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ECOLOGICAL AND PHYSIOLOGICAL INFLUENCES ON ALTRICIAL BIRD GROWTH AND DEVELOPMENT

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ECOLOGICAL AND PHYSIOLOGICAL INFLUENCES ON ALTRICIAL BIRD GROWTH AND DEVELOPMENT

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Ecological and physiological influences on altricial bird growth and development

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Rates of embryonic and post-natal growth vary extensively among species and geographic space. This variation is well represented in songbird offspring from different latitudes and can strongly influence organismal quality and fitness. However, environmental and evolutionary causes and consequences of variation in embryonic and post-natal growth remain unclear. Here we experimentally show in the field that, within the constraints imposed by physiological trade-offs, warmer incubation temperatures shortened embryonic period length among nine species of songbirds from two latitudes. Yet, the magnitude of the response varied and species-specific reaction norms of embryonic reduction in response to our treatment positively correlated with the natural temperature experienced during incubation. Furthermore, we found little evidence for potential metabolic costs imposed on offspring by faster development, but we detected benefits for size at hatching instead. These results question the generality of theories considering avian development to be strictly dictated by intrinsic trade-offs and suggest that shorter embryonic periods caused by warmer temperature may not be as detrimental as traditionally thought. Costs of shorter development due to warmer embryonic temperature may appear later in life as stunted post-natal growth via influences on offspring metabolism and parental feeding and brooding effort. Our treatment increased metabolic rate without producing appreciable changes in parental care yielding slower post-natal growth rates in two species, faster growth in one and no effects for the majority of the species studied. These results suggest that shorter embryonic periods are not generally associated to costs paid during the post-natal stages but also question the role of metabolism for growth. We tested for the association between metabolism and growth using a comparative approach. We discovered that metabolic rate and body mass of nestlings predicted variation in post-natal growth rates among 59 species of songbirds at three latitudes. These results beg the question of what are the possible evolutionary bases of metabolic variation. We found that nest depredation may be a selective force favoring increased metabolic rate to achieve faster growth independently from the constraints of adult mortality. This study advances our understanding of ecological and physiological causes and consequences underlying variation in embryonic time and post-natal growth.

ECOLOGICAL AND PHYSIOLOGICAL INFLUENCES ON ALTRICIAL BIRD GROWTH AND DEVELOPMENT

Life history theory assumes that trade-offs constrain phenotypes in expression of traits (Roff 1992). Lengths of embryonic development and post-embryonic growth are traits that vary substantially among species and across latitudes, with tropical organisms generally showing slower trajectories compared to the north temperate (Case 1978; Arendt 1997). This variation has major implications for fitness (Stearns 1992 ; Lindén et al. 1992; Roff 1992), but ecological and physiological causes and consequences of interspecific differences in development and growth are still unclear and debated.

A major tenet of classic theory is that slower growth and development can allow enhanced individual quality and survival due to physiological trade-offs (Ricklefs 1992; Arendt 2000; Lankford et al. 2001; Shine & Olsson 2003; Brommer 2004; Lee et al. 2013). Yet, when rates of embryonic development are extended due to cooler temperatures, lower offspring quality is expected (Gorman & Nager 2004; Hepp et al. 2006; Olson et al. 2006) and these costs may also carry over to later life stages (Metcalfe & Monaghan 2001; Monaghan 2008) . Thus, experiments are needed to test the relative contribution of physiological trade-offs and temperature to variation in development and growth, together with costs and benefits within and across life stages.

The respective role of temperature and physiological trade-offs in explaining interspecific rate of growth might differ between life stages of an organism. For example, in organisms that become endothermic when transitioning from the embryonic to the post-natal stage, temperature becomes internally regulated. Therefore physiological processes, such as differences in metabolism, might become the primary

determinant of variation in rate of growth among species (West et al. 2001). Yet, the importance of variation in metabolism to variation in growth rate among species is not well tested, and existing results are unclear (Drent & Klaassen 1989; Konarzewsky 1995; Williams et al. 2010). Moreover, the selective pressures causing the evolution of variation in metabolism among offspring of different species remain elusive (Lovegrove 2000).

Songbird embryos and nestlings show extensive interspecific variation in rates of embryonic development and post-natal growth within, and especially among, latitudes (Bosque & Bosque 1995; Remeŝ & Martin 2002; Martin 2002; Martin et al. 2011) . Also, eggs are ectothermic and sensitive to temperature (Hepp et al. 2006; Olson et al. 2006), while nestlings develop full endothermy as they age (Ricklefs 1987; Cheng & Martin 2012). Therefore, songbirds provide a unique opportunity to examine the potential differences in the relative importance of temperature versus metabolism across two developmental stages of the same organism. Moreover, both embryonic and post-natal development rates are positively correlated with nest predation rates across songbird species (Bosque & Bosque 1995; Remeŝ & Martin 2002; Martin et al. 2011), such that predation may act as an important driver of natural selection that favors evolution of faster metabolism to allow faster development, as traditionally expected (Von Bertalanffy 1957).

Here we found that, within the constraints imposed by physiological trade-offs, experimentally increased embryonic temperature shortened development time for nine species of songbirds from two latitudes. Yet, the magnitude of the reduction in embryonic period varied and was positively correlated with the incubation temperature naturally experienced by each species. Contrary to theories that consider avian

development as strictly intrinsically regulated (Ricklefs 1992; Robinson et al. 2008), our results suggest that temperature can have a major role for interspecific variation in embryonic development. We also found no clear metabolic costs for the embryos, but detected benefits in terms of size at hatching for the majority of species. These data suggest that when variation in embryonic period length is caused by temperature, theory predicting costs associated to shorter development across species may require revisions.

Potential costs of faster development may be paid at later life stages in the form of smaller size at hatching and slower growth (Atkinson 1994; Metcalfe & Monaghan 2001). Warmer temperatures shortening development can also affect other intrinsic traits such as metabolic rate (Nord & Nilsson 2011) that is strongly related to post-natal growth (Ton & Martin 2015). Additionally, warmer temperature can reduce thermoregulatory costs for incubating adults (Bakken 1980) allowing extra energy available for parental care (Perez et al. 2008) that is also known to influence growth (Martin et al. 2011). How the effects of warmer embryonic temperature on metabolism and adult behavior may interact to influence interspecific variation in growth is unclear. Post-natal growth showed no changes in response to warmer embryonic temperatures among the majority of our species, but decreased in two and increased in one. These results suggest minimal costs associated to warmer temperature and faster development, contrary to what is expected (Zuo et al. 2012). Our treatment also caused little changes in parental feeding and brooding effort but yielded higher post-natal metabolic rate among all species. These results are important for the possible long-term effects of metabolism for longevity (Harman 2001) and question the predicted association between metabolism and growth (Glazier 2015).

We used a comparative approach to test the importance of metabolism for post-natal growth and found that body mass and metabolism explained broad interspecific and geographic differences in post natal growth rates among 59 species of songbirds at three latitudes. These results further contribute to the available evidence in favor of metabolism as the physiological pacemaker of life history variation (Ricklefs & Wikelski 2002) but beg the question of what selective pressures may underlie interspecific and latitudinal metabolic differences.

We tested the hypothesis that depredation rate at the nest in the post-natal stage may be a major force favoring the evolution of higher metabolism to achieve faster growth and reduce probabilities of time dependent mortality. However, since higher metabolism early in life can incur later costs for longevity (Harman 2001) we also hypothesized that probability of adult mortality may act as a constraint on metabolic increase. We found a positive correlation between metabolism and rate of depredation during the post-natal stage but not with probability of adult mortality. These results offer a rare example of ecological sources of variation in metabolic rates.

Overall the present study advances our understanding of the ecological and physiological causes and consequences of interspecific and geographic variation in embryonic development and post-natal growth.

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CHAPTER ONE

On the importance of temperature versus intrinsic constraints for embryonic development times in temperate and tropical songbirds

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Abstract

Embryonic development time varies greatly across species and latitudes. Yet, it remains unclear to what extent this variation reflects intrinsic constraints from physiological trade-offs versus extrinsic effects of temperature. This distinction is important because slow development due to trade-offs can benefit adult longevity, whereas slow development from low temperature does not. To separate these alternatives we experimentally increased incubation temperature in tropical and north temperate species of songbirds. Warmer temperatures shortened development time for all species with no apparent costs reflected in embryo mass or metabolism. Moreover, species with colder natural incubation temperatures, which is common in the tropics, showed greater reductions in development time than did species experiencing temperatures closer to their developmental optima. These results raise questions about traditional theory predicting that longer embryonic development times generally result in higher quality offspring. Instead, benefits to both embryos and parents may accrue from faster development associated with environmental warming, especially in the tropics.

Embryonic development time varies substantially among species and latitudes, with major implications for fitness (Roff 1992; Arendt 1997). Yet, the reasons for this variation are still unclear. Classic theory posits that slower development typical of many tropical organisms increases longevity via physiological trade-offs that enhance tissue differentiation (Arendt 2000), quality of immune responses (Brommer 2004), and locomotor abilities (Shine & Olsson 2003) . However, slower growth increases exposure to time-dependent mortality such as predation (Stearns 1992; Lack 1968) . Thus, species may face two opposing pressures: grow slow for quality or grow fast to avoid predators.

Benefits of slow development are thought to arise from intrinsic physiological trade-offs. However, within individual species, developmental periods of ectothermic embryos become slow also in response to colder temperatures (Deeming & Ferguson 1991; Booth et al. 2000) . Still, these intraspecific changes in embryonic development time are small compared to known variation among species (Fig. 1). This larger interspecific variation may reflect physiological trade-offs as predicted by traditional theory, but a major role of temperature cannot be discounted (Fig 1). Indeed, strong correlations of embryonic periods with temperature across diverse taxa indicate that temperature plays an important role in interspecific variation in development time (Gillooly et al. 2002; Martin et al. 2007; Martin et al. 2013) . Yet, experimental tests of these alternatives are needed to assess their relative importance.

Understanding the relative roles of these two potential causes of interspecific variation in rate of embryonic development is critical because they yield opposing consequences for offspring quality and survival. Physiological trade-offs that extend development time increase offspring quality (Metcalfe & Monaghan 2003; Ricklefs

2006; de Magalhaes et al. 2007) , whereas shorter embryonic periods can lead to smaller size at birth, higher metabolic rate and lower survival (Harman 1955; Atkinson 1994; Mortola 2006). In contrast, cooler temperatures yielding longer development generally reduce offspring quality and survival (Hepp et al. 2006; Olson et al. 2006) , while warmer conditions may yield beneficial effects (Kingsolver & Huey 2008) . Thus, causes and consequences of interspecific variation in embryonic period can differ depending on the relative roles that physiological trade-offs versus temperature play in determining development time.

Songbird embryos provide a particularly strong context in which to test the relative effects of intrinsic constraints, temperature, and their phenotypic consequences at different latitudes. Tropical songbirds typically have longer embryonic periods that may reflect benefits of physiological trade-offs to explain the commonly observed higher adult survival compared with north temperate species (Ghalambor & Martin 2001; Martin et al. 2015) . Yet, tropical birds also exhibit lower parental effort during incubation resulting in colder embryonic temperatures and this might explain the differences in length of embryonic period across species and latitudes independent of any physiological trade-offs (Martin et al. 2007) . Still, these possibilities are experimentally untested in a temperate-tropical context, and available evidence is contradictory.

Some correlative studies found support for the importance of temperature to interspecific and latitudinal differences in embryonic development (Martin 2002; Martin et al. 2013) while others did not (Tieleman et al. 2004; Ricklefs & Brawn 2013). Egg swapping experiments between species at the same latitude showed a combined effect of physiological trade-offs and temperature in determining the length of embryonic

development (Martin et al. 2007; Martin et al. 2015). Experiments amplifying perceived predation risk at the nest caused increased incubation effort by parents, leading to shorter embryonic periods, but incubation temperature was not measured and changes were relatively small. Other tests that held temperature constant during incubation, found that differences in embryonic periods remained between populations (Robinson et al. 2008) and species (Robinson et al. 2014). However, the latter studies used incubators to manipulate temperature, which can prevent embryos from experiencing natural conditions that are critical to normal development (Olson et al. 2008). Thus, the relative importance of physiological processes versus temperature as causes of interspecific and latitudinal differences in development time remains unclear (Tieleman et al. 2004; Robinson et al. 2008; Martin et al. 2015).

Here we conduct controlled heating and egg-swap experiments in tropical and north-temperate species that exhibited broad differences in embryonic development times and temperatures. We also compared differences in egg mass loss, metabolic rates, and hatching success between treatment and control nests to test for costs to embryos potentially associated with faster development at warmer temperatures.

Methods

Study Areas and Species— Data were collected for six songbird species between May and July 2011-2014 in a north temperate mixed forest at 2000-2350m elevation in Arizona, USA (33° N). We studied three additional species between February and May 2012-2014 in a tropical forest at 1450-1750 m elevation in Sabah, Malaysia (6° N) (Table S1).

Experimental Increase in Incubation Temperature — We increased incubation temperature at 42 treatment nests each paired with a control nest exposed to the same level of manipulation but experiencing natural incubation temperatures. Treatment and control nests were spatially and temporally matched in order to minimize differences in weather, seasonality, habitat and elevation. We also matched nests by clutch size, since the number of eggs can influence embryonic development rates (Biebach 1984). The experiment lasted for the full length of the embryonic period starting from the last egg laid and ending with the first egg hatching. One heating device (Kapton Heaters model #KHLV-105) was installed around the nest cup and powered by a 12V car battery that we replaced every second day. The heat output from the device was regulated by a thermostat connected to a probe placed in bottom of the nest (Pressure Tek, model# 3943) set at 37.5^{o} C (Fig. S1). This value represents the upper end of the optimum range for embryonic development (White & Kinney 1974) . Control nests were treated exactly the same, except that the heating device was wired to a cardboard box to simulate the battery.

The overall effect of the heaters was to raise the temperature of the nest during periods when the parents were absent or when incubation temperatures were sub-optimal. Thus average 24-hr egg temperature was increased while maintaining normal incubation rhythms (Fig. 2) and avoiding heat stress to the embryos (Webb 1987). Nests were monitored every 48 hours and up to four times a day as hatch dates approached. Embryonic period length was calculated as the time between the last egg laid and the first egg to hatch. To minimize loss of nests to predation, treatment and control nests of open-

cup-nesting species were caged with iron mesh that allowed normal movements of parents but prevented most predators from accessing the nest.

Egg Swap Experiment — At our tropical site during the 2014 season, we performed a second experiment that replicated methodology detailed in Martin et al. (2007). The goal was to test the relative contribution of temperature and physiological trade-offs for differences in embryonic period between two tropical species. Chestnut-crested Yuhina (*Yuhina Everetti*) and Bornean Stubtail (*Urosphena whitheadi*) have comparable egg mass but embryos of the former experience temperatures 5° C warmer on average than the latter during development because of differences in how often parents incubate the eggs (Martin et al. 2013). If development is caused by physiological trade-offs alone, swapped eggs should hatch at the same time as un-swapped controls of the same species. In contrast, if temperature is the sole cause of developmental period length, swapped eggs should show the same embryonic periods as their host nest species. Thus, by examining the percentage change in developmental time in swapped eggs, we can partition the relative importance of physiological trade-offs versus temperature. Eggs were transferred in the morning during the laying stage (i.e., prior to start of incubation) between nests of the same stage. Neither of these two species starts incubating before all eggs are laid, so embryos were undeveloped at the time of swapping. Nest predation is reasonably high, and despite protection provided by the cage many experimental nests were lost, but we were successful in hatching swapped eggs between four different pairs of nests.

Temperature measurements— We measured temperature in all nests (Fig 2) by placing a

thermistor in the center of an artificial egg positioned in the middle of the nest and connected to a HOBO Stowaway XTI datalogger (Onset Computer Corporation, Bourne, Massachusetts, USA; Fig. S1). Temperature was recorded every 12 seconds for three days halfway through the natural incubation period. We chose to record temperature at this stage for three reasons. First, intra and interspecific variation in the amount of time spent brooding is higher during the early stages of incubation (Deeming 2002) . Second, incubating parents tend to be more sensitive to disturbance such as adding an extra egg to the clutch in early than middle incubation (personal observation). Third, postponing measurements during late stages of incubation may have been unfeasible because of anticipated hatch among treatment nests. Fake eggs were made of plaster of paris and were formed to mimic the size, shape and color of the host species. We limited our measurements of temperature differences to three days because larger clutches can increase the energetic costs of incubation to parents (Haftorn & Reinertsen 1985) .

Egg Mass and Metabolic Measurements— We marked and weighed all eggs of treatment and control nests using an ACCULAB portable electronic scale (precision 0.001 g). A first measurement was taken the day of clutch completion followed by a second one two days before the expected hatch date. This repeated measurement allowed us to quantify the mass lost by each clutch during development.

We measured embryo metabolic rate as oxygen consumption rate [$\dot{V}O_2$ (mL h^{-1})] using a FoxBox field gas analyzer (Sable System, Las Vegas, NV, USA) for one egg only in order to ensure independence among samples. Metabolic measurements were executed following the protocol detailed in Martin et al. (2013). Eggs were removed from the nest

at 79.9 ± 0.72% (mean ± SE) of their development and were replaced with fakes. Eggs were weighed using an ACCULAB portable electronic scale (precision 0.001 g; Edgewood, NY, USA). During metabolic measurements, the eggs rested in a 60 mL syringe, connected to an open-flow system flushed with atmospheric air at a rate of 25 ml/min. The air was scrubbed of CO_2 and water vapor magnesium perchlorate, soda lime and drierite. To precisely control experimental temperature, the chamber was submerged in a water bath and held at 37.5 ^{o}C.

Oxygen consumption rate was measured continuously every 0.5 s, and $\dot{V}O_2$ (mL h^{-1}) was calculated as the difference in O_2 concentration between the air input and output flowing through the chamber during the most stable three minutes of measurements. We used the formula $V_{O2} = FR_i(F_iO_2 - F_eO_2)/(1-F_eO_2)$ in ExpeData (ver. 1.3.2) software from Sable Systems. Where FR_i is the incurrent mass flow rate scrubbed from water vapor and CO_2, F_iO_2 is the incurrent fractional concentration of oxygen, and F_eO_2 is the excurrent fractional concentration of oxygen (Lighton 2008). Metabolic measurements lasted between 90 and 110 minutes, with larger eggs taking longer. After completion each egg was returned unharmed to the nest of origin.

Statistical Analyses— We tested the effect that differences in incubation temperatures between treatment and control nests have on embryonic period using a linear mixed model with species as a random effect nested within site and year. Because no difference in temperature should yield no difference in embryonic period, we forced all our intercepts through zero (Eisenhauer 2003) to obtain a better fit (Table S2)

Because the temperature changes between treatment and control were not

identical among species, we standardized changes in embryonic period to allow more direct comparisons. This was achieved estimating species-specific coefficients (slopes) using a linear model with changes in embryonic period as dependent variable and changes in temperature as an explanatory variable. To test for evolved differences in reaction norms, we used slopes as dependent variable and the average temperature in control nests as the independent variable in a linear model that takes into consideration the phylogenetic history of species (package "caper"; Orme et al. 2013). To produce our phylogeny we built a majority-rule consensus tree with program Mesquite (Maddison and Maddison 2015) using 1,000 trees sampled from BirdTree.org (Jetz et al. 2012).

We quantified the relative contribution of temperature versus intrinsic constraints in determining differences in embryonic period between species in our swap experiment. We divided the change in embryonic period of the transferred egg by the observed difference in embryonic periods of the host versus natal nest x 100. We attributed this percentage change in embryonic periods between species to temperature and the remaining portion to physiological trade-offs and other unmeasured effects.

We tested for effects of heating on egg mass loss by fitting a linear mixed model with percent egg mass loss difference between treatment and control as dependent variable and species as a random effect nested within site and year. Using the same statistical approach we tested for differences in mass specific metabolic rate and hatching success between treatment and control nests (see supplemental methods). We also conducted separate ANOVA tests for each species to evaluate whether differences in egg mass loss and mass-specific metabolic rate were significantly different from 0 (Table S3). All analyses were performed using R version 3.1.2 (R Core Team 2014).

