

Thermal adaptation of maternal and embryonic phenotypes in a geographically widespread ectotherm

Michael J. Angilletta Jr.^{a,*}, Christopher E. Oufiero^a,
Michael W. Sears^b

^a*Department of Life Sciences, Indiana State University, Terre Haute, IN 47809, USA*

^b*Department of Biology, University of Nevada, Reno, NV 89557, USA*

Abstract. Current theories predict the thermal adaptation of both maternal and embryonic phenotypes such that the fitness of the entire life cycle is maximized. Our studies of the eastern fence lizard (*Sceloporus undulatus*) have generated evidence that maternal and embryonic phenotypes are designed to promote growth and development in cold environments. Females in colder environments allocate more energy per egg enabling offspring to grow faster and reach a larger size at hatching. Females in cold environments also nest exclusively in warm, open sites that maximize rates of embryonic growth and development, although this behavior involves risky migrations. Likewise, thermal adaptation of embryonic physiology also promotes growth and development in cold environments. When incubated in the laboratory under shared environmental conditions, embryos from colder environments developed faster and grew more efficiently than embryos from warmer environments, which is a pattern called counter-gradient variation. Because thermal adaptation can produce geographic variation in a suite of maternal and embryonic phenotypes, biologists should develop theories of coadaptation that consider costs and benefits of behavioral and physiological strategies at both stages of the life cycle. © 2004 Elsevier B.V. All rights reserved.

Keywords: Counter-gradient variation; Egg size; Embryo; Growth; Nesting; Temperature

1. Introduction

Spatial and temporal variation in environmental temperature is a challenge for all organisms. Growth and development are very sensitive to temperature such that extreme

* Corresponding author. Tel.: +1 812 237 4520; fax: +1 812 237 4480.

E-mail address: m-angilletta@indstate.edu (M.J. Angilletta).

temperatures have significant consequences for the life histories of ectotherms [1,2]. The evolution of behavior, physiology, morphology and life history has enabled many species to span large portions of our planet despite the thermal gradients they encounter. Such adaptations might be required at all ontogenetic stages if a widespread species is to complete its life cycle in environments that differ not only in temperature but also in many other respects.

Thermal adaptation produces strategies that speed growth and development in cold environments, including modifications of maternal and embryonic phenotypes (Fig. 1). Mothers can speed the growth of their offspring by provisioning eggs with additional nutrients [3]. Viviparous females can thermoregulate during pregnancy [4,5] and oviparous females can construct nests that provide embryos with relatively warm conditions [6–8]. Finally, offspring can make more efficient use of resources by improving assimilation and reducing energy expenditure [9]. Here we consider the life-historical, behavioral, and physiological strategies available to ectotherms, and present evidence from our studies of lizards that these strategies facilitate growth and development in cold environments.

2. *Sceloporus undulatus*: a model for studies of thermal adaptation

The *Sceloporus undulatus* species group—a monophyletic group of phrynosomatids that includes *S. undulatus*, *S. belli*, *S. caudatus*, *S. exul* and *S. woodi* [10]—is ideal for studies of thermal adaptation. This group is widely distributed in the United States and northern Mexico, where it occupies a diversity of habitats including forests, prairies and canyons. Consequently, *S. undulatus* encounters a broad range of environmental

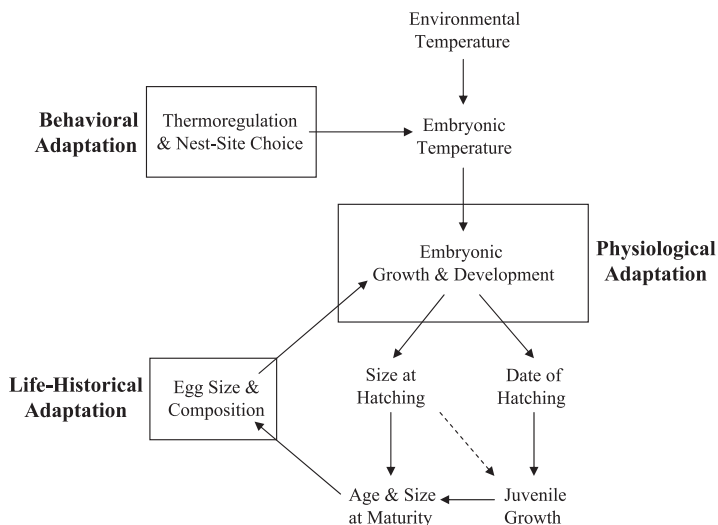


Fig. 1. A conceptual model relating life history, maternal behavior and embryonic physiology. Development and growth are enhanced by the thermal adaptation of these phenotypes.

temperatures and exhibits considerable variation in life history [11,12]. Phylogeographic evidence suggests *S. undulatus* expanded its range latitudinally at least four times [10], making it a good subject for comparative studies. Such studies have begun to shed light on the mechanisms by which this species has responded to thermal challenges.

