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Utilization of the Exotic Cladoceran *Daphnia lumholtzi* by *Gambusia affinis*

B.A. Metzke and C.L. Pederson
Eastern Illinois University Biological Sciences Department
Charleston, IL 61920

ABSTRACT

The exotic cladoceran, *Daphnia lumholtzi* Sars, possesses cyclomorphic features which are widely assumed to deter gape-limited predators. We presented adult *Gambusia affinis* with either *D. lumholtzi* or *Daphnia pulex* in effort to determine utilization of *D. lumholtzi* as a food source relative to a native prey item. Neither *D. lumholtzi* nor *D. pulex* were rejected by *G. affinis* more often than the other, but medium and large *Daphnia* were ingested more often than small individuals, regardless of prey species. These results suggest *G. affinis*, and therefore other gape-limited predators, may be able to utilize *D. lumholtzi* as a food source.

INTRODUCTION

As predator-prey interactions are a major driving force in freshwater community composition (Swaffar and O'Brien 1996), many zooplankters have developed mechanisms, both behavioral and morphological, to avoid predation by planktivores (Lysebo 1995). For instance, some taxa undergo diel vertical migration to escape predation (Zaret 1980, Williams and Pederson 2004). Others, particularly cladocerans, have developed cyclomorphological features to prevent ingestion by vertebrate, or gape-limited predators (Wetzel 2001). Gape-limited predators ingest their prey whole, and thus, maximum prey size is restricted to items that do not exceed the dimensions of the predator's mouth opening (Zaret 1980). *Daphnia lumholtzi* Sars is one such zooplankter that exhibits morphological features which may inhibit predation by gape-limited predators. In particular, *D. lumholtzi* possess lateral fornices, ventral spines, and pronounced anterior and posterior spines whose length can greatly exceed those of native *Daphnia* species (Swaffar and O'Brien 1996, Kolar et al 1997, Dzialowski et al 2000).

The natural distribution of *D. lumholtzi* includes southwestern Asia, Australia and most of Africa (Benzie 1988) where it can be found in habitats ranging from deep tectonic lakes to turbid temporary ponds. It was first reported in North America from Fairfield Reservoir, Texas in 1991 (Sorensen and Sterner 1992) and has since spread throughout much of the eastern half of the United States (U.S.G.S. 2001). The presence of *D. lumholtzi* in North American waters has been hypothesized to negatively impact planktivorous fish populations both by decreasing native prey abundance through competitive interactions (Kolar et al 1997, Lemke et al 2003) and by reducing predator feeding efficiency (Swaffar and O'Brien 1996). However, *in situ* experiments have concluded *D.*

lumholtzi can be utilized by planktivorous fish, but also suggest rejection of *D. lumholtzi* as a food source when gape limitation occurs, as in the case of juvenile fishes (Lemke et al 2003). Understanding the ecological relationship between gape-limited predators and *D. lumholtzi* is pertinent in that it considers the ability of planktivores to consume an non-native but potentially important food item.

Within this context we attempt to evaluate utilization of *D. lumholtzi* as a food source by a gape-limited predator. We also compare utilization of a native cladoceran, *Daphnia pulex* (Leydig) to that of *D. lumholtzi*. The western mosquitofish, *Gambusia affinis* (Baird and Girard, 1853) was used in this study as a model gape-limited predator because it remains planktivorous as an adult and is easily maintained in laboratory conditions (Carolina Biological Supply Company 2006, Manfield and McArdle 1998).

METHODS

Female *D. lumholtzi* were obtained by using vertical tows with an 80 µm mesh plankton net from Lake Barkley, an impoundment of the Cumberland River in western Kentucky. Individual females were placed in 30mL medicine cups filled with synthetic hard water (USEPA 1991) and incubated at 25°C on a 12-hour light/dark cycle. On alternate days, each female was fed approximately 1mL each of YCT (a mixture of yeast, powdered alfalfa leaves, and fish flake food) and a suspension of the green alga, *Selenastrum capricornutum* (USEPA 1991). Every third day the water in each medicine cup was replaced with fresh hard water. Both YCT and *S. capricornutum* as well as gravid *D. pulex* were obtained from Aquatic Biosystems, Inc., Fort Collins, Colorado. Female *D. pulex* were maintained in the same manner as was *D. lumholtzi*.

