

Anaerobic Metabolism and Behavior During Predatory Encounters Between Snakes (*Thamnophis elegans*) and Salamanders (*Plethodon jordani*)

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Summary. 1. We staged predatory encounters between snakes and salamanders in the laboratory.

2. Both snakes and salamanders accumulated significant quantities of lactic acid (0.3–2.3 mg/g) during predatory encounters. In a typical 14.4 min encounter, lactate concentration reached 260% of resting levels in snake predators and 880% of resting levels in salamander prey. This is one of the first demonstrations of anaerobiosis during natural ‘burst’ activity in both predator and prey.

3. Salamander responses to snake attack were surprisingly variable, and included writhing, thrashing, tail wrapping, tail autotomy, and biting. Antipredator responses were effective in nearly one third of trials. Lactate concentration was significantly correlated with duration of predatory encounters ($r=0.57$), snake mass, and salamander mass (Table 3).

4. Our calculations suggest that attack and ingestion of prey may cost snakes less than 1% of energy assimilated from prey.

the extent of anaerobiosis during burst activity. Although the causal basis of this correlation is poorly understood (Pough 1981; Taigen et al. 1982), behavior during predatory encounters is probably sufficiently vigorous to engender anaerobiosis. Accordingly, predatory encounters are especially suitable for demonstration of anaerobiosis during spontaneous natural activity, if it does occur.

A secondary aim was to examine variation in anaerobic energy mobilization and duration of predator defense as related to body size of both predator and prey. Pough (1977, 1978, 1981) has characterized ontogenetic increases in body size, stamina, and aerobic support systems and decreases in anaerobiosis in snakes (*Nerodia*, *Thamnophis*). These data suggest that metabolic patterns are an important determinant of prey choice and predator defense in snakes (see also Ruben 1976), and that ontogenetic changes in predatory and defensive behavior are coupled with changes in activity metabolism (Pough 1977, 1978, 1981). Furthermore, it may be possible to characterize relative prey sizes or breaking points above which snake metabolism is insufficient to subdue salamander prey, or below which salamander metabolism is insufficient to sustain escape from snake predators (Arnold 1982). To examine this possibility we varied both the relative and absolute size of snakes and salamanders in staged predatory encounters.

Materials and Methods

Garter snakes (*Thamnophis elegans*) were captured near Arcata, Humboldt Co., CA. Salamanders (*Plethodon jordani*) were captured near Mt. Rodgers, Giles and Smyth Co., Virginia. Snakes were maintained at 27° C and fed live fish. Salamanders were maintained at 15° C and fed *Drosophila*. All animals were maintained on a natural (Chicago) photoperiod and held in plastic boxes (17 cm × 31 cm × 9 cm depth) with paper towelling on the bottom.

Immediately prior to each predatory encounter, a plastic box containing a snake was transferred to a room at 20° C. An observer added a salamander (previously at 15° C) to the box and recorded the time and nature of all subsequent behavior of both snake and salamander. The transfer procedure produced no increase in lactate content of either predator or prey (see below). Trials in which no interaction occurred were terminated after 1 h. When snakes attacked salamanders, trials usually ended when the salamander was ingested. At that time the observer immediately forced the snake to regurgitate the salamander (Carpenter 1952), and froze both snake and salamander in liquid nitrogen. Regurgitation and freezing took no more than 5 s.

Introduction

Anaerobic metabolism, chiefly lactate production, is a major source of energy during strenuous ‘burst’ activity in lower vertebrates (Bennett 1978). Dependence upon anaerobiosis may liberate animals from the constraints of their oxygen delivery systems, but may likewise limit the variety and extent of behavior (Bennett 1980).

Despite the evident significance of physiological constraints upon foraging, predator defense, and reproduction, little is known of anaerobic metabolic rates in unrestrained animals in the field. Indeed, most reports to date suggest that reptiles avoid anaerobiosis during natural activity (Bennett et al. 1981; Gatten 1981). The possibility that anaerobiosis may be a laboratory phenomenon with little relation to field behavior lead us to examine lactate production during predatory encounters staged between snakes (*Thamnophis elegans*) and salamanders (*Plethodon jordani*). Likewise, Bennett et al. (1981) have investigated anaerobiosis during territorial encounters in free-ranging lizards.

Snake predation upon plethodontid salamanders elicits many stereotyped and apparently strenuous behaviors. These include writhing and thrashing movements that coat the predator with sticky secretions, tail autotomy (=tail loss), and biting (Arnold 1982). Bennett and Licht (1974) suggested that the particular antipredator behaviors of amphibian species are correlated with

Salamanders sometimes autotomized their tails. In these instances the escaping part (body or tail) was frozen immediately, and the part held by the snake frozen as in the above procedure. In one case an entire salamander escaped; it was frozen immediately.

