

33    Furthermore, accounting for differences in resource availability by considering

34    provisioning rate and a nest predation treatment did not reveal a trade-off that was

35    masked by variation in resources. Our results are most consistent with the idea that the

36    inter-specific association between development and feather quality arises from adaptive

37    specialization, rather than from a direct allocation trade-off.

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39    *Keywords:* plumage quality, trade-off, developmental time, resource allocation

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42    **INTRODUCTION**

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44        Understanding the co-expression patterns of ecologically important traits is a

45  primary goal of evolutionary ecology and life history evolution (Stearns 1992; Roff 2002;

46  Agrawal 2020). Theory suggests that trade-offs between different traits should be most

47  pronounced during periods of high demand or low resource availability (van

48  Noordwijk and de Jong 1986). For altricial birds, the early growth period presents a

49  particularly severe challenge, as nestlings must grow quickly to escape the nest and the

50  associated risk of predation while simultaneously developing morphological traits and

51  physiological systems that are critical for lifetime performance (Martin 1995; Martin et

52  al. 2011). Within species, growth rate and time spent in the nest are related to nest

53  predation rates and reduced adult survival, suggesting a resource allocation trade-off

54  (Metcalfe and Monaghan 2003; LaManna and Martin 2016). However, while the

55  relationship between growth and predation risk is also seen in inter-specific

56  comparisons (Bosque and Bosque 1995; Martin 1995; Remeŝ and Martin 2002), the

57  relationship between growth rate and adult survival is not (Martin et al. 2015).

58  Understanding when negative trait associations are similar at different scales (e.g.,

59  within- versus between-species) and when those associations occur only at one level,

60  will require a better understanding of the mechanisms that generate these patterns.

61        Comparative studies of trade-offs have the advantage of being potentially less

62  influenced by the masking effects of between-individual variation in resource

63  availability (van Noordwijk and de Jong 1986; Reznick et al. 2000). However, they also

64  have the disadvantage that the same pattern of inter-specific trait associations can arise

65  by more than one generating mechanism (Figure 1); without complementary intra-

66  specific studies it will often be impossible to distinguish between these possibilities. For

67  example, an apparent trade-off (negative trait association) between species could arise

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68    because of a strong constraint resulting in a resource allocation trade-off within each

69    individual of each species, or it could arise due to a "strategic trade-off" resulting from

70    niche specialization or biotic interactions that differ between species. While both of

71    these patterns are often referred to as trade-offs, the mechanism differs (Agrawal 2020).

72    Understanding how and when trait associations translate across scales—from

73    individuals to populations to species—requires both strengthening the scale-dependent

74    predictive framework (reviewed in Agrawal, 2020) and empirical studies that

75    investigate the same trait associations at different scales.

76        In the case of nestling growth, Callan et al. (2019) hypothesized that the apparent

77    difference between the growth and adult survival trade-off in comparative versus single

78    species studies might be explained by differences in relative investment in ephemeral

79    traits that contribute relatively little to fitness. They focus in particular on the growth of

80    body feathers in the nest because these feathers are often of lower quality than adult

81    feathers and are replaced shortly after fledging (Rohwer et al. 2005; Butler et al. 2008).

82    Thus, these nest-grown feathers may be relatively unimportant for long term

83    performance and disinvesting in their quality in the nest could ameliorate an otherwise

84    more severe trade-off between growth rate and adult survival. However, Agrawal

85    (2020) argues that negative trait associations often arise between species through

86    adaptive specialization and natural selection even when no direct resource allocation

87    link exists between the two traits; thus inter-specific analysis alone is insufficient to

88    infer within-species trade-offs. In fact, the inter-specific pattern described in Callan et al.

89    (2019) could result from four distinct intra-specific patterns that imply different

90    mechanisms operating at the within-species level (illustrated in Figure 1).

91        Here, we replicated the comparative analyses of Callan et al. (2019*a*) within a

92    single species—tree swallows (*Tachycineta bicolor*)—to ask whether the correlation

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93      between developmental speed and feather quality arises due to a direct resource

94      allocation trade-off. Tree swallow nestlings vary considerably in the exact age of

95      fledging and in the pace of development, even under similar ecological conditions.

96      While nest-grown feathers are quickly molted after fledging, they likely play an

97      important role in thermoregulation for young nestlings, and thermoregulation alone

98      can account for up to a third of all metabolized energy in altricial young (Weathers and

99      Sullivan 1991). Cold snaps are also a major source of mortality in developing tree

100     swallows (Shipley et al. 2020) and feather development in the nest may be particularly

101     important in this species. On the other hand, although predation rates are relatively low

102     in tree swallows, predation is still a common source of mortality, including in our study

103     population (Winkler et al. 2020*a*), and longer development periods lead to an extended

104     period of risk. Thus, it is plausible that a direct resource allocation trade-off could

105     operate on investment in nest-grown feather quality for thermoregulation versus rapid

106     development for predator avoidance.

107         To test for this trade-off, we measured both feather quality and indicators of

108     developmental rate (mass, wing length, and structural size) in nestling tree swallows.

109     We used a network of automated sensors at each nest to determine the exact age of

110     fledging for each individual nestling. When working at the intraspecific level, resource

111     allocation trade-offs can be masked by variation in resource acquisition (see discussion

112     above; van Noordwijk and de Jong 1986). Thus, we accounted for variation in resource

113     availability in several ways. First, the sensor network provided detailed data on

114     parental provisioning to ask whether the detectability of trade-offs depended on food

115     availability. Second, we took advantage of ongoing experiments in this population that

116     involved experimental predator treatments, which allowed us to assess whether any

117     trade-offs between development and feather quality differed in an environment with

118    high perceived predation risk. Finally, we cross-fostered eggs at each nest before

119    hatching. While cross-fostering was not strictly necessary to test for the trade-off of

120    interest, the ability to flexibly adjust resources during development is a prerequisite of

121    the within-individual trade-off being studied, and cross-fostering allowed us to

122    estimate the extent to which genetic versus environmental effects influenced feather

123    quality.

