2012

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Recommended Citation

Ligon, Day B., and Matthew B. Lovern. "Interspecific Variation in Temperature Effects on Embryonic Metabolism and Development in Turtles." ISRN Zoology 2012 (2012).

International Scholarly Research Network
ISRN Zoology
Volume 2012, Article ID 846136, 13 pages
doi:10.5402/2012/846136

Research Article

Interspecific Variation in Temperature Effects on Embryonic Metabolism and Development in Turtles

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Received 22 November 2011; Accepted 25 December 2011

Academic Editors: P. V. Lindeman and P. Scaps

We measured temperature-induced differences in metabolic rates and growth by embryos of three turtle species, *Macrochelys temminckii, Trachemys scripta*, and *Apalone spinifera*, at different, constant, temperatures. Oxygen consumption rate (VO_2) was measured during development and used to characterize changes in metabolism and calculate total O_2 consumption. Results from eggs incubated at different temperatures were used to calculate Q_{10}s at different stages of development and to look for evidence of metabolic compensation. Total O_2 consumption over the course of incubation was lowest at high incubation temperatures, and late-term metabolic rate Q_{10}s were <2 in all three species. Both results were consistent with positive metabolic compensation. However, incubation temperature effects on egg mass-corrected hatchling size varied among species. *Apalone spinifera* hatchling mass was unaffected by temperature, whereas *T. scripta* mass was greatest at high temperatures and *M. temminckii* mass was lowest at high temperatures. Hatchling mass : length relationships tended to correlate negatively with temperature in all three species. Although we cannot reject positive metabolic compensation as a contributor to the observed VO_2 patterns, there is precedence for drawing the more parsimonious conclusion that differences in yolk-free size alone produced the observed incubation temperature differences without energetic canalization by temperature acclimation during incubation.

1. Introduction

Although the suite of biochemical activities that contributes to an organism's metabolism is complex and therefore challenging to model [1], strong relationships exist between body temperature and whole-organism metabolic rate [2]. Various thermoregulatory mechanisms are employed by animals to dissociate body temperature from ambient temperature. Endothermy has evolved repeatedly as a physiological means of surviving suboptimal thermal conditions, but ectothermic species often rely primarily on behavioral strategies to regulate body temperature. In addition to behavior, however, ectotherms may exhibit physiological mechanisms to address thermal constraints of their environment. Solutions for surviving inhospitable temperatures may include manipulating biochemical reaction rates by varying enzyme concentrations or receptor densities, production of chaperone proteins to increase the range of temperatures over which target enzymes remain functional [3], changing the composition of cell membranes to affect permeability and (rarely) producing temperature-specific isozymes [4–6].

In comparison to other life stages when behavior can play an important role in overcoming thermal constraints, means for maintaining suitable body temperatures of oviparous animals during embryonic development are limited. Three factors contribute minimizing an embryo's exposure to or effects of suboptimal thermal conditions: (1) indirect behavioral temperature selection via maternal thermoregulation or nest-site selection; (2) developmental diapause during periods when thermal conditions are unsuitable; (3) utilization of one or more of the physiological strategies listed above. These strategies may function alone or in combination to circumvent thermal limitations on development. For example, because the efficacy of maternal nest-site choice may be limited by the stochastic nature of environmental temperatures [7], maternal nest-site selection seems likely to

function in combination with complementary physiological compensatory strategies.

Much attention has been paid to the effects of temperature on hatchling traits, particularly among taxa that exhibit temperature-dependent sex determination. In addition to effects on population sex ratios in many species [8–10], temperature has been demonstrated to influence hatchling size, posthatching growth, locomotor performance, metabolic rate, agility, and crypsis [11–28]. The ways in which temperatures during embryonic development affect postembryonic endpoints have received more attention among reptiles than have endpoints measured during embryonic development [29]. Thus, some aspects of how differences in the thermal environment experienced by embryos lead to morphological and performance differences remain poorly understood.

The finite quantity of energy and materials packaged in an egg must be budgeted to meet development and growth requirements. Therefore, temperature-induced differences in energy utilization could trickle down to affect posthatching condition of offspring by directly or indirectly influencing morphology, physiological performance, or postembryonic energy reserves. The effects of temperature on embryo metabolism have been studied in a variety of reptiles [30–36]. Patterns in the relationship between temperature and embryo energy expenditure have been fairly consistent. For example, Angilletta et al. [33] found that, in the lizard *Sceloporus undulatus*, energy expenditure over the course of incubation was similar at 30°, 32°, and 34°C, but 10–15% lower compared to embryos that developed at 28°C. Similarly, *Crocodylus johnstoni* embryos consumed 10% less oxygen at 31°C than at 29°C [37], and embryonic Nile soft shell turtles (*Trionyx triunguis*) incubated at 27° and 30°C exhibited similar energetic expenditure, but used 5% less oxygen at 33°C [38]. In contrast to this pattern of lower oxygen consumption at higher temperatures, no effect of incubation temperature was observed among *Emydura signata* embryos incubated at 24° and 31°C [39] or *Chelonia mydas* incubated at 26° and 30°C [40].

In combination, these studies suggest that embryos of many reptiles utilize less energy during development at higher incubation temperatures, but some species may exhibit a capacity to physiologically correct for suboptimally high or low body temperatures, commonly referred to as positive compensation [4, 41]. The goal of this study was to investigate differences in incubation temperature effects on embryonic development and metabolism among a sympatric but phylogenetically diverse assemblage of freshwater turtles [42]: *Trachemys scripta*; *Apalone spinifera*; *Macrochelys temminckii*. Our objectives were threefold. The first was to measure the capacity for metabolic compensation during embryonic development by comparing stage-specific VO_2 (oxygen consumption rate) of embryos exposed to different constant incubation temperatures. These measurements were then used to calculate differences in Q_{10} (the rate of change of a physiological process as a consequence of increasing the temperature by 10°C) at different stages of development. Second, we evaluated temperature effects on energetic cost of development by calculating the total volume of oxygen ($O_{2\text{total}}$) used over the course of incubation, and mass-conversion efficiency based on differences in hatchling mass after correcting for variation in initial egg mass. Third, we qualitatively assessed the degree of variation in different species' responses to temperature.

2. Materials and Methods

All procedures for this research were approved by the Oklahoma State University Institutional Animal Care and Use Committee (Protocol AS023), guaranteeing compliance with animal care guidelines described in The Guide for the Care and Use of Laboratory Animals, 7th edition (1996). *Trachemys scripta* and *M. temminckii* hatchling mass data were used in other analyses related to posthatching effects of T_{inc} (incubation temperature) [43].

2.1. Study Species. The three turtle species included in this study represent three different families and, although sympatric in parts of their range, they exhibit substantial ecological differences. *Trachemys scripta* (Family Emydidae) is medium in size and primarily aquatic but engages in frequent basking and terrestrial migrations between water bodies and conforms to the Type Ia pattern of temperature-dependent sex determination [44], wherein males are produced at low temperatures and females at high temperatures. *Apalone spinifera* (Family Trionychidae) exhibits many adaptations to a pelagic lifestyle, including a hydrodynamic form and substantial capacity for aquatic respiration. In contrast to most turtles, sex is determined genetically in members of this family [45]. Finally, *Macrochelys temminckii* (Family Chelydridae) is a very large-bodied, primarily bottom-dwelling species that seldom leaves water except to nest. It follows a type II pattern of temperature-dependent sex determination, characterized by development of females at low and high temperatures, and males or a mixed sex ratio at intermediate temperatures [44].

