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Combined effect of incubation and ambient temperature on the feeding performance of a small ectotherm

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Abstract Many ectothermic animals are subject to fluctuating environmental temperatures during incubation as well as post-birth. Numerous studies examined the effects of incubation temperature or ambient temperature on various aspects of offspring phenotype. We investigated whether incubation temperature and ambient temperature have an interactive effect on offspring performance. Our study animal, the ectothermic vertebrate *Lampropholis delicata* (common garden skink; De Vis 1888), experiences fluctuating environmental temperatures caused by differential invasion of an exotic plant *Vinca major* (blue periwinkle). In the laboratory, eggs from wild-caught females were assigned to different incubation temperatures that mimicked variation in natural nests. The feeding performance and digestion time of each hatchling was tested at ambient temperatures that represented environments invaded to different degrees by periwinkle. Incubation and ambient temperature interacted to affect a lizard's mobility, the time that it took to capture, subdue and handle a prey, and the number of handling 'errors' that it made while foraging. For a number of these characteristics, incubation-induced changes to a lizard's mass significantly affected this relationship. Irrespective of size, no interaction effect was found for digestion time: lizards digested food faster at warmer temperatures, regardless of incubation temperature. Thus, temperatures experienced during incubation may alter an animal's phenotype so that the surrounding thermal environment differentially affects aspects of feeding performance. Our results also demonstrate that incubation environment can induce changes to morphology and behaviour that carry over into a lizard's early life, and that in some cases these differences in phenotype interact to affect performance. We suggest that the immediate removal of exotic plants as part of a weed control strategy could have important implications for the foraging performance, and presumably fitness, of ectothermic animals.

Key words: adaptation, conservation, foraging, invasive species, phenotypic plasticity.

INTRODUCTION

In temperate environments, animals are exposed to thermal conditions that can fluctuate greatly in place and time. In particular, the life histories and behaviour of ectothermic animals are affected by this thermal variation (Hochachka & Somero 1984; Cossins & Bowler 1987).

Numerous studies have shown that an ectotherm's performance is influenced by ambient temperature (e.g. Van Damme *et al.* 1991; Marken Lichtenbelt 1993; Beyer & Spotila 1994; Ayers & Shine 1997; Angilletta *et al.* 2002; Forsman *et al.* 2002; Hilder & Pankhurst 2003; Rock & Cree 2003). It is also well established for ectotherms that the incubation of embryos at different temperatures can result in hatchlings with different morphologies (e.g. Phillips *et al.* 1990; Shine *et al.* 1997; Qualls & Shine 1998; Downes & Shine 1999; Braña & Ji 2000; Shine & Elphick 2001; Blumberg *et al.* 2002; Ji *et al.* 2002; Miranda *et al.* 2002; Du *et al.* 2003). More recently, studies showed that the temperature experienced during incubation can also significantly affect a hatchling's behaviour (thermoregulation, foraging, antipredator tactics), survivorship, and reproduction (e.g. Burger 1990, 1991; Nunney & Cheung 1997; Shine *et al.* 1997; Crews *et al.* 1998; O'Steen 1998; Downes & Shine 1999; Andrews *et al.* 2000; Flatt *et al.* 2001; Rodriguez-Munoz *et al.* 2001; Blumberg *et al.* 2002; Sakata & Crews 2003). Hence, the conditions experienced during incubation can affect various aspects of later life (carry-over effects, e.g. Pechenik *et al.* 1998; Lindström 1999). The first goal of this study is to examine how the temperature experienced by an ectothermic reptile during embryogenesis affected performance as a juvenile.

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Most previous studies focused on describing the effects of incubation and ambient temperatures separately, without considering a possible interaction (for two notable exceptions see Zamudio *et al.* 1995; Nunney & Cheung 1997). However, the effect of incubation temperature on an ectotherm's performance can depend on ambient temperature (Elphick & Shine 1998; reviewed in Scott *et al.* 1997). For instance, Nunney and Cheung (1997) found for *Drosophila melanogaster*, that the effect of incubation temperature on early fecundity depends on the ambient temperature at which fecundity was measured. If an animal performs best under conditions that are similar to their incubation temperatures, this can indicate an adaptive strategy to a thermal heterogeneous environment (Doughty & Reznick 2004). Alternatively, an 'optimal developmental temperature' may produce individuals that perform well at all ambient temperatures (for further information see Huey *et al.* 1999). The second and main goal of this study is to test for an interaction between incubation and ambient temperature on the performance of an ectothermic vertebrate.