3. Thermal adaptation of maternal and embryonic phenotypes

3.1. Life-historical adaptation: reproductive allocation across space and time

Reproductive allocation by females is an obvious mechanism by which the growth of offspring is promoted. A larger allocation of resources results in a larger hatchling [3] or a greater potential for growth after hatching [13]. Because low temperatures retard the growth of offspring, optimization theories predict females should allocate a greater quantity of energy per offspring in colder environments [14,15]. In seasonal environments, the same argument can be applied to temporal variation in temperature. Offspring produced later in the season will experience a shorter duration before environmental temperatures drop and brumation ensues; therefore, females should produce larger eggs later in the reproductive season [16].

We have investigated both spatial and temporal variation in the reproductive allocation of *S. undulatus*. Egg size varies considerably within each of the four major

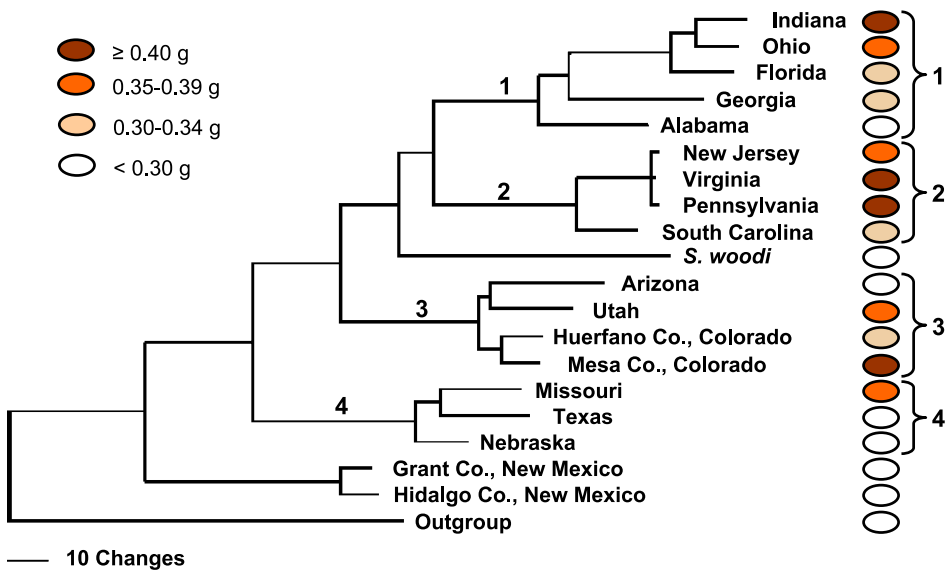


Fig. 2. In *S. undulatus*, egg mass varies considerably within four clades that span latitudinal thermal gradients. Egg masses from Niewiarowski et al. [11] and Oufiero et al. (unpublished) are mapped onto a cladogram from Angilletta et al. [12]. The phylogenetic signal (K), computed using the randomization procedure of Blomberg et al. [17], was only 0.1 (Niewiarowski et al., unpublished); a phylogenetic signal of 1.0 is expected for a trait that has evolved according to a model of Brownian motion.

clades of *S. undulatus* (Fig. 2), enabling powerful comparative studies of thermal adaptation. As predicted by theory, females in colder environments tend to produce larger eggs (Fig. 3). Lizards in Nebraska are a major exception to this trend because they produce relatively small eggs despite a very low environmental temperature. This exception is understandable considering females in Nebraska suffer unusually high rates of mortality as juveniles and thus mature early at an extremely small body size. The small body size of females in Nebraska probably constrains the size of eggs that can be produced [18].