124         If the inter-specific association between developmental speed and feather quality

125    results from a resource allocation trade-off that plays out within individuals of each

126    species, then we expected to find an intra-specific association within tree swallows that

127    paralleled the results of Callan et al.'s (2019) comparative study. Specifically, we

128    expected that individual nestlings that fledged at younger ages would pay a cost, as

129    indicated by lower quality nest-grown feathers. Furthermore, we predicted that this

130    trade-off would be most pronounced in nests with either experimentally increased

131    perceived predation pressure or naturally lower parental provisioning rates (*sensu* van

132    Noordwijk & de Jong, 1986). Alternatively, if the inter-specific association arises due to

133    strategic differences in developmental programs as a result of natural selection—rather

134    than a strict resource allocation trade-off—then we would not expect to find a direct

135    trade-off between developmental rate and feather quality within tree swallows.

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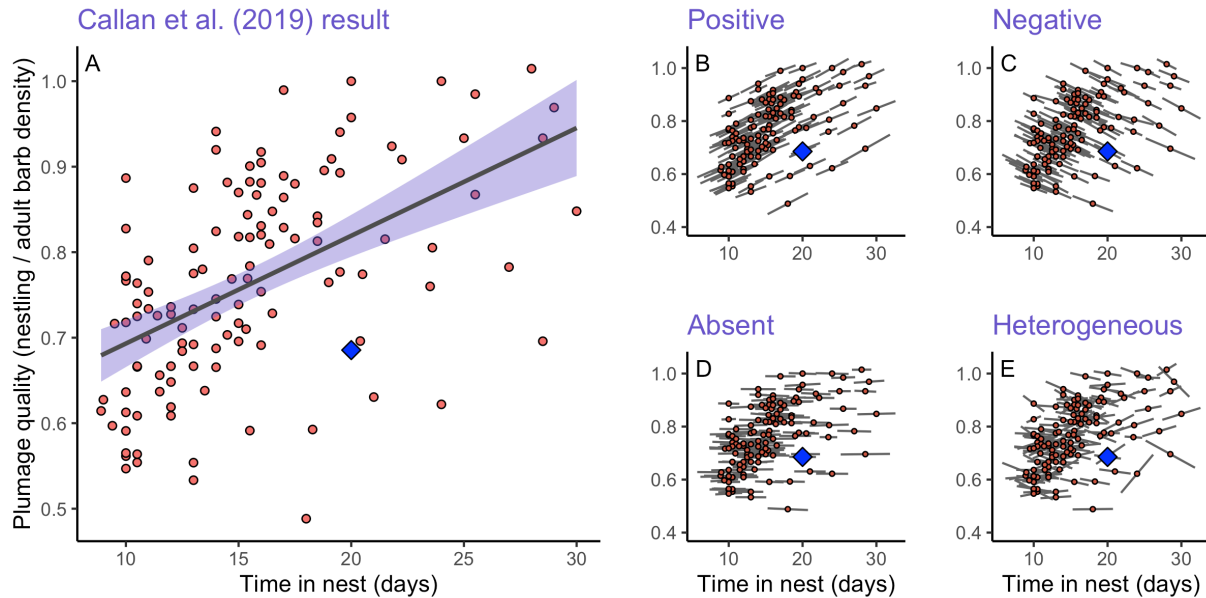
**Figure 1.** The between-species tradeoff reported in Callan et al 2019 (panel A; redrawn from publicly archived data: Callan et al. 2019*b*) could result from four distinct underlying within species patterns. We used the same mean species points to illustrate hypothetical within species patterns that are positive (B), negative (C), absent (D), or heterogeneous between species (E). These relationships are intended to illustrate the scaling problem when moving from between to within-species inferences and are not based on any biological information about each species shown. In all panels, the value for tree swallows is the large blue diamond.

**METHODS**

*General Field Methods*

We studied tree swallows breeding near Ithaca, New York, USA in 2018 and 2019 (42.503° N, 76.437° W). During each breeding season (May to July), we monitored every nest at the field sites following established protocols for this long-studied tree swallow population (Winkler et al. 2020*b*). Briefly, each nest box was checked every other day early in the season to determine clutch initiation and clutch completion date to within one day. Around the expected hatching date, we checked boxes every day so that an exact hatch date could be recorded.

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155    Adults at each nest were captured 1-3 times during incubation or provisioning.

156    At the first capture for each adult, we took morphological measurements (mass,

157    flattened wing-chord length, and head + bill length), a blood sample for paternity, and

158    6-8 feathers from two body regions—the center of the white breast and the rump just

159    above the tail—to measure barb density (see below). For individuals that were not

160    already banded from a previous year, we applied an aluminum USGS band and a

161    passive integrated transponder (PIT) tag that encoded a unique 10-digit hexadecimal

162    string.

163    Most nests in these two years were part of an experiment that involved targeted

164    manipulations of adult females. While these manipulations were not designed to have

165    any direct effects on nestlings, they may have had indirect effects on nestling resource

166    availability or developmental environment by changing adult provisioning rates,

167    reproductive investment, or antipredator behavior. Therefore, we conducted post-hoc

168    comparisons between nestlings raised in different treatment groups to ask whether

169    adult treatments influenced nestling trade-offs between development and feather

170    quality via changes in resource availability or perceived environmental risk.

171    The most relevant treatment for the purposes of this study involved 2-3

172    simulated predation events using a taxidermied mink (*Neovison vision*), a common

173    predator of tree swallows, that occurred shortly before (2018) or shortly after (2019)

174    hatching. In 2018, some females were subject to a challenge treatment that involved a

175    handicapping manipulation where 3 feathers on each wing were bound together with a

176    small plastic zip tie for approximately 5 days late in incubation, thereby reducing flight

177    efficiency and female foraging ability (described in Taff et al. 2019*b*). Finally, females

178    received a social signal manipulation (dulling or sham control of the white breast) in

179    each year (for details of plumage manipulation, see Taff et al. In Press*a*). For both the

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180  signal manipulation and challenges, we assumed that any effects on nestlings would

181  primarily occur through altered resources due to provisioning rates, and we therefore

182  analyzed provisioning rates directly from radio-frequency identification (hereafter

183  RFID) records. For the challenge treatments, we also fit models that included the

184  categorical treatments directly as predictors because there is evidence that perceived

185  predation risk can alter developmental trade-offs, independently of its effects on

186  resource availability (e.g., Clinchy et al. 2013; LaManna and Martin 2016).

