PREY-SIZE SELECTION BY *TRIOPS LONGICAUDATUS* (NOTOSTRACA: TRIOPSIDAE) FEEDING ON IMMATURE STAGES OF *CULEX QUINQUEFASCIATUS*

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ABSTRACT. The tadpole shrimp, *Triops longicaudatus*, was found to be a size-dependent predator of *Culex quinquefasciatus* larvae in the laboratory. However, changes in tadpole shrimp size were accompanied by changes in prey-size preference: larger-sized predators consumed an increasing proportion of larger prey items. Very large tadpole shrimp may be nonselective predators of this mosquito species. Quantified behavioral observations indicated that while second instar mosquito larvae were encountered significantly less frequently than were fourth instar larvae or pupae, they were captured at significantly greater rates and with shorter handling times. It is hypothesized that prey vulnerability has an important influence on tadpole shrimp prey size "preferences."

INTRODUCTION

In many predator-prey relationships, the body sizes of the predator and the prey play an important role dictating the size of prey items consumed (Krebs 1978). Prey vulnerability may be a more important cause of size-dependent predation than is active selection by invertebrate predators. As prey size increases, so does the efficiency of their escape responses. Invertebrate predators, termed size-dependent predators, have been found to prefer intermediate-sized prey items due to capture and handling tradeoffs (Allan et al. 1987, Pastorok 1981).

Pastorok (1981) found that *Chaoborus trivittatus* Saether larvae feed in an opportunistic manner on all sizes of *Daphnia pulicaria* Forbes; however, intermediate-sized prey were overrepresented in their diet. He found this disparity to be due to probabilities of encountering prey and prey escape capabilities and not active selection on the part of the predator. Molles and Pietruszka (1983) presented the stonefly, *Hesperoperla pacifica* (Banks) with two mayfly species, *Ephemerella altana* Allen and *Baetis tricaudatus* Dodds, and demonstrated that "preference" for *E. altana* was due to a better escape response (i.e., lower vulnerability) by *B. tricaudatus.*

Tadpole shrimp (Notostraca: Triopsidae) are crustaceans inhabiting transient fresh waters worldwide (Longhurst 1956). The diet and nutrition of tadpole shrimp remain relatively unstudied. Pont and Vaquer (1986) reported *Triops cancriformis* (Bosc.) to progress from juveniles feeding on phytoplankton to mature adults feeding on plant material, and only the largest individuals (>10 mm) are carnivorous. *Triops longicaudatus* LeConte was called polyphagous and found to feed upon invertebrates and plant material in both daylight and darkness (Scott and Grigarick 1979). They also noted that, in the laboratory, early instar mosquito larvae were consumed. In a more detailed study in the laboratory, this species was found to be a predator of mosquito larvae (*Culex quinquefasciatus* Say) (Tietze 1987) and is currently being considered as a biological control agent against mosquitoes. These studies investigated the feeding rates of two tadpole shrimp sizes on mosquito larvae; smaller tadpole shrimp (mean body length = 1.6 cm) consumed first and second instar mosquito larvae at higher rates than the third and fourth instars while larger tadpole shrimp (mean body length = 2.4 cm) consumed all mosquito instars at nearly equal rates (Tietze 1987). According to the classification scheme of Greene (1985), *Triops longicaudatus* are cruising raptorial predators.

Unlike floodwater mosquitoes (i.e., *Aedes* and *Psorophora*), stagnant water mosquitoes (*Culex* and *Anopheles*) may oviposit during the entire flooding period, thus producing an heterogeneous assemblage of larval instars and pupae. The ability of growing tadpole shrimp to feed upon various mosquito instars may determine their effectiveness as biological control agents of stagnant water mosquitoes. The goal of this study was to determine from laboratory experiments whether tadpole shrimp show significant size preference for stagnant water mosquito larvae and pupae and if these preferences change as the tadpole shrimp grow. The issue of whether active selection or vulnerability is involved in this predation scenario is addressed through limited behavioral observations.