2.2. Egg Collection. Eggs were obtained for all three species in May and June 2004. A single *A. spinifera* nest was excavated from a sand bar at Sequoyah National Wildlife Refuge (SNWR) in eastern Oklahoma, and transported to Oklahoma State University (OSU) within 15 h after deposition. Gravid *T. scripta* were trapped in an oxbow at SNWR using baited hoop nets. These turtles were transported to OSU where oviposition was induced using oxytocin (0.10 IU/kg IM) [46]. Turtles were placed individually in plastic tubs containing approximately 15 cm of water to minimize accidental destruction of eggs by the turtles. After eggs were obtained, the adult females were released at SNWR. Finally, *M. temminckii* eggs were obtained from a captive group maintained at Tishomingo National Fish Hatchery as part of a captive breeding/reintroduction program. The adult turtles which produced the eggs for this study originated from SNWR. Eggs were laid naturally and excavated from nests within two d following oviposition. No eggs of any of the three species showed signs of the white banding characteristic of early development [47] prior to arriving at OSU.

2.3. Incubation. Eggs from each species were measured (±0.1 mm) and weighed (±0.01 g), and then assigned to an incubation treatment in a randomized block design (block = clutch). Within each incubation treatment, eggs were distributed among 1–5 plastic shoeboxes (1.5 L) half-filled with damp vermiculite (1 : 1 vermiculite : water by mass; ~ −150 kPa water tension; [48]). Shoeboxes were then assigned to one of three constant-temperature incubators set at 26.5°, 28.5°, and 30.5°C. Boxes were rotated within each incubator daily to eliminate the possibility of position effects, and each box was weighed weekly and rehydrated as necessary to maintain its initial mass. Eggs were candled every 2-3 days during early development and eggs that failed to develop were discarded to eliminate substrate for invasion of mold.

2.4. Hatchlings. Upon pipping, each egg was placed in a plastic jar lined with dampened paper towels so that the identity of individuals could be determined after hatching. After emerging from the egg shell, hatchlings were kept in the plastic jars until residual yolks were completely internalized, a period that lasted 0–9 d and varied among species. *Apalone spinifera* were photographed and *T. scripta* and *M. temminckii* received unique markings to ensure future identification. Cuticle scissors were used to cut notches in unique combinations of marginal scutes on *T. scripta*. A small sewing needle was used to tie small loops of dental floss through unique combinations of scutes on *M. temminckii* [27]. Hatchlings were removed from the incubator to flow-through raceways.

2.5. Metabolic Rate. Metabolic rates were estimated by measuring changes in oxygen concentration in chambers via closed system respirometry [49] and calculating VO_2 [50]. Eggs were placed individually in metabolic chambers constructed from 169 mL plastic jars with screw-top lids. A stopcock was inserted through each lid and sealed in place with silicon. Initial air samples were drawn from each chamber into stopcock-equipped 30 cc syringes, and then sealed. The chambers were then placed into the incubators for 1–1.5 h (longer during early development when VO_2 was expected to be low). Chambers were then removed from the incubator, and final air samples were drawn into a second set of syringes. Eggs were weighed at the conclusion of each measurement and returned to the plastic shoeboxes.

Oxygen concentrations of all air samples were analyzed in 10 mL aliquots with a Sable Systems FC-1 oxygen analyzer. A stream of air was drawn from outside the building at a regulated flow rate of 100 mL/min. It passed through serial columns of Drierite and Ascarite to remove water and CO_2, respectively. Each aliquot was injected into the air stream, which passed through a small column of Drierite and Ascarite and then through the oxygen analyzer. VO_2 was calculated for each turtle as the difference between the initial and final volumes of oxygen after correcting for chamber volume [50].

VO_2 of *T. scripta* and *M. temminckii* embryos was measured at 7 d intervals starting 7 d after oviposition. *A. spinifera* embryo VO_2 was measured at 2-3 d intervals early and late in development, and on a 7 d schedule during the middle third of incubation.

The volume of oxygen used over the course of incubation was calculated for each turtle that successfully hatched by summing the trapezoidal areas created by adjacent VO_2 measurements using the equation:

$$O_{2\text{total}} = \frac{\sum^{i} 24(VO_{2n} + VO_{2n-1})(T_n - T_{n-1})}{2}, \quad (1)$$

where $O_{2\text{total}}$ was the oxygen consumed over the duration of incubation, VO_2 was the rate of oxygen consumption at age n measured in mL/h, T was the number of days since the beginning of incubation, n was the days on which VO_2 was measured, and p was day on which each turtle pipped. Because turtles hatched 0–7 d after the last embryo VO_2 measurement, VO_{2p} at the time of pipping was estimated based on the difference between VO_2 measurements prior to and after hatching. These estimates were calculated as:

$$VO_{2p} = \left[\left(VO_{2\text{hatchling}} - VO_{2\text{final}}\right)\left(T_{\text{hatchling}} - T_{\text{final}}\right)^{-1} \times \left(T_{\text{pip}} - T_{\text{final}}\right)\right] + VO_{2\text{final}}, \quad (2)$$

where $VO_{2\text{hatchling}}$ was measured at each hatchling's assigned incubation temperature following internalization of the residual yolk and $VO_{2\text{final}}$ was the last measurement prior to pipping. These values were used to calculate the final trapezoidal area of each turtle's $O_{2\text{total}}$ to produce a precise estimate of the volume of oxygen used between oviposition and hatching.

2.6. Statistics. Statistical analyses were conducted separately for each of the three species. Incubation temperature effects on three characteristics of VO_2 during embryonic development were analyzed: timing of initial VO_2 divergence; maximum VO_2 ($VO_{2\text{max}}$); developmental stage-specific Q_{10}.

The age at which VO_2 of embryos at different temperatures first diverged was assessed by performing post hoc comparisons using differences of least-squares means from a repeated measures ANCOVA across all measurements, with individual embryo's VO_2s repeated across measurement intervals and initial egg mass as a covariate.

$VO_{2\text{max}}$ was likely affected by timing of measurements; therefore, timing of $VO_{2\text{max}}$, expressed as a percentage of total incubation time, was included as a covariate in analyses comparing $VO_{2\text{max}}$ at different incubation temperatures.

