

Interaction of Sex and Size and the Standard Metabolic Rate of Paedomorphic *Ambystoma talpoideum*: Size Does Matter

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We measured the standard metabolic rate of paedomorphic Mole Salamanders (*Ambystoma talpoideum*) from South Carolina. Despite an absence of body size differences between females and males, analysis of covariance demonstrated metabolic rates (mL O₂/h) were significantly influenced by an interaction between sex and body size. The interaction appeared as a result of changes in the size-metabolism relationship in males (a steeper slope in the size-metabolism regression in small males as compared to large males), whereas the relationship remained constant in females, regardless of size. We hypothesize that the observed differences are attributable to differences in reproductive physiology between the sexes. The results of this study underscore the need to evaluate the potential interactions among relevant variables in studies of metabolism, which heretofore have been disregarded in studies of salamander physiology.

INTRASPECIFIC comparisons of basal metabolism in ectotherms are extremely sensitive to differences in body size. In certain amphibians, the relationship between body size and metabolism is particularly well established (Feder and Burggren, 1992). In addition to size, sex-specific differences in metabolic rates have been repeatedly documented among anurans, largely because of the increased energetic cost of calling behavior in breeding males (Taigen et al., 1985; Pough et al., 1992). However, such sex-based differences are lesser known among salamanders (Pough et al., 1992), even in the context of reproductive activity (Bennett and Houck, 1983).

This study was undertaken as part of a more comprehensive effort to understand the changes in metabolic requirements prior to, during, and after metamorphosis, in the Mole Salamander, *Ambystoma talpoideum*. This species exhibits a lifecycle polymorphism; some members of a population undergo metamorphosis prior to maturation as terrestrial adults (typical of temperate zone amphibians), whereas other individuals bypass metamorphosis and become mature, branchiate (aquatic) adults (Patterson, 1978; Semlitsch, 1985a). Although some of the costs and benefits of alternative lifecycle pathways are reasonably understood (Sprules, 1974; Whiteman, 1994; Ryan and Semlitsch, 1998), the physiological constraints and consequences of them are not as thoroughly explored. Herein we focus on the difference in standard metabolic rates between males and females of the adult branchiate (= paedomorphic) form; comparisons of other lifecycle phases will be presented elsewhere.

MATERIALS AND METHODS

We collected paedomorphic *A. talpoideum* from Ellenton Bay, a 10-ha Carolina bay located on the Savannah River Site near Aiken, South Carolina, using unbaited minnow traps set at a depth of 10–30 cm. We collected nine post-breeding individuals of each sex on each of three consecutive nights (28–30 March 1999) after the completion of the breeding season. Sex and status were determined by visual inspection of the cloacal and abdominal regions. Animals were housed individually in polyethylene containers (33.0 × 18.4 × 10.5 cm) with approximately 2 liters of 1:1 untreated well water to bay water and maintained in an environmental chamber (20 C, 80% humidity, 12L:12D). We determined standard metabolic rate (SMR), the metabolic rate of a postabsorptive animal at rest at a predetermined temperature, according to the methods of Rowe et al. (1998) and Hopkins et al. (1999). We held the animals at 20 C for 48 h prior to metabolic measurements to allow for gut clearance. In preparation for measurements, animals were transferred to respiratory chambers containing 300 mL of untreated well water at 20 C. Each of 20 respiratory chambers was devoted to an independent channel on a computer-controlled, closed-circuit, indirect respirometer (MicroOxymax, Columbus Instruments, Columbus, OH). The first chamber contained a medical battery that consumed a known amount of O₂/min (Procell, Bethesda, MD); the last chamber served as a blank control. We haphazardly assigned nine male and nine female *A. talpoideum* from a single collection night to the remaining 18 chambers. The array of chambers was maintained at 20 C for

TABLE 1. DESCRIPTIVE STATISTICS (MEAN, STANDARD ERROR, MINIMUM, AND MAXIMUM) FOR MASS AND OXYGEN CONSUMPTION FOR FEMALE AND MALE *Ambystoma talpoideum paedomorphs*. The raw differences in means are shown as the difference between male and female values, with the percent difference (difference/male value * 100) in parentheses.

| | N | Mass (g) | | | | O ₂ consumption (mL/h) | | | |
|---------------------|----|----------------|--------|------|------|-----------------------------------|--------|--------|--------|
| | | Mean | SE | Min | Max | Mean | SE | Min | Max |
| Females | 27 | 5.36 | 0.2069 | 4.12 | 8.58 | 0.3038 | 0.0193 | 0.1933 | 0.5411 |
| Males | 27 | 5.62 | 0.1837 | 4.23 | 7.38 | 0.3133 | 0.0131 | 0.1827 | 0.4782 |
| Difference in means | | 0.26 (4.63) | | | | 0.0095 (3.03) | | | |

the duration of each testing period; the amount of oxygen consumed (mL O₂) was determined at 2-h intervals over a 24-h period, beginning at approximately 1000 h. This procedure was repeated three times on consecutive nights, resulting in a total of 54 individuals tested (27 male, 27 female).

