COMPARATIVE LARVIVOROUS PERFORMANCES OF MOSQUITOFISH, *GAMBUSIA AFFINIS*, AND JUVENILE SACRAMENTO BLACKFISH, *ORTHODON MICROLEPIDOTUS*, IN EXPERIMENTAL PADDIES

JOSEPH J. CECH, JR. AND ALISON L. LINDEN

Department of Wildlife and Fisheries Biology, University of California, Davis, Davis, CA 95616

ABSTRACT. Mosquitofish, Sacramento blackfish, or combinations of both species were stocked in experimental paddies. Cultured *Culex tarsalis* larvae, stocked weekly into each paddy, and wild *Anopheles freeborni* larvae were counted by dipping. At the end of the 12 week experiment, paddies were drained and the remaining fish and visible invertebrates counted. Data show the highest number of mosquito larvae in the blackfish-stocked paddies. This low larvivorous ability of blackfish may result from their rapid growth to ca. 70 mm standard length and a consequent shift to a filtering mode of feeding. Although blackfish populations decreased from one-third to one-half of their stocked levels and mosquitofish populations increased to very high levels, mosquito control seemed to be a function of species rather than just the number of predatory fish. The lower number of larvae in the control paddies compared with the blackfish paddies might be attributable to predatory invertebrate communities.

INTRODUCTION

The mosquitofish, *Gambusia affinis*, has been employed for mosquito control in California for decades including areas such as rice fields (Hoy and Reed 1970, Hoy et al. 1971, Farley and Younce 1978). Mosquitofish are generally good larvivorous predators, even in dense stands of rice (Linden and Cech 1983). They reproduce readily in rice field habitats, thereby increasing their numbers for anopheline mosquito larval population surges late in the rice-growing season (Wainwright et al. 1985). However, because they are livebearers, their fecundity is rather small and the difficulties in mass-rearing these fish represent a major obstacle in increasing their rice field effectiveness in California. Specifically, problems with reproduction at low temperatures over the winter and with minimization of cannibalism of the young, restrict available numbers of mosquitofish for early rice season stocking.

Use of a second fish species during the early rice season may improve control of mosquito larvae. The Sacramento blackfish, *Orthodon microlepidotus*, is a native member of the Cyprinidae (minnow family) which is often found as an immigrant in some rice fields. In Clear Lake, California, it preys upon zooplankton and insect larvae as a young fish up to 40–75 mm standard length (SL) (Murphy 1950). Its fecundity is high (14.5×10^3 to 371.5×10^3 eggs for females 200 to 500 mm SL), due in part to its large size (Moyle et al. 1982). Ripe individuals have spawned in concrete-lined ponds at the University of California, Davis, (Cech et al. 1982) when provided brush or polyethylene strips as a substrate and even in laboratory aquaria (J. J. Cech, Jr., D. T. Castleberry, and A. L. Linden, unpublished data). Blackfish spawning has not been observed in rice fields.

The overall objective of the field experiments was to quantitatively compare the larvivorous ability of juvenile blackfish with that of mosquitofish against stocked and wild mosquitoes in experimental paddies. The small paddies (30.5 x 3.5 m) at the University of California, Davis, Rice Research Facility were considered "bridges" in scale between small laboratory tanks and large commercial rice fields. Subobjectives were to determine the effects of stocking density of each fish species and to assess the effects of species combinations. One of the important life history differences between the two species is that mosquitofish reproduce readily in paddies whereas the juvenile blackfish do not. Thus, mosquitofish populations typically show dramatic increases in numbers as their reproductive rates vastly overwhelm their mortality rates through the rice growing season. In contrast, blackfish show steady declines due to natural mortality (Cech and Linden 1986). One of the arguments for using blackfish concerned their potential availability in large numbers for rice field stocking at the beginning of the season. It was reasoned that combinations of the two species might function to best control rice field mosquito larvae throughout the growing season–blackfish providing early predatory abilities while mosquitofish would provide control later in the season.

