Suitability of the Arroyo Chub (*Gila orcutti*) for the Biological Control of Mosquitoes in a Southern California Treatment Wetland

Adena M. Why[1], Bonnie Johnson[2], Scott Nygren[2], Richard Zembal[2], Jennifer Thieme[3], and William E. Walton[1]

[1] *Department of Entomology, University of California, Riverside, CA 92521*
[2] *Orange County Water District, 18700 Ward Street, Fountain Valley, CA 92708*
[3] *West Valley Mosquito and Vector Control District, 1295 E. Locust Street, Ontario, CA 91761*
awhy001@ucr.edu; 951-827-3918

ABSTRACT: The construction of multipurpose constructed treatment wetlands for treating municipal wastewater, and providing much needed habitat for riparian and wetland species, has increased over the last few decades. The production of mosquitoes which can transmit pathogens of humans and companion animals is a potential drawback to utilizing these treatment wetlands. We evaluated the efficacy of the arroyo chub, *Gila orcutti*, as a biological control agent for larval mosquitoes in the Prado Wetlands, Riverside County, CA. The arroyo chub is native to southern California watersheds and has been designated by the California Dept. of Fish and Wildlife as a Threatened Species within its native range. Local vector control districts are in need of an alternative to stocking the invasive western mosquitofish, *Gambusia affinis*, for larval mosquito control in sensitive watersheds. Twelve enclosures were installed in the wetland and three stocking treatments used: control, 0 kg/ha (No fish); low stocking density, 1.5 kg/ha (2 fish); and high stocking density, 6 kg/ha (8 fish). Our results indicate that arroyo chubs did not adversely affect the diversity or abundance of macroinvertebrate and microinvertebrate taxa collected in the wetland over the course of the 5-week trial.

INTRODUCTION

The construction and use of multipurpose constructed treatment wetlands has proliferated over the past several decades (Walton 2002, Kadlec and Wallace 2008, Vymazal 2010). As well as water quality improvement, the projected benefits of multipurpose constructed treatment wetlands are numerous and varied; they include amenities for nearby housing developments, crucial wetland habitat for a variety of species, wildlife conservation and recreation (Cole 1998). The production of mosquitoes which can transmit pathogens to humans and companion animals is a potential drawback to utilizing multipurpose constructed treatment wetlands to treat municipal wastewater (Walton et al. 1998, CH2M Hill 1999, Russell 1999, Knight et al. 2003). In the southwestern United States, and particularly southern California, a major cause for concern is the spread of West Nile Virus by mosquitoes near human populations (Reisen et al. 2006). This issue is becoming more pronounced as continued human development encroaches on what was previously isolated wetland habitat, bringing humans in ever increasing contact with mosquitoes (Walton 2002).

Larvivorous fish can be an important component of mosquito abatement strategies in wetlands (Meisch 1985, Kramer et al. 1988, Walton and Mulla 1991, Walton 2007). The use of various fish species for the biological control of mosquito larvae began worldwide in the early 1800s (Walton et al. 2011). The western mosquitofish, *Gambusia affinis* (Baird and Girard), has been introduced widely for mosquito control and has subsequently caused significant impacts to the natural ecology of the river systems where it has been introduced outside its native geographic range (Moyle 2002). Negative effects attributed to *Gambusia* include consumption of non-target fauna (Sokolov and Chvaliova 1936, Washino 1968, Harrington and Harrington 1982) and competition with native fishes (Moyle 1995, 2002).

Moreover, studies in different habitats provide conflicting results as to whether *Gambusia* is truly effective at controlling mosquito larvae (Gratz et al. 1996). Mosquitofish seem to be very effective in habitats such as manmade pools, cattle troughs and areas with poor water quality and low oxygen levels, but their effectiveness for controlling mosquitoes in more natural conditions and habitats is less clear (Pyke 2008). Some studies have shown that in more heavily vegetated areas, *Gambusia* is not effective at maintaining low levels of mosquito production (Harrington and Harrington 1961, Pyke 2008, Walton et al. 2011).

Vector control districts tasked with keeping mosquito populations at low levels that prevent the spread of mosquito-transmitted diseases (OCVCD 2011) are left with few viable alternatives to the use of *G. affinis* for biological control of mosquitoes in ponds, lakes and streams. The arroyo chub, *Gila orcutti* (Eigenmann and Eigenmann) is native to southern California coastal watersheds (Moyle et al. 1995, Veirs and Opler 1998) and has been shown to be a potential alternative to the use of *Gambusia affinis* in habitats connected to the waters of U.S. (Van Dam and Walton 2007). Arroyo chubs typically inhabit pools and runs of headwater creeks and small to medium-sized rivers (Fishbase 2011). This fish have been maintained successfully in rearing ponds (Van Dam and Walton 2007), but its ability to proliferate in riverine wetlands is unknown. Currently, due to population declines and loss of habitat (Moyle et al. 1995, Veirs and Opler 1998), the arroyo chub is listed as a "Species of Special Concern" by the California Department of Fish and Wildlife and qualifies as a "Threatened Species" within its native range.

The objectives of this study were to evaluate: (1) Invertebrate community structure across a range of arroyo chub stocking densites in cage mesocosms, and (2) The suitability of a riverine constructed wetland as a habitat for conservation of the arroyo chub in the lower Santa Ana River.

