

EFFECTS OF TEMPERATURE AND TIME OF DAY ON METABOLIC RATE IN THE SALAMANDERS *DESMOGNATHUS OCHROPHAEUS* AND *PLETHODON WEBSTERI*

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ABSTRACT—Resting metabolic rates were measured in the salamanders *Desmognathus ochrophaeus* and *Plethodon websteri*. Metabolic rate increased significantly with temperature from 5 to 25°C for both species. *Desmognathus ochrophaeus* of ≤ 0.27 g had significantly higher mass-specific rates of oxygen consumption than conspecifics weighing 0.69 to 1.03 g. Time of day had a significant effect on metabolic rate for both species.

Salamanders of the family Plethodontidae, the lungless salamanders, are widespread throughout the eastern United States. Adults lack gills and lungs and rely on cutaneous respiration to supply their oxygen demands. These salamanders are most active on wet or humid nights. During the day, they can be found in burrows, under moist logs, or in wet areas (Conant and Collins, 1991).

Desmognathus ochrophaeus is a widespread salamander with a range extending from Georgia to New York and including eastern Tennessee (Conant and Collins, 1991). The effect of temperature and acclimation on metabolic rate have been studied in adults of this species (Fitzpatrick and Brown, 1975; Feder, 1985a, 1985b; Gatten et al., 1992) but, to our knowledge, not in juveniles. We were interested in determining if there were ontogenetic changes in metabolic rate in this species. We thought juveniles might have higher metabolic rates than expected to enhance rapid growth.

In contrast to *D. ochrophaeus*, *Plethodon websteri* is restricted to woodlands in Alabama and Georgia with isolated populations in South Carolina, Mississippi, and Louisiana (Conant and Collins, 1991). This species was recently described (Highton, 1979), and its life history and ecology have been studied (Semlitsch and West, 1983). However, the physiology of this species has not been investigated. Our purposes were to compare the effects of temperature and time of day on resting metabolic rates in two similarly-sized woodland salamanders, juvenile *D. ochrophaeus* and adult *P. websteri*, and to determine the effect of body size on metabolic rate in juvenile *D. ochrophaeus*.

MATERIALS AND METHODS

Juvenile *D. ochrophaeus* (0.43-1.11 g) collected in Macon Co., North Carolina, in June 1992 and *P. websteri* (0.52-0.72 g) collected in Cullman Co., Alabama, in March 1992 were maintained on a 13L:11D photoperiod at 15°C for a minimum of 12 days. The salamanders were housed with moist paper towels in clear 12- by 30-cm plastic boxes in groups of three to seven individuals. They were fed fruit flies (*Drosophila*) but were not fed for 7 days prior to experimentation.

The rate of oxygen consumption was measured with a Gilson Differential Respirometer (model ICRP-14). One milliliter of 10% KOH was placed in the sidearms of each reaction vessel to absorb

exhaled CO₂. Tap water was placed in the central well of each 15-ml reaction vessel. The salamanders were placed in the vessels 90 min prior to the beginning of measurements, and oxygen consumption was measured at 10-min intervals from 1300 to 1500 h. The lowest consecutive 30-min value was used as the resting metabolic rate for each animal and multiplied by 2 to give 1-h values. During equilibration and testing, the respirometer water bath was covered with a black cloth to reduce the effect human movements might have on the animals. After oxygen consumption was measured, each salamander was weighed to the nearest 0.01 g.

In the first experiment, we measured the effect of the dependent variable temperature on rate of oxygen consumption. Data were collected at 5, 10, 15, 20, and 25°C. Sample sizes varied from three to seven individuals per treatment. Data comparing rate of oxygen consumption at different temperatures were analyzed with a one-way analysis of variance and Student-Newman-Keuls multiple range test. Student's *t*-tests were used to compare data between *D. ochrophaeus* and *P. websteri* at each temperature. A linear regression of metabolic rate against temperature was conducted for each species to determine the slope of the relationship between the two variables.

In the second experiment, we measured the effect of the dependent variable mass on rate of oxygen consumption in *D. ochrophaeus* at 15°C. Data were gathered on seven small-sized (0.09-0.27 g; $\bar{X} = 0.19$ g) and five medium-sized individuals (0.69-1.03 g; $\bar{X} = 0.82$ g). Student's *t*-tests were used to compare data between small- and medium-sized *D. ochrophaeus*. A linear regression of the log of oxygen consumption (microliters per hour) against the log of body mass (grams) was used to determine the relationship between oxygen consumption and body mass in immature individuals.

In the third experiment, we measured the effect of the dependent variable time of day on rate of oxygen consumption in four *P. websteri* and four medium-sized *D. ochrophaeus* at 10°C. Oxygen consumption was measured for 30 min of each hour (except 1600 and 1900 h) of a 24-h period beginning at 1300 h. A two-way analysis of variance with repeated measures was used to compare the mean rates of oxygen consumption between the two species and hourly rates of oxygen consumption over the 24-h period.

