

THE ENERGETIC COST OF COURTSHIP AND AGGRESSION IN A PLETHODONTID SALAMANDER¹

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Abstract. The aerobic and anaerobic energetic contributions to courtship and aggression between males were measured in the plethodontid salamander *Desmognathus ochrophaeus* in the laboratory. Average rate of oxygen consumption and average lactate content were increased significantly above resting levels during both courtship (O_2 : $+0.029 \text{ mL} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$; lactate: $+0.058 \text{ mg/g}$) and aggression (O_2 : $+0.040 \text{ mL} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$; lactate: $+0.172 \text{ mg/g}$). However, these are rather small increments, representing rises of only 38–56% in oxygen consumption and only 8–23% of anaerobic capacity. The cost of a courtship bout is estimated to be 2.70 J/courting pair and is derived 90% aerobically. This value represents $\approx 1\%$ of the daily ingested energy and secondary productivity for this species, based on estimates in a previous study. It thus seems unlikely that the behavior patterns of courtship or aggression in this species are subject to energetic limitations.

Key words: aggression; anaerobic metabolism; courtship; *Desmognathus*; ecological energetics; lactic acid; metabolism; oxygen consumption; plethodontid; salamander.

INTRODUCTION

Do certain behaviors result in a relatively great expense in comparison with energetic intake or productivity, and thus form a severe drain on energy resources? Do some behaviors require energy utilization at rates that are close to the limits of energy delivery, and thus run the risk of entailing fatigue or inadequate performance? Although there has been much speculation over the answers to these kinds of questions concerning the energetic costs of behavior (e.g., Townsend and Calow 1981), there are relatively few actual measurements of costs of natural behaviors. In practice, such measurements are generally difficult at best.

Because courtship behavior plays such a crucial role in reproduction, determination of its energetic significance is particularly desirable. Is courtship expensive and does it require energy delivery at close to physiological limits? Most animals will not court and complete insemination under the restricted conditions necessary for metabolic measurements. However, salamanders will exhibit highly stereotyped courtship behavior under laboratory conditions (Organ 1961, Sawada 1963, Arnold 1972, 1976, Halliday 1977a). In particular, some terrestrial forms will court in small closed containers, making it possible to measure both aerobic and anaerobic energetic contributions to the courtship

bout. The former is measured by oxygen consumption, which supports low-level and sustainable activity. The latter is determined from increments in lactic acid content, which is virtually the sole anaerobically formed end product of vertebrate activity metabolism (Bennett 1978). Anaerobic metabolism is used under conditions of intense activity and when oxygen delivery is inadequate to support energy demand.

We have measured these costs in the plethodontid salamander *Desmognathus ochrophaeus*. This is a small (1–3 g adults), lungless salamander which is very common in the Appalachian region of the eastern United States. This species is easily maintained in large numbers and will court willingly in the laboratory from September through June. Females (in the field) store sperm until spring or early summer when ova are fertilized just prior to oviposition. The courtship pattern of *D. ochrophaeus* is typical of that of other plethodontids that court on land (Organ 1961, Arnold 1972, 1977). In a complete courtship sequence, the male typically initiates courtship by making physical contact with the female. During this preliminary stage, the male delivers courtship pheromones to the female (Arnold and Houck 1982). At first, the female usually attempts to flee or else remains relatively motionless. The female indicates receptivity by following the male, straddling his tail between her legs, and placing her chin on his tail base. The male then deposits a spermatophore on the substrate, the pair advances, and the female picks up the sperm mass in her vent. Males will not deposit a spermatophore unless the female is receptive and is in the tail-straddling position. This courtship behavior is complex, involving a series of

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interactions between the male and the female, but these interactions are relatively stereotyped and readily identifiable. The natural timing of courtship bouts is unknown as observations of complete courtships in the field are nonexistent or rare for plethodontid species. Courtship in the laboratory, however, usually lasts for >1 h. We measured oxygen consumption in courting and noncourting pairs of *D. ochrophaeus*. The latter served as a control for determination of the cost of courtship. Rates of oxygen consumption of aggressive male-male pairs and of individual males and females were also measured. Lactate contents were determined in these animals and in an additional group of exercised males. Lactate contents in the exercised animals permitted determination of the total possible lactate formation (the anaerobic capacity).

