Comparative Biochemistry and Physiology, Part A 154 (2009) 191–196

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Energetics of metamorphic climax in the pickerel frog (*Lithobates palustris*)

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ARTICLE INFO

Article history:
Received 13 December 2008
Received in revised form 29 May 2009
Accepted 1 June 2009
Available online 7 June 2009

Keywords:
Amphibian
Energetics
Metamorphosis
Respiration
Tadpole
Oxygen consumption

ABSTRACT

Anuran metamorphosis, the transition from aquatic larvae to terrestrial juveniles, is accompanied by significant morphological, physiological, and behavioral changes. Timing of metamorphosis and final size, which can influence adult fitness, may depend on sufficient energy accumulated during the larval period to support metamorphosis. However, only two species of anurans have been examined for energetic costs of metamorphosis, *Rana tigrina* and *Anaxyrus terrestris*. Based on these species, it has been hypothesized that differences in energy expenditure are related to duration of metamorphosis. To compare energetic costs of metamorphosis among species and examine this hypothesis, we quantified the total energy required for metamorphosis of *Lithobates palustris* tadpoles by measuring oxygen consumption rates over the duration of metamorphic climax using closed-circuit respirometry. Total energy costs for *L. palustris* were positively related to tadpole mass and duration of metamorphic climax. However, larger tadpoles completed metamorphosis more efficiently because they used proportionally less total energy for metamorphic climax than smaller counterparts. Costs were intermediate to *R. tigrina*, a larger species with similar metamorphic duration, and *A. terrestris*, a smaller species with shorter metamorphic climax. The results supported the hypothesis that amphibian species with more slowly developing tadpoles, such as ranids, require more absolute energy for metamorphosis in comparison to more rapidly developing species like bufonids.

1. Introduction

In anurans with complex lifecycles, metamorphosis marks an important transition from life in the aquatic larval environment to the terrestrial adult environment. The ecological aspects of amphibian metamorphosis have been studied in great detail (Wilbur and Collins, 1973; Travis, 1984; Werner, 1986; Alford and Harris, 1988). Length of the larval period and size at the completion of metamorphosis have important consequences for amphibian survival, growth, and ultimately fitness (Smith, 1987). These fitness-related traits are influenced by larval growth rate and growth history (Travis, 1984; Alford and Harris, 1988), which affect the amount of energy accumulated to support the significant changes associated with metamorphosis and the post-metamorphosis juvenile period (Crump, 1981; Scott et al., 2007).

Metamorphic climax is the period in anuran metamorphosis that includes a dramatic transformation of tadpole organs into their adult form, resorbtion of tadpole-specific structures, such as the tail, and completed development of adult-specific tissues such as limbs (Shi, 2000; Duellman and Trueb, 1986). Degeneration of larval mouthparts and development of adult jaws prevent feeding, which necessitates the use of energy accumulated during the larval period (Duellman and Trueb, 1986; Beck and Congdon, 2003). During larval development most variations in basal energy expenditure can be attributed to changes in mass rather than development, but at metamorphic climax considerable energy is allocated towards developmental costs (Funkhouser and Mills, 1969; Funkhouser and Foster, 1970; Feder, 1982; Crowder et al., 1998). However, total energetic costs associated with the morphological and physiological changes during metamorphic climax have been determined for only *Rana tigrina* (Pandian and Marian, 1985) and *Anaxyrus terrestris* (formerly *Bufo terrestris*, Beck and Congdon, 2003), which varied substantially in their body size, total energy requirements, and duration of metamorphic climax. Because only two species have been studied it remains unclear what physiological or life history traits contribute to differences in metamorphic energetics among species. Based on these two species it has been hypothesized that total energy expended during metamorphosis will be significantly greater for species with slowly developing tadpoles like ranids compared to tadpoles that undergo metamorphosis more rapidly like bufonids.