Conversely, temporal variation in egg size provides mixed support for theories of reproductive allocation. Angilletta et al. [16] observed seasonal variation in egg mass that was inconsistent with the prediction of optimization theory. Females in New Jersey produce two clutches of eggs per year, and the offspring from the second clutch have about 40% less time to grow before brumation [16,19]. Theory predicts that a female should allocate more energy per egg in her second clutch than she allocated per egg in her first clutch. Yet, eggs of the second clutch were 10% smaller than those of the first clutch [16]. In contrast, *S. woodi* produced larger eggs later in the season some years, but not others [20]. One possible cause of these discrepancies is temporal variation in food availability. The clutch size of a lizard is fixed during mid-vitellogenesis, after which no additional recruitment occurs and atresia is rare [21,22]. If a female encounters a surplus of resources between mid-vitellogenesis and the point at which eggs are shelled, she could not add follicles but could increase her reproductive output by allocating additional energy to each of her developing follicles. Indeed, females of *S. undulatus* that consumed more food during vitellogenesis produced larger eggs (Pringle and Angilletta, unpublished). Thus, temporal variation in resources can be a confounding factor that produces counterintuitive patterns of reproductive allocation.

3.2. Behavioral adaptation: selection of nesting sites

The embryonic growth and development of *S. undulatus* can be greatly enhanced by nesting behavior. When incubated at constant temperatures, embryos hatch successfully in

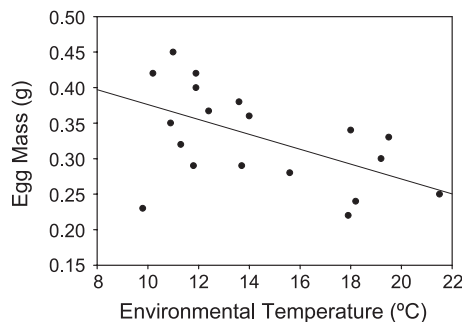


Fig. 3. Among 19 populations of *S. undulatus*, females in colder environments tend to produce larger eggs (robust regression: $F_{1,15,7}=6.90$, $P=0.02$, $r^2=0.30$). Egg sizes for 15 populations are from Ref. [11] and those for four populations are from Oufiero et al. (unpublished). Environmental temperatures are from Ref. [12].

the range of 23–34 °C [23–25]. Relatively high temperatures reduce the duration of incubation without affecting the body size at hatching [24,25], but survival is poor if the temperature is too high [23,25]. Angilletta et al. [25] combined data from the laboratory and the field to define sites that offered the most favorable thermal conditions for offspring. Specifically, they predicted that females in New Jersey—a locality at the northern limit of *S. undulatus*'s range—should nest in unshaded soil at a depth of ≥ 4 cm. Their reasoning was simple. Shaded soil, regardless of depth, offers relatively low temperatures that retard growth and development. By nesting at warmer sites, females could ensure that their offspring hatched earlier and had longer access to resources after hatching, potentially resulting in a higher survivorship, earlier reproduction, or a larger size at maturity [26]. Still, females that nest in unshaded soil must place their eggs sufficiently deep to prevent them from reaching lethally high temperatures.

In both natural and artificial environments, the nesting behavior of *S. undulatus* accorded extremely well with our expectation. Angilletta et al. (unpublished) used radiotelemetry to observe the nesting behavior of lizards in New Jersey. Nests were constructed at sites that were less shaded than randomly selected sights (canopy coverage of nests and random sites were $49 \pm 5.4\%$ and $79 \pm 3\%$, respectively). Moreover, eggs were placed at mean minimal and maximal depths of 4.7 and 6.4 cm, respectively. Nests provided mean temperatures between 28 and 32 °C for approximately 8 h per day, whereas the mean temperature at random sites was below 25 °C at all times of the day (Fig. 4). Although eggs of *S. undulatus* can hatch successfully at temperatures below 25 °C [24], incubation would be greatly extended, possibly having major consequences for the fitness of offspring. These observations correspond remarkably with those of Warner and Andrews [27], who observed the nesting behavior of *S. undulatus* in an artificial thermal gradient; females in their gradient selected mean temperatures between 23.8 and 28.2 °C, and females in New Jersey selected mean temperatures between 23.2 and 29.1 °C. Collectively, these observations are strong evidence that the nesting behavior of *S. undulatus* provides offspring with thermal conditions that speed embryonic growth and development.