All previous measurements were made in June and July 1992. In a fourth experiment, we measured rate of oxygen consumption in seven

medium-sized *D. ochrophaeus* in October 1992 and compared these measurements with those made in July to determine if there was an endogenous seasonal effect on the rate of oxygen consumption. Student's *t*-tests were used to compare data between months.

All values in each experiment were converted to microliters per gram times hour and adjusted to Standard Temperature and Pressure Dry. Values were considered significant at $P \leq 0.05$. Values are given as $\bar{X} \pm 1 SE$.

RESULTS

Temperature had a significant effect on the rate of oxygen consumption for both *P. websteri* ($F = 30.02$, $d.f. = 4$, $P < 0.001$) and *D. ochrophaeus* ($F = 22.99$, $d.f. = 5$, $P < 0.001$). There was a significant difference in rate of oxygen consumption between 10 and 15°C ($P < 0.05$, $d.f. = 6$) and between 20 and 25°C ($P < 0.05$, $d.f. = 6$) for *P. websteri* and between 15 and 20°C ($P < 0.05$, $d.f. = 9$) and between 20 and 25°C ($P < 0.05$, $d.f. = 11$) for *D. ochrophaeus* (Fig. 1). There was no significant difference in rate of oxygen consumption between species at any temperature. The linear regression equation showing the relationship between oxygen consumption and temperature was: oxygen consumption = 3.31 (temperature) - 10.3 ($r^2 = 0.83$) for *D. ochrophaeus*; and oxygen consumption = 3.52 (temperature) - 13.4 ($r^2 = 0.83$) for *P. websteri*.

In the second experiment, body mass had a significant effect on rate of oxygen consumption at 15°C in *D. ochrophaeus*. Small individuals had significantly higher ($P \leq 0.015$, $d.f. = 10$) mass-specific rates of oxygen consumption than did medium-sized conspecifics. Rate of oxygen consumption was $51.5 \pm 4.2 \mu\text{l/g}\cdot\text{h}$ for the small individuals and was $38.7 \pm 6.4 \mu\text{l/g}\cdot\text{h}$ for the medium-sized individuals. The linear regression equation for the relationship between oxygen consumption and mass in juvenile *D. ochrophaeus* was: $\log \text{ oxygen consumption} = 0.79 (\log \text{ mass}) + 1.56$ ($r^2 = 0.87$).

The mean rate of oxygen consumption over the entire 24-h test period in the third experiment was significantly higher ($F = 9.167$, $d.f. = 1$, $P = 0.023$) for *P. websteri* ($41.5 \pm 13.2 \mu\text{l/g}\cdot\text{h}$) than that for *D. ochrophaeus* ($20.3 \pm 4.4 \mu\text{l/g}\cdot\text{h}$). The values during mid-photophase (1300-1500 h) were not significantly different between the two species. Time of day had a significant effect ($F = 3.658$, $d.f. = 21$, $P \leq 0.001$) on rate of oxygen consumption for both species (Fig. 2). The pattern of oxygen consumption over time was significantly different ($F = 2.011$, $d.f. = 21$, $P \leq 0.01$) for the two species. The highest rates of oxygen consumption were recorded during scotophase for both species.

In the fourth experiment, season did not significantly affect rate of oxygen consumption in *D. ochrophaeus* at 15°C. In July, rate of oxygen consumption was $34.7 \pm 3.6 \mu\text{l/g}\cdot\text{h}$, and, in October, it was $33.3 \pm 4.8 \mu\text{l/g}\cdot\text{h}$.

DISCUSSION

An increase in the rate of oxygen consumption with temperature as shown in *P. websteri* and *D. ochrophaeus* occurs in many Plethodonids (Fitzpatrick et al., 1972; Feder, 1976b; Brown and Fitzpatrick, 1981; Gatten et al., 1992). Juvenile *D. ochrophaeus* and adult *P. websteri* have similar body mass, and, therefore, it was not surprising that rates of oxygen consumption were similar at each of the five test temperatures.

Until recently, *P. websteri* was included within the species *Plethodon dorsalis*. The two species are similar in size, color, and habitat but differ genetically (Highton, 1979). No physiological comparison has been made between these two species; therefore, we compared rates of oxygen consumption between *P. websteri* and literature values for *P. dorsalis*. *Plethodon dorsalis* acclimated to 15°C had higher mass-

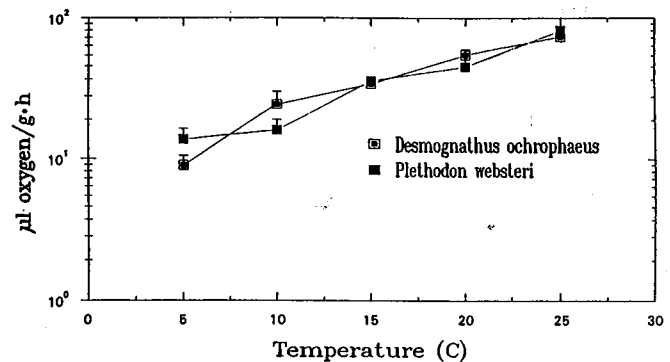


FIG. 1. Mean metabolic rate of *Desmognathus ochrophaeus* and *Plethodon websteri* at 5, 10, 15, 20, and 25°C. Vertical lines represent 1 SE.

specific rates of oxygen consumption at 10, 15, and 20°C (Brown and Fitzpatrick, 1981) than did *P. websteri*. Possibly, the morphologically similar *P. websteri* and *P. dorsalis* have physiological as well as genetic differences.

