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Consequences of complex environments: Temperature and energy intake interact to influence growth and metabolic rate

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ABSTRACT

The field of comparative physiology has a rich history of elegantly examining the effects of individual environmental factors on performance traits linked to fitness (e.g., thermal performance curves for locomotion). However, animals live in complex environments wherein multiple environmental factors co-vary. Thus, we investigated the independent and interactive effects of temperature and energy intake on the growth and metabolic rate of juvenile corn snakes (*Pantherophis guttatus*) in the context of shifts in complex environments. Unlike previous studies that imposed constant or fluctuating temperature regimes, we manipulated the availability of preferred thermal microclimates (control vs. relatively warm regimes) for eight weeks and allowed snakes to behaviorally thermoregulate among microclimates. By also controlling for energy intake, we demonstrate an interactive effect of temperature and energy on growth—relevant temperature shifts had no effect on snakes' growth when energy intake was low and a positive effect on growth when energy intake was high. Thus, acclimation to relatively warm thermal options can result in increased rates of growth when food is abundant in a taxon in which body size confers fitness advantages. Temperature and energy also interactively influenced metabolic rate—snakes in the warmer temperature regime exhibited reduced metabolic rate (O_2 consumption rate at 25 °C and 30 °C) if they had relatively high energy intake. Although we advocate for continued investigation into the effects of complex environments on other traits, our results indicate that warming may actually benefit important life history traits in some taxa and that metabolic shifts may underlie thermal acclimation.

1. Introduction

Organisms face increasingly dynamic and, arguably, stressful environments due to anthropogenic activities that include fossil fuel combustion, habitat modification, pollution, and the introduction of invasive species (reviewed in IPCC, 2014). The thermal effects of global climate change (GCC; e.g., 2–8 °C increase in mean global air temperature over the next 50–100 years) are expected to continue to alter ecological systems across multiple levels of biological organization (reviewed in Sears and Angilletta, 2011; IPCC, 2014). Temperature influences a myriad of biological processes—from an organism's enzymatic efficiency and metabolic rate (e.g., the rate at which O_2 is consumed [$\dot{V}O_2$]) to its behavior and reproduction—which has led to a large body of theory and empiricism on thermal biology (reviewed in Angilletta, 2009). To understand organisms' temperature sensitivity, most laboratory-based research has examined the effects of different constant temperatures on fitness-related performance traits (reviewed in Angilletta, 2009). Yet, performance in a constant environment is not always equivalent to performance in a fluctuating environment with the same mean temperature (reviewed in Angilletta, 2009; Niehaus et al., 2012; but see Michel and Bonnet, 2010). Further, thermal environments in which animals can behaviorally thermoregulate by moving among microclimates are even more realistic and ecologically relevant than fluctuating temperature regimes (Glanville and Seebacher, 2006; Sears et al., 2011).

An unprecedented shift in global temperatures (and, thus, microclimates) is not the only potential stressor encountered by animals. They must also deal with rapidly changing complex environments wherein temperature and other environmental factors vary simultaneously (reviewed in Todgham and Stillman, 2013). For example, animals require energy to fuel their activities, and food availability is influenced by climatic variation (Both and Visser, 2005; Altermatt, 2010; Pearce-Higgins et al., 2010; Gardner et al., 2011; Allan et al., 2013; Cahill et al., 2013). Although animal traits are influenced by the independent effects of food (energy) availability and shifts in temperature (e.g., Guderley, 2004: effects on locomotor performance and muscle metabolism; LeMoine et al., 2008: effects on mitochondrial enzyme activity and transcript levels of metabolic enzymes), few studies have examined the interactive effect of food and energy (but see; Adamo et al., 2012: effects on reproduction and survival).

Juveniles may be particularly sensitive to shifts in complex environments for a host of reasons, many of which are related to body size (Peters, 1986) and variation in energy budgets due to ontogeny (sensu the ontogenetic growth model: Hou et al., 2008; Moses et al., 2008;

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West et al., 2001). Animals at this stage are typically smaller and, as a result, have a relatively high surface area to volume ratio. Therefore, the body temperature (T_{body}) of smaller ectotherms more quickly conforms to environmental temperature, and smaller animals have relatively high mass-specific rates of energy expenditure (Peters, 1986). In addition, energy intake may be constrained by the body size of juveniles (e.g., smaller snakes are gape limited and, thus, have fewer prey options than adults: King, 2002). Juveniles also have fewer energy stores, which may be required for periods of reduced food availability. In addition to energetic constraints, smaller juveniles exhibit a higher risk of predation relative to adults because they can be eaten by a larger array of predators and exhibit a reduced capacity to escape predators (e.g., slower sprint speed: Cejudo and Marquez, 2001; Peters, 1986).

