RESEARCH ARTICLE

A parasite reduction conservation intervention does not improve fledging success or most condition metrics for Purple Martins

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ABSTRACT

Eastern Purple Martins (*Progne subis subis*) have an unusually close relationship with humans, as they nest exclusively in man-made nest boxes. Current conservation policy directly promotes further interaction with this species by advocating regular replacement of nest materials during the nestling phase to reduce ectoparasite load and increase nestling fitness. We conducted the first test of the efficacy of this recommendation and found that it was partially effective in reducing parasite abundance, but had no effect on nestling fledging success, body mass, leukocyte count, or triglyceride or uric acid concentration. We found a small but significant increase in nestling hematocrit associated with nest material replacement, implying that parasites may induce nestling anemia. Contrary to our expectations, we also found elevated heterophil/lymphocyte ratios in nestlings with replacements, possibly indicating elevated physiological stress associated with nest replacements. Based on our results, we do not recommend nest material replacements to combat routine parasite infestations.

Keywords: body condition, cavity nesting, conservation, ectoparasites, Purple Martin

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LAY SUMMARY

- Purple Martins are believed to have a high abundance of blood-feeding parasites (fleas, mites, and blowfly larvae) in their nests.
- A current conservation policy recommends that nest box managers regularly remove and replace nest materials during the nestling phase to reduce parasite abundance. We tested the efficacy of this policy.
- Nest replacements reduced flea and blowfly larvae abundance, but mite abundance rebounded quickly.
- There was no difference in fledging rate, body mass, white blood cell count, or triglyceride or uric acid levels for nestlings with or without nest replacements.
- Nestlings with nest replacements were less anemic and had a higher ratio of heterophils to lymphocytes.

Una intervención de conservación de reducción de parásitos no mejora el éxito de emplumamiento o la mayoría de las métricas de condición para *Progne subis subis*

RESUMEN

Progne subis subis tiene una relación inusualmente cercana con los humanos, ya que nidifica exclusivamente en cajas nido hecha por humanos. La política actual de conservación promueve directamente una mayor interacción con esta especie, recomendando el reemplazo regular de materiales del nido durante la fase de polluelo para reducir la carga de ectoparásitos y aumentar la adecuación biológica de los polluelos. Realizamos el primer test sobre la eficacia de esta recomendación y encontramos que fue parcialmente efectiva en reducir la abundancia de parásitos, pero no tuvo efecto en el éxito de emplumamiento del polluelo, en la masa corporal, en el recuento de leucocitos, o en las concentraciones de triglicéridos o de ácido úrico. Encontramos un aumento pequeño pero significativo en el hematocrito de los polluelos asociado con el reemplazo del material del nido, implicando que los parásitos pueden inducir anemia en los polluelos. Contrariamente a nuestras expectativas, también encontramos cocientes elevados de heterófilos/linfocitos en los polluelos con reemplazos, indicando posiblemente un estrés fisiológico elevado asociado con los reemplazos del nido. Basados en nuestros resultados, no recomendamos el reemplazo de los materiales del nido para combatir las infestaciones rutinarias de parásitos.

Palabras clave: anidación en cavidades, condición corporal, conservación, ectoparásitos, *Progne subis subis*

INTRODUCTION

Humans often encourage interactions with birds through nest box provisioning, or bird feeding, through which birds can benefit (Robb et al. 2008, Libois et al. 2012), and humans report reduced stress through an increased connection to nature (Cox and Gaston 2016). Even these seemingly positive interactions may, however, have unintended negative consequences; supplemental feeding can spread wildlife disease (Dhondt et al. 2007, Becker and Hall 2014, Murray et al. 2016) and poorly situated nest boxes could become ecological traps (Mänd et al. 2005, Klein et al. 2007). From a conservation perspective, understanding which human–wildlife interactions are truly beneficial, and which pose risks to either humans or wildlife, is an essential step in defining practical policy.

Purple Martins (*Progne subis*) are migratory aerial insectivores. Like many species in their guild, their populations are in decline across their range (Tautin et al. 2008, Nebel et al. 2010, Sauer et al. 2017). The species has an unusually close association with human settlements, with the eastern subspecies (*P. subis subis*) having shifted from nesting in natural cavities to provisioned nest boxes (Raleigh et al. 2019, Brown and Tarof 2020). The abundance and impact of hematophagous ectoparasites in nest boxes are generally thought to be high (Moss and Camin 1970, Hill 1994) and possibly exacerbated by the derived colonial habit of *P. subis subis.* Also, their relatively long nestling period and cavity nesting itself lead to a favorable microclimate for parasite population growth and transmission between individuals (Møller et al. 1990). Parasite species and abundances likely vary across the range of the Purple Martin, but nest-associated hematophagous ectoparasites commonly include bird blowfly larvae (*Protocalliphora* spp.), mesostigmatid mites (*Dermanyssus* spp.), and fleas (*Ceratophyllus* spp.) (Moss and Camin 1970, Kostka and Hill 1994).

The emergence of human-dependence in Purple Martins implies that effective conservation management during the breeding season has a high potential to affect nesting success (Raleigh et al. 2019). The Purple Martin Conservation Association (PMCA) is the leading conservation group for the species and is dedicated to using scientific research, public education, and management techniques to increase Purple Martin populations across North America (http://www.purplemartin.org/). To mitigate a perceived fitness cost of nest ectoparasites on martin nestlings, the PMCA advises martin "landlords" to replace nest material when nestlings are 10 days old (or earlier in the case of severe infestations) and again at 20 days old (nestlings fledge at 28 days on average) (Kostka and Hill 1994). To complete a nest replacement, landlords must carefully remove nestlings from their cavity, remove all nesting material and

scrape the base of the nest box to remove parasite larvae and eggs, replace nesting material with wood shavings or dry pine needles, and replace the nestlings.

Uptake of this policy has been relatively high with almost 20% of respondents to a recent PMCA member survey (*n* = 1,694; http://www.purplemartin.org/) stating that they both perceived a high parasite load in their colonies and undertook regular nest changes to combat them. This policy of manual parasite removal is easily achieved by Purple Martin landlords and has intuitive appeal, especially since heavy nest parasite infestations have often been associated with fitness costs for nestlings, particularly in cavity-nesting species (Møller et al. 1990). High abundance of ectoparasites has been associated with decreased hatching success (Oppliger et al. 1994), decreased nestling survival (Merino and Potti 1995, Edworthy et al. 2018), and decreased nestling mass (Moss and Camin 1970, Fitze et al. 2004). Beyond the relatively coarse survival and mass metrics, researchers have also often used blood-based measures to show an effect of nest-dwelling invertebrates on birds. Ectoparasitism has been associated with decreased hematocrit levels indicative of infection, anemia, and nutritional deficiency (Potti et al. 1999), and with elevated leukocyte counts (Dufva and Allander 1995) and heterophil/lymphocyte (H/L) ratios (Gross and Siegel 1983, Maxwell and Robertson 1998, Moreno et al. 2002, Owen 2011), indicative of immunological challenge. Parasitism may also result in altered nutritional status of nestlings, which can be determined through plasma metabolites, such as triglycerides and uric acid; high concentrations are associated with fat and protein absorption in the hours prior to blood sampling (Jenni-Eiermann and Jenni 1991, Lopez-Arrabe et al. 2015) and often positively correlate with other body condition metrics (Masello and Quillfeldt 2004, Quillfeldt et al. 2004, Lopez-Arrabe et al. 2015). Plasma metabolites can also provide an index of feeding rate (Zajac et al. 2006), and as such may also reflect an adult's provisioning rate in response to nest ectoparasitism, whether that be through a compensatory increase in feeding (Johnson and Albrecht 1993) or through a reduced rate associated with their own parasite-induced decline in body condition (Wagner et al. 1997, Tomas et al. 2007).

However, while nest ectoparasites often have a deleterious effect on their nestling hosts, results of other investigations are equivocal or do not show negative effects on nestlings (Møller et al. 1990, Rogers et al. 1991, Sanchez et al. 2018), raising the possibility that nest changes for Purple Martins are an unnecessary intervention. In the absence of a fitness cost of parasitism, nest changes may represent an unnecessary stressor to nestlings, as handling of wildlife by untrained individuals is generally not advised because of the potential for causing stress or injury (Sockman and Schwabl 2001). Finally, the humans undertaking nest

changes may be exposed to potential avian-associated zoonoses such as avian influenza, *Salmonella*, West Nile Fever, Lyme disease, *Campylobacter* (Abulreesh et al. 2007), and *Bartonella* (Williams and Dittmar 2020), which may be transmitted while handling the birds or their nesting materials, or when bitten by nest fleas or mites (du Feu 1987, George et al. 2015, Kavallari et al. 2018).

