PREY SELECTION BY MOSQUITOFISH (GAMBUSIA AFFINIS) IN CALIFORNIA RICE FIELDS: EFFECT OF VEGETATION AND PREY SPECIES

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ABSTRACT. Mosquitofish are widely used for biological control of mosquito larvae in rice fields, but the presence of different types of vegetation or alternative arthropod prey affects the performance of the fish. Feeding experiments were conducted in 4 rice field vegetation zones. Overall, predation was maximal in young and mature rice, moderate in the submerged naiad vegetation, and minimal in areas of open water. A preference for fourth instar Culex tarsalis mosquito larvae over Daphnia pulex and the amphipod Hyalella azteca was demonstrated. Second nymphal stage corixid bugs, Cenocorixa sp., were avoided; a similar notonectid, Buenoa sp., was eaten in amounts comparable to the D. pulex and H. azteca. No preference between Culex tarsalis and Anopheles freeborni mosquito larvae was detected.

INTRODUCTION

The mosquitofish, Gambusia affinis (Baird and Girard), is native to Central America and eastern North America (Rosen and Bailey 1963), and since the early 1900s has been stocked around the world to control mosquito larvae (Krumholz 1948). The natural diet of mosquitofish consists of insects and other invertebrates and a small amount of plant matter (Moyle 1976). Mosquitofish are widely used for biological control of mosquitoes in California, especially in rice fields.

Rice fields are complex ecological systems containing a variety of plant species in addition to rice. California rice fields consist of a shallow pan for the rice surrounded by deeper borrow pits and exposed levees. Terrestrial plants grow on the levees, and emergent and submerged macrophyte species grow in the water with the rice. Water temperature is dependent upon the water source, its rate of passage through the fields, water depth, vegetation present and weather.

Invertebrates present in California rice fields vary during the growing season and from field to field. As in most natural aquatic systems, an abundance of microorganisms is present (Miura et al. 1984); insects and crustaceans are the most abundant macroinvertebrates. Several mosquito species breed in rice fields in California, the 2 major species being Culex tarsalis (Coquillett) and Anopheles freeborni Aitken (Hoy et al. 1971).

Stocking mosquitofish in rice fields usually results in a reduction in mosquito larvae, but there is great variability in the data (Hoy and Reed 1970, 1971; Cech and Linden 1986, 1987). Some possible explanations for this variability are: variations in vegetation, the presence of alternate prey, insufficient numbers of fish, presence of mosquitofish predators and impact of mosquitofish on other mosquito larvae-consuming organisms. Our study investigated the effects of vegetation and the presence of alternate prey on mosquitofish predation on larval Culex tarsalis.

METHODS

We conducted mosquitofish feeding experiments in rice fields during the summers of 1984 and 1985. Most fish were seined from Putah Creek, Yolo County, California, a month prior to the beginning of each experimental series. Females with standard lengths between 35 and 40 mm were used. This size range eliminated fish too small to consume fourth instar Culex tarsalis mosquito larvae easily (Wurtsbaugh et al. 1980) and those larger than are normally found in rice fields (Botsford et al. 1987). The narrow size range controlled for effects of fish size on prey selection (Hess and Tarzwell 1942, Wurtsbaugh et al. 1980). Male fish were not used because they were much smaller than the females.

Fish were maintained in 3 wooden 165-liter tanks in the laboratory at the University of California, Davis (UCD). Tanks were fitted with a continuous flow of atmospherically equilibrated unchlorinated well water, maintained at 20 ± 1°C in 1984 and 30 ± 1°C in 1985 to approximate anticipated rice field temperatures. Fish were fed daily to satiation with Tetramin® complete flake diet during acclimation. A 12L:12D photoperiod was maintained. When early season water levels had stabilized in the rice fields, fish were moved from tanks to a 122 × 61 × 30 cm holding cage constructed of aluminum window screening (mesh size = 1.6 mm) which was set in a nonvegetated edge of one of the UCD rice fields. Fish continued to be fed daily with Tetramin.

