The Influence of Water Quality and Vegetation on Mosquitofish in Mosquito Control Programs in Wastewater Wetlands

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ABSTRACT: The impact of water quality and emergent vegetation on the abundance, distribution and survival of mosquitofish (*Gambusia affinis*) was assessed for two wastewater wetlands with distinct habitat characteristics. In separate experiments, minnow traps and cage enclosures distributed across each wetland location revealed that mosquitofish abundance and survivorship was lower at sites with comparatively lower water quality. Thick stands of California bulrush (*Schoenoplectus californicus*) also likely limited the distribution of mosquitofish since centrally placed traps collected fewer numbers than traps closer to open water edges. Nevertheless, higher survivorship and larger collections associated with the presence of vegetation when compared to open water sites suggested that vegetation is a key requirement for mosquitofish proliferation. These experiments revealed the importance abiotic and biotic wetland factors to mosquitofish health and can be used as guidelines when considering the use of mosquitofish to suppress mosquito production in wastewater systems.

INTRODUCTION

Mosquitofish (*Gambusia affinis*) are widely used as biological agents in the control of mosquitoes, although introductions into complex habitats have met with limited success (Gratz et al. 1996). Poor water quality usually associated with mosquito-infested environments is one factor that can hinder the efficacy of *G. affinis* despite its well-known ability to tolerate and thrive in nutrient-rich, polluted environments. Extreme conditions related to temperature, dissolved oxygen, pH and ammoniacal-nitrogen can be detrimental to feeding, growth and reproduction (Swanson et al. 1996) and may ultimately result in higher mortality from pathogens, parasites and predators. Aquatic vegetation also is known to have conflicting effects on mosquitofish health. Indeed, macrophytes provide mosquitofish and their young refuge from predation, cannibalism and other environmental stresses and are essential to maintain mosquitofish populations in permanent habitats (Walton et al. 1990, Swanson et al. 1996). However, at high densities, oxygen deficits and physical barriers may confound mosquitofish

performance by limiting dispersal and prey detection rates (Swanson et al. 1996).

This paper examines ecological effects of water quality and emergent vegetation on mosquitofish that are suggested in the literature (e.g. Swanson et al. 1996) but largely lack experimental confirmation. Mosquitofish distribution, abundance and survivorship patterns were explored under variable chemical, physical and biological conditions in constructed treatment wetlands that commonly produce large numbers of pestiferous and disease-vectoring mosquitoes in close vicinity to human habitation (Knight et al. 2003). Specifically, the experiments were carried out at two large-scale artificial wetlands that have intensive Integrated Mosquito Management (IMM) programs that include mosquitofish colonization efforts (Walton 2002, Walton et al. 2006). Each wetland system was a spatially heterogenous composite of water quality gradients and vegetated patches, although climate and quality of wastewater influent (Table 1) differed between each geographical site. Therefore, ecological trends that were detailed could be applied to as broad range of situations as possible on a regional and local basis. Ultimately, this analysis hopes to improve mosquitofish management strategies and, as a result, bolster mosquito abatement efforts in man-made treatment wetlands.

MATERIALS, METHODS AND RESULTS

Mosquitofish Survivorship: Water Quality, Predation and Vegetation. Mosquitofish survivorship was assessed in a 6-ha wetland complex that pumps wastewater through a series of wetlands (A = most polluted, B = moderately polluted, C = least polluted) at Valley Sanitary District (VSD) in Indio, CA. Eighteen rectangular mesh cages (46 cm x 46 cm x 65 cm) were distributed equally in open water areas among the most, moderate and least polluted locations and stocked with 30 fish. Mortality of fish was assessed on a weekly basis. Half of the cages at each location were also covered with bird block to examine the influence of avian predation on mosquitofish survival in the cages. Mantel log-rank tests indicated that survivorship differed significantly by wetland location ($\chi 2 = 27.596$, $p < 0.0001$), bird exclosure treatment ($\chi 2 = 29.673$, $p < 0.0001$) and in the interaction of both factors ($\chi 2 = 121.050$, $p < 0.0001$). Mean survival rates (Figure 1a) were generally highest in bird-excluded cages at the least polluted location.