METHODS AND MATERIALS

Study Site. The experiment was carried out at the Prado Wetlands in Riverside County, California. The 186-ha wetlands are located 7 km northwest of Corona (33.9°N, 117.9°W) and consist of 47 interconnected marshes/ponds managed by the Orange County Water District (OCWD). The wetland complex receives approximately one-half of the flow (1.7 - 2.3 $m^3 \cdot s^{-1}$) of the Santa Ana River. A 0.9-ha wetland was used for this experiment (Figure 1). A channel approximately 0.5 m deep x 3 m wide x 15 m long was cut into the bottom of the wetland adjacent to the outlet weir using a backhoe to facilitate the collection of fish at the end of the experiment.

Inflow and outflow drop boxes (inflow: 1.2 m wide; outflow: 0.6 m wide) were located at the east and west sides of the wetland, respectively (Figure 1). Exclusion screens were placed in the drop boxes to prohibit invasive species [e.g., mosquitofish (*Gambusia affinis)*; green sunfish (*Lepomis cyanellus*)] from entering the wetland and to prevent arroyo chub from leaving during the experiment. The exclusion screens were composed of fiberglass window screen (mesh aperture = 1.5 mm) attached to a wooden frame. The fine mesh screening was supported on one side with 1.5 mm gauge metal wire fencing to prevent debris from puncturing holes in the fine mesh and to facilitate removal of debris from the screen. The screens were installed in the inflow and outflow weirs prior to inundation of the wetland.

Initial flooding of the wetland occurred in May 2009. Wetland vegetation (California bulrush, *Schoenoplectus californicus*, and cattail, *Typha latifolia*) was allowed to colonize the wetland naturally. Aquatic macroinvertebrates were also allowed to recolonize the system naturally.

Impact of *G. orcutti* on Invertebrate Community Structure Cages. Twelve 0.9 m x 0.8 m x 3.7 m cages were installed in the wetland on 6 October 2009 (Figure 1). Lumite screen (mesh aperture = 0.53 mm; BioQuip Corp., Rancho Dominguez, CA) was stapled onto four sides of the wood frame (1 in. x 2 in. pine furring strips, mounted to 2 in. x 2 in. wooden vertical posts). Fiberglass window screen (Model # 3003947; Phifer, Tuscaloosa, AL) was stapled across the bottom to prevent fish from entering the cages at deployment.

A stand of California bulrush 0.3 - 0.6 m in diameter (15 – 25 culms per stand) was placed into each cage to maintain a source of natural wetland vegetation for macroinvertebrate and microinvertebrate colonization and to provide refugia for the fish. One week after placing the live bulrush into the enclosures, bundles of dried bulrush (mean ± SD: 66.65 ± 7.68 g, n = 12) were placed into the cages to provide an oviposition attractant for female mosquitoes.

The experiment was conducted for five weeks until above-normal rains in southern California caused massive flooding on 8 December 2009. Debris associated with the flooding clogged the outflow weir box, causing the water level in the experimental wetland to rise. Cages were either lifted out of the sediments and tipped or completely submerged.

Fish. Arroyo chubs were stocked into the cages on 27 October 2009. Three stocking treatments were used: control, 0 kg/ha (No fish); low stocking density, 1.5 kg/ha (2 fish); and high stocking density, 6 kg/ha (8 fish). A completely randomized experimental design was used, and each treatment was replicated four times. The mean (± SD) wet weight and mean standard length of the 40 *G. orcutti* stocked into the cages were 4.28 ± 1.3 g per fish and 58.8 ± 6.9 mm, respectively. After stocking, the fish were monitored throughout the duration of the experiment using minnow traps lined with window screen (mesh opening = 1.5 mm) and baited with dog food. Despite the impact of the flooding event on the cages, all of the chubs that were stocked into the cages were removed at the end of the experiment and returned to the stock population maintained by the Orange County Water District.

Invertebrates. Immature mosquitoes, macroinvertebrate and microinvertebrate fauna were sampled weekly beginning 2 November until 1 December. Samples were taken using a standard 350-ml dip cup (Bioquip, Rancho Dominguez, CA). Three dips per cage were taken, combined in a concentrator cup (mesh opening = 153 μm) and preserved in 80% ethanol. Dip samples (3 dips per location) were also taken at six locations within the wetland (Figure 1).

Funnel activity traps were used to sample macroinvertebrate and microinvertebrate fauna leaving the benthos. Funnel traps were constructed by inserting and affixing the top one-third of a 2-liter clear plastic soda bottle into a second 2-liter clear soda bottle from which the bottom had been removed. One funnel trap was vertically attached to one corner within each cage with flagging tape, approximately 0.3 m below the surface of the water. The location of the funnel trap within each cage was rotated weekly among the corners of each cage. Funnel traps were deployed for at least 24 h. The organisms collected from each funnel trap were concentrated using a concentrator cup and preserved in 80% ethanol. Funnel traps were also deployed in the wetland at the same locations in which dip samples were collected. Funnel traps were attached to emergent vegetation using flagging tape approximately 0.3 m below the water surface.

