

Effects of Temperature and Time of Day on the Resting Metabolic Rates of Paedomorphic and Metamorphic Mole Salamanders, *Ambystoma talpoideum*

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Mole salamanders, *Ambystoma talpoideum*, are one of several species that exhibit facultative paedomorphosis. Many characteristics of paedomorphs and metamorphs have been compared in an effort to understand the ecological and evolutionary significance of facultative paedomorphosis, but few physiological comparisons have been made. We measured resting metabolic rates (oxygen consumption) of paedomorphic and metamorphic mole salamanders, under aquatic (both morphs) and terrestrial (metamorphs) conditions in an effort to compare maintenance energy expenditures of these phenotypes. There were no statistically significant differences in metabolic rates between morphs during aquatic trials. Under aquatic conditions, size adjusted oxygen consumption rates ranged from a low of 0.085 ml/h to 0.194 ml/h. There was a trend for oxygen consumption to increase with temperature, and at night compared to day, but significant interactions among time of day, temperature, and phenotype revealed complex responses. Most interesting, although metamorphs and paedomorphs had indistinguishable metabolic rates in water, both morphs in water consumed 2–4 times as much oxygen as did metamorphs in air. Our results suggest that metamorphs, while in a terrestrial environment, may expend significantly less energy for maintenance than aquatic paedomorphs.

IN the salamander genus *Ambystoma*, some species, including *Ambystoma talpoideum*, are facultatively paedomorphic; individuals may or may not undergo metamorphosis based on environmental cues (Semlitsch and Wilbur, 1989). Species that exhibit facultative paedomorphosis may have higher fitness than a genetically fixed phenotype as a result of their ability to respond appropriately to changing environmental conditions (Whiteman, 1994). In some populations of *Ambystoma*, both metamorphs and paedomorphs coexist (Semlitsch, 1985a; Semlitsch and Gibbons, 1985). However, there can be substantial variation in the frequency of paedomorphs (Patterson, 1978; Collins, 1981; Semlitsch, 1985a) that is correlated with environmental conditions.

Theoretical models using a cost-benefit approach have been used to predict when a salamander should become paedomorphic or metamorphose (Whiteman, 1994). Aquatic and terrestrial environments may differ in resource availability, predation pressure, and interspecific and intraspecific competition. Yet to be explored is how energetic demands, such as metabolic rate, may differ between these phenotypes and in aquatic versus terrestrial environments. Indeed, although resting and active metabolic rates have been compiled for adults and larvae of many amphibians (for a comprehensive review, see Gatten et al., 1992), we know

of no studies comparing metabolic costs of these two life-history phenotypes.

Organisms have a finite amount of energy that can be allocated toward maintenance, growth, storage, and reproduction (Congdon et al., 1982; Dunham et al., 1989; Beaupre, 1996). Furthermore, resting maintenance costs account for a high percentage of the total energy budget of most ectotherms (Congdon et al., 1982; Spotila and Standora, 1985). If aquatic and terrestrial environments make different demands on resting metabolic rates of salamanders (e.g., Jiang and Claussen, 1994), then variation in maintenance expenditure may be an important source of costs and benefits associated with metamorphic and paedomorphic phenotypes.

The purpose of our study was to compare the resting metabolic expenditure of metamorphic and paedomorphic phenotypes of the mole salamander *A. talpoideum* to determine whether there were differences in estimated maintenance costs. We specifically tested (1) whether metamorphs and paedomorphs have different resting metabolic rates in water and whether they exhibit similar diel and temperature sensitivity, and (2) whether the metabolic rate of aquatic paedomorphs is comparable to the metabolic rate of terrestrial metamorphs.

MATERIALS AND METHODS

Experimental organisms.—We obtained 19 metamorphic and 22 paedomorphic *A. talpoideum*

from a population located in Ellenton Bay, Aiken County, South Carolina, in January 1997. Salamanders were captured during migration to the breeding pond and based on body mass (average mass, gram, ± 1 SE; male metamorphs, 4.2 ± 0.3 ; male paedomorphs, 4.0 ± 0.3 ; female metamorphs, 5.1 ± 0.4 ; female paedomorphs, 3.2 ± 0.1) and were assumed to be sexually mature (Semlitsch 1985b, 1987).