Given these size-related risk factors, growth rate strongly influences fitness across animal taxa (reviewed in Dmitriew, 2011). Faster growing individuals reach reproductive maturity earlier, and they are at less risk of predation. The growth rates and/or body sizes of many taxa increase with rearing temperature in controlled, high-food conditions of the laboratory environment (e.g., reviewed in Angilletta, 2009; Montagnes et al., 2003; Seebacher and Grigaltchik, 2014). Yet, food availability varies in nature where warmer temperatures typically reduce body size in a wide array of animal taxa (e.g., Gardner et al., 2011; Caruso et al., 2014). Therefore, examining energy expenditure ($\dot{V}O_2$) in juveniles may elucidate the interplay among growth, food (energy) availability or intake, and temperature in the context of shifts in complex environments.

In addition to food availability, the net energy available to animals for growth may be influenced by digestive efficiency (the proportion of ingested nutrients that are absorbed). Although digestive efficiency can be temperature-dependent, some taxonomic groups exhibit high digestive efficiencies that are relatively insensitive to temperature (snakes: reviewed in Alexander et al., 2012). In these taxa, growth is putatively driven by food intake, resource allocation, and energy expenditure (rather than by digestive efficiency). Thus, we manipulated the availability of preferred thermal microclimates and food for 8 weeks in the juvenile corn snake (*Pantherophis guttatus*, Linnaeus) to address two alternative hypotheses based on the effects of rearing temperature and energy intake on growth (change in body length) and metabolic rate at two test temperatures (25 °C and 30 °C).

(1) Our first hypothesis proposes a lack of thermal acclimation whereby metabolic expenditure will be obligatorily tied to temperature—meaning snakes reared at warmer temperatures will expend more energy and, as a result, have less energy to allocate to growth (Fig. 1a, b). This hypothesis predicts the following: (a) The slope of the positive relationship between energy intake and growth will be steeper in snakes maintained under control conditions (those in which snakes can reach their preferred body temperature, T_{pref}) relative to those maintained under warmer conditions (those in which snakes only have thermal options higher than their T_{pref}) (Fig. 1a). (a) Energy intake will be positively related to $\dot{V}O_2$—well-fed snakes will be heavier and, thus, consume more O_2. However, this energy–$\dot{V}O_2$ relationship will not be influenced by temperature regime during rearing. That is, when snakes' metabolic rates are measured at a constant test temperature, they will be affected by energy intake but not by (rearing) temperature regime (Fig. 1b).
(2) Our second hypothesis proposes that snakes maintained in warm conditions will acclimate to warmer temperatures by exhibiting more efficient (lower) metabolic expenditure, and that these metabolic savings will help maintain growth (Fig. 1c, d). This hypothesis makes the following predictions: (c) The slope of the positive relationship between energy intake and growth will be similar in snakes reared under warm and control temperature conditions (Fig. 1c). (b) The positive relationship between energy intake and $\dot{V}O_2$ *will* be influenced by (rearing) temperature regime wherein the offset or intercept will be lower in snakes reared in warm conditions relative to those in control conditions due to metabolic acclimation (hypometabolism) by warm snakes (sensu Seebacher and Grigaltchik, 2014; Fig. 1d).