Macroinvertebrate and microinvertebrate faunal composition and abundance were determined at 25 - 50X magnification using a stereo dissecting microscope. Macroinvertebrates were identified to at least the family level according to the taxonomic classification of Merritt et al. (2008). Additional aquatic taxa (non-insects) were identified according to the taxonomic classification of Pennak (1989). If a high density of microinvertebrate taxa (cladocerans, ostracods and copepods) was encountered in a sample, collections were sub-sampled using a fixed-area count. In that case, a 19-cm Petri dish (Fisher Scientific, Pittsburgh, PA) was divided into 16 equally sized units. Four of the sections were randomly chosen, and all microinvertebrates located within the boundaries of those sections were enumerated and taxonomically identified to at least the class or order level. The remaining sections of the Petri dish were then scanned for macroinvertebrate and non-planktonic taxa (e.g., Mollusca), which were counted and taxonomically identified to the family or order level. A list of all taxa identified can be found in Table 1.

Invertebrate group	Taxa Collected in Dip/Funnel Trap Samples	Common/Rare*
Macroinvertebrate	Aeshnidae	Rare
	Amphipod	Common
	Anopheles	Rare
	Callibaetis	Common
	Coenagrionidae	Common
	Ceratopogonidae	Common
	Chironomidae	Common
	Culex	Rare
	Ephydridae	Rare
	Gastrostricha	Common
	Hebridae	Rare
	Hymenoptera	Rare
	Leech	Common
	Libellulidae	Rare
	Mollusca	Common
	Muscidae	Common
	Oligochaeta	Common
	Platyhelminthes	Common
	Ram's Horn snail	Rare
	Veliidae	Rare
	Cladocera	Very Common
Microinvertebrate	Copepoda	Very Common
	Ostracoda	Common

*Rare is < 20 individuals; Common is 20 to < 10,000 individuals; Very Common is >10,000 individuals

Statistical Analyses. Repeated-measures MANOVA (SAS Version 9.2; SAS Institute Inc., Cary, NC) was used to determine if fish stocking density significantly affected invertebrate communities. Arroyo chub stocking density was the between-subject variable, while sampling date and taxon were the within-subject dependent variables. Rare taxa, defined as less than 20 individuals of a given taxon, were removed from the analysis. Abundances of the invertebrate taxa were log-transformed ($x + 1$) prior to analysis.

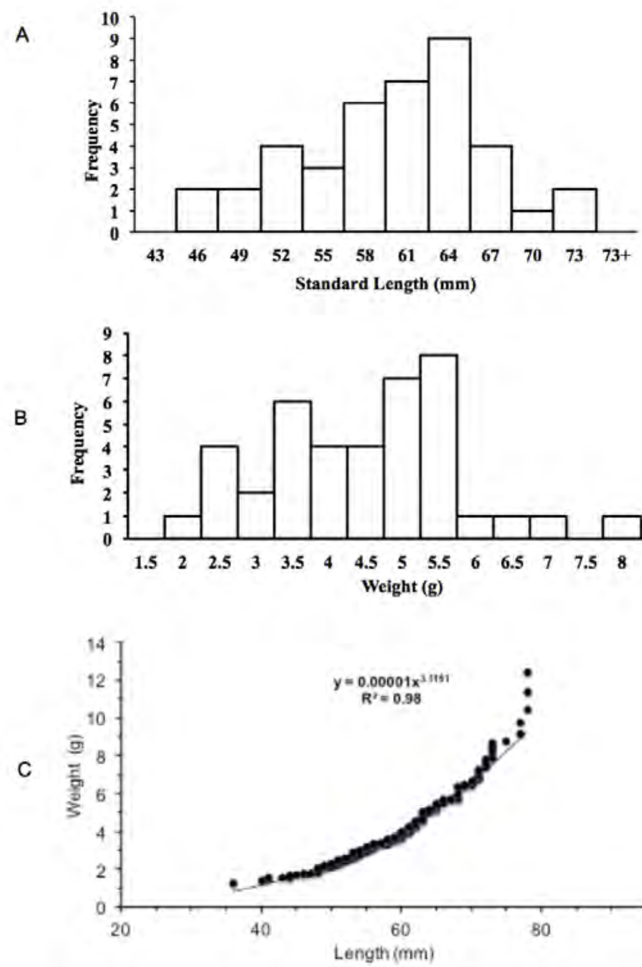
The impact of arroyo chub stocking density and other factors on invertebrate community structure was analyzed using ordination (CANOCO for Windows 4.5, ter Braak and Šmilauer 2002, Lepš and Šmilauer 2003). A detrended correspondence analysis (DCA) performed on the log-transformed abundance of taxa in the invertebrate community indicated that the lengths of axis 1 and axis 2 of the ordination was <2 standard deviations. Based on this result, linear ordination methods (principal components analysis: PCA) were used to examine the macroinvertebrate and microinvertebrate community structure across arroyo chub stocking densities. The species included in the ordination analyses are listed in Table 1. Rare taxa, which we defined as being less than 5 individuals of a given taxon, were removed from the analysis.

Forward stepwise regression was used to assess the proportion of the variation of the invertebrate community in the PCA ordination explained by arroyo chub stocking density, sampling date and physico-chemical variables. The conditional effect of adding a particular variable to the regression model was tested using a partial Monte Carlo permutation test (499 permutations/test) using CANOCO.

Suitability of a Riverine Constructed Wetland for *G. orcutti*:

Fish. *Gila orcutti* used in the experiment were obtained from a stock population maintained by the Orange County Water District. The parental-stocks were wild-caught fish that had been collected from the Santa Ana River within the city of Riverside, CA (Van Dam and Walton 2007). At the time of the experiment, the fish had been in aquaculture for no more than four generations.