In a subsequent experiment, mosquitofish survival in California bulrush (*Schoenoplectus californicus*) was analyzed independent of predatory effects. Half of the cages at each wetland site were placed in nearby stands of bulrush, and bird block was draped over every cage to control for avian predation. Mosquitofish survival comparisons indicated significantly (Mantel log-rank test: $\square^2 = 62.695$, P,< 0.0001) greater survivorship in cages encircled by bulrush compared to those exposed to open water conditions (Figure 1b). After survivorship trials were completed, around 1,600 mosquitofish (0.5 kg / ha) were released into the wetlands in May 2007. Thirty minnow traps were deployed over 12-hour periods on a weekly basis to monitor population numbers (Table 1).

From 2004 to 2006, adult mosquito production was analyzed with 60 floating emergence traps (Walton et al. 1999) distributed throughout the wetlands. Each sampling site consisted of transects of 5 traps spaced 2 m apart that began 2 m from the shore and extended perpendicularly into the

water. Mosquitoes collected were quantified (Table 1) and identified primarily as *Culex tarsalis* (79.8%) and *Culex quinquefasciatus* (19.8%) in the laboratory.

Mosquitofish Distribution Patterns in Vegetation. Mosquitofish and mosquito distribution patterns in California bulrush were analyzed in a 9.9 ha Demonstration Wetland (Walton 2002) at the Hemet-San Jacinto Regional Wastewater Reclamation Facility (HSJRWR) in San Jacinto, CA. Mosquitofish abundance at 30 different sites was assessed in 12-hour periods with minnow traps deployed throughout thick (20 m wide x 40 m long) and narrow (3 m wide x 40 m long) bands of bulrush in two wetland arms. Traps were positioned in open water and at 3 distances from open water (1.5m, 5m, and 10m) in thick bands of vegetation. Traps in the narrow bands were centrally located at 1.5 m within the vegetation. Mosquitofish abundance varied significantly among positions (RM-ANOVA: $F_{4, 25} = 3.123$, $P = 0.033$) such that the lowest and highest averages (Figure 2a) were from traps 10 m and 1.5 m from open water edges, respectively. Intermediate numbers of mosquitofish were generally associated with traps 5 m within bulrush and at open water sites.

Adult mosquito production was analyzed with floating emergence traps (Walton et al. 1999) deployed alongside minnow trap sites over 4 day periods. Mosquitoes collected were quantified (Table 1, Figure 2b) and identified to species in the laboratory. *Culex tarsalis* (50%) was the predominant species trapped, followed by *Culex stigmatosoma* (33%) and *Culex erythrothorax* (16%). Significant numbers of mosquitoes were evident at all vegetated sites (Figure 2b), although the greatest average emergence was at the center of the wide bulrush bands.

CONCLUSIONS

Our findings described significant ecological barriers likely to hamper mosquitofish as biological control agents for mosquitoes in wastewater wetlands. High rates of mortality that would hinder establishment and limit population numbers occurred in an acutely polluted, desert-climate wetland. An increased rate of predation was another factor suggested to restrict mosquitofish vitality and, combined with poor water quality, lessened survival. Emergent vegetation was both beneficial and harmful to mosquitofish success. For example, it may have provided refuge from adverse conditions to increase survivorship in cage trials and maintain populations along bulrush margins, but also restricted movement into mosquito-infested areas far from open water.

These trends are especially alarming for the future use of mosquitofish in these types of eutrophic habitats since biological control has been reported to be dependent on population density (Walton and Mulla 1989). Moreover, improving conditions to help self-sustaining populations of mosquitofish thrive may not be the answer because peak mosquito populations preceded peak mosquitofish abundance at the San Jacinto wetland (data not shown). Asynchronous phenologies between mosquitoes and mosquitofish would certainly lessen the probability of predatory control needed during periods of high mosquito and arbovirus activity.

The bottom line at each wetland site was that pathogen-transmitting mosquitoes (such as *Culex tarsalis,* a key

vector of the West Nile Virus) persisted in spite of mosquitofish colonization efforts complemented by vegetation management and larvicide/adulticide treatments. Our analysis did demonstrate that water quality and vegetation design can significantly alter mosquitofish population trends, although the direct impact on mosquito control, relative to the other IMM strategies, was uncertain. What is certain is that multipurpose wetlands must be engineered to limit mosquito production (e.g., by source reduction of pollutants and restriction of vegetation patch size) to remain viable technology for treating wastewater in water reclamation projects.