Results

Heating Experiment— Our heating experiments at 42 nests increased incubation temperatures as hoped (mean ± SE = 1.32 ± 0.13 °C). Temperature differences between treatment and control were associated with an overall decrease in treatment embryonic period among the nine species considered here (Fig. 3a; mean effect size ± SE = -1.26 ± 0.16 d, $P < 0.001$). However, individual species reactions to treatment varied substantially. Cordilleran flycatcher showed almost no reduction in embryonic period (mean ± SE = -0.2 ± 0.08 d) despite increased temperature (Fig. 3a). Differently, Mountain Wren-babbler showed impressive shortening in development time (mean ± SE = 5.33 ± 1.2 d) with a relatively small change in average temperature (mean ± SE = 2.36 ± 0.72 °C). The differences in reaction norm slopes of the nine species were correlated with the average normal (control) temperature (Fig. 3b; mean effect size ± SE = 0.3 ± 0.08, $r^2 = 0.69$, $P = 0.005$). In short, embryonic periods of tropical species with colder normal temperatures exhibited stronger responses (steeper reaction norms) to heating compared to north temperate species that normally develop under warmer conditions.

Egg Swap Experiment— Bornean Stubtail eggs placed in Chestnut-crested Yuhina nests experienced higher average temperatures (mean ± SE = 4.06 ± 0.39 °C) and showed a 25% shortening in development time (mean ± SE = 6 ± 0.4 d) compared to controls (fig. 4). This reduced the average gap in development time between the two species from nine (mean ± SE = 9 ± 0.4 d) to three (mean ± SE = 3 ± 0.4 d) days. Therefore temperature

alone accounted for 67 ± 5.8% of the difference in average embryonic period between natal and host species, while the remaining 33 ± 5.8% can be attributed to intrinsic constraints or other unmeasured variables (Fig. 4). Physiological trade-offs also acted on the other end of the temperature gradient limiting the extent to which development can be delayed. Indeed, eggs of chestnut-crested Yuhina that were transferred to the colder temperature conditions of stubtail nests extended their embryonic period by two days on average (mean ± SE = 2 ± 0.4 d). A substantial difference from the host nest remained (mean ± SE = 8 ± 0.4 d). Thus, physiological trade-offs act asymmetrically among species at the slow (cold) versus fast (warm) ends.

Consequences of increased temperature for egg mass and metabolism— Egg mass naturally decreases over the embryonic period due to water loss associated with metabolic processes underlying development. Average reduction in egg mass in our study was 14.6% ± 0.41 for all samples ($F = 2.618$, $P = 0.014$) but interspecific differences were substantial, ranging between 10-22%. Embryos of seven species lost less mass when exposed to heating, with three of these being significant (Fig 5a). The remaining two species lost more mass when incubated at warmer temperatures but the effect was not significant (Fig5a; Table S3). Overall, warmer temperatures during development resulted in higher embryonic size prior to hatching across the nine species in our experiment (Fig 5a; $F = 9.91$, $P = 0.003$).

Heated embryos had higher mass-specific metabolic rates than control embryos in six species, two of which showed a significant effect of treatment. Mass-specific metabolic rate was lower in three species and one of those showed a marginally

significant effect of the heating experiment. This resulted in an overall lack of significant effect of our treatment on metabolic rates (Fig 5b; $F = 0.813$, $P = 0.372$).

Hatching success is typically about 90% in natural nests (Briskie & Mackintosh 2004) . In our study, 93 ± 0.7% of eggs hatched successfully, with no differences between treatment and control clutches ($F = 0.213$, $P = 0.69$).

Discussion

The relative importance of temperature and physiological trade-offs in determining interspecific variation in the length of embryonic development has been extensively debated (Martin 2002; Tieleman et al. 2004; Martin et al. 2007; Ricklefs & Brawn 2013; Martin et al. 2013; Martin et al. 2015) . Our results suggest that temperature and physiological trade-offs play important but unequal roles in determining interspecific and latitudinal variation in embryonic development. The substantial reduction in embryonic period in response to artificial heating (Fig. 3a) concurs with previous correlational studies that include a much larger sample of species from different latitudes (Martin et al. 2007, 2015). This correspondence supports the idea that, within the constraints imposed by physiological trade-offs, temperature can strongly influence interspecific variation in embryonic period (Gillooly et al. 2002).

Ultimately, physiology appears to limit how short development time can be. The shortest period of embryonic development in birds is 10-11 days (Rahn & Ar 1974) , when average incubation temperature is close to 37 °C (Martin et al. 2007). Thus, embryos experiencing stable natural incubation temperatures near 37 °C may have

evolved development periods close to their physiological limits. Species near their physiological maxima at the warm end reduced their embryonic periods very little during warming experiments (Fig. 3). Rather, heating shortened embryonic period much more in tropical species with colder incubation temperatures and longer development (Fig. 3). This result is important because it provides experimental evidence that refutes a long-held view that tropical species are relatively insensitive to temperature and that their long embryonic periods reflect physiological trade-offs that provide benefits (i.e., Ricklefs 1992; Tieleman et al. 2004; Robinson et al. 2008, 2014).

Faster development may lead to reduced phenotypic quality like smaller size at hatching in lizards (Van Damme et al. 1992), and faster metabolic rates in chickens (Mortola 2006), both of which decrease survival (Allen et al. 2008). Yet, we found that eggs developing at warmer temperatures were heavier prior to hatch date compared to control eggs in seven out of nine species studied here (Fig. 4a). Similarly other studies found that cooling eggs caused reduced egg yolk mass in birds (Olson et al. 2006) and smaller size at birth in insects (Walters & Hassall 2006) and reptiles (Elphick & Shine 1998) . This effect may be explained by warmer temperature favoring higher efficiency in cell differentiation and proliferation and by lower temperatures diverting resources to respiration and self-maintenance. Intriguingly, our reported effects of temperature on egg mass support the hypothesis that, in the tropics, parents lay larger eggs to provision their embryos with extra resources that compensate for the maintenance costs of low average incubation temperatures (Martin 2008). Also, we did not detect an overall change in mass-specific embryonic metabolic rate due to heating (Fig. 5b). The possibility remains that higher incubation temperatures underlying faster development may produce other

costs unmeasured here, but we did not detect costs related to mass and metabolism.

Our results undermine the idea that longer embryonic periods of tropical species improve organismal quality and increase longevity. Instead, long development times caused by low incubation temperatures may impose a cost on young rather than provide a benefit (Ardia et al. 2010; DuRant et al. 2012). Slow development at lower temperatures extends the time of exposure to sources of mortality experienced during the vulnerable stage of incubation. For example, Bornean Stubtail eggs have a 24-day incubation period and are exposed to a daily predation probability of 0.045 (Martin et al. 2015). Yet, our experiments show that this species has the potential to shorten embryonic development by about six days, which translates into a 24% reduction in predation risk. Why then do songbirds not increase incubation effort to keep eggs warmer and shorten the incubation period so that their offspring benefit from reduced predation risk?

A possible answer is that costs accrue to parents rather than to offspring. In lon-lived tropical species selection may favor reduced parental energy expenditure by parents so that they enhance their own probability to breed in the future (Martin 2002; Martin et al 2015). Our experiments certainly demonstrate that long-lived tropical species with lower incubation temperatures had stronger responses to heating indicating that their long embryonic periods are due to low parental effort in warming eggs. Nevertheless, this species maintained a longer embryonic development compared to the host indicating an influence of intrinsic constraints. This suggests that selection may favor longevity in two ways: by acting on intrinsic trade-offs, and by reducing parental effort that affects extrinsic embryonic temperature.

The effects that developmental temperature has on phenotypic variation are

especially important in light of global climate changes (Griffith et al. 2016) . Our data show that “cold” tropical embryos shorten development time more than “warm” north temperate species for an equivalent increase in temperature. This suggests that small rises in temperature due to global warming predicted at low latitudes (Parry 2007) may benefit tropical embryos by shortening their incubation period and reducing exposure to predation without phenotypic costs to the offspring. Conversely increased temperatures in north temperate zones may yield smaller effects for development but could still impact species close to their physiological maxima (Somero 2010). Therefore songbird embryos may represent a major exception to the hypotheses that detrimental impacts on ectotherms from global warming should be stronger at lower latitudes (Deutsch et al. 2008; Dillon et al. 2010)

Our study shows that, within the constraints imposed by physiological trade-offs, extrinsic temperature plays a stronger role on interspecific and latitudinal variation in embryonic period. However the effect of higher temperatures varied as a function of the thermal conditions normally experienced during development, leading to questions about possible latitudinal differences in the effect of global warming on ectothermic embryos. Additionally we found benefits rather than costs associated to shorter embryonic periods. These results support the view that when caused by lower temperatures, extended development may not be as beneficial as traditionally thought.

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Figure 1. Conceptual graph representing the correlation between temperature and embryonic development. Each symbol and color represents a different species. At the intraspecific level, temperature (dashed lines) can explain differences in development period (small bracket). However it is still unclear if temperature (solid lines) or physiological constraints are the main determinant of interspecific variation in embryonic period (large bracket).

Figure 2. Thermal conditions experienced during 24 hours of incubation by a control and a treatment clutch of Red-faced Warbler (*Cardellina rubifrons*) of the same age. Measurements were recorded on the same date and nesting habitat; temperature oscillations reflect parental incubation bouts.

Figure 3. (a) Correlation between measured differences in egg temperature, and incubation period differences between treatment and control among 42 paired nests belonging to nine species at two latitudes. Each point represents a nest pair and each symbol and color a different species. Individual regression lines provide the intraspecific response of embryonic period to experimental heating and warmer colors are associated to warmer natural incubation temperature. Dashed lines denote tropical species. Names in figure legend are reported in order of ascending slope. (b) Correlation between average (± 1 SE) differences in control incubation temperature and change of embryonic period with treatment temperature (slope ± 1 SE) for nine songbird species at two latitudes. Tropical species are denoted as ~. The gray horizontal line intercepting zero represents the physiological threshold for development where further temperature increases produces no

changes in embryonic period.

Figure 4. Results of the swap experiment for eggs of Bornean Stubtail (cold species) transferred in nests of Chestnut-crested Yuhina (warm species), and for eggs of Chestnut-crested Yuhina transferred in nests of Bornean Stubtail. Arrows indicate the effect of temperature changes on embryonic development length. Segments indicate the effect of physiological trade-offs in limiting the reduction (cold species swapped to warm nest) or extension (warm species swapped to cold nest) of the embryonic period.

Figure 5. Mean differences between treatment and control (± 1 SE) in (a) % egg mass, and (b) mass-specific metabolic rate (mL O_2 h^{-1}) for nine bird species exposed to increased incubation temperature in a tropical (Malaysia) and north temperate (Arizona) site. Significant ($p < 0.05$) and marginally significant ($p < 0.1$) effects within species are denoted respectively as * and □.

Figure 1.

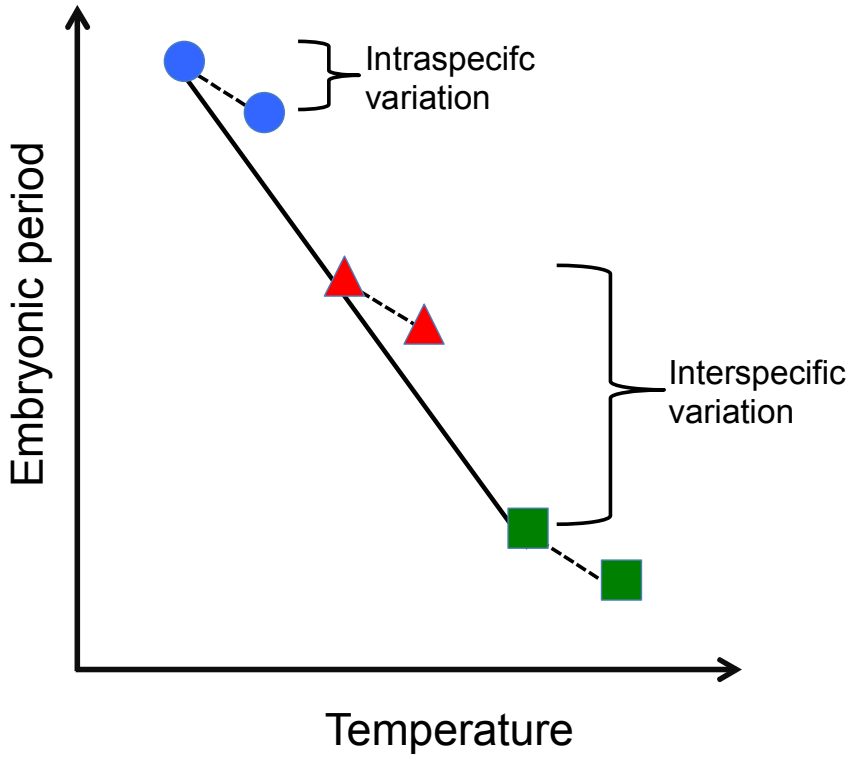

Intraspecifc
variation
Interspecific
variation
Embryonic period
Temperature

Figure 2.

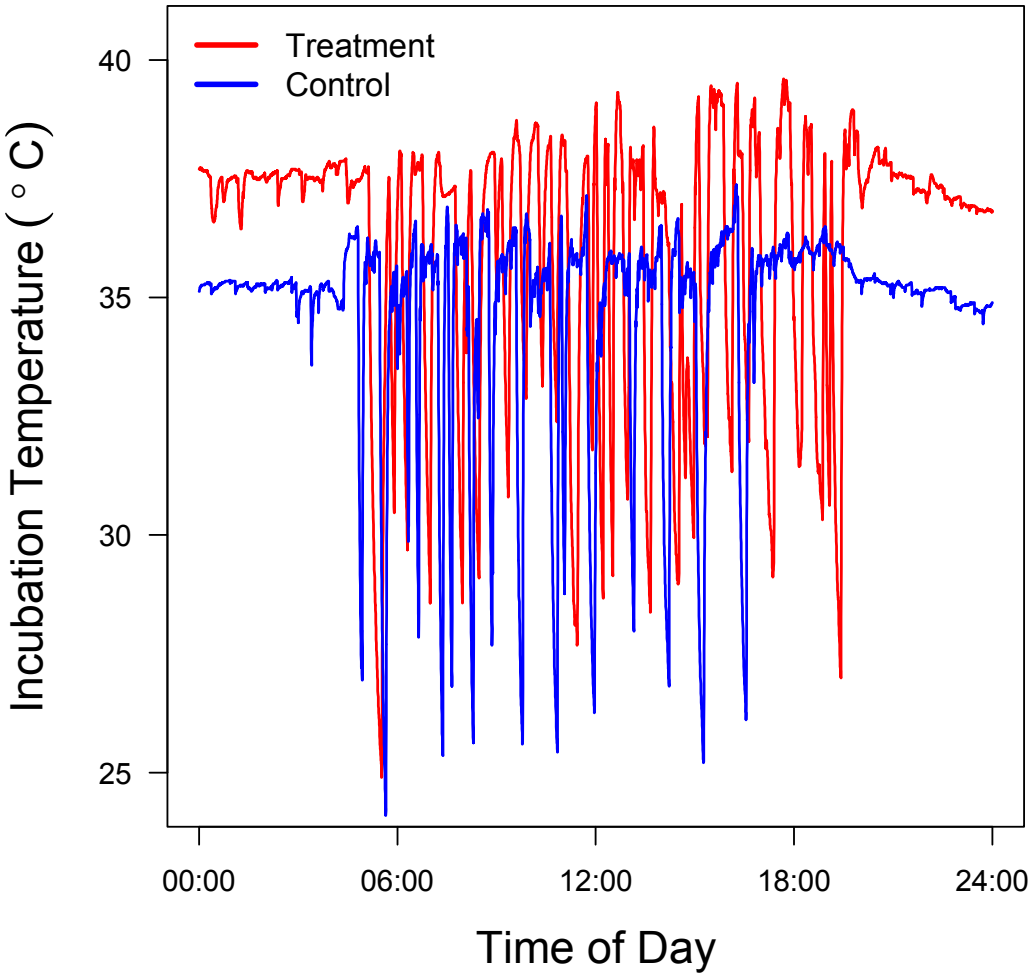

Treatment
Control
40
35
30
25
Incubation Temperature (° C)
00:00
06:00
12:00
18:00
24:00
Time of Day

Figure 3.

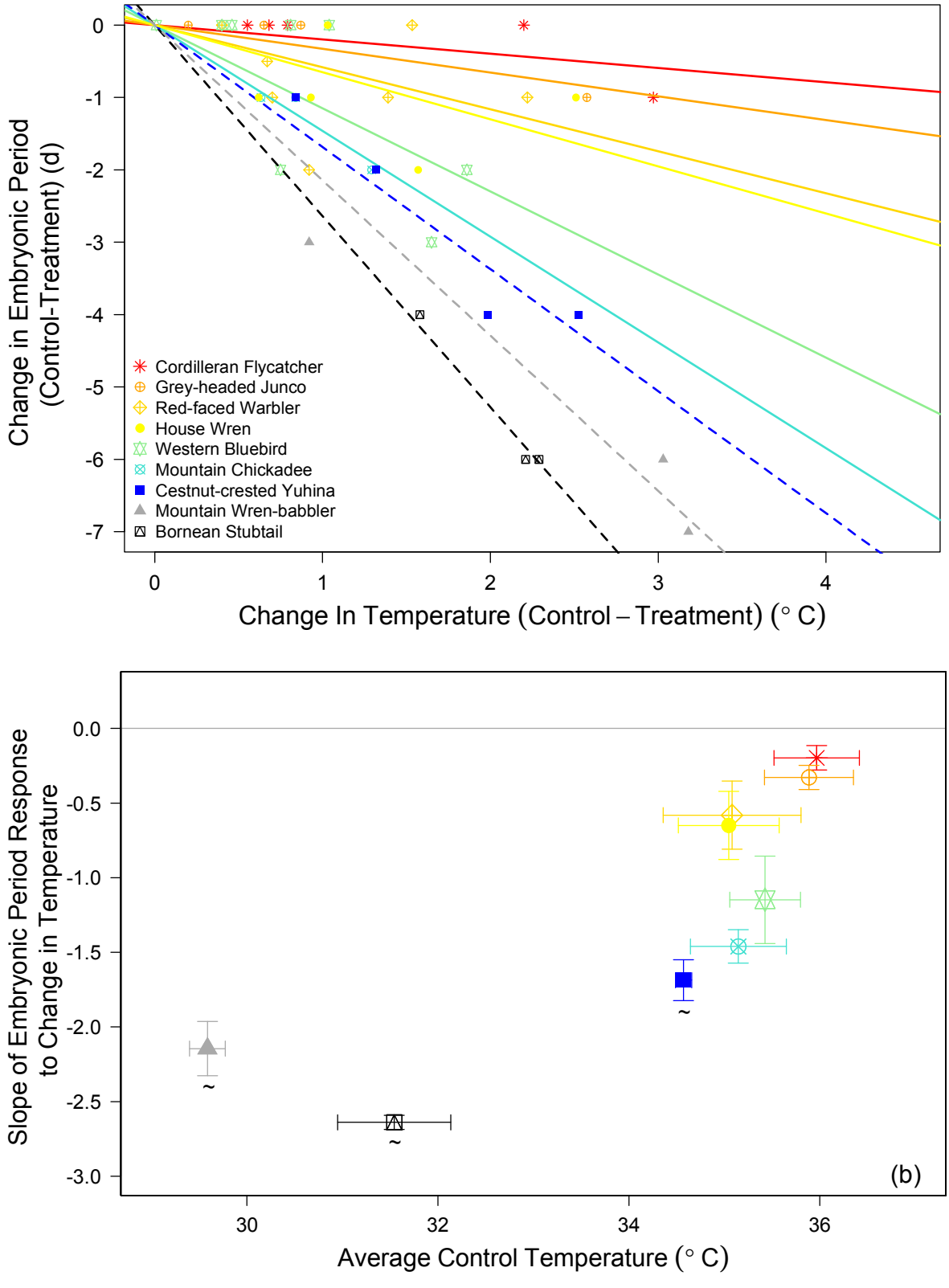

Change in Embryonic Period (Control-Treatment) (d)
0
-1
-2
-3
-4
-5
-6
-7
Cordilleran Flycatcher
Grey-headed Junco
Red-faced Warbler
House Wren
Western Bluebird
Mountain Chickadee
Cestnut-crested Yuhina
Mountain Wren-babbler
Bornean Stubtail
0
1
2
3
4
Change In Temperature (Control – Treatment) (° C)
Slope of Embryonic Period Response to Change in Temperature
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
(b)
30
32
34
36
Average Control Temperature (° C)

Figure 4.