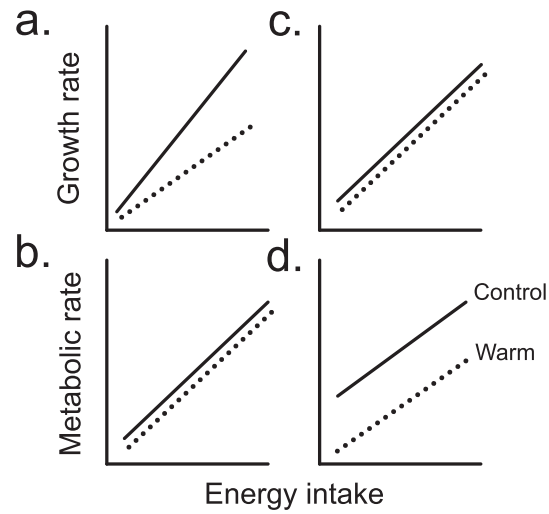

Fig. 1. Predicted effects of temperature regime (solid line: control; dotted line: warm) and energy intake on metabolic rate measured at a constant temperature (e.g., 30 °C) and growth rate based on our first (a., b.) and second hypotheses (c., d.). See text for details.

2. Materials and methods

2.1. Study species and husbandry

Pantherophis guttatus is a medium-sized, non-venomous snake native to the southeastern United States (Gibbons and Dorcas, 2005). To address our hypotheses, we used a captive colony of *P. guttatus* ($n = 66$) that were the progeny (1st–4th generation, with no known inbreeding) of wild caught snakes in Beaufort County, South Carolina, USA. All snakes were 5–6 months old at the onset of the study, and we individually housed them in translucent plastic enclosures (270 mm × 410 mm × 150 mm) with *ad libitum* access to water in a room with a 12:12 light:dark cycle that was maintained at 20–22 °C. Prior to the study, we offered each snake a prey item (sub-adult mouse that was 15–20% of weight of the snake to which it was offered) two times per week, which represents a high-food diet for colubrid snakes (Byars et al., 2010). We thawed commercially available mice that were pre-killed and previously frozen prior to offering. To facilitate behavioral thermoregulation, we placed subsurface heating at one end of each snake's enclosure to create a thermal gradient that ranged from approximately 24.5 °C at the cool end up to approximately 33 °C at the warm end ('control' temperature regime). Because refuge availability can influence thermorgulatory decisions in snakes (Tsai and Tu, 2005), we kept folded newspaper in place on the cooler end of each enclosure to create a cool refuge similar in concept to natural conditions (e.g., a subterranean burrow). All procedures were approved by the Institutional Animal Care and Use Committee at Georgia Southern University (protocol #I14004).

2.2. Experimental design

Over the course of our 8-wk study, we manipulated temperature and food availability. We individually housed approximately half of the snakes ($n = 32$) at the control temperature regime described above (Fig. 2). This temperature range reflects thermal options in the natural range of *P. guttatus* during the active season (spring–fall) (Howze and Smith, 2012), and it also accommodates the preferred temperature for

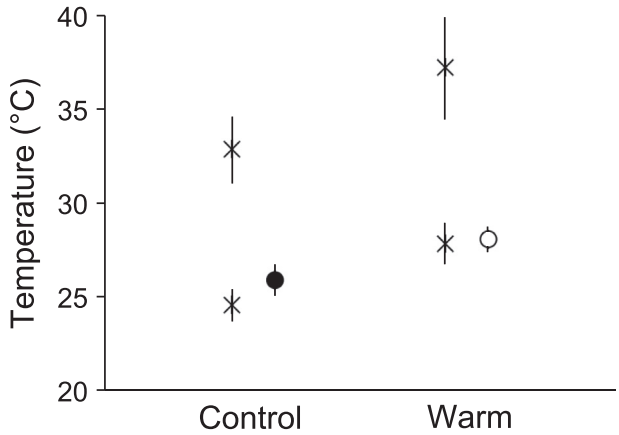

Fig. 2. Temperature ranges available to juvenile *P. guttatus* kept at either control or warm regimes (low and high temperatures are denoted by ×), as well as the estimated body temperature of snakes kept in each regime (circles). All values are displayed as mean ± 1 s.d.

P. guttatus (26 °C; Fig. 2). To create an ecologically relevant 'warm' temperature regime for the remaining snakes ($n = 34$), we increased the magnitude of thermal options by 3–4 °C, which is the predicted increase in temperature in the natural range of *P. guttatus* by the year 2100 (IPCC, 2013). Therefore, we gave individually housed snakes in the warm regime thermal options ranging from approximately 28 °C to 37 °C (Fig. 2). The temperature ranges we offered snakes (approx. a 9 °C gradient) were constrained by the dimensions of the enclosures and the temperature of the rooms in which enclosures were housed. However, the temperature gradients we used were similar to the range of temperatures in which a sympatric colubrid (*Lampropeltis getula*) can be found during the active season (difference between average minimum and maximum daily air temperatures: 11 °C) (Howze and Smith, 2012).