METHODS

All *Desmognathus ochrophaeus* were collected near the top of Unaka Mountain (elevation 1500 m), Unicoi County, Tennessee, in August of either 1980 or 1981. Salamanders were brought to the laboratory within 1 wk of collection and were placed in individually labelled plastic containers (31 × 17 × 9 cm). Salamanders were maintained at 15°C on a natural (Chicago) photoperiod with food (*Drosophila* spp.) available ad libitum. All animals used were adults that recently had shown courtship activity in the laboratory; 69 males (mean mass ± SE = 2.05 ± 0.045 g) and 39 females (mean mass ± SE = 1.48 ± 0.049 g) were used in this study, which was undertaken from September through November of 1981.

Measurements of oxygen consumption or whole-body lactate (analyses described below) or both were made for animals under five experimental situations. Unless noted otherwise, the following conditions apply to all experiments. Salamanders were placed in clear plastic boxes (≈15 × 9.5 × 4 cm) of measured volume, lined with moist paper toweling. Each box was also fitted at one end with a three-way stopcock valve through which air samples could be withdrawn into a syringe for analysis of oxygen content. Twenty millilitres of air were withdrawn at the beginning and end of a measurement period. Samples were taken in 20-mL glass syringes and stored with metal syringe caps. When air samples were taken, ambient air was allowed to replace each sample in the chambers.

Experiments were staged in the same temperature-controlled room (15°C) in which the salamanders were housed. Light was provided by two or three 25-W red light bulbs. Observations were made between ≈1700 and 2300 (CST), because these salamanders are normally active at night. To minimize effects of handling, salamanders were introduced to their experimental boxes at least 30 min prior to air sampling. Salamanders were observed continuously during all experiments, and activity levels and interactions were re-

corded. Body mass of each salamander was measured to the nearest 0.01 g within 24 h of each experiment.

The following five experimental groups were observed.

- 1) Courting pairs. Encounters were staged between one female and one male, and air samples were taken at the beginning and end of a courtship sequence for 15 pairs of animals. Courtship was never initiated during the 30-min equilibration period. The beginning of courtship was defined as the time when the male first showed sustained interest in the female and consistently made (or attempted to make) physical contact that eventually led to mutual courtship interest and spermatophore deposition. The end of courtship was taken as the moment shortly after spermatophore deposition when the female had picked up or attempted to pick up the sperm mass and moved away from the male. Males and females from 9 of the 15 pairs were sacrificed immediately after the termination of courtship for whole-body lactate analysis.
- 2) Noncourting pairs. Oxygen consumption was measured for 11 male-female pairs that showed no courtship activity and minimal locomotory behavior during the observation period. Both the male and the female of each pair remained essentially inactive after being placed in the experimental box.
- 3) Male-male pairs. An aggressive encounter between two males was defined as a period of 20 min characterized by sustained interactions including biting, chasing, and aggressive displays such as open-mouth threats. To maximize the occurrence and intensity of aggression, one male of each pair (the "resident") was placed in the experimental box (along with food and extra toweling for shelter) for 7 d prior to the staged encounter. During the actual encounter, the extra toweling and food were removed, and a second male (designated the "intruder") was introduced into the resident's box. Observations began immediately, but the initial air sample was not taken until the two males first showed sustained aggressive interactions. At the end of 20 min, a final air sample was taken, and each male was sacrificed immediately for lactate analysis. Sample size was 12 pairs.
- 4) Individual animals. Measurements of resting oxygen consumption were taken for single individuals (six males and six females) over a period of 1 h, while the salamander remained motionless. Immediately after the final air sample was taken, each male was sacrificed for lactate analysis.
- 5) Exercised males. Estimates of maximal values for whole-body lactate were obtained for six males, each of which was manually stimulated with blunt-nosed forceps for 5 min. During this time, the salamander struggled intensely and remained constantly in motion until it was close to losing its righting response.

TABLE 1. Oxygen consumption ($\text{mL} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) by the salamander *Desmognathus ochrophaeus* during courtship and aggression.

	Individuals			Male-female pairs		Male-male pairs
	Males	Females	Average	Noncourting	Courting	Aggression
Mean	0.088	0.092	0.090	0.072	0.099	0.112
SE	0.0113	0.0154	0.0098	0.0066	0.0071	0.0096
n	6	6	12	11	15	12

Oxygen consumption during the measurement intervals was determined by analysis of gas composition of the air samples. Samples were injected into a model S-3A oxygen analyzer (Applied Electrochemistry, Sunnyvale, California) with a syringe pump (Razel, Stamford, Connecticut). Injections were made through a column of Ascarite and Drierite for removal of carbon dioxide and water vapor, respectively. Each pair of samples was preceded and followed by a sample of outdoor air for standardization. Oxygen consumption was calculated according to the formula for closed-system oxygen analysis with sequential air replacement given by Bennett and Gleeson (1976). All volumes reported are corrected to standard temperature and pressure, dry (STPD) conditions.

