

## Effects of Temperature, Sex, and Gravidity on the Metabolism of Small-Mouthed Salamanders, *Ambystoma texanum*, during the Reproductive Season

MICHAEL S. FINKLER

*Department of Natural, Information and Mathematical Sciences, Indiana University Kokomo,  
P.O. Box 9003, Kokomo, Indiana 46904-9003 USA; E-mail: mfinkler@iuk.edu*

**ABSTRACT.**—Amphibians that breed in early spring may encounter considerable variation in ambient temperature. In this study, I investigated how the metabolic rate of male and female Small-Mouthed Salamanders (*Ambystoma texanum*) is influenced by variation in temperature (5–20°C) during the reproductive season. As expected, both rates of resting oxygen consumption ( $\text{VO}_2$ ) and resting carbon dioxide production ( $\text{VCO}_2$ ) increased with increasing temperature. Both measures of respiration were significantly higher in gravid females than in males and postgravid females across all temperatures. Moreover, the  $\text{VO}_2$  of gravid females increased more with increasing temperature than did those of either males or postgravid females. These findings suggest that variation in ambient temperature may have an important influence on the energetic cost of reproductive activity in males and females in this species and in other spring-breeding amphibians.

Many species of amphibians from northern latitudes, including most ambystomatid salamanders, breed in late winter and early spring. Often, it is the occurrence of warm, rainy weather that prompts migration to breeding areas and subsequent reproduction (Minton, 2001). During this migration and while in the breeding areas, amphibians may be exposed to a wide range of temperatures. Thus, these ectothermic animals may be susceptible to marked fluctuations in their metabolism during the breeding period (Rome et al., 1992).

Metabolic rate of spring breeding ambystomatids can differ considerably between sexes and with reproductive condition. In particular, there appears to be a considerable increase in the resting metabolic rate of gravid females compared to both males and postgravid females at 15°C (Finkler and Cullum, 2002; Finkler et al., 2003). However, it is unclear how variation in temperature might influence this difference in metabolic rate between males and females and between gravid and postgravid females. Fitzpatrick (1973) noted that gravid females of the plethodontid *Desmognathus fuscus* had higher oxygen consumption rates than did males at 15°C but not at 5°C. Data on other taxa are lacking. Given that the males of many ambystomatids tend to emerge from hibernation and migrate to breeding areas at temperatures lower than do the females (Downs, 1989; Petranka, 1998; Minton, 2001), differences in temperature at the time of emergence from the hibernaculæ and overland migration could contribute to differences in the energetic cost of reproduction beyond gamete formation.

The present study examined the influence of temperature on oxygen consumption and carbon dioxide production in male, gravid female, and post-gravid female Small-Mouthed Salamanders (*Ambystoma texanum*) to ascertain the effect of temperature on the differences in metabolic rate associated with sex and reproductive condition.

### MATERIALS AND METHODS

Male ( $N = 7$ ), gravid female ( $N = 5$ ), and postgravid female ( $N = 5$ ) *Ambystoma texanum* were collected via

dip net from breeding areas in the Salamonie River State Forest, Wabash County, Indiana, and Lost Bridge State Recreation Area, Huntington County, Indiana, between 22 and 29 March 2003. Animals in all three groups were collected throughout the collection period. Animals were housed in pairs in plastic shoe boxes containing moistened paper towels at a constant 15°C for two days prior to respirometry testing.

Measurements of resting oxygen consumption ( $\text{VO}_2$ ) and carbon dioxide production ( $\text{VCO}_2$ ) rates at four different test temperatures (5, 10, 15, and 20°C) were conducted using a Columbus Instruments Micro-Oxymax respirometry system. Each animal's gas exchange was measured at all four temperatures over a four-day period, with the sequence of temperatures randomized for each animal. After the mass of each animal was recorded, it was placed into a 250 mL sample bottle connected via tubing to the multiplexer of the respirometer, which was then submerged in a circulating water bath at the appropriate test temperature. After a 2-h adjustment period, the system automatically measured STPD-corrected  $\text{VO}_2$  and  $\text{VCO}_2$  in each chamber hourly for a 6-h period, with a refresh interval after 3 h. Hourly measurements were averaged for each animal to provide a single measure of  $\text{VO}_2$  and  $\text{VCO}_2$  at each temperature. As this species only rarely ingests food prior to breeding (Plummer, 1977), I assumed that all salamanders were in a postabsorptive state.