We evaluated the efficacy of Purple Martin nest replacements during the nestling stage through a controlled experiment over a 3-yr period. Parasite load in nests with and without nest replacements was assessed to test the hypothesis that replacements significantly reduce the in-nest abundance of the 3 most prevalent and abundant hematophagous ectoparasites in this system, namely, *Ceratophyllus idius*, *Dermanyssus prognephilus*, and *Protocalliphora sialia* (fleas, mesostigmatid mites, and bird blowfly, respectively). We also tested the hypothesis that nest changes are associated with increased fledging rate or improved body condition through a wide variety of metrics (body mass, hematocrit, leukocyte count, H/L, triglyceride concentration, and uric acid concentration). By conducting a multi-year study, assessing multiple condition metrics, and obtaining a relatively large sample size, we provide a powerful and rigorous test of an active conservation policy and evaluate the case for promoting human–wildlife contact in this context.

METHODS

Field Site

Fieldwork was carried out at Iroquois National Wildlife Refuge and Tonawanda and Oak Orchard Wildlife management areas in Genesee County, New York State, in the summers of 2016, 2017, and 2018 (Figure 1A,B). Nine Purple Martin colonies, with a total of 140 individual nest sites, were included in each study year. Nest box designs included wooden houses (Troyer T14) (*n* = 84) and artificial gourds (Troyer horizontal gourds, McKean, Pennsylvania, USA) (*n* = 56) (Figure 1C). All nest boxes had predator guards and modified entrance holes to exclude European Starlings (*Sturnus vulgaris*) and were managed for other local nest site competitors including House Sparrows (*Passer domesticus*). All colonies in the study were used by Purple Martins for at least 2 yr prior to the start of the study and have been maintained and managed by Fish and Wildlife Service and Department of Environmental Conservation staff and volunteers. Nest boxes were cleaned out at the end of each nesting season and were washed with diluted bleach, then rinsed and dried before the following season.

Nest Replacement Protocol

Individual nests were assigned to a control group (no nest replacements) or an experimental group (nest replacements) in a within-colony alternating pattern such that each control nest was neighbored by an experimental nest on either side. This applied to both the individual gourds and to the multi-unit boxes. We sealed small gaps between nest boxes in the multi-unit boxes with caulk before the first field season began to prevent parasites moving between cavities, and we coated the open edge of each nest box with petroleum jelly to prevent them moving between the hinged door and the edges of nest box trays (Figure 1C).

Nest material in experimental nests was replaced every 6–8 days while nestlings were aged between 5 and 21 days old, resulting in 2 or 3 replacements per nest depending on the exact age of nestlings at the first change (range: 5–11 days old). Handling nestlings older than 21 days is not recommended as it can result in premature fledging. In a nest change, all loose nest material was removed and placed into a sealable polythene bag. We used paintbrushes and scrapers to remove the remaining nest material and parasites into our collection bag. Nest material was replaced with ~50 g of dried pine needles. Samples of pine needles were examined prior to each field season for the presence of parasites, but none were ever found. Due to the alternating study design, all nests within a colony were disturbed and prevented from feeding for the same duration regardless of whether they received nest replacements. Although all nestlings in the study were handled (for banding, mass measurement, and blood draw), nestlings in the experimental group were handled more extensively than the control nestlings during nest replacements as part of the experimental protocol. We collected nest material from both experimental and control nests as soon as possible after the birds fledged using the same protocol.

All collected samples of nest material were processed using Berlese funnels. Nest material was placed under a heat lamp for 2–3 hr, and parasites were collected in ethanol. Preliminary work indicated that 3 hematophagous parasite species were by far the most abundant and prevalent: *Ceratophyllus idius* (hereafter "flea"), *Dermanyssus prognephilus* (hereafter "mite"), and *Protocalliphora sialia* (hereafter "blowfly larva"). Parasites were identified via morphology following Traub et al. (1983), Sabrosky et al. (1989), and Moss (1968), respectively, using a Zeiss microscope, White Plains, New York, USA. Using a dissecting microscope, we counted adult fleas, blowfly larvae, and adult mites in each ethanol sample. When counts of a single species in a single nest reached 200 individuals, we stopped counting individuals and estimated total abundance based on the proportion of nest material processed. We sorted through the dry nest materials to recover blowfly pupal cases. Other hematophagous parasite species such as chewing lice and biting midges did occur in some nests but had low prevalence and abundance in comparison with the fleas, mites, and blowfly, and were omitted from analysis.
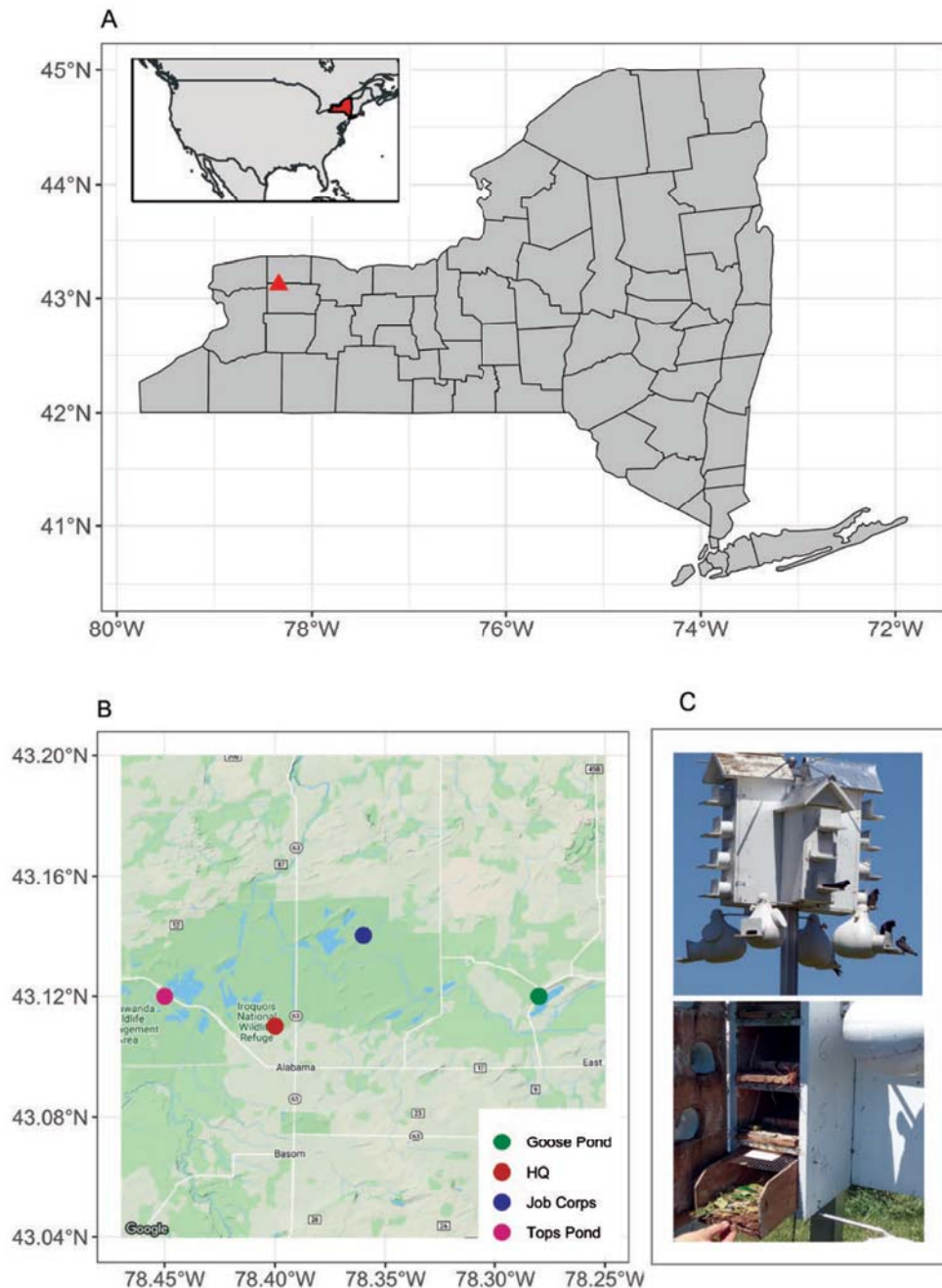
FIGURE 1. (**A**) Map showing the of the study site (Iroquois National Wildlife Refuge and Tonawanda and Oak Orchard Wildlife Management Areas) within New York State. The inset map shows the location of New York State within North America. (**B**) Map showing the location of Purple Martin (*Progne subis*) colonies within the study site. (**C**) View inside the multi-unit T-14 trays with the door opened (upper) and Purple Martin housing with the single-unit artificial gourds and the multi-unit T14 wooden houses (lower).

