

## A Quantitative Approach to Antipredator Performance: Salamander Defense against Snake Attack

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A factorial experiment was used to evaluate the antipredator performance of salamanders (*Plethodon cinereus*) attacked by garter snakes (*Thamnophis elegans*). Large salamander size, antipredator behavior and the presence of a tail each produced a significant delay in ingestion rate. These three components of the salamander defense system showed simple additive interactions when antipredator performance was measured on a logarithmic scale. When performance was measured on an arithmetic scale, these three components showed multiplicative effects or synergisms.

THE growing literature on antipredator responses by salamanders has revealed a surprising array of tactics. Under the duress of predator attack, salamanders may remain motionless, autotomize a writhing tail, bound away with saltatory movements, immobilize the predator with neurotoxins or adhesive secretions (Fig. 1), vocalize and bite the predator, rasp the predator with protruding ribs, or assume postures that impede ingestion (Hubbard, 1903; Mosher et al., 1964; Lanza, 1967; Wake and Dresner, 1967; Brodie and Gibson, 1969; Brodie, 1968, 1977, 1978; Brodie et al., 1974a, b; Dodd and Brodie, 1976; Maiorana, 1977; Nowak and Brodie, 1978; Shaffer, 1978). No species of salamander seems to use the full array of known tactics, but no species has been tested with a diverse series of predators in the laboratory, and so we probably do not know the complete antipredator repertoire of any species. Undoubtedly many tactics remain to be discovered. Furthermore, stimulus control of antipredator responses has received only passing attention and it may be possible, with extensive observations, to deduce algorithms specifying the rules for transition between tactics for particular species. While the behavioral and tactical diversity within species and its stimulus control are outstanding problems, we also need techniques to evaluate the performance of antipredator tactics. The latter topic is the subject of the present report.

The antipredator performance of the pletho-

dontid salamander *Plethodon cinereus* was evaluated in feeding trials with the garter snake *Thamnophis elegans*. Although *P. cinereus* never encounters *T. elegans* in nature, since their geographic ranges do not overlap, this salamander encounters comparable predators and the garter snake encounters comparable prey. Thus *P. cinereus* is preyed on by other garter snakes (Hamilton, 1951) and *T. elegans* preys on a variety of plethodontid salamanders (Fox, 1951, 1952).

Feeding trials were arranged in a factorial design since the goal of the experiment was to evaluate the effectiveness of three aspects of salamander defense (body size, tail and antipredator behavior) and their interactions. For simplicity the size of the snake predator was held constant. Weight specific ingestion rate was used as a measure of antipredator performance. The rationale for this measure is developed in the discussion. The design permitted statistical analysis of antipredator performance by means of a three-way analysis of variance.

### MATERIALS AND METHODS

The subjects of the experiment were the salamander *P. cinereus* (N = 56) and the garter snake *T. elegans* (N = 56). The *P. cinereus* were collected in Parke Co., Indiana, and the *T. elegans* were the laboratory born progeny of four gravid females collected at Scott Creek, Santa