The mean (± SD) wet weight and mean standard length of the 209 *G. orcutti* stocked in early summer 2009 were 4.04 ± 2.00 g per fish (Figure 2A), and 58.59 ± 8.77 mm (Figure 2B), respectively. The exponent for relationship between length and weight of the stocked fish exceeds 3 (Figure 2C), which indicates that the chub were healthy when stocked into the wetland.



Monitoring of fish populations. After stocking, the fish were monitored throughout the duration of the experiment using minnow traps lined with window screen (mesh opening = 1.5 mm) and baited with dog food. Minnow traps were deployed for 24 h and tied to emergent vegetation. Floats were placed within the traps to maintain a position just below the surface of the water, and visual inspections within the wetland were also carried out to monitor for distressed or dead fish.

The wetland was drained during a one-week period in late August and early September 2010 (16 months after stocking) and was searched for isolated standing water that might have contained fishes. Fish retained within the channel were collected by seine and hand net on 2 September 2010. The individuals collected were identified to species and the wet weight and standard length were determined for all specimens except for mosquitofish. More than 3,100 mosquitofish were collected; the length and weight of a representative sample (n = 39) of the fish collected was measured.

Water quality. Water quality variables were measured bi-weekly in the channel near the cages and adjacent to the outflow weir of the wetland using a potentiometric sensor array (YSI model 6920 sonde; YSI Incorporated, Yellow Springs, OH). Dissolved oxygen concentration (sensor #6562), turbidity (sensor #6136), temperature and specific conductance (sensor #6560) and pH (sensor #6361) were stored on a YSI 650 MDS data logger (YSI Incorporated, Yellow Springs, OH).

Nutrient concentrations in the wetland were measured by taking a 1-liter composite water sample. Three samples were collected near the outflow weir of the wetland and combined. The composite sample was placed on ice in the field and brought back to the laboratory. Ammonium nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N) and phosphate (PO_4) concentrations were determined colorimetrically (Hach DR 5000 spectrophotometer; Hach Company, Loveland CO) using TNT test kits (NH_3 = TNT 830, NO_3 = TNT 835, NO_2 = TNT 839, PO_4 = TNT 844; Hach Company, Loveland, CO).

RESULTS

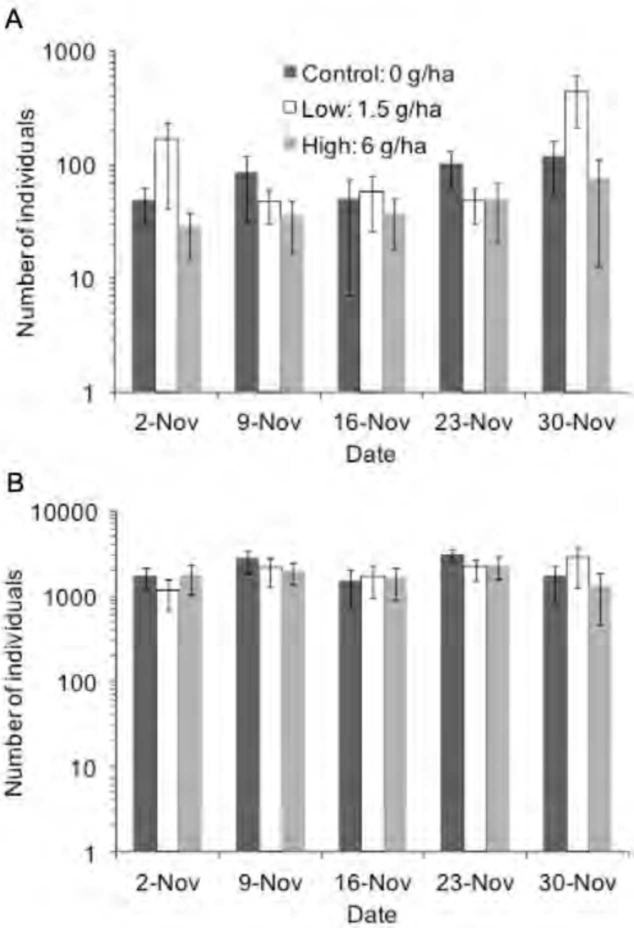
Impact of *G. orcutti* on Invertebrate Community Structure:

