MOSQUITO CONTROL IN WASTEWATER: A CONTROLLED AND QUANTITATIVE COMPARISON OF PUPFISH (*CYPRINODON NEVADENSIS AMARGOSAE*), MOSQUITOFISH (*GAMBUSIA AFFINIS*) AND GUPPIES (*POECILIA RETICULATA*) IN SAGO PONDWEED MARSHES

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ABSTRACT. We compared the abilities of pupfish, mosquitofish and guppies to control mosquitoes in wastewater marshes. All species of fish reduced mosquito emergence. When fish population densities were similar, fish reduced emergence to similar levels. As experiments progressed, guppies developed greater population densities and provided better mosquito control than mosquitofish, which developed greater densities and better control than pupfish. Fish also reduced numbers of zooplankton, and guppies increased total plant biomass, suggesting fish may influence the ability of wastewater marshes to treat wastewater.

INTRODUCTION

Vascular aquatic plants can provide a remarkably efficient and inexpensive means of treating municipal, agricultural and industrial wastewater (Wolverton et al. 1976, Hauser 1984). Unfortunately, mosquito control is difficult in artificial marsh environments (Mortenson 1982). Biological control, using fish as predators, is potentially an inexpensive and environmentally safe means of mosquito control (Haas and Pal 1984, Meisch 1985).

The fish most commonly used for mosquito control, mosquitofish (*Gambusia affinis*) (Meisch 1985), has shown limited success in wastewater (Schaefer and Miura 1985, Bay 1985, Carlson et al. 1986). This prompted us to consider Amargosa pupfish (*Cyprinodon nevadensis amargosae*) and guppies (*Poecilia reticulata*) because, in addition to being larvivorous, they reproduce in marsh environments, tolerate a wide range of temperatures and low dissolved oxygen levels and are available in California (Castleberry and Cech, unpublished data; Moyle 1976). These traits are necessary for a fish to provide mosquito control in California wastewater. Pupfish and guppies have been considered for mosquito control in the past but have had varying results (Danielson 1968[1], Coykendall 1980, Hiscox 1980, Haas and Pal 1984, Bay 1985, Meisch 1985, Mian et al. 1986). Our objective was to determine which of these species provides the best mosquito control in wastewater marshes.

[1] Danielson, T. L. 1968. Differential predation on *Culex pipiens* and *Anopheles albimanus* mosquito larvae by two species of fish (*Gambusia affinis* and *Cyprinodon nevadensis*) and the effects of simulated reeds on predation. Ph.D. dissertation. Univ. of California, Riverside.

METHODS

We compared the abilities of pupfish, mosquitofish and guppies to control mosquitoes in wastewater using tanks (Fig. 1) as controlled and replicated experimental systems. We conducted 2 sets of experiments: July–October 1985 and August–September 1986. *Culex tarsalis* Coq. failed to survive early larval stages after day 25 in 1985 even though they survived well in wastewater during preliminary tests and early in the experiment. Consequently, we switched to *Cx. pipiens* Linn., a mosquito more tolerant of wastewater (Bohart and Washino 1978). Because this change required several weeks, mosquitoes did not start emerging until after fish were well established in the tanks. The 1986 experiment was conducted to collect information on the early effects of fish introduction and to minimize effects of differences in population densities of fishes.

Both experiments began by planting Sago pondweed (*Potamogeton pectinatus*) tubers (Kester's Wildgame Food Nursery, P.O. Box 5, Omro, WI) in each tank. In 1985, we planted 10 tubers, equally distributed throughout the tank, in builders' sand (Fig. 1). In 1986, we planted 20 tubers per tank in potting soil contained in 0.95-liter plastic pots. Potted tubers were buried in builders' sand. More tubers were planted in pots of rich soil in 1986 because 1985 results showed slow plant growth and Anderson and Bissel (1985) had demonstrated the importance of soil nutrients to the growth of Sago pondweed in wastewater.