Nestling Measurements

Nest checks were conducted weekly at each colony and the progress of each nest noted in terms of the number and age of nestling present, number of fledged nestlings, and number of dead nestlings. Nestlings were considered to have successfully fledged when missing from the nest after day 25 (Purple Martins usually fledge between day 26 and day 32; Allen and Nice 1952, Brown and Tarof 2020). Dead nestlings were removed from nests. Nestlings were banded to allow individual identification between 12 and 20 days old (mean = 14). We weighed each nestling at the time of banding on a digital scale. In 2017 and 2018 we also took scaled digital photographs of nestling tarsi to enable accurate measurement (Williams et al. 2020) and

calculation of scaled body mass (Brown 1996). A subset of nests was fitted with in-nest video cameras as part of a parallel study (Williams and DeLeon 2020). Using footage from these cameras we determined the time in minutes prior to blood sampling that all nestlings in a brood were fed (e.g., the time for 5 provisioning events in a brood of 5 nestlings with the assumption that all nestlings are fed one prey item before a second round of provisioning begins).

Blood Sampling

Blood was drawn from a subset of nestlings in 2017 and 2018 when nestlings were between 16 and 20 days old. We aimed to sample the largest and smallest nestling from each nest selected for blood sampling. In each case blood was drawn from the brachial vein using a 27.5-gauge needle to collect a maximum of 140 µL of blood in heparinized microhematocrit capillary tubes. Blood samples were kept on ice in the field until being stored at −70°C later in the day until samples were processed. Blood samples were used to determine hematocrit, triglyceride, and uric acid concentrations, leukocyte count and H/L ratio. Not all assays were performed with all samples due to limited blood volumes.

We measured hematocrit by centrifuging one microhematocrit capillary tube full of blood at 12,000 revolutions per minute for 8 min (Potti et al. 1999). We used digital calipers to measure the total height of the sample and the height of the packed cell layer. Hematocrit was then calculated as the proportion of packed cells to total blood volume.

Plasma metabolites (triglyceride and uric acid concentrations) were measured using a colorimetric endpoint assay using a microplate spectrophotometer (Bio Tek EON and Gen5 2.01 software). Sample concentrations were measured alongside known standard concentrations following Guglielmo et al. (2005) and Smith and McWilliams (2009) for triglyceride and uric acid concentrations, respectively. Measurements were repeated until the coefficient of variation was <10% between all duplicates, except for 3 samples for uric acid and 5 triglyceride samples, which were <15% (Seewagen et al. 2015).

Finally, we used one drop of blood from each nestling sample to make a smear slide. Slides were stained with Wright-Giemsa 3-step stain (VWR Hematology Quick Stain Category No. 10143–224). Using the oil immersion lens of a compound microscope at 1,000x magnification, we counted the total number of leukocytes per 100 fields of view (Horak et al. 1999) with separate counts of heterophils and lymphocytes to estimate the H/L ratio.

Analysis of Parasite Load

Comparison of parasite load between experimental and control nests is not straightforward due to differences in collection regimes. For example, there are likely to be unquantified differences between the study groups in how long a parasite has been in the nest at the time of collection and how many parasites have escaped the nests prior to collection.

To allow comparison of flea and mite loads across experimental conditions over a controlled duration, in 2018 we put a 125 × 75 mm sticky insect sheet (BioQuip Products, Item 2872) into a subset of nest cavities such that fleas and mites might adhere to the surface, but it should not have come into contact with the birds. We left these strips in the nests for 1 week from the time the nestlings were old enough for nest replacements (≥5 days) and continued to replace them weekly until each nest had fledged. By counting the parasites stuck to these strips we were able to directly compare parasite loads between control and experimental nests with a controlled exposure duration.

We log-transformed the daily counts of mites and fleas from each insect sheet to improve their adherence to a normal distribution. In addition to experimental condition, these counts could be affected by factors intrinsic to each nest, including the number of hatchlings (4–6), the age of nestlings during the collection, and the collection date. To include these factors, we used general linear mixed models to predict the number of fleas and mites with nest box as the random factor to account for the repeated collections from the same nest. We used backwards stepwise regression for model selection and considered an experimental effect to be significant when experimental condition was included in the final model with $P < 0.05$.

Unlike mites and fleas, blowfly abundance can be directly compared between experimental and control nests (despite the differences in collection regimes), because of its life cycle and using counts of pupal cases. The blowfly life cycle begins when adult females oviposit in a bird's nest, generally soon after bird eggs hatch (Sabrosky et al. 1989). Development is temperature-dependent (Dawson et al. 2005), but larvae (the only blood-feeding life stage) typically emerge from eggs after 2 days and remain in the blood-feeding larval stage for 11 days before pupating (Gold and Dahlsten 1983, Bennett and Whitworth 1991). After adults emerge from these pupae, pupal cases remain in the nests. This means that a pupa or pupal case counted in our collection implies an 11-day impact on nestlings, whereas a larva may have affected nestlings for a maximum of 11 days depending on its age at the time of collection. This window of larval action can be further constrained where a nest change or eggs have hatched less than 11 days ago (under the assumption that all larvae, but not necessarily eggs, are removed during nest changes and female blowfly only oviposit after bird eggs have hatched). We measured each blowfly larva to estimate its body volume and combined this with a growth

curve to estimate their age in days (Williams and DeLeon 2020). The number of blowfly larvae in each nest collection was then scaled by their age, such that a 5-day-old blowfly was considered to have caused 5/11 of the impact of a blowfly pupae as it had only completed 5 of its 11 blood-feeding days. We then compared total estimated blowfly larval equivalents (sum of pupae and pupal cases + scaled sum of blowfly larvae) between experimental and control nests using a Mann–Whitney U-test (due to a non-normal distribution).

Analysis of Nestling Fitness

Fledging rate. Fledging rate was defined as the proportion of nestlings per nest which successfully fledged out of the number of nestlings still alive when nest replacements began. This definition avoids including nestling mortality that occurred before nest replacements began in the analysis. For control nests the "number of nestlings still alive when nest replacements began" was defined as brood size at 5 days old. As nests with mortality were relatively rare in the dataset, and nests with at least one nestling mortality were more likely to experience further mortalities, we used nest fledging success as a binary response variable where we differentiate between nests with no mortality after nest replacements began and nests where one or more nestlings died.

Fledging success was thus used as the response variable in a binomial general linear model. The global model included experimental condition (control or experimental), site (Headquarters, Tops Pond, Job Corps or Goose Pond; see Figure 1B), nest box type (gourd or cavity), brood size, study year, ordinal hatch date, and the interaction between year and ordinal hatch date as candidate predictors. We ranked all possible combinations of these predictors (such that each predictor is included in models the same number of times) with AIC_c and ranked their importance by summing the Akaike weights over all models in which a parameter appears using the R package *MuMIn* (Bartoń 2018). We based our inference on parameter estimates from the models with ΔAIC_c values of <2 in each case (Burnham and Anderson 2002, Symonds and Moussalli 2011). We conducted post-hoc Tukey's honest significant difference (HSD) tests to check whether experimental condition was a significant predictor of fledging success after correcting for important covariates. Specifically, we ran a second regression model including all variables with a parameter importance greater than 0.5, and then used the residuals of this relationship as the dependent variable in a Tukey HSD test.

Body mass. Nestling body mass was squared to improve adherence to a normal distribution and then used as the response variable in a general linear mixed model. The global model included experimental condition, nestling age, site, nest box type, brood size, study year, ordinal hatch date,

and the interaction between year and ordinal hatch date as fixed predictors with nest identity as a random factor to account for multiple nestlings from the same nest. We used the same AIC_c model selection procedure as detailed above for analysis, and marginal and conditional pseudo R^2 was calculated for the model with the lowest AIC_c. We used the same post-hoc test procedure as used for fledging success to check for a significant effect of experimental condition after correcting for covariates. A parallel analysis was conducted using scaled body mass (where the response variable was the residuals from the mass ~ tarsus regression) with the subset of mass samples matched with tarsus. As parasites are often aggregated among their potential hosts (Christie et al. 1998, Poulin 2007), we tested whether there was a significant difference in the within-nest range of nestling masses between control and nest replacement tests using a T-test.

Blood-based condition metrics. Hematocrit, leukocyte count, H/L, and triglyceride and uric acid concentrations were used as response variables in a set of general linear mixed models. Leukocyte count, H/L, and triglyceride and uric acid concentrations were log-transformed to improve adherence to a normal distribution prior to analysis.

The global model for hematocrit, leukocyte count, and H/L included experimental condition, study year, time of day that blood was drawn, minutes of disturbance prior to blood sampling, site, nest box type, ordinal hatch date, brood size, nestling age, nestling mass, the maximum temperature of the previous day, and the total rainfall over the previous day as fixed predictors, with nest identity as a random factor to account for multiple nestlings from the same nest. The global model for triglyceride and uric acid concentrations included the same candidate predictors as the other blood metrics, with the exception that temperature and rainfall were included over the 3 hr (rather than 24 hr) prior to blood sampling as plasma metabolite level is expected to respond more quickly to environmental conditions (Jenni-Eiermann and Jenni 1991). We used the same AIC protocol for model selection and estimation and the same post-hoc test protocol to check for the effect of experimental condition, as detailed above. Finally, we conducted parallel analyses of triglyceride and uric acid concentrations for the subset of samples for which we have provisioning rate data.

All statistical analyses and figures were produced using R Studio (RStudio Team 2015) in the R statistical environment, 3.5.2 (R Development Core Team 2018).