Upon maturity, females of both *Daphnia* species produced a clutch of one to fifteen neonates every 2-3 days. Offspring were incubated in their own individual medicine cups and allowed to mature for a specific number of days to produce sufficient individuals of a given size class as needed for predation experiments. In addition, ten individuals from each day class were removed and body length and total length measurements were obtained using an ocular micrometer. Body length was measured from the base of the head spine to the anterior end of the tail spine, while total length was measured as the distance between the termini of the head and tail spines. Age specific lengths were determined to ensure standard prey sizes in single and mixed species prey communities during predation experiments.

Approximately 100 *G. affinis* were obtained from Carolina Biological Supply Company, Burlington, North Carolina. Fish were divided equally among 5 10-gallon aquaria and fed tropical fish flakes daily as per label directions. For predation experiments, a single female *G. affinis* was randomly chosen and placed in a glass 2L trial chamber (2L cylindrical beaker) filled with 1 L of water gathered from the aquaria. Each fish was starved for 24 hours prior to the feeding trial and allowed to acclimate to laboratory conditions for 12 hours. At the conclusion of the acclimation period, each fish was offered 20 *Daphnia* from one of nine possible prey compositions, randomly chosen before trial initiation. Experimental prey composition could be 100% *D. lumholtzi*, 100% *D. pulex*, or 50% of each species and was composed of one size class that included either small (1-day old), medium (5-day old for *D. lumholtzi* and 7-day old for *D. pulex*), or large (11-day old) individuals. Trials with a single prey item were used to assess the impacts of prey size on predation rates, while 50/50 trials were used to evaluate predator preference for a given species of prey. A piece of cardboard was placed behind the trial chamber to facilitate observation of fish behavior during trails. To avoid unnecessary disturbances, only the observer was present in the laboratory during each trial. All trials were conducted for a maximum of 5 minutes.

At the conclusion of each predation trial, *G. affinis* was removed from the trial chamber, euthanized, and placed in 70% ethanol. Body length, vertical gape length, and horizontal gape length were recorded for each fish. The water remaining in the trial chamber at the conclusion of each predation trial was filtered to remove any uneaten prey. These remaining *Daphnia* were identified and the number rejected was recorded. This process was repeated three times for each possible prey composition for a total of 27 trials.

We used 1-way ANOVA to compare frequency of rejection of individual prey items with size as the independent variable. In combined prey trials, we used 2-way ANOVA to determine significant differences in mean number of prey items remaining at conclusion of the combined prey trials. Significance of main effect variables and interaction of prey species and prey size were considered at a level of $\alpha = 0.05$. All statistical analyses were performed using SPSS 13.0 for Windows.

RESULTS

Mean *D. lumholtzi* body length ranged from 0.60 mm as neonates to 1.76 mm as adults, while mean total length ranged from 1.12 to 2.64 mm. Mean body length of neonate *D. pulex* was similar to that of neonate *D. lumholtzi* (0.65 mm), but upon reaching the adult instar, *D. pulex* had a mean body length of 2.19 mm. Mean total length of neonate *D. pulex* was 0.88 mm and 2.64 as adults, which is the same mean total length as adult *D. lumholtzi* (Fig. 1). With respect to general patterns of growth, body length of both *Daphnia* species were similar throughout the juvenile instars, although *D. pulex* body length surpassed that of *D. lumholtzi* upon reaching adulthood. *D. lumholtzi* possessed a longer total length throughout the juvenile instars, but adult individuals of both species were similar length (Fig. 1). Upon reaching the adult instar, total length of both prey species exceeds *G. affinis* gape length resulting in the potential for gape-limitation. Mean vertical and horizontal gape length of *G. affinis* was 2.48 and 2.22 mm, respectively.

Size classes of prey were established such that similar sized individuals of the two prey species were used during predation experiments. Small prey were 1-day old individuals with corresponding mean total lengths of 1.12 mm for *D. lumholtzi* and 0.88 mm for *D. pulex*. Individuals used for the medium prey size category had mean total lengths of 1.75 mm for *D. lumholtzi* (which corresponds to an age of 5-days old) and 1.77 mm for *D. pulex* (7-days old). For the large size class, both *D. lumholtzi* and *D. pulex* were 11-days old and had mean total lengths of 2.64 mm.

For 100% *D. lumholtzi* predation trials, *G. affinis* rejected a mean of 15.67 (± 0.67), 0.00 (± 0.0), and 3.00 (± 1.57) prey for small, medium, and large size classes, respectively (Fig. 2). *G. affinis* rejected small *D. lumholtzi* more often than either medium or large individuals (F=552.22, $P<0.01$ for medium prey; F=57.76, $P<0.01$ for large prey), but there was no significant difference in rejection rates between medium and large size classes (F=3.86, P=0.12).