Following the last respirometry measurement, animals were anesthetized by submergence in 0.67% MS<sub>222</sub> solution. Total body length, snout-vent length, and live mass were recorded; then the animals were dissected to remove eggs from the oviducts of gravid females and to harvest organs for another study. The wet mass of the carcasses with eggs removed was recorded.

$\text{VO}_2$  and  $\text{VCO}_2$  were analyzed using the MIXED procedure on SAS 8.2. Differences in body size measurements (live mass, total length, snout-vent length, and wet carcass mass) among different groups (male, gravid female, or postgravid female) were analyzed using one-way ANOVAs. Since the sequence of temperatures tested was randomized for each animal, I analyzed the respiration data in two separate sets of

TABLE 1. Size comparisons among male, postgravid female, and gravid female *Ambystoma texanum*.

Parameter	Group	Mean $\pm$ SEM	$F_{2,14}$	$P$
Live mass (g)	Male	8.95 $\pm$ 0.54	3.67	0.052
	Postgravid female	10.92 $\pm$ 0.58		
	Gravid female	10.98 $\pm$ 0.76		
Total length (mm)	Male	139.3 $\pm$ 3.2	1.17	0.34
	Postgravid female	147.2 $\pm$ 4.1		
	Gravid female	142.5 $\pm$ 4.5		
Snout-vent length (mm)	Male	79.5 $\pm$ 1.9	2.3	0.14
	Postgravid female	85.4 $\pm$ 2.3		
	Gravid female	84.3 $\pm$ 2.6		
Wet carcass mass (g)	Male	8.75 $\pm$ 0.70	2.03	0.17
	Postgravid female	10.70 $\pm$ 0.79		
	Gravid female	9.88 $\pm$ 0.56		

analyses (see Finkler and Claussen, 1999). First, an ANOVA was conducted where individual salamanders were nested within Group to account for the multiple measurements taken at different temperatures from each animal. Where appropriate, pairwise differences among groups were evaluated using Bonferroni-adjusted  $t$ -tests between least-squares means. In addition,  $Q_{10}$ -values ( $[R_2/R_1]^{10/(T_2-T_1)}$ , where  $R_2$  and  $R_1$  are rates at the higher temperature [ $T_2$ ] and the lower temperature [ $T_1$ ], respectively) for  $VO_2$  and  $VCO_2$  were calculated for each 5°C interval and for the entire 15°C range of temperatures. Respiratory quotients (RQ, the amount of  $CO_2$  liberated/amount of  $O_2$  consumed) were also calculated for each group at each temperature. In a second set of analyses, a series of ANCOVAs were performed within each temperature with Group as a fixed effect to assess the importance of body size on metabolic rate. Because ambystomatid eggs are not fertilized until oviposition (Downs, 1989) and presumably have low metabolic activity, we used Wet Carcass Mass (not including eggs dissected from gravid females) as a covariate accounting for differences in body size among the salamanders.

## RESULTS

There was little difference in body size among males, gravid females, and postgravid females (Table 1). Although the live mass of gravid females was marginally significantly greater than that of males, total length, snout-vent length, and wet carcass mass did not differ significantly among the three groups.