MATERIALS AND METHODS

Mosquitofish for the field experiments were obtained from the Sacramento-Yolo Mosquito Abatement District, whereas juvenile Sacramento blackfish were produced by the natural spawning of ripe adults in two campus ponds. Adult Sacramento blackfish in spawning condition were obtained by seining in Clear Lake during April and May. These adult blackfish were stocked in concrete-lined ponds which had been equipped with anchored polyethylene strips which floated up to simulate rooted aquatic plants at the University of California, Davis (UCD). Young of the year blackfish from these wild broodstock were collected from the ponds by seining and stocked variously into 21 experimental paddies (30.5 × 3.5 m) at the UCD Rice Research Facility in early June 1985. Three of the paddies were stocked with 1,320 Sacramento blackfish; three were stocked with 10 mosquitofish; three with 30 mosquitofish; three with a 30:30 combination; one with a 64 blackfish:30 mosquitofish combination; three with a 300 blackfish:30 mosquitofish combination; and five controls had no fish stocked. In the data analysis, the data from the 64:30 combination paddy were combined with those from the 30:30 combination paddy.

Sampling and stocking of mosquitoes were conducted during the entire 12-week rice growing season. *Culex tarsalis* mosquitoes were cultured en masse at the UCD Ecology Institute Building so that between 3,000 and 10,000 larvae could be stocked weekly into each paddy. Dipper samples were made weekly during the early season, 60 dips per paddy, in the rice two days after mosquito stocking. During the last two weeks of the season, we dipped three times weekly. Dipper samples were concentrated using a fine mesh strainer cup and transferred to a graduated sample cup of water. Mosquito larvae were identified by species and larval stage and counted within 6 hours. Significant development of filamentous algae was noted in the paddies during the ninth week of the season. Algae were identified as *Cladophora, Spirogyra* and *Zygnema* by Professor Norma Lang of the UCD, Department of Botany. The algae were quantified by the amount collected in the regular larvae dipper samples as ml per 60 dips, as read from graduations on the sample cup, during the last four sampling days. At the end of the season, water was drained from the paddies and the remaining fish counted in the outflow traps and borrow pits. Also, a count of visible water striders (Gerridae) and notonectids (Notonectidae) was made in the low water remaining in the borrow pits of each paddy.

Data were compared using analysis of variance and correlation statistics. We defined the level of significance to be $P \leq 0.05$.

RESULTS

Figure 1a shows that the number of mosquito larvae was significantly higher in the blackfish-stocked paddies than in any of the other paddies (208 ± 25 SE). These data are from the last four dipper sampling dates when larval densities were highest primarily because of large numbers of wild anopheline mosquitoes. Figure 1a also shows that the mean larval numbers were lowest in the "heavily stocked" mosquitofish paddies (57 ± 25) and next lowest in the 30:30 combination paddies (74 ± 22), although these numbers were not significantly different from the control paddies (82 ± 20), the 300:30 combination paddies (89 ± 25), or the "lightly stocked" mosquitofish paddies (104 ± 25). Part of the reason for this low larvivorous ability of the blackfish at the end of the season may concern their rapid growth to ca. 70 mm SL in the paddies and a predicted shift to a filtering mode of feeding (Murphy 1950). However, dipper sampling data from earlier in the rice growing season showed very low larval counts (<5 per 60 dips) in all the paddies and did not reveal a dramatically different trend or evidence of a threshold where a particle-to-filtering feeding mode shift may have been obvious. The reason for the low larval counts is unknown, but good larval survival in fish exclusion cages during a similar phenomenon in 1984 argues against poor water quality. Overall, the stocking of blackfish without mosquitofish, even at very high stocking rates, contributed to significantly worse mosquito predation efficiency in these experiments, even compared with the control paddies.

The quantity of algae in the paddies was inversely related to the number of mosquito larvae in the rice paddies during the last four sampling days. The reason for this relationship is unknown. Since 99% of these mosquitoes were wild *Anopheles*, there may have been paddy selection by the female *Anopheles* mosquitoes for oviposition or differential survival of mosquito larvae. Although there was no apparent geographical pattern (i.e., east to west) to the algae concentrations, there did seem to be an interaction with fish species (Fig. 1b). With the two paddies at the ends of the experimental rice field excluded from the analysis there was significantly more algae in the "heavily stocked" mosquitofish paddies than in any of the other paddy categories. The lowest mean algal volume was found in the blackfish paddies and the next lowest algal volume was found in the "heavily stocked blackfish" plus mosquitofish combination paddies (Fig. 1b). Thus, the paddies containing the most blackfish seemed to contain the least algae. It is known that juvenile blackfish can eat and grow on strictly plant-based diets (Cech et al. 1982). It is possible that the blackfish were cropping some of the algae in their paddies.