Lactate contents of individual animals were determined by homogenization and analysis of animal carcasses, according to the technique of Bennett and Licht (1972). Individual salamanders were decapitated and homogenized in 20 mL of 0.6 mol/L perchloric acid with a model PCU-2-110 Polytron (Brinkmann Instruments, Westbury, New York). Homogenates were stored at 4°C for subsequent analysis. Lactate contents of filtered homogenates were measured with lactate analysis kits (No. 149993; Boehringer-Mannheim, Mannheim, Germany) on a model 191 digital spectrophotometer (Hitachi, Mountain View, California). Lactate concentrations are expressed as milligrams lactate per gram body mass.

RESULTS

Behavior

Behavior was variable among salamanders exposed to experimental conditions. Among male-female pairs, for example, we observed instances in which the male would begin to court the female but would persist only intermittently with significant intervals of noncourtship behavior. Likewise, among encounters staged between two males, there were cases in which little or no aggressive behavior occurred. Because of this variability, observations of many encounters were eliminated from analysis as being ambiguous. The observations reported include only those in which the activity was sustained throughout the observational period.

Courtship encounters averaged 1.23 h (± 0.15 h SE, range = 0.37–2.55 h). Measurements of oxygen con-

sumption in noncourting pairs were made over periods of similar duration.

Oxygen consumption

Levels of oxygen consumption (Table 1) were significantly different among groups (one-way ANOVA, $F = 2.75$, $P = .04$). Courting pairs have a significantly greater metabolic rate than do noncourting pairs (Student's $t = 2.62$, $P = .014$), but this represents a relatively small elevation of only 38%. Aggression in male-male pairs likewise increases metabolic rates an average of 56% above noncourting levels ($t = 3.40$, $P = .003$). Males and females measured individually do not have different rates of resting metabolism ($t = 0.17$, $P = .86$). Levels of oxygen consumption are not significantly different between the groups of individual animals and individuals of the noncourting pairs ($t = 1.49$, $P = .14$).

Neither the inactive individuals nor those in the noncourting pairs maintained minimal (standard) rates of oxygen consumption, which have been measured to be about half the values obtained in these experiments (Fitzpatrick 1973). Experimental conditions were not designed to attain minimal metabolic levels (i.e., animals were not fasted, darkness was not complete, equilibration time was short).

Lactic acid formation

Lactate concentrations (Table 2) were significantly different among groups (one-way ANOVA, $F = 11.97$, $P < .001$). There are no differences in the lactate concentrations in males and females in courting pairs (paired $t = 1.17$, $P = .28$) or in residents and intruders during aggression (paired $t = 0.28$, $P = .78$). Data are combined within each of these groups for respective comparisons with inactive individuals or members of noncourting pairs.

Both courtship ($t = 2.69$, $P = .013$) and aggression ($t = 2.72$, $P = .011$) involve a significant amount of anaerobic metabolism, lactate levels being double and quadruple resting levels, respectively. However, this lactate formation is small in comparison to the anaerobic capacity of these animals. Courtship and aggression utilized only 8 and 23%, respectively, of the potential anaerobic energy formation, as judged by the values for the exercised males. During the experiments on aggression, two animals attained levels of

TABLE 2. Lactate concentrations (mg/g body mass) of the salamander *Desmognathus ochrophaeus* after rest, exercise, courtship, and aggression.

	Rest	Exercised	Postcourtship			Postaggression males		
	Males	Males	Males	Females	Average	Resident	Intruder	Average
Mean	0.061	0.795	0.130	0.108	0.119	0.242	0.225	0.233
SE	0.0066	0.1053	0.0208	0.0124	0.0121	0.0457	0.0442	0.0311
n	6	6	9	9	18	12	12	24

lactate content, 0.73 and 0.69 mg/g, that are not significantly different ($t = 0.22$ and 0.38 , $P > .7$) from those of the exercised group. The full anaerobic capacity is, thus, occasionally utilized during combat by some individuals.