The purpose of this study was to quantify the energetic costs associated with metamorphosis in the pickerel frog, *Lithobates palustris*, (formally *Rana palustris*) to compare energetic costs among previously studied species including *R. tigrina*, a species with similar size and metamorphic duration, and *A. terrestris* a smaller, more rapidly metamorphosing species. To determine energetic costs of metamorphosis we measured oxygen consumption rates of individual tadpoles during the entire period of metamorphic climax. In contrast to earlier studies on *R. tigrina*, which determined energy costs of metamorphosis

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doi:10.1016/j.cbpa.2009.06.001

using bomb calorimetry (Pandian and Marian, 1985), we used closed-circuit respirometry. This technique was previously used with *A. terrestris* (Beck and Congdon, 2003). Our main objectives were to determine the total energy consumed during metamorphosis, to estimate the proportion of energy allocated to development compared to maintenance, and to describe the relationships between energy use, tadpole body mass, and the duration of metamorphic climax.

2. Materials and methods

2.1. Animals

On 25 March 2007 ten freshly laid *L. palustris* egg masses were collected from a pond in Arcadia, VA (Botetourt County), USA, and transported to the laboratory. Each egg mass was temporarily stored in shallow pond water at 8 °C. Pond water was gradually replaced with a 3:1 mix of dechloraminated tap water and well water.

Ten fertilized eggs with intact jelly coats were separated from each clutch and mixed to produce a 100 egg composite. This procedure was repeated ten times to produce ten composites with similar genetic variation. The egg composites were maintained in 2 L glass beakers. After hatching on 05 April 2007, surviving tadpoles were examined for malformations. Ten groups of 66 morphologically normal tadpoles were then transferred to ten outdoor mesocosms (1500 L stock tanks) where they were maintained until they reached developmental stages (Gosner, 1960) suitable for experiments. Twenty animals were drawn from these mesocosms for the respirometry experiments described below, but the majority of the animals were used for other research purposes (Orlofske and Hopkins, *unpublished data*). The twenty animals used in this study were divided into 12 tadpoles for larval energetics measurements and 8 tadpoles for energetics measurements during metamorphic climax.

2.2. Estimates of standard metabolic rate

Mesocosms were searched daily during which time twelve tadpoles (stages 39–40; Gosner, 1960) with well-developed hind limbs were collected for estimation of standard metabolic rate (SMR). Metabolic rates were quantified from oxygen consumption rates (O_2 mL/h) measured on a computer controlled, indirect, closed-circuit respirometer (Micro-Oxymax, Columbus Instruments, Columbus, OH, USA) using techniques similar to those used on eastern mosquitofish (*Gambusia holbrooki*; Hopkins et al., 2003), southern toad tadpoles (*A. terrestris*; Beck and Congdon, 2003), and bullfrog tadpoles (*L. catesbeiana*; Rowe et al., 1998).

Tadpoles were fasted for 48 h prior to respirometry to reduce metabolic contributions from digestion (Crowder et al., 1998). Tadpoles were blotted to remove excess moisture and wet mass recorded to the nearest ±0.1 mg. Respirometry chambers consisted of 100 mL sealed glass culture bottles filled with 80 mL well oxygenated, dechloraminated tap water. One standard control containing a medical battery (Duracell Procell Zinc Air Medical, DA 146 8.4 Volts) that consumed a known amount of O_2, and one chamber filled only with water serving as a blank were used to verify the proper instrument function. The instrument was calibrated using a certified air mixture prior to each trial. Micro Oxymax software automatically measured chamber volumes, while chamber leaks were measured manually. Prior to measuring O_2 consumption rates (mL/h), every air sample was dried using a hygroscopic drier containing nafion tubing (Columbus Instruments, Columbus, OH, USA). Chambers were placed in an environmental cabinet maintained at 25 °C during the 24 h trial. Software was programmed to measure oxygen consumption within the chambers every 66 min and corrected for standard temperature and pressure. Air within the headspace of the chambers was completely refreshed every 2.5 h to maintain normoxic conditions. The influence of natural circadian rhythms on tadpole respiration was standardized by starting each trial at approximately the same time each day (1000–1200 h; Roe et al., 2004).

Prior to estimating SMR, O_2 consumption rates (mL/h) of each tadpole were plotted by time and examined for activity peaks, which bias SMR estimates. Based on visual inspection of these plots, the lowest quartile value was used to estimate SMR for each individual (Hopkins et al., 2004). Mass and SMR were $\log_e$ transformed prior to analysis to meet assumptions of normality. The relationship between SMR and mass (g) was described using a simple linear regression (JMP 7.0, SAS Institute, Cary, NC USA). The relationship was approximated using the allometric equation: $\ln(\mathrm{SMR}) = a + b\ \ln(m)$, where SMR is the rate of oxygen consumption in mL/h, m is mass (g) and a and b are coefficients determined from the regression analysis.