To verify our temperature regimes, we recorded the temperature at the cool and warm ends of two empty enclosures (one in a control enclosure and one in a warm enclosure) on an hourly basis throughout the entirety of the study using small temperature data loggers (HOBO Pendant, Onset Computer, Corp., Bourne, MA) that were factory-calibrated. These two thermal regimes do not replicate the complex temporal and spatial dynamics of available microclimates in nature (e.g., temperature ranges shift from day to night). However, they are likely more realistic than commonly used regimes in which animals cannot thermoregulate (e.g., constant or fluctuating temperature regimes).

We initially manipulated food availability by randomly assigning half of the snakes in each temperature regime to a 'low-food' diet (one prey item offered per week) and the remaining snakes to a 'high-food' diet (two prey items offered per week). Because snakes fed a high-food diet ate more frequently, they also increased in body mass quickly—thus, they were offered larger (higher energy) prey items more frequently relative to snakes fed a low-food diet. Also, some snakes in the high-food group frequently refused prey items. Therefore, categorically separating snakes into low-food and high-food groups became problematic. To address this issue, we estimated the amount of energy ingested by each snake over the 8-wk study using recently published data on the energy content of each of the types of prey items we used in our study (Crocker-Buta and Secor, 2014).

Because thermal plasticity can be genetically constrained (e.g., Seebacher et al., 2012), inter-individual variation may obscure the relative importance of thermal acclimation versus that of thermal constraint. We addressed this potential confounding factor through two aspects of our experimental design. First, study animals were the offspring of nine female *P. guttatus*, so we evenly distributed snakes from each female into four treatment groups to account for potential maternal effects. Second, we accounted for inter-individual variation by initially measuring dependent variables (body size and mass, and metabolic rate) < 1 wk prior to the study. We then measured the same variables at the end of the study (approx. 8 wks later), and we used initial measures as effects (covariates) in our statistical models (see below).

2.3. Estimated body temperature

We serially estimated the body temperature of each snake throughout the study to verify that our temperature regimes influenced snakes' T_{body}. Each week between 09:00 and 12:00, we used a factory-calibrated infrared thermometer (ProTemp 12, Jewell Instruments, Manchester, NH; range: −50–550 °C; accuracy: 1.5%; resolution: <0.1 °C; emissivity: 0.95, which approximates the emissivity of snake skin: Tattersall et al., 2004) to estimate T_{body} of snakes 3 d post-feeding from a distance of 100–200 mm (measurement diameter: 8–17 mm). Because surface temperature and T_{body} are highly related to one another in reptiles (Lagarde et al., 2012), this method provides an indirect estimate of T_{body} that is most useful in quiescent, coiled snakes as it records the surface temperature of a circular area. Our study animals typically remain in quiescent, coiled positions for extended periods of time (≥1 h), which putatively allows adequate time for them to come to temperature equilibrium (ZRS unpublished). Therefore, we discarded measurements of moving, agitated, and/or non-coiled snakes. Our estimates of T_{body} in *P. guttatus* were similar to the actual T_{body} of *P. guttatus* (Roark and Dorcas, 2000). We used the mean value of each snake's weekly estimate of T_{body} for our analyses (described below).

2.4. Morphology

To determine changes in morphology (e.g., growth rate), we measured the body mass (±0.1 g) and body size (snout-to-vent length, SVL [±5 mm]) of each snake immediately prior to the study and 54 d into the study. We measured each snake's SVL by gently stretching it along a measuring stick.