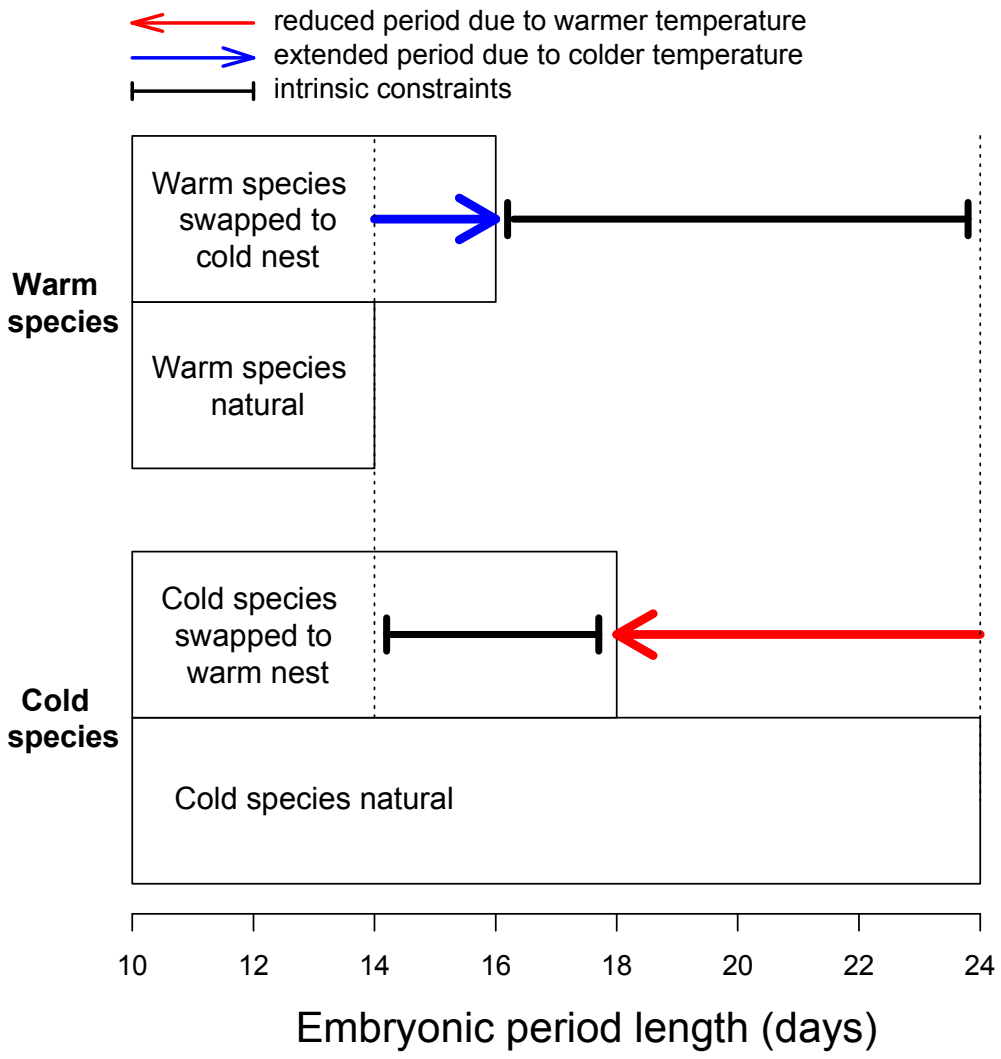
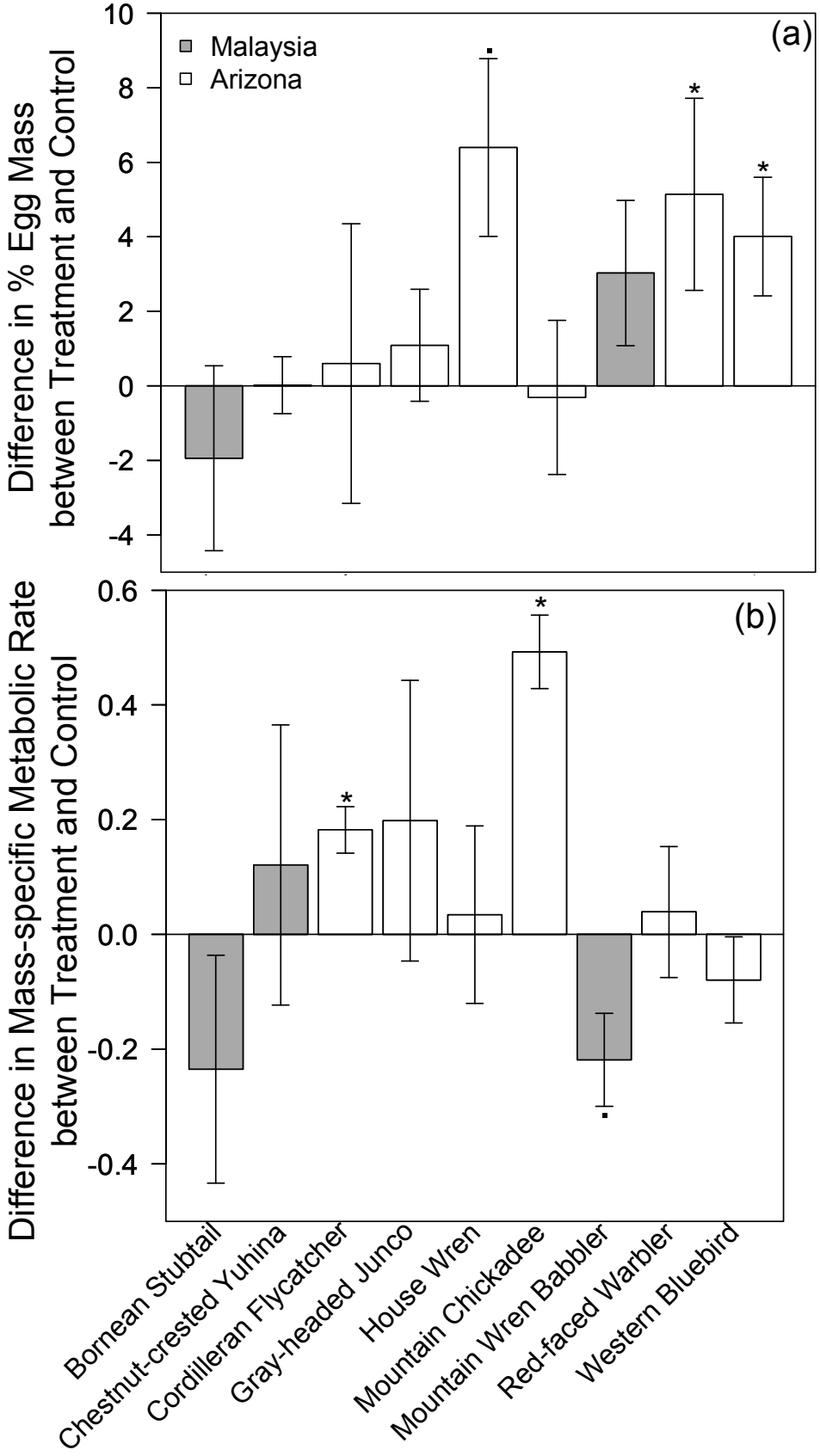

reduced period due to warmer temperature
extended period due to colder temperature
intrinsic constraints
Warm species
Warm species swapped to cold nest
Warm species natural
Cold species
Cold species swapped to warm nest
Cold species natural
10
12
14
16
18
20
22
24
Embryonic period length (days)

Figure 5.


(a)
Malaysia
Arizona
Difference in % Egg Mass between Treatment and Control
10
8
6
4
2
0
-2
-4
(b)
Difference in Mass-specific Metabolic Rate between Treatment and Control
0.6
0.4
0.2
0.0
-0.2
-0.4
Bornean Stubtail
Chestnut-crested Yuhina
Cordilleran Flycatcher
Gray-headed Junco
House Wren
Mountain Chickadee
Mountain Wren Babbler
Red-faced Warbler
Western Bluebird

On the importance of temperature and intrinsic constraints for embryonic development times in temperate and tropical songbirds.

Riccardo Ton and Thomas E. Martin

Supplemental Material

Table S1. Common and scientific names for 9 bird species breeding at our tropical (Malaysia) and a north temperate (Arizona) field sites. Total number of paired treatment and control nests (*n*) are shown for our heating and swap experiments.

Site	Common Name	Scientific Name	Heating Experiment *n*	Swap Experiment *n*
Malaysia	Bornean Stubtail	*Urosphena whiteadi*	3	4
Malaysia	Mountain Wren-babbler	*Napothera crassa*	3	---
Malaysia	Chestnut-crested Yuhina	*Yuhina everetti*	4	4
Arizona	Cordilleran Flycatcher	*Empidonax occidentalis*	5	---
Arizona	Grey-headed Junco	*Junco hyemalis*	4	---
Arizona	House Wren	*Troglodytes aedon*	5	---
Arizona	Mountain Chickadee	*Poecile gambeli*	3	---
Arizona	Red-faced Warbler	*Cardellina rubifrons*	7	---
Arizona	Western Bluebird	*Sialia mexicana*	8	---
	Totals		42	8

Figure S1. Picture of the set up needed for the heating experiment here uncovered from camouflaging materials for showing purposes. The heating device (solid red arrow) wraps around the nest cup where a fake egg connected to a probe (red star) records temperature experienced by the embryos during incubation. A thermostat connected to the circuit (dashed red arrow) regulates the energy input from the battery to the heating device. Notice the iron mesh surrounding the nest to reduce sample loss due to predation.

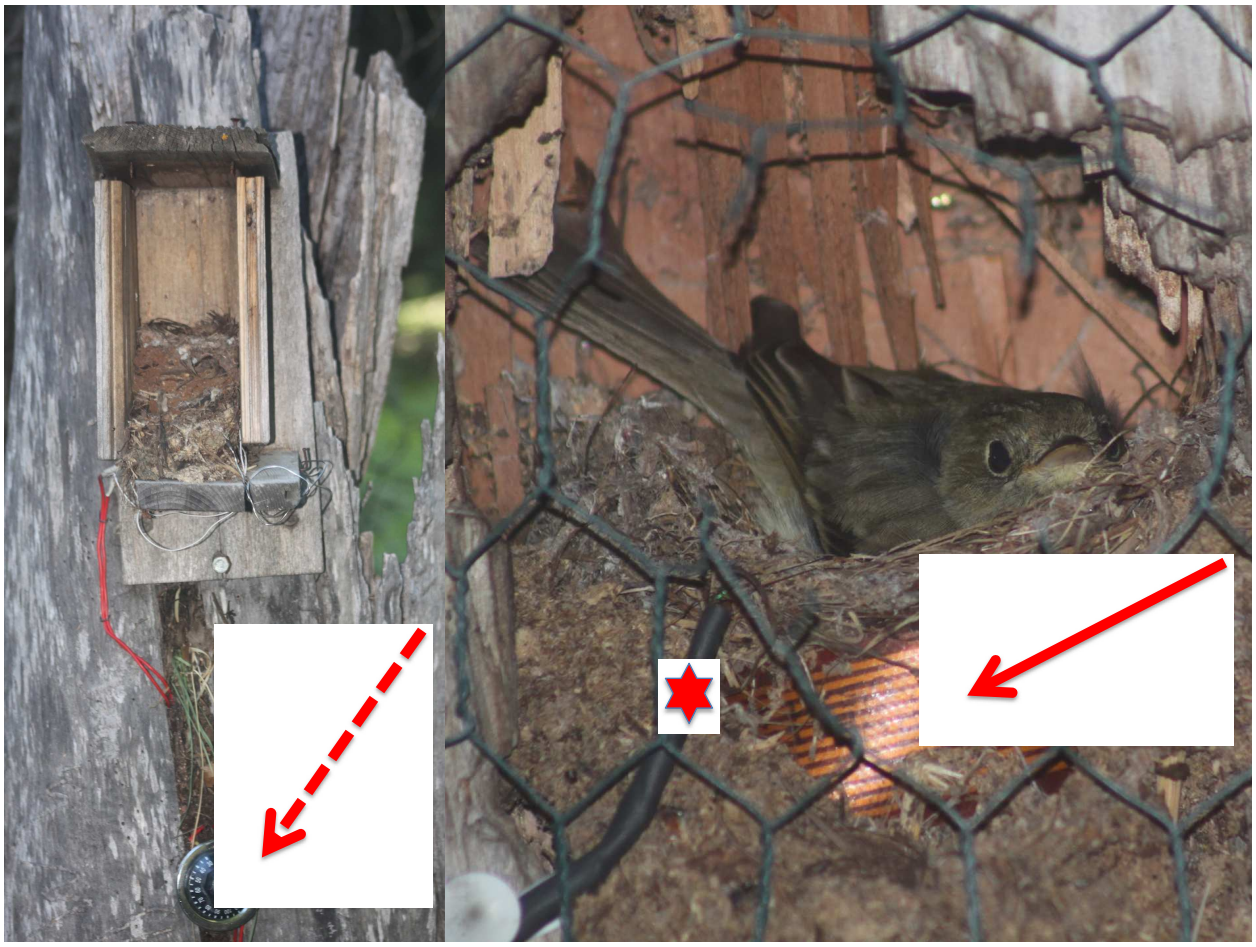


Figure S2. Phylogenetic relationships among north temperate (red) and tropical (blue) bird species used in the present study. The majority rule consensus tree was computed in program Mesquite using 1,000 trees obtained from BirdTree.org.

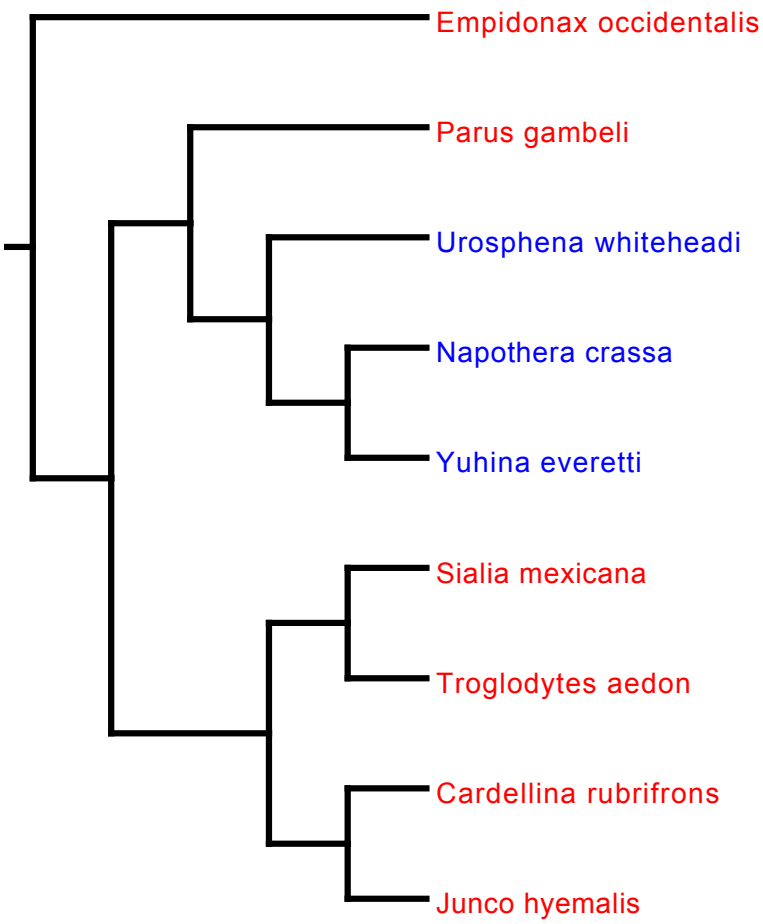


Table S2. Comparison of fit between linear mixed models testing the effect of incubation period differences on embryonic period changes between treatment and control nests for nine species of breeding songbirds at two latitudes (see methods). Models were tested (a) with intercept values free to vary and (b) forcing all intercepts through zero.

Selection criterion	Model with free varying intercepts	Model with intercepts forced through 0
AIC	154.61	153.67
BIC	161.57	158.88
r^2	0.91	0.88
SE	0.23	0.16
F-statistic	20.10	60.75

Table S3. Summary output of individual tropical (blue) and north temperate (red) species tested for differences between treatment and control nests in egg mass loss (g) and mass-specific metabolic rate (mL O_2 h^{-1}) using ANOVA with a random factor of year. Significant and marginally significant differences are denoted with (*) and (`) respectively.

Species	**Dependent variable**	***F* value**	***P* value**
Bornean Stubtail	Egg mass	0.612	0.515
	Mass-specific metabolic rate	0.910	0.440
Mountain Wren-babbler	Egg mass	2.393	0.261
	Mass-specific metabolic rate	4.26	0.090`
Chestnut-crested Yuhina	Egg mass	0.0005	0.983
	Mass-specific metabolic rate	0.676	0.471
Cordilleran Flycatcher	Egg mass	0.730	0.440
	Mass-specific metabolic rate	9.102	0.037*
Grey-headed Junco	Egg mass	0.520	0.545
	Mass-specific metabolic rate	0.929	0.407
House Wren	Egg mass	7.458	0.034*
	Mass-specific metabolic rate	1.016	0.352
Mountain Chickadee	Egg mass	0.023	0.888
	Mass-specific metabolic rate	79.65	0.012 *
Red-faced Warbler	Egg mass	4.666	0.083`
	Mass-specific metabolic rate	0.362	0.568
Western Bluebird	Egg mass	6.340	0.032*
	Mass-specific metabolic rate	1.444	0.26

CHAPTER TWO

Consequences of warmer temperature during embryonic development for metabolism, parental care, and post-natal growth among songbird species.

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ABSTRACT

Environmental conditions early in life can have substantial impacts on subsequent life stages. Higher temperatures during development can shorten embryonic period, which may reduce offspring size at maturity and fitness. Additionally, warmer embryonic temperatures can influence offspring metabolism. Lastly, warmer temperatures can lower thermoregulatory costs for the adults, and the energy saved may be invested into higher parental care for offspring. Faster embryonic development, changes in metabolism, and variation in parental care can affect the expression of important traits, like post-natal growth, and responses may differ across species depending on evolved life history differences. However, interspecific studies testing carry-over effects of warmer embryonic temperature on post-natal growth are lacking. Here, we found that experimentally increased temperatures during embryonic development increased post-natal metabolic rates among seven species of songbirds. Higher embryonic temperatures did not significantly alter the overall rates of parental food delivery or brooding effort. We also found no changes in growth rates in four of the species studied, faster growth in one species and slower growth in two. These results suggest that carry-over effects of warmer temperature causing faster embryonic development can have some reflection on growth that may not be as costly as traditionally thought. Yet, further studies should elucidate possible long-term consequences of increased metabolism for adult longevity.

