Is there a trade-off between ephemeral feather growth and time in the nest in tree swallows? [need a better title]

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Possible coauthors need to decide who should be on this

* Cedric: contributions to general field work but really not anything specific to this project, I guess probably OK to leave him off of this one…
* Tom: contribution to field work both years, field sample processing, not much involvement in the measurement/analysis
* Jenny: contribution to rfids both years, more field help in 2019, lots of help with field sample processing, some help coordinating students measuring feathers
* David: mainly working on water both years, but consistent help when needed on land, more help with land boxes at unit 1 in 2019, no involvement in post-season measure/analysis
* Alli: a lot of help with general field stuff and logistics in 2019 although not much directly at units 1/2, not here in 2018, no involvement in post-season measure/analysis
* Jenn: limited help with a few captures in field in 2019 but not much, limited help in post-season measurement just coordinating some students doing measurements, did most (all?) of the nestling sexing reactions so if we include that she should go on
* Sabrina & Colleen: minimal help with a few captures in the field

Student help:

* 2018 crew: Raisa, Jeremy, Allison, Audrey, Brianna, Kwame, Callum, Emma
* 2019 crew: Kai, Alex, Alex, Alyssa, Christine, Zapporah, Bella, Yusol, Raquel, Paige, Bashir, Jabril
* Help with photos of feathers: Brittany
* Some feather measurements of adults? Bella, Raquel, Sungmin, Paige, Kai

**ABSTRACT**

***Keywords:***

**INTRODUCTION**

1. Intro paragraph
   1. Set up general idea of life history trade-offs, limited resources, etc
2. Ephemeral traits in general
   1. Build directly from the intro and justification of the Callan paper
3. Cross species vs. within species comparisons
   1. When do we expect trade-offs that appear in cross species comparisons (Callan paper) to hold within a single species? Are there reasons to think they should or shouldn’t hold for within species comparisons?
   2. Ecological fallacy/simpson’s paradox/jensen’s inequality,
4. Context dependence of trade-offs
   1. Could depend on underlying resources -> predators/energy treatments
   2. Predators change lots of things about nestling development (see Tom Martin papers)
   3. Van noordwijk & de jong resource allocation paper
5. Importance of feathers and molting in general
   1. Could go here or up in #2 depending on how this gets written
   2. Why are feathers important: thermoregulation, signaling, flight, etc
   3. But early feathers are molted quickly so potentially big investment for a short time
6. Tree swallow system
   1. Low predation so relatively relaxed time to develop?
   2. But sensitive to resource availability and allocating to different types of development?
   3. Feathers could be an unnecessary cost, but could also be important for thermoregulation
7. Specifics of this study and hypotheses/predictions
   1. Cross fostered nestlings monitored in two years
   2. Treatments on adults that altered development time/resources
   3. Remote system to monitor exact fledging age with multiple time point development measures
   4. Some predictions: read through Callan and think about how to rephrase their cross species hypothesis for within a species
      1. Trade-off more likely to occur in resource limited (predator) treatments

**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). 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 day, we checked boxes every day so that an exact hatch date could be recorded.

Adults at each nest were captured during incubation or provisioning as part of an experiment focused on female responses to social and ecological challenges (Taff et al., In prep). For adults, we took morphological measurements (mass, flattened wing-chord length, and head + bill length), a blood sample for paternity, and 6-8 feathers each from the center of the white breast and the rump just above the tail to measure barb density (see below). For individuals that were not already banded from a previous year, we applied an aluminum USGS band and a passive integrated transponder (PIT) tag that encoded a unique 10-digit hexadecimal string.

Females at most nests in these two years were part of an experiment in which they received a social signal manipulation (dulling or sham coloring of the white breast) coupled with an ecological challenge (simulated predation attempts or a flight efficiency reduction). In 2018, the signal manipulation occurred first and was followed by the ecological challenge, while in 2019 the order was reversed. These treatments were focused entirely on the adult females at each nest, but they had indirect effects on nestlings resulting in lower overall fledging success at the predation treatment groups in each year (Taff et al., In prep). It is unclear what mechanism drove those differences, as females in each group provisioned nestlings at a similar rate, but presumably some difference in female behavior or reproductive investment translated into a harsher developmental environment for nestlings (Taff et al., In prep). In our analyses here, we accounted for female treatment by looking for overall differences in fledging time and feather quality among nestlings raised with social mothers in each treatment group and by asking whether any trade-off between feather quality and development differed between the treatment groups.