Figure 2 shows the number of fish remaining in each paddy when the water was drained at the end of the rice growing season. The control

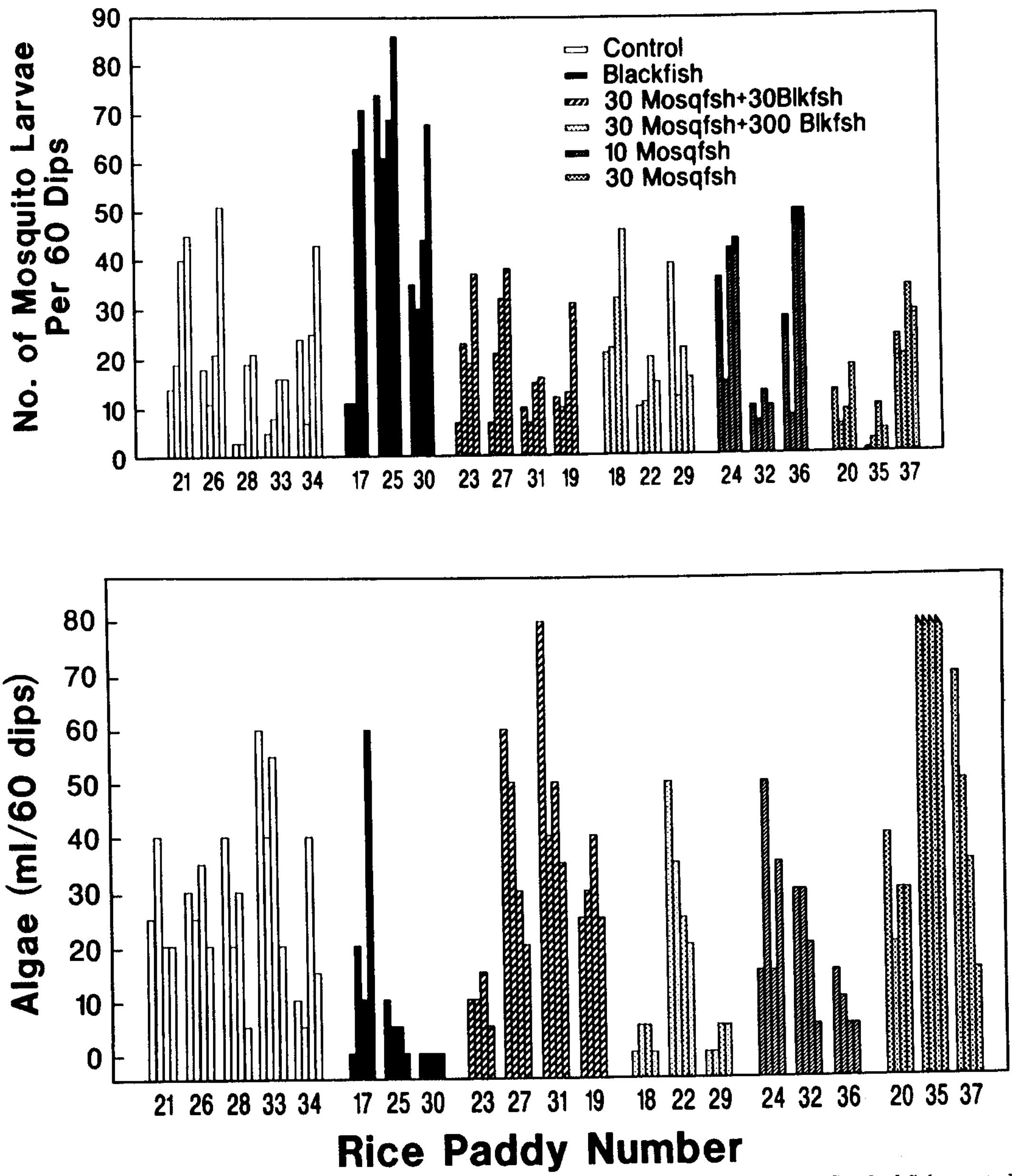

Fig. 1a. (Upper). Sampled mosquito larvae for last four sampling days from each paddy. Stocked fishes noted by shaded or stippled pattern. 1b. (Lower). Algae volumes from dipper samples for last four sampling days from each paddy.

paddies were virtually devoid of fish, indicating that our inflow and outflow screens and levees remained intact through the experiment. Also notable is that the blackfish populations were reduced to about one-third to one-half of the stocked levels, regardless of stocked level. In contrast to the blackfish, the mosquitofish population numbers all increased to very high levels. The paddies stocked with 30 mosquitofish (alone) and those stocked with 30 mosquitofish plus 30 blackfish had the highest mean mosquitofish populations: 1,720 and 1,840, respectively, even though the data from paddy 36 (10 mosquitofish stocked) may be an underestimate in that a leaking irrigation pipe prevented the borrow pits from drying sufficiently for a complete fish count. These two paddy categories had significantly more mosquitofish at the end of the season than the "lightly stocked" (10 fish) mosquitofish paddies and the 300:30 combination paddies. These results imply a more restrictive reproductive base number of fish (10 vs. 30) as