INTRODUCTION

Environmental conditions experienced early in life can have major repercussions on phenotypic expression later in life (Harrison et al., 2011;Pigliucci, 2001). Thermal fluctuations have pervasive effects on all levels of biological organization (Huey and Berrigan 2001; Jiang and Morin, 2004), and may be especially important during the sensitive ectothermic phase of embryonic development (Gilbert and Epel, 2009). Warmer temperatures experienced early in life can shorten embryonic periods within (Deeming and Ferguson, 1991) and among species (Ton and Martin 2016). Faster embryonic development may impose future costs such as smaller size (Atkinson, 1994), which is known to negatively impact fitness (Brown et al., 1993). However, faster embryonic development facilitated by warmer embryonic conditions can increase hatchling size across different taxa thus yielding benefits (Eiby and Booth, 2009; Hutton, 1987; Olson et al., 2006). Despite these opposing consequences for post-natal growth, the effects of warmer embryonic development on phenotypic expression in later life stages among species remain elusive.

Our understanding of the potential costs of carry over effects (i.e. changes in trait expression that transfer from one life stage to the next) (Lindström, 1999) may be limited by the complexity of the interactions occurring between temperature and other traits influencing growth. For example, offspring experiencing warmer embryonic conditions have higher post-natal metabolic rates in reptiles (Steyermark and Spotila, 2000), but not in birds (Olson et al. 2006; Nord and Nilsson 2011). Variation in post-natal metabolism is positively correlated with cellular proliferation and post-natal growth (Ton and Martin, 2015; West et al., 2001). Surprisingly, available experiments on the effects of embryonic temperature on post-natal growth within species produced both, faster (Durant et al., 2010) and slower growth (O'Steen, 1998), but metabolic rates were not measured. Furthermore, the effects of warmer embryonic temperature on metabolism are important to test because higher metabolic rate can yield higher levels of oxidative damages (Alonso-Alvarez et al., 2006), which can ultimately reduce longevity (Harman, 2001). These oxidative damages may be additive to potential costs of faster embryonic development, further impacting offspring growth.

Changes in post-natal metabolism also affect the energetic requirements of offspring regarding food and heat (Nagy et al., 1999). Interestingly, higher temperature during the embryonic stage can also reduce costs of thermoregulation for adults (Bakken, 1980). These energy savings can allow higher investment in offspring in species with parental care. Increased parental care in the form of food or heat can accelerate growth and can give higher offspring quality (Criscuolo et al., 2008; Lindström, 1999; Metcalfe and Monaghan, 2001). Those "silver spoon" advantages (Madsen and Shine, 2000) can potentially ameliorate costs of faster embryonic periods, but the magnitude of the behavioral responses to environmental changes can vary based on evolved interspecific differences in life history strategies (Ghalambor et al., 2013). Thus embryonic temperatures, embryonic development time, metabolic rate and parental care may all interact in determining rates of post-natal growth. Yet, interspecific studies investigating the interactions and relative importance that these traits can have for offspring growth are lacking.

The carry over effects of higher temperature during development on offspring growth are particularly important to test among altricial species, like songbirds, for multiple reasons. First, offspring of songbirds are ectothermic, immobile, and relatively small compared to the mass of adults, which reduces their thermal inertia. Thus, plastic metabolic adjustments of offspring exposed to warmer embryonic temperature can be important for their survival (Somero, 2010). Second, eggs of songbirds experience wide interspecific variation in embryonic period (Martin, 2002; Martin et al., 2015) and this variation is mostly due to temperature differences during incubation (Ton and Martin 2016). Thus, the consequences of carry over effects due to experimentally increased temperature may also differ among species. Third, parental care plays a critical role for passerine nestlings since they completely depend on the food and heat provided by adults for their growth and thermoregulation. Therefore, songbirds are ideal for testing the potential effects of increased temperature during embryonic development on variation in metabolism, parental care and post-natal growth (Figure 1).

Here we experimentally increased incubation temperatures in the field for seven songbird species, six temperate and one tropical, encompassing a broad range of variation in average incubation temperatures and parental care (Martin et al. 2015). We tested carry

over effects of higher incubation temperature on nestling metabolic rate, food delivery and brooding rates of adults, and their influences on post-natal growth.

MATERIAL AND METHODS

Study areas and species

Data were collected in a high elevation (2350m) mixed forest in north-temperate Arizona, USA for six songbird species and in a tropical mid-elevation forest (1450-1950 m) in Malaysia for one species (Table 1).

Experimental Increase in Incubation Temperature

Following the procedure described in Ton and Martin (2016), we increased incubation temperature in 46 treatment nests, each paired with a control nest that was not exposed to the artificial temperature increase. Because 13 nests were depredated during the early nestling stage they were not included in analyses. We raised the temperature experienced by the embryos throughout incubation while maintaining normal incubation rhythms and avoiding heat stress to the embryos (Ton and Martin 2016).

To quantify the temperature increase in treatment compared to control nests we placed a thermistor in the center of an artificial egg positioned in the middle of the nest and connected to a HOBO Stowaway XTI datalogger (Onset Computer Corporation, Bourne, Massachussets, USA). Temperature was recorded every 12 seconds for three days in the middle of the incubation period (Ton and Martin 2016).

Metabolic measurements

We measured metabolic rates of nestlings from our control and treatment nests following the procedure described in Ton and Martin (2015). Using a Foxbox field gas analyzer (Sable System, Las Vegas, NV, USA), we recorded oxygen consumption [V_{O2} (mL h^{-1})] at 37.0 ºC for one nestling per clutch to ensure independence among samples within a species.

Metabolic rates were measured at pin break, a standardized developmental stage when primary feathers break their sheaths and thermoregulatory capacities are comparable among species (Cheng and Martin, 2012; Pereyra and Morton, 2001; Sogge

et al., 1991). Therefore recording V_{O2} at pin break allowed us to control for interspecific variation in thermoregulation and its effect on metabolism during growth.

Each nestling was put in a 2.1L stainless-steel airtight metabolism chamber inside a large, dark, insulated cabinet with a Peltier device (Pelt-4; Sable Systems) maintaining temperature at 37 ± 0.1°C. The chamber was connected to an open-flow system and flushed with 200-300 milliliters per minute flow of atmospheric air scrubbed of CO_2 and water vapor. Air was filtered through scrubbers with soda lime, magnesium perchlorate and Drierite (Lighton, 2008). V_{O2} was measured continuously every 0.5s and was calculated as the most stable five minutes of oxygen consumption during measurements. After completion of V_{O2} measurements, nestlings were returned to their nest unharmed. V_{O2} (mL h^{-1}) was calculated in ExpeData (ver. 1.3.2) software from Sable Systems using the formula $V_{O2} = FR_i(F_iO_2 - F_eO_2)/(1-F_eO_2)$. Where FR_i is the incurrent mass flow rate scrubbed from water vapor and CO_2, F_iO_2 is the incurrent fractional concentration of oxygen, and F_eO_2 is the excurrent fractional concentration of oxygen (Lighton, 2008).

Feeding rate and brooding rate

We collected information on rate of offspring brooding and food delivery by videotaping nests for 6 - 8 hr starting within 30 min of sunrise(Martin et al., 2011). Hi-8 video camcorders (Sony Corporation, Tokyo, Japan) were concealed with surrounding vegetation 4 to 15 m from the nest and were left unattended. Per-nestling feeding rate was measured as feeding-trips $nestling^{-1}$ h^{-1} and calculated dividing the total feeding rate by the number of young in the nest. Brooding effort was calculated as percentage of time spent by the parents sitting on the nest to heat their offspring. Video recordings were made 2-3 days after hatch date, on the day that pin feathers broke their sheaths, and 2-3 days prior to the expected fledging date (Martin et al., 2000)

Growth rate

To calculate nestling growth trajectories, we measured body mass (g), wing chord length (mm), and tarsus length (mm) for the first three days after hatch and every other day thereafter. Mass measurements were taken with GemPro electronic scales (0.001 g resolution; model 250, MyWeigh, Phoenix, Arizona, USA). Other biometrics were taken

with Mitutoyo digital calipers (0.01 mm resolution; model 500-196-30, Mitutoyo, Aurora, Illinois, USA).

Statistical Analyses

We tested for effects of heating during development on the physiological and behavioral variables studied here by fitting linear mixed-effects models (R package lmer). Tests among species were performed including the difference between treatment and control for the trait of interest (i.e. mass specific metabolic rate, feeding rate, and brooding time) as response variables, and comparing the value of the intercept to zero. Species were included as a random effect nested within year. Furthermore, we fit separate ANOVA models with a random factor of year for each species to test for differences in mass-specific metabolic rate, brooding rate, and per-capita feeding rate between treatment and control nests.

For nestling mass, wing chord length, and tarsus size, we examined changes in growth rates (K), the timing of growth (inflection time, or t_i), and asymptotic size (A) using nonlinear mixed models (R package nlme) that estimated differences in growth between treatment and control (Sofaer et al., 2013). All analyses were performed using R version 3.1.2 (R Core Team 2014).

RESULTS

Effect of the experiment on metabolism—A relatively minor increase in average temperature during embryonic development (mean ± SE = 1.32 ± 0.13 °C) caused higher mass-specific metabolic rates in the post-natal stage across all seven species studied here ($F = 13.645$, $P < 0.001$, Figure 2). However this difference was only significant within two species (Figure 2, Table 1).

Effect of the experiment on parental care— Warmer temperatures during incubation did not change the overall level of care provided by parents to offspring in the form of feeding rates ($F = 1.01$, $P < 0.318$) or brooding ($F = 0.131$, $P < 0.718$) among species. Still, parents of six of the seven species showed a tendency for higher per capita food delivery rate under the treatment (Figure 3), while one species (mountain chickadee) instead showed a significant reduction in feeding rate compared to the control (Table 2).

Experimental increases in embryonic temperature produced minimal or no changes in the time parents spent brooding nestlings in five north temperate species (Figure 4). Yet, the species that reduced feeding (mountain chickadee) also showed a significant decrease in brooding effort. Only the tropical species increased both brooding time and feeding rate (Table 2, 3, Figure 3, 4), but this increase was not significant.

Consequences for growth rate— Growth rates of body mass, tarsus, and wing chord were higher for embryos exposed to warmer temperature in our tropical species alone (Table S1 Figure 5). Conversely, two species showed reduced growth rates under the treatment; one had slower growth in tarsus and wing chord (house wren), and the other in body mass and wing chord (mountain chickadee). One species showed reduced body mass growth but higher wing chord growth in the treatment compared to the control (cordilleran flycatcher). The remaining three species showed no significant difference in the rate of growth between heated and control nests (Figure 5, Table S1).

DISCUSSION

Faster development in ectothermic embryos is thought to impose fitness costs, including reductions in size at birth and post-natal growth (Atkinson, 1994; Zuo et al., 2012). Yet, when faster development is due to warmer temperatures costs for offspring may not be present. Our study shows that the magnitude and direction of growth rate responses to warmer embryonic temperatures differ among species. Two species showed slower growth in response to our treatment for at least one of the biometrics measured. This follows the expectations of the temperature size rule, which predicts smaller cellular size and lower body mass in ectotherms developing at warmer temperature (Atkinson, 1994). The majority (4) of species showed no apparent growth related costs from warmer development. One had a significant increase in rate of growth for all the body parts measured. These results emphasize that consequences of faster development from warmer temperature may not be as detrimental as generally thought.

Experimental increase in embryonic temperature also yielded higher mass-specific metabolic rate during the post-natal stage for all the species considered here. For six of these species, our results were consistent with higher embryonic metabolism

reported in a previous study (Ton and Martin 2016), indicating that the "physiological imprint" of temperature persists across life stages. Higher metabolism may produce increased accumulation of oxidative damages and reduce longevity (Harman, 2001). Higher metabolism is also normally associated with faster growth within and among species (Ton and Martin, 2015; West et al., 2001). Intriguingly, not all species that showed increased metabolism due to our treatment also grew faster, raising questions about the causal relationship between these two traits (Glazier, 2014).

The effects of warmer temperatures on parental care may explain the absence of faster growth despite increased metabolism in nestlings. Parents did not generally increase effort in favor of young. A significant reduction in feeding and brooding rate shown by one species was associated with a decrease in growth rate. Slower or similar growth rates compared to the control nests were detected in those species that increased feeding but not brooding. The only species that increased both rate of feeding and brooding in response to the treatment showed substantially higher growth rates. Thus, for species that show no positive effect on growth rates but had increased metabolism, parents may have not matched the energy requirements of the offspring by a sufficient increase in parental care.

The carry over effects of warmer temperature during embryonic development for metabolism, parental care and growth detected here are especially important considering the variety of ecological factors that may affect embryonic temperature. For example, exposure to higher perceived risk of predation or food available for incubating parents can lead to increased embryonic temperature (LaManna and Martin, 2016). Also, increased environmental temperatures expected with global warming can influence thermal conditions and development of embryos (Griffith et al., 2016; Scott and Johnston, 2012). We show that small changes in temperature (e.g. 1C) during embryonic development can have downstream repercussions for post-natal rates of offspring growth. However, species vary in their growth response to warmer embryonic conditions; some species benefit, some suffer, and others are unaffected. This interspecific variation in our study can be explained by integrating intrinsic (e.g. metabolism) and extrinsic (e.g. parental care) components of growth rate and ultimately questions the generality of

theories predicting detrimental consequences of faster development due to warmer embryonic temperature.

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AUTHOR CONTRIBUTIONS

RT and TEM conceived the study. RT performed the experiment and collected the data. RT and TEM analyzed the data and wrote the manuscript together.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

DATA ACCESSIBILITY

Data reported in this publication are available in the supplementary material.

ETHICS STATEMENT

This study was conducted under the auspices of the University of Montana IACUC protocol #059-10TMMCWRU.

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Figures

1.

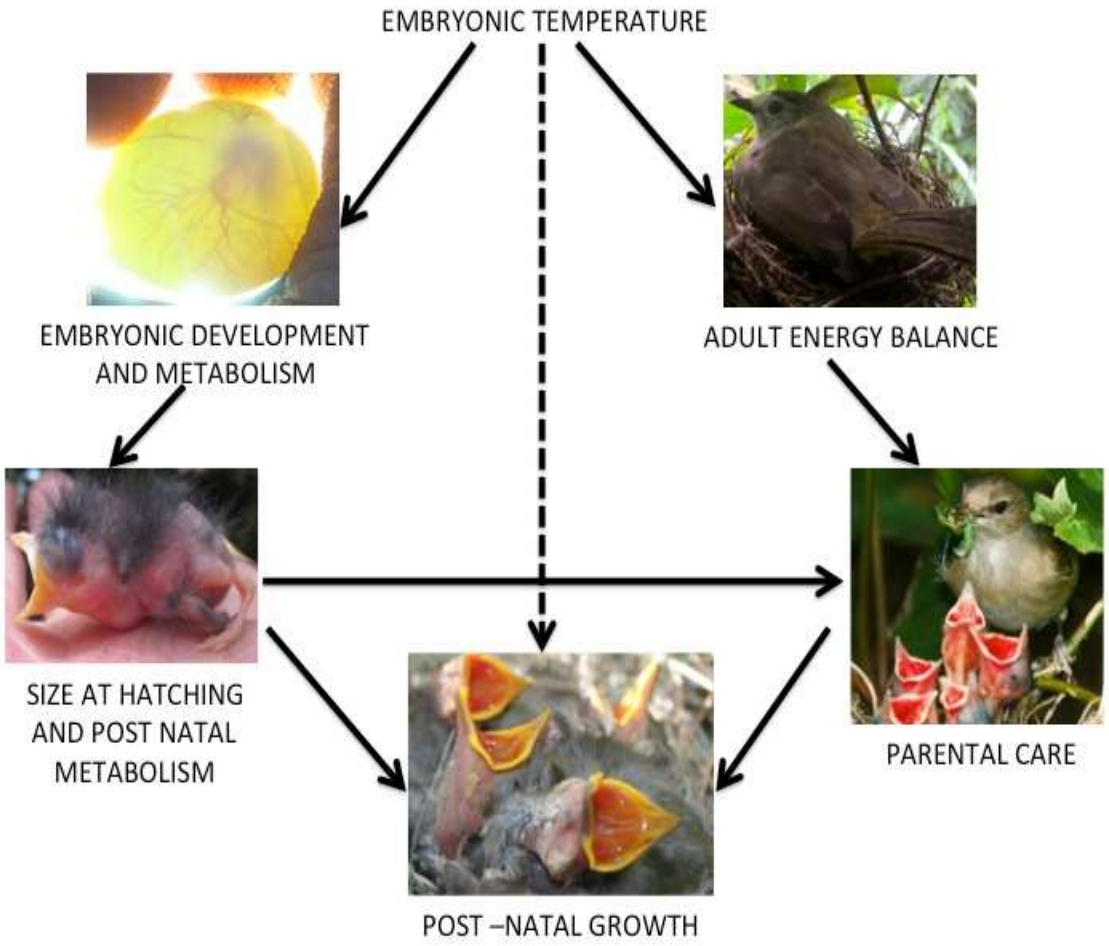

Figure 1: Conceptual representation of the direct (solid arrows) and indirect (dashed arrows) effects of increased temperature during embryonic development on post-natal growth.

2.

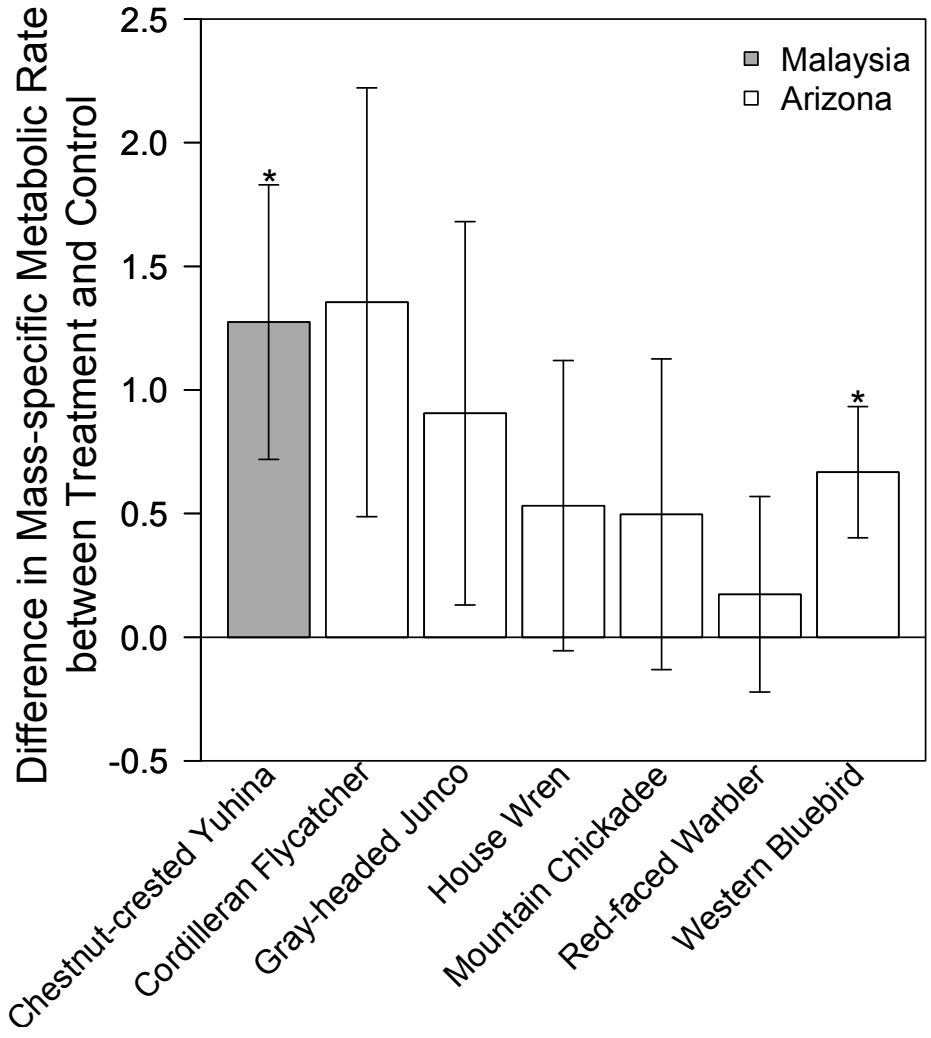

Figure 2: Differences between treatment and control means (± 1 SE) in post-natal mass-specific metabolic rate (mL O_2 h^{-1}) for seven bird species exposed to increased incubation temperature in a tropical (Malaysia) and north temperate (Arizona) site. Significant ($p < 0.1$) effects are denoted as *.