Although the benefits of nesting behavior are clear, the potential costs have not been documented. A major cost of nesting could be a greater exposure of gravid females to

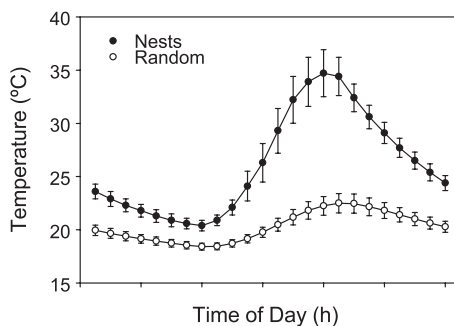


Fig. 4. At all times of the day, nests of *S. undulatus* were much warmer than randomly selected locations. Error bars are 95% confidence intervals. Data are from Angilletta et al. (unpublished).

predators. Nesting involved rapid migrations between habitats, which appeared risky for females. Just prior to nesting, females in New Jersey made sudden shifts from the forest to open terrain. These excursions appeared to be solely related to nesting because females returned to the forest immediately after nesting. On average, only 1% (range=0–8%, $n=19$ females) of the area used by a female during nesting was contained within her home range (i.e., the area used before and after nesting). Similar shifts in habitat have been observed for gravid fence lizards in Virginia (Andrews, pers. comm.). Gravid females probably experience a higher risk of mortality because of poor locomotor capacity [28], and migrations associated with nesting could further amplify this risk. Nesting behavior is possibly characterized by a considerable degree of philopatry because females from various regions of the forest migrated directly and rapidly to a rather restricted nesting area. If females were homing in on a known location [29], the risk of migration might have been less than if the nesting area was completely unfamiliar.

3.3. Physiological adaptation: embryonic growth and development

In many species, individuals from colder environments exhibit faster intrinsic rates of growth and development, which tend to counteract the effects of environmental temperature on these physiological processes [2,30]. Rapid growth can be achieved by higher rates of consumption, higher efficiencies of production, or both [31]. Embryos cannot use both of these strategies because their mother supplies them with nutrients and this supply cannot be supplemented through feeding. Still, the growth efficiency of embryos can be increased through modifications of metabolic functions that lead to greater assimilation and less expenditure of available energy [32].

Common garden experiments have revealed thermal adaptation of embryonic physiology in *S. undulatus*. The existence of multiple clades distributed along a latitudinal gradient enabled Oufiero and Angilletta (unpublished) to investigate parallel evolution of growth and development. They incubated eggs from populations belonging to two clades separated by the Appalachian Mountains (clades 1 and 2 in Fig. 2). Incubation temperatures were chosen to match thermal cycles of natural nests (see Section 3.2). In both clades, embryos from colder environments grew more efficiently and hatched earlier when incubated in the laboratory at realistic thermal cycles (Fig. 5). More efficient growth enabled embryos to reach a larger size before hatching, and a shorter incubation period provided more time for growth after hatching. Thus, embryos of *S. undulatus* exhibited counter-gradient variation in growth and development similar to that observed in juveniles of other ectothermic species [31]. The parallel evolution of counter-gradient variation in *S. undulatus* indicates strong selection of body size in cold environments.

Why don't lizards in all environments grow as efficiently and develop as rapidly as possible? We presume that rapid embryonic growth and development impose some cost to the embryo that arises from one or more tradeoffs [31,33]. Rapid growth might come at the expense of cellular maintenance, including protein turnover, ion transport and other ATP-consuming functions [34]. Since development is thought to occur at the expense of growth [35], the faster growth and development of embryos from colder environments must impose a complex set of tradeoffs, which presumably results in a lower survivorship of embryos or hatchlings. The consequences of these tradeoffs for fitness can be tested

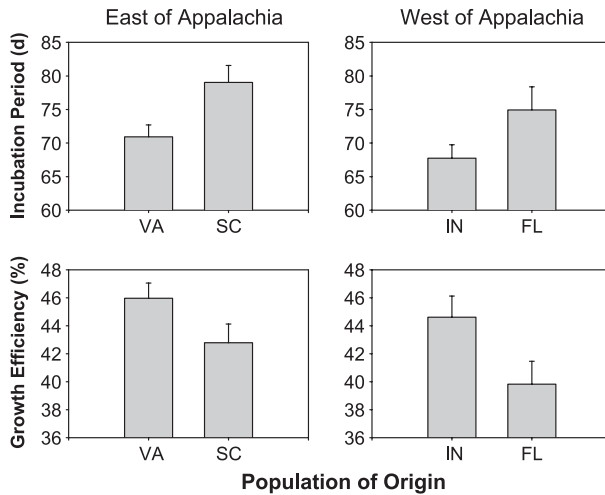


Fig. 5. Embryos of *S. undulatus* exhibited counter-gradient variation in growth and development. Top panels: embryos from colder environments (Virginia and Indiana) had shorter incubation periods than those from warmer environments (South Carolina and Florida). Bottom panels: embryos from colder environments grew more efficiently than those from warmer environments. Error bars are 95% confidence intervals. Data are from Oufiero and Angilletta (unpublished).