2.3. Energetics during metamorphic climax

Tadpoles that reached the onset of metamorphic climax at stage 42 (Gosner, 1960) were identified by the emergence of at least one front limb. Eight metamorphosing tadpoles were collected from mesocosms and transported to the lab for respirometry using the same equipment and software parameters described above. Unlike the previous SMR measurements, 24 h respirometry trials were repeated until the completion of metamorphosis when the tail was fully resorbed at stage 46 (Gosner, 1960). Prior to O_2 consumption (mL/h) measurements, tadpoles were blotted to remove excess moisture and wet mass recorded to the nearest ±0.1 mg. During metamorphosis tadpoles cease feeding while the mouthparts and digestive tract undergo substantial remodeling (Duellman and Trueb, 1986), which eliminated the need to fast metamorphosing individuals before O_2 (mL/h) measurements. Respirometry chambers consisted of 100 mL sealed glass culture bottles filled with 6 mL well aerated, dechloraminated tap water. In addition, a 3.8 cm × 3.8 cm piece of fiberglass window screen was placed against the side of the chamber forming a ramp on which the tadpole could emerge to facilitate air breathing. In order to monitor the development of metamorphosing individuals and add new individuals (stage 42) as they became available, trials were terminated and restarted daily. After each 24 h respirometry trial, tadpole developmental stage and mass were recorded. Before beginning the next 24 h trial the water in each chamber was replaced. Each trial was started at approximately the same time (1000–1200 h). When tadpoles completed metamorphosis at stage 46, noted by the complete resorbtion of the tail, they were removed from the chamber, blotted to remove excess moisture and weighed to the nearest ± 0.1 mg. The duration of metamorphic climax in hours was recorded.

Oxygen consumption rates were interpolated between consecutive respiratory trials for each individual to generate a continuous resiration profile that covered the entire metamorphic period (3–6 days). The first measurement of each respirometry trial was discarded because it may have been inflated due to stress caused by handling between trials. To estimate the total O_2 consumed (mL) during the break between daily respirometry trials (3–5 h), the O_2 consumption rate (mL/h) was assumed to remain constant between consecutive trials. Total oxygen consumed ($\dot{V}O_2$ mL) during metamorphosis was calculated by multiplying the O_2 consumption rate (mL/h) by the corresponding time interval (h) and calculating the sum of O_2 (mL) for the entire respiration profile. Total oxygen consumption was converted to J using a conversion factor of 18.8 J/mL O_2 (Schmidt-Nielsen, 1990). Total energy expenditure was divided into maintenance costs and developmental costs following the procedure described in Beck and Congdon (2003). Briefly, the slope and intercept of the regression of the SMR and mass of late stage tadpoles provided the values for the constants used in an integration to calculate maintenance costs over time. The integration assumed a linear decrease in mass over the course of metamorphosis and an exponential relationship between mass and metabolic rate. Energy allocated to development was calculated by subtracting maintenance costs from total energy expenditure during metamorphic climax.

A series of simple linear regressions (JMP 7.0, SAS Institute, Cary, NC, USA) was performed to describe the significance of the relationships between mass, change in mass, duration of metamorphic climax, total energy expenditure, and energy allocated to development. To examine the relationship between variation in climax duration and metabolic rate, we performed a regression of duration of metamorphosis (h) by metabolic rate (J/h).

3. Results

3.1. Standard metabolic rate

Tadpoles ranging in mass from 1.58–3.22 g were used for estimation of SMR. The average metabolic rate of all tadpoles was 0.156 ± 0.070 mL O_2/h. Mass and SMR were positively correlated ($r^2 = 0.54$, $p = 0.007$, Fig. 1.). The equation approximating the relationship between mass and SMR followed the form: $\ln(\mathrm{SMR}) = -3.484 + 2.142\ \ln(m)$.

3.2. Energetics during metamorphic climax

All tadpoles exhibited a substantial decline in mass over the course of metamorphic climax (Table 1). The range of mass loss was quite large; the maximum percent reduction in mass was approximately twice the minimum percent reduction in mass. The change in mass and the final mass after tail resorbtion were both positively correlated with mass at initiation of metamorphic climax ($r^2 = 0.51$, $p = 0.047$; $r^2 = 0.77$, $p = 0.004$, respectively).

