



Original Article

Context-dependent effects of complex environments on behavioral plasticity

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Although individual environmental factors influence behavioral plasticity, animals live in complex environments wherein multiple environmental factors vary simultaneously. Here, we investigated whether temperature and food intake independently or interactively affected the boldness and feeding behavior of juvenile corn snakes (*Pantherophis guttatus*, Linnaeus). Because thermal environments in which animals can behaviorally thermoregulate by moving among microclimates are likely more realistic and ecologically relevant than fluctuating or constant temperature regimes, we manipulated the availability of preferred thermal microclimates (control vs. warm regimes) for 8 weeks and allowed individuals to move among microclimates. By also manipulating food intake and controlling for phenotypic repeatability of behavior, we demonstrate context-specific effects of temperature and food intake on behavioral plasticity. Temperature and food intake independently affected feeding behavior (i.e., unidimensional plasticity), but these factors interactively affected boldness in an open arena (i.e., multidimensional plasticity). Clearly, complex environments can exert multifaceted effects on behavior. Therefore, 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.

Key words: boldness, climate change, corn snake, feeding, food availability, temperature.

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 that span 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 to its behavior and reproduction—which has led to a large body of theory and empiricism on thermal biology (reviewed in Angilletta 2009). The vast majority of laboratory research focused on understanding organisms' temperature sensitivity has examined the effects of constant temperatures on fitness-related performance traits. 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). Furthermore, 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 for animals. They must deal with rapidly changing environments that are complex, in which temperature and other environmental factors vary simultaneously (reviewed in Todgham and Stillman 2013). For example, animals require food to fuel their activities, and food availability is influenced by climatic variation and GCC (Both and Visser 2005; Altermatt 2010; Pearce-Higgins et al. 2010; Allan et al. 2013; Cahill et al. 2013). Yet, few studies have examined how animals may be affected by the independent and interactive effects of food (energy) availability and shifts in temperature (but see Guderley 2004; LeMoine et al. 2008; Adamo et al. 2012; Stahlschmidt et al. 2015).

Individual environmental factors, such as temperature or food availability, drive variation in behavior across an array of animal taxa (Dingemanse et al. 2004; Quinn and Cresswell 2005; Johnson and Sih 2007; Briffa et al. 2008; Martin and Réale 2008; Schofield et al. 2009; Biro et al. 2010; Rodríguez-Prieto et al. 2011; Betini and Norris 2012; Briffa et al. 2013; Montiglio et al. 2014; Stahlschmidt et al. 2014; Manciocco et al. 2015). Recently, attention has shifted to the ways in which complex environments (characterized by covariation of multiple environmental variables)

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influence behavioral variation (e.g., nest temperature and humidity interact to influence python egg-brooding behavior: [Stahlschmidt and Denardo 2010](#); temperature and predation risk interact to influence oviposition behavior in crickets: [Stahlschmidt and Adamo 2013](#); food odors, mating odors, and predation risk interactively influence antipredator behavior in squirrels: [Dosmann and Mateo 2014](#)). Thus, we are beginning to gain an appreciation for how specific environmental factors interact to affect behavioral plasticity (“multidimensional plasticity”: [Dosmann and Mateo 2014](#)) in the short term. However, the behavioral outcomes of prolonged exposure to shifts in complex environments remain poorly understood.

Despite environmental effects, behavior may exhibit among-individual repeatability, within-individual repeatability, or a combination thereof (phenotypic repeatability) along a shy–bold continuum ([Sih et al. 2004](#); [Dall et al. 2004](#); [Sih and Bell 2008](#); [Sih et al. 2012](#)). Accounting for this repeatability requires repeated examination of behavior in different functional categories (“contexts”: [Sih et al. 2004](#)), and these contexts should be ecologically relevant ([Réale et al. 2007](#)). For example, an animal’s behavior in response to novel stimuli is a common context to assess fitness-related boldness or risk taking (reviewed in [Biro and Stamps 2008](#); [Smith and Blumstein 2008](#); [Carter et al. 2013](#)), and boldness may be particularly relevant for juveniles whose lack of size, strength, and experience increases their risk of predation. Yet, foraging is a risky behavior that is also under strong selection in juveniles given their energetic demands of growth, high mass-specific rates of energy expenditure, and reduced energy stores ([Peters 1986](#)). Understanding the trade-off between foraging and predation risk has long been a goal of behavioral ecologists (e.g., [Charnov 1976](#); [Brown and Kotler 2004](#); [Stahlschmidt et al. 2014](#)). Therefore, combining assays of boldness and feeding behavior in juveniles while controlling for phenotypic repeatability of behavior may provide new insight into the effects of complex environments on behavioral plasticity across contexts.