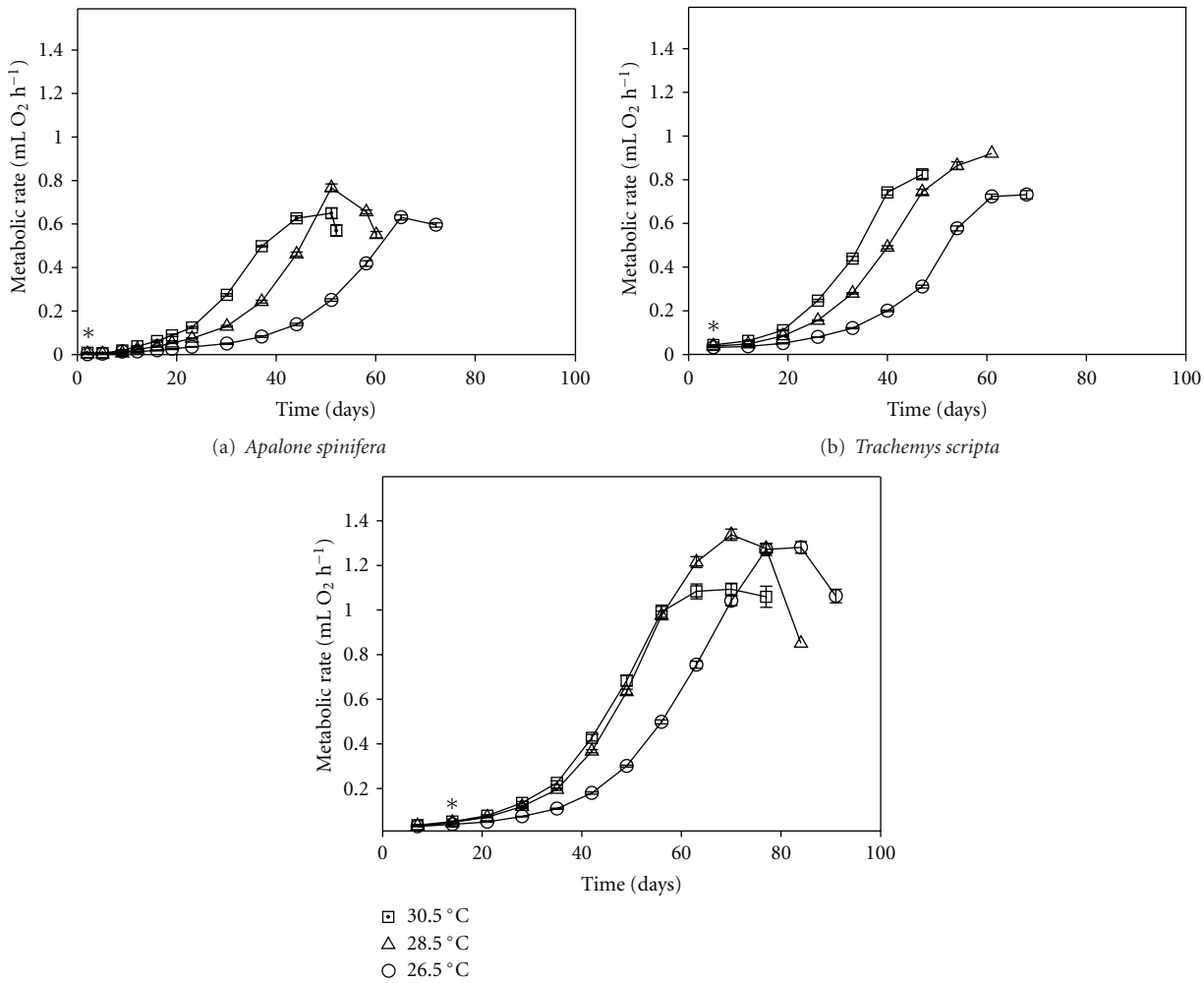
Q_{10} values were calculated at four different stages of development and were based on VO_2 of embryos at 26.5 and 30.5°C. Developmental stages were expressed as a percent of incubation time and were distributed such that Q_{10} was compared near the beginning, first and second thirds, and end of incubation.

Hatchling size was analyzed in two different ways: (1) mass was analyzed in an ANCOVA with initial egg mass as a covariate to assess incubation temperature effects on egg-to-tissue mass conversion efficiency; (2) differences in body

Table 1: Clutch, egg, and hatchling data for turtles included in this study (mean ± SE). Mean incubation periods are reported for eggs incubated at 26.5, 28.5, and 30.5°C, respectively.

Species	Number of Clutches	Number of eggs[a]	Hatchling success (%)	Incubation period (d)	Egg mass (g)	Hatchling mass (g)	Hatchling length (mm)[b]
A. spinifera	1	18 (18)	89	77, 61, 53	6.7 ± 0.06	4.4 ± 0.06	25.3 ± 0.25
M. temminckii	4	109 (17–33)	66	93, 82, 79	26.1 ± 0.18	16.4 ± 0.17	35.2 ± 0.17
T. scripta	5	58 (10–14)	93	71, 59, 52	11.5 ± 0.08	8.9 ± 0.09	32.1 ± 0.11

[a] Number of eggs of each species used in this study, with clutch size range in parentheses.
[b] Plastron length reported for *A. spinifera*, carapace length reported for *M. temminckii* and *T. scripta*.



(a) *Apalone spinifera*
(b) *Trachemys scripta*
(c) *Macrochelys temminckii*

Figure 1: Changes in VO_2 during embryonic development at three constant temperatures. Asterisks indicate first measurement at which VO_2 diverged across all three incubation temperatures. Error bars = ±1 SE.

length (carapace length was used for *T. scripta* and *M. temminckii* and plastron length was used for *A. spinifera*) were compared across incubation temperatures with hatchling mass as a covariate to assess differences in body composition (hereafter Body Condition Index, BCI).

In all of the above analyses, homogeneity of slopes among treatments was tested by comparing interaction terms that included the covariate. In all cases homogeneity was confirmed, and interaction terms were removed prior to final analyses. Also, nonsignificant covariates ($P > 0.05$) were removed to increase degrees of freedom for the error term. All statistical tests were conducted using SAS v. 9.1 Proc Mixed after testing the homogeneity of variance assumption using Proc GLM (SAS Institute 2002). All metabolism,