The duration of metamorphic climax ranged from approximately 3 to 6 days (Table 1) and did not explain a significant amount of the variation in the change in mass ($r^2 = 0.33$, $p = 0.136$). However, the lack of a significant relationship was likely the result of low statistical power (post-hoc power analysis: power = 0.301). Individual tadpoles showed irregular patterns of metabolism over the course of metamorphic climax, but patterns did not correspond to time of day (e.g., circadian rhythms) or particular developmental stages (Fig. 2A). The cumulative amount of oxygen consumed increased linearly (Fig. 2B), but the rate of increase for each individual differed contributing to the differences in the total energy required to complete metamorphosis. There was no significant relationship between the duration (h) of metamorphosis and metabolic rate (J/h) ($r^2 = 0.00$, $p = 0.95$), showing that more rapid metamorphosis was not associated with higher metabolic rates.

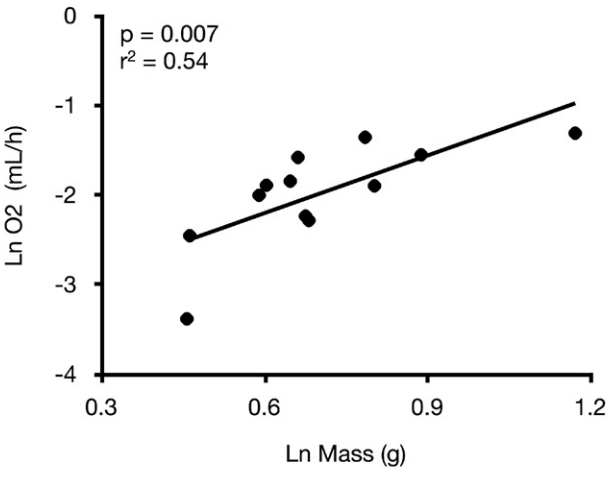

Fig. 1. Regression of late stage *Lithobates palustris* tadpoles ($n = 12$) standard metabolic rate (mL O_2/h) and mass (g).

Table 1
Descriptive statistics for changes of *L. palustris* tadpoles ($n = 8$) during metamorphic climax and the associated energy requirements.

Variable	Mean	SD	Minimum	Maximum
Change in wet mass (g)	− 1.566	0.317	− 0.943	− 1.954
% Change in wet mass	− 28.47	7.26	− 19.49	− 39.78
Duration of climax (h)	102	30.8	72.0	144.0
Metabolic rate (mL O_2/h) during climax	0.233	0.048	0.162	0.285
Total oxygen consumed (mL O_2)	22.58	7.57	10.76	30.85
Total energy used (J)	424.46	142.32	202.34	579.89
Maintenance costs (J)	224.49	113.60	46.17	359.13
Developmental costs (J)	199.97	48.87	117.08	249.19
% of energy allocated to development	50.24	14.67	35.27	77.18

There was a significant positive relationship between the duration (h) to complete metamorphosis and the total amount of energy (J) expended ($r^2 = 0.70$, $p = 0.010$, Fig. 3). The total amount of energy expended during metamorphosis was also positively related to initial body mass ($r^2 = 0.51$, $p = 0.048$, Fig. 4.).