We manipulated the availability of preferred thermal microclimates and food intake for 8 weeks in the juvenile corn snake (*Pantherophis guttatus*, Linnaeus) to address 3 hypotheses based on the effects of temperature and food intake on behavior in 2 contexts, boldness in an open arena and feeding behavior. First, we hypothesize correlated plasticity between behavioral contexts. From this hypothesis, we predict individuals that exhibit high feeding scores will also tend to be relatively bold in an open field. Second, we hypothesize that temperature regime and food intake will have independent effects on behavior. From this hypothesis, we predict additive (or unidimensional) effects whereby snakes chronically exposed to the least favorable environment (warmer-than-preferred temperature regime with low food intake) will be bolder and exhibit a greater feeding response relative to snakes exposed to other environments—that is, there will be a linear relationship between environmental quality and behavior ([Figure 1](#)). Contrary to our second hypothesis, our third hypothesis posits a significant temperature \times food effect on behavior (“multidimensional plasticity” sensu [Dosmann and Mateo 2014](#)). From this hypothesis, we predict that snakes in the least favorable environment (warmer-than-preferred temperature regime with low food intake) and most favorable environment (preferred temperature regime and high food intake) will be bolder and exhibit a greater feeding response relative to snakes in moderately favorable environments ([Figure 1](#))—that is, there will be a nonlinear (U-shaped) relationship between environmental quality and behavior. By examining a behavior linked to fitness (foraging and food acquisition) and an established behavioral assay

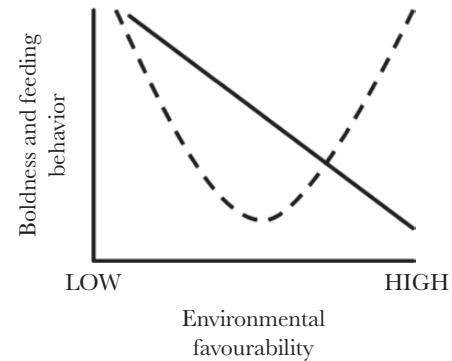


Figure 1

Predicted effects of environmental favorability on behavior based on our second hypothesis (additive effects of temperature and food intake: solid line) and third hypothesis (interactive effects of temperature and food intake: dashed line). High environmental favorability is characterized by the control temperature regime and abundant food intake, whereas low environmental favorability is characterized by the “warm” temperature regime and low food intake. See text for details.

(response to novel stimuli: e.g., [Boissy 1995](#); [Hedrick 2000](#); reviewed in [Smith and Blumstein 2008](#); [Carter et al. 2013](#)), we provide new insight into the independent and interactive effects of temperature and food availability on behavior in the context of GCC-relevant shifts in complex environments.

METHODS

Study species and husbandry

Pantherophis guttatus is a medium-sized, nonvenomous 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 (first–third generation) of wild caught snakes from Beaufort County, SC. All snakes were 5–6 months old at the onset of the study. We individually housed snakes in translucent plastic enclosures (27 cm \times 41 cm \times 15 cm) with ad libitum access to water in a room with a 12:12 light:dark cycle. 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) 2 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. We considered this temperature range to be the “control” temperature regime because it 1) reflects thermal options in the natural range of *P. guttatus* during the active season (spring–fall) ([Howze and Smith 2012](#)) and 2) accommodates the preferred temperature range for *P. guttatus* in the laboratory (27–29 °C, depending on digestive state: [Roark and Dorcas 2000](#)). To create a cool refuge similar in concept to natural conditions (e.g., a subterranean burrow), we kept folded newspaper in place on the cooler end of each enclosure.

Experimental design

Over the course of our 8-week study, we manipulated temperature and food availability. We individually housed approximately half of the snakes ($n = 32$) at the control temperature regime described

above (Figure 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 28–37 °C (Figure 2). To verify our temperature regimes, we recorded the temperature at the cool and warm ends of 2 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).