RESULTS

Analysis of Parasite Load
Nest changes did not significantly reduce the abundance of mites in the nest over a 7-day period after controlling
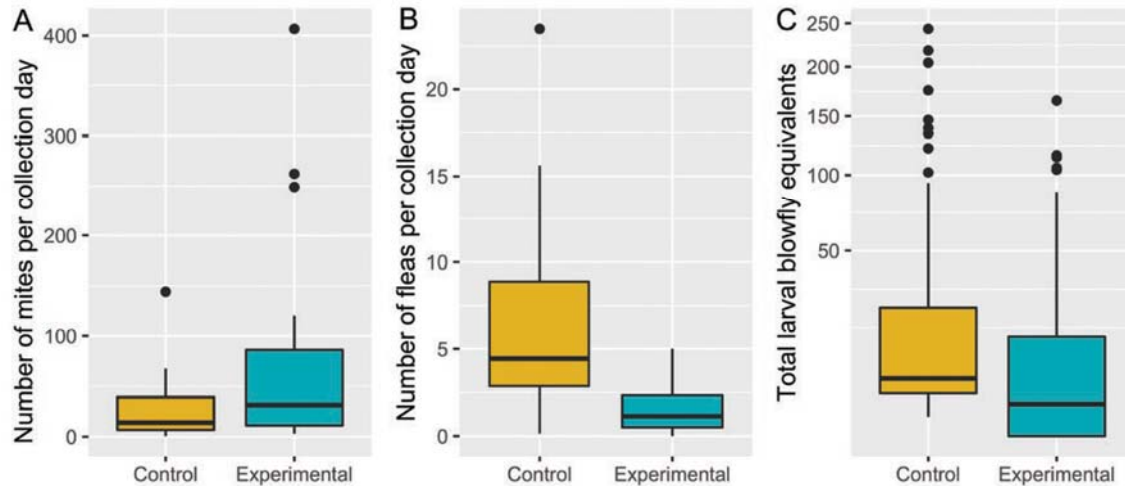
FIGURE 2. Effect of nest material replacements for Purple Martin (*Progne subis*) nestlings in western New York State on parasite abundance of (**A**) mites, (**B**) fleas, and (**C**) blowfly larvae in 3 breeding seasons (2016–2018). Lower and upper hinges on boxes represent the 25th and 75th percentile of data, respectively, with bold horizontal line showing the median value. Lower and upper whiskers show the lowest and highest values (excluding outliers), respectively, with upper outliers (values more than 1.5 times the interquartile range) shown as black points. Note the square root transformation on the *y*-axis in (**C**).

for other factors (estimate = −0.13, standard error [SE] = 0.65, $T_{(9.97)} = -0.20$, $P = 0.85$, $n_{\text{control}} = 17$ from 7 nests, $n_{\text{parasite reduction}} = 25$ from 7 nests) (Figure 2A). Conversely, flea abundance was halved by nest changes (estimate = −1.25, SE = 0.44, $T_{(11.34)} = -2.83$, $P = 0.02$) (Figure 2B). Finally, nest changes significantly reduced the exposure of nestlings to the blood-feeding larval stage of blowfly from a mean of 25.4 (SD = 45.36) to 15.3 (SD = 29.90) complete larval stages ($z_{215} = 4515$, $P < 0.001$, $n_{\text{control}} = 151$ nests, $n_{\text{parasite reduction}} = 160$ nests) (Figure 2C).

Analysis of Nestling Fitness

Our analysis was based on a maximum sample size of 704 nestlings from 160 nests in the control condition and 705 nestlings from 151 nests in the parasite-reduction condition. Sample sizes for specific analyses vary and are detailed in the relevant sections below.

Fledging success. Overall fledging rate was 95% (1,283 fledglings of 1,356 nestlings) after day 5 of nestling life with just 15% (45 of 311) nests in the study experiencing any mortality (Table 1). Causes of mortality for nestlings are unknown, but we presume predation to be low due to the predator exclusion features of nest boxes and the fact that dead nestlings were generally found in nest boxes rather than missing from brood counts. Summed Akaike weights indicated that hatch date (estimate = −0.15, SE = 0.04, Z = −3.63, $P < 0.001$), year (estimate = −34.95, SE = 12.63, Z = −2.77, $P = 0.006$; estimate = −15.94, SE = 8.13, Z = −1.96, $P = 0.05$), the interaction between year and hatch date and brood size (estimate = −0.39, SE = 0.17, Z = −2.25, $P = 0.02$) were the

most important determinants of fledging success, with nest box type, site, and experimental condition having lower importance (Table 2, Supplementary Material Table S1A). Fledging success was higher in 2017 and 2018 than in 2016 (Supplementary Material Figure S1A), decreased with brood size (Supplementary Material Figure S1B), and decreased with hatch date in 2016 and 2017, but not in 2018 (Supplementary Material Figure S1C). Post-hoc tests did not show any difference in fledging success by experimental condition after correcting for informative variables (Table 3).

Body mass. We obtained mass data for 1,409 nestlings from 311 nests ($n_{\text{control}} = 704$, $n_{\text{parasite reduction}} = 705$) (Table 1). Summed Akaike weights indicated little variation in the relative importance of parameters included in the model (Table 2), implying all explanatory variables considered had a small but distinct role in determining body mass. Accordingly, the global model was shown to be the most parsimonious according to AIC_c rankings, and the only model with $\Delta\text{AIC}_c < 2$ (pseudo-$R^2_{\text{marginal}} = 0.26$, pseudo-$R^2_{\text{conditional}} = 0.63$) (Supplementary Material Table S1B). Nestling body mass increased slightly with nestling age (Supplementary Material Figure S2A); was lower in 2018; varied by site; decreased with ordinal hatch date (Supplementary Material Figure S2B); was greater in gourds than cavities, decreased with brood size (Supplementary Material Figure S2C); and was greater in experimental nests (although this difference was not statistically significant) (Table 2). Post-hoc tests also did not show any difference in body mass by experimental condition after correcting for important variables (Table 3). Results for the parallel study in which body mass was scaled by

TABLE 1. Fledging success and uncorrected summary metrics of body mass and condition indices for Purple Martin (*Progne subis*) nestlings between the experimental (parasite reduction treatment) and control treatments in 3 yr of study (2016, 2017, and 2018) at colonies in western New York State.

| | Experimental | | Control | |
| | 85% ($n = 151$) | | 86% ($n = 160$) | |
Fledging success	Mean (SD)	Range	Mean (SD)	Range
Nestling body mass (g)	52.06 (6.22)	25.9–65.4	52.35 (6.01)	22.2–65.2
Hematocrit	0.38 (0.06)	0.21–0.48	0.33 (0.05)	0.20–0.43
Leukocytes per 10,000 erythrocytes	427 (213)	146–649	445 (243)	140–1304
Heterophil/lymphocyte ratio	7.21 (4.89)	1.69–24.36	4.23 (3.43)	0.52–13.34
Triglyceride (mmol L^{-1})	1.65 (0.79)	0.39–4.23	2.18 (1.32)	0.63–6.50
Uric acid (mmol L^{-1})	1.27 (0.57)	0.07–2.19	1.52 (1.22)	0.19–6.16

tarsus were qualitatively similar, the top model explained a similar amount of variation (pseudo-$R^2_{marginal} = 0.19$, pseudo-$R^2_{conditional} = 0.61$, $n = 724$) and did not support a strong effect of experimental condition (Supplementary Material Table S2). There was no evidence for an increased range of within-brood body mass in control nests compared with nest replacement nests ($T_{(294.4)} = -1.22$, $P = 0.22$, mean$_{control} = 7.02$ g, SD$_{control} = 4.61$, mean$_{parasite reduction} = 7.69$ g, SD$_{parasite reduction} = 4.83$, $n = 298$ nests).

Hematocrit. We obtained hematocrit measurements for 102 nestlings from 56 nests ($n_{control} = 54$, $n_{parasite reduction} = 48$) (Table 1). Summed Akaike weights indicated that experimental condition was by far the most important predictor for hematocrit (Table 2) and was the only retained predictor in the most parsimonious model, although there was a relatively low predictive power (pseudo-$R^2_{marginal} = 0.13$, pseudo-$R^2_{conditional} = 0.53$), and the second most parsimonious model only included the intercept and random effects of nest identity (Supplementary Material Table S1C). Mean hematocrit increased from 0.33 in control nests to 0.38 in parasite reduction treatment nests (Figure 3). Post-hoc tests supported this difference after correcting for nest level variation (Table 3).

Leukocyte count. We obtained leukocyte counts for 98 nestlings from 52 nests ($n_{control} = 49$, $n_{parasite reduction} = 49$) (Table 1). The most parsimonious model (pseudo-$R^2_{marginal} = 0.10$, pseudo-$R^2_{conditional} = 0.72$) retained time of blood draw and brood size as predictors, where leukocyte count increased when blood was drawn later in the day (Table 2, Supplementary Material Figure S3A), and decreased with increased brood size (Supplementary Material Figure S3B, Supplementary Material Table S1D). However, there was considerable model uncertainty with 4 models having $\Delta AIC_c < 2$ (Supplementary Material Table S1). Post-hoc tests did not show any difference in leukocyte by experimental condition after correcting for important variables (Table 3).