Maintenance of organisms.—Salamanders were maintained under a light:dark cycle of 10:14 h for 1 week in water-filled aquaria at 16 ± 1 C prior to experimentation and then housed in the same fashion throughout the duration of the experiment. Individuals were toe-clipped for identification and randomly assigned dates for experimental trials. We fed the salamanders crickets (*Acheta*) and African clawed frog larvae (*Xenopus laevis*) ad libitum, except during a three-day fasting period in preparation for metabolic measurements (Ryan and Hopkins, 2000). There are very few studies on the effect of feeding on metabolic rates of amphibians (see Powell et al., 1999), and the fasting regime we chose may or may not have resulted in elevated postprandial metabolic rates. Nevertheless, all animals were treated identically so that they would be in a comparable digestive state. This feeding and fasting regime was followed throughout the duration of the experiment.

Experimental procedures, aquatic conditions.—Aquatic oxygen consumption was measured with a Yellow Springs Instrument® field oxygen probe and meter. Salamanders were sealed into metabolic chambers that consisted of water-filled 1-liter glass bottles with Polyseal® caps and a 25-mm octagonal stir bar. We aged water for 24-h prior to use in a trial to ensure that the water was free from chlorine. Glassware was washed with a mild soap and autoclaved before each trial. All water was saturated with air by bubbling with an aquarium pump for 1 h immediately prior to initiation of trials. A single chamber without a salamander was run with each set of experiments to act as a control for background changes in oxygen concentration. The control chamber never showed detectable changes in oxygen concentration during the course of our experiments.

Oxygen consumption trials were conducted over a 2-week period. Consumption rates were measured both day and night at two temperatures in a randomly determined order, with all animals being treated identically in preparation for experiments. Artificial light was used to simulate daylight conditions for day trials. Day trials

occurred from 0830–1830 h (10 h) and night trials took place from 2000–0700 h (11 h) Eastern Standard Time. Day and night trials were each carried out at temperatures of 10 and 15 C. This allowed for four combinations of temperature and time: 10 C day, 15 C day, 10 C night, and 15 C night. Each salamander group, comprised of both metamorphs and paedomorphs of each sex, was tested during the day and night trials successively with a one-day rest between changes in temperature treatment. Metabolic chambers were placed in a Revco® digital temperature controlled incubator that maintained internal air temperature within ± 0.2 C. At the termination of each trial, metabolic chambers were placed on a stir plate and stirred for 10 sec to ensure homogeneity. Chambers were subsequently opened and a 300 ml sample decanted for measurement.

Experimental procedures, aerial conditions.—To measure aerial metabolism of metamorphic salamanders, we developed a protocol to measure oxygen consumption without inducing high water loss. In a preliminary test, individual salamanders were weighed and placed inside metabolic chambers constructed from 30 cc syringes. Salamanders rested inside chambers on a thin, moist sponge that spanned the length of the syringe. In each of 19 trials, three salamanders served as controls (no forced air flow through the chamber), while one salamander was the experimental organism (air flow of 90 ml/min). The control chambers had a 5-mm entrance and exit port to allow for an adequate air supply. Air was dried prior to entering the chambers (for the purposes of measuring flow rates accurately) by passing it through two tubes of Drierite®. Trials were conducted at 20 C ± 1 C, and lasted an average of 6 h. At the end of a trial, we removed and reweighed salamanders to the nearest 1 mg. Change in mass was used as an estimate of an individual's water loss. Salamanders exposed to dry air flowing through respirometer chambers lost the same amount of water (3.17% of total body mass) as animals in the control chambers (4.01% of total body mass; control $n = 57$; experimental $n = 19$; $P = 0.6488$). Therefore, we concluded from this experiment that there was little if any physical stress (i.e., excessive mass loss through desiccation) placed on *A. talpoideum* in our flow-through respirometry system and proceeded with aerial oxygen consumption trials. In these measurements, the metabolic chambers were filled with air rather than water but otherwise treated identically as in the aquatic trials.