We initially manipulated food availability by creating “low-food” and “high-food” diets wherein we offered snakes in both temperature regimes either 1 or 2 prey items (described above) per week, respectively. Because snakes fed a high-food diet ate more frequently, they also increased in body mass more quickly—thus, they were offered larger 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 intake ingested by each snake over the 8-week 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).

Body temperature

We serially estimated the body temperature (T_{body}) of each snake throughout the study to verify that our temperature regimes influenced snakes' T_{body} . Weekly, we used a factory-calibrated infrared thermometer (ProTemp 12, Jewell Instruments, Manchester, NH; range: –50 to 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-day postfeeding 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 measurement of T_{body} that is most useful in quiescent, coiled snakes as it records the surface temperature of a circular area. Therefore, we discarded measurements of moving, agitated, and/or noncoiled

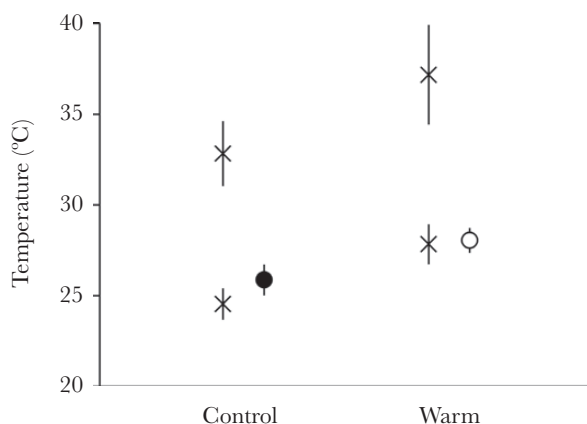


Figure 2

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

snakes—thus, we recorded 3–8 estimates of T_{body} for each snake over the 8-week study. To further improve the validity of our measurements, we compared infrared temperature readings with those measured by a thermocouple placed at the midpoint inside a temperature model (a proxy for T_{body}). Our temperature model was a water-filled vinyl tube with dimensions similar to the average size of *P. guttatus* used in our study (mass: 41 g; length: 49 cm). We took infrared and thermocouple readings simultaneously ($n = 7$) in a nonoccupied snake enclosure. The relationship between infrared and thermocouple measurements was strong ($R^2 = 0.99$), so we used the equation of the best-fit line to estimate T_{body} from infrared readings. Furthermore, our estimates of T_{body} in *P. guttatus* were similar to the actual T_{body} of *P. guttatus* as measured via temperature-sensitive tags that were implanted into live snakes (Roark and Dorcas 2000).

Feeding behavior

To measure feeding behavior, we offered each snake a prey item (frozen/thawed mouse that was 15–20% of the snake's body mass) in its enclosure. We dropped each prey item in approximately the same location (center of cool end of enclosure) and gently closed each enclosure. We then checked each snake's feeding progress at several sampling points—10 min, 1, 5, and 24 h after offering. We assigned feeding scores ranging from 0 (did not eat) to 4 (completely consumed meal within 10 min). We also assigned half scores for occasions in which snakes were in the process of eating during a sampling point. For example, a feeding score of 3.5 was assigned to a snake that was eating its mouse 10 min after offering and that had completely consumed the mouse by 1 h after offering.

We measured feeding behavior at 4 time points, each of which occurred 4 days after a previously offered meal. We measured feeding behavior 1 week prior to the study to establish a baseline or initial level. We then measured feeding behavior early and late in the study (4 days and 7 weeks after start of treatment) to determine the acute and chronic responses to treatment, respectively. Last, we measured feeding behavior 1 week after the study to determine if any effects of treatment carried over after the end of the study.

Data from feeding behavior trials were strongly skewed to the left (negative skew) given the propensity of most snakes to eat quickly. Thus, we created 2 categories of response for each snake: 1) active engagement of the prey item within 10 min of offering (feeding score ≥ 3.5; high-response or “high” feeding) and 2) all remaining responses (feeding score ≤ 3; low-response or “low” feeding).