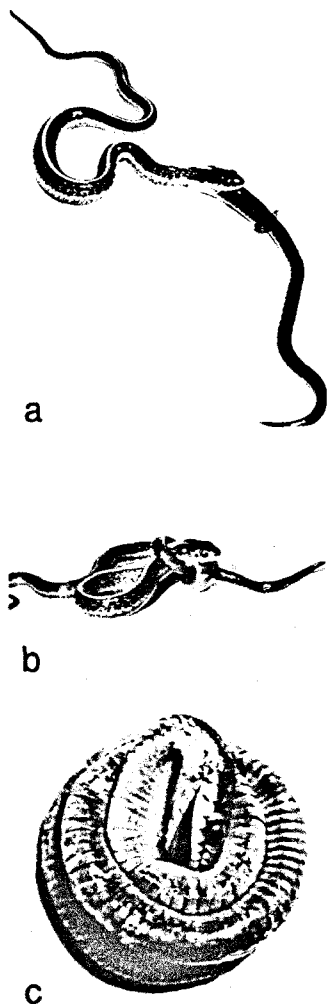


Fig. 1. Effects of antipredator responses of the plethodontid salamander *Batrachoseps attenuatus* on garter snakes (*Thamnophis*). (a) A small *T. couchi* beginning to ingest a large *B. attenuatus*. (b) Same subjects as in Fig. 1a. The *B. attenuatus* has thwarted ingestion by looping its tail around the snake's head. Note that the salamander's tail forms a knot. (c) A small *T. elegans* completely immobilized by the adhesive secretions of a large *B. attenuatus*. During a struggle with the salamander, the snake's body was coated with adhesive secretions. The snake coiled around the salamander during the attack, but the salamander flipped free and escaped. The snake was left glued to itself (the mouth is glued open) and was unable to free itself even after 48 hr.

Cruz Co., California. At the time of the experiment the snakes were 5–8 wks old and weighed  $4.62 \pm 0.51$  g (mean  $\pm$  standard deviation). Thus the snakes were of rather uniform size in order to minimize variation due to predator size in the experiment. They had not encountered salamanders prior to the experiment.

The experiment consisted of three treatments, with two levels each, in factorial design with seven replicates per cell (Table 1). Thus 56 salamanders were used in the experiment and, since each salamander was exposed to a different snake, the observations were independent. In order to obtain two sizes of salamanders for the first treatment, salamanders with complete tails were selected from a large field sample on the criteria that they fell in ranges 0.20–0.49 g or 0.90–1.19 g. The resultant means, standard deviations and ranges for the two treatment groups of 28 salamanders each were: small,  $0.33 \pm 0.07$  g (0.21–0.47) and large,  $1.02 \pm 0.09$  g (0.90–1.19). In order to achieve two levels for the second treatment, behavior, halves of each size group were selected at random and killed by freezing. Thus the behavior treatment consisted of two levels: a live group, capable of antipredator behavior, and a dead group, incapable of behavioral defense. Two levels of the third treatment, tail, were achieved by severing the tails of half of the salamanders in each of the four groups already described. Tails were severed just posterior to the vent with a razor blade. The severed tails were weighed to determine their relative contribution to total weight:  $0.05 \pm 0.02$  g in small salamanders or 15% of total weight and  $0.20 \pm 0.06$  g in large salamanders, or 20% of total weight. There was negligible loss of blood when tails were severed.

The presentations were staged inside plastic boxes ( $15 \times 30 \times 9$  cm) with clean, dry paper towel substrates. The snakes were individually housed in these same containers prior to the presentations. The salamanders were simply placed in the staging boxes, approximately half the length of the box away from the snake's head, and the snake was allowed to discover the prey. Room temperature was 28 C during the presentations. Live and dead salamanders were allowed to equilibrate at this temperature on damp paper towels for several min prior to the experiment.

The temperature during the trials, 28 C, is within the normal activity range of *T. elegans*

but higher than field temperature normally experienced by *P. cinereus* (Spotila, 1972). This temperature is well below thermal maximum for *P. cinereus* (Spotila, 1972; Burke and Pough, 1976).

Ingestion times were measured to the nearest 0.1 sec with a stopwatch from the time of attack (actual contact with the snake's mouth) until the last portion of the salamander disappeared inside the snake's jaws. Weight specific ingestion rates were then calculated by dividing the actual weight of each salamander by its ingestion time. In the case of the groups without tails, the weight used in these calculations was the body weight after the tail was severed.

In two instances salamanders escaped after the initial attack but were subsequently attacked and ingested. In these instances the tabulated ingestion time did not include the interval between the two attacks, during which the snake searched for the salamander, but only the durations of the two ingestion episodes.

The data were analyzed with a Model I, three-way analysis of variance (Simpson et al., 1960; Sokal and Rohlf, 1969). In order to satisfy the assumption of homogeneous variances, the natural logarithm of weight specific ingestion rate was used. The effect of this transformation will be discussed in the next section.