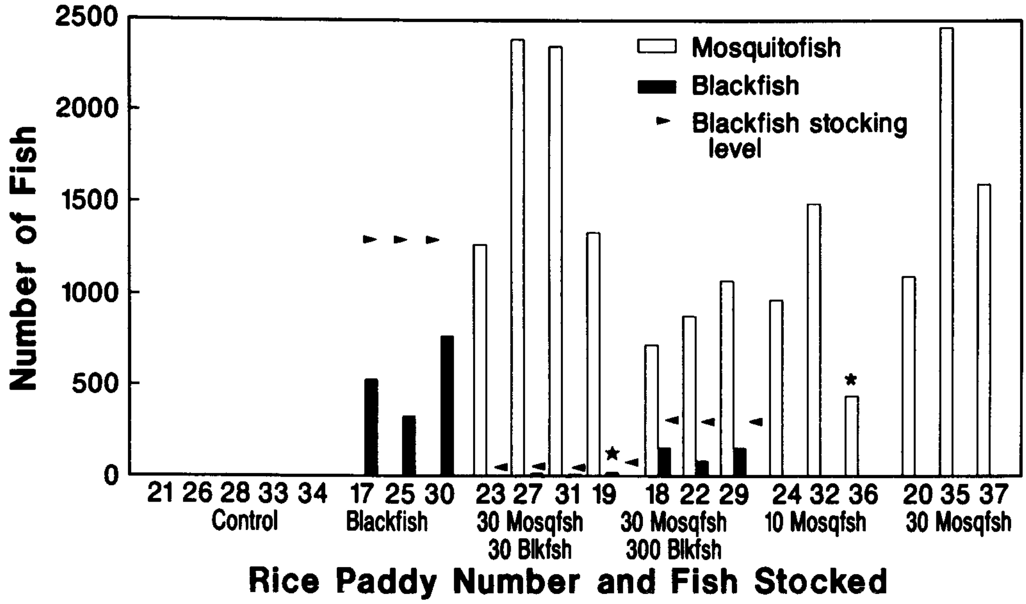

Fig. 2. Fish remaining at end of rice growing season. Arrows denote stocking level for blackfish. Star denotes paddy (No. 19) receiving 64 blackfish at stocking instead of 30. Asterisk denotes paddy (No. 36) with leaking irrigation pipe which prevented a complete fish count from dry borrow pits.

compared with density-dependent limitations of food resources for the young fish in these paddies. In contrast, when the 300 blackfish were stocked with the 30 mosquitofish density-dependent interactions (e.g., competition for food, predation) may have had importance towards the beginning of the rice growing season.

There was no significant difference between total fish numbers in the blackfish paddies and both the 300:30 combination paddies and the "lightly stocked" mosquitofish paddies, yet mosquito larval numbers were significantly higher in the blackfish paddies (Figs. 1a, 2). As a result, mosquito control seemed to be a function of species rather than just the number of predatory fish.

The lower number of larvae in the control paddies compared with the blackfish paddies might be attributed to the predatory invertebrate communities which developed in the control paddies. Figure 3 shows that the control and blackfish paddies had significantly more water striders than all other paddies. Importantly, the control paddies had significantly more notonectids than all of the other paddies, including the blackfish paddies. Notonectids are known invertebrate predators on mosquito larvae, and mosquitofish are known to prey upon notonectids (Bence and Murdoch 1982). Thus, notonectids and, perhaps, other invertebrate predators and parasites may have provided predation equivalent to that of the mosquitofish and combination paddies.

DISCUSSION

These experiments indicate that blackfish exert significantly less predatory control on mosquito larvae in experimental paddies than do mosquitofish. These data generally support results from similar field experiments during 1984, which compared mosquitofish, blackfish and hitch (*Lavinia exilicauda*). Mosquitofish reduced larval numbers significantly greater than either blackfish, hitch or controls in the 1984 studies (Cech and Linden 1986). However, mosquitofish populations had grown to contain 8–10 times the number of fish compared with the various minnow paddies. Consequently, a strong negative correlation (r value of -0.83) was calculated between total fish and total mosquito larvae samples in each paddy (Cech and Linden 1986). A more direct comparison can be made between the fishes' effectiveness in the present study (1985 season data), in which the correlation between total fish and total mosquito larvae (last four sampling dates) was -0.355.