G. affinis rejected a mean of 15.33 (± 4.18), 0.00 (± 0.0), and 0.58 (± 0.33) prey for small, medium, and large size classes, respectively, during 100% *D. pulex* predation trials (Fig. 2). *G. affinis* rejected small *D. pulex* more often than either medium or large individuals (F=13.48, P=0.02 for medium prey; F=12.25, P=0.03 for large prey), but, as with *D. lumholtzi*, there was no significant difference in predation on medium and large size classes (F=4.00, P=0.12).

In order to test species-specific predation rates within size classes, comparisons of prey rejection were made for 50/50 predation trials (Fig. 3). When presented with small prey, *G. affinis* rejected a mean of 4.73 (± 2.73) *D. lumholtzi* and 3.51 (± 2.03) *D. pulex*. *G. affinis* rejected a mean of 1.53 (± 0.88) *D. lumholtzi* and 1.15 (± 0.67) *D. pulex* during medium size class trials. Finally, during large size class trials, *G. affinis* rejected a mean of 3.61 (± 2.08) *D. lumholtzi* and 0.58 (± 0.33) *D. pulex*. Neither the main effect of prey species (F=1.452, p=0.251) nor the interaction between main effect variables (F=0.368, p=0.70) were significant. However, predator rejection of small-sized individuals was greater than that the medium sized prey (F=4.342, p=0.038).

DISCUSSION

Regardless of prey species, we observed that *G. affinis* rejected small prey more often than medium or large prey. This may have resulted from difficulty in detection of small individuals, rather than from gape limitation. Gape-limited predators utilize visual senses to detect prey (Mills et al 1986, Wetzel 2001, Wissel et al 2003) and small *Daphnia* occupy less of the predator's visual field and possess a less conspicuous pattern of movement. Medium and large *Daphnia*, however, may not have escaped detection, and accordingly, were preyed upon at a significantly higher rate.

As *G. affinis* gape size was smaller than the total length of large sized individuals of both *Daphnia* species, one would expect large prey to be rejected more often than medium prey (Zaret 1980). However, there was no significant difference in predation on medium and large prey during this study. Even though total length of large prey items exceeded gape dimensions of *G. affinis*, capture of daphnids along their long axis would permit ingestion. Although not formally measured during predation trials, handling time of large prey appeared longer than that of small or medium prey, perhaps contributing to the high variability of rejection rates between individual trials.

Predation trials with 50/50 prey composition confirmed that *G. affinis* does not reject one prey species more often than the other. Similarity of total body length between the two prey species within a given size class likely accounts for this result. Although gape-limited predators often exhibit selectivity for certain prey (Kasumyan and Doving 2003, Keast and Eadie 1985, Link 2004), similar-sized individuals of *D. lumholtzi* and *D. pulex* may be similar with respect to other characteristics (e.g., movement patterns, overall visibility) that determine selection of prey by fish (Siefert 1972).

Previous studies (Lemke et al 2003, Swaffar and O'Brien 1996) focused on the potential impacts of cyclomorphic features on gape-limited predators, but did not consider preference of gape-limited predators when presented with both *D. lumholtzi* and native prey items. In this study we have determined predation rates may not differ between native daphnids and *D. lumholtzi* and that preference may be more dependant upon prey size as it relates to detectibility. This conclusion suggests *D. lumholtzi* may be utilized by planktivorous fish as a food source.

In our study, as with other laboratory experiments (Havel and Hebert 1993, Kappes and Sinsch 2002), cultured *D. lumholtzi* did not develop spines to a length typical of their wild counterparts. Since spine development in cladocerans often is triggered by the presence of both invertebrate and vertebrate predators, a lack of predatory cues in laboratory conditions often results in underdeveloped cyclomorphic features (Dzialowski et al 2003, Kappes and Sinsch 2002, Lysebo 1995, Work and Gophen 1995). Larger spines may augment total body length to the point where it exceeds predator gape size resulting in an overall reduction in available prey (Kolar et al 1997). Further analysis of interactions between *D. lumholtzi* with completely developed spines and gape-limited predators is necessary before the ecological impacts of this exotic zooplankter can be fully assessed.

SUMMARY

The widely accepted paradigm for evaluating impact of exotic species introductions is that they may interfere with ecological processes in the habitats into which they are introduced. In the case of *Daphnia lumholtzi*, concern derives from the potential displacement of native cladocerans with subsequent reduction in food resources available for planktivores, especially fish. This paper documents that extreme morphology of this exotic species does not deter a planktivore such as *G. affinis*, which preyed effectively on both *D. lumholtzi* and the native cladoceran, *D. pulex*. For either species, the only size class of individuals that were not preyed upon were those small enough to escape detection. A slight, but insignificant decrease in predation occurred with respect to those individuals with total body length approaching the gape dimensions of *G. affinis*. Based on these results, we conclude that pronounced head and tail spines of *D. lumholtzi* may not be sufficient to reduce risk to predation.