Our study organism is a small diurnal lizard, *Lampropholis delicata* (common garden skink; De Vis 1888). At our study site in urban Sydney, New South Wales, Australia, this species experiences a wide range of nest and ambient temperatures due to differential invasion of open microhabitats by the exotic plant, *Vinca major* (blue perwinkle). This plant significantly reduces temperatures both in the soil and surrounding environment (see below), by preventing sunlight from reaching the soil. More than 60% of the habitat of *L. delicata* in eastern Australia has been colonized by *V. major* (Twyford & Baxter 1999; see also McClintock 1985). At our study site skinks are distributed evenly throughout grass and periwinkle habitats: during a 2-day census of lizard abundance across the entire area 53% were observed in weeded habitats ($n = 257$ individuals). Weed invasion is one of the most concerning environmental changes in Australia: alien plants have led to the extinction of at least four species of Australian native plants, and between 300 and 450 weed species have gone on to cause widespread ecological problems (Hinde & Perry 2000). Effective control techniques are essential for the long-term conservation of Australia's biodiversity. An efficient strategy requires knowledge of how weeds affect ecosystem processes. To date, research in this area has focused almost exclusively on the impact of weed infestation on vegetation (e.g. Mitchell & Tur 1975; Dodkin & Gilmore 1984; Walker & Smith 1997; Woods 1997). A third objective of this study is to gain insight into how weeds may influence the ecology of native animals.

In summary, the main aim of this study is to test the interactive effect of incubation and ambient temperature on the feeding performance and digestion time of *L. delicata*. In doing so, we will also gain insight on carry-over effects and the potential for invasive plant species to influence the ecology of native animals. We focused on feeding and digestion because these traits critically impact on ability to assimilate energy and growth (e.g. Pough *et al.* 2001), and hence are directly related to fitness.

MATERIALS AND METHODS

Collection and husbandry of gravid female lizards

As part of another experiment, during the 2001 summer adult male and female garden skinks were translocated from an urban area in Sydney to outdoor enclosures (diameter 4 m, comprised of sheet metal) located at The Australian National University (ANU), Canberra. From November 2002 to February 2003, 59 gravid female lizards were collected from these enclosures and transported to a laboratory on the ANU campus. The lizards were individually housed in plastic tubs ($18 \times 12 \times 6$ cm) that were filled with soil (to 1 cm) and contained a cardboard refuge. These boxes were maintained in a room set to 18°C with a photoperiod at L : D of 11:13 h. Lizards had access to thermoregulatory opportunities during the day, provided by a heat tape underneath their tub. Water and crickets (*Acheta domestica*) were provided *ad libitum.*

Egg incubation and husbandry of hatchling lizards

Each gravid female was checked daily. Fifty-one of these females produced viable eggs, that is fertilized and turgid (mean ± SE number of eggs: 3.2 ± 0.12). Upon oviposition the eggs were weighed and placed separately into a 64-mL glass jar containing vermiculite (water potential = –200 kPa; for details on calibration see Shine 1983) and covered to prevent water loss. The eggs of each female were randomly assigned to one of three different incubation treatments; however, for the purpose of this study only two of the incubation treatments will be discussed ('hot' and 'cold' incubator, see below). By using such a split clutch design, we accounted for potential maternal effects on the foraging behaviour of hatchlings. There was no significant variation in the oviposition dates or masses of the eggs that were assigned to the two different incubation treatments (in both cases: ANOVA, $F_{1,97} < 2.87$, $P > 0.09$).

We selected incubation temperatures that represented the regimes experienced by lizards in open grassland *versus* blue periwinkle microhabitats. *L. delicata* deposit their eggs in shallow (1–2-cm)

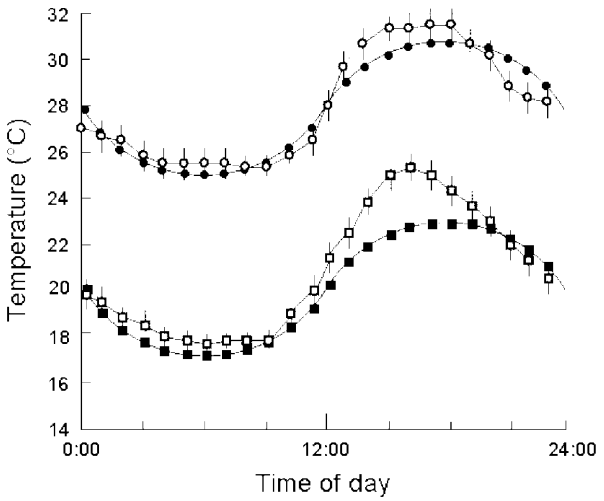

Fig. 1. Mean daily temperature regimes in natural nests in open grassland ($n = 4$) and blue periwinkle ($n = 4$) microhabitats and the thermal regimes used to incubate eggs in the laboratory. Circles represent open grassland profiles, squares represent blue periwinkle profiles, open symbols represent field nest temperatures (error bars indicate 1 SE each side of the mean), filled symbols represent laboratory incubation regimes. The location of the site was an urban cemetery (33°53′S 151°10′E) from which we collected the lizards that were used in this study.

depressions in the ground layer of soil that are covered by rock, stone or wood. During the laying season of 2002/03, we located four natural nests in each of the two microhabitats (eight nests in total) by identifying suitable sites and then turning debris to uncover the eggs. We used miniature data loggers (Thermocron, iButton, DS 1921L-F51) to measure temperatures (±1°C) in natural nests at 35-min intervals for 23 consecutive days. We pooled nest data for each time of the day at the replicate sites to give the average daily temperature regime in nests over this time, and based the incubation treatments on these mean figures (Fig. 1). One incubator mimicked the mean temperatures experienced by nests located in open grassland (~28°C, hereafter 'hot incubator': see also Qualls & Shine 1998). The incubation curve was adjusted slightly to approximate a sinusoidal shape. While the temperatures in the hot incubator closely followed the regime measured in the field, we programmed the incubator that mimicked periwinkle temperatures (hereafter 'cold incubator') 8°C lower than the hot incubator at all times (Fig. 1). This difference represents the discrepancy between nests in open grassland areas *versus* areas infested with periwinkle. Only mean temperatures and not thermal variance differed between the treatments. If the shape of the curves differed as well as the mean temperatures, interpretation of the data could have become very complicated if both thermal variance and mean temperature influenced hatchling phenotype.