Both  $VO_2$  and  $VCO_2$  (Fig. 1, Table 2) increased with temperature in all three groups.  $VO_2$  was influenced by a significant Group  $\times$  Temperature interaction.  $VO_2$  increased more with increasing temperature in gravid females than in either males or postgravid females (Fig. 1, Table 2), so that although there was no difference in

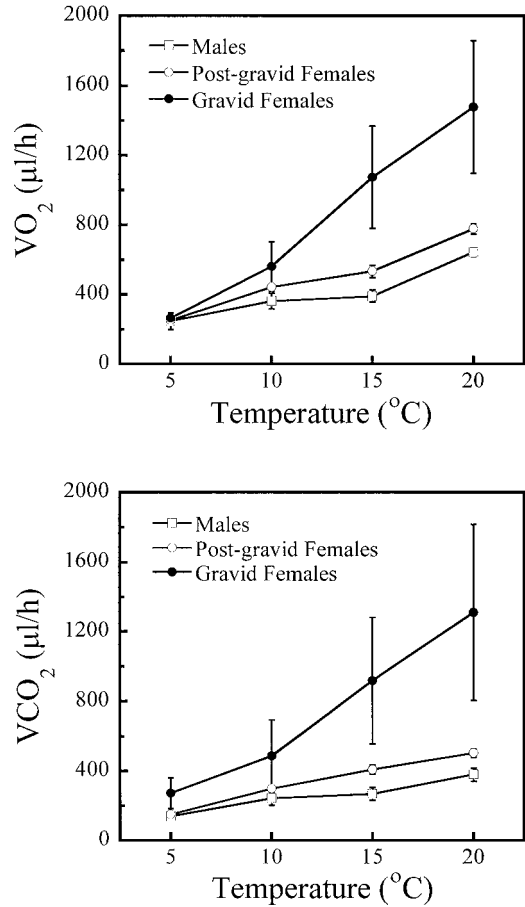


FIG. 1. Variation in STPD-corrected oxygen consumption ( $VO_2$ ) and carbon dioxide production ( $VCO_2$ ) rates with temperature in *Ambystoma texanum* males ( $N = 7$ ), gravid females ( $N = 5$ ), and postgravid females ( $N = 5$ ). Data are presented as unadjusted means  $\pm$  SEM.

$VO_2$  among the three groups at 5°C and 10°C,  $VO_2$  was significantly higher in gravid females than in the other two groups at 15°C and 20°C (pairwise  $P < 0.017$ ). There was no difference between male and postgravid female  $VO_2$  at any temperature. Across temperatures,  $VCO_2$  was higher in gravid females than in the other two groups, but there was no significant difference in  $VCO_2$  between males and postgravid females. Overall  $Q_{10}$  values for both  $VO_2$  and  $VCO_2$  over the 15°C range of temperatures tended to be higher in gravid females than in either males or postgravid females (Table 3). Respiratory quotients (Table 4) also tended to be higher in gravid females than in either males or postgravid females at all temperatures. Neither  $VO_2$  nor  $VCO_2$  was significantly influenced by carcass mass at any temperature ( $F_{1,13} \leq 0.94$ ,  $P \geq 0.35$ ).

## DISCUSSION

Similar to the findings of previous examinations of the influence of sex and gravidity on metabolic rate (Finkler

TABLE 2. ANCOVA results for the effects of temperature, group and body size on  $VO_2$  and  $VCO_2$  in *Ambystoma texanum* males, postgravid females, and gravid females.

Parameter	Factor	F	df	P
$VO_2$	Group	27.45	2, 42	<0.0001
	Temperature	38.83	3, 42	<0.0001
	Group $\times$ Temperature	5.66	6, 42	0.0002
	Individual (Group)	4.93	14, 42	<0.0001
$VCO_2$	Group	30.71	2, 42	<0.0001
	Temperature	20.12	3, 42	<0.0001
	Group $\times$ Temperature	4.45	6, 42	0.0014
	Individual (Group)	8.31	14, 42	<0.0001

and Cullum, 2002; Finkler et al., 2003), gravid females had greatly elevated metabolic rates compared to either males or postgravid females. For example,  $VO_2$  at 15°C was 101% higher in gravid females than in postgravid females (comparable to the 87% higher  $VO_2$  of gravid *A. texanum* reported in Finkler and Cullum, 2002). The degree to which gravidity affects metabolic rate, however, decreases with decreasing temperature, such that at 5°C mean  $VO_2$  in gravid females is only 4% higher than that of postgravid females. This suggests that the metabolic rate of gravid females is much more sensitive to temperature fluctuations than are those of either males or postgravid females. This is reflected in the higher  $Q_{10}$ -values for gravid females as well; overall  $Q_{10}$ -values for respiration in males and postgravid females are typical of those for amphibians (generally 1.8–2.2 as reviewed in Rome et al., 1992), but those for gravid females tend to be considerably higher than expected.

