PREDATORY BEHAVIOR OF LARVAL SMALL-MOUTHED SALAMANDERS (*AMBYSTOMA TEXANUM*)

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ABSTRACT: The predatory behavior of larval small-mouthed salamanders (*Ambystoma texanum*) was quantitatively studied in the laboratory. A split-plot Latin square design was used to test for differences in behavior between two size classes of larvae foraging on six types of prey. Size-specific differences in foraging behavior were observed. Small larvae had a higher frequency and duration of swimming-affiliated behaviors, while large larvae exhibited more crawling-affiliated behaviors. Shifts in foraging behavior associated with different prey types were not as obvious. Transitions between different sequential pairs of feeding behaviors were never random, regardless of prey type. Larvae showed similarly ordered behavior sequences with *Daphnia*, small isopods, and even when no prey were present. Large isopods and the two size classes of amphipods elicited more complex behavioral sequences, especially for attack sequences. Thus, changes exist in larval foraging behavior in response to prey. These behavioral shifts are responses to general characteristics of the prey (behavior and/or size) and are not species-specific.

Key words: Urodele; *Ambystoma texanum*; Larvae; Foraging; Predation; Behavior; Ontogeny

STUDIES of foraging strategies often involve collecting animals in the field and documenting the types of food eaten by individuals. When this approach is combined with characterizations of the prey available to the predator, indications of preference and selection can be made (Hyslop, 1980; Ivlev, 1961). These types of data have provided valuable information on diet and habitat requirements but have supplied less conclusive results regarding whether predators are seeking or avoiding particular prey types (Maiorana, 1978).

More recently, behavioral ecologists have attempted to identify the mechanisms of predation. This type of research has often involved controlled laboratory studies that provide choices of prey or patches and the recording of the animal's decisions in these environments (Jaeger and Barnard, 1981; Krebs, 1978; Leff and Bachmann, 1986).

Some field studies report anecdotal observations of behavioral sequences involved in larval predation. Hassinger et al. (1970) described variability in prey capture attempts with degree of larval stratification by depth and ontogenetic development for *A. opacum* and *A. tigrinum*. Premetamorphic *A. macrodactylum* and *A. tigrinum* larvae have been observed using short lunges to capture prey (Anderson, 1968). However, these field observations do not separate factors (e.g., water temperature, prey activity and abundance, developmental stage of the larvae) potentially responsible for differences in feeding behavior. Leff (1985) and Leff and Bachmann (1986) provided one of the few studies analyzing feeding behavior of larval salamanders in conditions where access to prey was varied systematically. Little is known regarding possible variation in predatory behaviors with prey type.

Our experiment was designed to determine whether the behavior of larval salamanders varies with prey type. By observing how larvae capture different types of prey, an assessment can be made of the relative costs and benefits associated with foraging on these prey types. The experiment complements a field study undertaken in the same year which documented the diet of larval *Ambystoma texanum* and availability of the prey species (McWilliams, 1986; McWilliams and Bachmann, 1988, 1989).

This study specifically addresses three questions. (1) What are the sequences of behaviors which constitute predation by

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larval *Ambystoma texanum*? (2) Do larvae of different sizes vary in predatory behavior? (3) Are unique behavioral sequences used for capturing particular prey types?

MATERIALS AND METHODS

Larvae were collected from a flooded woodland pond at Flaming Prairie Preserve, Louisa County, Iowa (Sec. 29 Port Louisa Twp., T-75N, R-2W) in early May 1984 approximately 10 wk after courtship and oviposition by the adults. Larvae were immediately transported to Iowa State University and placed in 38 l aquaria in an environmental chamber maintained at 16 C, with a 12:12 L:D photoperiod. Approximately 100 larvae were housed in each aquarium. Larvae were fed ad libitum a variety of invertebrates collected from local marshes and acclimated to the laboratory for at least 2 wk prior to being tested.

Individual larvae were given a single prey type and the larva's behavior was serially recorded. Two size classes each of an isopod, amphipod, and cladoceran were used: a *Asellus* sp. ($\bar{x} = 3.0 \pm 0.7$ mm and 1.5 ± 0.3 mm), a *Hyallela* sp. ($\bar{x} = 3.1 \pm 0.6$ mm and 1.4 ± 0.3 mm), and a *Daphnia* sp. ($\bar{x} = 2.1 \pm 0.7$ mm and 0.7 ± 0.3 mm) (all measurements are longest linear distances ± 1 SD). All three species of prey are eaten by larvae under natural conditions (McWilliams and Bachmann, 1988) and each exhibits different behavioral and morphological characteristics. In addition, two size classes of larvae were tested to see if any size-specific differences existed. Small larvae were 11–18 mm SVL ($\bar{x} = 14.0$, SD = 2.1), and large larvae were 21–31 mm SVL ($\bar{x} = 26.3$, SD = 2.75).