Acknowledgements

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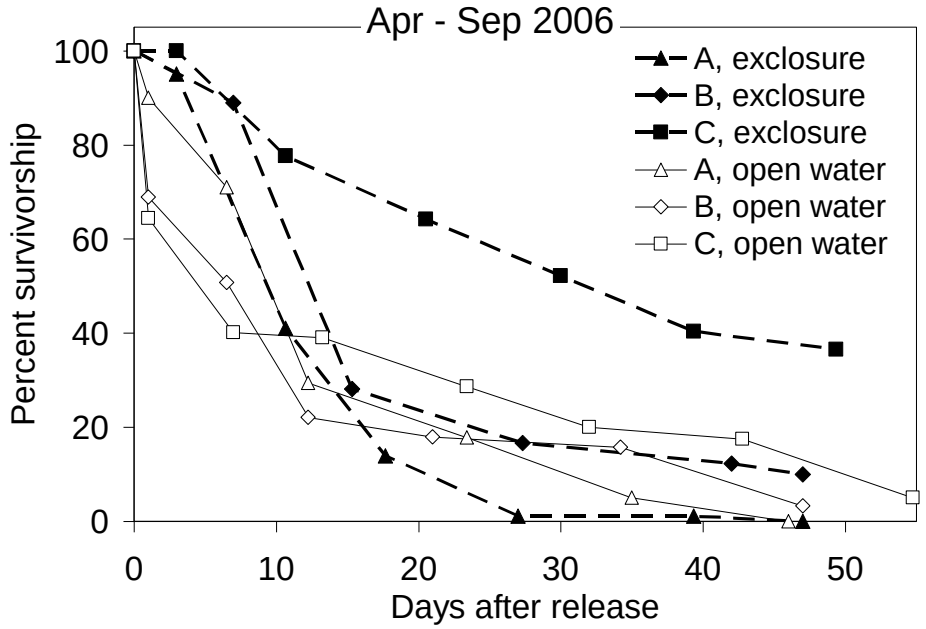
(a)



(b)

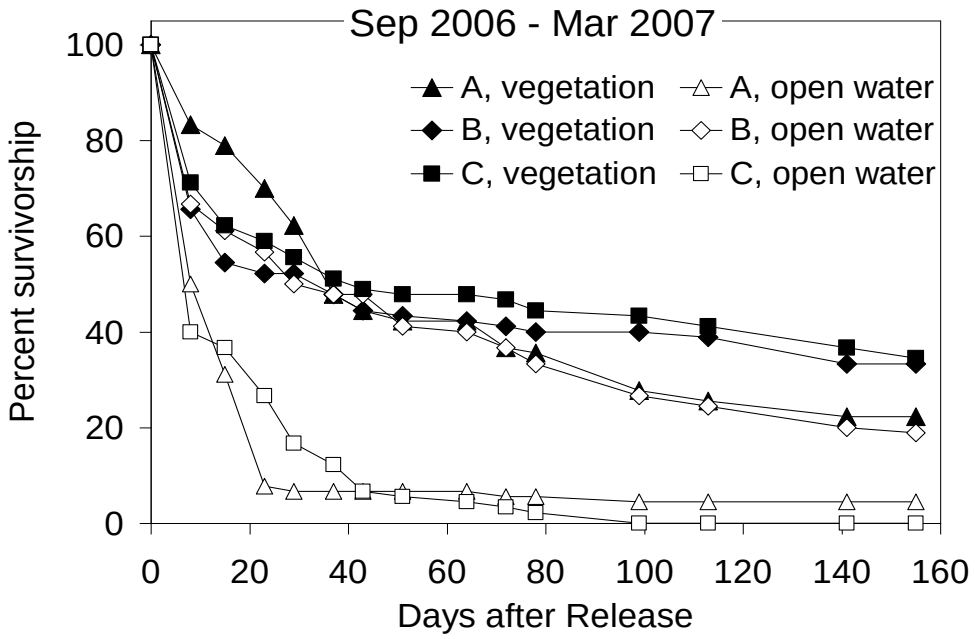
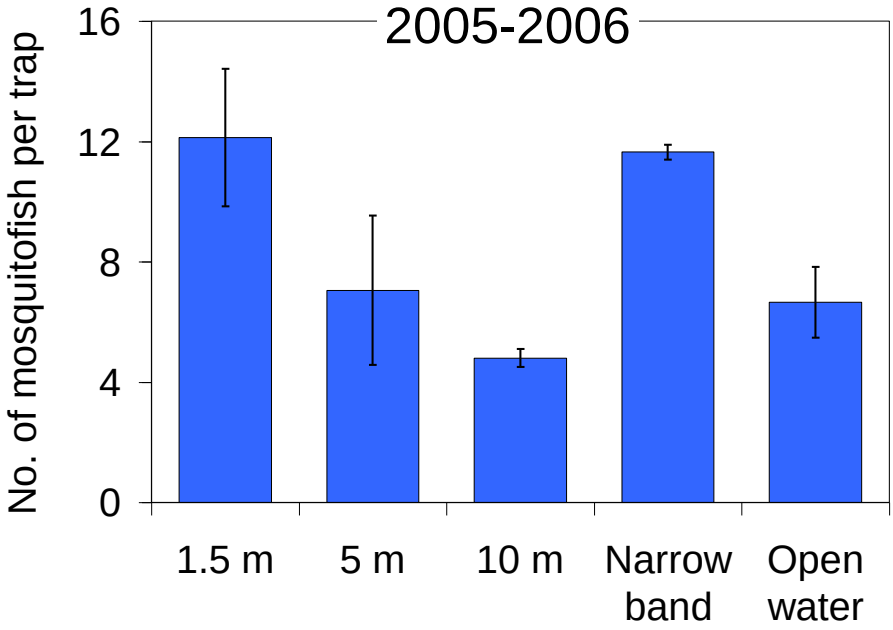