Aerial oxygen consumption was measured by

TABLE 1. LEAST-SQUARE MEAN OXYGEN CONSUMPTION RATES (ML/H; ADJUSTED FOR DIFFERENCES IN BODY SIZE BY ANCOVA) OF METAMORPHIC AND PAEDOMORPHIC SALAMANDERS IN WATER AND METAMORPHS IN AIR.

Temperature (C)	Phenotype	n	Day	SE	Night	SE
Aquatic						
10	Metamorph	18	0.0916	0.0075	0.1477	0.0145
10	Paedomorph	12	0.0848	0.0042	0.1808	0.0104
15	Metamorph	18	0.1648	0.0036	0.1940	0.0054
15	Paedomorph	12	0.1704	0.0050	0.1576	0.0084
Air						
10	Metamorph	9	0.0453	0.0161	0.0349	0.0092
15	Metamorph	9	0.0353	0.0085	0.0436	0.0084

a Sable TR-3 flow-through system (Sable Systems International, Henderson, NV) that includes an oxygen (Ametek S-3A, Pittsburgh, PA) and a CO₂ analyzer (LiCor 6251, Lincoln, NE). Our setup followed specifications in the technical documents, allowing automated data collection and analysis of oxygen consumption rates (expressed as ml/min, VO_2) estimated by the following equation (Withers, 1977):

$$VO_2 = [FR \cdot 60 \cdot (FiO_2 - FeO_2/100) - (VCO_2 \cdot FiO_2)] / (1 - FiO_2)$$

where *FR* is flow rate measured in ml/min using a mass flow meter; *FiO₂* is fractional concentration of O₂ entering the metabolic chamber; *FeO₂* is fractional concentration of O₂ leaving the metabolic chamber; *VCO₂* is the rate of CO₂ production (ml/min), given by:

$$VCO_2 = [(FeCO_2 - FiCO_2)/100] \cdot FR \cdot 60$$

Statistical analysis.—Aquatic oxygen measurements were recorded in parts per million (ppm) and later converted to ml of O₂/l using the conversion factor 0.70 (22.4 l/32 g; Dejours, 1981). We used a temperature-dependent oxygen solubility chart to correct for elevation. We compared metabolic rates using ANCOVA and repeated-measures MANCOVA with body mass as a covariate. A repeated-measures design was used because each individual was measured repeatedly at all combinations of temperature and time of day (Keppel, 1982; Beaupre, 1993; Niewiarowski et al., 2000). Covariance analysis adjusts for effects of body mass without introducing statistical artifacts associated with a ratio approach (for discussion and references, see Beaupre and Duval, 1998). In all analyses, we first transformed total oxygen consumption and mass by the natural logarithm (ln) to linearize the relationship between oxygen consumption and mass (Beaupre and Duval, 1998). For the

comparison of results between aquatic and aerial metamorphs, we used a repeated-measures MANCOVA, with metamorphs measured in air and those measured in water coming from two randomly chosen samples (i.e., individuals were scored for aerial or aquatic consumption, but not both), and “time of day” as the repeated measure. Residuals were checked for departures from normality using the Shapiro-Wilk statistic (Jmp 3.2.5, Statistical Analysis Systems Institute, Inc., Cary, NC, 2000, unpubl.). Homogeneity of variance and slopes were always verified prior to use of parametric statistics. Results were judged to be statistically significant at $P < 0.05$.

RESULTS

At the conclusion of each trial, salamanders appeared to be at rest within their chambers and did not move when the temperature cabinet was opened at the end of the trial. We assume that activity above resting levels was infrequent and random with respect to trial conditions.