All incubators were checked twice daily for hatchling lizards. Out of the 100 eggs assigned to one or other treatment, 73 successfully hatched a lizard. Egg mortality during embryogenesis was not significantly different among incubation treatments (failed/incubated = cold, 16/51; hot, 11/49; Fisher's Exact Test: d.f. = 1, $\chi^2 = 1.01$, $P = 0.32$). Newborn lizards were weighed (to 0.01 g) and then housed individually in plastic boxes (15 × 10 × 7 cm) at 21°C. The plastic boxes were filled with sand (to 1 cm) and contained a cardboard shelter as well as structural complexity in the form of vertical strips of card. Before commencing the experiments all hatchlings were provided with 5 *Tribolium* larvae (i.e. mealworms) that comprised a similar proportion of their body mass (mean ± SE percentage of hatchling mass = 15.9 ± 0.3). By adopting this procedure we controlled for variation in the early feeding experience of hatchlings from the different incubation treatments. To control for the level of hunger of experimental animals, lizards were fed their last mealworm 2 days before commencing the first experiment.

Testing procedure

At the beginning of our experiment, cold-incubated hatchlings were significantly heavier than hot-incubated hatchlings (mean + SE g: cold, 0.13 ± 0.01; hot, 0.11 ± 0.01: $t_{60} = 6.14$, $P < 0.001$). To control for any effect that the variation in mass may have on feeding and/or digestion performance, prey *Tribolium* larvae that were 3.0 ± 0.5% of the mass of test lizards were used for the experiments. Pilot trials showed that this proportional mealworm size was appropriate for revealing differences in the efficiency of feeding by lizards; anything smaller was always consumed rapidly, and lizards could not handle larger worms.

The test temperatures selected for our experiments represented the coolest (20°C) and warmest (28°C) environments during the summer invaded to different degrees by periwinkle (Fig. 1), and were obtained in two different rooms. The experimental tubs were plastic containers (15 × 10 × 7 cm) filled with sand (to 1 cm depth) that contained vertical card strips and small pebbles. To mimic the lizards' natural thermal environment as closely as possible, a basking spot (2 cm diameter) was available. In all test conditions the basking spot was heated to 30°C, which is approximately the mean body temperature of hatchling lizards that had access for 1 h to a gradient ranging 18–35°C (mean ± SE selected temperature cold-incubated hatchlings = 28.9 ± 0.5°C, $n = 15$; hot-incubated hatchlings = 30.3 ± 0.3°C, $n = 15$). This mean body temperature was assumed to reflect the preferred body

temperature of the lizards. The hot spot was generated using heat tape underneath the floor and a light bulb suspended directly above. In nature, even skinks that experience an ambient environment averaging 20°C can attain preferred body temperatures by climbing up plants and basking on leaves (Downes & Hoefer submitted). Thus, we focused entirely on mimicking the thermal aspects of habitat infested (20°C) and not infested (28°C) by periwinkle, but removed any structural differences.

Every hatchling was tested twice, once at each of the test temperatures. Each test condition was presented first to one half of the cold- and hot-incubated animals (16 and 17 individuals respectively). The lizards were tested at only one temperature per day and we allowed 1 day of rest between subsequent tests with the same individual. Hence, in the two trials lizards were maintained at the same hunger level (i.e. 2 days since eating one worm).

Before the trials we tested whether the mobility of the worms used as prey for lizards in our experiments varied between test temperatures. The amount of time that a solitary worm was stationary (did not move), mobile (its head and forebody were moving), and very mobile (its whole body was moving) did not vary significantly among the different test temperatures ($F_{2,187} = 0.99$, $P = 0.44$).

Lizards began experiments when they were 9–11 days old. Two days before the first experiments, they were introduced into experimental tubs and allowed to acclimate. On every test day, hatchlings were weighed and then transported within their experimental tubs, with the card refuge removed, to the room set to the appropriate test temperature. Lizards were left undisturbed to acclimate to this thermal environment for 1.5 h. Subsequently we began our observations of the mobility, feeding performance, basking behaviour and digestion time of the hatchlings.

Mobility

The mobility of the lizards was measured to estimate the rate at which they might encounter prey. During the 5 min prior to the feeding trials, one of us (IB) watched the lizard from the top of the cage and counted the number of times the lizard crossed the lines of a grid (1.5 cm × 2 cm, comprised of string across the top of the tub). We assumed that more mobile lizards had a greater chance of encountering prey.