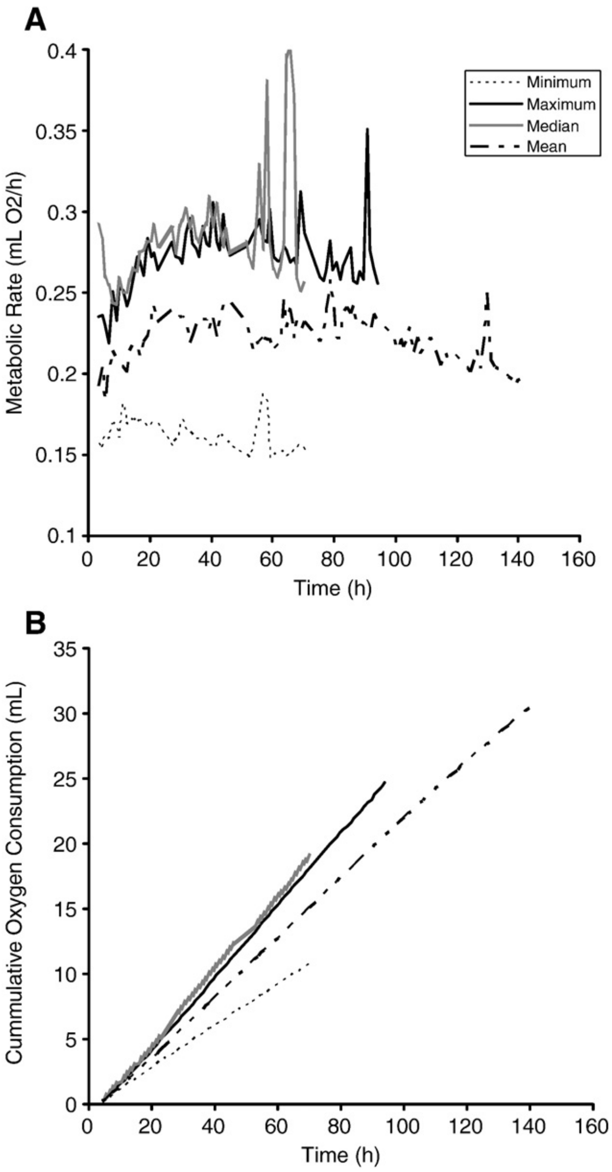

Fig. 2. Energy metabolism during metamorphic climax of pickerel frogs (*Lithobates palustris*). Metabolic rate (A) and cumulative oxygen consumption (B). Data are presented for a subset of tadpoles that represent the range of body masses of individuals used: *Minimum* 0.9431 g; *maximum* 1.9544 g; *median* 1.4733 g; *mean* 1.5887 g.

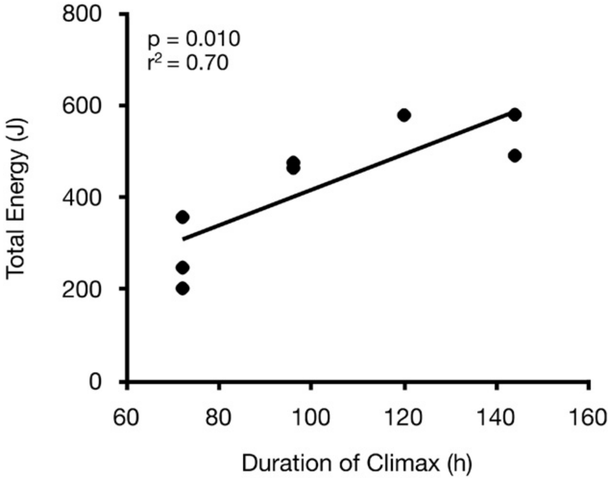

Fig. 3. Regression of the relationship between the duration of climax (h) of *Lithobates palustris* tadpoles ($n=8$) undergoing metamorphosis and the total energy (J) expended.

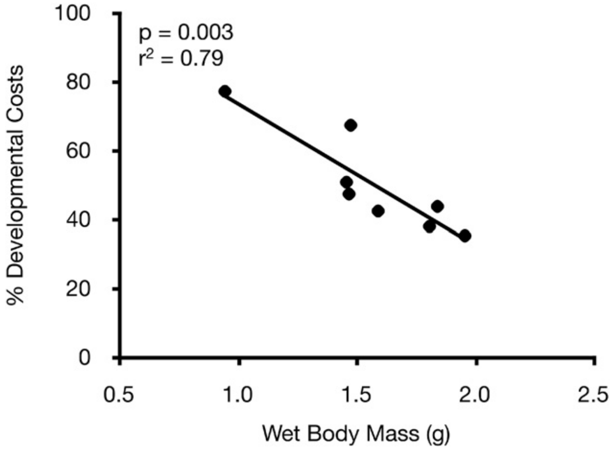

Fig. 5. Regression of initial wet body mass (g) and the percentage of total energy used during metamorphic climax that was allocated to developmental costs for *Lithobates palustris* tadpoles ($n=8$).