MATERIALS AND METHODS

Laboratory selection experiments: Tadpole shrimp (*Triops longicaudatus*) were reared from eggs in fiberglass microcosms at the UCR (University of California, Riverside) Aquatic and Vector Control Research Facility. The microcosms were 0.84 m^2 in area and equipped with float valves to maintain the proper water depth (30 cm). Five microcosms were flooded at different times over a 5-day period to produce a range of tadpole shrimp sizes. Larval and pupal stage mosquitoes of the species *Cx. quinquefasciatus* were provided from a laboratory colony maintained at UCR.

Predator and prey sizes were measured using a dissection microscope fitted with calibrated ocular micrometer. These measurements were subsequently converted to millimeters. Due to telescoping of the abdomen, tadpole shrimp size was measured as the length of the carinal suture that divides the carapace along the longitudinal body axis (Linder 1959). Longhurst (1956) found the carapace length of *T. cancriformis* to be closely correlated (growth coefficient $k = 1.02$) to total body length. Immature mosquito size was measured from the apex of the head capsule to the base of the air siphon for larvae and from the anterior of the head to the paddles for pupae.

Preference tests were performed in a Gibson environmental chamber. Temperature was maintained at 26 ± 2°C and measured using a continuous hygrothermograph. Illumination within the chamber was produced by one 60 watt cool white fluorescent bulb. Arenas were plastic containers filled with 200 ml of well water and a water depth of 4 cm. Tadpole shrimp were acclimated to the arena and temperature by placing individuals into arenas inside the environmental chamber for 1 h pretreatment. The shrimp were starved during the acclimation period. Immature mosquitoes were acclimated to the same test temperature by placing them into the environmental chamber for 1 h pretreatment. Ten representatives of each larval instar and pupae were preserved in 70% EtOH and were later measured in body length. Tadpole shrimp size was measured immediately before placing them into the arena for acclimation. Preference tests consisted of exposing individual tadpole shrimp in the arena to five of each mosquito larval instar and pupae (25 total). The prey items were simultaneously added to the arena and the time of addition noted. After 1 h of predation each arena was inspected for numbers of prey consumed. Six replicates were made for each predator size class. Due to the short period of exposure (1 h), replacement of prey consumed was not implemented.

Prey selection was calculated using an electivity indice, Chesson's alpha (å) (Manly et al. 1972, Chesson 1983):

$$\mathring{a}_i = \frac{\ln\ [(n_i - r_i)/n_i]}{\Sigma\ \ln\ [(n_j - r_j)/n_j]}$$

where n_i is the number of prey items of type i present at the beginning of a foraging bout, r_i is the number of prey items of type i consumed, n_j and r_j are symbols having the same definition as n_i and r_i, respectively, but are used to sum over the various prey categories. Chesson's alpha can be used for calculating prey selection in constant or changing prey densities (Chesson 1978). Alpha was calculated for each prey size class in each arena (replicate) and each predator size group. A multivariate analysis of variance (SAS Institute Inc. 1982) was performed to test the null hypothesis: there were no significant differences in selection between predator size groups. Separate ANOVAs and Duncan's multiple range tests (SAS Institute Inc. 1982) were performed to determine whether significant differences exist for prey size preferences within each predator size group.

Quantified predator-prey interactions: Behavioral observations were made to quantify encounters, captures and handling times of a large tadpole shrimp (carapace length = 7.0 mm). Attacks could not be quantified due to the cruising predation strategy used by tadpole shrimp. An encounter was defined as the prey's coming within contact distance of the predator or the length of the shrimp's first thoracic appendage which is held anteriad, like an antenna. Handling time was the duration between a capture and subsequent searching. Due to difficulty in visually distinguishing between consecutive larval instars, only three of the original five prey groups could be analyzed: three of each second and fourth instar larvae and pupae were simultaneously added, and the ensuing behaviors were recorded for 30 min. This was done four times using different shrimp each time.