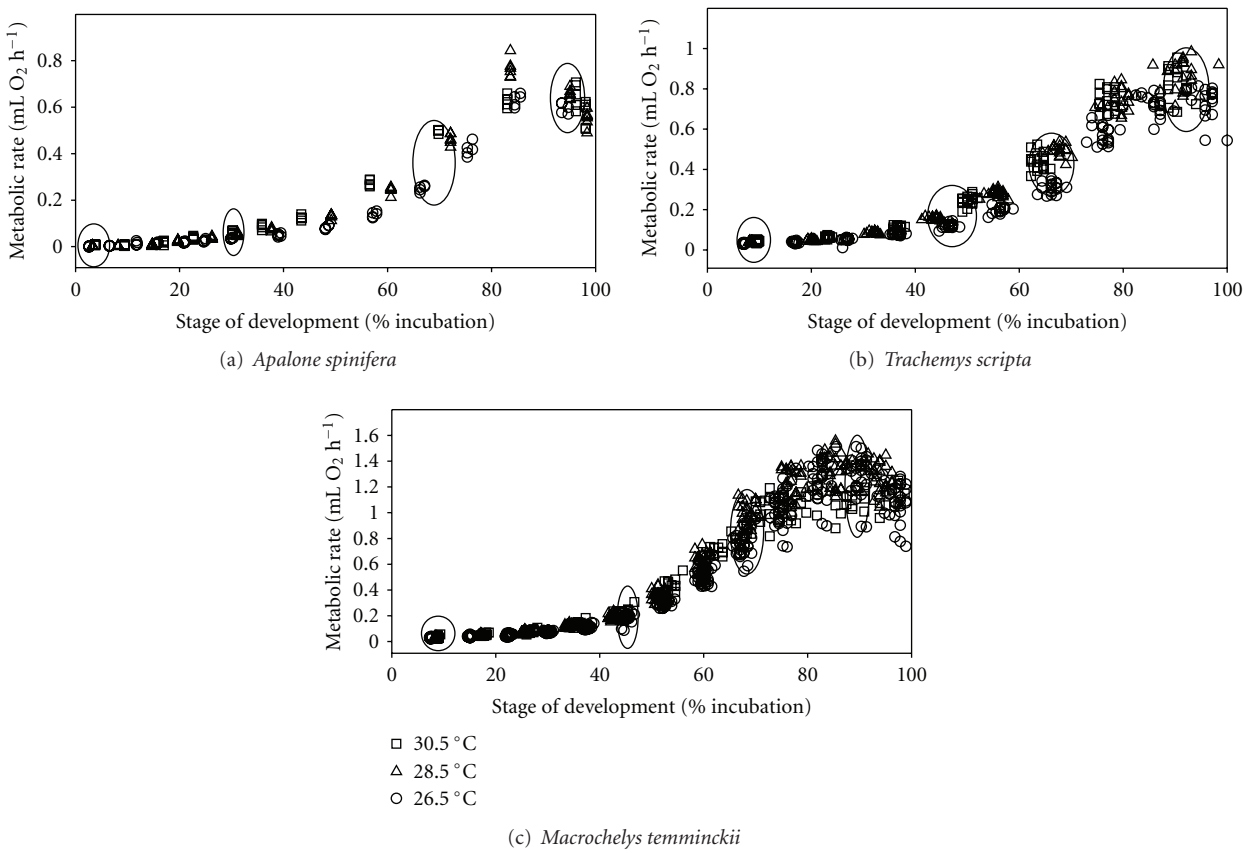


Figure 2: Comparison of changes in VO_2 of embryos maintained at different temperatures, with time expressed as a percentage of total incubation duration. Ovals superimposed on each figure indicate samples used to calculate developmental stage-specific Q_{10} values (see text).

mass, and length values were $\log_{10}$ transformed prior to analysis to improve data distribution. Results are expressed as mean ± 1 SE.

3. Results

3.1. Species Comparisons. Eggs used in this study comprised one *A. spinifera*, five *T. scripta*, and four *M. temminckii* clutches (Table 1). Egg mass varied among clutches in the latter two species ($P < 0.0001$), but because a randomized block experimental design was employed, it did not vary among incubation temperatures (*A. spinifera*: $P = 0.507$; *M. temminckii*: $P = 0.145$; *T. scripta*: $P = 0.953$).

Temperature affected incubation duration (Table 1), embryo VO_2 (Figures 1, 2) and total O_2 consumed (Figure 3) in all three species. However, temperature affected incubation duration differently among the three species. The 4°C difference experienced by eggs incubated at 26.5° compared to 30.5°C produced 31% and 28% differences in incubation time in *A. spinifera* and *T. scripta*, respectively, compared to just a 15% difference in *M. temminckii*. The effect of incubation temperature on *M. temminckii* incubation duration was an even smaller 4% across the 2°C span between 28.5–30.5°C, compared to 19% and 12% in *A. spinifera* and *T. scripta*, respectively.

Despite low VO_2 values during early development, precision of measurements was sufficient to detect differences among all three incubation temperatures at the first measurement in *A. spinifera* and *T. scripta* (days 2 and 5, resp.). Among *M. temminckii* embryos, mean VO_2 at 26.5° differed from that at 28.5° and 30.5°C by day 7 and differed among all three temperatures by day 14 ($P < 0.001$; Figure 1).

Patterns in the magnitude of VO_{2max} among different incubation temperatures varied among species. *Macrochelys temminckii* embryos maintained at 26.5° and 28.5° had similar VO_{2max} ($P = 0.353$) and were both greater than those incubated at 30.5°C ($P < 0.0001$; Figure 4(c)). In contrast, VO_{2max} among *T. scripta* embryos was lowest at 26.5° ($P < 0.0001$) and did not differ between 28.5° and 30.5°C ($P = 0.054$; Figure 4(b)). Finally, *A. spinifera* VO_{2max} was greatest at the intermediate incubation temperature (28.5°: $P < 0.0003$) and did not differ at the extremes (26.5–30.5°C: $P = 0.157$; Figure 4(a)).

The timing of VO_{2max} (expressed as % total incubation duration) was unaffected by temperature for all three species (*M. temminckii*: 87.5%, range = 82–97%, $P = 0.521$; *T. scripta*: 91%, range = 84–97%, $P = 0.661$; *A. spinifera*: 87%, range = 83–96%, $P = 0.060$). These values were no doubt dependent on the timing of VO_2 measurements, however. It should be noted that whereas *M. temminckii* and *A. spinifera*