Blackfish stocking density may have had a greater indirect effect than direct effect on mosquito control. There were lower numbers of blackfish in larger paddies during the 1984 season (fish density $\approx$ 1 fish$\cdot m^{-2}$), and mosquito larval numbers in blackfish paddies were indistinguishable from those in control paddies (Cech and Linden 1986). During the 1985 experiments, blackfish density approximated 5 fish$\cdot m^{-2}$ and mosquito larval numbers were significantly

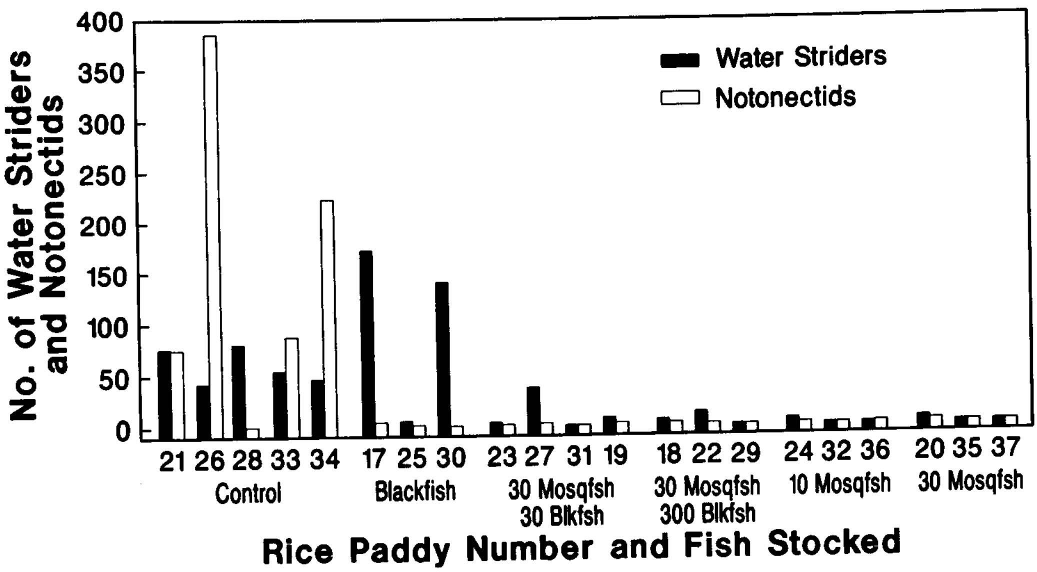

Fig. 3. Number of observed water striders (Gerridae) and notonectids (Notonectidae) in borrow pits of draining paddies.

higher than control paddies (Fig. 1a). Also in 1985, notonectid numbers were significantly lower in the blackfish paddies than in the control paddies (Fig. 3). The invertebrate survey of 1984 was limited to taxa identified in 50 dipper samples per paddy on one day when the paddies had begun to drain at season's end. Although the 1984 invertebrate sample yielded more taxa (Cech and Linden 1986), the method used in the present study yielded better estimates of notonectids and water striders. Blackfish mortality rates approximated $1\% \cdot d^{-1}$ during both field seasons.

In contrast with the blackfish densities, mosquitofish densities were remarkably comparable between the 1984 (16 $fish \cdot m^{-2}$) and 1985 (16 $fish \cdot m^{-2}$) field seasons. This consistency does not explain why mosquitofish displayed significantly better larval control over control paddies in 1984 (Cech and Linden 1986), but not in 1985. It is possible that invertebrate predators were more effective in control paddies in 1985, but the lack of comparable invertebrate data in 1984 precludes this analysis.

Blackfish may prey on fewer mosquito larvae than mosquitofish because of either reduced metabolic rates or a preference for alternative prey. However, routine metabolic rates for <1 g wet body weight mosquitofish and blackfish are roughly comparable. Mosquitofish (0.2 g) used 60, 130, and 200 $\mu g\ O_2 \cdot h^{-1}$ at 15, 25, and 30°C, respectively (Cech et al. 1985). Blackfish of 0.5, 0.2, and 0.4 g used 98, 179, and 327 $\mu g\ O_2 \cdot h^{-1}$, respectively at the same three temperatures (J. J. Cech, Jr., A. L. Linden and K. R. Marine unpublished data). The somewhat higher rates of the blackfish would be expected at the generally larger body sizes (Heusner 1984).