Behavior was recorded using a Sony Beta I SLO-340 video recorder, an RCA CC 0-1-0 camera and a Tamron 90 mm telemacro lens. The recordings were reviewed by playing back on a Sony Beta I SLO 323 and viewed on a Sony SVM-1010 Motion Analyzer. Number of encounters, captures and handling time was determined by careful replay of each predation sequence. Capture success rate was calculated by dividing number of captures by number of encounters. These values were arcsine transformed and the three prey groups were compared using ANOVA and Duncan's multiple range test (SAS Institute Inc. 1982). Handling times and encounter rates were similarly compared between the three prey groups.

RESULTS

Prey selection: Significant differences ($P < 0.05$) in prey size preference (å) were found for first, third and fourth instar larvae and pupae when comparing six predator (tadpole shrimp) size classes; preference for second instar larvae was not significantly different ($P > 0.05$) between predator size classes (Table 1).

Prey size preferences of tadpole shrimp changed dramatically with their growth (Table 2 and Fig. 1a–f). The smallest tadpole shrimp size group (2.2 mm) preferred first instar larvae significantly more than second instar larvae and did not consume larger instars or pupae (Fig. 1a). The second predator size group (3.0 mm) fed upon first and second instar larvae without a significant difference ($P > 0.05$) in preference (Fig. 1b). Members of all prey size classes were taken when the tadpole shrimp had carapace lengths of 4.0 mm or greater. Medium-sized tadpole shrimp (4.1 mm) showed significant preference for earlier instar larvae but did feed upon third and fourth instar larvae and pupae (Fig. 1c). Aside from between fourth instar larvae and pupae, larger-sized tadpole shrimp (4.7 mm) showed distinct preferences between prey size classes (Table 2 and Fig. 1d). Tadpole shrimp with 5.75 mm carapace length exhibited similar preferences for third and fourth instar larvae and pupae but significantly greater preference for third instar larvae over pupae (Fig. 1e). Finally, while the largest tadpole shrimp (9.9 mm) had no significant difference in preference between first, third and fourth instar larvae and pupae, there was significant differences between these and second instar larvae (Table 2 and Figure 1f).

Table 1. Analysis of preference for immature *Culex quinquefasciatus* between six tadpole shrimp (*Triops longicaudatus*) size groups.

Instar/stage (mean size ± SD)[a]	MANOVA results		
	F	*df*	*P*
L1 (1.2 ± 0.10)	18	5	0.0001
L2 (2.3 ± 0.13)	2	5	0.0818
L3 (4.1 ± 0.26)	7	5	0.0003
L4 (5.6 ± 0.19)	8	5	0.0001
Pupae (3.3 ± 0.62)	6	5	0.0011

[a] Mean size (mm) of mosquito larvae (n = 10) measured from apex of head capsule to base of siphon; size of pupae was measured from apex of head to base of paddles.

Behavioral observations: The capture success rate of second instar *Culex quinquefasciatus* was significantly greater than that of fourth instars or pupae (Table 3). Tadpole shrimp (7.0 mm) capture success rate on second instar larvae was five times greater in magnitude than on fourth instar larvae and six times greater than that of pupae. Similarly, handling time of second instar mosquito larvae was significantly less than that of fourths and pupae (Table 3). Comparing the numbers of encounters between the tadpole shrimp and the three prey items, fourth instar larvae and pupae had significantly more encounters than did second instar mosquito larvae (Table 3).

DISCUSSION

Tadpole shrimp were found to be size-dependent predators of mosquito larvae and generally displayed decreasing preferences for increasing larval size. As tadpole shrimp become larger, their relative preference for larger prey sizes increases. Distinct prey-size preferences are evident if tadpole shrimp are separated into arbitrary size groups (i.e., small, medium and large). Small tadpole shrimp consumed early instar (first and second) mosquito larvae. Medium-sized tadpole shrimp consumed all available instars but preferred early instar larvae. As the predators grew larger, the relative preference for larger prey items increased. The largest tadpole shrimp size group appeared to feed upon most prey sizes in a nonselective manner.