## RESULTS

*Qualitative.*—Behavioral responses to attack often occurred in rapid sequence during a trial. Since the main goal was to accurately measure ingestion times, this sometimes interfered with the recording of behavioral responses. Consequently it was impossible to tally the absolute frequency of various behaviors. However, it was possible to rank recorded responses in order of frequency. After the onset of snake attack, live salamanders showed the following antipredator behaviors, in order of decreasing frequency of occurrence: 1) writhing or thrashing movements, which commonly delayed ingestion and infrequently led to escape, 2) tail autotomy and 3) biting the snake. Of these three major categories of response, the first was by far the most frequent and also the most substantial cause of delayed ingestion. Writhing or thrashing movements sometimes occurred prior to attack (e.g., in response to contact with the snake's flicking tongue) and caused the salamander to move away from the snake in saltatory fashion. These

TABLE 1. WEIGHT SPECIFIC INGESTION RATE (G/MIN) BY *T. elegans* AS A FUNCTION OF SALAMANDER BODY SIZE, ANTIPREDATOR BEHAVIOR AND PRESENCE OF THE TAIL IN *P. cinereus*. The mean of seven observations is shown in each cell. Means are back transformed from calculations using  $\ln$  weight specific ingestion rate. Standard deviations, shown in parentheses, are for transformed data.

Behavior	Tail	Size	
		Small	Large
Dead	absent	1.58 (0.53)	0.70 (1.00)
	present	1.28 (0.64)	0.33 (0.70)
Alive	absent	0.92 (0.52)	0.32 (0.98)
	present	0.24 (1.07)	0.09 (0.39)

movements seemed to be accomplished by sudden lateral flexures of the body and tail.

Writhing and thrashing movements invariably occurred once the snake had attacked and seized a live salamander in its jaws. The movements included violent lateral flexures of the head and body, undulations of the tail, and sometimes rotations about the long axis of the body. These movements had three major consequences: 1) they directly delayed ingestion, 2) they applied adhesive secretions to the snake's jaws, head and body and 3) they sometimes caused loops to form around the snake's head, which considerably delayed ingestion. Adhesive secretions, released from the surface of the salamander's body and tail, appeared to delay ingestion in nearly all trials with live salamanders. On two occasions the snake's head adhered to the staging box and/or its own body after the completion of ingestion (in both instances large salamanders with complete tails were the subjects of attack).

On nine occasions ingestion was considerably impeded because writhing and thrashing movements resulted in loops of the salamander's body and tail that encircled the snake's head. In most instances these were simple loops, but sometimes complex loops were formed as in Fig. 1b. Of the nine instances of delayed ingestion due to formation of stable loop-like configurations, seven were due to large salamanders (all but two of these had complete tails), and two were due to small salamanders with complete tails. In one of these instances, the formation of a loop resulted in the momentary escape of the attacked salamander. This sala-

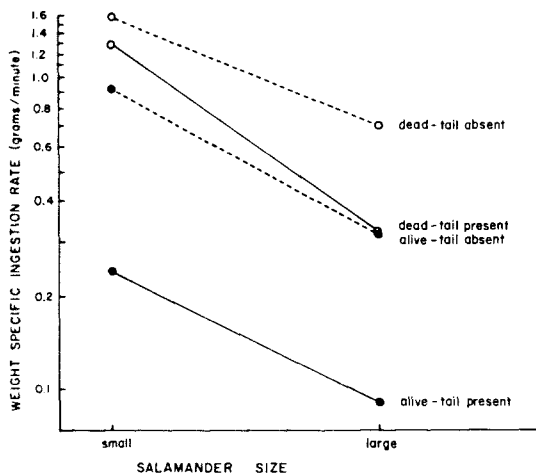


Fig. 2. Ln weight specific ingestion rate as a function of salamander body size, antipredator behavior and the presence of a tail. Each point is the mean of seven observations.

mander, large with a complete tail, formed a loop around the snake's snout with its body. This configuration delayed ingestion for nearly 14 min (893.7 sec), during which time the snake gaped and twisted in an apparent attempt to dislodge the salamander from its snout. Finally the snake disgorged the still living salamander. The salamander was attacked some 90 sec later and ingested. This second attack led to one of the only two instances of tail autotomy in the experiment. The snake attacked the salamander's tail, which autotomized, and then immediately attacked and ate the salamander.