2.5. Oxygen consumption rate

To estimate metabolic rate, we measured the oxygen consumption rate ($V\dot{O}_2$, ml $O_2 \cdot h^{-1}$) of each snake 1–2 d prior to the study and 55–56 d into the study. At both time points, we measured $V\dot{O}_2$ three times per day at one of two test temperatures (25 ± 0.5 °C or 30 ± 0.5 °C), the order of which was randomized. The following day, we determined each snake's $V\dot{O}_2$ at the other test temperature. For each snake, we used the lowest $V\dot{O}_2$ value on each day for subsequent analyses because this value best represented resting metabolic rate. Thus, we acquired two values from each snake ($V\dot{O}_2$ at 25 °C and 30 °C) before the study and at the end of the study. We required these data to determine each snake's metabolic sensitivity to temperature by calculating the temperature coefficient (Q_{10}) for $V\dot{O}_2$, which is the rate at which $V\dot{O}_2$ increases due to an increase in temperature of 10 °C (Prosser, 1991: see below).

$$Q_{10} = \left(\frac{VO_2 \text{ at } T_2}{VO_2 \text{ at } T_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

where T1 = 25 °C and T2 30 °C.

To determine $V\dot{O}_2$, we used closed respirometry (Vleck, 1987) on snakes fasted for 5–6 d to eliminate the hyper-metabolic effects of feeding in *P. guttatus* (Sievert et al., 2013; Crocker-Buta and Secor, 2014). Because *P. guttatus* is active during day and night (Gibbons and Dorcas, 2005) and all trials occurred between 7:00 and 16:00, we cannot be certain that we measured snakes' true resting metabolic rates. We placed each snake into a nearly opaque respirometry chamber (0.6 l) fitted

with incurrent and excurrent air ports, each connected to a three-way stopcock. To habituate snakes to trial conditions, we placed respirometry chambers in darkened rooms maintained at either 25 °C or 30 °C overnight and constantly pumped ambient air through the chambers. The following morning, we started metabolic trials by first pulling a 50–60 ml air sample (baseline sample) from each chamber's excurrent port and then closing both ports. We next opened the excurrent port and pulled another 50–60 ml air sample (end sample) after 45–90 min, depending on snake body mass and test temperature (e.g., snakes that were larger and/or measured at 30 °C required shorter trials). We pulled all samples in darkened conditions using the red light from a small headlamp to see. We pumped air samples at a rate of 70 ml·min^{-1} through a small column of water absorbent (Drierite, W.A. Hammond Drierite Co., Xenia, OH) into an O_2 analyzer (Q-S102, Qubit Biology Inc., Kingston, ON, Canada) that we calibrated ≤ 15 min prior to analysis of samples. The oxygen concentration of snakes' chambers never dropped below 19%. We determined $V\dot{O}_2$ corrected for standard pressure and temperature using Equation 6b in Vleck (1987).

3. Statistics

We performed all analyses with SPSS (version 21, IBM Corp.), and all data met the assumptions of parametric statistics or were logarithmically transformed to achieve normality. To compare mean T_{body} and the mean temperature of the cool end of the enclosure for each treatment group, we used one-sample *t* tests. We used an unpaired *t* test to determine the effect of temperature regime on mean T_{body}. We used linear mixed models to determine the main and interactive effects of temperature regime and energy intake on dependent variables (late-study $V\dot{O}_2$, SVL, body mass, and Q_{10} for $V\dot{O}_2$). We included treatments in our model: temperature regime as a fixed effect and energy intake as a covariate. To account for inter-individual variation, we included snake ID number as a random effect and initial variables (e.g., pre-study SVL when analyzing effects on late-study SVL) as covariates. We also included each snake's body mass <1 d prior to trial as a covariate and test temperature (25 °C or 30 °C) as a fixed effect in $V\dot{O}_2$ mixed models.

4. Results

4.1. Body temperature

Estimated T_{body} was higher in snakes maintained in the warm regime (mean: 28.0 °C) relative to snakes in the control temperature regime (mean: 25.9 °C) ($t_{65} = 12$, $P < 0.001$; Fig. 2). The estimated T_{body} of snakes in the control temperature regime significantly varied from temperature of the cool end of the enclosure (mean: 24.5 °C; $t_{31} = 7.3$, $P < 0.001$; Fig. 2). However, estimated T_{body} of snakes in the warm regime did not differ from the temperature of the cool end of the enclosure (mean: 28 °C; $t_{33} = 0.73$, $P = 0.47$; Fig. 2).