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Figure 1. Mean (n=10) total (body + spine) and body length of *Daphnia lumholtzi* and *Daphnia pulex*.

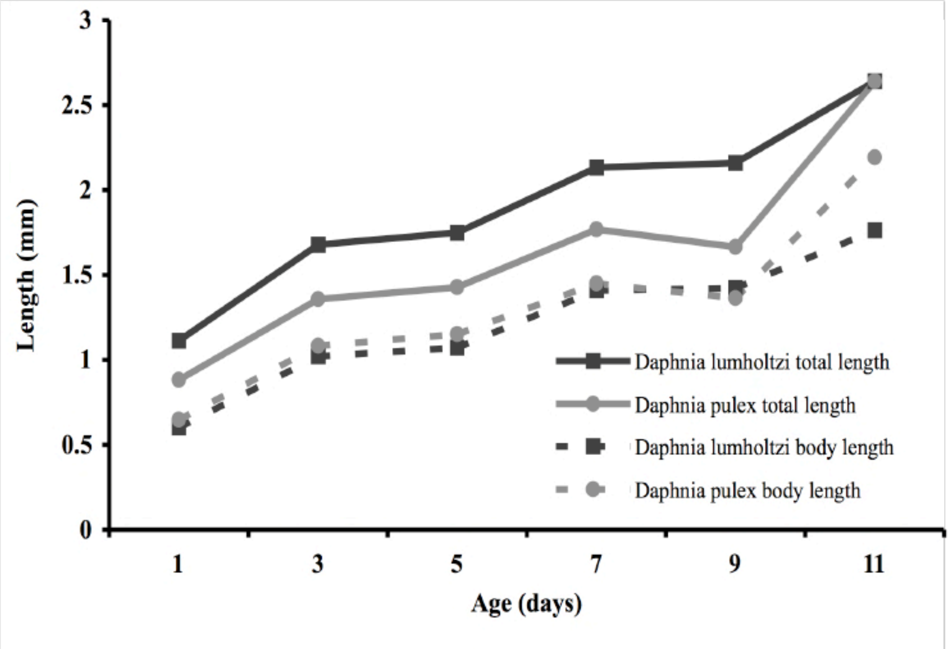

Figure 2. Mean (±1 SE) number of prey rejected during 100% predation trials (n=3).

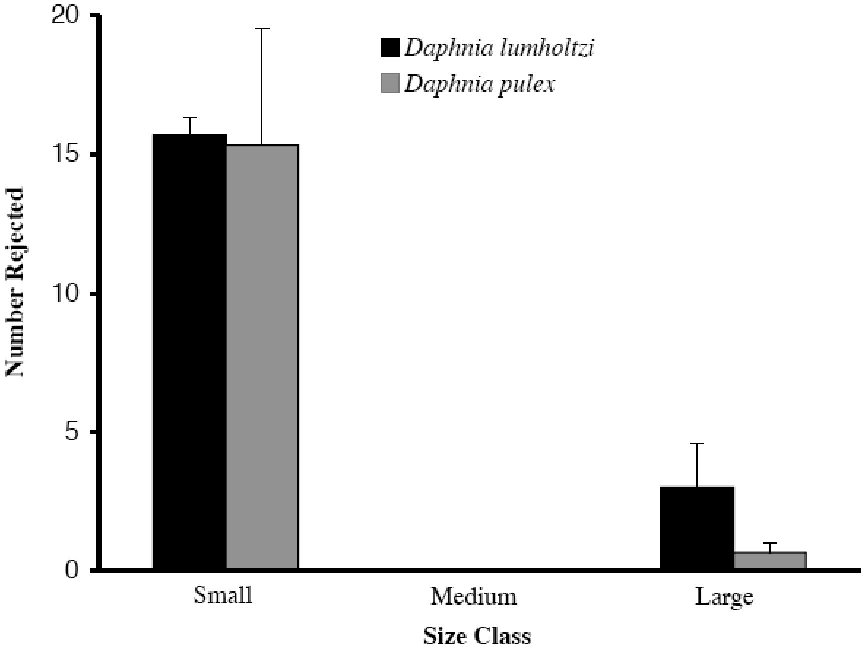

Figure 3. Mean (±1 SE) number of prey rejected during 50/50 predation trials (n=3).