The cost of courtship

The average cost of a courtship bout of activity may be calculated from the foregoing data. The oxygen consumption of the noncourting pair group is used as the comparative standard to assess the cost of courtship, rather than that of the individually measured animals. Its use permits the calculation of the energy associated with the physical activities associated with courtship per se. The aerobic contribution (as O_2 consumption, 0.122 mL/pair) is the product of the differential between the average oxygen consumption of courting and noncourting pairs ($0.027 \text{ mL} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$), the average mass of each pair (3.66 g), and the average duration of the courtship bout (1.23 h). This quantity is equivalent to 2.44 J (at 20.1 J/mL O_2 consumed). The anaerobic contribution (as lactate production, 0.21 mg/pair) is the product of the average differential between postcourting and resting lactate contents (0.058 mg/g) and the average mass of the courting pair. This lactate formation represents the release of 0.26 J/courting pair (calculated as the energy released during catabolism of glycogen to lactate in glycolysis = 109.8 J/mole lactate formed [Mahler and Cordes 1966]). The combined courtship cost is 2.70 J/courting pair, with aerobic metabolism accounting for $\approx 90\%$ of the total and anaerobic metabolism supplementing with an additional 10%.

DISCUSSION

Both courtship and aggression among males involve significant aerobic and anaerobic metabolic costs. These increments are, however, low in both the absolute amounts of energy involved and the proportion of potential energy utilization that they represent. Small plethodontid salamanders may increase oxygen consumption ≈ 10 -fold above resting levels during stimulated activity (Bennett and Licht 1973, Hillman et al. 1979), but the *Desmognathus ochrophaeus* in the encounters reported here did not even double oxygen consumption. Likewise, an average of less than a quarter of the anaerobic capacity was utilized even

during aggression, although 2 animals among 24 did sustain very high lactate levels. Thus, energy expenditure by the salamanders during our experiments did not rise greatly above resting levels. The activity capacity of salamanders may be more fully exploited during predator avoidance. Lactate concentrations in the salamander *Plethodon jordani* rose to very high levels (1.05 mg/g) during attempts to escape from snake predators (Feder and Arnold 1982).

The amount of energy expended by a courting pair may be compared to various components of the energy budget of this species. A pair of salamanders acclimated to 15°C and fed ad libitum in the laboratory ingests $\approx 3.98 \text{ mW}$, assimilates 3.50 mW, and produces 2.77 mW (Fitzpatrick 1973). Thus, the energy utilized in a courtship bout, 2.70 J, is $<1\%$ of the energy ingested daily and is only $\approx 1\%$ of the potential secondary productivity of a courting pair. The expended energy could be recovered by the ingestion of $<0.2 \text{ mg}$ dry mass of food. Even if standard metabolic rate (as measured by Fitzpatrick 1973) is used instead as a comparative baseline measurement, the cost of a courtship bout is still only 6 J or only $\approx 2\%$ of the energy ingested daily or productivity. The energetic costs of courtship to the salamanders, thus, appear to be minimal within the context of their daily energy budgets. The cost of formation of the reproductive products (sperm mass and spermatophore by the male, ova by the female) is not known.

Direct measurements of energetic relations of reproductive activities are rare for vertebrates. A few determinations have been made for other species of amphibians. In the leptodactylid frog *Physalaemus pustulosus*, calling by males more than doubles their rate of oxygen consumption and building foam nests quadruples metabolic expenditure above resting levels (Bucher et al. 1982). Experiments on the newt *Triturus vulgaris*, which courts underwater, indicate that males may experience oxygen depletion during courtship (Halliday and Sweatman 1976, Halliday 1977b). Males postpone breathing because they may fail to locate the female after surfacing. Thus in *Triturus*, the duration and intensity of courtship are directly affected by the physiological capacities of the males. Our data indicate, however, that reproductive activity and even intraspecific aggression in a small terrestrial salamander

need not be expensive, even if the activity is protracted. These activities are carried out well within the physiological limits of energy utilization.

We conclude that the courtship patterns in *D. ochrophaeus* are not subject to energy limitations. A male will court a female persistently until the female indicates a willingness to accept a spermatophore. The long and intricate courtship serves to synchronize the readiness of the female to be inseminated during the actual act of spermatophore deposition. Arnold (1976) has shown for another terrestrial plethodontid (*Plethodon jordani*) that this synchronization is critical. Unlike the situation with pond-breeding ambystomatid salamanders, plethodontid females will not pick up the sperm mass from a spermatophore except during a tail-straddling walk. Our results show that the cost of this courtship activity is so low that priority can be according to determining the certainty of female receptivity rather than to energetic conservation.

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