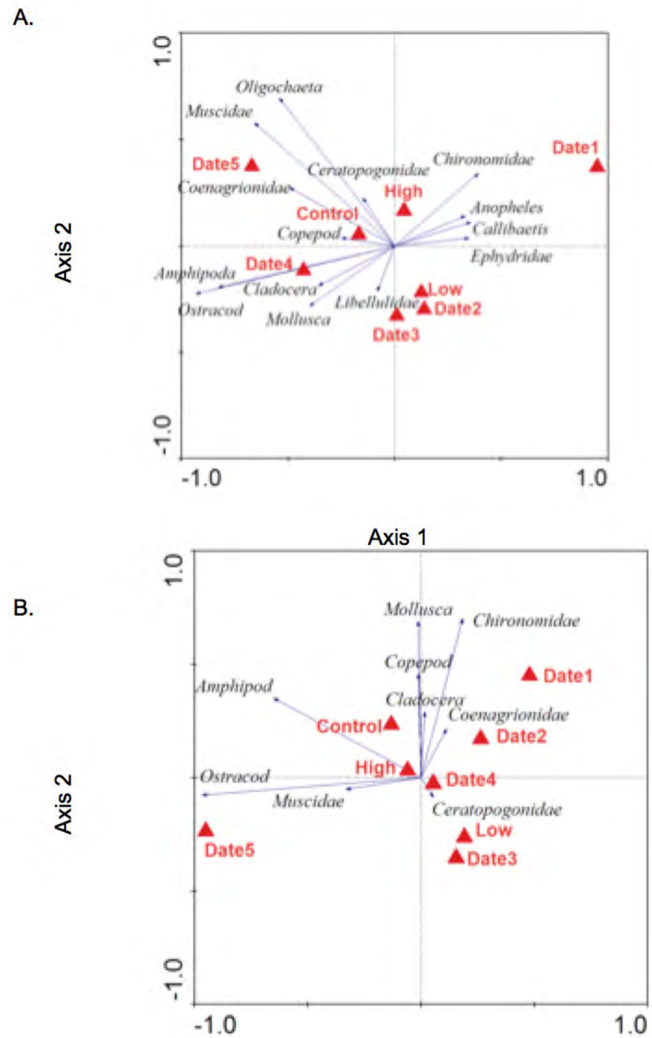
Minnow traps. Arroyo chubs were collected in the minnow traps during the 5-week cage experiment in order to monitor the overall health of the stocked population. However, fewer than five fish were collected across the eight cages containing fish on each sample date, except for the last collection date. The number of fish collected by minnow traps on each sampling date was therefore not representative of the differences in the two stocking treatments. Nevertheless, the initial stocking densities were maintained throughout the experiment; all of the stocked fish were collected from the cages at the end of the experiment. Minnow trap collections indicated that no additional fish species entered the cages and that the arroyo chub did not reproduce during the five-week study.

Dip Samples. Arroyo chub did not affect the abundances of taxa present, even at the highest stocking level of 8 fish per cage (Wilks' Lambda: $F_{8,12}$ = 1.07, P = 0.444). The interaction between arroyo chub stocking density and the taxa collected (stocking density x taxon interaction: $F_{11,22}$ = 1.99, P = 0.08) and between sample date and fish stocking density level ($F_{4,8}$ = 0.73, P = 0.66) were not statistically significant, indicating that the invertebrate community in the three stocking levels of fish did not respond differently across sample dates. However, sample date had a significant effect on the taxa present in the cages ($F_{44, 88}$ = 3.18, P < 0.0054). This finding indicates that variables within the wetland, other than arroyo chub stocking density, were the major determinant of taxon abundance and diversity (Table 2).

Source	df	MS	F	Pr>F
Between Subject Effects				
Stocking Density	2	65020.36	1.21	0.34
Error	9	53547.90		
Within Subject Effects				
Taxon	11	12699996.4	395.94	0.001
Taxon*Stocking Density	22	63214.7	1.99	0.08
Error (Taxon)	99	31823.5		
Week	4	155694.96	1.88	0.14
Week*Stocking Density	8	60537.79	0.73	0.66
Error (Week)	36	82800.16		
Taxon*Week	44	156200.52	3.18	0.0054
Taxon*Week*Stocking Density	88	34516.38	0.70	0.98
Error (Taxon*Week)	396	49125.08		

Taxa were spilt into macroinvertebrates (aquatic insects, annelids and mollusks) and microinvertebrates (crustacean zooplankton) to determine if arroyo chub stocking density affected abundances based on prey size. No significant difference in the abundance of either prey category in dip samples was found (macroinvertebrates: Wilks' Lambda: $F_{4,6}$ = 1.42, P = 0.51; microinvertebrates: Wilks' Lambda: $F_{4,6}$ = 4.84, P = 0.24; Figures 3A and 3B) across the three fish stocking densities.





The first principal component was associated with changes in taxon abundance across the experiment. Chironomidae, *Callibaetis,* Ephydridae and *Anopheles* decreased in abundance from Date 1 compared to the last week of the experiment, Date 5. The abundance of taxa in dip samples collected on the first date was positively associated with axis 1; dip samples collected on the last date were negatively associated with axis 1 (Date1: $r = 0.649$; Date 5: $r = -0.458$; Figure 4A). Muscidae, Oligochaeta, Amphipoda and Ostracoda showed the greatest increase in abundance during the 5-week experiment, with abundances peaking at the end of the 5-week trial. Mollusca, Cladocera, Coenagrionidae, Ceratopogonidae, Copepoda and Libellulidae increased in abundance to varying degrees over the course of the experiment (Figure 4A).