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188  *Cross-Fostering and Nestling Measurements*

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190          One of the goals of this study was to determine the degree to which

191  environmental conditions versus genetic contributions drove differences in feather

192  quality and nestling development. Therefore, we cross fostered eggs from each nest

193  before incubation began so that any contribution to nestling feather development

194  driven by developmental environment (e.g., incubation, provisioning rate) was

195  decoupled from genetic inheritance or maternal effects associated with investment in

196  the egg contents.

197          We paired nests based on timing of clutch initiation and on day 4 of egg laying

198  we swapped half of the brood between the pair and marked all eggs in each nest with a

199  pencil on the bottom of each egg. At half of the nests, we returned on the following day

200  and swapped the 5$^{th}$ (unmarked) egg between the two nests. This two-step process

201  ensured that some eggs from early and late in the laying order were swapped in case

202  there were differences in yolk contents associated with laying order. In cases where

203  there was not an appropriate nest to swap, we sometimes paired three nests together for

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204    cross fostering. A few late season nests did not have any compatible pairs and were not

205    cross fostered.

206        We banded nestlings when they were 12 days old, collected a blood sample for

207    paternity assignment, and took morphological measurements (mass, wing length, and

208    head + bill length). When nestlings were 15 days old, we once again took a mass

209    measurement, applied a unique PIT tag, and collected feathers exactly as described

210    above for adults to measure barb density. After day 15 we avoided visiting the nest to

211    prevent forced fledging. Final nestling fate and exact fledging date were determined

212    using RFID records and a check of the nest on day 24 to find any nestlings that had died

213    in the nest after day 15.

214

215    *RFID Sensor Network*

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217        We installed an RFID system at each nest box in the study no later than day 4 of

218    incubation (as in Vitousek et al. 2018). The system consisted of an RFID board held in a

219    waterproof container on the bottom of the nest box (Bridge and Bonter 2011), an

220    antenna that circled the nest box entrance, and a 12-volt battery that powered the

221    system. We programmed the readers to record PIT tags within range (~2 cm) of the

222    entrance hole every second from 5am to 10pm each day of the breeding season. From

223    raw RFID records, we extracted female and—when possible—male provisioning rates

224    at each nest following the algorithm described in Vitousek et al. (2018).

225        We also used RFID records to determine the exact age of fledging for each

226    nestling in the population. For each individual nestling, we considered the latest record

227    at the nest box to be the time of fledging. While it is possible that nestlings could leave

228    and then return to the box, we saw no evidence for this behavior in our RFID data even

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229     when the sensors were left running long after we had confirmed fledging. Occasionally,

230     RFID units failed because of software problems or dead batteries and we are therefore

231     missing some records from parts of the provisioning period or fledging times for some

232     nestlings.

233

234     *Feather Measurements*

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236     We measured the density of feather barbs for adults and nestlings following the

237     method developed by Butler et al. (2008) as described in Callan et al. (2019*a*), except that

238     we modified their approach for use with photographs rather than measuring with a

239     dissecting scope. To take photographs, we spread each feather on a microscope slide

240     that had been covered in contrasting cardstock paper with a scale bar. We used black

241     paper as a background when photographing white breast feathers and white paper

242     when photographing brown, green, and blue rump feathers. The feather was pressed

243     down flat with a second clear microscope slide and photographed using a digital

244     camera with a macro lens held in place on a document-scanning platform with diffuse

245     lights. The camera mount ensured that photographs were in sharp focus and always

246     taken at a direct 90° angle from the slide surface to avoid parallax issues when

247     measuring. For each individual, we photographed two breast and two rump feathers.

248     From the digital photographs, we measured the density of feather barbs using

249     ImageJ (Schneider et al. 2012) . We first set the scale for each image using the scale bar

250     that was included in every photograph. Next, we identified the section of the rachis to

251     be measured and marked those points with the annotation tool in ImageJ. For the start

252     point, we chose the most distal point on the feather rachis where a pennaceous barb

253     could be seen branching off from the rachis. For the end point, we chose the most

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254    proximal point on the rachis where pennaceous barbs could be clearly seen branching

255    off of the rachis before becoming plumulaceous.

256         We next measured and recorded the length of the rachis between these two

257    points using the segmented line tool. Finally, we counted the number of pennaceous

258    barbs between the two points and recorded the left and right side barbs separately. We

259    calculated a single barb density measure for the feather by dividing the average count

260    of barbs from the two sides by the length of feather rachis and expressed density in

261    terms of barbs per centimeter of rachis. We repeated this procedure for the two breast

262    and two rump feathers and then averaged the two replicate measurements from each

263    region together to arrive at a single breast density and rump density measurement.

264         We used multiple feathers from the same bird to estimate biological repeatability

265    across feathers within a subject and multiple measurements of the same photograph by

266    different observers to estimate inter-observer measurement repeatability. In some cases,

267    we did not have complete measurements because we were missing feathers or had only

268    a single feather. Feather measurements were also not available for any nestlings that did

269    not survive to 15 days old.

270

271    *Determining Nest of Origin*

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273         Adult and nestling blood samples were stored in lysis buffer (Seutin et al. 1991)

274    in the field and DNA was extracted using Qiagen DNeasy Blood & Tissue Kit spin

275    columns following the standard kit protocol. We amplified a set of 9 variable

276    microsatellite loci that have been previously used in this population (Makarewich et al.