Feeding performance

To begin a trial the mealworm was placed with tweezers into the experimental tub approximately 3 cm away from the head of the lizard; pilot studies determined that this was within the detection distance of hatchling skinks. One of us (IB) then continuously watched the lizard from the top of the cage and recorded with a stopwatch the following measures of feeding performance: *capture time* (the time between introducing the prey and the first successful seizure of the prey), *subdue time* (the time between the first successful seizure of the prey and the beginning of swallowing the prey), and *handle time* (the time between the beginning of swallowing the prey and its complete disappearance into the mouth of the lizard). We also counted the number of *failures* (unsuccessful attempts to capture prey), *drops* (subsequent releases of the prey after capture), and *shakes* (quick jerky movements of the head and/or body of the lizard after prey capture and before swallowing the prey) made by the lizard.

Basking

Basking frequency was recorded to check if lizards showed different basking behaviour at different ambient temperatures. During the 5 min prior to the feeding trials and the feeding trials themselves, we counted the number of times a lizard basked on the hot spot (i.e. rested under the light bulb with its ribs spread laterally).

Digestion time

The digestion time of the hatchlings was defined as the time between the complete disappearance of prey into the mouth of the lizard and the appearance of the first faeces. Preliminary studies using mealworms marked with a fluorescent elastomer (North-west Marine Technology Inc., Washington, USA) showed that at all test temperatures digestion was completed within 40 h. We assumed that the first faeces contained the remains of the worm that was consumed in the preceding trial because at least 48 h elapsed between subsequent foraging trials with the same lizard. Immediately after the trial we moved the lizard, still within its experimental tub, to a room at the same temperature as the feeding trial. The card refuge was replaced and a basking spot was provided as in the feeding trials. The tubs were placed directly underneath video surveillance cameras (model #1020 CCD). The lizard was filmed continuously in real time for the following 40 h (using a Sharp VHS video cassette recorder) to determine the exact time that the faeces appeared.

Data analysis

We excluded data collected from four hot-incubated lizards and two cold-incubated lizards because they

showed no interest in pursuing prey in one or more trials. These lizards originated from six different mothers. Data on 33 cold-incubated and 34 hot-incubated lizards were included in our final analyses.

Mobility, capture time, subdue time, handle time, shakes and digestion time were treated as continuous variables. We redefined the two variables failures and drops as two binary variables (with '1' = hatchling showed at least one failure/drop during trial and '0' = no failure/drop was observed) to be able to test for interaction effects (see below).

First, we tested for an interaction effect between incubation and ambient temperature on each of the response variables.

For *mobility* we initially fitted a mixed model (using the proc MIXED procedure in SAS: Littell *et al*. 1996) with 'incubation temperature', 'test temperature' and the interaction between incubation and test temperature as fixed factors, 'test order' and 'sex' as covariables, and 'lizard' as the repeated measure. A repeated measures design was used because each hatchling was measured twice: once at the cold ambient temperature, and once at the hot ambient temperature.

For each of the other continuous response variables (capture time, subdue time, handle time, shakes and digestion time), the same procedure was used. In all of the models, a significant four- or three-way interaction between either test order or sex *versus* incubation and test temperature did not exist, therefore these covariables were omitted. All of the continuous response variables were square-root transformed so that the residuals of the final models were normally distributed (Shapiro–Wilks tests).

For the binary variable *failures* we used the GENMOD procedure in SAS (Stokes 2000) to fit models. Because the response variable is binary, we used the logit link. We fitted a model with 'incubation temperature', 'test temperature' and the interaction between incubation and test temperature as predictors, 'test order' and 'sex' as covariables and 'lizard' as the repeated measure. For the binary variable *drops*, the same procedure was used. The covariables 'test order' and 'sex' were removed from the final models, because the models with these covariables did not fit the data significantly better than the simpler models without these covariables (the models were compared using the likelihood ratio statistic (=$-2 \times$ (log likelihood (model without covariables) – log likelihood (model with covariables)): *failures*: Likelihood Ratio = 9.22, $P = 0.32$; *drops*: Likelihood Ratio = 12.28, $P = 0.14$).

Second, we fitted again for each response variable (mobility, capture time, subdue time, handle time, shakes, digestion time, drops and failures) the final models, but with the continuous variable 'lizard mass' added as a covariable. These additional tests were performed because a strong relationship was found between incubation temperature and the mass of the lizards, that is, cold-incubated lizards were significantly heavier than hot-incubated lizards (see *Methods*). Hence with these additional tests we investigate if the mass of the lizards also plays a role in affecting feeding performance. Because of collinearity among incubation temperature and lizard mass, the mass data were centred (i.e. from each observation the mean mass of all observations (0.12 g) was subtracted) and scaled (i.e. the centred mass data were divided by the standard deviation (0.02 g)) (Kutner *et al*. 2005).

Basking was observed in only 19 of the 134 feeding trials, therefore variation in the rate of this behaviour was not analysed statistically.

RESULTS

Mobility

Incubation and ambient temperature interacted to affect the mobility of the hatchling lizards during the 5-min pretrial observation periods ($F_{1,65} = 6.96$, $P = 0.01$). Cold-incubated lizards were more mobile (i.e. crossed the grid more often) when tested at the cold temperature *versus* the hot temperature, whereas hot-incubated lizards were more mobile when tested at the hot temperature *versus* the cold temperature (Fig. 2a).