ANALYSIS AND RESULTS

Mass and oxygen consumption (Table 1) were tested for normality and homoscedasticity using normal probability plots; both were log-transformed to meet the assumptions of parametric tests for all subsequent statistical analyses. Following visual inspection of the raw data, the highest 50% of the O₂ consumption values for each individual were removed from the dataset prior to analysis to eliminate the potentially inflationary effects of unobserved periods of activity on SMR (Rowe et al., 1998; Hopkins et al., 1999). We used analysis of variance (ANOVA)

to compare the mass of test subjects. There was no significant difference in mass between sexes ($F_{1,52} = 1.13$, $P = 0.2911$). We used analysis of covariance (ANCOVA) to evaluate differences in metabolic rates (ml O₂/h) between sexes, with size (mass) as a covariate. There was a significant effect of sex ($F_{1,50} = 4.30$, $P = 0.043$) and a sex-by-size interaction ($F_{1,50} = 4.31$, $P = 0.041$; Fig. 1). The difference between the sexes in mean oxygen consumption rate and mean SMR is negligible (Tables 1–2, respectively), suggesting that the significant effect of sex in this analysis is a statistical artifact of the sex-by-size interaction. To further characterize the sex-by-size interaction, we recategorized our dataset for illustrative rather than statistical purposes. We calculated the mean size of each sex, and grouped all individuals of a given sex below the mean [small: females = 4.71 ± 0.075 (mean \pm SE); males = 4.82 ± 0.072] and all individuals of that sex above the mean (large: females = 6.31 ± 0.328 ; males = 6.48 ± 0.162). This recategorization showed that females demonstrated a higher SMR at large body sizes relative to similar-sized males (Fig. 2). Small males and females exhibited similar-size SMR relationships.

DISCUSSION

Although the difference in mean SMR between the sexes was minor (Table 1), the interaction between size and sex proved to be a significant source of variation. Our crude recategorization illustrates that the interaction results from changes in the size-metabolism relationship in males whereas the relationship remains consistent in females, regardless of size. We are unaware of another reported instance of this relationship, and the biological significance of our result is unclear at present. We hypothesize, however, that differences in reproductive physiology contribute to the sex-by-size interaction we observed in this study.

Males and females have different metabolic

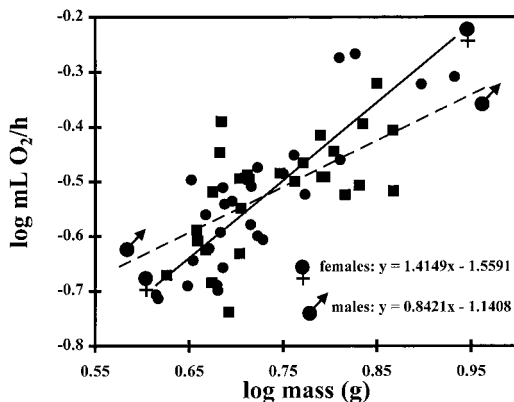


Fig. 1. The relationship between body size and metabolic rate in paedomorphic male and female *Ambystoma talpoideum*. Females are represented by circle symbols and a solid regression line; males are represented by square symbols and a dashed regression line.

TABLE 2. COMPARISON OF AND DIFFERENCES BETWEEN MALE AND FEMALE METABOLIC RATES IN THREE SPECIES OF SALAMANDERS. The values in the table represent mean metabolic rates (mL O₂/g*h) with measurements of variation (SE for this study and Bennett and Houck, 1983; SD for Fitzpatrick, 1973) in brackets. The raw difference is the mean value of male–female; negative values therefore demonstrate higher metabolic rates in females. Percent difference is the raw difference divided by the male value *100.