through reciprocal transplants of offspring. In cold environments, residents should have higher survivorship than lizards transplanted from warmer environments. However, lizards transplanted from cold environments to warmer environments should have lower survivorship than residents. Importantly, experimental reduction of yolk can be used to equalize the energy available to embryos from different populations, enabling one to tease apart the contributions of reproductive allocation and embryonic physiology to the success of offspring.

4. Conclusion

Using *S. undulatus* as a model, we have shown that ectotherms use a suite of strategies to enhance growth and development in cold environments. These strategies are manifested at both embryonic and adult stages of the life cycle. Because the survivorship and fecundity of a genotype is determined by its performance at all stages, fitness is a multivariate function of embryonic and maternal phenotypes [36,37]. Natural selection should produce a combination of strategies that maximizes fitness given genetic, developmental and functional constraints [38]. Because thermal adaptations impose tradeoffs, particular suites of strategies should arise in particular environments [31]. Although our work has highlighted potential responses to a thermal challenge, we should like to know why certain strategies evolve in a given environment and why others do not. Undoubtedly, we shall only achieve this goal through detailed studies of the costs and benefits of life-historical, behavioral and physiological strategies.

Acknowledgments

We thank the Indiana Academy of Sciences for supporting our research, and Indiana State University for an International Travel Grant, which enabled the senior author to participate in this symposium.

References

- [1] R.B. Huey, R.D. Stevenson, Integrating thermal physiology and ecology of ectotherms: a discussion of approaches, *Am. Zool.* 19 (1979) 357–366.
- [2] M.J. Angilletta, P.H. Niewiarowski, C.A. Navas, The evolution of thermal physiology in ectotherms, *J. Therm. Biol.* 27 (2002) 249–268.
- [3] B. Sinervo, The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance, *Evolution* 44 (1990) 279–294.
- [4] J. Rock, A. Cree, R.M. Andrews, The effect of reproductive condition on thermoregulation in a viviparous gecko from a cool climate, *J. Therm. Biol.* 27 (1) (2000) 17–27.
- [5] M.B. Charland, Thermal consequences of reptilian viviparity: thermoregulation in gravid and nongravid garter snakes (*Thamnophis*), *J. Herpetol.* 29 (3) (1995) 383–390.
- [6] R. Shine, P.S. Harlow, Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard, *Ecology* 77 (6) (1996) 1808–1817.
- [7] T. Madsen, R. Shine, Life history consequences of nest-site variation in tropical pythons (*Liasis fuscus*), *Ecology* 80 (3) (1999) 989–997.
- [8] R. Shine, Some like it hot: effects of forest clearing on nest temperatures of montane reptiles, *Ecology* 83 (10) (2002) 2808–2815.
- [9] S.J.W. Robinson, L. Partridge, Temperature and clinal variation in larval growth efficiency in *Drosophila melanogaster*, *J. Evol. Biol.* 14 (2001) 14–21.
- [10] A.D. Leaché, T.W. Reeder, Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood and Bayesian approaches, *Syst. Biol.* 51 (2002) 44–68 [2001].
- [11] P.H. Niewiarowski, M.J. Angilletta, A. Leaché, Phylogenetic comparative analysis of life history variation among populations of the lizard *Sceloporus undulatus*: an example and prognosis, *Evolution* 58 (2004) 619–633.
- [12] M.J. Angilletta, et al., Bergmann's clines in ectotherms: illustrating a life-historical perspective with sceloporine lizards, *Am. Nat.* 164 (2004) (in press).
- [13] J.D. Congdon, Proximate and evolutionary constraints on energy relations of reptiles, *Physiol. Zool.* 62 (1989) 356–373.
- [14] N. Perrin, Why are offspring born larger when it is colder? Phenotypic plasticity for offspring size in the cladoceran *Simocephalus vetulus* (Müller), *Funct. Ecol.* 2 (1988) 283–288.
- [15] L.Y. Yampolsky, S.M. Scheiner, Why larger offspring at lower temperatures? A demographic approach, *Am. Nat.* 147 (1996) 86–100.
- [16] M.J. Angilletta, M.W. Sears, R.S. Winters, Seasonal variation in reproductive effort and its consequences for offspring size in the lizard *Sceloporus undulatus*, *Herpetologica* 57 (2000) 365–375.
- [17] S.P. Blomberg, T. Garland Jr., A.R. Ives, Testing for phylogenetic signal in comparative data: behavioral traits are more labile, *Evolution* 57 (2003) 717–745.
- [18] J.D. Congdon, J.W. Gibbons, Morphological constraint on egg size: a challenge to optimal egg size theory? *Proc. Natl. Acad. Sci.* 84 (1987) 4145–4147.
- [19] P.H. Niewiarowski, Understanding geographic life history variation in lizards, in: L.J. Vitt, E.R. Pianka (Eds.), *Lizard Ecology: Historical and Experimental Perspectives*, Princeton University Press, Princeton, 1994, pp. 31–49.
- [20] V.G. DeMarco, Annual variation in the seasonal shift in egg size and clutch size in *Sceloporus woodi*, *Oecologia* 80 (1989) 525–532.
- [21] B.A. Shanbhag, B.S.K. Prasad, Follicular dynamics and germinal bed activity during the annual ovarian cycle of the lizard *Calotes versicolor*, *J. Morphol.* 216 (1993) 1–7.