Feeding experiments were conducted in a 50-cm cubic frame of welded 0.63-cm steel rods covered on 4 sides with 253-μm zooplankton screening leaving the top and bottom open. The nylon mesh was dyed olive green (similar to natural rice field background coloration) to
avoid highlighting the prey organisms. The frame was pressed down several centimeters into the rice field sediment in a chosen vegetation type for each feeding trial and covered with a transparent plastic top.

The 4 vegetation zones used in the experiments were: 1) bare sediment covered with clear water, 2) submerged aquatic macrophyte (primarily southern naiad, Najas sp.), 3) early season young rice (30-50 cm tall, with 1 plant/100 cm² density and 4) mature, dense rice stand (two 10-12 cm wide solid rows of stems that filled the water column).

Fourth instar Culex tarsalis mosquito larvae were the prey item of primary interest. Five other arthropods were chosen to present the fish with alternative prey that inhabited different parts of the water column—surface, midwater and bottom. Prey items were individually matched in size by length and girth to minimize size selectivity and temperature-adjusted prior to use in a feeding experiment.

Each feeding experiment used 250 individual prey items of one species or 125 each of 2 species. Prey were: 1) fourth instar larvae of the mosquito, Culex tarsalis, which were obtained from UCD laboratory colonies; 2) adult Daphnia pulex (Cladocera) which were collected from local ponds; 3) second instar Cenocorixa sp. bugs (Corixidae, Hemiptera) which were collected from local ponds; 4) adult Hyalella azteca (Amphipoda) which were obtained from Carolina Biological Supply Co. and a local pond; 5) fourth instar Anopheles freeborni mosquito larvae which were obtained from UCD laboratory colonies; and 6) second instar Buenoa sp. bugs (Notonectidae, Hemiptera) which were collected from local ponds. These prey occur naturally in California rice fields and represent taxa that have been found in the intestinal tracts of wild mosquitofish (Miura et al. 1979, Farley 1980).

Experimental fish were collected from the holding cage with an aquarium net. The first 12 individuals caught were used, unless a fish was too gravid. Parturitive females may not feed normally and young mosquitofish constitute alternative prey (Dionne 1985). Twelve fish were used in each feeding trial because mosquitofish typically school and may not behave normally when isolated. Fish were not reused.

The 12 fish were placed in the sampling frame 24 h before feeding experiments to clear their guts and consume any enclosed, naturally occurring prey items. At the start of the experiment, no naturally occurring prey were observed in the frame, and the 250 experimental prey items were concentrated into a 0.5-liter jar to ensure good mixing (i.e., of 2 species) and gently poured into the enclosure, distributing them evenly. An investigator watched discreetly until the first prey item was consumed, started a timer, and moved out of view of the fish. After 10 min the trial was terminated and the fish removed. Fish were immediately preserved in 70% alcohol until gut dissections could be performed. Water temperature was recorded after fish removal. Sometimes one or more fish were not recovered from the sampling frame, having apparently hidden in the mud or vegetation. Fish not recovered within 3 min after the experiment ended were not used in analyses of the results as regurgitation or progression of digestion could have altered stomach contents.

To eliminate some of the natural variability in field conditions, feeding experiments were only conducted on clear days and at the same time of day (1400 h). The field sampling frame was moved to a new spot in the rice field for each experiment to avoid any remaining prey items and because the submerged vegetation was often severely damaged or disturbed when the fish were netted out.

Accurate identification of the organisms in the gut could be made because fish did not macerate the food appreciably. The stomach was dissected from the fish, slit carefully, and individual prey items teased from the ingested mass and identified. For each fish the total number of prey items of each species was recorded and standard length was measured. Forty feeding experiments used a total of 480 fish of which 456 were recovered and dissected.

Data were first analyzed for effects due to differences in fish length or temperature. Multiple regression analysis was employed on the total number of prey items eaten vs. temperature, standard length and indicator variables for the vegetation and prey types used. There was a small but statistically significant effect due to temperature on total prey eaten within the range encountered (18.9-31°C). Data showed an increase of 0.1 prey items per degree centigrade increase in temperature. Because field temperatures were confounded with vegetation type due to shading, prior to analyzing for the effects of vegetation, all data were standardized to the temperature of the warmest trial by adding 0.1 prey items per degree centigrade difference in temperature.