We define the duration of predatory encounters as the time from the onset of attack until the salamander (exclusive of tail) either disappeared within the profile of the snake's jaws or escaped.

We varied this procedure in several ways. We performed multiple trials with some snakes by not freezing them at the end of a bout. These snakes fed regularly despite repeated forced regurgitation. We stimulated both snakes and salamanders to vigorous burst activity by prodding them with forceps for 2 min, and then froze them. Undisturbed snakes and salamanders were also frozen as controls.

Frozen carcasses were weighed and analyzed for lactic acid content according to the method of Bennett and Licht (1972). Carcasses were shattered, and the pieces homogenized in ice-cold 0.6 N perchloric acid. Lactate content of the homogenate was analyzed enzymatically with clinical reagents (Single Vial Lactate, Biodynamics/BMC, Indianapolis, IN) and expressed in mg/g. In most cases body and tail of salamanders were analyzed separately.

Results

Behavioral Activities During Predation

The outcome of predatory encounters was variable. Snakes successfully ingested salamanders in 15 trials (68%) lasting 0.7–35.0 min. Salamanders (i.e., the body portion) escaped from snakes' jaws in 7 trials (32%).

Salamanders responded to snake attack with a variety of antipredator behaviors (Table 1). All salamanders writhed and thrashed vigorously (Arnold 1982) in immediate response to attack, and this was probably the major cause of anaerobiosis. Usually each bout of this activity lasted only about 10–90 s and was interspersed with much longer intervals (1–5 min) during which the salamander was motionless. These movements applied adhesive secretions to the snake's head and body and seemed to delay ingestion. In addition some salamanders escaped from the snake's jaws as a direct consequence of these movements. Ingestion was also delayed by tail wrapping, in which the tail, covered with adhesive secretions released from epidermal glands, coiled around the snake's head, neck or body. Six salamanders attempted to bite the snake while writhing and thrashing but this did not seem to frustrate ingestion. Salamanders (and some snakes) ceased respiratory movements during predatory encounters.

Tail autotomy occurred in six trials and was usually associated with attacks to the tail or posterior body. Tail autotomy resulted from writhing and thrashing of the salamander as the snake held the salamander's tail in its jaws. Five of six salamanders ran off after twisting free from their restrained tails. Whether snakes could recapture and ingest salamanders after 'escape' is uncertain because we froze salamanders immediately after 'escape'. However, in one instance a snake was clearly unable to pursue an escaping salamander because the salamander's secretions glued the snake to the substrate.

Snakes swallowed salamanders in three distinct ways: head first, tail first, and by folding the salamander so that some point on the trunk was the first to be ingested. The mode of swallowing probably varied according to the site of initial attack, but our

Table 1. Behavioral responses by salamanders and outcome of predatory encounters as functions of the site of initial attack on the salamander

	Site of initial attack on salamander				
	Head <i>n</i> = 7	Front body <i>n</i> = 5	Rear body <i>n</i> = 6	Tail <i>n</i> = 4	Total <i>n</i> = 22
Behavioral responses by salamander:					
Writhing & thrashing	7	5	6	4	22
Tail wrapping	6	5	3	0	14
Tail autotomy	0	1	2	3	6
Biting	1	3	1	1	6
Outcome of encounter:					
Prey ingested					
Head first	5	1	1	0	7
Tail first	0	0	0	2	2
Folded	1	3	2	0	6
Prey escaped					
	1	1	3	2	7

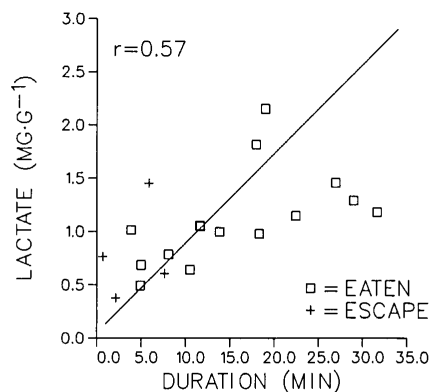


Fig. 1. Relationship between duration of predatory encounters between snakes and salamanders and salamander lactate concentration at the end of encounters. Values for salamanders that were ingested and salamanders that escaped are plotted separately; all values were included in calculation of the regression line

data are too few to demonstrate a statistical association (Table 1). No association was evident between site of attack and probability of escape (*Chi-square* = 3.59, 3 df; $0.2 < P < 0.3$).

In one encounter, tail autotomy occurred 2 s after attack, and the salamander 'escaped'. This trial was omitted from subsequent analyses.