The small *D. ochrophaeus* had significantly higher mass-specific rates of oxygen consumption than did medium-sized, conspecific salamanders. The difference in mass between the small- and medium-sized *D. ochrophaeus* was about four-fold. The smallest individuals had higher oxygen-consumption values per gram of body weight than did the medium-sized salamanders. The linear regression of \log_{10} oxygen consumption against \log_{10} mass in the present study was the same as the linear regression equation calculated for lungless salamanders at 15°C (Feder, 1976a). Feder's (1976a) equation ($\log \text{ oxygen consumption} = 0.817 [\log \text{ mass}] + 1.61$) included data from many species of lungless salamanders, and most of his data were from animals larger than those in the present study. Although the small-sized salamanders had significantly higher mass-specific oxygen consumption rates than did the medium-sized individuals, both groups had mass-specific oxygen consumption rates that were predicted from the regression equations for oxygen consumption versus mass for lungless salamanders.

Even under resting conditions, rates of oxygen consumption often are higher during the normal activity period of an amphibian. The nocturnal toad *Bufo marinus* increased rates of oxygen consumption for the first 2-3 h of scotophase regardless of whether it was on an 8L:16D, 12L:12D, or 16L:8D photoperiod (Hutchison and Kohl, 1971). Newly metamorphosed *Xenopus laevis*, a nocturnal frog, had a higher rate of oxygen consumption at night than during the day (Abel et al., 1992). Resting rates of oxygen consumption of the diurnal frogs *Dendrobates auratus* (at 25°C) and *Colostethus inguinalis* (at 20°C) were higher during photophase than scotophase (Taigen and Pough, 1983). *Ambystoma tigrinum* at 15°C showed an increase in rate of oxygen consumption at the onset of scotophase and a decrease at the onset of photophase (Hutchison et al., 1977). A significant diel pattern in rate of oxygen consumption was observed in *P. websteri* and *D. ochrophaeus* in the present study. Oxygen consumption at night was significantly higher than that observed during the day (Fig. 2).

Not all amphibians show significant diel cycles in metabolic rate. Diurnal Dendrobatid frogs (Taigen and Pough, 1983), nocturnal scorpions (*Centruroides sculpturatus*; Hadley and Hill, 1969), and carp (*Cyprinus carpio*; Chakraborty et al., 1992) maintained on a 12L:12D photoperiod lack diel cycles in metabolic rate.

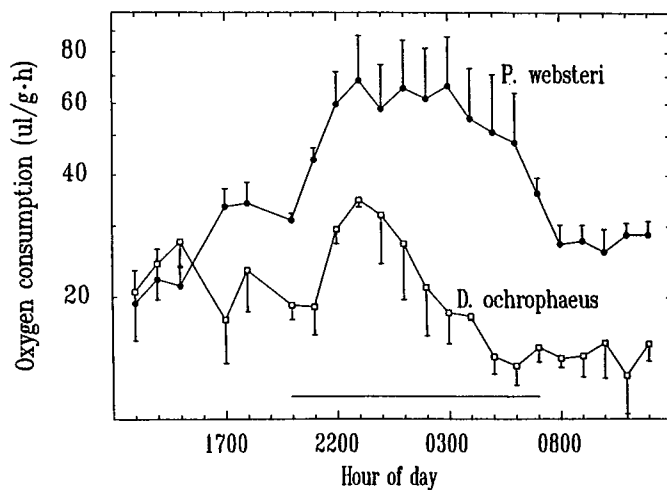


FIG. 2. Mean metabolic rates of *Desmognathus ochrophaeus* and *Plethodon websteri* over a 24-h period at 10°C. Vertical lines represent 1 SE. The horizontal line indicates scotophase.

Woodland salamanders are active at night. For *Desmognathus fuscus*, a close relative of *D. ochrophaeus*, evening movement peaks between 2100 and 2400 h (Barbour et al., 1969). Activity of *D. ochrophaeus* occurs throughout the night under humid conditions but is restricted or completely inhibited by dry conditions (Hairston, 1949). In our study, where humidity remained constant, peak rates of oxygen consumption were between 2200 and 0200 h. Moisture probably controls nocturnal activity in *P. websteri* (Semlitsch and West, 1983). In the laboratory where humidity levels did not restrict activity, the patterns of oxygen consumption during the nocturnal activity period of these two Plethodontid species varied greatly.

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