When total energy expended during metamorphic climax was divided into energy used to support maintenance and development costs, we found that nearly equal quantities were allocated to these two energy budget compartments (Table 1). The positive correlation between the amount of energy used for development and the duration of metamorphic climax approached statistical significance but was limited by low statistical power ($r^2=0.46$, $p=0.066$; power = 0.470). There was a highly significant negative relationship between initial mass and the percentage of total energy expended that was allocated to development ($r^2=0.79$, $p=0.003$, Fig. 5). The relationship between the percent developmental cost and the duration of metamorphic climax was not significant and also limited by low statistical power ($r^2=0.26$, $p=0.18$; power = 0.240). Energy expenditure and metabolic rates associated with metamorphic climax for *L. palustris* are compared to two other anuran species, *R. tigrina* and *A. terrestris* in Table 2.

4. Discussion

Metamorphic climax is a period of profound change in the morphology and physiology of anuran larvae (Shi, 2000) that is entirely supported by stored energy (Crump, 1981). Although this critical window of development has important implications for future fitness, the energy requirements of metamorphic climax have previously been examined for only two species. It is necessary to quantify the energy costs of metamorphosis to understand the constraints they place on tadpoles with insufficient energy reserves and to explore the variation among different anuran species.

The average and maximum total energy expenditure during metamorphosis of *L. palustris* were intermediate to *R. tigrina* and *A. terrestris* (Table 2). Most of the variation among these studies may be related to the use of tadpoles in different mass ranges. In our study, tadpoles of *L. palustris* were within the lower to middle size range of *R. tigrina* tadpoles, but were considerably larger than *A. terrestris*. Because allometric relationships among mass, climax duration, and energy expenditure were not reported in all three of these studies, we cannot make rigorous quantitative comparisons that account for differences in mass and duration of climax. However, qualitative comparisons using isometric relationships with mass and time (i.e., rate calculated as total energy expenditure divided by body mass and duration of climax [J/g/h]) reveal that the total energy expenditure for *L. palustris* was very similar to that calculated for *R. tigrina*. In contrast, mass-specific energy use was substantially higher for *A. terrestris* due primarily to their much smaller body sizes (Table 2) (Beck and Congdon, 2003).

By partitioning total energy of metamorphosis into maintenance costs and developmental costs, we determined that approximately 50% of the energy used during metamorphic climax was allocated to development. Essentially, a tadpole undergoing metamorphic climax consumes twice the amount of energy as a tadpole of the same mass at rest during the developmental stages immediately preceding forelimb emergence. For comparison, *A. terrestris*, the only other species for which data on development costs during climax are available,

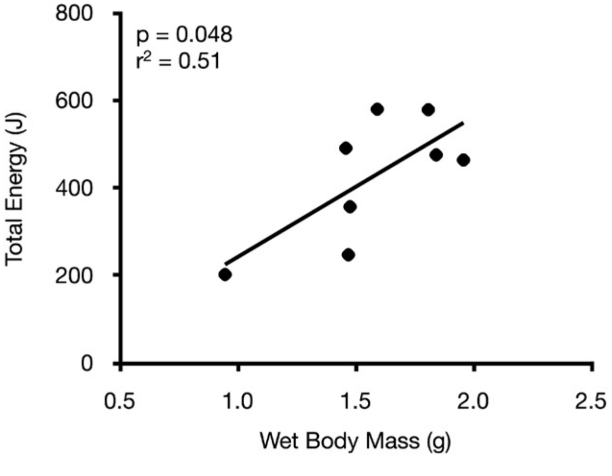

Fig. 4. Regression of the relationship between the initial wet body mass (g) of *Lithobates palustris* tadpoles ($n=8$) undergoing metamorphosis and the total energy (J) expended.

Table 2
Comparison of energy requirements associated with metamorphic climax among three anuran species.

Species	Mean mass g (range)	Mean duration of climax h (±SD)	Temperature °C	Mean total Energy used J (±SD)	Maximum total energy used J (±SD)	Isometric metabolic rate J/g/h
Lithobates palustris[a]	1.57 (0.94–1.95)	102 ± 30.8	25	424.5 ± 142.3	579.9	2.67
Rana tigrina[b]	1.8 (0.64–3.20)	144	27	–	904.0	2.63
Anaxyrus terrestris[c]	0.115 (0.094–0.256)	57	25	50.3 ± 12.5	72.2	6.76

[a] Present work.
[b] Pandian and Marian 1985.
[c] Beck and Congdon 2003.

allocated 40% of total energy expended towards development (Beck and Congdon, 2003). The metabolic rates of *L. palustris* did not vary in response to particular times or stages within metamorphic climax, which was similar to the lack of distinct pattern reported for *A. terrestris* (Beck and Congdon, 2003).