*Cross-Fostering and Nestling Measurements*

One of the goals of this study was to determine the degree to which environmental conditions versus genetic contributions drove differences in feather quality and nestling development. Therefore, we cross fostered eggs from each nest before incubation began so that any contribution to nestling feather development driven by developmental environment (e.g., incubation, provisioning rate) was decoupled from genetic inheritance or maternal effects associated with investment in the egg contents.

We paired nests based on timing of clutch initiation and on day 4 of egg laying we swapped half of the brood between the pair and marked all eggs in each nest with a pencil on the bottom of each egg. At half of the nests, we returned on the following day and swapped the 5th (unmarked) egg between the two nests. This two-step process ensured that some eggs from early and late in the laying order were swapped in case there were differences in yolk contents associated with laying order. In cases where there was not an appropriate nest to swap, we sometimes paired three nests together for cross fostering. A few late season nests did not have any compatible pairs and were not cross fostered.

We measured nestlings at several time points. First, we took a full brood mass measurement at day 6 with all nestlings at each nest counted and weighed together. Second, we banded nestlings when they were 12 days old, collected a blood sample for paternity assignment, and took morphological measurements (mass, wing length, and head + bill length). Finally, when nestlings were 15 days old we once again took a mass measurement, applied a unique PIT tag, and collected feathers to measure barb density exactly as described above for adults. After day 15 we avoided visiting the nest to prevent forced fledging. Final nestling fate and exact fledging date were determined using RFID records and a check of the nest on day 24 to find any nestlings that had died in the nest after day 15.

*RFID Sensor Network*

We installed an RFID system at each nest box in the study on day 4 of incubation (as in Vitousek et al., 2018). The system consisted of an RFID board held in a waterproof container on the bottom of the nest box (Bridge & Bonter, 2011), an antenna that circled the nest box entrance, and a 12-volt battery that powered the system. We programmed the readers to record PIT tags within range of the entrance hole every second from 5am to 10pm each day of the breeding season. From raw RFID records, we extracted female and—when possible—male provisioning rates at each nest following the algorithm described in Vitousek et al. (2018). We also used RFID records to determine the exact age of fledging for each nestling in the population. For each individual nestling, we considered the latest record at the nest box to be the time of fledging. While it is possible that nestlings could leave and then return to the box, we saw no evidence for this behavior in our RFID data even when the sensors were left running long after we had confirmed fledging. Occasionally, RFID units failed because of software problems or dead batteries and we are therefore missing some records from periods of the provisioning period or fledging times for some nestlings.

*Feather Measurements*

We measured the density of feather barbs for adults and nestlings following the method developed by Butler et al. (2008) as described in Callan et al. (2019), except that we modified their approach for use with photographs rather than measuring with a dissecting scope. To take photographs, we spread each feather on a microscope slide that had been covered in contrasting cardstock paper with a scale bar. We used black paper as a background when photographing white breast feathers and white paper when photographing brown, green, and blue rump feathers. The feather was pressed down flat with a second clear microscope slide and photographed using a digital camera held in place on a document-scanning platform with diffuse lights. The camera mount ensured that photographs were in sharp focus and always taken at a direct 90° angle from the slide surface to avoid parallax issues when measuring. For each individual, we photographed two breast and two rump feathers.

From the digital photographs, we measured the density of feather barbs using ImageJ (Schneider et al., 2012). We first set the scale for each image using the scale bar that was included in every photograph. Next, we identified the section of the rachis to be measured and marked those points with the annotation tool in ImageJ. For the start point, we chose the most distal point on the feather rachis where a pennaceous barb could be seen branching off from the rachis. For the end point, we chose the most proximal point on the rachis where pennaceous barbs could be clearly seen branching off of the rachis before becoming plumulaceous.