- [22] B. Sinervo, P. Licht, Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards, *Science* 252 (1991) 1300–1302.
- [23] O.J. Sexton, K.R. Marion, Duration of incubation of *Sceloporus undulatus* eggs at constant temperature, *Physiol. Zool.* 47 (1974) 91–98.
- [24] R.M. Andrews, T. Mathies, D.A. Warner, Effect of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*, *Herpetol. Monogr.* 14 (2000) 420–431.
- [25] M.J. Angilletta, R.S. Winters, A.E. Dunham, Thermal effects on the energetics of lizard embryos: implications for hatchling phenotypes, *Ecology* 81 (2000) 2957–2968.
- [26] F.J. Qualls, R. Shine, Post-hatching environment contributes greatly to phenotypic variation between two populations of the Australian garden skink, *Lampropholis guichenoti*, *Biol. J. Linn. Soc.* 71 (2000) 315–341.
- [27] D.A. Warner, R.M. Andrews, Nest-site selection in relation to temperature and moisture by the lizard *Sceloporus undulatus*, *Herpetologica* 58 (2002) 399–407.
- [28] D.B. Miles, B. Sinervo, W.A. Frankino, Reproductive burden, locomotor performance, and the cost of reproduction in free ranging lizards, *Evolution* 54 (2000) 1386–1395.
- [29] E.W. Hein, S.J. Whitaker, Homing in eastern fence lizards (*Sceloporus undulatus*) following short-distance translocation, *Great Basin Nat.* 57 (1997) 348–351.
- [30] D.O. Conover, E.T. Schultz, Phenotypic similarity and the evolutionary significance of countergradient variation, *Trends Ecol. Evol.* 10 (1995) 248–252.
- [31] M.J. Angilletta, et al., Tradeoffs and the evolution of thermal reaction norms, *Trends Ecol. Evol.* 18 (2003) 234–240.
- [32] B.L. Bayne, Phenotypic flexibility and physiological tradeoffs in the feeding and growth of marine bivalve molluscs, *Integr. Comp. Biol.* 44 (2004)2001 (in press).
- [33] K. Gotthard, Growth strategies of ectothermic animals in temperate environments, in: D. Atkinson, M. Thorndyke (Eds.), *Environment and Animal Development: Genes, Life Histories, and Plasticity*, BIOS Scientific Publishers, Oxford, 2001, pp. 287–303.
- [34] W. Wieser, Cost of growth in cells and organisms: general rules and comparative aspects, *Biol. Rev.* 68 (1994) 1–33.
- [35] J.D. Arendt, Allocation of cells to proliferation vs. differentiation and its consequences for growth and development, *J. Exp. Zool.* 288 (2002) 219–234.
- [36] C.A. Beuchat, S. Ellner, A quantitative test of life history theory: thermoregulation by a viviparous lizard, *Ecol. Monogr.* 57 (1987) 45–60.
- [37] S.J. Arnold, C.R. Peterson, A model of optimal reaction norms: the case of the pregnant garter snake and her temperature-sensitive embryos, *Am. Nat.* 160 (2002) 306–316.
- [38] S.J. Arnold, Constraints on phenotypic evolution, *Am. Nat.* 140 (1992) S85–S107.