277    2009; Hallinger et al. 2019). Our amplification protocol exactly followed that described

278    in (Hallinger et al., 2019) and details on primer sequences, reaction volumes, cycling

279    conditions, and fragment analysis can be found there. We determined nest of origin by

280    comparing nestlings to their putative mothers (the females from the 2-3 nests in each

281    cross-fostering pair). Nestlings that matched only one putative mother at 8 of 9 loci

282    were considered to have been laid by that female. Using these criteria, we were able to

283    assign definitive genetic mothers to 275 of 313 sampled nestlings. The remaining 38

284    nestlings were either missing blood samples, missing maternal genotype information,

285    mismatched at more than two loci, or had two putative mothers that were so similar

286    that the genetic mother could not be determined definitively. Those nestlings are

287    excluded from all analyses that included nest of origin but included in summary

288    statistics.

289

290    *Data Analysis*

291

292        We assessed repeatability between observers and between multiple feathers from

293    the same individual by estimating the intra-class correlation coefficient (ICC) in linear

294    mixed models with no covariates as implemented by the 'rptR' package version 0.9.22

295    in R (Stoffel et al. 2017). Overall differences in adult and nestling feather barb density

296    measurements were assessed using linear mixed models with age (adult or nestling) as

297    a fixed effect and nest as a random effect. To assess the extent to which environment

298    (nest identity) and genetics (genetic mother) jointly contributed to variation in nestling

299    feather barb density, we fit LMMs using the 'rptR' package that included random

300    effects for both the nest identity and the genetic mother. We report the ICC for each of

301    these effects as an estimate of the amount of variation explained by nest and genetic

302    mother.

303    To evaluate the evidence for a trade-off between the speed of development and

304    feather quality, we fit a series of models that included proxies of developmental pace as

305    response variables (exact age at fledging, mass and morphological measurements on

306    day 12 or 15). For fixed effects, these models included breast and back feather density, a

307    categorical effect for the challenge treatment that the female received (control, handicap,

308    or predator), and two-way interactions between feather density measurements and

309    challenge treatment. For simplicity, we present reduced models that exclude

310    interactions receiving no support. These models also included nest and genetic mother

311    as random effects to account for the non-independence of nestlings from the same nest

312    or mother.

313    Finally, we fit models to ask whether variation in female provisioning rate

314    predicted nestling back or breast feather density (male provisioning was not considered

315    because not all males had PIT tags). We first estimated standardized female

316    provisioning rate by fitting a model of daily provisioning that included brood size,

317    nestling age, a quadratic effect of nestling age, and random effects for the day of year

318    (to account for weather differences) and nest identity (to account for repeated

319    observations at each nest). We extracted a provisioning rate for each female from this

320    model and standardized to a mean of 0 and standard deviation of 1. This standardized

321    provisioning rate was then used as a predictor of nestling feather barb density in an

322    LMM with nest identity and genetic mother included as random effects as described

323    above.

324    All LMMs apart from those used to calculate ICC were fit with the 'lme4'

325    package version 1.1-26 in R (Bates et al. 2015). Predictors were considered to be

326    meaningful if the confidence interval did not cross zero. In tables presenting mixed

327    model details, we also include p-values based on the Satterthwaite approximation as

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328   implemented by 'lmerTest' version 3.1-3 (Kuznetsova et al. 2017). All figures and

329   analyses were produced in R version 4.0.2 (R Core Development Team 2020).

330

331   **RESULTS**

332

333        In total, our analysis included 313 nestlings raised in 85 nests with at least one

334   feather region measurement and 274 adults with at least one feather region

335   measurement. In a validation dataset, inter-observer repeatability of barb density

336   measurements from the same feather photograph was high ($n$ = 149 measurements of 38

337   photographs by 8 observers; repeatability = 0.96; 95% CI = 0.94 to 0.98). Measurements

338   of two independent feathers from the back or the breast of the same bird were also

339   repeatable (breast: $n$ = 1123 measures of 572 individuals; repeatability = 0.78; CI = 0.74

340   to 0.81; back: $n$ = 1021 measures of 572 individuals; repeatability = 0.68; CI = 0.63 to

341   0.72).

342        Overall, there was a moderate positive correlation between barb density of back

343   and breast feathers within an individual (Figure 2). This relationship was observed for

344   both adults and nestlings with a similar slope in each group (Pearson's correlation for

345   adults and nestlings combined: $r$ = 0.66, CI = 0.61 to 0.70; adults $r$ = 0.34, CI = 0.23 to

346   0.45; nestlings $r$ = 0.24, CI = 0.13 to 0.35).

347        As expected, adults had substantially higher barb density for both back and

348   breast feathers (Figure 2). For back feathers, nestlings had an overall barb density of

349   17.9 barbs per cm, while adults had 24.3 barbs per cm (LMM with nest as a random

350   effect, ® for nestlings = -6.39, CI = -6.97 to -5.81). For breast feathers, nestlings had an

351   overall barb density of 20.4 barbs per cm, while adults had 28.2 barbs per cm (LMM ®

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352   for nestlings = -7.79, CI = -8.33 to -7.26). Thus, nestlings had on average 73.7 % (back)

353   and 72.4 % (breast) of the barbs per cm as adults.

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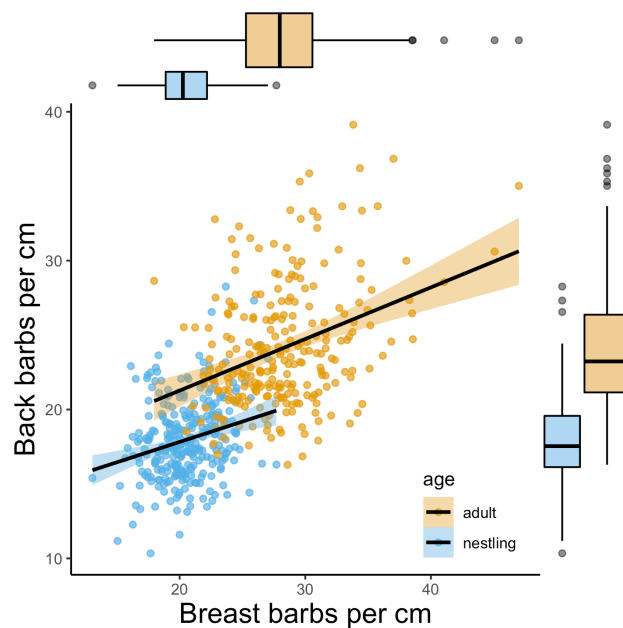


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356

357   **Figure 2.** Relationship between breast and back barb density for feathers measured from the same individual for adults (orange)

358   and nestlings (blue). Box and whisker plots in the margins show the distribution of barb density measurements for each body

359   region and age group.