The only other instance of tail autotomy also involved a large salamander with a complete tail. In this trial the salamander was abruptly released only 2.7 sec after the onset of the initial attack. About 5.5 min later the still intact salamander flipped and thrashed about as the snake approached it. The attacking snake grabbed the salamander's tail, which immediately autotomized. After swallowing the tail, the snake immediately attacked and ate the now tailless salamander. These two trials in which tail autotomy occurred were the only instances in which salamanders escaped, even momentarily, after initial attack.

*Quantitative.*—The results of the experiment, using ln weight specific ingestion rate, are tabulated in Table 1 and graphed in Fig. 2. Using untransformed weight specific ingestion rates,

TABLE 2. THREE-WAY ANALYSIS OF VARIANCE FOR LN WEIGHT SPECIFIC INGESTION RATE OF SALAMANDERS (*P. cinereus*) ATTACKED BY GARTER SNAKES (*T. elegans*). (\*\* denotes  $P < 0.01$ ; n.s. denotes  $P > 0.05$ ).

Source of variation	Sums of squares	df	M.S.	F
Size (A)	15.343	1	15.343	26.091**
Behavior (B)	15.684	1	15.684	26.671**
Tail (C)	11.166	1	11.166	18.988**
AB interaction	0.022	1	0.022	0.037n.s.
BC interaction	2.281	1	2.281	3.879n.s.
AC interaction	0.192	1	0.192	0.326n.s.
ABC interaction	0.361	1	0.361	0.614n.s.
Error	28.227	48	0.588	
Total	73.276	55		

standard deviations were found to be correlated with means ( $r = 0.97$ ,  $df = 6$ ,  $P < 0.001$ ). A logarithmic transformation removed this correlation ( $r = 0.06$ ,  $df = 6$ ,  $P > 0.1$ ) and produced homogeneous variances for analysis.

The results of a three-way analysis of variance are reported in Table 2. The three main effects were statistically significant, but the four interaction effects were not. Thus large size, performance of antipredator behavior and the presence of a tail all had a significant effect in delaying ingestion. Thus a simple additive model describes the data on the logarithmic scale, and this implies that there are multiplicative interactions, i.e., positive synergisms, between the three main factors (size, behavior and tail) on the linear scale.

## DISCUSSION

Although a simple catalog of antipredator responses can provide valuable insights, the study of performance is crucial to an understanding of adaptive significance. The present study illustrates the advantages of using factorial experiments to dissect the contributions of the various components of the defense system and weight specific ingestion rate as a measure of antipredator performance.

The main advantage of a factorial experimental design is that it permits statistical tests for synergisms as well as for the main effects of separate components on performance. Salamander body size, presence of the tail and antipredator behavior could have been varied in three separate experiments, but this would not have permitted a test for, say, equal contribu-

tions of antipredator behavior to performance in salamanders of different size. In the present experiment no statistically significant interactions or synergisms were found between the components of the defense system when performance was measured on a logarithmic scale. Thus large body size, behavior and the presence of a tail each made a significant contribution to antipredator performance, but the tail made an equal contribution to performance in salamanders of different size when performance was measured on a log scale, and likewise for the other kinds of interaction (Table 2, parallel lines in Fig. 2). This additive system of effects is useful since it permits straightforward tests for contributions of the separate components (Simpson, Roe and Lewontin, 1960). But additive effects on a logarithmic scale imply that the components have multiplicative effects when performance is measured on an arithmetic scale. The biological basis for such multiplicative effects or synergisms are easy to imagine.