Tadpole body size was important in determining total energy expenditure and percent development costs. We found a positive relationship between body size at initiation of climax and total energy expenditure during metamorphosis, supporting similar findings of Beck and Congdon (2003) and Pandian and Marian (1985). However, larger tadpoles had a significant physiological advantage over smaller tadpoles during the metamorphic period because they completed metamorphosis more efficiently (i.e., they used proportionally less total energy for metamorphic climax) than their smaller counterparts. Beck and Congdon (2003) found similar negative relationships between percent developmental costs and size at the initiation of metamorphic climax in *A. terrestris.*

Our results provide support for the hypothesis that amphibian species with more slowly developing tadpoles, such as ranids, will require more absolute energy for metamorphosis in comparison to more rapidly developing species like bufonids (Beck and Congdon, 2003). Comparisons among existing studies show that species with long climax periods such as *L. palustris* and *R. tigrina* expend more energy during metamorphosis than species with more rapid metamorphic climax such as *A. terrestris* (Table 2). Importantly, comparisons among individuals within our study demonstrated that duration of metamorphic climax was positively related to the total energy expenditure (Fig. 3). A better understanding of the relationship between energy expenditure and duration of metamorphosis across species may address how metamorphic duration may be limited by energetic constraints (Downie et al., 2004). Such energy constraints may complement other factors, such as increased risk of predation during a period of vulnerability caused by reduced locomotor ability (Wassersug and Sperry, 1977; Arnold and Wassersug, 1978) and risk of desiccation (Downie et al., 2004), as strong selection pressures for a shorter metamorphic period. The strength of such selection pressures may vary substantially based on the life history and developmental ecology (e.g., hydroperiod of developmental environment) of amphibians, and may help explain much of the variability in climax duration observed among species.

Individual *L. palustris* tadpoles were highly variable in energy expenditure and duration of metamorphic climax. This variability was also seen in the *A. terrestris* study (Beck and Congdon, 2003), but is greater than the variability reported for *R. tigrina* (Pandian and Marian, 1985). Individual variation could be attributed to differences in larval metabolism and energy allocation during earlier development. For example, *R. tigrina* tadpoles that were larger at the onset of metamorphosis or had higher larval metabolic rates had lower mass-specific metabolic rates during metamorphic climax (Pandian and Marian, 1985). At the population level, such variability may present differential advantages to individuals that can attain a larger size before initiating metamorphosis because larger individuals reduce the percentage of their energy expenditure during climax that must be allocated to developmental costs. Better body condition of the tadpole could also allow for faster metamorphosis (Downie et al., 2004), and result in better metamorphic body condition than those with longer metamorphic climax and/or higher developmental costs (Deban and Marks, 2002). Because aspects of metamorphic body condition can influence survival during the critical transition to terrestrial life (Scott et al., 2007), variability in energy expenditure during metamorphic climax may be an important physiological factor that ultimately influences early survival.

In conclusion, our results provide an important comparison to previous studies, and provide increased support for the hypothesis that body size and duration of metamorphic climax are among the key determinants of variation in energy costs of metamorphosis among species. By examining the energetics of metamorphic climax among a greater diversity of species (e.g., hylids), it might be possible to develop generalized allometric scaling relationships that would be predictive of energy expenditures for a broad range of species based on their size and the duration of climax. Future research should investigate energetic costs of metamorphosis in anuran taxa with various larval traits such as alternative tadpole feeding morphologies in spadefoot toads (Pelobatidae), developmental periods (i.e., permanent water breeding species compared to ephemeral pond breeding species), and metamorphic duration. Temperature, larval resource availability and quality, and competition all could potentially influence intraspecific variation in energetic costs of metamorphosis, warranting further study.

Acknowledgements

This study was supported in part by startup funding from Virginia Tech and by the National Science Foundation (NSF # IOB-0615361) to WAH and an NSF Graduate Research Fellowship to SAO. The authors thank Dr. Kenneth Hannsgen for assistance with formulas and performing the integration. The authors also thank Robert C. Jadin for assistance in daily searches of the mesocosms and comments on the manuscript. This manuscript was further improved by comments from Jessica M. Orlofske, Kristine L. Grayson, Christopher W. Beck, and two anonymous reviewers. This research was conducted under 07-021-FIW approved by the Virginia Tech Institutional Animal Care and Use Committee.