The six prey types and one control with no prey were organized into a 7 × 7 Latin square design, enabling time-of-day (TOD) and day-of-week (DOW) to be blocked. The DOW factor was blocked to control for variation in disturbance between days. The two size classes of larvae were then added to this design as a split-plot component (Cochran and Cox, 1957), providing each larval size class with exposure to each treatment combination. The seven DOW periods corresponded to each day

of the week. The seven TOD periods began with a 0800–0900 h and a 1100–1200 h period with subsequent 1 h time blocks every other hour concluding with a 2100–2200 h time period.

The first four TOD periods were conducted under white light conditions using a 60-W light bulb. The last three TOD periods were conducted under red light conditions using a 60-W light bulb covered with red acetate placed approximately 1 m away from the aquaria. These red light conditions are thought to simulate nighttime conditions since salamanders apparently have low sensitivity to red light (Grusser-Cornehls and Himstedt, 1976).

We randomized the order in which sizes of larvae were tested for the first day. Individual larvae (total $n = 98$) were tested only once. These larvae were randomly selected from approximately 500 larvae collected in early May 1984.

A single trial consisted of randomly selecting a specific-sized larva which had not been fed for 24 h, and placing it in one side of a 30 × 15 cm glass aquarium filled to a depth of 8 cm with dechlorinated water. One individual of the designated prey species and size to be tested was placed on the opposite side of a removable partition which divided the aquarium in half. Only one prey individual was used in an attempt to elicit an active predatory response (versus passive capture which is potentially similar across prey types).

After a 10 min acclimation period, the partition separating the larva from the prey was removed. Using a Datamyte 1000 recorder (DataMyte Corp., Minnetonka, Minnesota), 12 components of the feeding behavior and activity of the larva (defined in Table 1) were serially recorded (EXIT was recorded only when no feeding was observed). These 12 components were defined initially by watching videotapes of larvae preying on a variety of prey types and represent all common behaviors exhibited by larvae while feeding. Terminology is consistent with, though not exactly the same as, that found in Lindquist and Bachmann (1980) and Leff and Bachmann (1986). Here we delineate two modes of prey capture, STRIKE and LUNGE

(Table 1). STRIKE has been kinematically described for mid-water prey capture by premetamorphic *Ambystoma* larvae (Hoff et al. 1985). LUNGE is associated with capture attempts initiated while the larva is on the substrate typically motionless. This type of prey capture has been kinematically described for aquatic *Ambystoma* adults (Lauder and Shaffer, 1985; Shaffer and Lauder, 1985*a,b*).

The Datamyte recorded the time a behavioral component occurred, so durations of each of the behaviors in a given trial could be calculated. In addition, the sequences and frequencies of components could be determined for every predatory sequence. The trial ended either 5 min after the larva had captured the prey or after 10 min had elapsed without an encounter between the larva and prey.

Statistical analysis was conducted using Statistical Analysis System (SAS Institute Inc., 1985). Sequence analysis (Chatfield and Lemon, 1970; Lewis and Gower, 1980) was used to compare larvae foraging on different prey types and to compare the foraging behaviors of the two size classes of larvae. Transition matrices were constructed documenting the frequency with which each behavior preceded every other behavior. A total of 10 transition matrices were constructed; one matrix was constructed by pooling across all trials, seven matrices represent larvae foraging on six different prey types and one control (with no prey), and two matrices were constructed by pooling across all trials with either large larvae or small larvae. Each matrix was square and at most 10 by 10 in dimension, because CONSUME, MISS, and EXIT were excluded from the sequence analysis. These three behaviors were excluded because they are sequels to other behaviors rather than independent behaviors. Smaller matrices result when one or more behaviors are not exhibited during a given set of trials.

A chi-square test was performed for each matrix to test whether the matrix was significantly different from one constructed assuming all behaviors were independent. The diagonal of each matrix consisted of all zeros, because only transitions in be-

TABLE 1.—Behavioral components of larval salamander feeding behavior.

READY—motionless; in contact with substrate.
FLOAT—motionless; not in contact with substrate.
SWIM—active movement of tail fin with legs and gills pulled back alongside body.
BRAKE—vertical or horizontal movement, with legs and gills outstretched and no movement of the tail fin.
CRAWL—limbs used in coordination to move body along substrate.
LUNGE—rapid, short distance movement in the direction of prey; initiated by limb movement (see text for details).
STRIKE—rapid, longer distance movement in the direction of prey; initiated by thrust of the tail (see text for details).
SNAP—quick opening and closing of the mouth.
CONSUME—prey in mouth, not visible to observer
MISS—unsuccessful prey consumption.
GULP—quick movements of the jaw, but mouth stays relatively closed compared to SNAP.
AIR GULP—snout breaks surface of the water and larva snaps air.
EXIT—documents 10 min without predator/prey encounter or 5 min after prey consumption.

haviors were counted. Expected values were calculated for this type of matrix using methods outlined in Lewis and Gower (1980) and Appendix I of Lemon and Chatfield (1971). Simulation studies indicate that if 10 behaviors are used in the analysis, a minimum of 500 behavioral transitions must be recorded and preferably no less than 1000 (Fagen and Young, 1978). All transition matrices used in this study, except for the control matrix with no prey, had more than the prescribed 1000 transitions. The control matrix had 995 total behavioral pairs.