Metamorphs and paedomorphs under aquatic conditions.—Oxygen consumption rates adjusted for body size ranged from a low of 0.0848 ml/h to 0.1940 ml/h (Table 1). In general, least-square mean oxygen consumption rates increased from 10 C to 15 C and from day to night (Table 1). Full-factorial MANCOVA on whole animal oxygen consumption with time of day and temperature as repeated measures, sex and phenotype as main effects, and body mass as a covariate, explained a significant portion of the variance in oxygen consumption (Table 2). Oxygen consumption was higher at 15 C compared to 10 C (Table 2). Time of day was not a significant effect in the model, but there was a significant time of day \times temperature interaction (Table 2). Furthermore, the response of salamanders to time of day and temperature effects was com-

TABLE 2. COMPOUND MANCOVA OF METAMORPHIC AND PAEDOMORPHIC *Ambystoma talpoideum* UNDER AQUATIC CONDITIONS. Each salamander was tested at all combinations of time of day (day or night) and temperature (10 or 15 C). Time of day, temperature, and the time of day \times temperature interactions are repeated measures. If a repeated-measure effect was not significant, then tests of effects associated with that repeated measure were not considered further because they did not explain a significant portion of variance in the model. Interactions not shown were not significant at $P \leq 0.05$.

Source	df Num	df Den	Exact F	P
Temperature	5	24	3.1466	0.0253
Ln mass	1	24	2.7057	0.1130
Sex	1	24	7.7000	0.0105
Phenotype	1	24	3.0981	0.0911
Phenotype \times sex	1	24	6.7058	0.0205
Time of day	5	24	0.5565	0.7321
Temperature \times time of day	5	24	2.9649	0.0319
Ln mass	1	24	1.8841	0.1826
Sex	1	24	0.9787	0.3324
Phenotype	1	24	0.8941	0.3538
Phenotype \times sex	1	24	5.2859	0.0305

plex, as indicated by significant phenotype \times sex interactions under the temperature and time of day \times temperature interaction repeated measures effects (Table 2). Both interactions were driven by a reduced rate of oxygen consumption by male metamorphs at 10 C during the night relative to the other groups (contrast $F_{1,24} = 10.089, P = 0.0041$).

Metamorphs under aerial versus aquatic conditions.—Average oxygen consumption of metamorphs in water was from 2.03 to 4.67 times that in air, depending upon temperature and time of day (10 C day and 15 C day, respectively; Table 1). Furthermore, the sensitivity of oxygen consumption to temperature and time of day was greater in water than in air (Fig. 1, Table

1). Underlying these simple trends was a statistically complex response of oxygen consumption by metamorphs to temperature and time of day as a function of medium (Table 3). Full factorial compound MANOVA revealed that the repeated measures time of day and temperature \times time of day interaction were significant (Table 3), but temperature as a simple effect was not (Table 3). Other simple effects (medium and sex) and two and three-way interactions accounted for a significant amount of variation in oxygen consumption rates as well. In particular, medium had a significant overall effect on oxygen consumption as a function of time of day (Table 3). Although males and females had similar responses to time of day in aquatic trials (contrast $F_{1,10} = 0.8479, P = 0.3788$; Fig. 1), they did not in aerial trials (contrast $F_{1,10} = 8.7699, P = 0.0143$; Fig. 1). Finally, medium was significant across the temperature \times time of day interaction (Table 3), but its effect is complicated by the three-way interaction including the covariate, body mass (Table 3).