360

361   *Environmental and Genetic Influence on Barb Density*

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363         For breast feathers, variation in nestling barb density was explained by both the

364   genetic mother and the nest environment that a nestling was raised in. The adjusted

365   ICC of nest environment controlling for genetic mother in an LMM was 0.33 (CI = 0.2 to

366   0.442). The adjusted ICC of genetic mother controlling for nest environment in an LMM

367   was 0.27 (CI = 0.14 to 0.39). For back feathers, genetic mother explained some variation

368   in nestling barb density, but nest environment explained little. The adjusted ICC of

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369    genetic mother for back feather barb measurements controlling for nest environment

370    was 0.20 (CI = 0.05 to 0.34). The adjusted ICC of nest environment controlling for

371    genetic mother was 0.07 (CI = 0.0 to 0.19).

372         For breast measurements, unadjusted ICC estimates were nearly identical, but

373    for back measurements, unadjusted estimates were higher for both categories,

374    suggesting that nest environment and genetic mother explained much of the same

375    variation. Unadjusted ICC for genetic mother on back barbs was 0.26 (CI = 0.12 to 0.39)

376    and for nest environment was 0.22 (CI = 0.08 to 0.34).

377

378    *Barb Density by Time in the Nest*

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380         Nestlings fledged an average of 22.6 days after hatching (standard deviation =

381    1.5, range = 18 to 29 days). There was no apparent relationship between age at fledging

382    and the density of feather barbs for either back or breast feathers (Figure 3). There was

383    also no indication that the (lack of) relationship differed by treatment group (Table 1;

384    unsupported interactions are not shown). However, there was a main effect of the

385    predator treatment with nestlings from the predation treatment fledging at older ages

386    than those from control or handicap nests. Nest identity explained much of the

387    variation in fledging age and genetic mother explained little additional variation (Table

388    1; marginal $R^2$ for reduced model = 0.095, conditional $R^2$ including random effects =

389    0.657).

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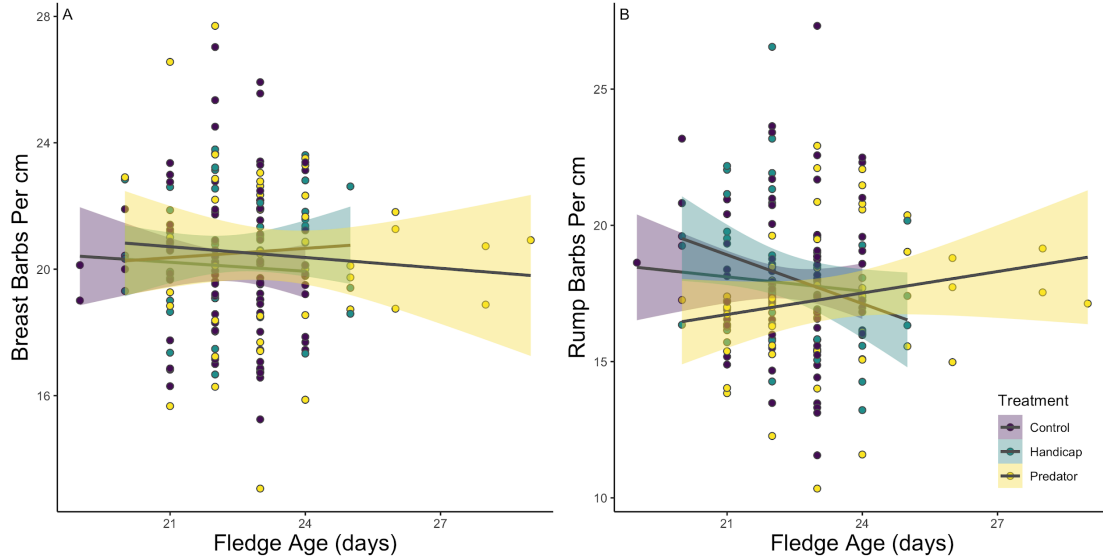
**Figure 3.** Fledging age was not related to breast (A) or back (B) feather barb density per cm for nestlings in the three different treatment groups.
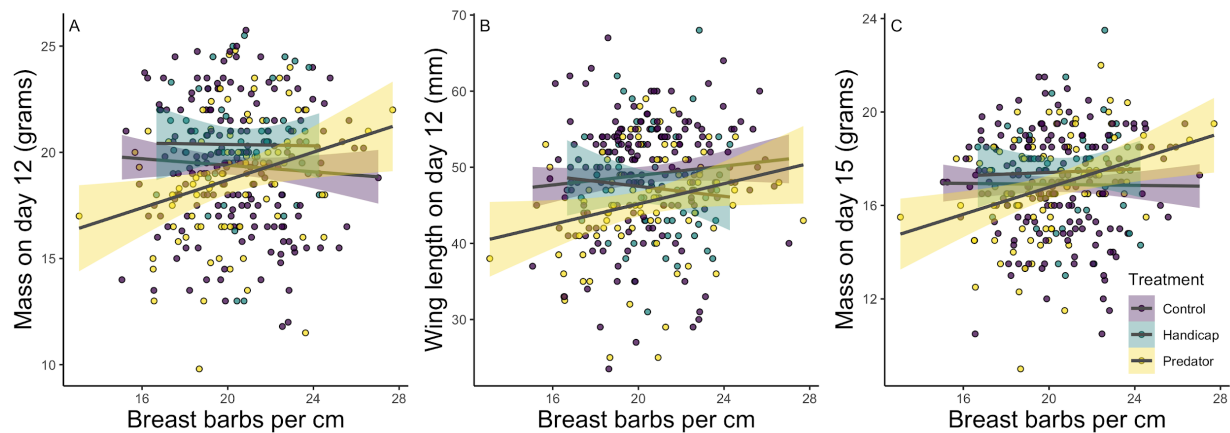
**Table 1.** Feather barb density and nest treatment as predictors of fledging age.