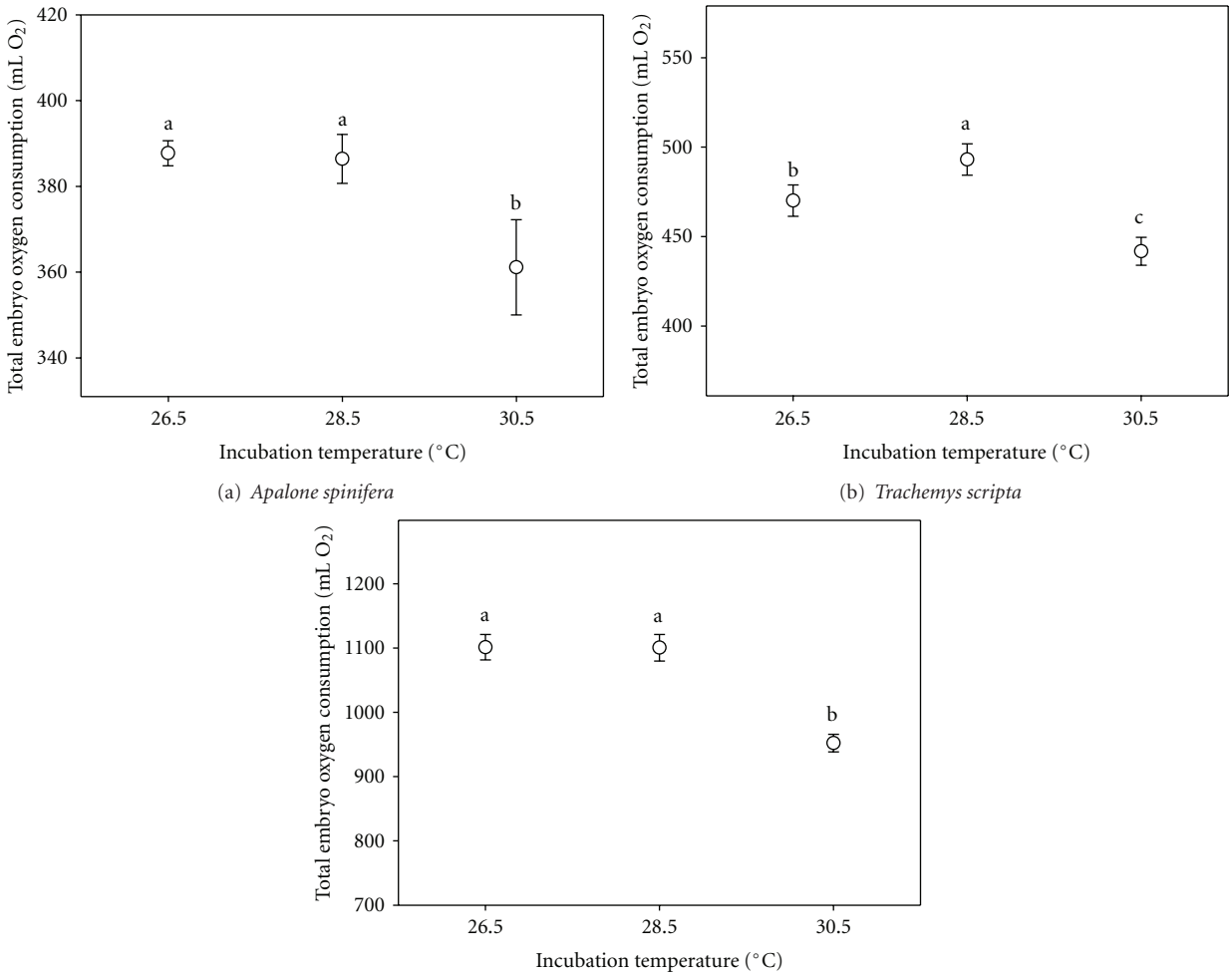


Figure 3: Comparison of the total volume of O_2 consumed over the duration of incubation by embryos incubated at different constant temperatures. Lower case letters indicate treatment differences ($P < 0.05$). Error bars = ±1 SE.

exhibited distinctly peaked embryo VO_2 patterns, VO_{2max} among *T. scripta* tended to occur at the last measurement prior to hatching (Figure 1). The exceptions to this pattern were five out of 18 embryos incubated at 26.5°C that peaked at the penultimate VO_2 measurement.

Total O_2 consumption over the course of embryonic development was similar at 26.5° and 28.5°C and lower at 30.5°C in *A. spinifera* and *M. temminckii*. By comparison, total O_2 consumption in *T. scripta* was highest at 28.5°C, intermediate at 26.5°C and lowest at 30.5°C (Figure 3).

3.2. Metabolic Compensation. VO_2 measurements used for calculating Q_{10} values were selected based on their timing (targets: 0, 33, 67, and 100% of incubation) and degree of overlap in the timing of 26.5° and 30.5°C measurements when expressed as a proportion of incubation duration in order to closely match the developmental stages compared among embryos at different incubation temperatures (Figure 2 and Table 2). Among *A. spinifera*, Q_{10} was 4.0–69.5 for the first three stages. However, at approximately 95% development Q_{10} = 1.2, lower than expected in the absence of positive compensation. In comparison, *T. scripta* exhibited Q_{10}s between two and three during the initial two-thirds of development, and a similar decrease close to hatching (Q_{10} = 1.4 at approximately 88% development). In contrast, *M. temminckii* registered Q_{10}s less than two throughout development. VO_2 measurements at 89% development produced a Q_{10} = 0.7.

3.3. Cost of Development. Although hatchling mass was not significantly different among incubation temperatures (*A. spinifera*: $P = 0.983$; *T. scripta*: $P = 0.065$; *M. temminckii*: $P = 0.200$; Figure 5), temperature-induced differences were evident in mass conversion efficiency (Figure 6) and hatchling BCI (Figure 7) in *T. scripta* and *M. temminckii*, but not *A. spinifera*.

Hatchling mass scaled positively to egg mass in all three species. *Trachemys scripta* hatchling mass fit the equation $M_{hatchling} = -0.04M_{egg}^{0.93}$ ($r^2 = 0.32$, $P < 0.0001$), *M. temminckii* fit the equation $M_{hatchling} = -0.05M_{egg}^{0.93}$

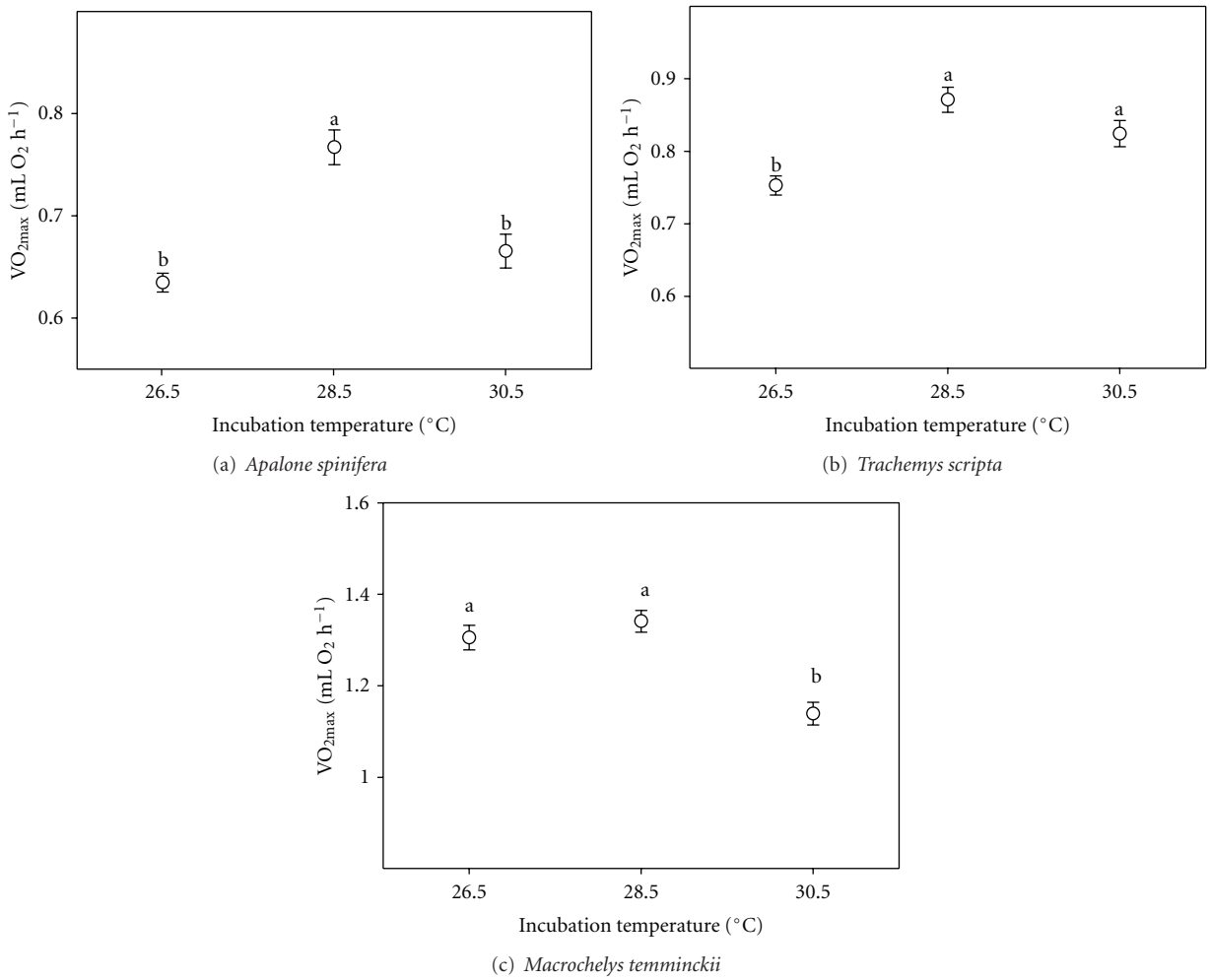


FIGURE 4: VO_{2max} of turtle embryos incubated at three temperatures. Lower case letters indicate treatment differences ($P < 0.05$). Error bars = ±1 SE.