References

Alford, R.A., Harris, R.N., 1988. Effects of larval growth history on anuran metamorphosis. Am. Nat. 131, 91–106.

Arnold, S.J., Wassersug, R.J., 1978. Differential predation on metamorphic anurans by garter snakes (*Thamnophis*): social behavior as a possible defense. Ecology 59, 1014–1022.

Beck, C.W., Congdon, J.D., 2003. Energetics of metamorphic climax in the southern toad (*Bufo terrestris*). Oecologia 137, 344–351.

Crowder, W.C., Nie, M., Ultsch, G.R., 1998. Oxygen uptake in bullfrog tadpoles (*Rana catesbeiana*). J. Exp. Zool. 280, 121–134.

Crump, M.L., 1981. Energy accumulation and amphibian metamorphosis. Oecologia 49, 167–169.

Deban, S.M., Marks, S.B., 2002. Metamorphosis and evolution of feeding behaviour in salamanders of the family Plethodontidae. Zool. J. Linn. Soc. 134, 375–400.

Downie, J.R., Bryce, R., Smith, J., 2004. Metamorphic duration: an under-studied variable in frog life histories. Biol. J. Linn. Soc. 83, 261–272.

Duellman, W.E., Trueb, L., 1986. Biology of Amphibians. McGraw-Hill.

Feder, M.E., 1982. Effect of developmental stage and body size on oxygen consumption anuran larvae: a reappraisal. J. Exp. Zool. 220, 33–42.

Funkhouser, A., Mills, K.S., 1969. Oxygen consumption during spontaneous amphibian metamorphosis. Physiol. Zool. 42, 15–21.

Funkhouser, A., Foster, S.A., 1970. Oxygen uptake and thyroid activity in *Hyla regilla* tadpoles. Herpetologica 26, 366–371.

Gosner, K.L., 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16, 183–190.

Hopkins, W.A., Tatara, C.P., Brant, H.A., Jagoe, C.H., 2003. Relationships between mercury body concentrations, standard metabolic rate, and body mass in eastern mosquitofish (*Gambusia holbrooki*) from three experimental populations. Environ. Toxicol. Chem. 22, 586–590.

Hopkins, W.A., Roe, J.H., Philippi, T., Congdon, J.D., 2004. Standard and digestive metabolism in the banded water snake, *Nerodia fasciata fasciata.* Comp. Biochem. Physiol. A 137, 141–149.

Pandian, T.J., Marian, M.P., 1985. Time and energy costs of metamorphosis in the Indian bullfrog *Rana tigrina.* Copeia 1985, 653–662.

Roe, J.H, Hopkins, W.A., Snodgrass, J.W., Congdon, J.D., 2004. The influence of circadian rhythms on pre- and post-prandial metabolism in the snake *Lamprophis fuliginosus*. Comp. Biochem. Physiol. A 139, 159–168.

Rowe, C.L., Kinney, O.M., Nagle, R.D., Congdon, J.D., 1998. Elevated maintenance costs in an anuran (*Rana catesbeiana*) exposed to a mixture of trace elements during the embryonic and early larval periods. Physiol. Zool. 71, 27–35.

Schmidt-Nielsen, K., 1990. Animal Physiology — Adaptation and Environmentfourth ed. Cambridge University Press.

Scott, D.E., Casey, E.D., Donovan, M.F., Lynch, T.K., 2007. Amphibian lipid levels at metamorphosis correlate to post-metamorphic terrestrial survival. Oecologia 153, 521–532.

Shi, Y., 2000. Amphibian Metamorphosis: from Morphology to Molecular Biology. Wiley-Liss.

Smith, D.C., 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology 68, 344–350.

Travis, J., 1984. Anuran size at metamorphosis experimental test of a model based on intraspecific competition. Ecology 65, 1155–1160.

Wassersug, R.J., Sperry, D.G., 1977. The relationship of locomotion to differential predation on *Pseudacris triseriata* (Anura: Hylidae). Ecology 58, 830–839.

Werner, E.E., 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. Am. Nat. 128, 319–341.

Wilbur, H.M., Collins, J.P., 1973. Ecological aspects of amphibian metamorphosis. Science 182, 1305–1314.