Heterophil/lymphocyte. We obtained H/L for 98 nestlings from 52 nests ($n_{control} = 49$, $n_{parasite reduction} = 49$) (Table 1). The most parsimonious model (the only

model with $\Delta AIC_c < 2$) (pseudo-$R^2_{marginal} = 0.36$, pseudo-$R^2_{conditional} = 0.69$) retained time of blood draw and experimental condition as predictors, where H/L increased when blood was drawn later in the day (Table 2, Supplementary Material Figure S4), and was higher in the experimental parasite reduction treatment brood size (Figure 4, Supplementary Material Table S1E). Visualization of data highlighted one potential outlier (H/L ratio = 24.36). When analysis was repeated with the exclusion of this outlier the results were qualitatively the same, so we maintained the data point in the analysis. Post-hoc tests supported an increased H/L in experimental parasite reduction nests after correcting for important variables (Table 3).

Triglyceride concentration. We obtained measurements of plasma triglyceride concentration for 68 nestlings from 46 nests ($n_{control} = 34$, $n_{parasite reduction} = 34$) (Table 1). The most parsimonious model (the only model with $\Delta AIC_c < 2$) (pseudo-$R^2_{marginal} = 0.24$, pseudo-$R^2_{conditional} = 0.79$) retained only these factors in the model, where triglyceride concentration increased when blood was drawn later in the day (Supplementary Material Figure S5A), and with temperature (Table 2, Supplementary Material Figure S5B, Supplementary Material Table S1F). When the effect of provisioning rate on triglyceride concentration was considered ($n = 30$), it was not highlighted as an important predictor. Post-hoc tests did not show any difference in triglyceride concentration by experimental condition after correcting for important variables (Table 3).

Uric acid concentration. We obtained measurements of plasma uric acid concentration for 95 nestlings from 53 nests ($n_{control} = 50$, $n_{parasite reduction} = 45$) (Table 1). The most parsimonious model (the only model with $\Delta AIC_c < 2$) (pseudo-$R^2_{marginal} = 0.63$, pseudo-$R^2_{conditional} = 0.84$) retained only these factors in the model, where uric acid concentration increased when blood was drawn later in the day (Supplementary Material Figure S6A), and declined with increasing rainfall (Supplementary Material Figure S6B, Table 2, Supplementary Material Table S1G). When the effect of provisioning rate on uric acid concentration was considered ($n = 30$), its inclusion did not

TABLE 2. Relative parameter importance of variables explaining fledging success, nestling body mass and condition indices for Purple Martins (*Progne subis*) based on the Akaike sum of weights. Parameters retained in the most parsimonious model are shown in bold. The direction of the relationship is indicated with + or – for continuous predictors maintained in the most parsimonious model analysis.

Candidate predictors	Fledging success	Body mass (g)	Hematocrit	Leukocytes per 10,000 erythrocytes	Heterophil/ lymphocyte ratio	Triglyceride (mmol L⁻¹)	Uric acid (mmol L⁻¹)
Experimental condition	0.27	**0.98**	**0.63**	0.10	**0.99**	0.19	0.14
Site	0.17	**1.00**	0.01	0.10	<0.01	<0.01	<0.01
Year	**0.95**	**1.00**	0.02	0.12	<0.01	<0.01	<0.01
Ordinal hatch date	**1.00(–)**	**1.00 (–)**	<0.01	0.07	<0.01	<0.01	<0.01
Year*Ordinal hatch date	**0.88**	**1.00**	NA	NA	NA	NA	NA
Nest box type	0.46	**0.98**	0.04	0.10	<0.01	<0.01	<0.01
Brood size	**0.81 (–)**	**1.00 (–)**	<0.01	**0.53 (–)**	0.05	0.16	0.04
Age	NA	**1.00 (+)**	<0.01	0.30	0.11	0.02	0.02
Time of blood sampling	NA	NA	0.11	**0.51 (+)**	**1.00 (+)**	**0.79 (+)**	**0.99 (+)**
Minutes disturbed before blood draw	NA	NA	<0.01	0.02	<0.01	<0.01	<0.01
Mass	NA	NA	<0.01	0.12	0.01	<0.01	0.01
Maximum temperature over 24 hr	NA	NA	<0.01	0.02	0.02	NA	NA
Total rainfall over 24 hr	NA	NA	<0.01	0.23	0.24	NA	NA
Maximum temperature over 3 hr	NA	NA	NA	NA	NA	**0.90 (+)**	0.03
Total rainfall over 3 hr	NA	NA	NA	NA	NA	0.02	**1.00 (–)**

outcompete the most parsimonious model. Post-hoc tests did not show any difference in uric acid concentration by experimental condition after correcting for important variables (Table 3).

DISCUSSION

We found that nest replacements resulted in significantly reduced abundance for 2 of the 3 most prevalent and abundant ectoparasite species of Purple Martins, but did not result in significant differences in fledging success, nestling body mass, leukocyte count, or plasma metabolite concentrations. Increased nestling hematocrit and H/L were, however, associated with nest material replacements.

While nest changes were associated with a significant and enduring decline in the abundance of fleas and blowfly larvae, the procedure did not eliminate these parasites. Furthermore, there was no evidence for a reduction in mite abundance over a 1-week time period. In practice, not all individual parasites were removed during a nest change as some were inaccessible in tight corners of nest cavities and underneath gourd traction strips. Similarly, it is possible that eggs of any or all of the 3 parasite species were missed during nest changes. Given the short life cycles of mites (6–10 days) (Tucci et al. 2008) in particular, any nymphs or eggs missed during a nest change could potentially develop and multiply within 1 week, limiting nest changes to a short-term abundance reduction for this parasite species. Finally, our alternating study design means that control and parasite reduction nests were in close proximity, raising the

TABLE 3. Results of post-hoc Tukey HSD test for effect of experimental condition after correcting for the effects of parameters with a variable importance of at least 0.5. Statistically significant comparisons are shown in bold. Mean and standard deviation values are based on the residuals from the regressions adjusting for parameters with a variable importance of at least 0.5 (not including experimental condition).

|	F	df	P	Mean residual value (SD): Control	Mean residual value (SD): Experimental
Fledging success	0.226	309	0.635	0.11 (2.79)	–0.07 (3.74)
Body mass (g)	0.155	1407	0.694	–3.53 (343.72)	3.53 (330.39)
Hematocrit	**4.940**	**100**	**0.029**	**–0.006 (0.03)**	**0.007 (0.04)**
Leukocyte count per 10,000 erythrocytes	0.033	96	0.856	–0.004 (0.24)	0.004 (0.18)
Heterophil/lymphocyte	**5.344**	**96**	**0.023**	**–0.07 (0.34)**	**0.07 (0.27)**
Triglyceride (mmol L⁻¹)	0.644	66	0.425	0.02 (0.15)	–0.02 (0.19)
Uric acid (mmol L⁻¹)	0.375	93	0.542	0.02 (0.27)	–0.02 (0.28)
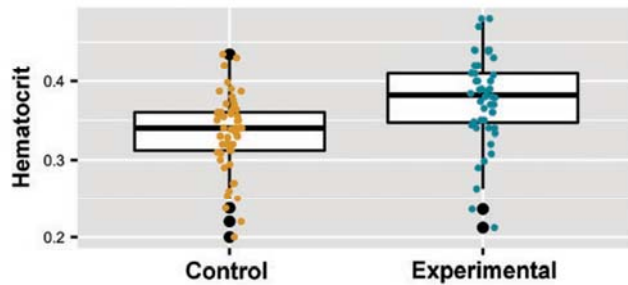
FIGURE 3. Effect of nest material replacements on Purple Martin (*Progne subis*) nestling hematocrit over 2 breeding seasons (2017–2018) in western New York State. Yellow and turquoise points show raw data from the control and experimental groups, respectively. Lower and upper hinges on boxes represent the 25th and 75th percentile of data, respectively, with the bold horizontal line showing the median value. Lower and upper whiskers show the lowest and highest values (excluding outliers), respectively, with outliers (values above or below 1.5 times the interquartile range) shown as black points.

FIGURE 4. Effect of nest material replacements on Purple Martin (*Progne subis*) nestling heterophil/lymphocyte ratio over 2 breeding seasons (2017–2018) in western New York State. Yellow and turquoise points show raw data from the control and experimental groups, respectively. Lower and upper hinges on boxes represent the 25th and 75th percentile of data, respectively, with the bold horizontal line showing the median value. Lower and upper whiskers show the lowest and highest values (excluding outliers), respectively, with outliers (values above or below 1.5 times the interquartile range) shown as black points.

possibility of parasite movement between conditions (although we took steps to reduce this risk), which might particularly affect mite populations, because they are the most mobile of the 3 parasite species. The reduction in blowfly larva, the largest parasite in this system with the highest potential for blood draw (Gold and Dahlsten 1983) was, however, more marked, meaning nestlings in nests with nest changes likely had a substantial reduction in overall blood loss compared with those in the control nests.