Nonselective predation occurs when the relative abundance of prey size classes in the diet is not different from that of the environment. Five prey groups of equal abundance have a theoretical nonselective value of 0.2. When the prey size preferences of the largest tadpole shrimp size class (9.9 mm) are compared to 0.2 (t-test), only second instar larvae are significantly ($P < 0.05$) different.

Tadpole shrimp had an overall preference for second instar mosquito larvae. Preference for

Table 2. Analyses of preference for immature *Culex quinquefasciatus* within six tadpole shrimp (*Triops longicaudatus*) size groups (ANOVA; Duncan's multiple-range test: unshared letters indicate significant differences).

TPS size[a] (mm)	ANOVA results			Prey size class				
	F	*df*	*P*	L1	L2	L3	L4	P
2.20	833	4	0.0001	0.684a	0.316b	—	—	—
3.00	37	4	0.0001	0.518a	0.482a	—	—	—
4.10	26	4	0.0001	0.411a	0.373a	0.137b	0.047b	0.030b
4.70	24	4	0.0001	0.424a	0.311b	0.193c	0.072d	—
5.75	17	4	0.0001	0.332a	0.323a	0.196b	0.110bc	0.042c
9.90	4	3	0.01	0.207b	0.289a	0.175b	0.168b	0.141b

[a] Mean (n = 6) tadpole shrimp size measured as carapace length.

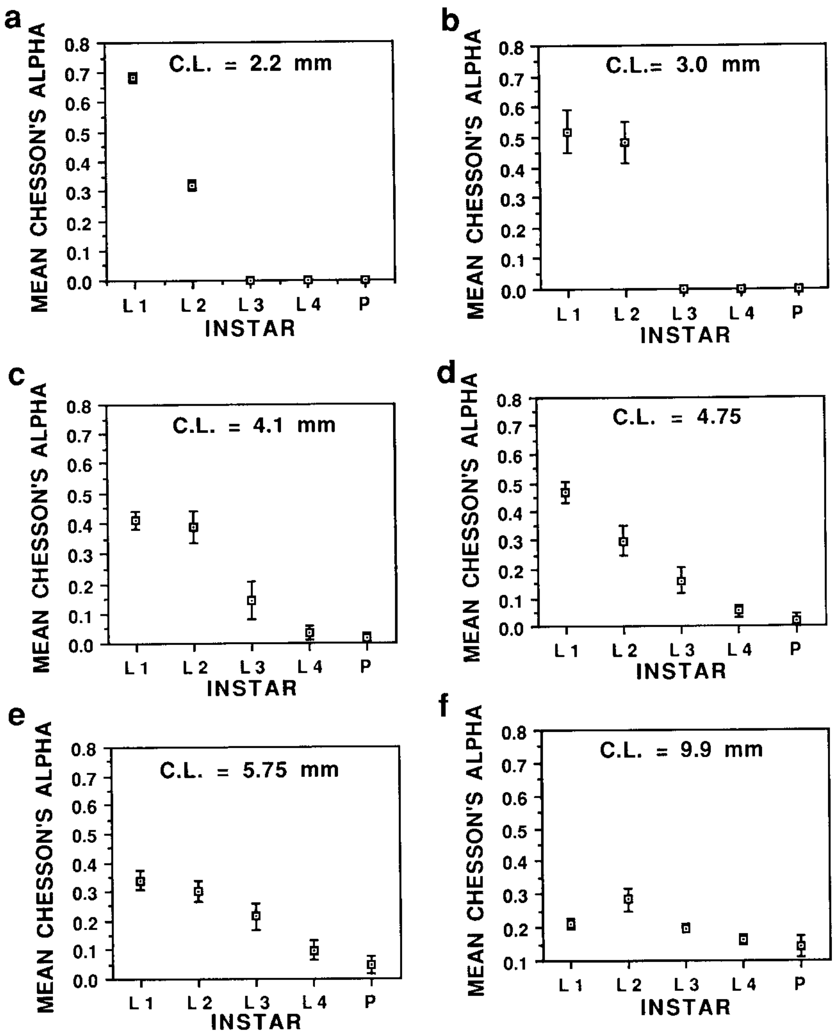

Fig. 1a–f. Mean preference (Chesson's alpha) ±SE for four *Culex quinoquefasciatus* larval instars and pupae by predatory tadpole shrimp (*Triops longicaudatus*) at six size classes (C.L. = carapace length).