| Predictors | Fledge Age (Days) | | |
| --- | --- | --- | --- |
| | Estimates | CI | p |
| Intercept (Control) | 22.40 | 20.35 – 24.45 | **<0.001** |
| Back Barb Density | 0.04 | -0.04 – 0.11 | 0.328 |
| Breast Barb Density | -0.04 | -0.13 – 0.05 | 0.410 |
| Handicap | -0.06 | -0.99 – 0.88 | 0.905 |
| Predator | 1.17 | 0.33 – 2.02 | **0.006** |
| **Random Effects** | | | |
| $\sigma^2$ | 1.00 | | |
| $\tau_{00 \text{ Mother}}$ | 0.01 | | |
| $\tau_{00 \text{ Nest}}$ | 1.63 | | |
| ICC | 0.62 | | |
| $N_{\text{Nest}}$ | 60 | | |
| $N_{\text{Mother}}$ | 75 | | |
| Observations | 177 | | |
| Marginal $R^2$ / Conditional $R^2$ | 0.095 / 0.657 | | |

*Correlation Between Nestling Feather Barb Density and Morphology*

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401   Breast feather barb density was positively related to day 12 and day 15 nestling

402  mass, but only in the predator treatment group (Figure 4, Table 2). Back barb density

403  was not related to nestling wing length, head plus bill length, or mass in any treatment

404  group. Overall, nestlings raised in the predator treatment group had shorter wings on

405  day 12 and lower mass on day 12 and 15 than did nestlings raised in either the handicap

406  or control group (Table 2). However, the amount of variation in mass explained by

407  feather measurements was small compared to that explained by random effects fitted

408  for the nest environment and the genetic mother (Table 2; marginal $R^2$ of main effects =

409  0.02 to 0.09; conditional $R^2$ including random effects = 0.32 to 0.70).

410



411

412 **Figure 4.** Nestling mass on day 12 (A), flattened wing chord length on day 12 (B), and mass on day 15 (C) in relation to breast

413 feather barb density by treatment group.

414

415

416 **Table 2.** Linear mixed models showing relationship between barb density and nestling morphology by treatment group. Genetic

417 mother and nest of development are included as random effects.

| Predictors | Day 12 Mass | | | Day 12 Head + Bill | | | Day 12 Flat Wing | | | Day 15 Mass | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Estimates | CI | p | Estimates | CI | p | Estimates | CI | p | Estimates | CI | p |
| Intercept | 21.92 | 18.26 – 25.57 | **<0.001** | 24.20 | 22.80 – 25.60 | **<0.001** | 48.71 | 39.91 – 57.51 | **<0.001** | 17.75 | 14.76 – 20.74 | **<0.001** |
| Back Barb Density | -0.04 | -0.14 – 0.07 | 0.495 | 0.01 | -0.04 – 0.06 | 0.813 | 0.05 | -0.20 – 0.30 | 0.677 | -0.02 | -0.11 – 0.07 | 0.649 |
| Breast Barb Density | -0.10 | -0.26 – 0.06 | 0.221 | 0.03 | -0.03 – 0.09 | 0.281 | -0.03 | -0.42 – 0.35 | 0.864 | -0.03 | -0.16 – 0.11 | 0.711 |
| Handicap | 3.43 | -4.55 – 11.41 | 0.399 | -0.03 | -0.55 – 0.49 | 0.913 | 7.73 | -11.30 – 26.75 | 0.426 | -0.69 | -7.24 – 5.87 | 0.838 |
| Predator | -6.27 | -12.15 – -0.39 | **0.037** | -0.34 | -0.77 – 0.10 | 0.132 | -15.83 | -30.01 – -1.64 | **0.029** | -4.72 | -9.38 – -0.06 | **0.047** |
| Breast Barb * Handicap | -0.11 | -0.48 – 0.27 | 0.578 | | | | -0.40 | -1.29 – 0.49 | 0.376 | 0.07 | -0.24 – 0.38 | 0.674 |
| Breast Barb * Predator | 0.28 | 0.00 – 0.56 | **0.049** | | | | 0.53 | -0.14 – 1.21 | 0.122 | 0.23 | 0.01 – 0.46 | **0.041** |
| **Random Effects** | | | | | | | | | | | | |
| $\sigma^2$ | 3.35 | | | 0.99 | | | 18.47 | | | 2.50 | | |
| $\tau_{00}$ | 1.33 Mother | | | 0.02 Mother | | | 5.14 Mother | | | 0.72 Mother | | |
| | 3.61 Nest | | | 0.42 Nest | | | 33.02 Nest | | | 1.33 Nest | | |
| ICC | 0.60 | | | 0.31 | | | 0.67 | | | 0.45 | | |
| N | 84 Nest | | | 84 Nest | | | 84 Nest | | | 82 Nest | | |
| | 90 Mother | | | 90 Mother | | | 90 Mother | | | 89 Mother | | |
| Observations | 290 | | | 290 | | | 288 | | | 286 | | |
| Marginal $R^2$ / Conditional $R^2$ | 0.048 / 0.615 | | | 0.020 / 0.320 | | | 0.085 / 0.702 | | | 0.031 / 0.467 | | |

418

419

420

421    After controlling for nestling age and brood size, female provisioning rate was

422    not associated with nestling back feather barb density (LMM with nest and genetic

423    mother as random effects; full model marginal $R^2$ = 0.008; effect of standardized

424    provisioning rate = -0.26; CI = -0.66 to 0.15). However, a higher rate of female

425    provisioning was associated with decreased nestling breast feather barb density (Figure

426    5, LMM full model marginal $R^2$ = 0.04; effect of provisioning rate = -0.49; CI = -0.87 to -

427    0.10).