We found no evidence for a beneficial (or detrimental) effect of nest replacements for nestling fledging success, body mass, leukocyte count, or triglyceride or uric acid concentrations. Nor did we find evidence of an increased within-brood range of body mass in control nests, implying that ectoparasites in control nests are not aggregating on one nestling in a clutch. Nest changes resulted in increased nestling hematocrit, from a mean of 0.33 in control nests to a mean of 0.38. Although a modest increase, this increase takes nestlings beyond the commonly defined level of anemia (0.35) to a healthy level (Campbell and Dein 1984, Mitchell and Johns 2008), and implies that blood loss from hematophagous ectoparasites is not being completely compensated by increased nestling erythropoiesis (Johnson and Albrecht 1993, Merino and Potti 1995, Potti et al. 1999). However, we caution against placing too much weight on this result for 2 reasons. Firstly, considering both the AIC$_c$ and log likelihood, the inclusion of experimental condition in the hematocrit model was only marginally more supported than a null model accounting only for nest identity (Arnold 2010). Secondly, hematocrit as a measure of body condition has been questioned as it has not always been found to correlate with other condition metrics and may also covary with nestling workload (Dawson and Bortolotti 1997, Cuervo et al. 2007, Fair et al. 2007, Potti 2007), meaning this result does not
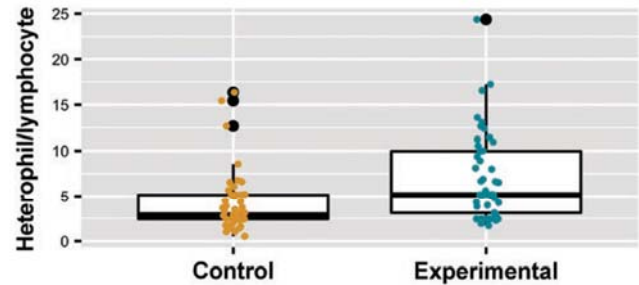
stand alone as compelling evidence of a parasite-mediated fitness effect.

The ratio of heterophils to lymphocytes is a widely accepted indicator of stress, where a relatively high heterophil count is generally associated with fighting infection and inflammation (Maxwell and Robertson 1998, Davis et al. 2008) and thus indicates poor body condition. Previous studies have associated a high ectoparasite load with a high H/L in nestling birds (Moreno et al. 2002, Lobato et al. 2005, Müller et al. 2011) and in reptiles (Knapp et al. 2019). Surprisingly, we found evidence for the opposite trend, where H/L was highest in the nests with decreased parasite load. One possible explanation for this is that the increased handling of nestlings in the parasite reduction treatment increased stress. Heterophil production increases, while lymphocyte production decreases in response to elevated corticosterone (Davis et al. 2008), which is, itself, frequently associated with handling stress (Sockman and Schwabl 2001). It may thus be that any beneficial decrease in H/L in the parasite reduction treatment nests due to reduction in the inflammatory response caused by ectoparasitism was negated by increased physiological stress associated with handling. A second possibility is that the original unchanged nest materials were conferring a benefit to the nestlings that was removed in nest changes. Purple Martins often line their nests with green leaves prior to egg laying (Brown and Tarof 2020). These leaves remained in the nests in the control group but were removed in the parasite reduction treatment. Although the function of the green leaves has not been tested in Purple Martins, the "drug hypothesis" suggests that aromatic properties of the plants could have a direct positive benefit on nestlings through mediation of the immune responses (Gwinner et al. 2000), or reduction of nest bacterial load

(Gwinner and Berger 2005), plausibly explaining our unexpected finding of increased H/L where the green leaves were removed with the parasites. This hypothesis has gained experimental support with the findings of reduced bacterial load, increased nestling body mass and decreased lymphocyte count in European Starlings (Gwinner et al. 2000, Gwinner and Berger 2005), and increased rates of mass gain and feather development in Blue Tits (*Cyanistes caerulus*) incorporating aromatic green leaves in their nests (Mennerat et al. 2009). Further research could consider whether this behavior in the Purple Martins fulfills this function, or whether it is more associated with courtship (Viega et al. 2006) or reduction of nest ectoparasite load (Scott-Baumann and Morgan 2015). Finally, it should be noted that the mean H/L measurements in both control (4.23) and experimental groups (7.21) were high compared with literature results for other species (e.g., 0.45 and 0.62 in nestling Pied Flycatchers [*Ficedula hypoleuca*] [Moreno et al. 2002, Lobato et al. 2005], 1.2 in Eurasian Kestrels [*Falco tinnunculus*] [Müller et al. 2011], 0.8–1.4 in Red-tailed Tropicbirds [*Phaeton rubricauda westralis*] [Dehnhard et al. 2011]), and with our own results from Eastern Bluebirds (*Sialia sialis*) (mean H/L = 1.26) and Tree Swallows (*Tachycineta bicolor*) (mean H/L = 0.79) breeding sympatrically with these Purple Martins (H. M. Williams personal observation). This suggests that the Purple Martins in both groups may be stressed with an elevated immune response. In this scenario, the more heavily parasitized nestlings in the control group may have started with the same high H/L as in the nest replacement group, but were unable to sustain the cost of maintaining this elevated immune system as heterophils were depleted following ectoparasites triggering the immune response (Dufva and Allander 1995, Owen and Moore 2008).

That we found no effect of parasite load on fledging success, body mass, or leukocyte count implies that parasitized nestlings are able to tolerate the effect of these parasites (DeSimone et al. 2018) and that nest changes are neither beneficial nor detrimental to nestlings in terms of these metrics. Other studies have found only equivocal evidence for parasite effects in these measures (Rogers et al. 1991, Roby et al. 1992, Saino et al. 1998, Hannam 2006). We likewise found no effect of parasite load on nestling triglyceride or uric acid concentrations. This partially supports the findings of Quillfeldt et al. (2004) and Lopez-Arrabe et al. (2015), who found no effect of ectoparasite load on triglyceride concentration, but did associate reduced total plasma protein with increased ectoparasite load. This implies that the feeding rate for nestlings is similar across differing parasite loads, and was supported by a parallel study which showed only a small increase in provisioning rate for highly parasitized nests (Williams and DeLeon

2020). We did, however, find that plasma metabolite concentrations decrease in wet and cool weather which, given that provisioning responded closely to temperature in this population (Williams and DeLeon 2020), supports the use of these metrics as an index related to feeding rate (Zajac et al. 2006).

Our test of an active conservation policy aimed at reducing presumed fitness costs of ectoparasites on nestling birds yielded little evidence for its efficacy across a wide range of condition metrics (Brown 1996). Although there are likely cases of high parasite abundance, which may cause negative nestling fitness consequences, and would thus justify the nest replacement policy, it is not clear that they were reached in 3 yr of study at our study site. Based on our results and given the additional potential for human exposure to nest borne parasites and zoonoses associated with nest changes, we do not recommend that Purple Martin "landlords" complete nest changes for routine infestations. Our results may have management implications for other nestbox breeding species with similar assemblages of ectoparasites, such as Western Bluebirds (*Sialia mexicana*), Eastern Bluebirds, and Tree Swallows, for which nest replacements have also historically been promoted (Mason 1944). We further suggest that future research may consider whether there is a "tipping point" of parasite abundance that does result in negative fitness consequences in Purple Martins, how to recognize it, and whether nest changes can prevent it.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *The Condor: Ornithological Applications* online.

and special use permit (#2016-002); US Fish and Wildlife Service scientific collecting permit (MB83070B-0); Federal bird banding permit (#08066); New York State Department of Environmental Conservation temporary revocable permit; New York State Department of Environmental Conservation license to collect or possess (scientific) (#1976); and New York State Department of Environmental Conservation license to collect or possess (banding) (#179). **Author contributions**: H.M.W. and K.D. conceived the experiment. H.M.W. and S.S.P. performed experiments, developed methods, and analyzed data. H.M.W. wrote the paper and all the authors edited the manuscript.

Data availability: Analyses reported in this article can be reproduced using the data provided by Williams et al. (2020).

LITERATURE CITED

Abulreesh, H. H., R. Goulder, and G. W. Scott (2007). Wild birds and human pathogens in the context of ringing and migration. Ringing & Migration 23:193–200.

Allen, R. W., and M. M. Nice (1952). A study of the breeding biology of the Purple Martin (*Progne subis*). The American Midland Naturalist 47:606–665.

Arnold, T. W. (2010). Uninformative parameters and model selection using Akaike's Information Criterion. The Journal of Wildlife Management 74:1175–1178.

Bartoń, K. (2018). MuMIn: Multi-model inference. R package version 1.42.1. https://cran.r-project.org/package=MuMIn.