second instar larvae did not change significantly with growth of the tadpole shrimp (Table 1) and the largest predator group did show greater preference for these than for first, third and fourth instar larvae and pupae (Table 2). Behavioral analysis gave similar results: high capture success and short handling times for second instar larvae. Second instar larvae may be the most vulnerable mosquito larval instar to tadpole shrimp predation.

Altering of prey body shape is known to influence predator capture and handling success (Kerfoot 1975, 1977). Mosquito pupation produces drastic changes in body shape; from a linear fourth instar larva to a smaller, round pupa. The latter stage was generally the least "preferred" by tadpole shrimp, or similarly, the least vulnerable to predation. Behavioral analysis indicated slightly lower capture success and greater handling times for mosquito pupae than fourth instar larvae.

Although this study is not a definitive test of whether apparent prey size preference is due to active predatory selection or vulnerability of the prey, behavioral observations tend to support the latter hypothesis. The behavioral analysis showed that although the number of encounters between tadpole shrimp and second instar larvae were fewer in frequency than with fourths and pupae, capture success of second instars was far greater. Since second instar larvae were observed (but not quantified) on the videotaped predation sequences to consistently display weaker escape responses (in velocity and dis-

Table 3. Capture success rates, handling times (sec) and encounter frequencies (no./30 min) of *Culex quinquefasciatus* second and fourth instars and pupae by *Triops longicaudatus* (carinal length = 7 mm) from 30-min video recordings (n = 4) in the laboratory (ANOVA; Duncans multiple-range test: unshared letters indicate significant differences).

	ANOVA results			Instar/stage		
Quantified behaviors	*F*	*df*	*P*	L2	L4	P
Capture success	49	2	0.0001	0.26a	0.05b	0.04b
Handling time	16	2	0.0000	31a	73b	175b
Encounters	5	2	0.0356	15a	48b	58b

tance) when encountering a predator, they may be more vulnerable to being captured. These observations suggest vulnerability to have an important influence on size-dependent predation. However, lower encounter rates of second instar larvae may be in part due to their smaller size, swimming behavior and position in the water column.

Prey-size selection is an important factor to consider when evaluating new biological control agents. The proper degree of control may only be realized when there are appropriate relative sizes of predator and prey. For example, in this study we found that smaller tadpole shrimp (carapace length <4.0 mm) do not consume third or fourth instar *Cx. quinquefasciatus* larvae. In the field, some stagnant water mosquito larvae produced from ovipositions during the first days after flooding may escape predation by becoming third and fourth instars before the tadpole shrimp have grown to 4.0 mm in carapace length. Similar situations may occur where prey growth rates are very high, as in the floodwater mosquito, *Psorophora columbiae* Dyar and Knab, whose eggs hatch immediately upon hydration. These mosquitoes may completely escape predation by rapid development and emergence.

Whether similar prey-size "preference" relationships exist for other prey species of *Triops longicaudatus* remains to be investigated. What happens if tadpole shrimp are offered a variety of plants and invertebrates as a food source? The presence of an alternative food source (TetraMin® fish flakes) was found to significantly ($P < 0.05$) reduce the consumption of second instar *Cx. quinquefasciatus* larvae by *Triops longicaudatus* in the laboratory (unpublished data). Studies concerning tadpole shrimp relative preference for various prey taxa and possibly prey-switching between these taxa deserve future investigation.

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