Consider the ramifications of increased size on antipredator performance. An increase in size during ontogeny or phylogeny could lead to more effective defense for a variety of reasons. First, increased size will delay ingestions for the trivial reason that larger objects are harder to swallow. Second, and more interesting, larger size can have synergistic effects on behavioral aspects of defense. The thrashing and writhing movements of larger salamanders are undoubtedly more forceful and hence more effective as defensive maneuvers. In addition, larger salamanders probably release a greater volume of adhesive secretions, and these play a major role in defense. Furthermore, larger salamanders are more likely to encircle the snake's head with stable body loops that inhibit ingestion. Third, larger size can have synergistic effect on the value of a tail. We can expect larger tails to yield more secretion and accomplish more forceful movements. In addition, tail autotomy (which was not an important component of defense in the present experiment) might be more effective in larger salamanders since a larger tail could distract or engage the predator longer and increase the opportunity for escape. For all these reasons larger salamanders are more likely to survive a predatory attack. While larger size promotes survival once attack has occurred, it might be a disadvantage during the detection stage of predation. Larger salamanders might be more conspicuous to

predators and they might not be able to use as many retreats as small salamanders.

There are a number of reasons for using the logarithm of weight specific ingestion rate (ln WSIR) as a measure of antipredator performance. For many purposes this measure is more useful than simpler alternative measures, like the proportion surviving attack or survival time. 1) Since ln WSIR is a continuous variable, it will give better resolution of performance than discontinuous measures like the proportion surviving. Discontinuous measures may also require larger sample sizes to resolve differences in performance, say between salamanders of different size. 2) The use of ingestion rate rather than survival time removes an awkward scaling problem, since a salamander that survives attack has a survival time score of infinity but an ingestion rate of zero. 3) Weight specific ingestion rate is useful since simple ingestion time is bound to be longer for larger salamanders for obvious physical reasons. 4) A logarithmic transformation of ingestion rate is desirable if standard deviations are correlated with means. This transformation will often remove this association and produce the homogeneous variances required for analysis of variance. Slightly more complicated transformations are sometimes desirable (Wright, 1968:232). If ln WSIR is used and some animals survive (giving ingestion rates of zero), one should add a positive constant (like 1.0) to all observations before taking logarithms. This transformation simply avoids a scaling problem and will not distort statistical tests (Sokal and Rohlf, 1969:384). 5) A final justification for ln WSIR is that it may produce a good fit to a linear model, as in the present analysis. This too is a desirable feature since it greatly simplifies the interpretation of the analysis of variance table (Simpson et al., 1960).

Although weight specific ingestion rate has many statistical virtues as a measure of antipredator performance, is it a biologically meaningful measure? After all, a prey that simply delays ingestion is just as dead as a prey that is eaten quickly. The key to the significance of ingestion rate is that the prey may survive if it delays ingestion long enough. However, delay of ingestion will promote survival, and hence fitness, only if the snake predator simply overpowers the prey rather than kills it by constriction or envenomation. For such overpowering predators, like *Thamnophis* and other natricine snakes, it is useful to conceive of the predator's

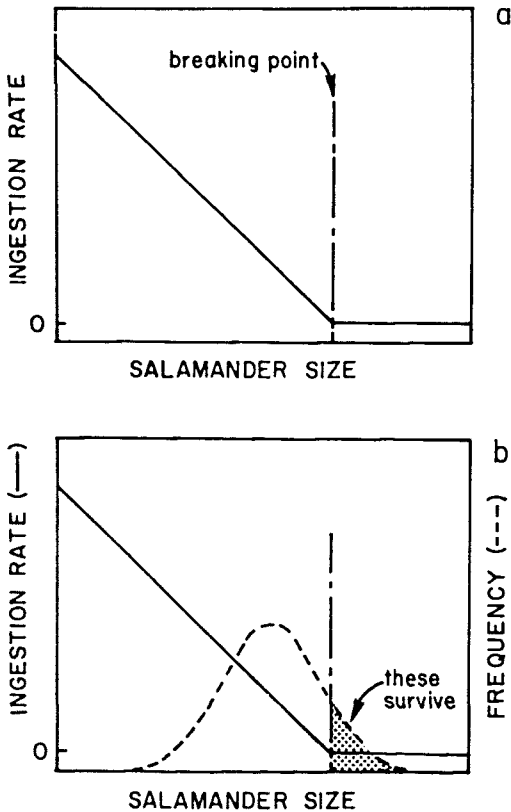


Fig. 3. The expected relationship between ingestion rate, survivorship and salamander body size. (a) Ingestion rate as a function of body size. (b) Survivorship as a function of body size.