Two months prior to the study, we validated the phenotypic repeatability (combined among- and within-individual variation) of this feeding behavior assay. We measured feeding behavior in *P. guttatus*, and we then measured it again 1 week later in the same individuals. Based on a Pearson's correlation test, the relationship between these 2 sampling points was significant ($n = 54$, $R = 0.56$, $P < 0.001$).

Behavior in an open field (boldness)

We performed open-field behavioral trials on snakes at 2 time points (4 days prior to the study and again 7 weeks into the study), each of which occurred 3 days after a meal offering. For 2–6 h prior to trials, we acclimated snakes in the room in which trials occurred. To minimize disturbance, we kept snakes in their enclosures under darkened conditions during this acclimation period. All trials were performed at 27 ± 1 °C.

As in other taxa (reviewed in Carter et al. 2013), we used a modified open arena to characterize snakes' behavior in an open field (Figure 3). The arena's floor was 86-cm long and 61-cm wide and

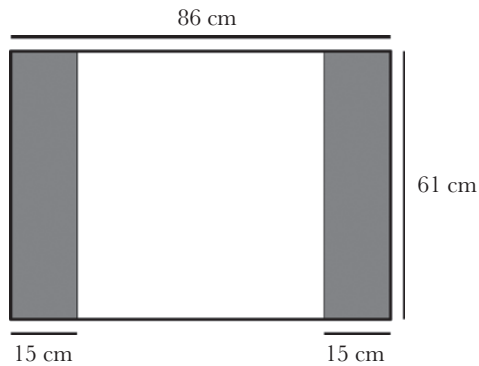


Figure 3

Top-view schematic of the open-field arena. Each snake began a given trial in the center of the arena and its behavior was monitored for 10 min. Gray areas indicate shelters, which were boards situated 3 cm off of the arena's floor. Detailed information can be found in the Methods.

constructed of white linoleum tile. The arena's walls were 91-cm high and constructed of white-painted plywood. The arena consisted of an open center region ($56 \times 61 \text{ cm}^2$) and 2 sheltered ends ($15 \times 61 \text{ cm}^2$ plywood boards situated 3 cm off of the arena's floor). We carefully transferred each snake from its individual enclosure into an opaque plastic cup (height: 13 cm; diameter of opening: 5 cm). We then capped the cup with a sheet of cardboard before gently inverting the cup and placing it on the floor of the center of the arena. Next, we carefully removed the cardboard from the rim of the cup. After 2 min, we gently raised the cup to expose the snake to the open-field arena.

For 10 min, we characterized 7 behaviors associated with the bold-shy continuum or behavioral activity. To maintain consistency, only 1 observer (L.M.H.) quantified behavior in the open field. We measured "freeze time," which is the duration of time between a snake's exposure to the open field (the raising of the cup) and any motion by a snake (e.g., tongue flicking or locomotion). Freezing is a stereotypic antidetection response associated with predation avoidance across taxa (Chelini et al. 2009; reviewed in Stynoski and Noble 2012; Stahlschmidt et al. 2014). Next, we measured the number of times each snake entered/exited the sheltered ends ("shelter exploration") and the total amount of time each snake spent under shelter ("shelter time"). Snakes should seek the arena's sheltered ends to reduce their risk of predation—thus, individuals that repeatedly entered and exited shelters were presumably bolder (higher risk taking) than those that simply stayed within a single shelter during the 10 min trial period. Related, we measured the number of times a snake poked its head out from inside a shelter it occupied ("head poking"). We also measured how many times each snake reared up onto the walls of the arena ("rearing") and whether it climbed on top of either shelter ("shelter climbing")—we presumed that snakes exhibiting "rearing" and "shelter climbing" were higher risk takers. Last, we determined the amount of time each snake spent locomoting in the open field ("locomotion") as a proxy for behavioral activity. After each trial, we cleaned the arena with 70% ethanol and returned each snake to its enclosure.