| Reference | Species | Temperature (C) | Male | Female | Raw difference | % difference |
|-------------------------|----------------------------------|-----------------|--------------------|--------------------|----------------|--------------|
| This study | <i>Ambystoma talpoideum</i> | 20 | 0.0560 [0.0019] | 0.0557 [0.0020] | 0.0003 | 0.54 |
| Bennett and Houck, 1983 | <i>Desmognathus carolinensis</i> | 15 | 0.088 [0.0113] | 0.092 [0.0154] | -0.0040 | -4.54 |
| Fitzpatrick, 1973 | <i>Desmognathus ochrophaeus</i> | 5 | 0.0139 [0.0068] | 0.0165 [0.0107] | -0.0026 | -18.70 |
| Fitzpatrick, 1973 | <i>Desmognathus ochrophaeus</i> | 15 | 0.0341 [0.0068] | 0.0320 [0.0095] | 0.0021 | 6.16 |

demands with regard to reproductive physiological requirements, primarily because of the investment in gamete production. Ova generally require more energy to produce than do sperm (Trivers, 1972), and larger females produce proportionally more eggs than smaller females (Kaplan and Salthe, 1980; Semlitsch, 1985a). Thus, the size-metabolism relationship is likely to differ between the sexes, with (1) females demonstrating a steeper slope in log-log plots of metabolism regressed on body size and (2) the disparity between the sexes being greatest at large body sizes. This is the pattern observed in our study, and our interpretation is consistent with general trends among the Caudata. The slope of the log-log regression of metabolism on body size for males is quite similar to values gen-

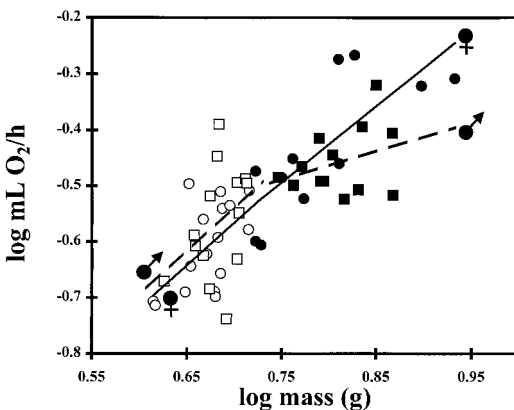


Fig. 2. The relationship between body size and metabolic rate in small (< mean) and large (> mean) paedomorphic male and female *Ambystoma talpoideum*. Symbols as in Figure 1; for each sex, small and large individuals are represented by open and closed symbols, respectively.

erated using other caudates (range 0.80–0.81; see Gatten et al., 1992: table 12.2, p. 330), whereas the slope of the same regression for females in our study is noticeably elevated. Our tests were run in April, well after the end of the breeding season (Semlitsch, 1985b; Semlitsch et al., 1993). Following reproduction, relatively few ova remain in the reproductive tract (Semlitsch, 1985a), indicating that an entire compliment of ova must be produced for each reproductive bout. Because reproduction among female *A. talpoideum* is frequently annual (Semlitsch et al., 1988) and females are ready to breed as early as late September or early October (Krenz and Sever, 1995), ova production is believed to commence in the spring. Therefore, we hypothesize that the observed disparity between males and females is attributable to the production of ova for the upcoming reproductive season, increased energetic deficit because of the production and oviposition of eggs in the previous reproductive season, or some combination of these factors.

Sex-based differences in basic physiology are frequently unaccounted for in experimental designs or descriptive studies when sexes are pooled or grouped together for analyses. Therefore, considering sexes collectively may overlook significant differences in metabolic parameters and obscure interactions with other variables such as age, growth rate, reproductive status, and temperature (see below). There are, however, a few reports in the literature that consider the sex-specific metabolic rates of salamanders. Fitzpatrick (1973) found that gravid female *Desmognathus ochrophaeus* had higher rates of oxygen consumption than did either males or nongravid (spent) females but that males and nongravid females did not differ apprecia-

bly. Fitzpatrick's results were supported by Bennett and Houck (1983) in *D. carolinensis*; they detected no differences in resting metabolism between sexes early in the mating season, presumably prior to ova development. Neither of these studies, however, considered the potential interaction between size and sex in their analyses.