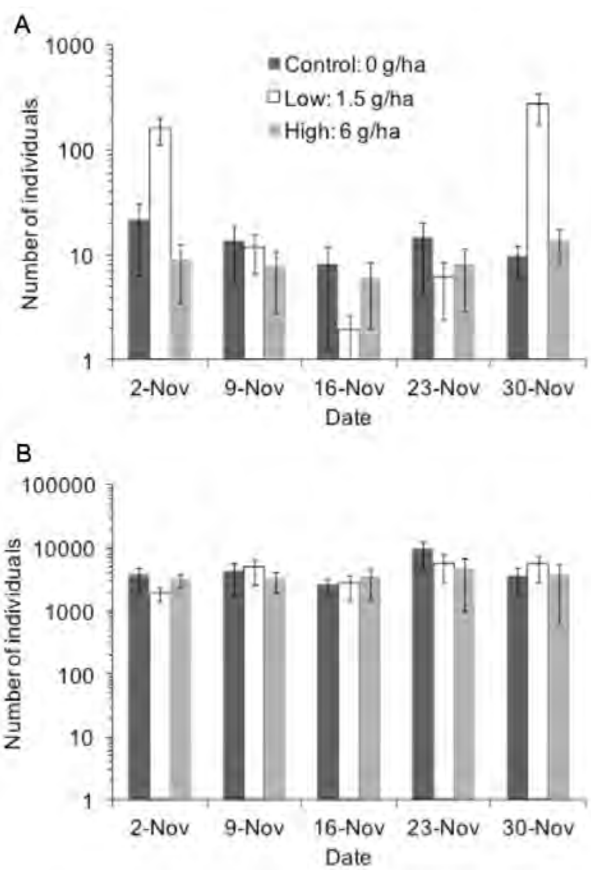
The second PCA axis was associated with differences in the invertebrate communities on first and last sample dates (Date 1 and Date 5) versus the intermediate sample dates (Date 2 and Date 3) as well as the Low stocking level of arroyo chubs. Date 2, Date 3, and the Low stocking level of chubs were associated with increases of abundances of Mollusca, Cladocera, amphipods, ostracods and Libellulidae.

The first PCA axis accounted for 39.8% of the total variability in the species data, and together with the second axis explained 52.7% of the total variability in species data present in the model. Sample date explained 24% of the variability in the species data (Monte Carlo permutation test: $F_{1,499} = 18.32$, $P < 0.002$), while the fish-stocking level only explained an additional 2% of the variability in the invertebrate community ($F_{1,499} = 1.42$, $P > 0.192$).

Funnel Traps. The abundance of taxa collected in the funnel trap samples over the 5-week trial did not differ among arroyo chub stocking treatments (Wilks' Lambda: $F_{8,12} = 1.97$, $P > 0.14$). There was no interaction found between the levels of chub stocked into the cages and taxa collected ($F_{5,10} = 0.14$, $P = 0.99$). There was also no interaction between sample date and fish stocking treatment ($F_{4,8} = 0.87$, $P > 0.52$), indicating that the invertebrate communities responded similarly across time to each of the three fish stocking treatments . Sample date had a marginally significant effect on the invertebrate community present in the cages ($F_{20,40}$ = 3.15, P = 0.054; Table 3). No significant difference among stocking treatments was detected when taxa were grouped into macroinvertebrates or microinvertebrates (macroinvertebrate: Wilks' Lambda: $F_{4,6}$ = 3.17, P = 0.32; microinvertebrate: Wilks' Lambda: $F_{4,6}$ = 5.37, P = 0.22; Figs. 5A and 5B). **INSERT TABLE 3 HERE**

The first principal component was associated with changes in taxon abundance from Date 1 to Date 5 (Date 1: r = 0.649; Date 5: r = -0.458). The second axis was weakly associated with invertebrate taxa present at the start of the experiment and inversely associated with invertebrate community on Date 3 and in the Low fish stocking treatment. Mollusks, chironomids and copepods were abundant at the start of the study, and the abundances of ostracods and amphipods increased towards the end of the experiment (Figure 4B). Higher abundances of Cladocera, Copepoda, Mollusca, Chironomidae and Coenagrionidae were associated with the Control treatment as compared to the low fish stocking density. The first PCA axis accounted for 35.8% of the total variability in the species data, with the second axis explaining an additional 18.2% of the total variability present in the model.

Sample date explained 19% of the variability in the invertebrate community (Monte Carlo permutation test: $F_{1,499}$ = 13.31; $P < 0.002$), and treatment level only explained an additional 1% of the variability in the data ($F_{1,499}$ = 0.88; $P > 0.506$) in funnel trap collections (Figure 5).



Wetland. Minnow traps that were deployed in the wetland collected a diverse range of taxa, most notable were *G. affinis, L. cyanellus* and *Xenopus*. Arroyo chubs were not collected in the wetland over the course of the 5-week cage experiment. The numbers of invertebrates collected increased from week 2 through week 4 and showed a similar pattern to the collections of taxa from within the cages (see Why 2012). Mollusca and amphipods exhibited the highest overall abundances during the experiment. As was observed in the collections within the cages, cladocerans were collected in much larger numbers (thousands) compared to less than a hundred individuals per taxa of all other groups collected.

Suitability of a Riverine Constructed Wetland for *G. orcutti*:

Fish and other aquatic vertebrates. Approximately 16 months after stocking *G. orcutti* into the wetland, 3,689 fish were collected; however, no arroyo chubs were recovered. All of the fish collected were invasive species in the Santa Ana River system (Table 4). *Gambusia affinis* and *L. cyanellus* were predominant among the collections, making up about 86% and 12%, respectively, of the individuals collected. By wet mass, *C. carpio*, *M. salmoides* and *L. cyanellus* were the dominant species in the fish community.

Table 4. Fish collected from the test wetland at the end of the experiment in late summer 2010.

Species	Total no. collected	Length (mm) mean ± S.D. (minimum, maximum)	Weight (g) mean ± S.D. (min.,max)
Cyprinus carpio	23	360.4 ± 94.9 (105, 480)	4.51 ± 938.94 (35.6, 3175.15)
Ameiurus melas	40	54 ± 9.43 (34, 74)	4.1 ± 2.03 (0.7, 9.2)
Gambusia affinis	3184	23.46 ± 6.10* (7, 35)	0.49 ± 0.56* (0.1, 2.9)
Lepomis cyanellus	432	69 ± 32.48 (21, 160)	18.58 ± 23.92 (0.4, 127.3)
Micropterus salmoides	8	214.63 ± 62.8 (72, 275)	311.61 ± 198.4 (9.0, 680.39)
Menidia beryllina	1	61	1.8
Ameriurus natalis	1	171	90.9
Total Weight of Fish Collected			52,079.69 (114 lbs.)