A lizard's mobility was not significantly affected by the relationship between lizard mass, incubation temperature and ambient temperature ($F_{1,65} = 0.20$, $P = 0.66$). There was no significant main effect of lizard mass on the tendency of a lizard to cross the grid ($F_{1,65} = 0.49$, $P = 0.49$).

Capture time

Incubation and ambient temperature interacted to affect the time that it took hatchlings to capture their prey ($F_{1,65} = 21.02$, $P < 0.0001$). Cold-incubated lizards captured worms most quickly at the cold ambient temperature, whereas hot-incubated lizards captured worms most quickly at the hot ambient temperature compared (Fig. 2b).

Lizard mass interacted with incubation temperature and ambient temperature to affect the time that it took hatchlings to capture prey (significant 3-way interaction: $F_{1,65} = 4.22$, $P = 0.044$). Figure 3 depicts the relationship between incubation temperature and ambient temperature separately for light lizards (mass up to 0.12 g; $n = 36$) and heavy lizards (mass greater than 0.12 g; $n = 31$). It showed that ambient temperature has little effect on the capture times of lighter cold-incubated lizards, whereas heavy cold-incubated lizards took longer to capture a mealworm at 28°C

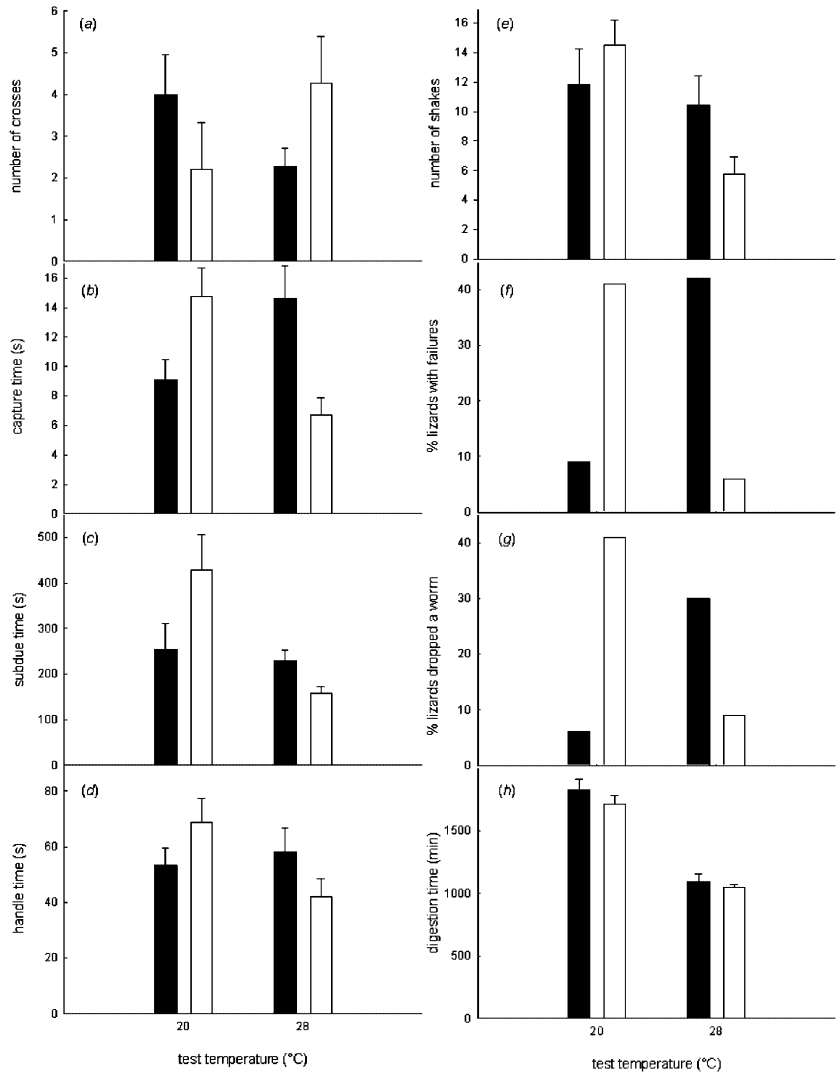

Fig. 2. Mean ± SE (a) mobility time, (b) capture time, (c) subdue time, (d) handle time, and (g) digestion time of *Lamproholis delicata;* and percentage of lizards showing at least one (e) failure, (f) drops, incubated at 20°C (black bars) and 28°C (white bars); at low (20°C) and high (28°C) test temperatures.

compared with at 20°C. A hot-incubated lizard's mass did not affect its ability to capture prey at different ambient temperatures (Fig. 3a).

Subdue time

Incubation and ambient temperature interacted to affect the time that it took hatchlings to subdue their prey ($F_{1,65} = 15.65$, $P = 0.0002$). Hot-incubated lizards took less time to subdue prey at the hot test temperature, whereas the subdue time of cold-incubated lizards was not significantly affected by test temperature (Fig. 2c).