Experiments conducted in both laboratory and rice paddy enclosures show a consistent preference of mosquitofish for *Culex tarsalis* mosquito larvae over similar sized *Daphnia pulex* and *Hyalella azteca*.[1] In contrast, preliminary experiments with juvenile blackfish in the laboratory show no clear difference between *Culex tarsalis* and *Daphnia pulex* (J. J. Cech, Jr., A. L. Linden, and J. C. Chiu, unpublished data). Klein[2] showed that the blackfish gut lengthens from 1.5–2 body lengths at a fish standard length of 40 mm to 5–7 body lengths at 300–350 mm SL. Gut samples from Clear Lake-caught blackfish show a corresponding transition from cladocerans in the smaller juveniles to a "detritus" in larger fish (Murphy 1950; Jacobs, unpublished data). In Clear Lake this "detritus" was primarily algae and was not significantly different in composition than organisms samples from the surrounding water, indicating non-selective filter feeding (Murphy 1950; Jacobs, unpublished data). In the present study, blackfish up to 70 mm SL were recovered at the end of the season, perhaps suggesting a diverse diet from

[1] Linden, A. L. 1986. Prey selection by the mosquitofish, *Gambusia affinis*, in California rice fields: Effect of vegetation and prey species. M.S. Thesis. Univ. Calif., Davis. 89 pp.

[2] Klein, K. 1978. Comparative gut morphology of three California cyprinid fishes. Ph.D. Dissertation, University of California, Davis. 78 pp.

filtering including algae. Algae cropping by blackfish may have contributed to the low algae volumes found in the blackfish paddies (Fig 1b). Juvenile blackfish have shown the ability to feed and grow on an exclusively plant-based diet (Cech et al. 1982). No trend in mosquito larval numbers was noted in the blackfish paddies through the season indicating a shift from individual predation to filtering, except that which may have been masked by the rise in anopheline mosquitoes near the end of the season.

The differential abundance of algae among the paddies may have influenced ovipositional patterns of the wild anopheline mosquitoes during their peak abundance in the last two weeks of the season. Mosquitoes choose oviposition sites based on the presence or absence of vegetation, and the particular plant species (Angerilli and Beirne 1980). Plant species can function as attractants or repellants (Angerilli 1980). The presence of certain aquatic plant species can also inhibit growth and development of the mosquito larvae—the green alga *Chara* sp., which occurs in California rice fields, can cause 100% mortality of larvae (Angerilli and Beirne 1974). The variable distribution of the algae may also have affected colonization of the rice paddies by other invertebrate predators. Notonectids, water striders, predaceous diving beetles, damselflies and dragonflies have been shown to select habitats with certain aquatic vegetations over others (Angerilli and Beirne 1980).

Haas and Pal (1984) suggest "the need for more careful evaluation" of fish species besides *Gambusia*, "especially those which already occur in the geographical area where their systematic use has promise." The present study predicts serious drawbacks to the stocking of blackfish in California rice fields. However, non-significant differences among the various combination-stocked paddies and the mosquitofish-stocked paddies recommend against deliberate screening attempts to remove blackfish larvae and fry from the rice field irrigation inflows. Preliminary laboratory studies on juvenile hitch show a more aggressive feeding behavior and, possibly, a greater preference for *Culex tarsalis* larvae than shown by juvenile blackfish (J. J. Cech, Jr., A. L. Linden and J. C. Chiu, unpublished data). However the fish culture, transport and stocking requirements of using any non-reproducing species potentially place a higher cost on mosquito control. Mortality rates averaging $1\% \cdot d^{-1}$ (Cech and Linden 1986) imply massive fish stocking efforts which may have to be repeated through the rice season, after a suitable larvivorous species is found. Possible alternatives to these costs include the stocking of broodstock fish in a reservoir which supplies adequate numbers of surviving young-of-the-year fish along with the irrigation water.

ACKNOWLEDGMENTS

We gratefully acknowledge the support of the University of California Mosquito Research Program. Mr. Stuart McAllister assisted in the field and Dr. Norma Lang identified the algae. We benefitted from useful discussions with Mr. Leon Blaustein, Mr. Dan Castleberry, Mr. Robert Coykendall, Dr. Russell Fontaine, and Mr. Steve Mitchell. Experimental facilities were made available at the UCD Rice Research Facility and rearing facilities were accommodated by the UCD Institute of Ecology and Department of Zoology. The manuscript was typed by Mrs. Donna Raymond.

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