Flow rates of secondary wastewater, pumped from a secondary clarifier at the University of California, Davis, Wastewater Treatment Facility, were set at 4–6 ml/sec to achieve < 1-day detention time in each tank. Our objective in setting flow rates (reset every 2 days) was to maintain consistent water quality within and

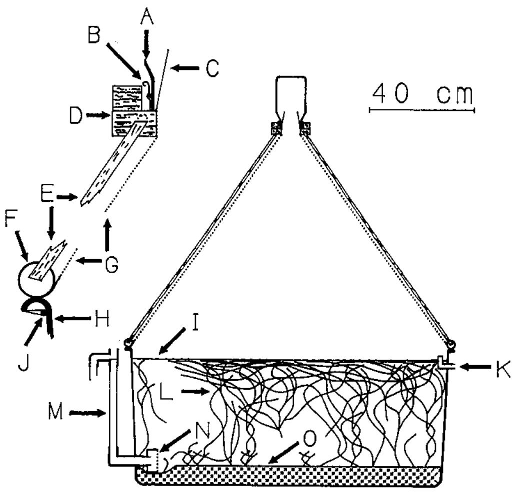

Fig. 1. Cutaway, scale diagram of a tank. Insets provide detail on jar attachment (top) and drawstring tie-down (bottom). The fiberglass window screen (G) formed a funnel attached at the bottom by a drawstring (J) to the tank lip and supported by four hardwood dowels (E). Dowels extended from a ring of polyethylene pipe (F), which rested on the outside edge of the round, 210-liter fiberglass tank (H), to a round plywood jar support (D). The top of the window screen funnel was sandwiched between 2 pieces of plywood supporting the jar. The 0.95-liter collection jar (A) screwed into a canning jar lid (B) attached to the jar support. A plastic cup with its bottom removed (C) inhibited escape from the jar. Water flowed in through polyvinylchloride (PVC) pipe (K) and out through a screened PVC pipe (N). Water level (I) was maintained by an exterior standpipe (M). Sago pondweed (L) grew from 7 cm of builder's sand (O) and provided a thick canopy just below the water surface.

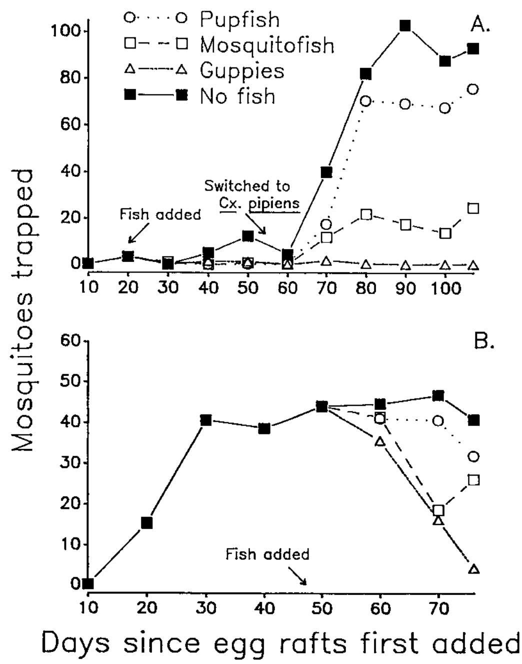

Fig. 2. A. (Upper) Mean of the 10 preceding days' mean numbers (n = 5) of mosquitoes emerged daily for the 1985 experiment (*Culex tarsalis* were used at the start of the experiment; *Cx. pipiens* were used once *Cx. tarsalis* proved unsuitable). The last mean presented is for the days following the last 10 day mean. Means prior to fish being added are for all tanks combined (n = 20). B. (Lower) Similar data for the 1986 experiment (*Cx. pipiens* were used throughout this experiment).

among tanks. After thick growths of pondweed developed, we placed 8 large mosquito egg rafts every 2 days in each tank throughout the experimental periods. These egg rafts were produced during the 2 previous days in our blood-fed laboratory colony at the University of California, Davis.

Both experiments used a randomized block design in which we split our 20-tank facility into 5 blocks of 4 adjacent tanks. We placed fish in 3 of 4 tanks per block, giving 5 replicates of each fish species and 5 controls (no fish). In 1985, we introduced 10 fish after mosquito adult emergence started (Fig. 2a) and in 1986, 30 fish after emergence stabilized (Fig. 2b). In both years, we kept sizes of fish similar among replicates and among species.

Enclosures on tanks (Fig. 1) allowed us to control the number of mosquitoes and mosquito predators and competitors that entered and to monitor the numbers of mosquito adults that emerged in each tank. Counts of mosquitoes captured in collection jars (Fig. 1) were made each morning to provide a daily measure of mosquito emergence. Previous studies (Castleberry 1986, Castleberry et al. 1989) showed that these counts provide precise and unbiased estimates of mosquito emergence.