Becker, D. J., and R. J. Hall (2014). Too much of a good thing: Resource provisioning alters infectious disease dynamics in wildlife. Biology Letters 10:20140309. https://dx.doi.org/10.1098%2Frsbl.2014.0309.

Bennett, G. F., and T. L. Whitworth (1991). Studies on the life-history of some species of *Protocalliphora* (Diptera, Calliphoridae). Canadian Journal of Zoology 69:2048–2058.

Brown, C. R., and S. Tarof (2020). Purple Martin (*Progne subis*), version 1.0. In Birds of the World (A. F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.purmar.01.

Brown, M. E. (1996). Assessing body condition in birds. In Current Ornithology (V. Nolan, Jr. and E. D. Kettersons, Editors). Plenum Press, New York, NY, USA. pp. 67–135.

Burnham, K. P., and D. R. Anderson (2002). Model Selection and Multimodel Inference: A Practical Information-Theoretical Approach, 2nd edition. Springer Verlag, New York, NY, USA.

Campbell, T. W., and F. J. Dein (1984). Avian hematology, the basics. Veterinary Clinics of North America: Small Animal Practice 14:223–248.

Christie, P., A. P. Møller, and F. de Lope (1998). Immunocompetence and nestling survival in the House Martin: The tasty chick hypothesis. Oikos 83:175–179.

Cox, D. T., and K. J. Gaston (2016). Urban bird feeding: Connecting people with nature. Plos One 11:e0158717.

Cuervo, J. J., A. P. Møller, and F. de Lope (2007). Haematocrit is weakly related to condition in nestling Barn Swallows *Hirundo rustica*. Ibis 149:128–134.

Davis, A. K., D. L. Maney, and J. C. Maerz (2008). The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. Functional Ecology 22:760–772.

Dawson, R. D., and G. R. Bortolotti (1997). Are avian hematocrits indicative of condition? American Kestrels as a model. The Journal of Wildlife Management 61:1297–1306.

Dawson, R. D., K. K. Hillen, and T. L. Whitworth (2005). Effects of experimental variation in temperature on larval densities of parasitic *Protocalliphora* (Diperta: Calliphoridae) in nests of Tree Swallows (Passeriformes: Hirundinidae). Environmental Entomology 34:563–568.

Dehnhard, N., P. Quillfeldt, and J. C. Hennicke (2011). Leucocyte profiles and H/L ratios in chicks of Red-tailed Tropicbirds reflect the ontogeny of the immune system. Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology 181:641–648.

DeSimone, J. G., E. D. Clotfelter, E. C. Black, and S. A. Knutie (2018). Avoidance, tolerance, and resistance to ectoparasites in nestling and adult Tree Swallows. Journal of Avian Biology 49:jav-01641. https://doi.org/10.1111/jav.01641.

Dhondt, A. A., K. V. Dhondt, D. M. Hawley, and C. S. Jennelle (2007). Experimental evidence for transmission of *Mycoplasma gallisepticum* in House Finches by fomites. Avian Pathology: Journal of the W.V.P.A 36:205–208.

du Feu, C. R. (1987). Some observations on fleas emerging from tit nestboxes. Ringing & Migration 8:123–128.

Dufva, R., and K. Allander (1995). Intraspecific variation in plumage coloration reflects immune response in Great Tit (*Parus major*) males. Functional Ecology 9:785–789.

Edworthy, A. B., N. E. Langmore, and R. Heinsohn (2018). Native fly parasites are the principal cause of nestling mortality in endangered Tasmanian pardalotes. Animal Conservation 22:96–103.

Fair, J., S. Whitaker, and B. Pearson (2007). Sources of variation in haematocrit in birds. Ibis 149:535–552.

Fitze, P. S., B. Tschirren, and H. Richner (2004). Life history and fitness consequences of ectoparasites. Journal of Animal Ecology 73:216–226.

George, D. R., R. D. Finn, K. M. Graham, M. F. Mul, V. Maurer, C. V. Moro, and O. A. Sparagano (2015). Should the poultry red mite *Dermanyssus gallinae* be of wider concern for veterinary and medical science? Parasites & Vectors 8:178.

Gold, C. S., and D. L. Dahlsten (1983). Effects of parasitic flies (*Protocalliphora* spp.) on nestlings on Mountain and Chestnut-backed Chickadees. The Wilson Bulletin 95:560–572.

Gross, W. B., and H. S. Siegel (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Diseases 27:972–979.

Guglielmo, C. G., D. J. Cerasale, and C. Eldermire (2005). A field validation of plasma metabolite profiling to assess refueling performance of migratory birds. Physiological and Biochemical Zoology 78:116–125.

Gwinner, H., and S. Berger (2005). European Starlings: Nestling condition, parasites and green nest material during the breeding season. Journal of Ornithology 146:365–371.

Gwinner, H., M. Oltrogge, L. Trost, and U. Nienaber (2000). Green plants in starling nests: Effects on nestlings. Animal Behaviour 59:301–309.

Hannam, K. (2006). Ectoparasitic blow flies (*Protocalliphora* sp.) and nestling Eastern Bluebirds (*Sialia sialis*): Direct effects and compensatory strategies. Canadian Journal of Zoology 84:921–930.

Hill, J. R. I. (1994). What's bugging your birds? An introduction to the ectoparasites of Purple Martins. Purple Martin Update. https://www.purplemartin.org/uploads/media/5-1-buggingyourbirds-340.pdf.

Hõrak, P., L. Tegelmann, I. Ots, and A. P. Møller (1999). Immune function and survival of Great Tit nestlings in relation to growth conditions. Oecologia 121:316–322.

Jenni-Eiermann, S., and L. Jenni (1991). Metabolic responses to flight and fasting in night-migrating passerines. Journal of Comparative Physiology B 161:465–474.

Johnson, L. S., and D. J. Albrecht (1993). Effects of hematophagous ectoparasites on nestling House Wrens, *Troglodytes aedon* — Who pays the cost of parasitism? Oikos 66:255–262.

Kavallari, A., T. Küster, E. Papadopoulos, L. S. Hondema, Ø. Øines, J. Skov, O. Sparagano, and E. Tiligada (2018). Avian mite dermatitis: Diagnostic challenges and unmet needs. Parasite Immunology 40:e12539.

Klein, Á., T. Nagy, T. Csörgő, and R. Mátics (2007). Exterior nest-boxes may negatively affect Barn Owl *Tyto alba* survival: An ecological trap. Bird Conservation International 17:273–281.

Knapp, C. R., C. Perez-Heydrich, T. T. Zachariah, J. Jollay, A. N. Schnelle, S. D. Buckner, C. R. Lattin, and L. M. Romero (2019). Host sex, size, and hemoparasite infection influence the effects of ectoparasitic burdens on free-ranging iguanas. Ecology and Evolution 9:1946–1956.

Kostka, K., and J. R. I. Hill (1994). How and why do nest replacements for Purple Martins. Purple Martin Update. https://www.purplemartin.org/uploads/media/how-why-to-do-nest-r-332.pdf.

Libois, E., O. Gimenez, D. Oro, E. Mínguez, R. Pradel, and A. Sanz-Aguilar (2012). Nest boxes: A successful management tool for the conservation of an endangered seabird. Biological Conservation 155:39–43.

Lobato, E., J. Moreno, S. Merino, J. J. Sanz, and E. Arriero (2005). Haematological variables are good predictors of recruitment in nestling Pied Flycatchers (*Ficedula hypoleuca*). Ecoscience 12:27–34.

López-Arrabé, J., A. Cantarero, L. Pérez-Rodríguez, A. Palma, C. Alonso-Alvarez, S. González-Braojos, and J. Moreno (2015). Nest-dwelling ectoparasites reduce antioxidant defences in females and nestlings of a passerine: A field experiment. Oecologia 179:29–41.

Mänd, R., V. Tilgar, A. Lõhmus, and A. Leivits (2005). Providing nest boxes for hole-nesting birds – Does habitat matter? Biodiversity and Conservation 14:1823–1840.

Masello, J. F., and P. Quillfeldt (2004). Are hematological parameters related to body condition, ornamentation and breeding success in wild burrowing parrots *Cyanoliseus patagonus*? Journal of Avian Biology 35:445–454.

Mason, E. A. (1944). Parasitism by *Protocalliphora* and management of cavity nesting birds. The Journal of Wildlife Management 8:232–247.

Maxwell, M. H., and G. W. Robertson (1998). The avian heterophil leucocyte: A review. World's Poultry Science Journal 54:155–178.

Mennerat, A., P. Perret, P. Bourgault, J. Blondel, O. Gimenez, D. W. Thomas, P. Heeb, and M. M. Lambrechts (2009). Aromatic plants in nests of Blue Tits: Positive effects on nestlings. Animal Behaviour 77:569–574.

Merino, S., and J. Potti (1995). Pied Flycatchers prefer to nest in clean nest boxes in an area with detrimental nest ectoparasites. The Condor 97:828–831.