We next measured and recorded the length of the rachis between these two points using the segmented line tool. Finally, we counted the number of pennaceous barbs between the two points and recorded the left and right side barbs separately. We calculated a single barb density measure for the feather by dividing the average count of barbs from the two sides by the length of feather rachis measured and expressed density in terms of barbs per centimeter of rachis. We repeated this procedure for the two breast and two rump feathers and then averaged the two replicate measurements from each region together to arrive at a single breast density and rump density measurement. We used multiple feathers from the same bird to estimate repeatability and multiple measurements of the same photo to estimate inter-observer measurement error (see supplementary methods). In some cases, we did not have complete measurements because we were missing feathers or had only a single feather. For some nestlings, feathers were so under-developed by day 15 that they were impossible to measure, and those nestlings are excluded from most analyses.

*Nestling Paternity and Molecular Sexing*

Adult and nestling blood samples were stored in lysis buffer (Seutin et al., 1991) in the field and DNA was extracted using Qiagen DNeasy Blood & Tissue Kit spin columns following the standard kit protocol in the lab. We amplified a set of 9 variable microsatellite loci that have been previously used in this population (Hallinger et al., 2019; Makarewich et al., 2009). Our amplification protocol exactly followed that described in Taff et al. (In prep) and details on primer sequences, reaction volumes, cycling conditions, and fragment analysis can be found there. We determined nest of origin by comparing nestlings to their putative mothers (the females from the 2-3 nests in each cross fostering pair). Nestlings that matched a female at 8 of 9 loci were considered to have been laid by that female. In total, x of x nestlings matched at 9 loci and x matched at 8 loci but the mismatch could be explained by a null allele. The other x nestlings could not be definitively assigned to a mother and are excluded from analyses that consider nest of origin (see Taff et al., In prep for discussion; note that this study uses a subset of nestlings from Taff et al. because not all nestlings had feather measurements).

We also determined the sex of each nestling in this study by amplifying an intron of the *CHD1* gene that differs in length on the sex chromosomes in birds (Griffiths et al., 1996). We used the P8 and P2 primers and the cycling conditions described in Whittingham & Dunn (2000). The difference in length of the *CHD1* intron in tree swallows is too small to score reliably on a gel. We followed Whittingham & Dunn (2000) by digesting our PCR product [how long] with the *HaeIII* restriction enzyme (product number). This enzyme cuts at a restriction site in the *CHD1-Z* fragment and removes about 45 bp from that fragment while leaving the *CHD1-W* unchanged. After digestion, we ran products on a x% agarose gel at xx xx. Females were identified by the presence of two clear bands while males had only one. All runs included positive and negative controls with adults of known sex used as positive controls.

*Data Analysis*

Fill in based on analyses done.

All figures and analyses were produced in R version 4.0.2 (R Core Development Team, 2020). All data and code required to reproduce the analyses presented here is available for review in a public GitHub repository (https://github.com/cct663/tres\_feather\_density; data and code will be archived according to journal policies upon acceptance).

**RESULTS**

**DISCUSSION**

1. Summary of main results and their implication
2. Compare/contrast with Callan paper results
3. Is spending a longer time in the nest good because of slow/controlled development, or bad because of lower resources that force slow development? Or maybe quadratic?
4. Multiple stressors/challenges/climate change and ephemeral traits: maybe can get away with skimping on these traits in some situations, but if things are hard that might not work out (e.g., cold snaps – connect to Ryan’s new PNAS paper and their hand raised paper).
5. Caveats or things that aren’t clear from our paper

**ETHICAL NOTE**

We received approval for all of the procedures described here from the Cornell University Institutional Animal Care & Use Board (IACUC protocol # xxx). Sampling and capture in the field were approved by federal and state scientific collecting permits to MNV (federal permit # xxx; state permit # xxx).

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**AUTHOR CONTRIBUTIONS**

Maybe wait and see if the journal we submit to requires this or not.

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