We used Principal components analysis (PCA) to generate an index of open-field behavior at 2 time points (4 days prior to the study and 7 weeks into the study) using all 7 behaviors as initial variables. We used several test statistics (e.g., the Bartlett's and Kaiser–Meyer–Olkin measures) to verify that our data set was appropriate for PCA. By removing 1–2 initial variables (prestudy: "freeze time";

late study: "freeze time" and "rearing"), our 2 data sets satisfied the assumptions of having significant and compact patterns of correlations (reviewed in Stahlschmidt and Adamo 2015). For subsequent analyses (see Statistical analyses for details), we included the principal components (PCs) that accounted for the most variance and that had eigenvalues >1 . Prior to the study, the first PC accounted for 49% of the variance, and it loaded positively on "shelter exploration" (0.42), "rearing" (0.81), "locomotion" (0.86), and "shelter climbing" (0.81). This PC loaded negatively onto "shelter time" (−0.66) and "head poking" (−0.52). That is, a relatively high PC score reflected relatively more exploration, rearing, locomotion, and climbing, and it reflected relatively less time in shelter and head poking. Thus, we herein refer to this PC as prestudy "boldness." Near the end of the study, the first PC accounted for 40% of the variance, and it loaded positively on "shelter exploration" (0.42), "locomotion" (0.82), and "shelter climbing" (0.62). This PC loaded negatively onto "shelter time" (−0.48) and "head poking" (−0.27). Again, a relatively high PC score reflected a tendency toward risk-taking behaviors, so we herein refer to this PC as late-study "boldness." We used the first PCs from initial and late-study PCAs (i.e., initial boldness and late-study boldness) for our statistical analyses (described below).

Two months prior to the study, we validated the phenotypic repeatability of this open-field behavior assay. We measured open-field behavior in *P. guttatus*, and we then measured it again 1 week later in the same individuals. Based on a Pearson's correlation test, the relationship between boldness values determined at these 2 sampling points was significant ($n = 17$, $R = 0.59$, $P < 0.001$).

We accounted for the effects of phenotypic repeatability by initially measuring dependent variables (open-field and feeding behavior) ≤ 1 week prior to the study. We used initial (prestudy) measurements as fixed effects (covariates) in our statistical models (see below) to determine the effects of temperature and food intake on late-study measurements.

Statistical analyses

We performed all analyses with SPSS (version 21, IBM Corp.), and we determined 2-tailed significance at $\alpha < 0.05$ for independent and interactive effects. All data met the assumptions of parametric statistics or were logarithmically transformed as necessary. To compare estimated mean T_{body} and the mean temperature of the cool end of the enclosure for each treatment group, we used 1-sample *t*-tests. We used an unpaired *t*-test to determine the effect of temperature regime on estimated mean T_{body} . To examine whether feeding behavior and boldness exhibited correlated plasticity, we used 2 univariate analyses of variance tests to determine whether high-feeding snakes exhibited higher boldness at each time point (prestudy PC1 and late-study PC1). We used a linear model to determine the main and interactive effects of temperature and food intake on boldness. Our model included treatments (temperature regime and food intake) and initial (prestudy) boldness as fixed effects and snake ID number as a random effect. When a covariate was significant (e.g., covariation between food intake and feeding behavior), we examined its regression coefficient to determine the sign of the correlation. To determine the main and interactive effects of temperature and food intake on feeding behavior (response variable: low feeder vs. high feeder), we used a binary logistic generalized linear model with temperature regime, food intake, temperature \times food, time point (early study, late study, or poststudy), and prestudy feeding score as fixed effects and snake ID as a random effect.

RESULTS

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) ($t_{65} = 12$, $P < 0.001$; Figure 2). The estimated T_{body} of snakes in the control temperature regime significantly varied from temperature of the cool end of the enclosure ($t_{32} = 7.3$, $P < 0.001$; Figure 2). However, estimated T_{body} of snakes in the warm regime did not differ from the temperature of the cool end of the enclosure ($t_{32} = 0.73$, $P = 0.47$; Figure 2).

Relationship between boldness and feeding behavior

Snakes that were high feeders did not exhibit greater boldness than low feeders before the study ($F_{1,65} = 0.24$, $P = 0.63$) or late in the study ($F_{1,65} = 0.20$, $P = 0.66$). That is, boldness was not associated with feeding behavior.

Feeding behavior

Initial (prestudy) feeding behavior significantly affected late-study feeding behavior ($\chi^2 = 29$, degrees of freedom [df] = 1, $P < 0.001$). The feeding behavior of *P. guttatus* was also significantly affected by temperature ($\chi^2 = 4.2$, df = 1, $P = 0.041$) and time ($\chi^2 = 7.3$, df = 2, $P = 0.026$) (Figure 4). Feeding behavior was also affected by food intake ($\chi^2 = 7.7$, df = 1, $P = 0.005$) where food intake positively covaried with high feeding, but it was not affected by a temperature \times food interaction ($\chi^2 = 0.98$, df = 1, $P = 0.32$).