3.

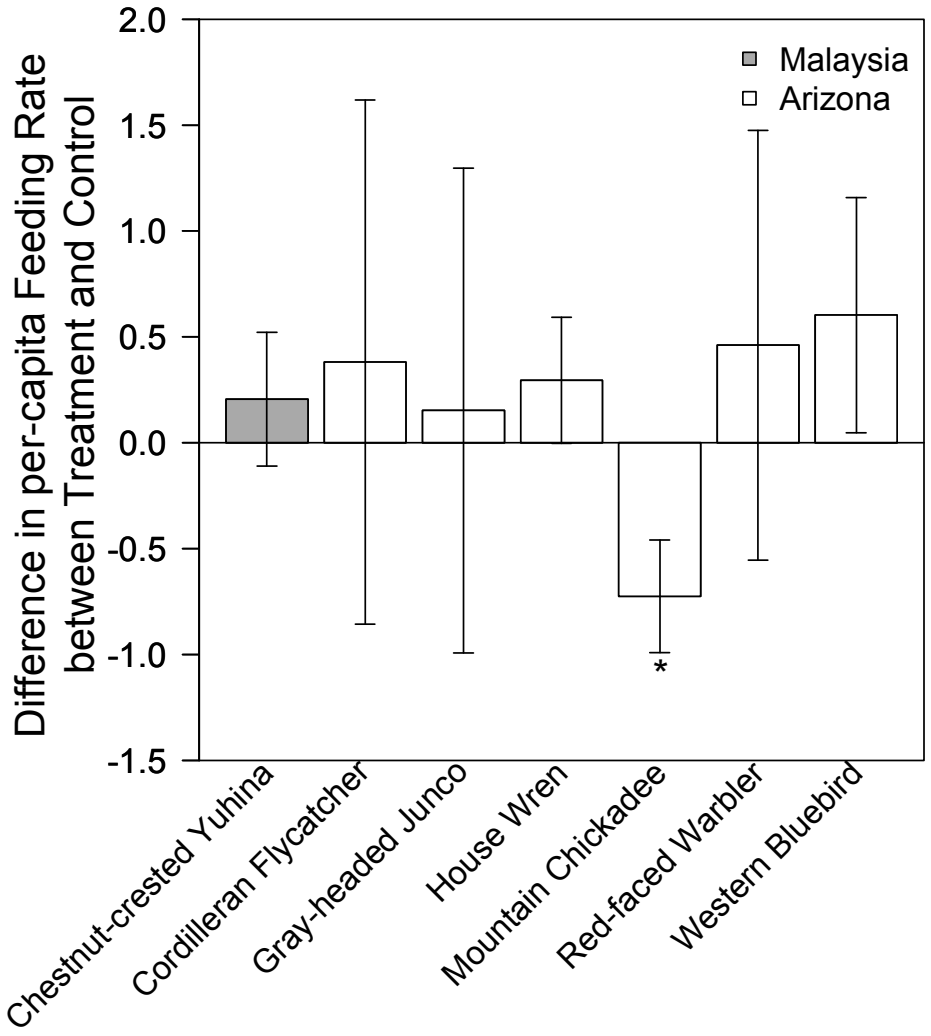

Figure 3: Differences between treatment and control means (± 1 SE) in per-capita feeding rate (feeding trips nestling^{-1} h^{-1}) for seven bird species exposed to increased incubation temperature in a tropical (Malaysia) and north temperate (Arizona) site. Significant ($p < 0.1$) effects are denoted as *.

4.

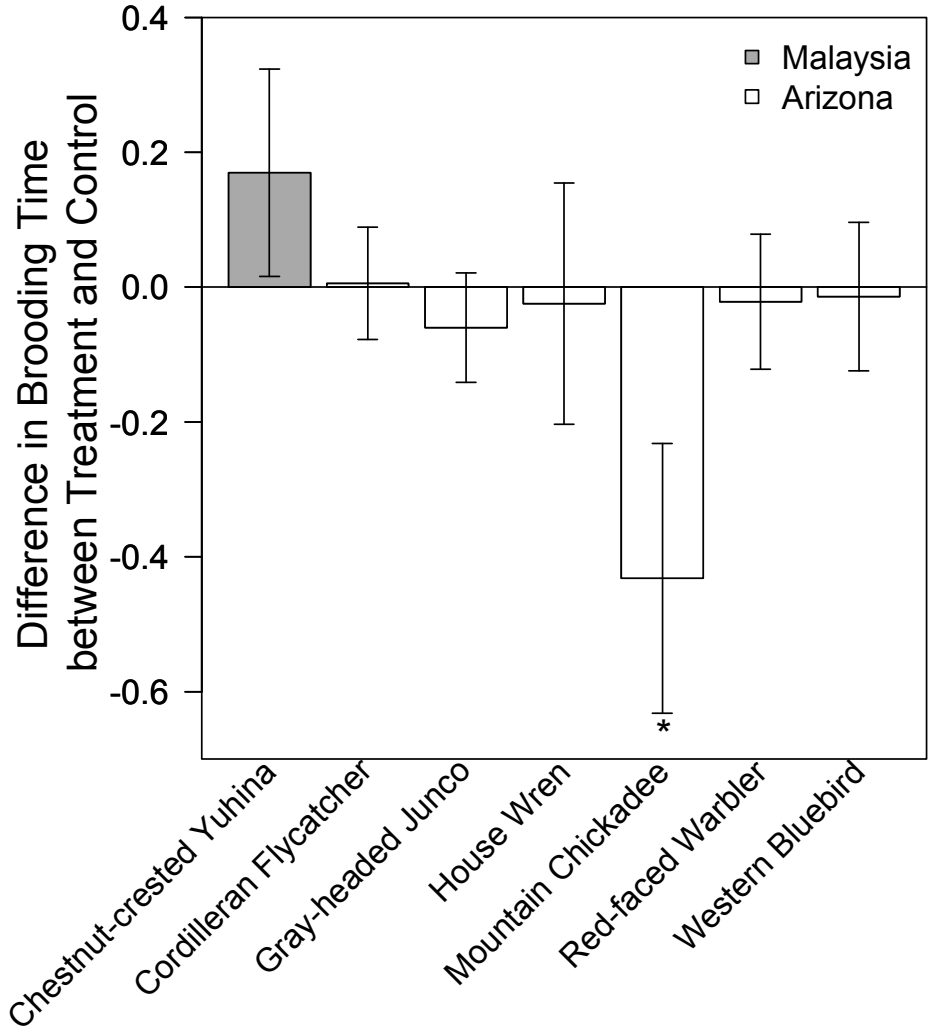

Figure 4: Differences between treatment and control means (± 1 SE) in brooding attentiveness (% of time spent brooding) for seven bird species exposed to increased incubation temperature in a tropical (Malaysia) and north temperate (Arizona) site. Significant ($p < 0.1$) effects are denoted as *.

5.

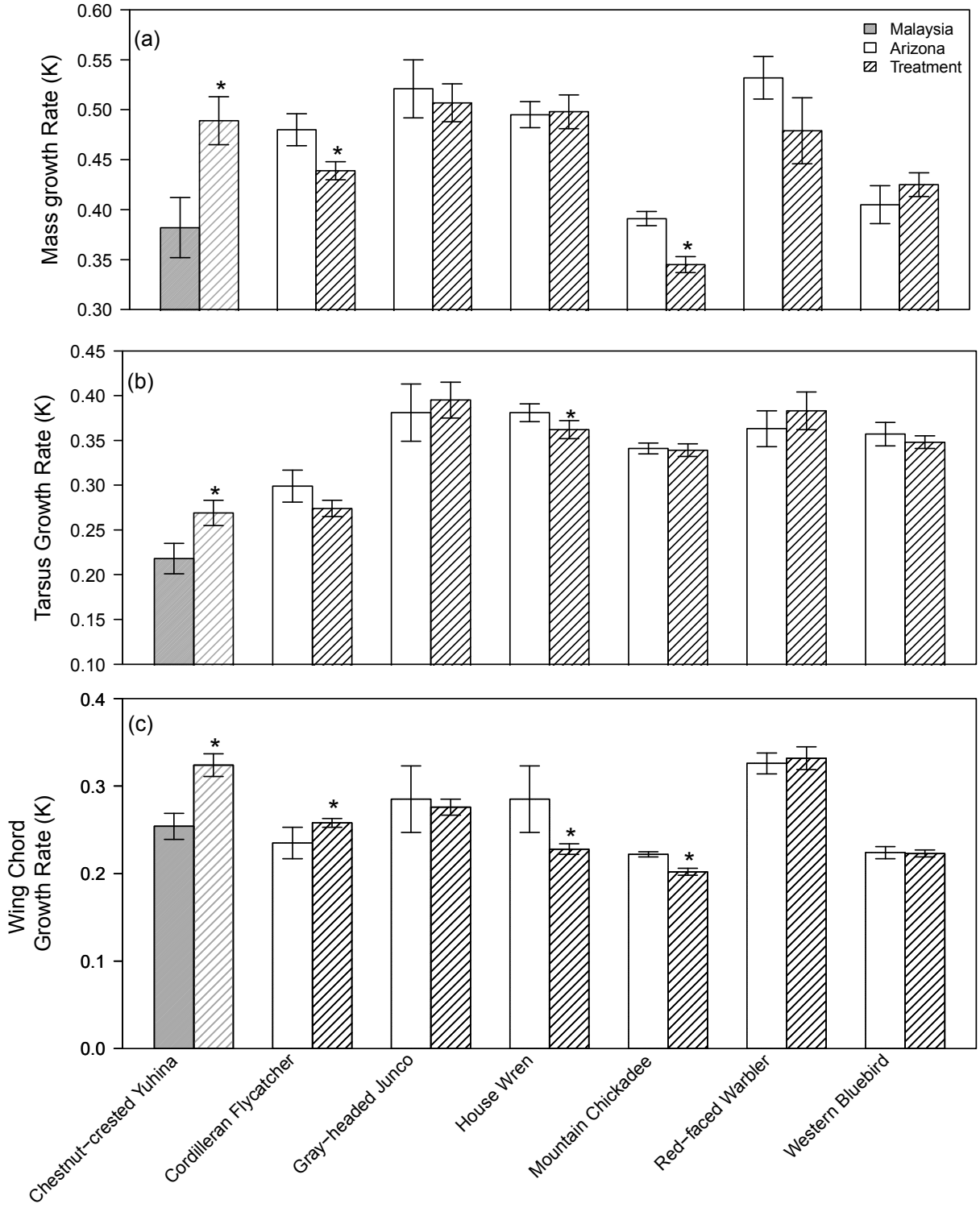
(a)
Malaysia
Arizona
Treatment
Mass growth Rate (K)
0.60
0.55
0.50
0.45
0.40
0.35
0.30
(b)
Tarsus Growth Rate (K)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
(c)
Wing Chord
Growth Rate (K)
0.4
0.3
0.2
0.1
0.0
Chestnut-crested Yuhina
Cordilleran Flycatcher
Gray-headed Junco
House Wren
Mountain Chickadee
Red-faced Warbler
Western Bluebird

Figure 5: Treatment and control means (± 1 SE) for nestling growth rate of (a) mass, (b) tarsi, and (c) wings of seven bird species exposed to increased incubation temperature in a tropical (Malaysia) and north temperate (Arizona) site. Significant ($p < 0.1$) effects within species are denoted as *.

Table 1. ANOVA tests for differences in mass-specific metabolic rate (mL O_2 h^{-1}) between treatment and control nests for one tropical (in bold) and six north temperate species. Sample sizes, means, standard errors (SE), *F* statistics, and *p*-values are provided. Significant effects ($p \leq 0.10$) are denoted as *.

Variable: mass-specific metabolic rate (mL O_2 h^{-1})

Species	Treat *n*	Control *n*	Diff. Mean	Diff. SE	*F*	*p*
Chestnut-crested Yuhina	5	5	1.27	0.55	5.27	0.105*
Cordilleran Flycatcher	3	3	1.35	0.86	2.44	0.259
Grey-headed Junco	3	3	0.90	0.77	1.36	0.363
House Wren	8	8	0.53	0.58	0.82	0.399
Mountain Chickadee	3	3	0.49	0.62	0.62	0.512
Red-faced Warbler	3	3	0.17	0.39	0.19	0.704
Western Bluebird	8	8	0.66	0.26	6.65	0.042*

Table 2. ANOVA tests for differences in per-capita feeding rate (feeding trips nestling^{-1} h^{-1}) between treatment and control nests for one tropical (in bold) and six north temperate species. Means, standard errors (SE), *F* statistics, and *p*-values are provided. Significant effects ($p \leq 0.10$) are denoted as *.

Variable: per capita feeding rate (feeding trips nestling^{-1} h^{-1})

Species	Diff. Mean	Diff. SE	*F*	*p*
Chestnut-crested Yuhina	1.27	0.20	1.65	0.235
Cordilleran Flycatcher	0.38	0.86	0.186	0.677
Grey-headed Junco	0.15	0.77	0.01	0.916
House Wren	0.29	0.58	0.234	0.634
Mountain Chickadee	-0.72	0.62	4.351	0.092*
Red-faced Warbler	0.17	0.46	0.18	0.678
Western Bluebird	0.66	0.60	1.43	0.112

Table 3. ANOVA tests for differences in brooding rate (% time spent on the nest) between treatment and control nests for one tropical (bolded) and six north temperate species. Means, standard errors (SE), *F* statistics, and *p*-values are provided. Significant effects ($p \leq 0.10$) are denoted as *.

Variable: brooding rate (% time spent on the nest)

Species	Diff. Mean	Diff. SE	*F*	*p*
Chestnut-crested Yuhina	0.17	0.15	1.21	0.351
Cordilleran Flycatcher	0.005	0.08	0.004	0.951
Grey-headed Junco	-0.06	0.08	0.55	0.594
House Wren	-0.02	0.17	0.01	0.896
Mountain Chickadee	-0.43	0.20	4.66	0.090*
Red-faced Warbler	-0.02	0.10	0.04	0.836
Western Bluebird	-0.01	0.01	1.43	0.899

Consequences of warmer temperature during embryonic development for metabolism, parental care, and post-natal growth among songbird species.

Riccardo Ton and Thomas E. Martin

Supplemental Material

Table S1. ANOVA tests for differences between treatment and control nests in asymptotic size (A), growth rate (K), and time of growth curve inflection (ti). Mean values are provided for treatment and control nestlings, followed by SE, *F* statistics, and *p*-values. Significant ($p < 0.1$) and highly significant ($p < 0.005$) effects are denoted as * and ** respectively. Results are presented for mass growth, wing growth, and tarsus growth. Species used for the experiment are reported in alphabetical order (Chestnut-crested Yuhina, Cordilleran Flycatcher, Gray-headed Junco, House Wren, Mountain Chickadee, Red-faced Warbler, Western Bluebird).

Species: **Chestnut –crested Yuhina**

Variable	Treat. Mean	Treat. SE	Control Mean	Control SE	*F*	*p*
Mass Growth:						
A (asymptote; g)	10.46	0.91	10.98	1.23	1.13	0.288
K (growth rate)*	0.459	0.02	0.457	0.03	7.61	0.006
t_i (inflection time; days)	3.91	0.30	4.21	0.45	0.66	0.414
Wing Growth:						
A (asymptote; mm)	49.63	8.45	45.07	4.69	0.18	0.659
K (growth rate) **	0.333	0.02	0.317	0.01	11.46	0.001
t_i (inflection time; days) *	7.17	0.92	9.23	6.62	0.49	0.074
Tarsus Growth:						
A (asymptote; mm)	21.22	1.89	18.84	0.87	< 0.01	0.953
K (growth rate)*	0.275	0.02	0.242	0.01	4.82	0.030
t_i (inflection time; days)	3.74	0.78	2.47	0.33	0.46	0.497

Species: **Cordilleran Flycatcher**

Variable	Treat. Mean	Treat. SE	Control Mean	Control SE	*F*	*p*
Mass Growth:						
A (asymptote; g)	12.99	0.09	13.24	0.35	< 0.01	0.966
K (growth rate)*	0.432	0.008	0.469	0.17	6.45	0.012
t_i (inflection time; days)*	5.08	0.21	4.43	0.35	4.03	0.047
Wing Growth:						
A (asymptote; mm)	58.51	1.43	71.66	9.17	2.00	0.159
K (growth rate)*	0.284	0.008	0.235	0.02	5.64	0.019
t_i (inflection time; days)	8.84	0.21	10.16	1.10	1.37	0.244
Tarsus Growth:						
A (asymptote; mm)	18.75	0.26	18.27	0.49	0.76	0.384
K (growth rate)	0.269	0.007	0.299	0.01	2.23	0.131
t_i (inflection time; days)	3.56	0.27	2.76	0.44	2.26	0.135

Species: **Gray-headed Junco**

Variable	Treat. Mean	Treat. SE	Control Mean	Control SE	*F*	*p*
Mass Growth:						
A (asymptote; g)	18.74	0.39	18.97	0.49	0.13	0.720
K (growth rate)	0.507	0.02	0.52	0.03	0.17	0.676
t_i (inflection time; days)	4.07	0.16	3.94	0.24	0.19	0.665
Wing Growth:						
A (asymptote; mm)	76.19	14.19	69.15	6.80	< 0.01	0.938
K (growth rate)	0.289	0.02	0.285	0.04	< 0.01	0.931
t_i (inflection time; days)	8.68	1.26	8.96	1.94	0.01	0.901
Tarsus Growth:						
A (asymptote; mm)	23.91	0.58	23.82	0.36	< 0.01	0.926
K (growth rate)	0.395	0.02	0.381	0.011	0.12	0.722
t_i (inflection time; days)	2.76	0.19	2.715	0.21	0.01	0.899

Species: **House Wren**

Variable	Treat. Mean	Treat. SE	Control Mean	Control SE	*F*	*p*
Mass Growth:						
A (asymptote; g)	11.12	0.24	11.13	0.24	0.08	0.779
K (growth rate)	0.503	0.01	0.49	0.012	0.59	0.440
t_i (inflection time; days)	4.73	0.14	4.88	0.14	0.07	0.277
Wing Growth:						
A (asymptote; mm)	51.49	1.32	51.52	1.38	< 0.01	0.938
K (growth rate)*	0.228	0.006	0.24	0.007	4.01	0.031
t_i (inflection time; days)	10.90	0.26	10.75	0.26	0.01	0.901
Tarsus Growth:						
A (asymptote; mm)	18.45	0.18	18.58	0.36	0.06	0.808
K (growth rate)*	0.358	0.007	0.387	0.011	5.31	0.021
t_i (inflection time; days)	3.45	0.13	3.65	0.21	0.42	0.513

Species: **Mountain Chickadee**

Variable	Treat. Mean	Treat. SE	Control Mean	Control SE	*F*	*p*
Mass Growth:						
A (asymptote; g)	12.39	0.25	12.63	0.23	2.19	0.139
K (growth rate) **	0.345	0.007	0.391	0.007	18.05	<0.001
t_i (inflection time; days)**	7.00	0.24	6.00	0.18	16.08	<0.001
Wing Growth:						
A (asymptote; mm)**	73.08	2.28	62.99	1.98	33.17	<0.001
K (growth rate)**	0.202	0.004	0.222	0.003	14.75	<0.001
t_i (inflection time; days)**	14.15	0.21	12.56	0.14	37.57	<0.001
Tarsus Growth:						
A (asymptote; mm)	19.43	0.14	19.64	0.10	1.76	0.183
K (growth rate)	0.339	0.007	0.341	0.006	0.006	0.806
t_i (inflection time; days)	4.28	0.22	4.20	0.15	0.08	0.769

Species: **Red-faced Warbler**

Variable	Treat. Mean	Treat. SE	Control Mean	Control SE	*F*	*p*
Mass Growth:						
A (asymptote; g)	10.07	0.32	10.02	0.36	< 0.01	0.925
K (growth rate)	0.501	0.13	0.53	0.012	0.02	0.280
t_i (inflection time; days)	3.77	0.09	3.80	0.14	0.14	0.895
Wing Growth:						
A (asymptote; mm)	54.10	2.26	59.59	3.97	1.44	0.233
K (growth rate)	0.333	0.01	0.326	0.01	0.19	0.661
t_i (inflection time; days)	6.95	0.27	7.68	0.41	2.08	0.152
Tarsus Growth:						
A (asymptote; mm)	19.78	0.50	19.71	0.65	< 0.01	0.924
K (growth rate)	0.381	0.01	0.363	0.02	0.44	0.507
t_i (inflection time; days)	2.73	0.14	2.90	0.21	0.47	0.494

Species: **Western Bluebird**

Variable	Treat. Mean	Treat. SE	Control Mean	Control SE	*F*	*p*
Mass Growth:						
A (asymptote; g)	26.76	0.18	26.30	0.46	1.20	0.273
K (growth rate)	0.440	0.007	0.405	.007	0.79	0.373
t_i (inflection time; days)	5.7	0.09	5.52	0.10	0.16	0.405
Wing Growth:						
A (asymptote; mm)	87.93	1.80	89.39	3.17	0.15	0.690
K (growth rate)	0.223	0.004	0.224	0.007	0.01	0.896
t_i (inflection time; days)	12.67	0.21	12.87	0.36	0.21	0.645
Tarsus Growth:						
A (asymptote; mm)	21.34	0.15	21.08	0.22	1.33	0.248
K (growth rate)	0.350	0.005	0.357	0.008	0.95	0.329
t_i (inflection time; days)	3.3	0.11	3.12	0.18	1.01	0.313

CHAPTER THREE

Metabolism Correlates with Variation in Post-Natal Growth Rate among Songbirds at three Latitudes

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Summary

1. Variation in post-natal growth rates is substantial among organisms and especially strong among latitudes. Tropical species typically have slower growth than north-temperate relatives. Metabolic rate is thought to be a critical mechanism underlying post-natal growth (after accounting for the effect of body mass). However, comparative tests on a large spatial scale are lacking, and the importance of metabolism for growth rates remains unclear both within and particularly across latitudes.