Lactate Concentration

Both snakes and salamanders underwent marked increases in lactate concentration during predatory encounters (Fig. 1, Table 2). The mean encounter duration was 14.4 min. Lactate increased to 260% of resting levels in snakes and 880% of resting levels in salamanders. In salamanders, lactate levels at the end of predatory encounters were approximately equal to levels in salamanders that were exercised for 2 min by the experimenter. The latter salamanders fatigued in 2 min of exercise. By contrast, lactate levels in snakes at the end of the predatory encounters were only 58% of levels after 2 min of forced activity, in which snakes fatigued.

Table 2. Body mass (g) and lactate concentration (mg/g) in experimental subjects. In each cell, upper value is mean \pm standard error; lower value is sample size

Treatment:	Mass (g)			Lactate concentration (mg/g)		
	Snout-vent mass	Tail mass	Total mass	Snout-vent	Tail	Whole body
<i>Plethodon jordani</i> :						
Snake-salamander encounters	1.79 \pm 0.12 20	0.40 \pm 0.03 19	2.17 \pm 0.14 20	1.00 \pm 0.11 18	1.31 \pm 0.12 18	1.05 \pm 0.10 19
Resting	1.89 \pm 0.19 5	0.48 \pm 0.07 3	2.19 \pm 0.22 5	0.12 \pm 0.01 4	0.14 \pm 0.01 3	0.12 \pm 0.01 5
In experimental box for 1 h	2.13 \pm 0.28 5	0.66 \pm 0.19 5	2.79 \pm 0.45 5	0.10 \pm 0.04 5	0.46 \pm 0.36 5	0.15 \pm 0.09 5
Forced activity for 2 min	1.61 \pm 0.17 3	0.34 \pm 0.07 3	1.95 \pm 0.24 3	1.14 \pm 0.20 3	1.31 \pm 0.26 3	1.17 \pm 0.21 3
<i>Thamnophis elegans</i> :						
Snake-salamander encounters			20.55 \pm 2.50 5			0.82 \pm 0.09 5
Resting			14.90 \pm 2.10 4			0.31 \pm 0.04 4
Forced activity for 2 min			13.37 \pm 3.52 2			1.40 \pm 0.13 2

Table 3. Partial correlation coefficients between duration of predatory encounters, whole body lactate concentration of salamander prey, and sizes of predator and prey. Degrees of freedom are given in parentheses; * = $P < 0.05$; ns = not significant

	Encounter duration	Lactate concentration
Salamander mass		
Controlling for snake mass	0.38 (18)*	0.41 (16)*
Controlling for snake mass and encounter duration	—	0.19 (15) ns
Snake mass		
Controlling for salamander mass	-0.47 (18)*	-0.23 (16) ns
Controlling for salamander mass and encounter duration	—	-0.06 (15) ns

In salamanders attacked by snakes, the tails showed significantly greater lactate concentrations than the bodies of these salamanders ($P < 0.01$; t test for paired comparisons). Tail and body lactate concentrations did not differ significantly in any other experimental treatment.

As expected, lactate concentration of salamanders at the end of predatory encounters was correlated with the duration of encounters (Kendall's $\tau = 0.50$; $P = 0.001$; see also Fig. 1). This correlation was evident both for salamanders attacked by various snakes and among salamanders attacked by a single snake. For example, one snake attacked eight salamanders; Kendall's τ between lactate and encounter duration was 0.78 ($P = 0.016$).

The efficacy of antipredator tactics seemed related to the body sizes of both predator and prey. To discern size relationships, we calculated partial correlation coefficients (Table 3). The coefficients were mainly as expected, indicating that snakes ingest large salamanders more slowly than small salamanders and that large snakes feed more rapidly than small snakes. A significant positive correlation between lactate concentration and salaman-

der size became non-significant when controlled for encounter duration; this suggests that body size *per se* does not affect mass-specific lactate production. No correlation was evident between point of initial attack (Table 1) and either encounter duration or salamander lactate concentration (Kendall's τ ; $P > 0.25$).

We applied discriminant analysis to identify features associated with salamanders' 'escape' from snakes. Only the four escapes for which salamander lactate was measured were included in this analysis. Escape was not associated with any variables describing relative or absolute size of predator or prey. Rather, encounter duration and lactate concentration (which is correlated with encounter duration) weighed heavily in the discriminant function. Apparently escapes occurred before ingestion times expected for salamanders of similar size.

Discussion

Many workers have suggested that anaerobiosis should occur in 'life and death situations' in which rapid mobilization of energy reserves is essential (Bennett and Licht 1974; Bennett 1980). In such situations the advantages of anaerobiosis may outweigh disadvantages such as energetic inefficiency, acidosis, and limited stamina (Bennett 1980). Few observations are available to support these suggestions. Reptiles and amphibians apparently rarely show significant anaerobiosis during routine behavior in the field (Bennett et al. 1981). Moreover, reptiles appear to avoid anaerobiosis even during diving, except when stressed during observation (Bennett et al. 1981; Gatten 1981).