Gravid females also tended to have a higher mean RQ than did either males or postgravid females. This suggests a relatively heavy dependence on carbohydrates to fuel metabolism in gravid females, whereas males and postgravid females appear to rely more on lipids as their primary energy source (Kleiber, 1961). This is in contrast to Finkler and Cullum (2002) who found relatively uniform RQ measurements (~0.9) among the three groups. As measurements occurred over a longer period of time in this study (six days as opposed to two days in Finkler and Cullum, 2002), it is possible that animals may have become more accustomed to handling and other stresses of captivity that could elevate hormones such as corticosterone (Homan et al., 2003) that in turn influence metabolic substrate consumption. Endocrine differences could also account for the differences in RQ observed between gravid females and the other two groups in this study. Homan et al. (2003) found that female *A. maculatum* entering a breeding pool had higher plasma corticosterone concentrations than did males entering the pool, but there was no difference in plasma corticosterone between males and females leaving the pool. Elevated corticosterone could increase the catabolism of glycogen and protein to support maintenance and/or vitellogenesis (Wilson and Wingfield, 1994) and, thus, may contribute to the elevation in RQ in gravid females.

The findings of this study suggest that the differences in the energetic cost of reproduction between males and females could be heavily influenced by temperature,

TABLE 3.  $Q_{10}$ -values for changes in  $VO_2$  and  $VCO_2$  with increasing temperature in *Ambystoma texanum* males, postgravid females, and gravid females.

Parameter	Group	Overall			
		5–10°C	10–15°C	15–20°C	(5–20°C)
$VO_2$	Males	2.1	1.2	2.7	1.9
	Postgravid females	3.0	1.5	2.1	2.1
	Gravid females	4.5	3.6	1.9	3.2
$VCO_2$	Males	3.0	1.2	2.0	1.9
	Postgravid females	3.9	1.9	1.5	2.2
	Gravid females	3.2	3.6	2.0	2.9

particularly with respect to “indirect” energetic costs, such as the cost of transport during migration or metabolic costs associated with the perfusion of reproductive structures (Angilletta and Sears, 2000; Demarco and Guillette, 1992; Olsson et al., 2000). Males, in addition to having generally lower metabolic rates than females, have a tendency to emerge from their hibernacula at somewhat lower temperatures than do females (Downs 1989; Petranka, 1998; Minton, 2001), thus arriving at the breeding areas earlier. Protandrous arrival may help increase the chances of a male successfully encountering one or more females with which it can reproduce (Semlitsch et al., 1993), although it may also increase the chances of mortality caused by adverse environmental conditions such as freezing. Low temperatures during the early portion of the breeding season may help reduce energy expenditure by early arriving males. Gravid females, in contrast, are less likely to migrate into the breeding areas when temperatures are low. The higher temperatures may ensure that the females can rapidly migrate to the breeding areas and reduces the risk of freezing (see Morbey and Ydenberg, 2001) but may come at the cost of even greater energetic expenditure.

Although these findings suggest the energetic cost of breeding activities (e.g., migration) may be higher in females than in males, they also suggest that there may be relatively little difference between males and females in the indirect energetic cost of reproduction during the period prior to the breeding season, when low temperatures in the hibernaculae would keep the resting metabolic rate of individuals of both sexes uniformly low. Thus, the observed differences in the metabolic rate in previous studies (e.g., Finkler and Cullum, 2002; Finkler et al., 2003) may pertain only to the actual breeding period following emergence from hibernation.

*Acknowledgments.*—Animals for this study were collected with the permission of the Indiana Department

TABLE 4. Respiratory quotients in male, postgravid female, and gravid female *Ambystoma texanum* at four different temperatures.