Mitchell, E. B., and J. Johns (2008). Avian hematology and related disorders. The Veterinary Clinics of North America. Exotic Animal Practice 11:501–22, vi.

Møller, A. P., K. Allander, and R. Dufva (1990). Fitness effects of parasites on passerine birds: A review. In Population Biology of Passerine Birds, vol. 24 (J. Blondel, A. Gosler, J. D. Lebreton, and R. McCleery, Editors). Springer, Berlin, Heidelberg, Germany. pp. 269–280.

Moreno, J., S. Merino, J. Martinez, J. J. Sanz, and E. Arriero (2002). Heterophil/lymphocyte ratios and heat-shock protein levels are related to growth in nestling birds. Ecoscience 9:434–439.

Moss, W. W. (1968). An illustrated key to the species of the acarine genus *Dermanyssus* (Mesostigmata: Laelapoidea: Dermanyssidae). Journal of Medical Entomology 5:67–84.

Moss, W. W., and J. H. Camin (1970). Nest parasitism, productivity, and clutch size in Purple Martins. Science 168:1000–1003.

Müller, C., S. Jenni-Eiermann, and L. Jenni (2011). Heterophils/lymphocytes-ratio and circulating corticosterone do not indicate the same stress imposed on Eurasian Kestrel nestlings. Functional Ecology 25:566–576.

Murray, M. H., D. J. Becker, R. J. Hall, and S. M. Hernandez (2016). Wildlife health and supplemental feeding: A review and management recommendations. Biological Conservation 204:163–174.

Nebel, S., A. Mills, J. D. McCracken, and P. D. Taylor (2010). Declines of aerial insectivores in North America follow a geographic gradient. Avian Conservation and Ecology 5:1. http://www.ace-eco.org/vol5/iss2/art1/.

Oppliger, A., H. Richner, and P. Christe (1994). Effect of an ectoparasite on lay date, nest-site choice, desertion, and hatching success in the Great Tit (*Parus major*). Behavioral Ecology 5:130–134.

Owen, J. C. (2011). Collecting, processing, and storing avian blood: A review. Journal of Field Ornithology 82:339–354.

Owen, J. C., and F. R. Moore (2008). Relationship between energetic condition and indicators of immune function in thrushes during spring migration. Canadian Journal of Zoology 86:638–647.

Potti, J. (2007). Variation in the hematocrit of a passerine bird across life stages is mainly of environmental origin. Journal of Avian Biology 38:726–730.

Potti, J., J. Moreno, S. Merino, O. Frías, and R. Rodríguez (1999). Environmental and genetic variation in the haematocrit of fledgling Pied Flycatchers *Ficedula hypoleuca*. Oecologia 120:1–8.

Poulin, R. (2007). Are there general laws in parasite ecology? Parasitology 134:763–776.

Quillfeldt, P., J. F. Masello, and E. Mostl (2004). Blood chemistry in relation to nutrition and ectoparasite load in Wilson's Storm-Petrels *Oceanites oceanicus*. Polar Biology 27:168–176.

R Development Core Team (2018). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/

Raleigh, D., J. D. Ray, B. A. Grisham, J. Siegrist, and D. U. Greene (2019). Nest survival data confirm managed housing is an important componenet to the conservation of the Eastern Purple Martin. Wildlife Society Bulletin 43:93–101.

Robb, G. N., R. A. McDonald, D. E. Chamberlain, S. J. Reynolds, T. J. Harrison, and S. Bearhop (2008). Winter feeding of birds increases productivity in the subsequent breeding season. Biology Letters 4:220–223.

Roby, D., K. Brink, and K. Whittmann (1992). Effects of bird blowfly parasitism on Eastern Bluebird and Tree Swallow nestlings. The Wilson Bulletin 104:630–643.

Rogers, C. A., R. J. Robertson, and B. J. Stutchbury (1991). Patterns and effects of parasitism by *Protocalliphora sialia* on Tree Swallow nestlings. In Bird–Parasite Interactions: Ecology, Evolution and Behaviour (J. E. Loye and M. Zuks, Editors). Oxford Ornithology Series, Oxford, UK.

RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA.

Sabrosky, C. W., G. F. Bennett, and T. L. Whitworth (1989). Bird Blow Flies (*Protocalliphora*) in North America (Diptera: Caliphoridae), with Notes on the Palearctic Species. Smithsonian Institution Press, Washington D.C., USA.

Saino, N., S. Calza, and A. P. Moller (1998). Effects of a dipteran ectoparasite on immune response and growth trade-offs in Barn Swallow, *Hirundo rustica*, nestlings. Oikos 81:217–228.

Sánchez, C. A., D. J. Becker, C. S. Teitelbaum, P. Barriga, L. M. Brown, A. A. Majewska, R. J. Hall, and S. Altizer (2018). On the relationship between body condition and parasite infection in wildlife: A review and meta-analysis. Ecology Letters 21:1869–1884.

Sauer, J. R., D. K. Niven, J. E. Hines, D. J. Ziolkowski, K. L. Pardieck, J. E. Fallon, and W. A. Link (2017). The North American Breeding Bird Survey, Results and Analysis 1966–2015, version 2.07.2017. USGS Patuxent Wildlife Research Center, Laurel, MD, USA. https://www.mbr-pwrc.usgs.gov/bbs/

Scott-Baumann, J. F., and E. R. Morgan (2015). A review of the nest protection hypothesis: Does inclusion of fresh green plant material in birds' nests reduce parasite infestation? Parasitology 142:1016–1023.

Seewagen, C. L., M. Glennon, and S. B. Smith (2015). Does ex-urban housing development affect the physiological condition of forest-breeding songbirds? A case study of Ovenbirds (*Seiurus aurocapillus*) in the largest protected area in the contiguous United States. Physiological and Biochemical Zoology 88:416–424.

Smith, S. B., and S. R. McWilliams (2009). Dietary macronutrients affect lipid metabolites and body composition of a migratory passerine, the White-throated Sparrow (*Zonotrichia albicollis*). Physiological and Biochemical Zoology 82:258–269.

Sockman, K. W., and H. Schwabl (2001). Plasma corticosterone in nestling american kestrels: Effects of age, handling stress, yolk androgens, and body condition. General and Comparative Endocrinology 122:205–212.

Symonds, M. R. E., and A. Moussalli (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behavioral Ecology and Sociobiology 65:13–21.

Tautin, J., B. Cousens, K. Kostka, S. Kostka, and D. A. Airola (2008). Addressing regional declines in Purple Martin populations. In Fourth International Partners in Flight Conference: Tundra to Tropics (T. Rich, C. Arizmendi, D. Demarest, and C. Thompson, Editors). Partners in Flight. pp. 82–87.

Tomas, G., S. Merino, J. Moreno, J. Morales, and J. Martínez De La Puente (2007). Impact of blood parasites on immunoglobulin level and parental effort: A medication field experiment on a wild passerine. Functional Ecology 21:125–133.

Traub, R., M. Rothschild, and J. F. Haddow (1983). The Ceratophyllidae: Key to the Genera and Host Relationships with Notes on Their Evolution, Zoogeography and Medical Importance. Academic Press, Cambridge, UK.

Tucci, E. C., A. P. Prado, and R. P. Araújo (2008). Development of *Dermanyssus gallinae* (Acari: Dermanyssidae) at different temperatures. Veterinary Parasitology 155:127–132.

Viega, J. P., V. Polo, and J. Vinuela (2006). Nest green plants as a male status signal and courtship display in the spotless starling. Ethology 112:196–204.

Wagner, R. H., P. Davidar, M. D. Schug, and E. S. Morton (1997). Do blood parasites affect paternity, provisioning and mate-guarding in purple martins? The Condor 99:520–523.

Williams, H. M., and R. L. DeLeon (2020). Deep learning analysis of nest camera video recordings reveals temperature sensitive incubation behavior in the Purple Martin (*Progne subis*). Behavioral Ecology and Sociobiology 74:7.

Williams, H. M., and R. L. DeLeon (2020). Using artificial intelligence classification of nest box videos to examine the environmental, evolutionary and physiological constraints on nestling provisioning behavior. Journal of Avian Biology. In press.

Williams, H. M., and K. Dittmar (2020). Expanding our view of *Bartonella* and its hosts: *Bartonella* in nest ectoparasites and their migratory avian hosts. Parasites & Vectors 13:13.

Williams, H. M., K. Dittmar, and S. Smith Pagano (2020). Data from: A parasite reduction conservation intervention does not improve fledging success or most condition metrics for Purple Martins. The Condor: Ornithological Applications 122:1–14. doi:10.5061/dryad.m905qftzj

Williams, H. M., S. B. Wilcox, and A. J. Patterson (2020). Photography as a tool for avian morphometric measurements. Journal of Ornithology 161:333–339.

Zajac, R. M., D. J. Cerasale, and C. G. Guglielmo (2006). The rapid response of plasma metabolites to changes in feeding rate in a small passerine, Wilson's Warbler, *Wilsonia pusilla*. Journal of Avian Biology 37:405–408.