($r^2 = 0.50$, $P < 0.0001$), and *A. spinifera* fit the equation $M_{hatchling} = -0.13M_{egg}{}^{0.92}$ ($r^2 = 0.25$, $P = 0.04$). Mass conversion efficiency was unaffected by incubation temperature in *A. spinifera* ($P = 0.93$), was reduced at high temperatures in *M. temminckii* ($P = 0.03$), and was elevated at high temperatures in *T. scripta* ($P = 0.02$; Figure 6).

Hatchling BCI, expressed as the relationship between $\log_{10}$-transformed length and mass, did not differ across incubation temperatures in *A. spinifera* (Figure 7(a)). BCI of hatchlings was not different at 26.5° and 28.5°C in *T. scripta*, and was higher at both these temperatures in comparison to hatchlings from 30.5°C (Figure 7(b)). Among *M. temminckii*, BCI was greatest at 26.5°, intermediate at 28.5°, and lowest at 30.5°C (Figure 7(c)).

4. Discussion

Interspecific variation was evident in the effects temperature had on stage-specific Q_{10} values, O_{2total}, VO_{2max}, hatchling size, and hatchling composition (BCI). However, broad-scale consistency in the direction and timing of observed patterns suggest that incubation temperature influenced embryo energetics and growth similarly among the three species.

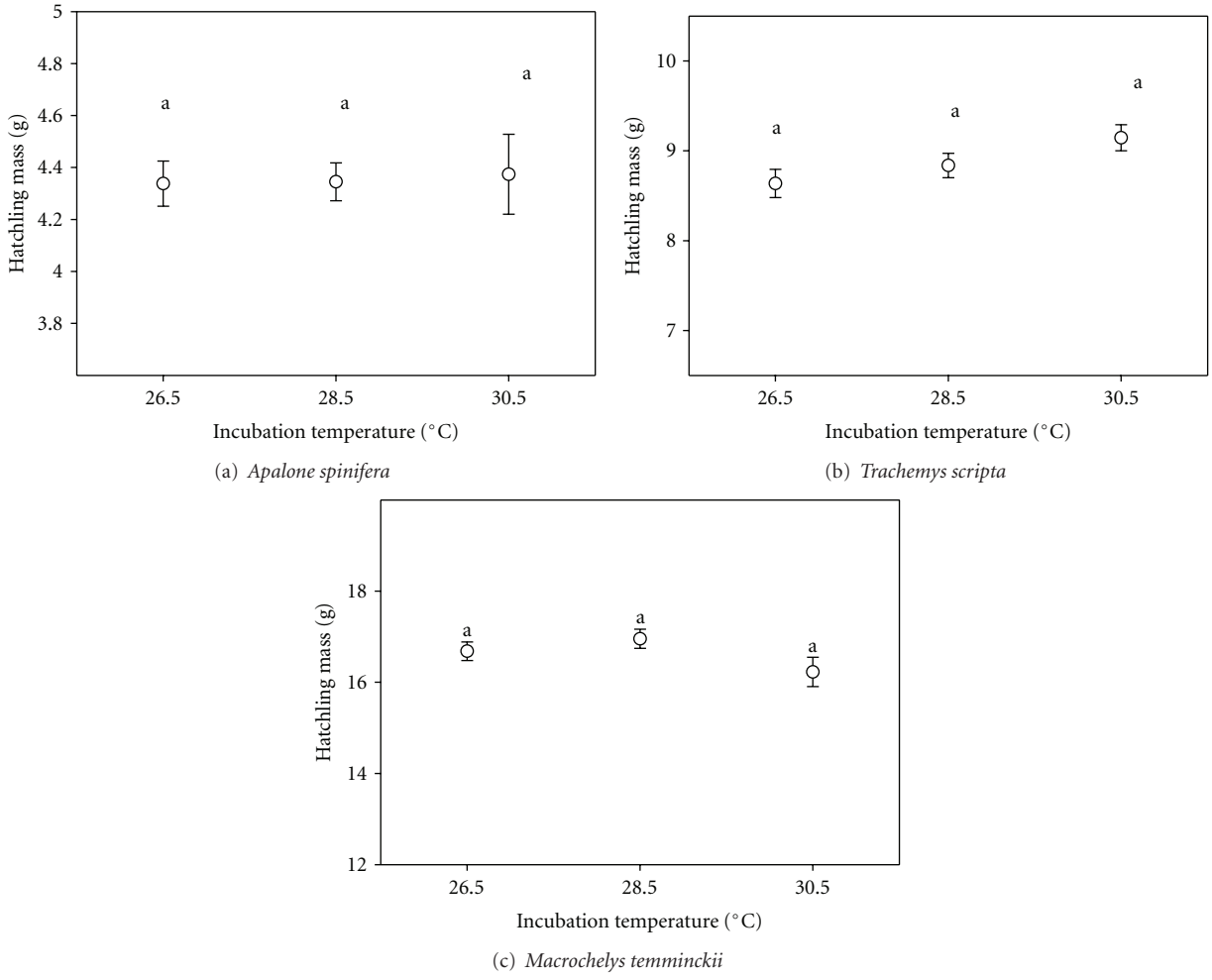
Chemical reaction rates typically exhibit Q_{10}s of 2-3. Biological rates that fall below this range are often interpreted to be indicative of temperature compensation, whereas higher values indicate inverse compensation, or hypersensitivity to temperature [51]. Progressively decreasing Q_{10}s over the course of embryonic development have been observed in several poikilotherms, including a turtle (*Emys orbicularis*; [35]), and two fishes (*Danio rerio* and *Salmo gairdneri*; [52, 53]. This pattern frequently has been proposed to reflect greater thermal compensation as development progresses.

Q_{10} values were highly variable both within and among species and did not neatly fit a negative correlation with embryonic development. However, as in previous studies, Q_{10} was consistently lower near the conclusion of embryonic development than during earlier stages in all three species.

Macrochelys temminckii incubated at 30.5°C consumed less oxygen than those at lower temperatures and exhibited VO_{2max} lower than that of turtles at 28.5°C and similar to

Table 2: VO_2 Q_{10} values calculated at different stages of embryonic development in three turtle species. Stages are expressed as % of total incubation duration, followed by the number of days since oviposition. Q_{10} values were calculated from VO_2s of turtles incubated at 26.5 and 30.5°C.