Mass and ambient temperature interacted to affect the subdue time of the lizards ($F_{1,65} = 15.06$, $P = 0.0002$), but this relationship was not significantly affected by incubation treatment (no significant 3-way interaction: $F_{1,65} = 0.10$, $P = 0.75$). Figure 3b shows that lighter lizards of both incubation treatments had longer subdue times at the coolest temperature, whereas for heavy lizards subdue times were similar for lizards from both incubation treatments and at both ambient temperatures.

Handle time

Incubation and ambient temperature interacted to affect the time that it took hatchlings to handle their prey ($F_{1,65} = 10.09$, $P = 0.002$). Hot-incubated lizards handled prey more quickly at the hot test temperature,

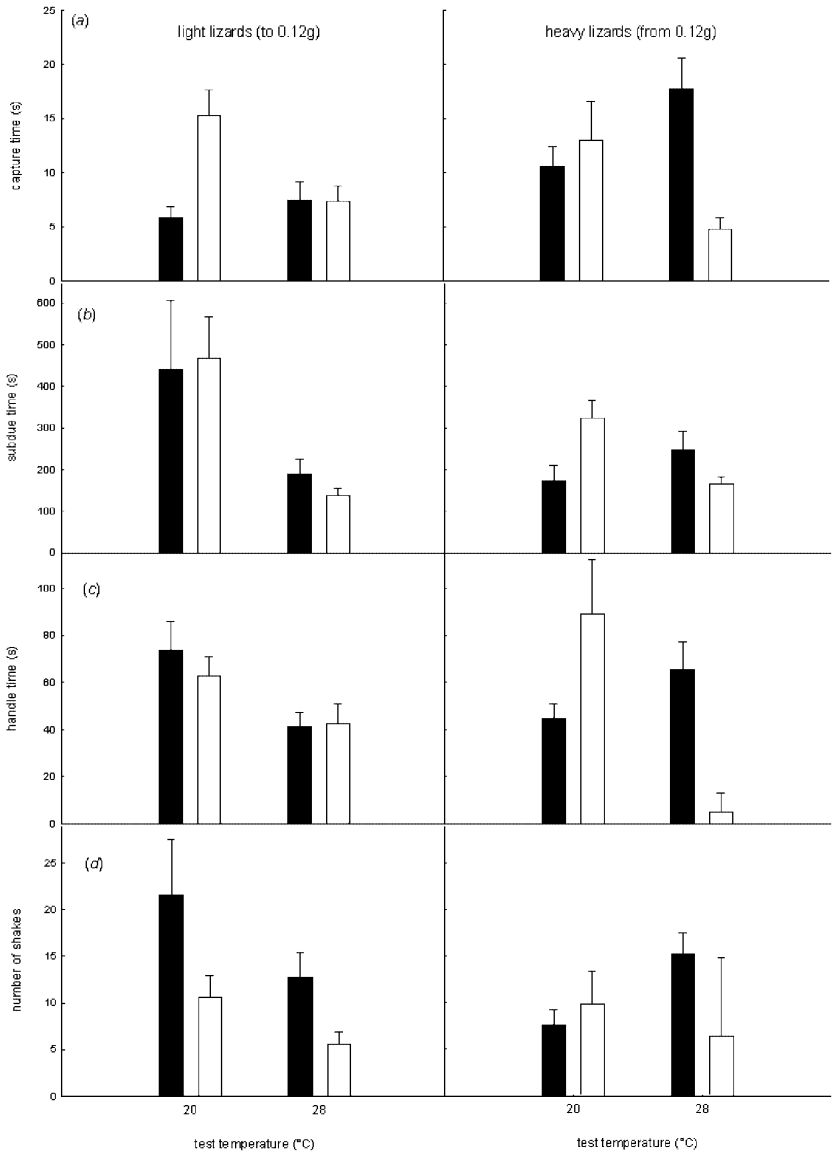

Fig. 3. Mean ± SE (a) capture time, (b) subdue time, (c) handle time, and (d) shakes of light (<0.12 g) and heavy (≥0.12 g) *Lamproholis delicata* incubated at 20°C (black bars) and 28°C (white bars); at low (20°C) and high (28°C) test temperatures.

whereas the handle time of cold-incubated lizards was not significantly affected by test temperature (Fig. 2d).

Lizard mass interacted with incubation and ambient temperature to affect the handle time of the hatchlings (significant 3-way interaction: $F_{1,65} = 10.61$, $P = 0.002$). Figure 3c shows that lighter lizards took less time to handle a mealworm at hot test temperatures compared with cold test temperatures, and that this trend is stronger for cold-incubated lizards compared with hot-incubated lizards. Heavy lizards took less time to handle a mealworm at test temperatures that were similar to their incubation temperatures; this trend is much stronger for hot-incubated compared with cold-incubated lizards.

Shakes

Incubation and ambient temperature interacted to affect the number of shakes that a hatchling made during foraging ($F_{1,65} = 14.71$, $P = 0.0003$). Hot-incubated lizards shook their prey at a lower rate at the hot test temperature, whereas the number of shakes made by cold-incubated lizards was not significantly affected by test temperature (Fig. 2e).