Boldness

Initial (prestudy) boldness significantly affected late-study boldness (Table 1). The boldness of *P. guttatus* at the end of the study was not independently affected by temperature regime or food intake (Table 1). However, late-study boldness was significantly affected by a temperature \times food interaction (Table 1). This result was driven by a significant (negative) relationship between food intake and boldness in snakes kept in the warm regime ($n = 34$, $R = -0.42$, $P = 0.015$) but not by snakes in the control temperature regime ($n = 32$, $R = 0.13$, $P = 0.48$).

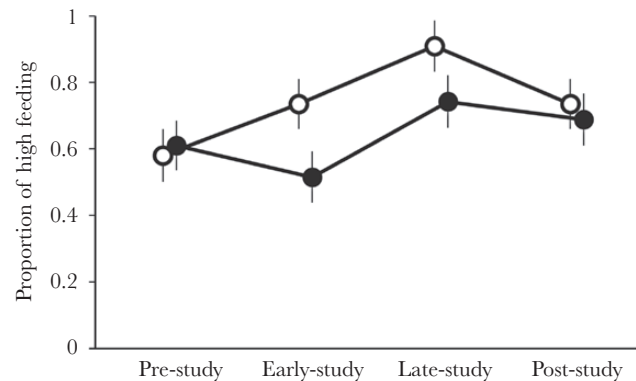


Figure 4 Significant effects of time and temperature regime on proportion of high feeding (propensity to engage a prey item within 10 min of offering) for juvenile *Pantherophis guttatus* kept at control (●) and warm (○) temperature regimes for an 8-week study. See text for details. All values displayed as mean \pm standard error of mean.

DISCUSSION

By using an ecologically relevant manipulation of temperature (varying the availability of preferred thermal microclimates) and accounting for variation in food intake, our study provides new insight into the effects of shifts in complex environments on animal behavior. Boldness and feeding behavior were related to one another in *P. guttatus*, which did not support our first hypothesis (correlated plasticity between behavioral contexts). We detected significant effects of rearing temperature and food intake on boldness and feeding behavior—however, the type of effect (independent vs. interactive effect) varied between behavioral contexts. Temperature and food intake independently affected feeding behavior in support of our second hypothesis (temperature regime and food intake have independent effects on behavior) and provided support for unidimensional plasticity. Yet, our prediction based on this hypothesis posited that the relationship between food intake and feeding behavior would be negative (Figure 1), not positive as our results demonstrate. Last, temperature and food intake interactively affected boldness (but not feeding behavior) in *P. guttatus* in support of our third hypothesis (a significant temperature \times food effect on behavior) and provided evidence of multidimensional plasticity. Clearly, complex environments can exert complicated effects on behavioral plasticity after accounting for phenotypic repeatability of behavior.

Previous research has demonstrated links between an animal's behavior in different contexts (reviewed in Carter et al. 2013). There continues to be an effort to combine behavioral contexts to form an integrated perspective on animal behavior that is similar to that of humans (reviewed in Nettle and Penke 2010; Carter et al. 2013) and/or that exhibits ecological relevance (reviewed in Réale et al. 2007). Others have cautioned against testing behavior in uninterpretable contexts (e.g., behavior in an open field may be meaningless for animals that are predated in closed habitats) (Carter et al. 2013; Niemelä and Dingemanse 2014). Yet, strong evidence indicates the link between shelter (closed habitats) and predation risk in reptiles (reviewed in Lelièvre et al. 2010). Furthermore, a snake's propensity to leave its shelter and expose itself to predation while foraging would intuitively be viewed as boldness because a snake cannot defend itself (e.g., by hissing or biting) while it is eating. Thus, our results contribute to a growing literature describing the complicated nature of “boldness” as inferred by behavioral ecologists (reviewed in Réale et al. 2007; Carter et al. 2013).