The cryptic habits of reptiles and amphibians often render 'life and death' situations inaccessible to the experimental biologist. We have circumvented this difficulty by staging predatory encounters in the laboratory. During these encounters both predators and prey underwent significant anaerobiosis; in salamanders lactate concentrations at ingestion were comparable to levels in exhausted salamanders (Table 1; Bennett and Licht 1974; Cushman et al. 1976; Hutchison et al. 1977; Feder and Olsen

1978; Hillman et al. 1979). Lactate levels in exhausted snakes were concordant with literature values (Ruben 1976; Gratz and Hutchison 1977; Pough 1977, 1978) but greater than lactate concentration in snakes that attacked salamanders (Table 1).

From these comparisons we suggest that significant anaerobiosis is associated with predatory encounters in the field, and that lactate concentrations after encounters are often comparable to concentrations achieved by mechanical or electrical stimulation in the laboratory. This is the first demonstration of significant anaerobiosis during natural burst activity in an amphibian, and one of only a few for reptiles (Jackson and Prange 1979; Bennett et al. 1981). Hillman et al. (1979) suggested that many studies underestimated aerobic metabolism relative to anaerobic metabolism in active amphibians because of physical artifact associated with measurement technique. Without commenting on their physical explanations, we suggest that normal responses to stress or attack may also reduce oxygen consumption in active amphibians. Salamanders (and snakes in some cases) ceased respiratory movements during encounters and manual stimulation. Even though plethodontid salamanders lack lungs, buccopharyngeal gas exchange accounts for a significant portion of total oxygen uptake (Whitford and Hutchison 1965). It may be that salamanders cease ventilation during intense struggling to escape, and thereby increase dependence upon anaerobiosis.

The simple arena used to stage encounters may have hampered salamander escape. On natural substrates in the field, salamanders may cling to objects or glue detritus to a predator's head and body. Staging of predatory encounters on more natural substrates would be a useful test of these suggestions.

Inasmuch as vigorous struggling and anaerobiosis are considered to be antipredator tactics (Bennett and Licht 1974; Arnold 1982), it is worth asking how effective these tactics are in allowing salamanders to escape predation. In more than two thirds of encounters, salamanders failed to escape from snakes (Table 1). In the remaining encounters, escape was apparently at random and by chance. These figures may underestimate the probability of successful ingestion because we terminated encounters before snakes had an opportunity to recapture escaping prey. In a similar study (Arnold 1982) all salamanders were eaten. In the present study, no obvious differences in absolute or relative size of predator and prey distinguish encounters in which salamanders escaped from those in which salamanders were eaten. Escapes occurred mainly during shifts of snakes' jaws or bodies to facilitate ingestion. Tail autotomy, of course, occurred most frequently when salamanders were seized by or near the tail. Accordingly, chance aspects of swallowing movements, point of capture, and individual variation may do more to determine escape than size relationships in metabolic rates and stamina.

Similarly, it may pay snakes to attack large or difficult prey even if such prey are ingested only occasionally. We have calculated metabolic rates of snakes and caloric contents of salamanders to examine this possibility. Our calculations in general overestimate the energetic cost of predatory behavior, especially because we assume maximal oxygen consumption throughout the predatory encounter. Also, our calculations neglect missed opportunities for thermoregulation and sexual behavior, as well as specific dynamic action, increased water loss and risk of attracting snake predators. Nonetheless, for a 16 g snake ingesting a 2.2 g salamander during a 14.4 min encounter (our mean values), the maximal increment in oxygen consumption would be 1.31 cc oxygen STPD (equation 16 of Bennett and Dawson (1976) and 20° C 'active' equation of Bennett (1981)) and the maximal increase in lactic acid would be 9.28 mg (Table 1). These metabolic increments are equivalent to 6.69 and 2.42 cal

(Hochachka and Somero, 1973) or 27.97 and 10.13 J, respectively. Additionally, in paying an oxygen debt after activity a snake might incur an additional energetic expense of 67% (25° C figure of Gratz and Hutchison 1977), or 25.53 J. These total 63.63 J. In a congeneric salamander, *Plethodon cinereus*, the caloric content is 910 cal/g wet mass (Feder, unpublished data). Hence a 2.2 g salamander represents 2 kcal or 8.39 kJ. Even if a snake assimilates only 87% of this energy (mean of two species from Smith 1976), the net energy intake is 7.30 kJ. Thus the estimated cost of predatory activity, 0.06 kJ, is less than 1% of the energy assimilated from the prey. In view of the huge reward for a successful attack, snakes should attack large prey, even if such attacks are commonly unsuccessful. A single success would more than compensate for multiple failures. Moreover, garter snakes require only a few successful captures of large prey each year to satisfy their energy requirements (Porter et al. 1975).

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