Lizard mass interacted with incubation temperature and ambient temperature to affect the number of shakes that a hatchling performed during foraging (significant 3-way interaction: $F_{1,65} = 6.86$, $P = 0.01$). Lighter lizards exhibited fewer shakes at the hot test

temperature compared with the cold test temperature, and this trend was stronger for cold-incubated lizards compared with hot-incubated lizards (Fig. 3d). Heavy cold-incubated lizards exhibited fewer shakes at the cold temperature compared with the hot temperature; the reverse trend was true for heavy hot-incubated lizards (Fig. 3d).

Failures

Incubation and ambient temperature interacted to affect the presence/absence of failure(s) during the feeding trial of *L. delicata* ($\chi^2_{(1)} = 19.74$, $P < 0.0001$). A smaller percentage of hot-incubated lizards failed to catch a worm at the hot test temperature compared with the cold test temperature; the inverse relationship was true for cold-incubated lizards (Fig. 2f).

The number of times a lizard failed to catch a worm was not significantly affected by the relationship between lizard mass, incubation temperature, and ambient temperature $\chi^2_{(1)} = 1.52$, $P = 0.22$). There was no significant main effect of lizard mass on the number of times a lizard failed to catch a worm ($\chi^2_{(1)} = 0.38$, $P = 0.54$).

Drops

Incubation and ambient temperature interacted to affect the presence/absence of drop(s) during the feeding trial of *L. delicata* $\chi^2_{(1)} = 12.62$, $P = 0.0004$). A smaller percentage of hot-incubated lizards dropped a mealworm at least once at the hot test temperature compared with the cold temperature; the inverse relationship was true for cold-incubated lizards (Fig. 2g).

The number of times a lizard dropped a worm was not significantly affected by the relationship between lizard mass, incubation temperature, and ambient temperature ($\chi^2_{(1)} = 0.14$, $P = 0.70$). There was no significant main effect of lizard mass on the number of times a lizard dropped a worm ($\chi^2_{(1)} = 1.49$, $P = 0.22$).

Digestion

Incubation and ambient temperature did not significantly interact to influence the digestion time of the lizards ($F_{1,62} = 0.22$, $P = 0.64$; Fig. 2h). At both test temperatures there was no significant variation among cold- *versus* hot-incubated lizards in the time required to digest a mealworm ($F_{1,62} = 2.06$, $P = 0.16$; Fig. 2h). Irrespective of incubation treatment, hatchlings digested faster at the hot test temperature compared with the cold test temperature ($F_{1,62} = 128.68$, $P < 0.001$; Fig. 2h).

A lizard's digestion time was not significantly affected by the relationship between lizard mass, incubation temperature and ambient temperature ($F_{1,62} = 1.20$, $P = 0.28$). There was no significant main effect of lizard mass on the time a lizard digested a mealworm ($F_{1,62} = 0.16$, $P = 0.69$).

DISCUSSION

Our study extends previous work on the independent effects of incubation temperature and ambient temperature for phenotypic expression, by demonstrating that these two influences can have an interactive effect on several key elements of feeding performance. Our data also demonstrate that incubation-induced changes in the mass of lizards can influence this relationship. No interactive effect of incubation temperature and ambient temperature was found on the time that it took lizards to digest their food.

Conditions (i.e. temperatures) experienced during incubation clearly affect some aspects of the feeding performance of *L. delicata*. The main result from our study is that, for all measures of feeding performance (except digestion time), the effects of incubation temperature depend on the temperature at which the lizards are tested, that is, a clear interaction exists between incubation and test temperature. Regarding mobility, capture time, drops and failures, each lizard performs best at temperatures similar to those experienced during incubation. With respect to subdue time, handle time and number of shakes during foraging, cold-incubated lizards are less sensitive to differences in ambient temperatures compared with hot-incubated lizards.

Variation in some of these traits might reflect differences in the basking behaviours of lizards. For instance, at temperatures cooler than those experienced during development, lizards may take longer to catch prey because they first must bask to reach their preferred body temperature. However, basking was rare during the feeding trials, and most lizards pursued prey immediately upon detecting it.

Our results demonstrate that incubation-induced differences in mass have the potential to play a significant role in defining the interaction between incubation temperature and test temperature. This factor was important in four of the eight behaviours that we measured (capture, subdue and handle time and shakes). In the case of subdue time, the interactive effect of incubation and ambient temperature disappears when controlling for mass: the way the subdue time of light and heavy lizards is affected by ambient temperature, does not depend on incubation temperature. However, in the other cases where mass covaried with incubation and test temperatures (capture and handle time and shakes), this influence was not necessarily responsible

for the significant interaction between these two factors. For instance, for both lighter and heavier lizards, the time taken to capture a prey was significantly greater for hot-incubated lizards tested in cooler *versus* warmer environments, and this trend was different from that of cold-incubated lizards in the same weight class (Fig. 3a). In other cases (e.g. handle time and shakes), a significant interaction between incubation temperature and test temperatures exists for heavier lizards (Fig. 3c,d).