breaking point. For a snake of any particular size there must be a size of prey that the snake is incapable of ingesting. This critical prey size constitutes the breaking point of the predator. The significance of this breaking point for the prey is that it may very well survive attack if it exceeds the critical size specified by the breaking point. The relationship between ingestion rate and the breaking point is shown diagrammatically in Fig. 3. If an ingestion rate of zero means survival for the prey, then ingestion rate, or  $\ln$  WSIR, is a biologically meaningful measure of antipredator performance. For under this condition, the slower the ingestion rate, the closer the prey has come to the predator's breaking point and hence to survival. In addition, the longer the ingestion time, the greater the opportunity for behavioral defense and escape.

If no prey survive attack in an experiment, this does not necessarily invalidate the concept

of breaking point or the use of ingestion rate as a measure of performance. Thus in the present experiment no *P. cinereus* survived attack, probably because none were large enough to exceed the breaking point of the garter snakes. Indeed no *P. cinereus* may ever be large enough to exceed the breaking point of a 4.5 g *Thamnophis*. Nevertheless, an imaginary 10 g *P. cinereus* would certainly survive attack, and the *Plethodon* size required to reach the breaking point could actually be determined by using larger congeneric species.

Snakes must have breaking points since some large prey completely frustrate ingestion and survive attack (Fig. 1). The important question is how salamanders and other prey push snakes past their breaking points. It is possible that the breaking point represents both mechanical and physiological constraints. Mechanical constraints alone could determine the maximum prey size, and this mechanical breaking point could be determined with a sequential sampling scheme in which the snake is offered dead prey in an increasing size series. But the breaking point could also be governed by physiological constraints. For example, Pough (1977) found that newborn garter snakes (*T. sirtalis*) could sustain strenuous activity for only 3–5 min, while adult snakes could sustain activity for 2–25 min at 25 C. The greater stamina of larger snakes was a consequence of greater capacity for aerobic metabolism, more effective pulmonary ventilation and an ontogenetic increase in blood oxygen capacity (Pough, 1977, 1978). It is conceivable that the behavioral defenses of prey could push garter snakes to a physiological breaking point. Whether predatory interactions can profitably be viewed as physiological battles between predator and prey is an outstanding research question.

The defensive value of plethodontid skin secretions may be due to adhesive, rather than toxic, properties. Although there is conclusive evidence that salamandrids produce toxic skin secretions (Mosher et al., 1964; Brodie, 1968; Brodie et al., 1974), there is no compelling evidence for toxicity of secretions in plethodontids. Nevertheless, plethodontid skin secretions may be toxic to some predators, since birds and shrews are known to abort attack on plethodontids and show signs of discomfort, and at least some plethodontid skin secretions produce an astringent or burning taste in humans (Hubbard, 1903; Brodie et al., 1979). Although the toxicity of plethodontid secretions remains problematical, the adhesive effect of these se-

cretions is abundantly revealed in feeding trials with snakes. Indeed, if the salamander is large enough, adhesive skin secretions can completely immobilize a snake predator (Fig. 1c). In the present experiment, snakes were temporarily immobilized by adhesive secretions. In other feeding trials with a variety of plethodontids, I have observed several additional effects. In *Ensatina eschscholtzi* these include: slapping the snake's head with the secretion-laden tail prior to attack (as described by Hubbard, 1903) and consequently gluing the snake's mouth closed; coating the snake's teeth with secretions so that subsequent attacks were ineffective; and anchoring body parts, particularly the tail, to the snake with secretions so that ingestion was thwarted (these observations will be detailed in a later report). Thus feeding trials with snakes suggest that the major defensive value of plethodontid skin secretions is adhesion rather than toxicity.

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