2. Songbirds exhibit strong interspecific variation in growth rates across geographic space, although within latitudes an association between metabolic rate and growth rate has not always been observed. Moreover, the hypothesis that differences in growth rates across latitudes reflect underlying differences in metabolism is untested. Here we investigate these possibilities across north temperate, south temperate and tropical study sites.

3. Phylogenetic analyses showed that, for a given body mass, metabolic rates of north temperate nestlings were higher than tropical relatives. Metabolic rates independent from body mass correlated with post-natal growth rates both within and among latitudes. Also, when accounting for interspecific differences in metabolic rates offspring body mass explained substantial variation in growth rates as expected under classic allometric theory.

4. Our results suggest that variation in metabolic rates has an important influence on broad patterns of avian growth rates at a global scale. We recommend further studies

that address the ecological and physiological costs and consequences of variation in metabolism and growth rates.

Key-words: body mass, life history theory, metabolic rate, physiology, temperate and tropical nestlings.

Introduction

Growth rates of offspring vary extensively among taxa, especially when comparing slow-growing tropical versus fast-growing north temperate organisms (Case1978; Roff 1992; Arendt 1997). Fast growth can decrease the risk of time-dependent mortality (Skutch 1949; Case 1978; Ricklefs 1993; Benrey & Denno 1997; Remeš & Martin 2002), allow earlier access to food and other resources (Conover & Present 1990), and increase opportunities for repeated reproductive events (Sibly & Calow 1986). However, fast growth can also negatively affect a broad array of traits related to organismal quality (Arendt 2003; Alonso-Alvarez *et al.* 2007; Arriero, Majewska & Martin 2013) and can reduce longevity (Metcalfe & Monaghan 2003; Hulbert *et al.* 2007; Lee, Monaghan & Metcalfe 2013; but see Martin *et al.* 2015). Despite these important ramifications for organismal quality and fitness (Starck & Ricklefs 1998), our understanding of the physiological mechanisms underlying broad interspecific and latitudinal variation in growth remains limited (Dmitriew 2011; Flatt & Heyland 2011).

Body mass, body temperature, and metabolic rate are thought to be responsible for extensive interspecific variation in rates of cellular proliferation (von Bertalanffy 1957). In particular, ontogenetic models predict that for a given temperature and body mass, the rate of somatic growth increases with metabolic rate (West, Brown & Enquist 1997; West, Brown & Enquist 2001). While this relationship has long been accepted, recent results have questioned the generality of metabolic rate as a pacemaker for growth within (Burton *et al*. 2011) and among species (Glazier 2014).

This uncertainty is highlighted in songbirds (Fig. 1) where the influence of metabolism and body mass on growth rates remains unclear.

Previous interspecific tests of the relationship between growth and metabolism in birds within latitudes have found no correlation (Konarzewsky *et al.* 2000), a weak positive correlation (Williams *et al.* 2007), and a positive correlation (Drent & Klassen 1989; Klassen & Drent 1991). However, the latter two studies did not directly measure metabolic rate but projected it from allometric equations. Moreover, reanalysis of their data yielded conclusions opposite to those of the original tests (Konarzewsky 1995), increasing the uncertainty surrounding the metabolism-growth relationship. The ability of metabolic rate to explain differences in growth rates of songbirds is further questioned by the absence of a correlation between offspring body mass and growth rate (Martin *et al.* 2011), opposite to expectations under scaling theory (West *et al.* 2001). Overall, this inconclusive evidence begs for studies directly testing if metabolism explains interspecific variation in avian growth.

While the role of metabolism in explaining growth rate variation within latitudes has been unclear, the role of metabolism in determining latitudinal differences in growth rates is untested. Metabolic rates of adults are lower in tropical songbirds compared to temperate relatives (Wikelski *et al.* 2003; Wiersma *et al.* 2007). If nestlings exhibit the same latitudinal pattern, then this may explain geographic variation in growth rates. Interestingly, embryonic metabolism did not differ among latitudes (Martin, Ton & Nicklison 2013), raising questions about latitudinal patterns of metabolism in offspring. Of course, metabolic rates can change across life stages (Glazier 2005), which emphasizes the need for direct measurements of post-natal metabolism. Here,

we test the hypothesis that metabolic rates underlie interspecific growth rates of songbirds both within and among latitudes.

Materials and Methods

STUDY AREAS AND SPECIES

Data were collected in a high elevation (2350m) mixed forest in north-temperate Arizona, USA (34° 34' N, 111° 14 W); in a tropical mid-elevation forest (1450-1950 m) in Malaysia (5° 59'N , 116° 34' E); and in a south-temperate dwarf shrubland located at sea level in South Africa (33° 41' S, 18° 26' E) (see Martin *et al.* 2015). Our sample included 59 species from 52 genera and 25 families within the order Passeriformes spanning substantial variation in body mass and rates of post-natal growth (see data accessibility).

METABOLIC MEASUREMENTS

We measured metabolic rates for 436 nestlings of 59 passerine species (see Table S1 in supporting information). Sample size varied between 1 and 13 with a mean ± SE of 7.4 ± 0.44 individuals measured per species. Only one nestling per clutch was tested to ensure independence among samples. Measurements were taken for 22 species in Arizona from May through July between 2011 and 2014; 23 species in Malaysia from February through April in 2012-2014; and 14 species in South Africa between August and November 2014. To account for possible effects of circadian rhythms, such as ambient temperature and rates of food delivery, on metabolic rates, measurements were taken between 11 a.m. and 5 p.m.

We recorded oxygen consumption [V_{O2} (mL h^{-1})] at 39.0 ºC in an open flow respirometry system using a Foxbox field gas analyzer (Sable System, Las Vegas, NV, USA). The temperature of 39.0 ºC was selected because it appears to match with the thermoneutral zone (temperature of lower oxygen consumption) for our species. We reached this conclusion based on extensive metabolic measurements we performed along a five steps Q10 interval of temperatures from 31 to 41 ºC (unpublished data). Also 39.0 ºC best approximated the average value of body temperature recorded in the field for the species studied here (38.95 ± 0.0586 ºC). We measured a nestling's internal body temperature as soon as it was removed from a nest and before the metabolic measurements using a HH506RA Multilogger Thermometer (Omega, Stamford, CT, USA). After inserting a 0.8 mm diameter thermocouple in the nestling's cloaca, we monitored core body temperature for at least 10 seconds and recorded the highest temperature value for each individual. Metabolic rates were measured at pin break, a standardized developmental stage when primary feathers break their sheaths and thermoregulatory capacities are comparable among species (Fig. 1, Sogge *et al*. 1991; Pereyra & Morton 2001). Therefore recording V_{O2} at pin break allowed us to control for interspecific variation in thermoregulation and its effect on metabolism during growth.

Each nestling was put in a 3.2L stainless-steel airtight metabolism chamber where it could sit on a cup-shaped piece of iron mesh that prevented extensive movements of the nestling while still allowing normal airflow. The chamber sat in a large, dark, insulated cabinet with a Peltier device (Pelt-4; Sable Systems) maintaining temperature at 39 ± 0.1°C. The chamber was connected to an open-flow

system and flushed with 200-300 milliliters per minute flow of atmospheric air scrubbed of CO_2 and water vapor. These flow rates guaranteed a stable proportion of oxygen available to birds within the sampled body range (Table S1). Air was filtered through scrubbers with Soda Lime, Magnesium Perchlorate and Drierite to remove water and CO_2 (Lighton 2008). After allowing the nestling to become accustomed to the chamber for 30 minutes, an initial baseline was recorded for about 10 minutes. Subsequently, V_{O2} was measured continuously every 0.5s until a plateau was reached (stable oxygen readings for at least 10 minutes). Lastly, a final baseline was recorded for 10 more minutes. The two baselines were later used to correct for potential drift in ambient O_2 during measurements and thus maximize the accuracy of our estimates. V_{O2} was calculated as the most stable five minutes of oxygen consumption within the plateau. The total amount of time needed to complete a measurement ranged from 70 to 110 minutes depending on nestling size. After completion of V_{O2} measurements, nestlings were fed with commercial food for altricial birds and returned to their nest unharmed. V_{O2} (mL h^{-1}) was calculated in ExpeData (ver. 1.3.2) software from Sable Systems using the formula $V_{O2} = FR_i(F_iO_2 - F_eO_2)/(1-F_eO_2)$. Where FR_i is the incurrent mass flow rate scrubbed from water vapor and CO_2, F_iO_2 is the incurrent fractional concentration of oxygen, and F_eO_2 is the excurrent fractional concentration of oxygen (Lighton 2008).

GROWTH RATE

Nestling growth rates were estimated for a total of 53 species. Small sample sizes prevented us from obtaining robust data on growth rates for two species in Arizona, two in Malaysia, and two in South Africa. Our growth rate estimates are based on

extensive sampling lasting 15 years (1999-2013) in Arizona, 6 years (2009-2014) in Malaysia, and 5 years (2000-2004) in South Africa. To calculate growth rates, we measured nestling body mass at the same time (± 1h) every day for the first three days after hatch and then every other day until fledge. We used *GemPro 250 portable electronic scales (MyWeigh, Phoenix, Arizona, USA) with an accuracy of ± 0.001 g*. We calculated the growth rate constant (k) for each species using logistic regression; a standardized and widely used method that allows interspecific comparison independent of absolute development time and body mass (Ricklefs 1968; Remeš & Martin 2002). Growth rates data are available online at the following link http://dx.doi.org/10.5061/dryad.ks62j.

STATISTICAL ANALYSIS

We produced our estimates for metabolic rate (V_{O2} at 39 ºC) and body mass using a linear mixed model to capture within and among species variation for each parameter of interest. To explain the scaling relationship between metabolic rate (V_{O2} at 39 ºC) and body mass, we ran a generalized linear model with body mass, site and the interaction between body mass and site as fixed factors and species' metabolic rate as the response variable. Both nestling metabolic rate and body mass were $\log_{10}$ transformed to meet assumptions of normality. To evaluate whether these relationships varied across sites, we conducted post hoc tests for differences in slopes among sites. To control for possible phylogenetic effects (Felsenstein 1985), we conducted a Phylogenetic Generalized Least Squares (PGLS) analysis using the Caper package in R (Orme 2013). To create the phylogenetic tree used to constrain the analysis, we sampled 1000 trees containing our study species from

www.birdtree.org (Jetz *et al.* 2012) using the Hackett backbone (Hackett *et al.* 2008). We then produced a majority-rules consensus tree using the program Mesquite (Appendix S1 in supporting information, Maddison & Maddison 2001). We used the scaling parameter Pagel's lambda (λ) as a measure of phylogenetic signal, which can range form 0 to 1 (Pagel 1999). A value closer to 0 indicates lower similarity in traits among species than strictly expected by their phylogenetic relationships based on a Brownian motion model of evolution (Pagel 1999). The λ values produced by our PGLS analysis were based on maximum likelihood optimization.

To assess how metabolic rates may influence growth rates, we ran an additional PGLS model, with growth rate as the response factor and metabolic rate, body mass, site, site × body-mass and site × metabolic rate interaction terms as fixed factors. We used backward-stepwise selection criteria to pick the best model and dropped non-significant variables from our analysis (Table S2). Full model outputs including all variables are listed in Tables S3 and S4. All statistical analyses were done in R v.3.0.3 (R Development Core Team 2014, Vienna, Austria).

Results

Metabolic rate increased with nestling body mass for 59 species of songbirds at all three latitudes (Fig. 2). The slope of this scaling relationship was 0.77 ± 0.022 (β ± SE), very close to the ¾ exponent expected under allometric theory (Kleiber 1932). For a given body mass, metabolic rates were significantly higher for north temperate species compared to those in the south temperate and tropics (Table 1). Also,

metabolic rates of nestlings in the south temperate were higher than in the tropics but the differences were not significant (Table 1).

The residual variation in metabolic rate unexplained by body mass strongly correlated with interspecific nestling growth rates (Fig 3b; Table 2). Moreover, latitudinal differences in growth disappeared as emphasized by the lack of significance of the "site" variable ($P = 0.814$) (Table S2). Allometric rules predict that growth rates should decrease with increasing body mass. When accounting for interspecific differences in metabolic rate nestling body mass explained 33% of interspecific variation in growth rate among sites (Fig. 3a; Table 2). None of the interactions terms tested in our models were significant indicating similar relationships among sites and were therefore dropped from the analysis and reported only in the supporting information (Tables S1 and S2). The λ values produced by our PGLS analyses were all greater than 0 but less than 1 (Table 1, 2) as seen in other comparative studies among birds and passerines in particular (Freckleton, Harvey & Pagel 2002)

Discussion

Predictions that body mass and metabolism should underlie growth rate variation have not been well supported in interspecific avian studies within latitudes (Dunn 1980; Klassen & Drent 1991; Konarzewsky 1995; Konarzewsky et al. 2000; Williams *et al.* 2007), and have never been tested across latitudes. We directly measured growth, body mass, and metabolic rates from three bird communities across the world, and documented strong correlations between these traits. Moreover we demonstrated that a geographic pattern in metabolism coincided with a known

latitudinal gradient in growth rates (Martin *et al.* 2011). North-temperate nestlings had higher metabolic rates and faster growth than south-temperate and tropical species. Previous studies have argued that metabolic variation in adult songbirds among latitudes reflected differences in "pace of life" (Wikelski *et al.* 2003; Wiersma *et al.* 2007). This argument revolves on theoretical expectations (Ricklefs & Wikelski 2002) and direct tests (Williams *et al.* 2010) suggesting that life history and physiological traits are expressed in coordination along a slow-fast continuum. Our study supported these previous findings by offering correlative evidence that a physiological trait (metabolic rate) underlies broad interspecific variation in a major life-history trait (rate of growth).

The latitudinal metabolic differences we found between tropical and north-temperate sites were not detected for embryonic metabolic rates for the same species (Martin *et al.* 2013). However, embryos are ectothermic while nestlings are first poikilothermic and finally endothermic as they grow. These radically different thermoregulatory conditions are known to be associated with equally different metabolic regimes (Peterson, Nagy & Diamond 1990). Therefore, dissimilarities between studies may reflect ontogenetic changes in metabolism across life stages of the same organism (White & Kearney 2013).

This stage-dependent nature of metabolic processes may also explain differences in the scaling relationship between metabolic rate and body mass among studies (Glazier 2005). Our slope for nestlings closely approximated the traditional ¾ exponent expected under classic scaling theories (Kleiber 1932, West *et al.* 1997, Banavar *et al.* 2014). However, this result did not match predictions of shallow slopes

close to ⅔ for organisms of small body size (Kolokotrones *et al.* 2010). Our scaling exponent was higher than for embryos (Martin *et al.* 2013) and adults (Wiersma *et al.* 2007) of songbirds, but lower than for growing young in other avian studies (Klassen & Drent 1991). Similar ontogenetic changes in allometric scaling have been previously documented (Czarnolesky *et al.* 2008; Peng *et al.* 2010; Glazier, Hirst & Atkinson 2015) and contribute to the debate over the existence of a universal slope for the metabolism-body mass correlation (West *et al.* 1997; White, Cassey & Blackburn 2007)

Previous studies have found weak effects or failed to detect the predicted negative relationship between body mass and growth rate (Remes and Martin 2002; Martin et al. 2011). Here we found that, when taking into account interspecific differences in metabolic rate, body mass explained a good part of growth rates variation. This result unveils the masking effect that metabolic rate can have on allometric relationships and further emphasizes the importance of interspecific differences in metabolism for broad geographic patterns of growth. Metabolic variation unexplained by body mass may be favored by environmental mortality during offspring growth (Rose 1991), but this possibility needs testing.

Understanding the selective pressures that influence metabolic variation would be fruitful because of the potential role of metabolism in life history evolution and "rate of living" theory (Pearl 1928). For example, high metabolic activity favoring fast growth may induce high production of reactive oxygen species (ROS) (Harman 1955). These metabolic byproducts have been hypothesized to carry physiological costs paid off later in life via oxidative damage that increase probability of adult

mortality (Harman 2001; Hulbert *et al.* 2007). Yet, the actual connection between high metabolism, ROS production, and costs for adult survival is still debated (Barja 2007). Some evidence also suggests that the intrinsic costs of growth rate variation may be of less importance than extrinsic sources of mortality in passerines (Martin *et al.* 2015). Future studies should elucidate if the variation in metabolic rate underlying post-natal growth is also a possible mediator of trade-offs between offspring and adult mortality.

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Data Accessibility

Metabolic data used in this manuscript can be accessed in the supplementary information (Appendix S1). Growth rate data are available at the following link http://dx.doi.org/10.5061/dryad.ks62j.