Other kinds of developmental plasticity may drive the interaction between incubation temperature and test temperature. Incubation-induced plasticity in developmental period, tail length, interlimb length and body shape has been demonstrated for *L. delicata* (Downes & Shine 1999). However, plasticity at a physiological level, for example, the muscle physiology of the head and jaws of the lizards, is also likely to cause differences in feeding performance. Thermal plasticity of skeletal muscles has been demonstrated for several ectothermic species (Johnston & Temple 2002; Seebacher *et al.* 2003), but has not been examined for *L. delicata*.

The better performance of some lizards under conditions resembling their incubation environment may represent adaptation to a thermally heterogeneous environment (Doughty & Reznick 2004). Although during the summer (i.e. when incubation occurs) *L. delicata* experiences a relatively constant temperature over time, areas invaded by blue periwinkle are significantly cooler that open grasslands (Fig. 1). This situation results in a spatially heterogeneous environment, both in terms of incubation (soil) temperature and ambient (environmental) temperature. If hatchling lizards are likely to forage at ambient temperatures that are similar to the thermal conditions experienced during incubation, it may be advantageous for them to perform best at temperatures that are similar to those in the nest. The home range of *L. delicata* is relatively small ($\leq$20 m: S. Burgin, unpubl. data 1995). In our study site the size of patches of microhabitat varies from 4 m^2 to 60 m^2 with more than 70% of the patches being greater than 20 m^2. Thus, resident *L. delicata* may remain in the same microhabitat for much of their life. Unfortunately, we do not know whether hatchlings disperse from their natal area, and therefore their chances of residing in the same microhabitat type experienced during incubation. This information is critical for evaluating whether the improved feeding performance of some lizards under conditions resembling their incubation environment may be an adaptation to thermally heterogeneous environments (see also Huey *et al.* 1999).

Previous studies (e.g. Stevenson *et al.* 1985; Van Damme *et al.* 1991) showed that different closely related traits might be influenced by temperature in different ways. Our finding that digestion time was not significantly influenced by an interaction between incubation temperature and test temperature may reflect the different ways that periwinkle microhabitats are used by lizards when they are foraging *versus* digesting. Foraging most often takes place on the ground layer, whereas even in weeded areas lizards can climb plants to reach the open canopy and bask at preferred body temperatures (Downes & Hoefer submitted). Hence, with respect to digestion, lizards may cope with their thermally heterogeneous environment by adjusting their behaviour.

An auxiliary finding from our work is that incubation environment can induce changes to several different aspects of phenotype (in the present case, morphology and behaviour) that may interact to effect performance in early life. *L.* delicate eggs incubated at cool temperatures took longer to hatch than those at warmer temperatures (ANOVA: $F_{1,98} = 9170.2$, $P < 0.0001$); this finding is consistent with virtually all findings from similar studies on reptiles (reviewed in Elphick & Shine 1998). As in our experiment, most studies on the effects of incubation temperature report larger hatchlings from lower incubation temperatures (reviewed in Elphick & Shine 1998). Hatchlings incubated on moist substrates are generally larger than individuals incubated on drier media (reviewed in Deeming & Ferguson 1991). Despite the fact that substrate water potentials were standardized at the start of our experiment, the vermiculite may have lost more water through evaporation in the 'hot' treatment than in the 'cold' treatment, to result in the observed difference is body size. Interestingly, this variation in body size in part explained some of the ambient-temperature-induced variation in feeding behaviour of lizards from different incubation environments. To determine how 'important' these carry-over effects are, we need to investigate the phenotypes of older animals to see if the trends for mass and foraging performance persist during the life of the lizards.

Our temperature treatments closely mimicked the microhabitats available to *L. delicata* in our study area, the coolest of which is caused by invasion by the exotic plant *V. major*. We therefore infer from our laboratory study that the presence of periwinkle can significantly influence the feeding performance of *L. delicata*, by altering incubation conditions and ambient temperatures. We presume that this situation could be created by any dense sprawling plant that invades open grassland to create localized shaded areas but acknowledge that differences in plant structure and associated invertebrate assemblages might vary among situations, and impact on feeding performance. Many weed control strategies involve the rapid and complete removal of plants from invaded areas. We expect the potential impact of immediate plant removal on animal ecology to depend on the mechanism

underlying the development of temperature-induced changes in phenotypes. For instance, in the present system, if adaptation over a relatively long period of time is the mechanism underlying the interaction between incubation conditions and ambient temperature, the immediate removal of weeds may have serious implications for the foraging ecology, and presumable fitness, of lizards.

In conclusion, our study confirms previous work which demonstrates that incubation temperature can affect important components of fitness in ectotherms. It shows that incubation temperature and ambient temperature can interact to affect some aspects of a hatchling lizard's behaviour, and that this interaction may be affected by incubation-induced differences in the body sizes of lizards. Therefore, testing the combined effects of incubation temperature and ambient temperature delivers a more accurate demonstration of the overall effect of thermal environments on the performance of ectotherms. In these studies it may be important to incorporate incubation-induced changes in morphology into statistical analyses. It is also critical to control for variation in developmental temperature when examining performance in behaviour (see also Qualls & Shine 1998). Our results indicate that the immediate removal of exotic plants as part of a weed control strategy could have important implications for the foraging performance, and presumably fitness, of ectothermic animals.

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