We continuously monitored dissolved oxygen level in one tank using a LG Nester Model 8500X portable meter attached to a Soltec strip chart recorder. Every 2 days we moved the oxygen probe to another tank in the first block of tanks. We also read minimum/maximum thermometers placed in 5 of the tanks every 2 days to follow fluctuations in temperature throughout the experiment. On several dates during the 1986 experiment, measurements of inflowing and outflowing water hardness, PO_4, NO_2, NO_3 and NH_4, dissolved oxygen, temperature, pH, turbidity and redox potential were made by chemical analysis and a Hydrolab Corporation multifunction meter.

Samples of invertebrates were taken and fish were removed and weighed at the end of both experiments (November 7 in 1985 and October 6 in 1986). Invertebrate samples were taken in 1985 by draining all the water in the tanks through fine-mesh nets. Samples were diluted to 50 ml and the invertebrates counted in five 1-ml subsamples. Invertebrate sampling was simplified in 1986 by taking a 1-liter core of water and pouring it through a fine mesh net. Net samples were then fixed in alcohol so that invertebrates could be later identified and counted under a dissecting microscope. Field and laboratory checks on both laboratory (1985) and field (1986) subsampling methods showed that these approaches yielded reliable estimates of total invertebrate population sizes. Fish were fixed in 10% formalin and measured and counted later.

Inflowing and outflowing water quality values were compared using ANOVA. Numbers of mosquitoes that emerged for each treatment were compared using a blocked ANOVA with date and a qualitative measure of wind speed as covariates. Fish mass and number and number of invertebrates in appropriate treatments were compared using a blocked ANOVA. The effect of number of fish in tanks on numbers of mosquitoes emerged during the last 5 days of an experiment were assessed for each fish species using ANOVA. Data were log-transformed ($\log[x+1]$) before analysis. The ANOVAs were followed by appropriate hypothesis tests with Bonferroni adjustments (Wilkinson 1988). In 1985, all mosquitofish died in one tank; therefore, this tank was not used in analyses.

RESULTS

Water quality values[2] were within the range of values reported for similar experimental and operational water treatment systems (Hauser 1984, Reddy and Debusk 1985, Owen 1988[3]). Inflowing and outflowing measures of water quality were not significantly different ($P \leq 0.05$) between or within tanks.

All fish species significantly reduced mosquito emergence below emergence levels in no fish (control) tanks ($P \leq 0.05$) in both the 1985 experiment (Fig. 2a) and in the 1986 experiment (Fig. 2b). In 1985, the 3 fish species reduced *Cx. tarsalis* emergence to similar levels ($P > 0.05$) before the switch to *Cx. pipiens* (day 19–65). After the switch to *Cx. pipiens* (day 66–107), guppies reduced mosquito emergence more than mosquitofish, mosquitofish more than pupfish, and pupfish more than tanks without fish ($P \leq 0.05$, Fig 2a).

Prior to fish being added in 1986 (day 10–48), there were no significant differences between tanks which would receive fish and those which did not ($P > 0.05$). After fish were added in 1986 (day 55–77), guppies reduced emergence to a greater extent than pupfish and mosquitofish ($P \leq 0.05$), especially near the end of the experiment (Fig. 2b) while pupfish and mosquitofish reduced emergence to similar levels ($P > 0.05$).

Reproduction during the longer 1985 experiment resulted in differences between fish numbers and weights by the end of the experiment (Table 1). Fish weights and numbers were more similar at the end of the 1986 experiment (Table 1). Length-frequency histograms plotted for mosquitofish and guppies in 1986 show bimodal distributions of lengths, with a first peak around 7 mm, suggesting recent reproduction resulted in greater numbers of mosquitofish and especially guppies.

Number of fish in a tank accounted for a significant amount of the variation in the number of mosquitoes emerged during the last 5 days of an experiment for pupfish in 1985 ($P \leq 0.05$) and for pupfish ($P \leq 0.1$) and mosquitofish ($P \leq 0.05$) in 1986. Mosquitofish and guppies in 1985 and guppies in 1986 eliminated mosquito emergence for several of the last 5 days, preventing similar analyses for those species.

Fish reduced numbers of invertebrates in both experiments, generally affecting larger invertebrates, such as ostracods, more than smaller invertebrates (Table 2). Reduction in numbers of invertebrates was greater in guppy and mosquitofish tanks than in pupfish tanks.