Group	5°C	10°C	15°C	20°C
Males	0.6	0.7	0.7	0.6
Postgravid females	0.6	0.7	0.8	0.6
Gravid females	1.0	0.9	0.9	0.9

of Natural Resources Division of Fish and Wildlife (Scientific Collecting Permit 2686), and the procedures in this study were approved by the Institutional Animal Care and Use Committee of Indiana University Kokomo (Protocol 99-1). I thank B. Brodman and the staff of the Salamonie Lake State Reservoir, especially T. Edwards, for their assistance in obtaining animals. This manuscript was improved with suggestions from A. Gillem and two anonymous reviewers.

## LITERATURE CITED

- ANGILLETTA, M. J., AND M. W. SEARS. 2000. The metabolic cost of reproduction in an oviparous lizard. *Functional Ecology* 14:39–45.
- DEMARCO, V., AND L. J. GUILLETTE JR. 1992. Physiological cost of reproduction in a viviparous lizard (*Sceloporus jarrovi*). *Journal of Experimental Zoology* 262: 282–390.
- DOWNS, F. L. 1989. Family Ambystomatidae. In R. A. Pflingsten and F. L. Downs, (eds.), *Salamanders of Ohio*. Ohio Biological Survey Bulletin 7:87–172.
- FINKLER, M. S., AND D. L. CLAUSSEN. 1999. Influence of temperature, body size, and inter-individual variation on forced and voluntary swimming and crawling speeds in *Nerodia sipedon* and *Regina septemvittata*. *Journal of Herpetology* 33:62–71.
- FINKLER, M. S., AND K. A. CULLUM. 2002. Sex-related differences in metabolic rate and energy reserves in spring-breeding Small-Mouthed Salamanders (*Ambystoma texanum*). *Copeia* 2002:824–829.
- FINKLER, M. S., M. T. SUGALSKI, AND D. L. CLAUSSEN. 2003. Sex-related differences in metabolic rate and locomotor performance in breeding Spotted Salamanders (*Ambystoma maculatum*). *Copeia* 2003:887–893.
- FITZPATRICK, L. C. 1973. Energy allocation in the Allegheny Mountain Salamander *Desmognathus ochrophaeus*. *Ecological Monographs* 43:43–58.
- HOMAN, R. N., J. M. REED, AND L. M. ROMERO. 2003. Corticosterone concentrations in free-living Spotted Salamanders (*Ambystoma maculatum*). *General and Comparative Endocrinology* 130:165–171.
- KLEIBER, M. 1961. *The Fire of Life*. Wiley, New York.
- MINTON, S. A. 2001. *Amphibians and Reptiles of Indiana*. 2nd ed. Indiana Academy of Science, Indianapolis.
- MORBAY, Y. E., AND R. C. YDENBERG. 2001. Protandrous arrival timing to breeding areas: a review. *Ecology Letters* 4:663–673.
- OLSSON, M., R. SHINE, AND E. BAK-OLSSON. 2000. Locomotor impairment of gravid lizards: is the burden physical or physiological? *Journal of Evolutionary Biology* 13:263–268.
- PETRANKA, J. W. 1998. *Salamanders of the United States and Canada*. Smithsonian Institution Press, Washington, DC.
- PLUMMER, M. V. 1977. Observation on breeding migrations of *Ambystoma texanum*. *Herpetological Review* 8:79–80.
- ROME, L. C., E. D. STEVENS, AND H. B. JOHN-ALDER. 1992. The influence of temperature and thermal acclimation on physiological function. In M. E. Feder and W. W. Burggren (eds.), *Environmental Physiology of the Amphibians*. University of Chicago Press, Chicago.
- SEMLITSCH, R. D., D. E. SCOTT, J. PECHMANN, AND J. W. GIBBONS. 1993. Phenotypic variation in the arrival time of breeding salamanders: individual repeatability and environmental influences. *Journal of Animal Ecology* 62:334–340.
- WILSON, B. S., AND J. C. WINGFIELD. 1994. Seasonal and interpopulational variation in plasma levels of corticosterone in the Side-Blotched Lizard (*Uta stansburiana*). *Physiological Zoology* 67:1025–1049.

Accepted: 17 November 2005.