The outcome of our experiment reemphasizes the need to consider potential confounding variables in analyses of metabolic rates. Primary among these should be size, temperature, and sex, with consideration always given to the potential interactions among these variables. Consider the following example. cursory comparisons of the size and oxygen consumption of the sexes in our study (Tables 1–2) do not reveal any differences in the parameters of interest. Nevertheless, we found a significant interaction between size and sex for the mass-specific consumption rate, despite a minute difference between the means. Fitzpatrick (1973) and Bennett and Houck (1983) also failed to detect significant differences between the means of males and nongravid females, despite the fact that the percent difference in mass-specific metabolic rates between the sexes reported in these studies is up to 34 times *greater* than what we report here (Table 2). Neither Fitzpatrick (1973) nor Bennett and Houck (1983) included a sex-by-size interaction term in their analyses. Furthermore, in Fitzpatrick's study, the direction of the sex-based difference changes under different temperature regimes (i.e., at low temperature, females had a higher metabolic rate, and at high temperatures, males had a higher metabolic rate), indicating that more complex interactions, such as temperature-by-sex-by-size, need consideration. Without including the interaction between size and sex, a potentially significant source of variation is overlooked, leaving sex-based physiological differences only partially resolved.

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LITERATURE CITED

- BENNETT, A. F., AND L. D. HOUCK. 1983. The energetic cost of courtship and aggression in a plethodontid salamander. *Ecology* 64:979–983.
- FEDER, M. E., AND W. W. BURGGREN. 1992. Environmental physiology of the amphibians. Univ. of Chicago Press, Chicago.
- FITZPATRICK, L. C. 1973. Energy allocation in the Allegheny Mountain salamander, *Desmognathus ocozophaeus*. *Ecol. Monogr.* 43:43–58.
- GATTEN JR., R. E., K. MILLER, AND R. J. FULL. 1992. Energetics at rest and during locomotion, p. 314–377. *In: Environmental physiology of the amphibians*. M. E. Feder and W. W. Burggren (eds.). Univ. of Chicago Press, Chicago.
- HOPKINS, W. A., C. L. ROWE, AND J. D. CONGDON. 1999. Elevated trace element concentrations and standard metabolic rate in banded water snakes (*Nerodia fasciata*) exposed to coal combustion wastes. *Environ. Tox. Chem.* 18:1258–1263.
- KAPLAN, R. H., AND S. N. SALTHER. 1979. The allometry of reproduction: an empirical view in salamanders. *Am. Nat.* 113:671–689.
- KRENZ, J. D., AND D. M. SEVER. 1995. Mating and oviposition in paedomorphic *Ambystoma talpoideum* precedes the arrival of terrestrial males. *Herpetologica* 51:387–393.
- PATTERSON, K. K. 1978. Life history aspects of paedomorphic populations of the mole salamander, *Ambystoma talpoideum*. *Copeia* 1978:649–655.
- POUGH, F. H., W. E. MAGNUSON, M. J. RYAN, K. D. WELLS, AND T. L. TAIGEN. 1992. Behavioral energetics, p. 395–436. *In: Environmental physiology of the amphibians*. M. E. Feder and W. W. Burggren (eds.). Univ. of Chicago Press, Chicago.
- ROWE, C. R., O. M. KINNEY, R. D. NAGLE, AND J. D. CONGDON. 1998. Elevated maintenance costs in an anuran (*Rana catesbeiana*) exposed to a mixture of trace elements during the embryonic and early larval periods. *Phys. Zool.* 71:27–35.
- RYAN, T. J., AND R. D. SEMLITSCH. 1998. Intraspecific heterochrony and life history evolution: decoupling somatic and sexual development in a facultatively paedomorphic salamander. *Proc. Nat. Acad. Sci. USA* 95:5643–5648.
- SEMLITSCH, R. D. 1985a. Reproductive strategy of a facultatively paedomorphic salamander *Ambystoma talpoideum*. *Oecologia* 65:305–313.
- . 1985b. Analysis of climatic factors influencing migrations of the salamander *Ambystoma talpoideum*. *Copeia* 1985:477–489.
- , D. E. SCOTT, AND J. H. K. PECHMANN. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology* 69:184–192.
- , ———, AND J. W. GIBBONS. 1993. Phenotypic variation in the arrival time of breeding salamanders: individual repeatability and environmental influences. *J. Anim. Ecol.* 62:334–340.
- SPRULES, W. G. 1974. The adaptive significance of

- paedomorphosis in North American species of *Ambystoma* (Amphibia: Caudata): an hypothesis. *Can. J. Zool.* 52:393-400.
- TAIGEN, T. L., K. D. WELLS, AND R. L. MARSH. 1985. The enzymatic basis of high metabolic rates in calling frogs. *Phys. Zool.* 58:719-726.
- TRIVERS, R. L. 1972. Parental investment and sexual selection, p. 136-179. *In: Sexual selection and the descent of man*. B. Campbell (ed.). Aldine, Chicago.
- WHITEMAN, H. H. 1994. Evolution of facultative paedomorphosis in salamanders. *Q. Rev. Biol.* 69:205-221.
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