Similar to other taxa (Casey 1993; Shimeta et al. 2004; Yee and Murray 2004), *P. guttatus* in the warm regime had a higher propensity to be high feeders, and this effect was particularly marked after acute exposure to temperature regime (4 days into the study; Figure 4). Given the positive relationship between T_{body} and metabolic rate in most animals, increased foraging in warmer-than-preferred environments may serve to offset the energetic costs obligated

Table 1 Effects of temperature regime, food intake, and initial (prestudy) boldness behavior on late-study boldness in an open arena

	Estimate	Standard error	df	F	P
Temperature	−0.51	0.78	61	0.78	0.39
Food intake (kJ)	−0.62	0.26	61	0.91	0.31
Temperature \times food	0.88	0.36	61	6.1	0.017
Prestudy boldness	0.39	0.11	61	13	0.001

See text for details.

by elevated temperatures (reviewed in Angilletta 2009). Snakes reared in the warm regime for this study also exhibited reduced metabolic rate (O_2 consumption rate) near the end of the study (Stahlschmidt et al. 2015), which may explain why “warm” snakes did not exhibit increased high feeding over time relative to “control temperature” snakes (Figure 4). Together, these results indicate a functional shift in thermal acclimation strategy—initially, animals rely on behavioral shifts (increased foraging propensity) and later turn to physiological adjustments (hypometabolism).

We anticipated *P. guttatus* consuming less food (energy) would be more motivated to eat and, thus, exhibit a greater propensity for high feeding. Unexpectedly, we observed the opposite effect—a positive relationship between food intake and feeding behavior. We interpret this result as the unexpected consequence of body size on feeding propensity. Snakes grew over the 8-week study (3–18 cm, largely depending on food intake: Stahlschmidt et al. 2015), and they also tended to increase their propensity for high feeding over time (Figure 4). The tendency for boldness or boldness-related behaviors (e.g., feeding behavior or dispersal distance) in larger animals occurs in some taxa (reptiles: Lopez et al. 2005; this study; birds: Saino et al. 2014) but not in other taxa (fishes: Brown and Braithwaite 2004; Harris et al. 2010; Nyqvist et al. 2012). Therefore, we encourage researchers to account for body size in studies on behavioral plasticity—particularly, when examining behavior in fast-growing juveniles.

Our third hypothesis posited a nonlinear effect of environmental favorability on boldness in an open field (Figure 1). Specifically, relatively high-quality environments would embolden individuals, whereas relatively low-quality environments would promote risky behavior in response to an impending (or occurring) “life-history emergency stage” (sensu Wingfield et al. 1998; Wingfield 2003). We found some support for this notion in “warm” snakes because they exhibited a negative relationship between food intake and boldness. This result adds to other work demonstrating the interactive effects of temperature and food intake on important traits, such as growth and metabolism in *P. guttatus* (Stahlschmidt et al. 2015) and survival, fecundity, and/or locomotor performance in other taxa (fish: Guderley 2004; crickets: Adamo et al. 2012). Therefore, we advocate for continued investigation into the consequences of shifts in complex (multifactorial) environments on animal traits (e.g., boldness or feeding behavior) and trait–trait interactions.

In addition to assays of boldness and feeding behavior, *P. guttatus* made other behavioral adjustments in response to the potential costs of elevated temperatures. Specifically, warm snakes selected the coolest available microclimates (Figure 2), which agrees with other studies that demonstrate the role of behavior in thermal acclimation (e.g., Refsnider and Janzen 2012; reviewed in Angilletta et al. 2006). 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 (Figure 2). A limited selection of suitable microclimate options may obligate ecological costs, such as increased predation risk, reduced prey availability, or increased competition (e.g., McAuliffe 1984; McIvor and Odum 1988; Kotler et al. 1991; Henle et al. 2004). Thus, we advocate for continued research examining the ecological effects of alterations in behavior due to shifts in complex environments.

By controlling for food intake and manipulating thermal microclimates, we demonstrate behavioral plasticity in response to GCC-relevant shifts in complex environments. Boldness in an open field and feeding behavior were not related to one another, and

temperature and food intake affected behavior in a context-specific manner (either independently or interactively, depending on the behavioral context or measured behavior). Together, our results yield further insight into the role of plasticity in behavioral adjustments to shifts in complex environments. 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 behavioral acclimation.

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