DISCUSSION

Both years showed much day-to-day variation in the numbers of mosquito adults that emerged (Fig. 2a, b). Even with this variation, tanks with fish consistently showed fewer mosquitoes emerged than tanks without fish (Fig. 2a, b). The 3 fish species showed similar mosquito control abilities when numbers of mosquitoes emerging were low (early in the 1985 experiment when *Cx. tarsalis* was used, Fig. 2a) or when numbers of fishes were similar (early in both experiments, Fig. 2a, b). When emergence was high or fish numbers dissimilar, guppies proved to be the best mosquito controllers (Fig 2a, b). Mosquitofish were second best and pupfish proved to be the poorest mosquito controllers.

Fish that established high population densities provided the best mosquito control. Ranking fish by reproductive success (high numbers at

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[2] Medians and low and high values for both experiments are reported in Castleberry, D. T. 1990. Environmental and energetic requirements and ecological interactions of selected larvivorous fishes suitable for mosquito control in wastewater marshes. Ph.D. dissertation. Univ. of California, Davis.

[3] Owen, C. R. 1988. Tertiary wastewater treatment by two submersed macrophytes, *Elodea canadensis* L. and *Potamogeton pectinatus* L. M.S. thesis. Univ. of California, Davis.

Table 1. Mean mass (g) and number (±SE) of fish for each species at the conclusion of both experiments.

	1985 experiment			1986 experiment		
	Pupfish	Mosquitofish	Guppy	Pupfish	Mosquitofish	Guppy
Mass[1]	8.1 ± 0.4	40.0 ± 15.0[a]	70.6 ± 7.8[a]	17.5 ± 0.8[a]	12.0 ± 1.5[a]	16.8 ± 1.8[a]
No.	11.0 ± 1.5	136.0 ± 26.9	677.6 ± 89.9	24.4 ± 1.0	47.6 ± 8.6	262.8 ± 13.0

[1] All tanks, except the no-fish controls, started with 10 fish at a mean total mass of 0.28 g in 1985 and 30 fish at a mean total mass of 15.3 g in 1986.

[a] Means within an experiment followed by the same superscripted lower-case letter are not significantly different ($P > 0.05$).

the end of the experiment, Table 1) matched their rank by mosquito control success in 1985 (Fig. 2a). Guppies established the highest population densities (Table 1) and provided the best mosquito control in 1986 (Fig. 2b). Over the last 5 days of each experiment, number of fish in a tank accounted for a significant amount of the variation in mosquito emergence, suggesting population density determined mosquito control success. Differences between numbers of fishes at the end of the 1986 experiment were less than differences in 1985 (Table 1) and so were differences in mosquitoes trapped (Fig. 2a, b), again suggesting population density determined mosquito control success.

Mosquitofish and guppies reproduced during both experiments, but guppies achieved higher population densities than mosquitofish (Table 1). Bay (1985) also observed that guppies reproduce more rapidly than mosquitofish. Pupfish rarely reproduced and never achieved high population densities. Their failure to reproduce may be due to their oviparous mode of reproduction. Pupfish eggs are tolerant of a narrower range of environmental conditions than are subsequent life history stages (Gerking 1981) and may be unable to survive in wastewater systems. Mosquitofish and guppies, on the other hand, are ovoviviparous. Their new-born young are mobile and grow rapidly in a wide range of conditions (Wurtsbaugh and Cech 1983). Both mosquitofish and guppies reproduced well in both experiments.

Mosquito control improved as both experiments progressed and fish numbers increased. No mosquitoes emerged from guppy tanks after day 74 of the 1985 experiment (Fig. 2a), and guppy tanks seemed to be moving toward zero emergence when the 1986 experiment ended (Fig. 2b). These results again suggest population density determined mosquito control success.

Bence (1985[4]) reviewed observations that demonstrated the relationship between high mosquitofish population densities and successful mosquito control in rice fields. Sasa et al. (1965) observed that mosquito control with guppies was dependent on population density and concluded that densities above $10/m^2$ result in elimination of *Cx. quinquefasciatus* Say in polluted waters in Bangkok. Our densities were always higher but control was not always complete, possibly due to vegetation densities. Phan-Urai et al. (1976) observed reduced efficacy when debris was present.

Bence (1988) observed that small mosquitofish provide the bulk of mosquito control while large mosquitofish prefer to eat invertebrate mosquito predators. If small guppies also eat more mosquitoes than large guppies, then the guppies' habit of establishing larger populations of smaller fish than mosquitofish may help guppies provide better mosquito control than mosquitofish.