4.2. Morphology

When accounting for initial body mass, energy intake strongly influenced the body mass of *P. guttatus* after 54 d of treatment ($F_{1,61} = 196$, $P < 0.001$). However, late-study body mass was not affected by temperature regime ($F_{1,61} = 0.045$, $P = 0.83$) or a temperature × energy interaction ($F_{1,61} = 0.013$, $P = 0.91$). When accounting for initial SVL, energy intake strongly influenced the late-study SVL of *P. guttatus* (i.e., growth rate) (mixed model: $F_{1,61} = 241$, $P < 0.001$; Fig. 3). Growth rate was not significantly affected by temperature regime (mixed model: $F_{1,61} = 3.0$, $P = 0.088$, Fig. 3), but it was significantly affected by a temperature × energy interaction (mixed model: $F_{1,61} = 4.1$, $P = 0.043$; Fig. 3) wherein warm snakes were relatively more efficient in converting energy intake (kJ) into growth (change in SVL).

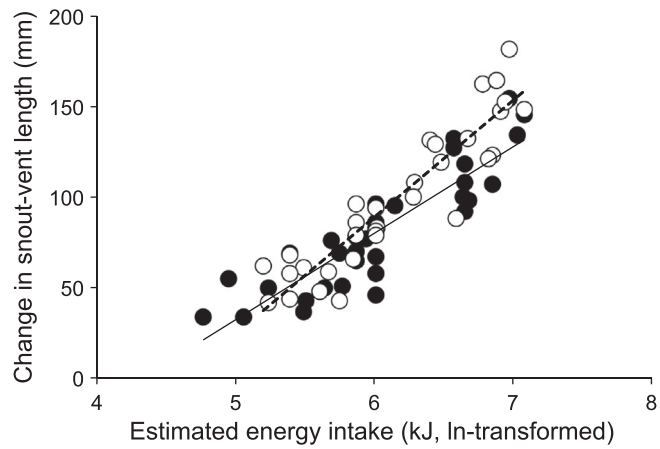

Fig. 3. Relationships between energy intake and change in body length (a proxy for growth) for juvenile *P. guttatus* kept at control (●) and warm (○) temperature regimes for approximately 8 wks. Growth was significantly affected by energy intake and a temperature × energy interaction (see text for details).

4.3. Oxygen consumption rate

Late-study $V\dot{O}_2$ was influenced by temperature regime ($F_{1,59} = 4.8$, $P = 0.033$) wherein snakes maintained in the warm regime had lower $V\dot{O}_2$ than those in the control regime (Fig. 4). Late-study $V\dot{O}_2$ was also affected by energy intake ($F_{1,59} = 62$, $P < 0.001$) and a temperature × energy interaction ($F_{1,59} = 6.3$, $P = 0.015$) wherein the late-study $V\dot{O}_2$ of snakes kept in the control temperature regime was more strongly influenced by energy intake relative to those kept in the warm regime (Fig. 4). We found no effect of pre-study Q_{10} ($F_{1,61} = 0.08$, $P = 0.78$) or treatments (temperature regime: $F_{1,61} = 0.47$, $P = 0.50$; energy intake: $F_{1,61} = 0.13$, $P = 0.72$; temperature × energy: $F_{1,61} = 0.40$, $P = 0.53$) on late-study Q_{10} (mean ± s.e.m.: 3.2 ± 0.2).

5. Discussion

5.1. Interactive effects of temperature and energy intake on growth

By using an ecologically relevant manipulation of temperature (varying the availability of preferred thermal microclimates) and accounting for variation in energy intake, our study provides new insight