		Stages (% incubation duration, day of incubation)							
Species	T_{inc}	1		2		3		4	
A. spinifera	26.5°	2.6%, 2	Q_{10} = 69.54	30.0%, 23	Q_{10} = 3.98	66.6%, 51	Q_{10} = 5.57	94.0%, 72	Q_{10} = 1.24
	30.5°	3.8%, 2		30.2%, 16		69.8%, 37		96.2%, 51	
T. scripta	26.5°	7.1%, 5	Q_{10} = 2.14	36.7%, 26	Q_{10} = 2.30	66.3%, 47	Q_{10} = 2.37	86.1%, 61	Q_{10} = 1.39
	30.5°	9.7%, 5		36.7%, 19		63.7%, 33		90.7%, 47	
M. temminckii	26.5°	7.6%, 7	Q_{10} = 1.38	37.5%, 35	Q_{10} = 1.72	67.5%, 63	Q_{10} = 1.98	89.8%, 84	Q_{10} = 0.67
	30.5°	8.9%, 7		35.7%, 28		71.1%, 56		88.9%, 70	



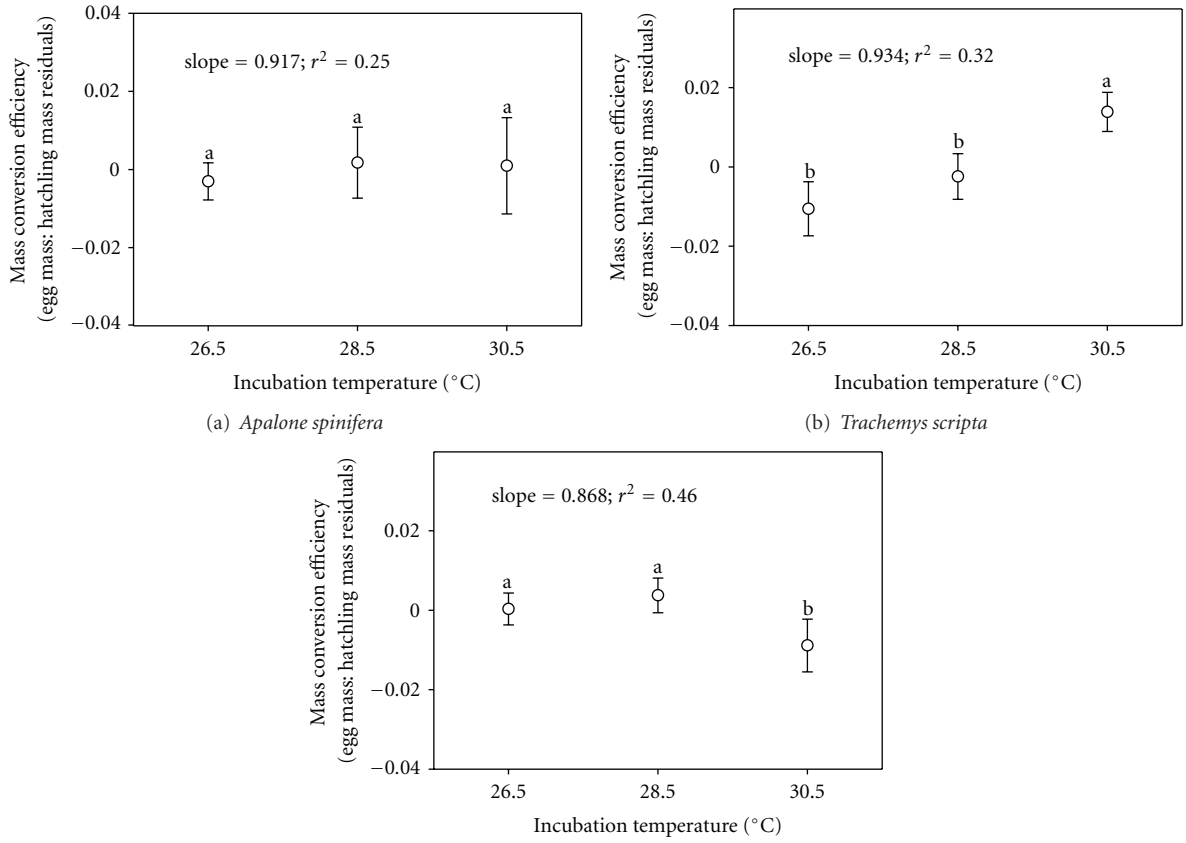
(a) *Apalone spinifera*

(b) *Trachemys scripta*

(c) *Macrochelys temminckii*

Figure 5: Mass of hatchlings incubated at different constant incubation temperatures. Lower case letters indicate treatment differences ($P < 0.05$). Error bars = ±1 SE.

those at 26.5°C. Such results could stem from metabolic compensation to temperature, ultimately resulting in greater efficiency in the conversion of yolk to metabolically active tissue. Although it was infeasible in this study to sacrifice embryos or hatchlings to directly measure yolk : tissue ratios, evidence from Q_{10}s and hatchling size support an alternative conclusion: though incubation temperature produced minimal differences in hatchling mass, those from the highest temperature exhibited lower BCIs and were, therefore, morphometrically smaller after correcting for variation in



(a) *Apalone spinifera*

(b) *Trachemys scripta*

(c) *Macrochelys temminckii*

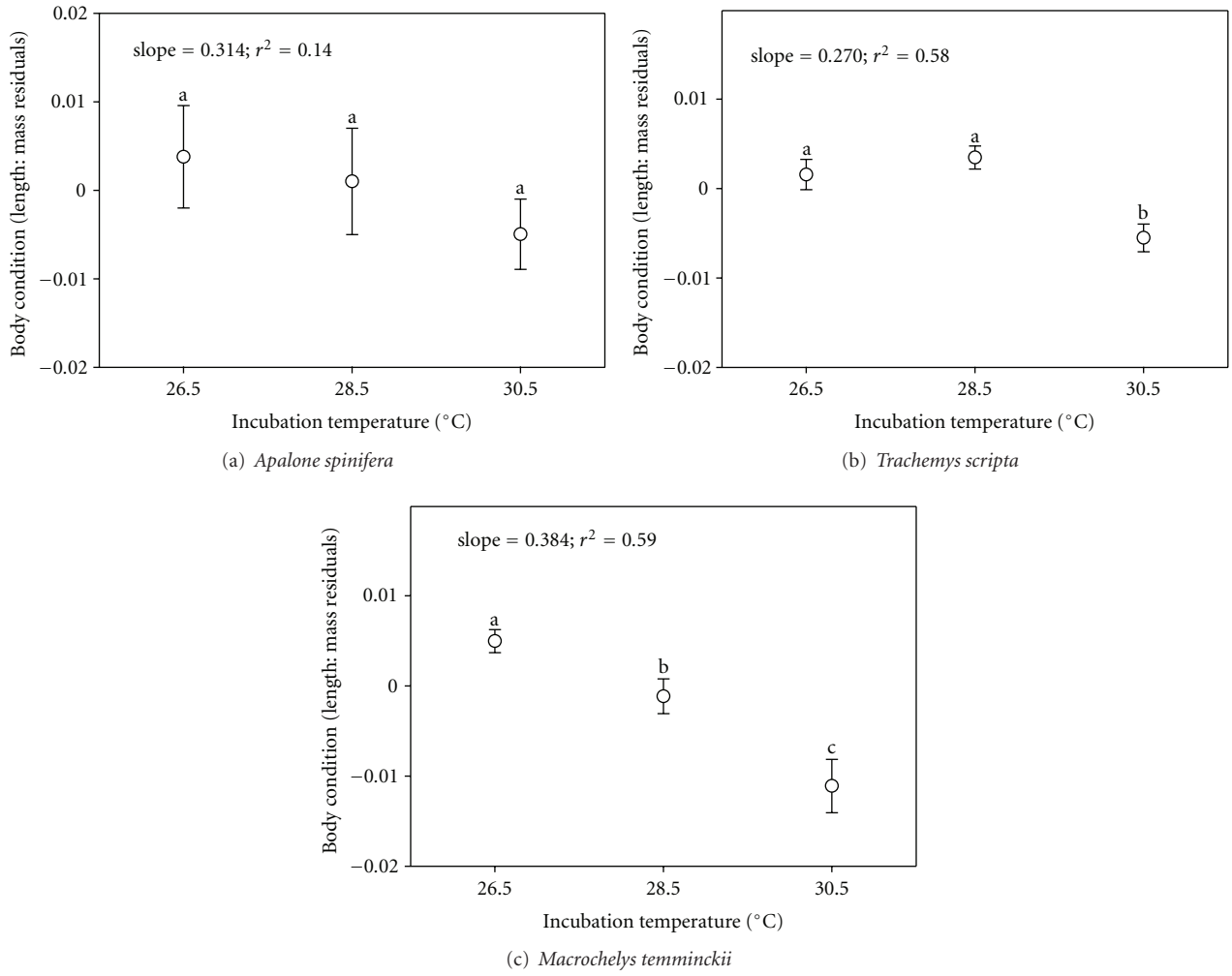
Figure 6: Differences in hatchling mass corrected for differences in initial egg mass. Values were calculated from L-S regression residuals from a log-log plot of hatchling mass against initial egg mass. Lower case letters indicate treatment differences ($P < 0.05$). Error bars = ±1 SE.