Figure 1. Mean mosquitofish survivorship impacted by water quality and either (a) avian predation or (b) the presence of vegetation at VSD.

(a)



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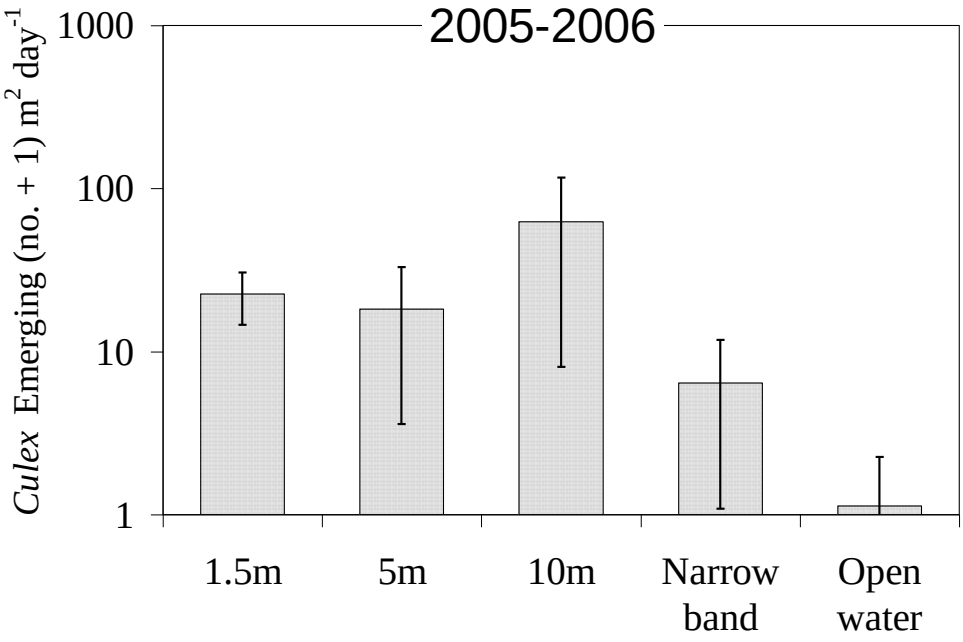

Figure 2. Abundances (mean ± SE) of (a) mosquitofish in minnow traps and (b) emerging mosquitoes among vegetation sampling sites at HSJRWR.

Table 1. Comparison of climate, wastewater quality (mean ± SD), and mosquitofish/mosquito activity (mean ± SE) of two constructed wetlands located in Southern California.

2005-2006		San Jacinto, CA[a]	Indio, CA
Air Temperature (° C)	Mean	15° C	24° C
	Max / Min	25 / 6	31 / 15
Total Precipitation (mm)		526	< 1
Relative Humidity (%)		63	40
NH_x-N (mg/L) [b]		10 ± 3	30 ± 16
TSS (mg/L) [b]		7 ± 3	51 ± 17
Emerging mosquitoes (m^2 day^{-1})		22 ± 11	7 ± 5
Mean mosquitofish per trap		20 ± 9	0.6 ± 0.4

[a] Meteorological data gathered from CIMIS station in nearby Winchester, CA.
[b] Ammoniacal-nitrogen (NH_x-N) and total suspended solids (TSS) wastewater levels were measured in the fall/winter of 2005-2006 at San Jacinto and in fall/winter of 2006-2007 at Indio.

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