Temperature-adjusted data were analyzed using a 2-way analysis of variance on the single-prey data to examine the effects of vegetation and prey type. Because sample sizes were unequal due to some unrecovered fish, these analyses of variance were done by the regression method. Single-way analysis of variance and Tukey or Bonferroni multiple comparison procedures were then performed within each series.

Data from the 2-prey experiments were converted to percentages and a Kruskal-Wallis one-way analysis of variance by rank run on each prey combination followed by Dunn's multiple
comparison procedure. Student's $t$-test compared the 2 $Cx. \text{tarsalis} + An. \text{freeborni}$ vs. $Cx. \text{tarsalis}$ experiments and a $Buenoa \text{sp.}$ vs. $Cenocorixa \text{sp.}$ experiment. A statistical significance level of $P \leq 0.05$ was adopted.

**RESULTS**

There was no effect on total number of prey items eaten due to fish length. This confirmed that the size range of fish used was sufficiently narrow to control for any effects due to variation in fish size, eliminating this factor from further consideration.

Analyses of single-prey experiments, within each prey type, showed a significant increase in number of prey items consumed with the presence of vegetation (Figs. 1 and 2). In Fig. 1, $Cx. \text{tarsalis}$ were eaten in significantly greater amounts in naiad and rice than in open water. $Daphnia \text{pulex}$ were eaten in significantly greater amounts in rice than in naiad or open water. In Fig. 2, $Cx. \text{tarsalis}$ were eaten in significantly greater amounts in all 3 vegetated zones compared to open water, and a significant difference also existed between naiad and young rice zones. Consumption of $D. \text{pulex}$ increased with vegetation, with significant differences between the open water and all vegetated zones and between naiad and rice zones. $Hyalella \text{azteca}$ were eaten in similar quantities in open water and naiad, but in significantly greater quantities in rice. $Cenocorixa \text{sp.}$ experiments were an exception to this trend in that few were eaten under all conditions.

Analyses within vegetation types and across prey types showed that $Cx. \text{tarsalis}$ were consumed in the highest numbers, $D. \text{pulex}$ and $H. \text{azteca}$ were not significantly different from each other in most cases and were consumed in intermediate amounts, and $Cenocorixa \text{sp.}$ were consumed the least (Figs. 1 and 2).

Specific interactions between particular vegetation types and particular prey species were also apparent from the single-prey data. $Culex \text{tarsalis}$, $D. \text{pulex}$ and $H. \text{azteca}$ were all eaten in similar amounts in open water (Figs. 1 and 2). In both young and mature rice $Cx. \text{tarsalis}$ was eaten in significantly larger numbers than $D. \text{pulex}$ or $H. \text{azteca}$; the latter 2 were eaten in equal amounts. Thus, rice would appear to provide a better refuge for $D. \text{pulex}$ and $H. \text{azteca}$ than $Cx. \text{tarsalis}$.

In the submerged macrophyte (naiad), $Cx. \text{tarsalis}$ were consumed in far greater quantities than $D. \text{pulex}$, which were consumed in greater quantities than $H. \text{azteca}$. The presence of naiad in the lower half of the water column apparently provided good refuge for the bottom and vegetation-associated $H. \text{azteca}$, moderate refuge for the mid-water swarming $D. \text{pulex}$ and no refuge for the surface-oriented $Cx. \text{tarsalis}$. $Buenoa \text{sp.}$ were preferred to the similarly shaped and behaving $Cenocorixa \text{sp.}$ Fish consumed $Buenoa \text{sp.}$ and $D. \text{pulex}$ in similar amounts. $Cenocorixa \text{sp.}$ may have been avoided because of its relative unpalatability due to the presence of distasteful scent glands (Usinger 1956), masking any effect due to vegetation.