When the chi-square method is used, it is assumed that the probabilities associated with each behavioral occurrence do not change over time (Lemon and Chatfield, 1971). Additionally, because we combine sequential data from more than one individual, we assume that variation between individuals is negligible. Because our data do not conform entirely to these assumptions, the levels of significance associated with the chi-square tests must be viewed as only rough indicators of the major sequential dependencies. Consequently, we have followed Lemon and Chatfield's (1971) suggestion and reject the null
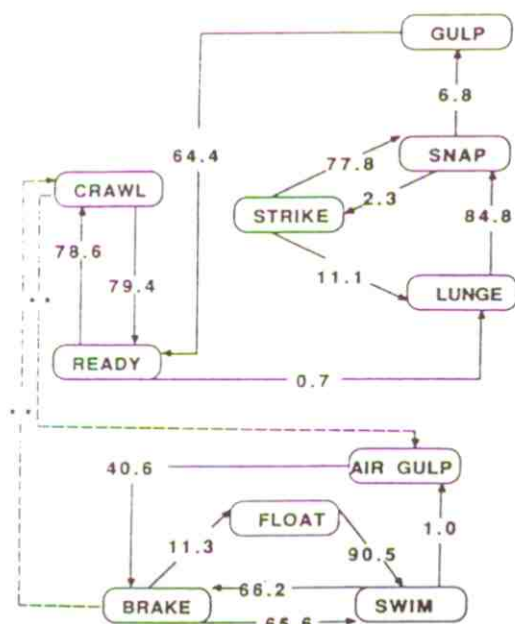
FIG. 1.—Flow diagram illustrating significant transitions (based on the binomial test) for general foraging behavior of larval A. texanum. Values are calculated by dividing the number of times the specific transition occurred by the row total for the preceding behavioral component. ** = frequent transitions which integrate the crawling and swimming sequences but which are not significant based on the binomial test.

hypothesis only if the test statistic is highly significant ($P < 0.01$). Nevertheless, conclusions based on chi-square analysis in this and similar studies of sequential behavior must be considered tentative when data do not conform entirely to the assumptions that observations are independent and that transition probabilities are constant throughout the behavioral sequences.

The large sample case of the binomial test (Siegel, 1956) was applied to each transition frequency in a given matrix to test whether the deviation of observed from expected frequency was statistically significant. Values used in the calculation of each z score were derived from the equations of Poole (1974).

A split-plot Latin square design analysis of variance (ANOVA) (SAS Institute Inc., 1985) was used to compare the number of transitions in each trial (standardized by trial length), the duration and arcsine transformed frequency of each behavior

with respect to TOD and DOW blocks, and prey type and larval size treatments. The duration and frequency values used in the ANOVA were standardized by trial length and the total number of behavioral acts in a given trial, respectively. All values used in the ANOVA were tested for normality and homogeneity of variance and found to conform to the assumptions of ANOVA.

RESULTS

The general foraging behavior of larval Ambystoma texanum was obtained by combining data for all prey types and larval sizes. The chi-square value for this transition matrix was significantly larger than expected ($P < 0.001$, df = 71), implying behaviors were not independent of one another. Four of the 10 categories of behavior constituted 95% of the 9873 individual behaviors observed in all 98 trials. READY (27.4%) and CRAWL (29.0%) were the two most frequently occurring behaviors, with SWIM (23.1%) and BRAKE (15.6%) constituting the remaining two predominant components. FLOAT (1.9%) was the only other behavior with greater than 0.5% frequency of occurrence.

Analysis of each transition in the matrix using the binomial test reveals two separate sets of significant behaviors (Fig. 1); one associated with mid-water activity, the other associated with substrate activity. Prey capture attempts always involved SNAP, although SNAP occurs equally as often when prey are not close. LUNGE and STRIKE are also integral components of prey capture, with SNAP following LUNGE or STRIKE at least 75% of the time.

Most of a larva's time during a trial was spent either alternating READY and CRAWL, or BRAKE and SWIM. It was obvious from observing larvae that this stop/start type of activity was the predominant mode of movement whether on the substrate or in mid-water. In particular, it was from this READY or BRAKE position that prey capture was often initiated. These transitions, plus the frequent transition from FLOAT to SWIM, occurred in all transition matrices.