DISCUSSION

To our knowledge, this study is the first to compare metabolic rates of paedomorphic and metamorphic salamanders of the same species in both air and water subject to temperature and diel effects. Because we manipulated many variables, the details of effects we measured are complicated. Nevertheless, oxygen consumption rates of these salamanders respond in ways expected of ectotherms; in particular, metabolic rates tend to increase with increasing tempera-

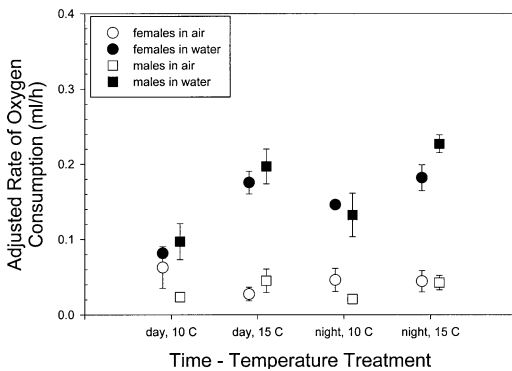


Fig. 1. Average oxygen consumption rates of metamorphic *Ambystoma talpoideum* during aquatic and aerial trials at 10 C and 15 C. Rates were adjusted for body size variation using covariance analysis. Error bars represent ± 1 SE. Symbols are offset for clarity.

TABLE 3. COMPOUND MANOVA FOR OXYGEN CONSUMPTION RATES OF METAMORPHS DURING AQUATIC AND AERIAL TRIALS. Medium is either water or air, and animals used in each medium were different. Time of day (night or day) and temperature (10 or 15 C) are treated as repeated measures because each salamander was measured at each combination of both factors. Results shown are for the reduced model; interactions not shown were not significant at the $P \leq 0.05$ level and were omitted.

Source	df Num	df Den	Exact F	P
Temperature	7	10	2.1154	0.1365
Time of day	7	10	4.8936	0.0123
Ln mass	1	10	10.3109	0.0093
Sex	1	10	3.0648	0.1106
Medium	1	10	11.8847	0.0063
Ln mass \times sex	1	10	11.7272	0.0065
Ln mass \times medium	1	10	16.3726	0.0023
Medium \times sex	1	10	8.3727	0.0160
Temperature \times time of day	7	10	5.7096	0.0072
Ln mass	1	10	23.0176	0.0007
Sex	1	10	8.9946	0.0134
Medium	1	10	12.8674	0.0050
Ln mass \times medium	1	10	7.3018	0.0222
Ln mass \times sex \times medium	1	10	13.6385	0.0042

ture. Rates of aquatic oxygen consumption by paedomorphs in our study are also comparable with rates reported in another study with the same species (Ryan and Hopkins, 2000). For example, a rough estimate of mass-specific metabolic rate of paedomorphs in our study (males and females combined; measured at 15 C) is $0.044 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, which is approximately 78% of the average resting mass-specific metabolic rate ($0.056 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) measured by Ryan and Hopkins (2000) at 20 C. Ryan and Hopkins (2000) did not measure metabolic rate at different temperatures, but the precise effect of temperature on oxygen consumption in our study was not uniform across all factors. For example, males and females did not always show identical sensitivity to temperature and time of day effects (Table 2), and gender-specific sensitivity may also depend upon phenotype (Table 2) as well as the medium in which oxygen consumption is measured (Fig. 1).

Our study demonstrated that metamorphs and paedomorphs have essentially indistinguishable rates of oxygen consumption in water (Tables 1–2) even though the two morphs rely on different respiratory surfaces for primary oxygen uptake. Paedomorphs use external gills as a primary site of oxygen uptake, whereas metamorphs rely solely on cutaneous methods for gas exchange when in water with no access to air. Therefore, metamorphs, without specialized gill structures, were still able to maintain a metabolic rate similar to those of paedomorphs while at rest in water.

What is more interesting, however, is that the metabolic rate of metamorphs is substantially higher in water than in air (Fig. 1, Table 3). Metamorphs in water consumed at least twice as much oxygen during the 10 C day trials as during the similar trials in air, and in some cases the difference was fourfold (Table 1). We had no specific a priori expectation that metamorph metabolic rates would be higher in water compared to air, but there is at least one other study that found differences between aquatic and aerial metabolic rates in a newt (Jiang and Clausen, 1994). Red-spotted newts (*Notophthalmus viridescens*) had roughly two-fold higher metabolic rates in water compared to air, similar to the minimum difference found here at 10 C during the day. Although two examples do not make a general trend, we believe there is compelling evidence now that such physiological differences, along with their potential energetic consequences, deserve more study.