Results of the 2-prey feeding experiments confirm and amplify the trends found in the single-prey experiments. When mosquitofish were fed $Cx. \text{tarsalis}$ in combination with either $D. \text{pulex}$ or $H. \text{azteca}$ in open water or rice (young or mature), approximately 60% of the prey items ingested were $Cx. \text{tarsalis}$ (Table 1). These vegetation types were structurally homogeneous throughout the water column. In the naiad which grew in the lower half of the water column, the percentage of $Cx. \text{tarsalis}$ ingested tended to be slightly higher (Table 1). There was no significant difference between the total number of prey items ingested of $Cx. \text{tarsalis}$ for the surface-oriented $Cx. \text{tarsalis}$. $Buenoa \text{sp.}$ were preferred to the similarly shaped and behaving $Cenocorixa \text{sp.}$ Fish consumed $Buenoa \text{sp.}$ and $D. \text{pulex}$ in similar amounts. $Cenocorixa \text{sp.}$ may have been avoided because of its relative unpalatability due to the presence of distasteful scent glands (Usinger 1956), masking any effect due to vegetation.

![Fig. 1. Feeding experiments (1984): number of prey eaten for 3 prey types in 4 vegetation types (mean ± SD). Horizontal lines connect prey types which are not significantly different (P > 0.05) from each other within vegetations. Prey types underlined with a single short line are significantly different from the other prey types in that vegetation.](image1)

![Fig. 2. Feeding experiments (1985): Explanation as given in Fig. 1.](image2)
Table 1. Mean ± SD prey consumed in 2-prey experiments, with (percent Culex tarsalis eaten) and number of fish.

<table>
<thead>
<tr>
<th>Vegetation type</th>
<th>Prey</th>
<th>Open</th>
<th>Naiad</th>
<th>Young rice</th>
<th>Mature rice</th>
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<tr>
<td></td>
<td>Cx. tarsalis</td>
<td>6.1 ± 2.2</td>
<td>15.1 ± 5.3</td>
<td>—</td>
<td>12.1 ± 2.7</td>
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<tr>
<td>+ D. pulex</td>
<td>3.8 ± 1.6</td>
<td>4.3 ± 2.9</td>
<td>—</td>
<td>6.4 ± 1.8</td>
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<tr>
<td></td>
<td>n = 12</td>
<td>n = 24</td>
<td>n = 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cx. tarsalis</td>
<td>8.3 ± 4.9</td>
<td>16.1 ± 2.7</td>
<td>—</td>
<td>12.3 ± 3.6</td>
</tr>
<tr>
<td>+ Cenocorixa sp.</td>
<td>0.9 ± 1.0</td>
<td>1.6 ± 1.1</td>
<td>—</td>
<td>2.8 ± 2.2</td>
<td></td>
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<tr>
<td></td>
<td>n = 12</td>
<td>n = 11</td>
<td>n = 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cx. tarsalis</td>
<td>5.3 ± 3.1</td>
<td>13.5 ± 4.1</td>
<td>8.9 ± 2.1</td>
<td>10.7 ± 2.2</td>
</tr>
<tr>
<td>+ D. pulex</td>
<td>3.4 ± 1.2</td>
<td>5.5 ± 2.8</td>
<td>6.2 ± 2.8</td>
<td>7.2 ± 1.8</td>
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<td>n = 12</td>
<td>n = 12</td>
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<tr>
<td></td>
<td>Cx. tarsalis</td>
<td>5.0 ± 2.9</td>
<td>16.2 ± 4.3</td>
<td>8.7 ± 3.9</td>
<td>10.2 ± 5.6</td>
</tr>
<tr>
<td>+ H. azteca</td>
<td>2.8 ± 1.5</td>
<td>3.2 ± 2.2</td>
<td>4.7 ± 5.4</td>
<td>7.3 ± 3.8</td>
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<td>n = 12</td>
<td>n = 12</td>
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</table>

alone or of Cx. tarsalis in combination with D. pulex or H. azteca in the 2-prey trials. When fed in combination with Cenocorixa sp., the amount of Cx. tarsalis eaten was closer to 80 or 90% under all vegetation conditions: the fish essentially ignoring the presence of the Cenocorixa sp. and feeding on Cx. tarsalis alone, as in the single-prey experiment.

Comparisons of Cx. tarsalis vs. Cx. tarsalis + An. freeborni in open water were not significant. The fish consumed 4.1 ± 2.5 (SD) Cx. tarsalis mosquito larvae compared with 4.6 ± 2.3 An. freeborni larvae (n = 12).