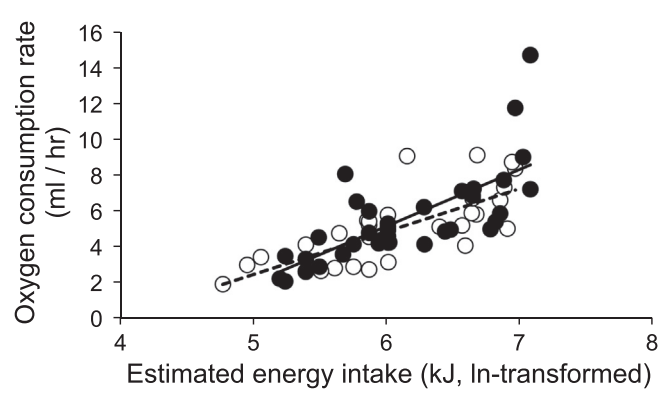

Fig. 4. Relationships between energy intake and metabolism ($V\dot{O}_2$: rate of O_2 consumption at 30 °C) for juvenile *P. guttatus* kept at control (●) and warm (○) temperature regimes for approximately 8 wks. After accounting for other effects (e.g., body mass), metabolic rate was significantly affected by temperature regime and a temperature × energy interaction (see text for details). For clarity, we show only the data for $V\dot{O}_2$ at 30 °C; however, the relationships between energy intake and $V\dot{O}_2$ at 25 °C for each temperature regime were similar.

into the effects of shifts in complex environments on animals. Our results (Figs. 3 and 4) fail to fully support either of our hypotheses (Fig. 1). However, we demonstrate interactive effects of rearing temperature and energy intake on growth and metabolism suggesting animals exhibit metabolic acclimation to elevated temperature (Figs. 3 and 4). Our results show that acclimation to relatively warm thermal options can result in increased rates of growth when food is abundant in a taxon in which body size confers fitness advantages (Bonnet et al., 2000; Brown and Shine, 2005) (Fig. 3). Although temperature generally influences growth across taxa (Angilletta, 2009), we show that growth rate was affected by a temperature × energy interaction, but not by temperature alone—thus, warm snakes more efficiently allocated resources to growth relative to control-temperature snakes at higher rates of energy intake. (Fig. 3).

The interactive effect of temperature and energy intake on growth rate begs the question—*why is the effect of temperature only significant in animals ingesting relatively more energy?* One explanation is that frequent feeding at warmer temperatures is particularly energy efficient, and that these energy savings are allocated to growth. Yet, work in another snake (Children's python, *Antaresia childreni*) shows that frequent feeding results in *reduced* assimilation of energy (Stahlschmidt et al., 2011). If available (absorbed) energy does not increase with frequent feeding, the improved energy efficiency of frequent feeding must be driven by a reduction in the energetic costs of processing food (the specific dynamic action, SDA). Recent work demonstrates that SDA relative to the amount of ingested energy (SDA coefficient) is not influenced by temperature in *P. guttatus*, but these authors only examined the energetic consequences of single feeding bouts (Crocker-Buta and Secor, 2014). Although others have examined components of the dynamic interplay among energy intake, feeding frequency, temperature, metabolic rate, and growth (e.g., Crocker-Buta and Secor, 2014; Glanville and Seebacher, 2006; Guderley, 2004; LeMoine et al., 2008; this study), to our knowledge no single study has examined all of these variables in concert. For example, researchers could use an experimental paradigm similar to ours in any number of animal taxa, and measure growth, energy intake, and integrated metabolic rate (e.g., doubly labeled water technique) over the duration of their study.

5.2. Thermal acclimation: Shifts in metabolism and behavior

We further show that snakes experiencing relatively warm options exhibit reduced metabolic rate (Fig. 4). Hypometabolism as an acclimating effect may be a fundamental aspect of thermal plasticity, and it should be considered when constructing animal energy budgets in the context of GCC. It may also be a universal response by organisms to prolonged shifts in temperature as it has been documented in a broad range of taxa (plants: Ow et al., 2008a; Ow et al., 2008b; fungi: Malcolm et al. 2008; crustaceans: Powell and Watts, 2006; insects: Lachenicht et al. 2010; fishes: Donelson et al. 2011, Strobel et al. 2012; amphibians: Seebacher and Grigaltchik, 2014; reptiles: this study; birds: Abdelqader and Al-Fataftah, 2014; mammals: Geiser et al. 2003). However, these studies did not control for variation in energy intake or food availability, which can covary with climatic temperature shifts (Both and Visser, 2005; Altermatt, 2010; Pearce-Higgins et al., 2010; Allan et al., 2013; Cahill et al., 2013). Thus, examining the effects of individual environmental factors (e.g., temperature *or* food availability) may be an overly simplistic approach to understanding how animals respond to rapidly changing environments. We advocate continued investigation into the consequences of complex (multi-factorial) environments on the plasticity of energy use across taxa (sensu Guderley, 2004; LeMoine et al., 2008).