Another potential explanation for the lower aerial metabolic rates compared to aquatic rates is that body temperatures of salamanders during aerial trials were lower than body temperatures of salamanders during aquatic trials because of evaporative heat loss. To minimize disturbance of salamanders during trials, we did not measure body temperatures. However, a simple analysis of water loss based on empirically derived relationships of water loss rates, respiratory surface area (Spight, 1968), and known air flow rates through chambers suggests that body temperatures would have been low-

ered by at most several tenths of a degree (at 10 C, a 3 g salamander exposed to dry air moving at 0.6 cm/s is expected to evaporate 0.0110 ml/h, equivalent to 54 cal/h or approximately 0.1 C). Even if rates of water loss were an order of magnitude higher than this (but we have no reason to expect they were), such differences in body temperature (on the order of 1 C or so) still would not be able to account for the two- to fourfold difference in metabolic rate.

If the metabolic differences between aquatic paedomorphs and terrestrial metamorphs seen here in the laboratory exist for these salamanders in nature, then metamorphosis may confer significant energetic advantages. Metamorphs spend a significant fraction of the year resident in terrestrial microhabitats, and assuming both morphs consume the same amount of calories while expending similar amounts of energy for locomotion to acquire food, avoid predators, and reproduce (i.e., all else is equal), it is possible that metamorphs would have more energy to allocate toward growth, storage, and reproduction than paedomorphs. However, there is a lack of data for caloric consumption and locomotor costs in paedomorphic and metamorphic *Ambystomatid* salamanders and further research is needed. Nevertheless, our suggestion of a potential energetic advantage is not without precedent. Jiang and Claussen (1994) inferred that a Massachusetts population of *N. viridescens* resting at 5 C could survive 2.5 times longer on land than in water. For mole salamanders, under the assumption that metamorphs remain inactive on land for long periods, it might be energetically advantageous for larval salamanders to metamorphose and live a largely terrestrial existence because of the possibility of lower maintenance costs.

Our results may also help explain observed differences in growth rates, body size, and reproductive output between metamorphic and paedomorphic salamanders. For example, Semlitsch (1985a) noted that one-year-old metamorphic individuals of *A. talpoideum* had longer SVL and produced more eggs than one-year-old paedomorphs. Whiteman et al. (1996) reported that metamorphic tiger salamanders (*Ambystoma tigrinum nebulosum*) grew more in SVL between years and gained more body mass within years than paedomorphs. Over a 3-yr study, paedomorphs did not increase in SVL or body mass, whereas metamorphs increased significantly in both categories. Whiteman et al. (1996) suggested that differences in growth rate might be best explained by the observation that the stomach contents of metamorphs were significantly higher in calories than paedomorph stomach

samples, because metamorphs were able to travel to areas of higher food quality than were paedomorphs. However, our results suggest an alternative (but not necessarily mutually exclusive) hypothesis. Perhaps the lower maintenance expenditures by metamorphs leads to a greater allocation of assimilated energy to growth, storage, and reproduction.

In summary, the results of our study suggest that metamorphs and paedomorphs have relatively equal maintenance costs under aquatic conditions. However, metamorphs on land appear to have large energetic savings because they expend less energy on maintenance than their aquatic paedomorphic counterparts. This should allow metamorphs to direct more energy towards growth, reproduction, and survival functions (e.g., storage and locomotion). Our study adds another potential mechanism to explain observed body size and growth rate differences between metamorphic and paedomorphic mole salamanders (Semlitsch 1985b; Whiteman et al. 1996) as a result of presumably lower maintenance demands in metamorphs. Because maintenance can consume a large portion of the daily energy budget of an ectotherm, differences between phenotypes in maintenance expenditure may be relevant to cost/benefit models that attempt to predict the conditions under which metamorphosis or paedomorphosis is the optimal life-history strategy.

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