428
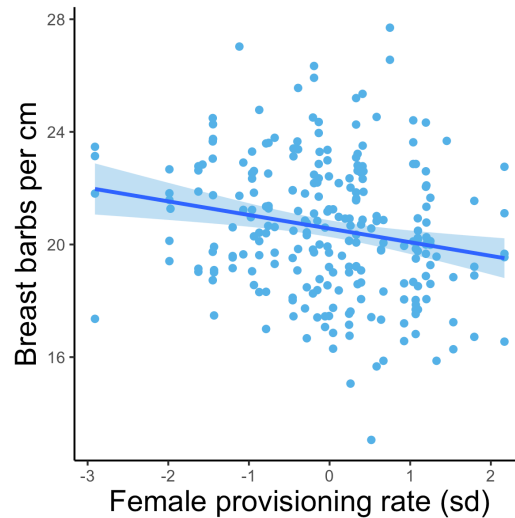
429

430  **Figure 5.** Nestling breast barbs per centimeter in relation to standardized female provisioning rate. Provisioning rate accounts for

431  nestling age and brood size (see methods) and is shown in units of standard deviations.

432

433  **DISCUSSION**

434

435      Consistent with the idea that ephemeral traits may be deprioritized during

436  development, we found that tree swallow nestlings grew body feathers that had lower

437  barb density than those of adults from the same body regions. Individuals varied

438  considerably in their barb density, but there was high repeatability within individuals

439  for feathers from the same body region and a moderate association between feathers

440  from different body regions. Cross fostering revealed that there was both a strong

441  environmental and genetic basis to variation in feather barb density. Despite the fact

442  that there was substantial variation in both feather quality and in the pace of

443  development—measured as either the exact age of fledging or body size—we found no

444  evidence for a within-individual resource allocation trade-off between feather quality

445  and rapid development. Moreover, accounting directly for variation in resource

446  acquisition by including maternal provisioning rates or experimental challenges on

21

447    parents did not reveal any hidden trade-off, as might be expected if variation in

448    resource acquisition had masked allocation trade-offs (sensu van Noordwijk & de Jong,

449    1986). In fact, higher female provisioning rates were associated with slightly lower

450    feather quality and, among nestlings in a nest predation treatment group, there was a

451    positive association between feather quality and body size measurements; these

452    patterns are opposite to those predicted if reduced resources had forced differential

453    investment in feathers versus growth. Our results highlight the difficulty of inferring

454    individual level resource trade-offs from comparative data alone.

455         In a comparative study of feather barb density from nest-grown feathers

456    including 123 species, Callan et al. (2019) found a strong relationship between time in

457    the nest and feather quality (Figure 1A). Species that develop more rapidly in the nest

458    have consistently lower quality feathers—as compared to adults of the same species—

459    than do those that develop more slowly in the nest. The authors hypothesize that this

460    relationship arises from a resource allocation trade-off, with species that must develop

461    rapidly in order to leave the nest and avoid predation shunting resources away from

462    other traits. They argue that these trade-offs may be especially apparent in ephemeral

463    traits, like nest-grown feathers, because the fitness consequences of lower quality

464    ephemeral traits are relatively low since they are quickly replaced after fledging. How

465    can we resolve this evidence for an apparently strong trade-off between development

466    and feather quality among species with the lack of evidence for a trade-off found within

467    a single species in our study? Agrawal argues that often, "trade-offs at one level of

468    organization will provide little insight into what may occur at other levels" (pg. 3;

469    Agrawal, 2020) without additional consideration of the mechanism that generates trait

470    associations. His review goes on to develop a conceptual framework for considering

471    what types of trait associations (or trade-offs) are likely to scale across levels or to only

472    be apparent at a single level.

473            Interpreted in light of this framework, our results in tree swallows and the

474    comparative results presented by Callan et al. (2019) are most consistent with the idea

475    that the inter-specific association between rapid development and feather quality arises

476    from adaptative specialization to different developmental strategies by each species,

477    rather than from a direct resource allocation trade-off that plays out within species or

478    individuals (Figure 1D). This is perhaps unsurprising given the nature of the proposed

479    trade-off. Direct resource allocation trade-offs are most likely to arise with strong

480    constraints and between life history traits that are directly associated with fitness when

481    all else is held equal (Agrawal, 2020; Roff, 2002; Stearns, 1992). In contrast, negative trait

482    associations among species often represent the outcome of adaptive specialization

483    based on strategic optimization (Futuyma and Moreno 1988; Agrawal 2020). In the case

484    of ephemeral feathers, the direct developmental costs of feather barbs may not be severe

485    enough to generate a within species resource allocation trade-off and may not share a

486    direct mechanistic link with the overall rate of development (e.g., a shared

487    developmental pathway). Additionally, plastic responses are only favored when

488    relevant information is available (Pigliucci 2005), and if nestlings cannot reliably assess

489    their own developmental pace, there may be no realized benefit to modifying the

490    allocation of resources to faster development over feathers. In contrast, when

491    comparing different species, strong selection for particular developmental trajectories

492    (e.g., fast fledging to avoid predation) may result in subsequent selection to optimize

493    investment in secondary traits, such as ephemeral feather quality, without the need to

494    invoke a direct allocation trade-off.

23

495   Alternatively, it is possible that some species do face direct resource allocation

496   trade-offs between feather quality and developmental rate, but that tree swallows are

497   not representative of a more general mechanism (Figure 1E). Several aspects of tree

498   swallow life history might make them less likely to show this particular allocation

499   trade-off. First, as cavity nesters, tree swallows experience relatively low predation rates

500   and spend an unusually long time in the nest for their size (Winkler et al. 2020*b*), which

501   may mitigate the need to redirect resources towards rapid development. Second, cold

502   snaps create strong selection events for tree swallow nestlings because the parents are

503   entirely dependent on flying insects (Winkler et al. 2013). When flying insects are scarce,

504   parents travel far from the nest and are absent for long periods (Stocek 1986). These cold

505   snaps can lead to mass mortality events for nestlings, but after feathers are grown

506   nestlings are much less vulnerable because they can thermoregulate independently

507   (Shipley et al., 2020). Thus, for tree swallows in particular, growing effective feathers in

508   the nest might be especially important even when resources are scarce. It is possible that

509   direct resource allocation trade-offs might be observed in other species that face

510   different life history challenges.