mass, than hatchlings from lower temperatures. Thus, we conclude that high temperatures negatively affected embryo growth, but that those smaller hatchlings may have emerged with large quantities of unmetabolized yolk, thus accounting for the lack of large differences in mass. The combination of these factors suggests that less metabolically active tissue was present in high incubation temperature hatchlings, resulting in lower VO_2 even in the absence of metabolic compensation.

The effects of high incubation temperature on VO_2 and O_{2total} in *T. scripta* and *A. spinifera* were similar to those in *M. temminckii*. At 30.5°C, VO_{2max} was lower than at 28.5°C (though not significantly so among *T. scripta*) and hatchlings at 30.5°C exhibited lower O_{2total} than conspecifics at lower temperatures. Whereas *M. temminckii* hatchlings from 30.5°C incubation temperature were slightly lighter than from lower temperatures, no such negative mass-incubation temperature relationship was evident in the two other species, and *T. scripta* from the highest temperature were actually slightly heavier. However, BCIs were lowest at 30.5°C in all three species (though not significantly so in *A. spinifera*), suggesting that, as appears likely in *M. temminckii*, body length was smaller and the proportion of mass composed of metabolically inactive yolk was higher among hatchlings that developed at high temperatures. Evidence supports such a negative correlation between quantity of residual yolk and the length-to-mass relationship in another turtle, *Chelonia mydas*, in which posthatching yolk mass and hatchling yolk-free mass were measured [40].

These results suggest, albeit indirectly, that incubation temperature produced differences in yolk-free mass. This conclusion is consistent with a number of turtle and squamate studies that have shown a negative correlation between incubation temperature and yolk-free tissue mass [37, 40, 48, 54–56]. Although we cannot reject positive metabolic compensation as an additional factor contributing to the observed VO_2 patterns, there is precedence for drawing the more parsimonious conclusion that differences in yolk-free size alone produced the observed incubation temperature differences without a compensatory response to temperature acclimation during incubation [55].

The differences in the effects of temperature on hatchling size and yolk reserves suggest that the costs and benefits of developing at relatively high temperatures differ among the three species studied. It has been demonstrated in several lizard species that large hatchling size increases survival, presumably by increasing foraging efficiency and decreasing susceptibility to predation [57–64]. Studies suggest that size affects aquatic turtle hatchling survival, as well [65–69]. However, hatchling size may affect fitness in subtler ways. It is also generally assumed that large postembryonic yolk



(a) *Apalone spinifera*

(b) *Trachemys scripta*

(c) *Macrochelys temminckii*

Figure 7: Hatchling body composition indices calculated as residuals from a L-S regression from a log-log plot of body length on mass. Lower case letters indicate treatment differences ($P < 0.05$). Error bars = ±1 SE.

reserves increase fitness, either by increasing the time a hatchling can survive before eating or, similarly, increasing winter survival in species that delay emergence until spring [70, 71].

Trachemys scripta exhibited evidence of retaining more residual yolk at 30.5°C than at lower incubation temperatures. Therefore, development at high incubation temperature may confer the benefits of additional energy reserves, even at the cost of smaller body length. Because females are produced exclusively at 30.5°C [42], and female *T. scripta* attain sexual maturity at a larger size than do males [42], this difference in hatchling composition lends support to the hypothesis that temperature-dependent sex determination evolved due to asymmetrical benefits to the sexes of developing at different temperatures [9]. Similar patterns were observed in *A. spinifera*, and so the benefits of developing at high incubation temperatures are likely similar. However, males and females do not differentially benefit, as this species exhibits genetic sex determination.

As with *T. scripta* and *A. spinifera*, evidence suggests that *M. temminckii* that were incubated at high temperatures retained relatively large yolk residuals. This potential benefit to survival was offset by the fact that hatchlings were morphometrically smaller at 30.5°C. Additionally, *M. temminckii* embryo mortality was high at 30.5°C [43], whereas hatching success was unaffected by incubation temperature in the two other species. Therefore, it is unlikely that the phenotype expressed at high temperatures by *M. temminckii* hatchlings enhances fitness, but is instead the result of limitations on growth at high temperatures.

Future research investigating the ways that incubation temperature affects embryo energetics and hatchling body composition is needed to differentiate costs associated with growth and maintenance during development and to assess the relative benefits to hatchlings of maximizing body size versus retaining yolk for sustaining posthatching metabolism. A large body of literature exists on the effects of incubation temperature on hatchling turtle size and growth [11–15], but more detailed analyses of temperature's effect on the proportions of tissue and yolk that comprise hatchling mass will lend greater insight into the role incubation temperature plays in affecting survival and, ultimately, fitness.

Acknowledgments

The authors thank Sequoyah National Wildlife Refuge and Tishomingo National Fish Hatchery for providing turtle eggs for this research. S. Watkins, P. Widder, and E. Ligon assisted with measuring metabolic rates. The paper benefited greatly from comments by J. Bidwell, S. Fox, and M. Payton. Financial support was provided by the Oklahoma State University Environmental Institute, Association for the Study of Ichthyology and Herpetology, Chicago Herpetological Society, Chelonian Research Foundation, Society of Integrative and Comparative Biology, and Sigma Xi. Experiments reported in this study comply with the current United States laws on animal experimentation.

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