* N = 39

The abundance and total mass of fish present in the wetland at the end of the experiment was most likely underestimated because birds, predominantly ardeids, were observed consuming fish as the wetland was being drawn down prior to seining (A. Why pers. observation). In addition to the fish collected, African clawed frogs [*Xenopus laevis* (Duadin)], American bullfrog tadpoles (*Lithobates catesbeianus* Shaw) and red swamp crayfish [*Procambarus* (*Scapulicambarus*) *clarkia* (Girard)] were collected.

Water quality. Water quality remained consistently high in the wetland throughout the duration of the experiment (Table 5) and therefore should not have affected the overall health of the *G. orcutti* population.

Water quality variable	Mean ± SD (*N* = 18)
Dissolved oxygen [DO]	4.3 ± 1.91 mg L^{-1*}
Specific conductance	1.03 ± 0.11 mS/cm^{-1}
Temperature	19.3 ± 4.45 °C
pH	7.2 ± 0.25
NO_3-N	1.4 ± 2.0 mg L^{-1}
NO_2-N	0.4 ± 0.7 mg L^{-1}
NH_4-N	0.2 ± 0.3 mg L^{-1}
PO_4^{-3}	2.6 ± 1.5 mg L^{-1}

DISCUSSION

Arroyo chub did not adversely affect the diversity or abundance of macroinvertebrate and microinvertebrate taxa collected in enclosures in the wetland during the 5-week experiment. Even at the highest stocking level of 6 kg/ha (8 fish) per cage, arroyo chub had no discernable impact on abundances and composition of animal taxa in lower trophic levels.

Cladoceran abundance in the cages was high (> 100 individuals/liter) and, even if *G. orcutti* was consuming cladocerans, a small change in cladoceran abundance might not have been detectable. Greenfield and Deckert (1973) found that cladocerans comprised a small proportion of the arroyo chub's overall diet, even when cladocerans were dominant in the system. Van Dam and Walton (2007) showed that arroyo chub had no effect on microinvertebrate abundances during two 6-week studies conducted in earthen ponds. The abundance of microinvertebrates in ponds containing arroyo chub was 14 times higher than in ponds containing mosquitofish, *G. affinis* (Van Dam and Walton 2007).

A decrease in abundance of ephydrid larvae in cages containing *G. orcutti* was observed over the course of the experiment. It is possible that consumption of brine fly larvae might have been incidental when the chubs were consuming plant material. Brine fly larvae generally inhabit the littoral areas of lentic habitats but can be benthic algivores and also are associated with vascular hydrophytes (Merritt et al. 2008). Ephydrid pupae were found within the thallus of duckweed which was ubiquitous on the surface of the cages and the wetland. Greenfield and Deckert (1973) showed that 60 - 80% of the stomach contents of adult arroyo chub consisted of algae. They also found that arroyo chubs are opportunistic feeders and the composition of their diet changes seasonally and with availability of insect and other aquatic fauna.

Arroyo chub adults tend to occur low in the water column (A. Why pers. observation), and this may be related to the decline seen in Chironomidae abundance over the course of the experiment. Chironomid larvae are typically benthic in nature, feeding on detritus at the bottom of a lake or stream. Chironomid larvae also feed on a variety of other organic substances (Merritt et al. 2008). It is probable that the chub were more likely to consume chironomid larvae as they remained lower in the water column, as well as incidental consumption as the fish consumed plant material such as algae.

Arroyo chub fry tend to stay at the surface of the water column where they can provide effective control of mosquito larval populations in some aquatic habitats. Henke and Walton (2009) found that immature mosquito abundance in mesocosms containing bulrush (*Schoenoplectus californicus*) and arroyo chubs was lower than in vegetated mesocosms lacking *G. orcutti*; however, the effectiveness of mosquito control provided by *G. orcutti* appeared to differ seasonally (Jennifer Henke pers. comm.). Van Dam and Walton (2007) found that mosquitofish populations grew at a much higher rate than arroyo chub populations, after initially being stocked at equivalent levels, but that greater reproduction of *Gambusia* did not translate into significantly better control of larval mosquito populations when compared with the smaller population of arroyo chubs.

We were not able to assess the effect of predation by immature arroyo chubs on the invertebrate community because reproduction did not occur during the study. The cage experiment in the Prado Wetlands was performed after the peak period of reproduction for *G. orcutti,* which occurs in late spring and early summer (Tres 1992). Adult *G. orcutti* were not caught in the floating minnow traps deployed in the wetland during the 5-week cage experiment; this was most likely due to the fact that the fish tended to remain close to the benthos (A. Why pers, observation). It is therefore unlikely that adult *G. orcutti* would have a strong negative, direct effect on necktonic invertebrates and on invertebrates residing near the water surface. The changes detected in the composition of the invertebrate community can be attributed to the physicochemical changes in the wetland during the 5-week cage study, rather than to *G. orcutti*.