By measuring the temperature sensitivity of metabolic rate to acute changes in temperature (Q_{10} for $\dot{V}O_2$), our results also provide insight into the specificity of hypometabolism associated with thermal acclimation in snakes. If hypometabolism was specific, for example, the metabolic rate of warm snakes may be less sensitive to increases in temperature (lower Q_{10}) relative to that of control snakes. However, we show that Q_{10} was not affected by temperature regime, energy intake, or a temperature × energy interaction. This result implies that hypometabolic effects associated with thermal acclimation are general rather than specific—that is, warm-reared snakes exhibited hypometabolism generally (at both cooler and warmer temperatures: 25 °C and 30 °C, respectively) rather than specifically (at warmer temperatures only). If thermal acclimation results in general adjustments in metabolism, it may also obligate a reduction in other traits (e.g., immune or antioxidant defenses, locomotor capacity, or digestion) that require energetically expensive enzymatic performance. In other words, the peak performance in the thermal reaction norm of some physiological traits in warm-reared animals may be lower relative to control or cool-reared animals (e.g., muscle performance: Langfeld et al., 1991; Seebacher and James, 2008). Such consequences of thermal acclimation may be appreciable. In fact, the notion that an adaptive shift in optimal temperature due to thermal acclimation is widespread and has a net benefit (sensu the *beneficial acclimation hypothesis*) has garnered only equivocal support (reviewed in Angilletta et al., 2006; Angilletta, 2009). Further, mounting evidence demonstrates that thermal acclimation appears incapable of eliminating all of the costs of GCC (e.g., Sinervo et al., 2010; Gardner et al., 2011; Caruso et al., 2014).

In addition to metabolic shifts (Fig. 4), *P. guttatus* made behavioral adjustments in response to the potential costs of elevated temperatures. Specifically, snakes in the warm regime selected the coolest available microclimates (Fig. 2), which agrees with other studies that demonstrate the role of behavior in thermal acclimation (e.g., reviewed in Angilletta et al., 2006; Refsnider and Janzen, 2012). Yet, snakes in the warm regime had higher estimated T_{body} relative to those in the control temperature regime. Together, these results suggest snakes in the warm regime were trapped in environments with warmer than preferred microclimates (Fig. 2) despite exhibiting enhanced growth when energy intake was high (Fig. 3). The apparent mismatch between preferred T_{body} and optimal T_{body} due to temperature shifts is widespread (e.g., locomotor performance in amphibians: Wilson et al., 2000; Wilson and Franklin, 2000; crustaceans: Diaz et al., 2002; fish: Perez et al., 2003; reptiles: Yang et al., 2008; but for reptiles see Glanville and Seebacher, 2006). The importance (fitness consequences) of the trait(s) of interest and non-measured variables are critical to understanding these mismatches. Our study did not examine the fitness outcome of increased SVL in *P. guttatus*—although, growth and its result (large body size) are typically selected upon in snakes (Bonnet et al., 2000; Brown and Shine, 2005) and many other taxa (reviewed in Dmitriew, 2011). We also did not measure the other effects of limited and/or warmer microclimate options. A limited selection of suitable microclimate options may obligate ecological costs (e.g., increased predation risk, reduced prey availability, increased competition, or reduced foraging opportunities), and warmer microclimates may obligate detrimental shifts in a suite of physiological traits (see above).

6. Conclusion

By accounting for energy intake and manipulating thermal microclimates, we demonstrate that relevant temperature shifts had a positive effect on growth when resources were relatively abundant (Fig. 3). This interactive effect of temperature and energy intake was accompanied by a similar effect on metabolic rate (Fig. 4). Thus, our results indicate that warming may actually benefit important life history traits in some taxa (but see Gardner et al., 2011; Caruso et al., 2014) and that metabolic shifts may underlie thermal acclimation. We provide further support for the utility of multifactorial experimental designs in making biological inferences about the impacts of GCC (reviewed in Todgham and Stillman,

2013). In the future, researchers should continue to examine the effects of complex environments to better understand the specific metabolic mechanisms underlying thermal acclimation (e.g., changes in the regulation of insulin-like growth factor: Dantzer and Swanson, 2012). We also advocate for research examining the ecological effects of GCC-induced shifts in metabolism and behavior.

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