Guppies have been used as mosquito control agents in a wide variety of systems, but particularly in wastewater systems, primarily because they seem to be more tolerant of wastewater conditions than mosquitofish (Bay and Self 1972, Bay 1985, Mian et al. 1986, Sasa et al. 1965). Their mosquito control success has been variable and seems to be related to their ability to maintain dense populations and enter areas of poor water quality that would otherwise provide refuge for mosquito larvae. In our study, guppies developed dense populations, inhabited the entire water volume, and provided excellent mosquito control. Mosquitofish, on the other hand, did not establish high populations, and in one tank, all fish died, possibly indicating a lower tolerance of wastewater than guppies.

Mian et al. (1986) compared guppies and desert pupfish (*Cyprinodon macularis*) as mosquito control agents in wastewater and found greater mosquito control with guppies. In their study, guppies established higher population densities than pupfish, and guppies congregated at the influent end of their systems and pupfish at the effluent end, suggesting guppies are more tolerant of wastewater than pupfish.

Fish generally decreased zooplankton population densities (Table 2). Fish never increased

[4] Bence, J. R. 1985. Selection of prey by the mosquitofish and its predatory impact on invertebrates. Ph.D. dissertation. Univ. of California, Santa Barbara.

Table 2. Mean number (±SE, no./liter) of mosquito larvae, ostracods, copepods, and cladocerans and mean total plant mass (±SE, g) for each fish treatment ($n = 5$) at the conclusion of both experiments.

	1985 experiment				1986 experiment			
	Pupfish	Mosquitofish	Guppy	No fish	Pupfish	Mosquitofish	Guppy	No fish
Mosquitoes[1]	1.1 ± 0.4^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.7 ± 0.2^{a}	$10.6 \pm 5.7^{a,b}$	$14.6 \pm 5.5^{a,b}$	1.2 ± 0.4^{b}	17.0 ± 5.6^{a}
Ostracods	0.6 ± 0.4^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	12.2 ± 4.8	468.4 ± 290.0^{a}	26.6 ± 17.2^{a}	15.2 ± 7.7^{a}	126.2 ± 83.8^{a}
Copepods	31.1 ± 11.6^{a}	0.2 ± 0.1^{b}	0.0 ± 0.0^{b}	4.7 ± 2.5^{a}	118.6 ± 93.2^{a}	478.2 ± 351.7^{a}	48.8 ± 25.3^{a}	101.2 ± 33.6^{a}
Cladocera	25.4 ± 16.4^{a}	0.01 ± 0.01^{a}	0.0 ± 0.0^{a}	3.6 ± 2.0^{a}	66.4 ± 30.0^{a}	$5.4 \pm 1.8^{b,c}$	2.0 ± 1.0^{c}	$26.4 \pm 8.2^{a,b}$
Plant mass	*	*	*	*	$1{,}050.9 \pm 38.8^{a,b}$	892.3 ± 77.1^{a}	$1{,}240.6 \pm 125.5^{b}$	912.7 ± 58.4^{a}

[1] Mosquito pupae and larvae were counted.
[a, b, c] Means for an organism within an experiment followed by the same superscript lower-case letter are not significantly different ($P > 0.05$).
* Plant mass was not measured at the conclusion of the 1985 experiment.

the numbers of any zooplankton group and had the greatest effect on zooplankton when fish numbers were high. They had no effect on cladocerans in 1985, but that year cladocerans were absent in most tanks. In contrast, fish in the 1986 experiment only reduced numbers of cladocerans. Other authors (Bence 1988, Hurlbert et al. 1972) have observed reductions in zooplankton in the presence of mosquitofish.

Zooplankton consume small organisms and particulate organic matter, possibly making nutrients available to plants. Fish may reduce the water treatment efficiency of marshes if they reduce the contribution zooplankton make to nutrient cycling and if this contribution is not compensated for elsewhere in the system. Plant mass comparisons from the 1986 experiment show tanks with guppies developed greater plant mass than all other treatments (Table 2), indirectly suggesting that fish may affect water treatment. We also observed guppies feeding on solid waste, as did Sasa et al. (1965), and suspect this behavior may affect wastewater treatment. High flow rates through our tanks maintained similar inflow and outflow water qualities, obscuring differences that might have existed between treatment capabilities of tanks. A fish species that improves or impairs the water treatment abilities of a marsh would be more or less desirable, respectively, than species which do not. Further research is needed to determine the effects of fish presence on wastewater treatment.

Guppies appear to be the best choice for mosquito control in wastewater marshes. Although all 3 species provided similar mosquito control when their population densities were similar, guppies established higher population densities than mosquitofish and pupfish, and this seemed to result in better mosquito control.

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