**DISCUSSION**

Mosquitofish exhibit selection in their feeding behaviors by consuming prey species in proportions that differ from the availability of those items in the environment (Hess and Tarzwell 1942, Miura et al. 1979). There are several possible reasons for these differences: some species of prey may be inaccessible to fish in their microhabitat; others may have distasteful chemicals, unmanageable spines, successful escape behavior or be well-camouflaged. There may also be some positive gustatory cues involved. Major items reported in the intestinal tracts of wild mosquitofish are crustaceans and the aquatic stages of insects (Sokolov and Chvaliova 1936, Farley 1980).

The increase in prey consumption with increasing temperature that we found might be expected because the growth and metabolic rates of mosquitofish increase with increasing temperature (Wurtsbaugh and Cech 1983, Cech et al. 1985). Reddy (1975) found that mosquitofish ate 150–200% as many larvae at 30°C than at 20°C during a 10-h period. However, it is highly questionable whether the 10-min feeding period in our study would allow this energetic need to be demonstrated. The increased consumption was more likely due to the greater hunger state of fish starved for the same length of time (24 h) at a warmer temperature as increased hunger state can alter prey capture rates (Mathaven et al. 1980).

Most literature reports that the presence of vegetation reduces the success of fish predators. Prey can be less visible in vegetation, reducing the reactive distance and increasing search time (Cook and Streams 1984). Prey are often camouflaged to match the vegetation. Fish may swim slower through dense vegetation while either searching or pursuing prey (Vince et al. 1976), resulting in longer search and handling times. Handling time is often increased as prey captured in vegetation or on sediment must be sieved from the detritus before being swallowed (Cook and Streams 1984). When a predatory fish is not successful in its first attempt to capture a prey item, the likelihood of the prey being able to elude successive attempts is increased when vegetation is present (Cook and Streams 1984).

In contrast, the presence of vegetation increased the number of prey items ingested by mosquitofish in our study, possibly due to prey highlighting against the vegetation. Naturally occurring mosquito larvae in the rice fields were often varying shades of green, perhaps due to diet. The laboratory-reared larvae used in these studies were brown, similar to the diet they ingested. However, the alternative D. pulex and H. azteca were a similar color to the laboratory-reared mosquito larvae.

Mosquitofish behavior with and without vegetation may also have been a major factor con-
tributing to the general trend of increased consumption in vegetation. Mosquitofish naturally inhabit the vegetated shallow fringes of ponds and streams (Stearns 1976). Casterlin and Reynolds (1977) found that when given a choice, mosquitofish selected submerged vegetation rather than open water and preferred vegetation which afforded them lateral concealment.

Mosquitofish are able to penetrate dense vegetation. Armstrong (1977) showed that they could swim at speeds of up to 106 m/h through dense cattails. Davey and Meisch (1977) found that stocked mosquitofish rapidly dispersed throughout rice fields. Hence, it is unlikely that inaccessibility of the prey was a problem for our fish except in the mature rice in the field. There, such dense walls of rice may have had the simple effect of confining both prey and fish to a smaller volume of water.

The prey items we used actively associated with the vegetation to varying degrees, but none had much time to orient themselves when they were introduced prior to the start of a feeding experiment. Mosquito larvae may be able to reduce their visibility to predaceous fish by inhabiting the meniscus associated with the air-water interface around many plant stems. Wurtsbaugh et al. (1980) found that Cx. tarsalis larvae sought refuge in the meniscus around the edges of aquaria. However, close examination of rice plants has shown that the rice stems actually are hydrophobic, causing a reverse meniscus around the stem (Raskin and Kende 1985). Although Cx. tarsalis larvae and An. freeborni larvae orient differently with respect to the water surface, experiments offering Cx. tarsalis + An. freeborni in open water and in submerged macrophyte did not show differential predation by mosquitofish. A more complex habitat with varied surface vegetation might elucidate a difference in meniscus utilization between the 2 mosquito species.

Although rice fields contain a wide variety of vegetation types, we have shown that mosquitofish are able to prey successfully in several of these. However, the presence of much floating or surface vegetation would likely shift predation away from mosquito larvae to other more accessible prey. Presence of suitable alternate prey items will also reduce the number of mosquito larvae consumed. Thus, in rice fields containing much surface vegetation or other prey species, predation on mosquito larvae will be reduced but not eliminated.

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