511   We also found that feather barb density itself and morphological relationships

512   with barb density differed for breast versus rump feathers. In general, breast feathers

513   had higher barb density and were more clearly influenced by both nest environment

514   and genetic mother; feathers from this body region were also the only ones that showed

515   any relationship with nestling morphological measures. In adult tree swallows, feathers

516   in both of these body regions are putative social signals, but their function and color

517   patterns differ dramatically. Breast feathers are light gray to pure white and have been

518   implicated in social signaling and aggression (Beck et al. 2015; Taff et al. 2019*b*). Rump

519   feathers are brown to iridescent blue-green and have been associated with mate choice

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520    and extrapair paternity in males (Bitton et al. 2007; Van Wijk et al. 2016; Whittingham

521    and Dunn 2016). Adult female rump feathers go through a delayed plumage maturation

522    with one year old females displaying brown feathers that turn iridescent blue-green in

523    subsequent years and this process may also mediate social relationships and

524    performance (Berzins and Dawson 2016; Dakin et al. 2016). We do not know at present

525    whether the color of these patches has a function in fledgling tree swallows and, if so,

526    how that might relate to barb density. From a thermoregulation perspective, the

527    importance of breast and rump feathers may also differ. For nestlings huddling in a nest

528    cup, the rump feathers are more directly exposed to ambient air, but the breast feathers

529    insulate the pectoralis muscles, which is a major source of shivering thermogenesis in

530    birds (West 1965). We also observed substantial variation in the age of feathering in

531    between nestlings in our study; some nestlings had fully developed feathers across

532    most of the body by day 15 while others had only small pin feathers. Because feathers

533    are inert after exiting the follicle, the barb measurements we took from 15-day-old

534    nestlings represent the influence of conditions experienced earlier in development. It

535    seems likely that overall feathering is more important for thermoregulation than barb

536    density *per se*, but we do not know at present whether the quality of individual feathers

537    and overall rate of feather growth are mechanistically linked.

538          Although we did not find any evidence for an allocation trade-off, we did find

539    that predator treatments resulted in an association between feather barb measurements

540    and body size in some cases. In many species, experimentally increasing the perceived

541    threat of predation leads to faster nestling development (LaManna and Martin 2016). In

542    contrast, nestlings in our study fledged later when raised in a nest that experienced

543    predation treatments. While we cannot be certain about the cause of this discrepancy, it

544    seems likely that the details of our treatment may have contributed to the difference.

545    Because our predation treatments were targeted at adult females and occurred shortly

546    before or after hatching, there was little or no opportunity for nestlings to directly

547    perceive predator treatments. Rather, any perceived threat would have occurred

548    through the indirect effect of subsequent parental behavior. In contrast, previous

549    studies targeting nestlings have manipulated cues (e.g., auditory or visual

550    presentations) that nestlings could perceive directly (Hallinger et al., 2019; LaManna &

551    Martin, 2016). In our study, nestlings in the predation treatment group showed a

552    positive correlation between body size and breast feather density (the opposite to that

553    predicted if predation threat increased relative investment in growth or decreased

554    acquisition of resources). There are two possible explanations for this pattern. First,

555    parental behavior may have been altered in a way that impacted nestling

556    developmental trajectories. While we did not observe differences in overall

557    provisioning rate, parents may have changed brooding schedules, or the type or quality

558    of food delivered to nestlings. Second, as a result of altered parental behavior, the

559    feather relationships we observed in the predation group may be the result of

560    differential survival to day 15 rather than changes in relative resource allocation.

561    Because we only had feather measurements for nestlings that lived to day 15, we cannot

562    assess whether differential survival contributed to the patterns that we observed. In

563    either case, our results do not support a role for predation threat in revealing a hidden

564    resource allocation trade-off between development and feather quality in tree swallows,

565    although variation in predation risk is clearly an important driver of trait correlations in

566    inter-specific comparisons (Callan et al. 2019*a*).

567        Our results highlight the fact that strong trait-correlations among species do not

568    necessarily scale across levels of biological organization and that it is often difficult to

569    infer within-species mechanisms from among species associations. Moving forward, we

26

570 suggest that more studies are needed that integrate measures of trait covariation within

571 individuals and species with comparative analyses in order to parse the hierarchical

572 nature of variation in trait correlations. These approaches are complementary: within-

573 species measurements are needed to identify the proximate mechanisms producing trait

574 correlations, while comparative studies are needed to understand how evolution has

575 shaped population and species level strategies and to understand the conditions under

576 which trade-offs represent strong constraints on life history evolution. Combining these

577 approaches is a critical step towards developing a predictive framework for

578 understanding when and why trait-associations do or do not scale across levels of

579 biological organization.

580

581 **ETHICAL NOTE**

582 　　　We received approval for all of the procedures described here from the Cornell

583 University Institutional Animal Care & Use Board (IACUC protocols # 2001-0051 and

584 2019-0023). Sampling and capture in the field were approved by federal and state

585 permits.

586

587 **ACKNOWLEDGMENTS**

588

599

**DATA AND CODE ACCESSIBILITY**

601      The complete dataset and code required to reproduce all analyses and figures is

602      available on GitHub (https://github.com/cct663/tres_feather_density).

603

**AUTHOR CONTRIBUTION STATEMENT**

605      ATA, BAJ, CCT, and MNV contributed to the conceptualization of the study.

606      AMR, ATA, BAJ, CCT, JJU, and JLH contributed to data collection. AMR, ATA, and

607      BAJ, carried out measurements for the study. MNV contributed funding acquisition.

608      CCT conducted analysis and visualization with input from all authors. CCT wrote the

609      original draft of the manuscript with BAJ and with subsequent feedback and editing

610      from all authors. All authors approved the final version of the manuscript.

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