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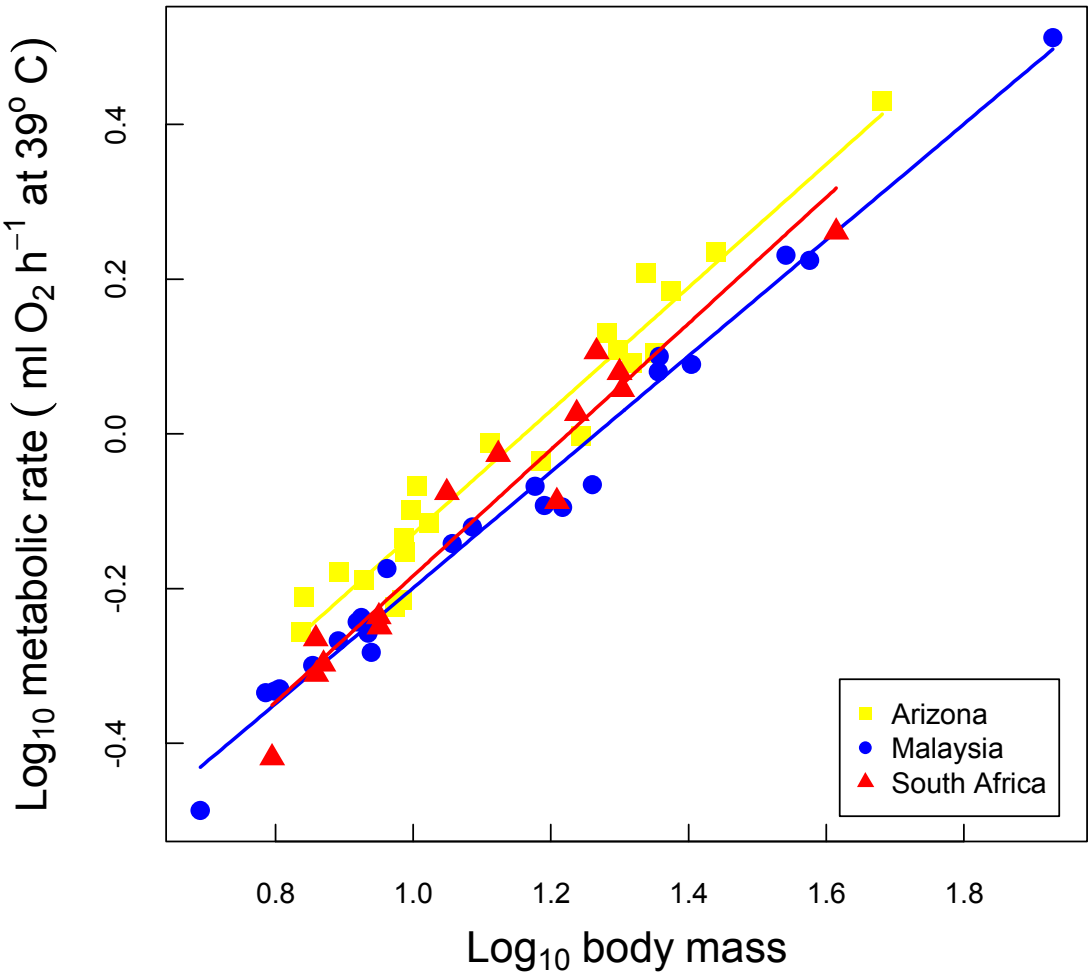
Figures

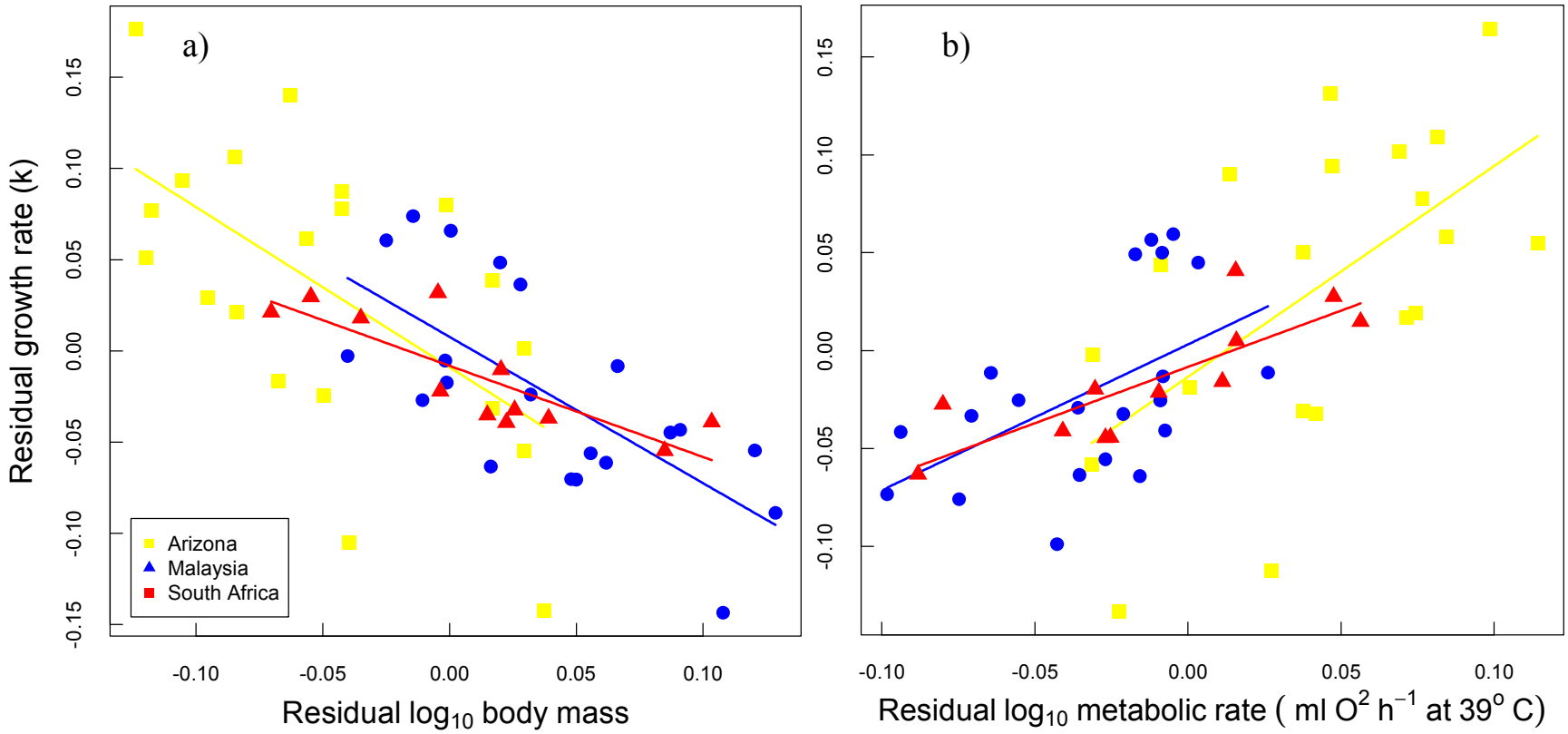
Fig. 1. Hermit thrush (*Catharus guttatus*) on pin break day. This is a species commonly breeding at our Arizona study site; the nestling just underwent metabolic measurements and after being fed it will be ready to re-join its nest of origin.

Fig. 2. Allometric scaling of metabolic rate for 59 species of songbirds nestlings. The lines represent a linear regression of body mass and metabolic rate (both $\log_{10}$ transformed) for each of the three latitudes investigated. For a given body mass, metabolic rate of north temperate species (Arizona) is significantly higher compared to that of species from tropical and south temperate regions (Malaysia and South Africa).

Fig. 3. Partial regression plots of growth rates for 52 species of songbirds' nestlings at three latitudes as a function of a) $\log_{10}$ body mass while accounting for metabolic rate and b) $\log_{10}$ metabolic rate when controlling for body mass. Bigger body sizes are associated with slower growth while higher metabolic rates yield faster growth within and among sites.

Figure 1.


Log10 metabolic rate (ml O2 h−1 at 39o C)
-0.4
0.0
0.2
0.4
0.8
1.0
1.2
1.4
1.6
1.8
Log10 body mass
Arizona
Malaysia
South Africa


a)
b)
Residual growth rate (k)
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
Arizona
Malaysia
South Africa
-0.10
-0.05
0.00
0.05
0.10
Residual log10 body mass
Residual log10 metabolic rate (ml O2 h−1 at 39o C)

Tables

Table 1. Summary of the model representing the scaling relationship between metabolic rate (ml O_2 h^{-1} at 39^o C), and log_{10} transformed body mass (g) with site as a fixed factor. Differences in metabolic rates between sites are listed as pair-wise contrasts. We report effect size (R^2), lambda values (λ), coefficients with standard error (β ,SE), *F-values*, degrees of freedom (*df*) , and significance (*P*) obtained from PGLS (Orme 2013) for 59 species of passerine nestlings in Arizona, Malaysia and South Africa.

Offspring metabolic rate as the dependent variable, (R^2=0.94), (λ = 0.710)				
Variable	β (SE)	*F*	*df*	*P-value*
Body mass	0.771 (0.022)	1240.4	1	<0.001
Site	---	9.347	2	<0.001
Malaysia vs Arizona	-0.065 (0.022)	---	-	<0.001
South Africa vs Arizona	-0.046 (0.015)	---	-	0.003
South Africa vs Malaysia	0.018 (0.013)	---	-	0.164
Error			55	

Table 2. Summary of the model for offspring growth rates (*k*) as a function of $\log_{10}$ transformed body mass (*g*), and $\log_{10}$ metabolic rate (ml O_2 h^{-1} g^{-1} at 39° C). We report effect size (R^2), lambda values (λ), coefficients with standard error (β ,SE), *F-values*, degrees of freedom (*df*) , and significance (*P*) obtained from PGLS (Orme 2013) for 53 species of passerine nestlings in Arizona, Malaysia and South Africa.

Offspring growth rate (*k*) as the dependent variable ($R^2 = 0.44$), ($\lambda = 0.573$).

Variable	β (SE)	*F*	*df*	*P-value*
Residual metabolic rate	0.780 (0.152)	24.95	1	<0.001
Body mass	-0.720 (0.125)	33.06	1	<0.001
Error			50	

Metabolism Correlates with Variation in Post-Natal Growth Rate among Songbirds at three Latitudes

Riccardo Ton and Thomas E. Martin

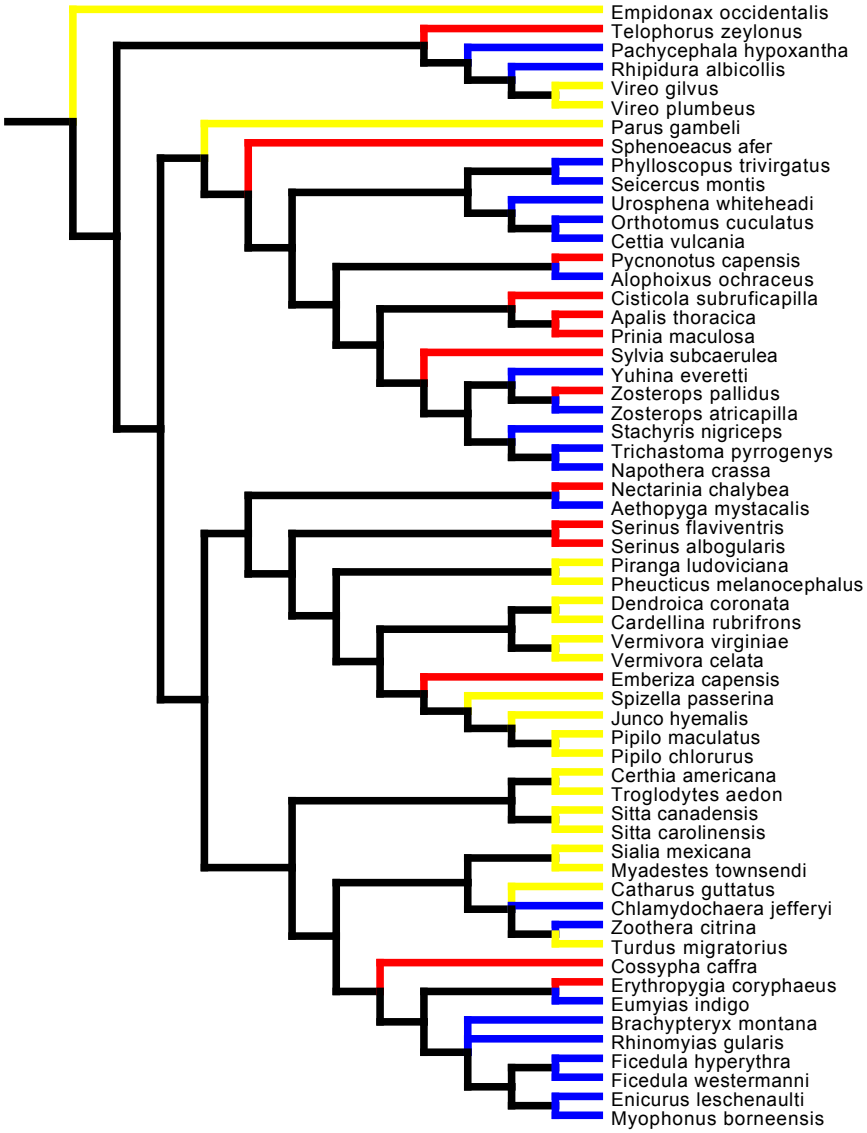
Supplemental material

Table S1. List reporting parameter estimates, standard errors (SE), and sample sizes (N = number of individuals) of body mass and metabolic rate for each of the 59 nestling species studied at our three sites (AZ=Arizona, ZA=South Africa, MY=Malaysia).

Species	Mass (g)	SE	Metabolic rate ($\dot{V}O_2$ (mL h^{-1}))	SE	N	Site
Empidonax occidentalis	10.53	0.227	0.700	0.031	11	AZ
Vireo plumbeus	12.94	0.505	0.965	0.064	5	AZ
Vireo gilvus	9.93	0.347	0.807	0.010	6	AZ
Sialia mexicana	22.42	0.571	1.309	0.034	11	AZ
Myadestes townsendi	27.48	-	1.615	-	1	AZ
Catharus guttatus	20.76	0.504	1.279	0.053	10	AZ
Turdus migratorius	47.99	1.899	2.570	0.125	10	AZ
Sitta canadensis	9.39	0.157	0.727	0.030	11	AZ
Sitta carolinensis	17.53	0.374	1.039	0.045	10	AZ
Certhia americana	6.86	0.162	0.568	0.020	10	AZ
Troglodytes aedon	9.62	0.231	0.615	0.034	13	AZ
Poecile gambeli	9.74	0.265	0.677	0.050	12	AZ
Junco hyemalis	15.32	0.263	0.962	0.025	10	AZ
Spizella passerina	9.68	0.251	0.773	0.023	10	AZ
Pipilo chlorurus	19.80	0.547	1.224	0.122	9	AZ
Pipilo maculatus	21.81	0.508	1.494	0.055	9	AZ
Oreothlypis celata	7.79	0.166	0.663	0.020	10	AZ
Oreothlypis virginiae	6.94	0.198	0.598	0.029	10	AZ
Setophaga auduboni	10.12	0.337	0.834	0.052	8	AZ
Cardellina rubrifrons	8.48	0.182	0.721	0.039	10	AZ
Piranga ludoviciana	19.11	1.106	1.424	0.042	6	AZ
Pheucticus melanocephalus	23.71	1.272	1.914	0.107	3	MY
Pachycephala hypoxantha	15.50	0.631	0.912	0.043	7	MY
Rhipidura albicollis	9.15	0.216	0.681	0.025	9	MY
Myophonus borneensis	84.94	5.939	2.977	0.189	7	MY
Geokichla citrina	37.65	-	1.932	-	1	MY
Chlamydochaera jefferyi	34.76	0.962	1.743	0.083	6	MY
Brachypteryx montana	16.47	0.491	0.810	0.028	10	MY
Vauriella gularis	22.77	0.877	1.306	0.043	9	MY
Ficedula hyperythra	8.41	0.266	0.624	0.018	10	MY
Ficedula westermanni	7.14	-	0.472	-	1	MY
Eumyias indigo	15.03	0.103	0.877	0.038	3	MY
Enicurus leschenaulti	25.36	1.407	1.166	0.026	4	MY
Alophoixus ochraceus	22.69	1.132	1.325	0.054	4	MY
Zosterops atricapilla	6.29	-	0.542	-	1	MY
Urosphena whiteheadi	7.78	0.340	0.480	0.020	6	MY
Horornis vulcania	8.60	-	0.573	-	1	MY
Phyllergates cuculatus	6.10	0.289	0.448	0.022	5	MY
Phylloscopus trivirgatus	8.29	0.196	0.549	0.021	8	MY
Seicercus montis	6.39	0.128	0.469	0.017	7	MY
Pellorneum pyrrogenys	11.40	0.654	0.769	0.029	10	MY
Napothera crassa	18.20	0.449	0.853	0.036	2	MY

Stachyris nigriceps	12.19	0.262	0.893	0.034	13	MY
Yuhina everetti	8.69	0.317	0.539	0.018	13	MY
Aethopyga temminckii	4.90	0.239	0.333	0.012	4	MY
Telophorus zeylonus	41.15	0.949	2.182	0.165	4	ZA
Dessonornis caffra	20.12	0.530	1.436	0.031	8	ZA
Tychadeon coryphaeus	16.17	0.846	0.941	0.028	9	ZA
Pycnonotus capensis	19.94	0.780	1.597	0.071	11	ZA
Cisticola subruficapilla	7.41	0.394	0.676	0.052	4	ZA
Prinia maculosa	7.23	0.102	0.618	0.014	12	ZA
Apalis thoracica	8.91	0.269	0.723	0.026	10	ZA
Zosterops capensis	7.22	0.395	0.665	0.041	8	ZA
Sphenoeacus afer	17.28	0.775	1.392	0.077	4	ZA
Curruca subcaeruleum	8.93	0.232	0.779	0.056	2	ZA
Anthobaphes chalybeus	6.24	0.225	0.511	0.018	9	ZA
Crithagra flaviventris	11.19	0.514	1.103	0.064	8	ZA
Crithagra albogularis	18.46	1.161	1.833	0.031	3	ZA
Emberiza capensis	13.28	0.691	1.367	0.064	5	ZA

Appendix S1.
Majority rules consensus tree based on 1000 trees from birdtree.org (Jetz et al. 2012) showing the phylogenetic associations among the 59 species studied. Branches are color coded as follow: Yellow=Arizona, Red=South Africa, Blue=Malaysia.



References

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Table S2. Description of the backward-stepwise criteria used for best model selection. Models are ranked according to Δ AIC and number of model parameters (*k*).

Metabolic rate as the dependent variable.

Model	Δ AIC	*k*
Body mass + site + site*body mass	1.90	4
Body mass +site	0.00	3
Body mass	12.94	2

Growth rate as the dependent variable.

Model	Δ AIC	*k*
Metabolic rate + body mass + site + site*body mass + site*metabolic rate	18.04	6
Metabolic rate + body mass + site + site*body mass	15.40	5
Metabolic rate + body mass + site	19.13	4
Metabolic rate + body mass	0.00	3

Table S3. Summary of the model representing the scaling relationship between metabolic rate (ml O_2 h^{-1} at 39° C), and log_{10} transformed body mass (g) with site as a fixed factor, and including a site*body mass interaction term. We report effect size (r^2), lambda values (λ), coefficients with standard error (β ,SE), *F-values*, degrees of freedom (*df*) , and significance (*P*) obtained from PGLS (Orme 2013) for 59 species of passerine nestlings in Arizona, Malaysia and South Africa.

Offspring metabolic rate as the dependent variable, (R^2=0.96), (λ = 0.447)				
Variable	β (SE)	*F*	*df*	*P-value*
Body mass	0.795 (0.046)	294.12	1	<0.001
Site	---	8.892	2	<0.001
Site*body mass	---	0.865	2	0.426
Error			53	

Table S4. Summary of the model for latitudinal comparisons of offspring growth rates (*k*) relative to $\log_{10}$ transformed body mass (*g*), and $\log_{10}$ residual metabolic rate (ml O_2 h^{-1} g^{-1} at 39^o C), with site as a fixed factor, and including a site*body mass and a site*metabolic rate interaction terms. We report effect size (r^2), lambda values (λ), coefficients with standard error (β ,SE), *F-values*, degrees of freedom (*df*) , and significance (*P*) obtained from PGLS (Orme 2013) for 53 species of passerine nestlings in Arizona, Malaysia and South Africa.

Offspring growth rate (*k*) as the dependent variable (R^2=0.53), (λ = 0.421).