Although one of the initial goals of this experiment was to evaluate whether *G. orcutti* could be an effective biological control agent of mosquitoes in a surface-flow treatment wetland, few mosquito larvae were collected during the five-week trial. The extremely low abundance of mosquito larvae in the system can be attributed to the treatment of the test wetland with Bti, *Bacillus thuringiensis israelensis*, a few weeks prior to the start of the experiment by the local vector control district. Mosquito abundance had increased dramatically and reached unacceptably high levels following vegetation management in which cuttings from the macrophytes remained in the wetland. Only five *Anopheles hermsi* larvae were collected during the experiment.

Even though fine-mesh screens were deployed to inhibit colonization of the test wetland by non-native fishes, non-native fishes were observed in the wetland prior to the start of the cage experiment. Mosquitofish, green sunfish and carp were visually confirmed or caught in minnow traps deployed within the wetland to monitor the chub population. Unforeseen difficulties maintaining water level in the test wetland were caused in part by backflow from the downstream wetland perhaps due to unauthorized manipulation of the boards in the weir boxes. Even though the exclusion screens remained intact, it is unknown whether backflow into the test wetland, movement of juvenile fish through the window screen mesh or some other factor(s) accounted for colonization of the test wetland by non-native competitors and piscivores.

Over 3600 fish were seined from the wetland at the end of the experiment, with 86% of the individuals being mosquitofish and another 12% comprised of green sunfish. While only small numbers of American bullfrog, African clawed frog and crayfish were seined out at the end of the experiment, over 40 bullfrog tadpoles had been seen previously in a single day in the wetland during the course of the experiment (A. Why pers. observation).

The extirpation of the arroyo chubs and the overwhelming abundance of invasive species recovered from the wetland at the end of the experiment raise the obvious issue of how to reintroduce native species to their historical ranges while mitigating for their survival. Although we cannot ascribe the disappearance of *G. orcutti* directly to piscivory or competition with the invasive species present in the test wetland, we feel these factors were likely important. The water quality in the wetland should have been conducive for the survival of *G. orcutti*. A massive die-off or dead individual *G. orcutti* was never observed in visual surveys of the wetland.

The persistence of *G. orcutti* in pond or wetland studies (Van Dam and Walton 2007, Henke and Walton 2009) that lacked invasive fishes, but permitted predation by avian predators such as ardeids, provides evidence that lentic ecosystems can be conducive for survival of arroyo chubs. If invasive fishes have a negative impact on *G. orcutti* in certain types of aquatic ecosystems associated with rivers within their native geographic range, and if vector control districts in southern California anticipate using arroyo chub as an alternative biological control agent to mosquitofish, then they will need to work in concert with agencies such as the California Department of Fish and Wildlife to remove invasive species, especially piscivorous fish, such as largemouth bass and green sunfish, from areas in which they hope to release chub. This will not be easy and periodic monitoring of the system will be needed to prevent both the reintroduction of invasive species and extirpation of the arroyo chub.

Additional studies need to be conducted investigating competition between mosquitofish and arroyo chub to see if *G. orcutii* can survive and reproduce in sufficient numbers within the same system. *Gambusia affinis* can currently be found throughout almost all of the watersheds in southern California, and the cost of trying to remove them would be astronomically prohibitive (Moyle et al.1995, Walton et al. 2011). Therefore studies need to be conducted to see if chub populations can adequately compete with mosquitofish given that arroyo chub have a much slower reproductive rate and require habitat conducive to egg laying.

Riverine and wetland systems within southern California that lack a high abundance of invasive species appear to provide the best habitat for using arroyo chub as an alternative biological control agent to *G. affinis*. However if measures are undertaken to reduce the abundance of large predatory fish, more habitat would become suitable, not only for the arroyo chub, but for other imperiled native fish species. Arroyo chubs are capable of withstanding seasonal temperature fluctuations and changes in flow rate, which makes them well suited to survive in a managed wetland habitat. Though their effectiveness at controlling larval mosquito populations could not be directly tested in this experiment, results of previous studies indicate that arroyo chub are a viable alternative to the use of mosquitofish for the biological control of mosquitoes in sensitive watersheds. However, additional studies looking at larval mosquito control by arroyo chub in natural systems and their interactions with other native species need to be conducted.

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FIGURE LEGENDS

Figure 1. An aerial view of the 0.9-ha test wetland in the Prado Wetlands, Corona, CA.

Figure 2. Relative abundance of (A) wet weight, (B) standard length classes and (C) the relationship between wet weight and standard length of arroyo chub (*Gila orcutti*) stocked into the test wetland on 24 June 2009.

Figure 3. Mean (±SE) abundance of (A) macroinvertebrates and (B) microinvertebrates collected in dip samples from three fish stocking densities.

Figure 4. Ordination (PCA) diagrams illustrating the variation in the abundances of invertebrate taxa in (A) dip samples and (B) funnel traps from three arroyo chub stocking densities. The centroids for the arroyo chub treatments (High, Low and Control) and the sampling dates (Date 1 – 5) are indicated by triangles.

Figure 5. Mean (±SE) abundance of (A) macroinvertebrates and (B) microinvertebrates collected in funnel trap samples from three fish stocking densities.

TABLE LEGENDS

Table 1. Macroinvertebrate and microinvertebrate taxa collected in the cage mesocosms in the Prado Wetlands during 2009 and 2010.

Table 3. MANOVA results for invertebrate abundance in funnel trap samples.

Table 4. Fish collected from the test wetland at the end of the experiment in late summer 2010.

Table 5. Water quality in the study wetland from June through November 2009.