Variable	β (SE)	*F*	*df*	*P-value*
Body mass	-0.051 (0.053)	11.48	1	0.001
Residual metabolic rate	0.858 (0.270)	13.02	1	<0.001
Site	---	2.93	2	0.064
Site*body mass	---	1.448	2	0.246
Site*residual metabolic rate		0.414	2	0.663
Error			44	

CHAPTER FOUR

The Roles of Nest Predation and Adult Mortality in the Evolution of Post-natal Metabolic Rate in Songbirds.

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Selection should drive the coevolution of life history strategies and associated physiological mechanisms but the sources of ecological mortality underlying this process remain unclear. High nest predation risk can be a major agent of selection favoring fast growth rates among songbird species and thereby may favor higher post-natal metabolic rates. Metabolism is strongly linked to post-natal growth rate but can also be an important determinant of adult mortality. Therefore, increases in metabolism may be constrained in species with lower adult mortality probability because of potential intrinsic costs of high metabolism on longevity. Here we test these possibilities among songbird species at three latitudes. We found that faster metabolic rate was associated with higher nest predation risk but not adult mortality probability. Our results provide a rare example of ecological sources of mortality acting on post-natal metabolic rate as a mechanism underlying offspring growth.

Keywords: offspring predation, adult mortality, metabolic variation, growth rate, songbird nestlings.

1. Introduction

Metabolic theory of ecology postulates that interspecific variation in metabolic rates underlay the slow-fast continuum in life history traits [1] . This link between physiology and life history has both bio-molecular and ecological causes [2] . However, while the former mechanisms are being actively investigated [1] , the latter are still poorly understood [3] . Predation is a major source of ecological mortality during vulnerable developmental stages and underlies variation in post-natal growth [4] . Since rapid growth in turn requires support from metabolism [5, 6] , high offspring predation rates might favor high metabolism as a way to achieve faster growth and enhance the probability of survival during early life stages. This hypothesis, however, remains untested, and as a result, limits our understanding of possible evolutionary causes of metabolic variation.

The evolution of fast metabolism from nest predation risk may be constrained by potential physiological costs on longevity (e.g., see [7]). Indeed, high metabolic rate can produce high oxidative damage [8] and result in trade-offs between fast growth and low organismal quality, both of which may increase adult mortality [9] . Thus, high offspring predation should favor fast metabolism while low adult mortality should favor slow metabolism to avoid oxidative damage and the detrimental consequences of fast growth. Here we provide a comparative test of the prediction that species under high predation during the post-natal stage should evolve fast metabolic rates, while species experiencing high adult mortality should evolve the opposite. We explore this possibility among songbird species at three latitudes encompassing substantial variation in post-natal metabolic rates.

2. Materials and Methods

(a) Study Area and Species

We measured metabolic rates, nest predation rates, and probability of adult mortality in 43 species of songbirds (order *Passeriformes*) at three latitudes (see supplementary material). We studied 16 species in high elevation (2300 m) mixed forest in Arizona (34° N latitude), 14 species in tropical mid-elevation forest (1450-1950 m) in Malaysia (6°N), and 13 species in coastal shrubland at sea level in South Africa [7] .

(b) Metabolic measurements

We recorded oxygen consumption [V_{O2} (mL h^{-1})] at 39.0 ºC, a typical temperature experienced by songbird offspring while being brooded by parents, for 370 nestlings in an open flow respirometry system using a Foxbox field gas analyzer (Sable System, Las Vegas, NV, USA). Measurements were made on only one nestling per clutch and at pin break, a standardized developmental stage among species when primary feathers break their sheaths. Nestlings were placed in a 2.3L stainless-steel airtight chamber within a dark temperature controlled cabinet set at 39 ± 0.1°C. The chamber was flushed with 200-300 milliliters per minute flow of atmospheric air scrubbed of CO_2 and water vapor [10] . After allowing 30 minutes for the sample to adjust, V_{O2} was measured every 0.5s until a plateau (maximum oxygen consumption) was reached and maintained for at least 10 minutes. V_{O2} (mL h^{-1}) was calculated as the O_2 concentration value observed during the most stable 5 minutes of oxygen consumption within the plateau using ExpeData software (ver. 1.3.2) from Sable Systems (see [7] for more details).

(c) Nest predation rates and adult mortality probability

We located and monitored large numbers of nests for 28 years in Arizona (1987-2014), six years (2009-2014) in Malaysia, and five years (2000-2004) in South Africa to obtain robust estimates of nest predation rates [4]. We calculated daily nest predation rates during the post-natal period with the logistic exposure method [11] .

Adult mortality probability was obtained from capture-recapture and resighting of color-banded birds in each site [12] for South Africa and [7] for Arizona and Malaysia).

(d) Statistical analysis

To account for phylogenetic effects, we sampled 1000 trees from www.birdtree.org [13] using the Hackett backbone [14] and produced a majority-rules consensus tree (see supplementary material) using the program Mesquite [15] . We then conducted Phylogenetic Generalized Least Squares (PGLS) analyses using the Caper package in R [16] .

Estimates for metabolic rates (V_{O2} at 39 ºC) and body masses of the 43 study species were obtained with a linear mixed model [4]. We used PGLS to test the ability of site as a fixed factor, and nest predation rate, adult mortality probability and body mass as covariates to explain variation in metabolic rate. We tested for differences in regression slopes among sites by including site × predation rate, site × adult mortality, and site × body mass interaction terms in the model. All statistical analyses were done in R v.3.0.3 for Macintosh (R Development Core Team 2014, Vienna, Austria).

3. Results

After accounting for nest predation, adult mortality, and site effects, body mass explained most of the variation in metabolic rate among species (table 1, figure 1 a), as expected under classic allometric theory [17] . Nest predation explained residual variation in nestling metabolic rates (table 1, figure 1 b). Adult mortality probability did not correlate with any residual variation in metabolic rate (table 1, figure 1 c). Metabolism differed among sites (table 1), confirming the presence of a latitudinal pattern in metabolic variation detected by previous studies [4]. None of the explanatory variables in our analysis exhibited significant interactions among sites ($p > 0.1$) therefore the interaction terms were dropped from the model.

4. Discussion

Metabolism can play an important role in many aspects of phenotypic variation among species [18] . However, examples of ecological mortality sources underlying interspecific and geographic variation in metabolism are rare [3]. Here we show that nest predation explains part of the post-natal metabolic variation among diverse passerine species across the world. In contrast, we did not detect any influence of adult mortality probability in constraining metabolic rates. These results suggest that songbirds may not face a conflict between higher metabolism to increase growth rate to reduce nest predation risk and lower metabolism to reduce intrinsic costs that may shorten life.

Songbirds appear to avoid this conflict by evolving physiological mechanisms that reduce the detrimental consequences of metabolic damage and fast growth. Indeed low rates of free radical production [19] , and membranes protecting cells from oxidative damages [20] are known to buffer the potentially negative effects of high oxygen consumption. These intrinsic defenses may relax possible constraints imposed by adult mortality probability while allowing nest predation to favor high metabolism to accelerate growth and minimize stage-dependent mortality.

Our results fit with previous evidence showing a strong effect of nest predation rate but not of adult mortality probability on post-natal growth rates [7] . Yet, the influence of mortality sources on intrinsic mechanisms such as metabolic rate that are responsible for growth were previously untested. Our results help link sources of environmental mortality and physiological mechanisms that may underlie phenotypic variation in post-natal growth.

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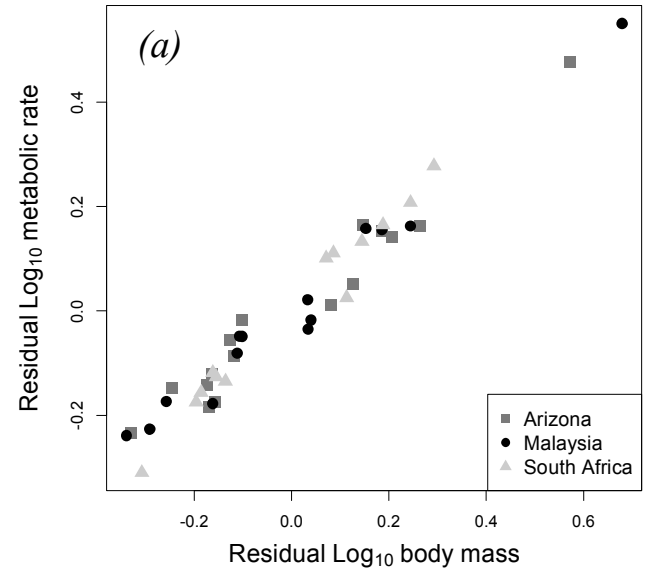
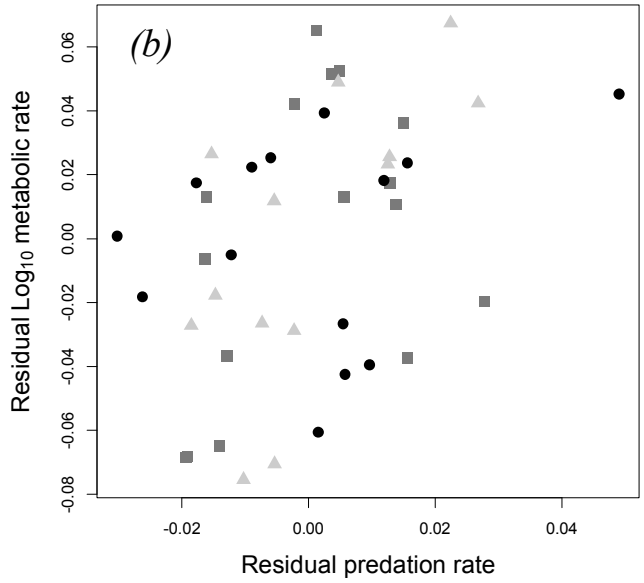
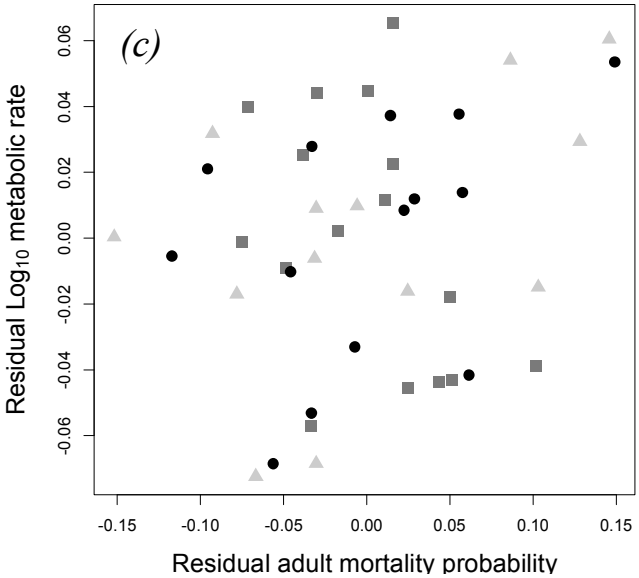
Figure legends

Figure 1. Partial regression plots of metabolic rate (ml O_2 h^{-1} at 39°C) as (*a*) a function of body mass after accounting for nest predation, adult mortality probability, and site, (*b*) as a function of nest predation rate after accounting for adult mortality probability, body mass, and site, and (*c*) as a function of adult mortality probability after accounting for nest predation rates, body mass, and site. Each point represents a species.


(a)
Residual Log10 metabolic rate
Residual Log10 body mass
Arizona
Malaysia
South Africa


(b)
Residual Log10 metabolic rate
Residual predation rate


(c)
Residual Log10 metabolic rate
Residual adult mortality probability

Tables

Table 1.

Linear model representing the relationship between metabolic rate (ml O_2 h^{-1} at 39^o C), rate of nest predation, adult mortality probability, and body mass for 43 species of songbirds at three latitudes. We report effect size (R^2), coefficients with standard error (β ,SE), *F-values*, degrees of freedom (*df*), and significance (*P*) obtained from PGLS.

Mass-specific offspring metabolic rate as the dependent variable. $R^2 = 0.42$				
Variable	β (SE)	*F*	*df*	*P-value*
Body mass	0.798 (0.026)	916.3	1	<0.001
Nest predation	1.027 (0.373)	7.562	1	0.017
Adult mortality	0.112 (0.087)	1.638	1	0.206
Site	---	3.174	2	0.052
Error			37	

The Roles of Nest Predation and Adult Mortality in the Evolution of Post-natal Metabolic Rate in Songbirds.

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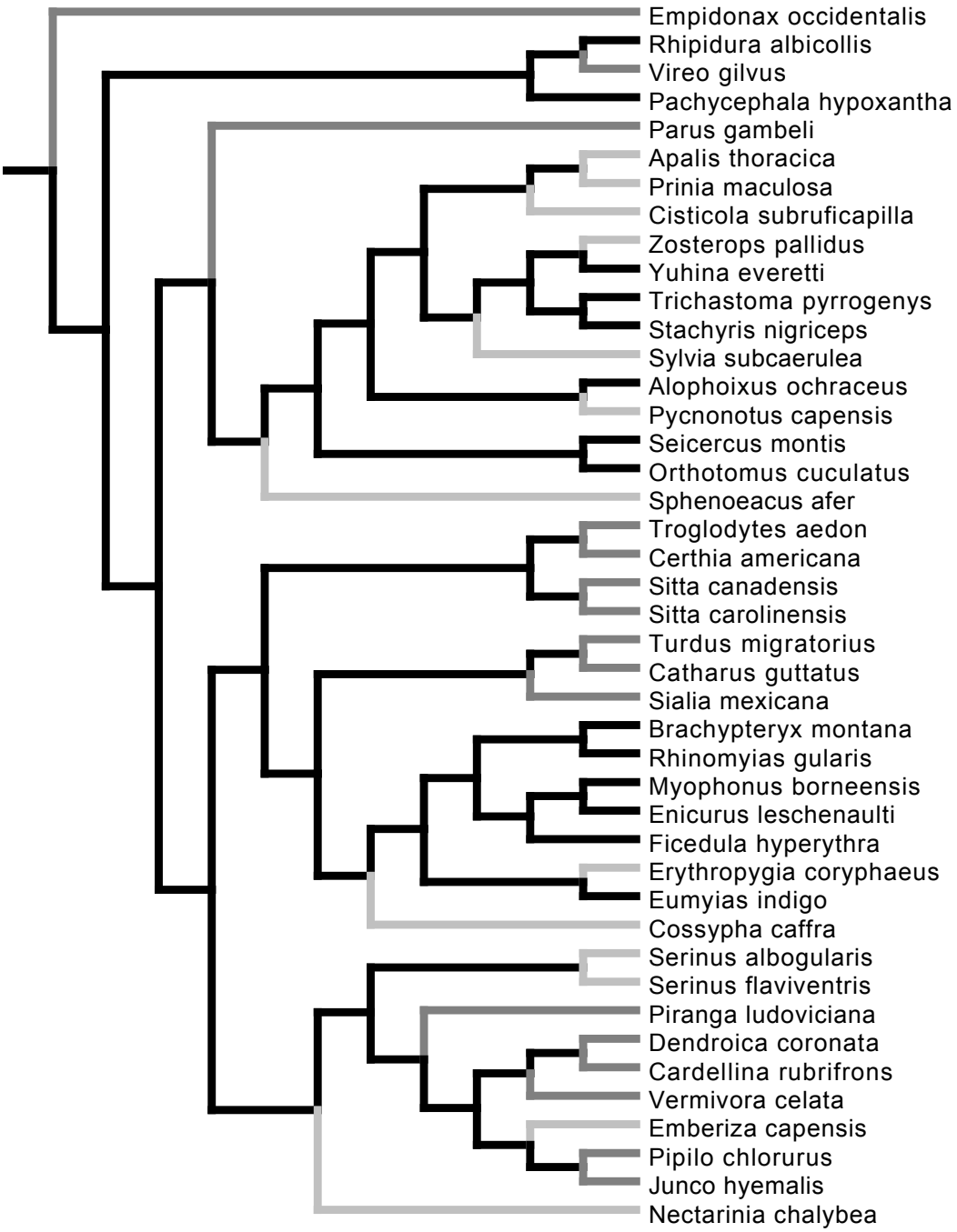
Supplemental material

Appendix S1. List reporting parameter estimates, standard errors (SE), and sample sizes (N = number of individuals) of body mass and metabolic rate for each of the 43 nestling species studied at our three sites (AZ=Arizona, ZA=South Africa, MY=Malaysia).

Species	**Mass (g)**	**SE**	**Metabolic rate ($\dot{V}O_2$ (mL h^{-1}))**	**SE**	**N**	**Site**
Empidonax occidentalis	10.53	0.227	0.700	0.031	11	AZ
Vireo gilvus	9.93	0.347	0.807	0.010	6	AZ
Sialia Mexicana	22.42	0.571	1.309	0.034	11	AZ
Catharus guttatus	20.76	0.504	1.279	0.053	10	AZ
Turdus migratorius	47.99	1.899	2.570	0.125	10	AZ
Sitta Canadensis	9.39	0.157	0.727	0.030	11	AZ
Sitta carolinensis	17.53	0.374	1.039	0.045	10	AZ
Certhia Americana	6.86	0.162	0.568	0.020	10	AZ
Troglodytes aedon	9.62	0.231	0.615	0.034	13	AZ
Poecile gambeli	9.74	0.265	0.677	0.050	12	AZ
Junco hyemalis	15.32	0.263	0.962	0.025	10	AZ
Pipilo chlorurus	19.80	0.547	1.224	0.122	9	AZ
Oreothlypis celata	7.79	0.166	0.663	0.020	10	AZ
Setophaga auduboni	10.12	0.337	0.834	0.052	8	AZ
Cardellina rubrifrons	8.48	0.182	0.721	0.039	10	AZ
Piranga ludoviciana	19.11	1.106	1.424	0.042	6	AZ
Pachycephala hypoxantha	15.50	0.631	0.912	0.043	7	MY
Rhipidura albicollis	9.15	0.216	0.681	0.025	9	MY
Myophonus borneensis	84.94	5.939	2.977	0.189	7	MY
Brachypteryx montana	16.47	0.491	0.810	0.028	10	MY
Vauriella gularis	22.77	0.877	1.306	0.043	9	MY
Ficedula hyperythra	8.41	0.266	0.624	0.018	10	MY
Eumyias indigo	15.03	0.103	0.877	0.038	3	MY
Enicurus leschenaulti	25.36	1.407	1.166	0.026	4	MY
Alophoixus ochraceus	22.69	1.132	1.325	0.054	4	MY
Phyllergates cuculatus	6.10	0.289	0.448	0.022	5	MY
Seicercus montis	6.39	0.128	0.469	0.017	7	MY
Pellorneum pyrrogenys	11.40	0.654	0.769	0.029	10	MY
Stachyris nigriceps	12.19	0.262	0.893	0.034	13	MY
Yuhina everetti	8.69	0.317	0.539	0.018	13	MY
Dessonornis caffra	20.12	0.530	1.436	0.031	8	ZA
Tychadeon coryphaeus	16.17	0.846	0.941	0.028	9	ZA
Pycnonotus capensis	19.94	0.780	1.597	0.071	11	ZA
Cisticola subruficapilla	7.41	0.394	0.676	0.052	4	ZA
Prinia maculosa	7.23	0.102	0.618	0.014	12	ZA
Apalis thoracica	8.91	0.269	0.723	0.026	10	ZA
Zosterops capensis	7.22	0.395	0.665	0.041	8	ZA
Sphenoeacus afer	17.28	0.775	1.392	0.077	4	ZA
Curruca subcaeruleum	8.93	0.232	0.779	0.056	2	ZA
Anthobaphes chalybeus	6.24	0.225	0.511	0.018	9	ZA
Crithagra flaviventris	11.19	0.514	1.103	0.064	8	ZA
Crithagra albogularis	18.46	1.161	1.833	0.031	3	ZA

Emberiza capensis	13.28	0.691	1.367	0.064	5	ZA

Appendix S2. Majority rules consensus tree based on 1000 trees from birdtree.org (Jetz et al. 2012) showing the phylogenetic associations among the 43 species studied. Branches are color coded as follow: Dark gray=Arizona, Light grey=South Africa, Black=Malaysia.



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