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Suitability of a Constructed Treatment Wetland as Conservation Habitat and the
Impact of the Arroyo Chub (*Gila orcutti*) on the Invertebrate Community and
Mosquito Oviposition

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Masters of Science

in

Entomology

by

Adena Mary Why

September 2012

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Dr. William E. Walton, Chairperson
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The Thesis of Adena Mary Why is approved:

Committee Chairperson

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Acknowledgements

I would like to acknowledge the following people for the immeasurable help they provided during my thesis work: Dr. William Walton, Dr. Margaret Wirth, Jennifer L. Thieme, Jesus R. Lara, David A. Popko, the other members of the Walton Lab, Dr. Timothy Paine, Dr. Richard Redak, Dr. Bradley Mullens, Varenka Lorenzi, Bonnie Johnson, Scott Nygren, Richard Zembal and the crew at the Prado Wetlands.

I would also like to thank the following entities for their financial support: Orange County Water District, Orange County Vector Control District, the Anderson Endowed Scholarship in Immature Insects, and the Entomology and Graduate Divisions of the University of California, Riverside.

Additionally, thanks to Dr. Peter B. Moyle for allowing me to use the Arroyo chub range map found within this thesis, as well as the Orange County Water District for the schematic of the Prado Wetlands.

I would like to thank the following people for all the love and support they have given to me during the course of my studies, I truly appreciate it: my mother, Lena Why; the Why and Chang families; my godmother, Kathleen Zeno as well as my other godparents. There are too many friends without whose support I could have not have done without. I would specifically like to thank the following: Peter J. Brabant III, Roger Burks, Andrew Ernst, Jesus R. Lara, Sanson B. Lin, Crystal May, James B. Munro and Jennifer L. Thieme.

Dedication

My thesis is dedicated to the memory of my grandmother, Louise Chang, and my father Donald Why. I would not be where I am today without their love and support. And to whom I promised to go as far in my studies as I could.

This is also dedicated to the loving memory of Franklin Thomas Hovore IV, without whom I would never have discovered the joys and wonder of Entomology.

ABSTRACT OF THE THESIS

Suitability of a Constructed Treatment Wetland as Conservation Habitat and the Impact of the Arroyo Chub (*Gila orcutti*) on the Invertebrate Community and Mosquito Oviposition

by

Adena Mary Why

Master of Science, Graduate Program in Entomology
University of California, Riverside, September 2012
Dr. William E. Walton, Chairperson

The arroyo chub, *Gila orcutti*, is native to the watersheds of southern California and has been proposed as a replacement for the non-native western mosquitofish, *Gambusia affinis*, as a biological control agent for mosquitoes in sensitive watersheds throughout the region. Because mosquito-eating fish are not strictly larvivorous, they also have the potential to impact non-target organisms in systems where they are introduced. The impact of arroyo chub stocking density on the invertebrate community and the suitability of a riverine constructed wetland as conservation habitat for the arroyo chub population in the Santa Ana River were evaluated at the Prado Wetlands in Riverside County, California. Invertebrate abundance and community structure did not differ

significantly among three stocking densities (0, 1.5 and 6 kg/ha) during a five-week study in cage mesocosms. Approximately 16 months after stocking *G. orcutti* into a 0.9-ha wetland, nearly 3,700 fish were collected; however, no arroyo chubs were recovered. All of the fish collected were invasive species in the Santa Ana River system and piscivory by non-native species most likely caused the extirpation of the arroyo chubs in the wetland.

The ovipositional responses of female *Culex tarsalis* to semiochemicals produced by aquatic organisms in three aquatic guilds (predaceous insects, algivorous fish and predatory/larvivorous fish) were examined in laboratory choice assays. Previous binary choice trials have shown that female mosquitoes avoid laying eggs on water that contains fish exudates and will instead choose to oviposit on water without kairomones released by fish. *Culex tarsalis* was not deterred from ovipositing in cups containing semiochemicals from invertebrate predators and algivorous fish, but was deterred from ovipositing in cups containing arroyo chub semiochemicals.

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Chapter 1

INTRODUCTION

Larvivorous fish are an important component of integrated mosquito management (IMM) programs worldwide. The use of various fish species for the biological control of mosquito larvae began worldwide in the early 1800s (Walton et al. 2011). Since then much research has been done investigating the efficacy of the mosquitofish, *Gambusia* spp., as a biological control agent of mosquitoes. Mosquitofish have been shown to reduce immature mosquito populations in many man-made aquatic habitats, but there is conflicting evidence on how well the fish controls larval mosquito populations in natural settings (Pyke 2005). The native range of *Gambusia* extends throughout parts of the eastern United States and it has become highly invasive in the many areas where it has been introduced (Walton et al. 2011). Vector control districts in areas where *Gambusia* is not endemic are now looking to use native fish species as alternative biological control agents of mosquitoes. The efficacy of native fishes for the biological control of mosquitoes and the effect of enhancing populations of native fishes on non-target fauna are unknown for many native fish species.

Recently, researchers have begun exploring whether decreases seen in larval mosquito populations that had previously been attributed solely to the feeding efficacy of fish, and other aquatic predators present in a given system (Blaustein 1999), should actually be attributed to changes in oviposition behavior

brought about by chemical cues emanating from the predators themselves (Petranka and Fakoury 1991; Blaustein et al. 2005; Van Dam and Walton 2007). My research addressed the ecological impacts of the fish, as well as the chemical ecology of aquatic taxa on mosquito larvae. First, I evaluated the efficacy of a native fish, the arroyo chub, *Gila orcutti*, as a biological control agent of mosquitoes in a surface-flow treatment wetland in southern California. Second, I studied the response of egg-laying *Culex tarsalis* to semiochemicals produced by aquatic taxa in different guilds.

Constructed wetlands: Study site

The creation and use of multipurpose constructed treatment wetlands has proliferated over the past several decades (Walton 2002; Vymazal 2010). Wetlands, which were once viewed as having no real benefit to humans, were drained, filled and/or paved over, under the guise of urban development. It is only within the last few decades that the true benefit of wetlands, and the services they provide to humans, has begun to be recognized and utilized (Walton 2002; Vymazal 2010).

Multipurpose constructed treatment wetlands have been built for a variety of uses in many parts of the world. Advancement in the design and management of these wetlands has seen their implementation in countries throughout Europe, Australia and North America, specifically in the arid Southwest of the United States (Cole 1998). The projected benefits that are gained from the construction

of these wetlands are numerous and varied; they include water quality improvement/reclamation, amenities for nearby housing developments, crucial wetland habitat for a variety of species, wildlife conservation and recreation.

The lower construction and annual operation costs of building and maintaining treatment wetlands have made them a viable alternative technology to conventional wastewater treatment facilities (Kadlec and Knight 1996). The operation of constructed treatment wetlands can be a cost-effective alternative for cities and municipalities required to treat wastewater and urban runoff in an era of financial constraints and increased environmental regulations (e.g. compliance with wastewater discharge requirements and enforcement of the Clean Water Act, Bastian 2007).

Although it might appear a win-win situation for municipalities, local landowners and wildlife, one potential major drawback to utilizing multipurpose constructed treatment wetlands, that function to treat municipal wastewater, is the production of mosquitoes which can be vectors of pathogens causing disease in humans, companion animals, and wildlife (Walton et al. 1998; CH2M Hill 1999; Russell 1999; Knight et al. 2003). The presence of these wetlands may alter the seasonal phenology of mosquito populations in several ways. Nutrient-rich municipal wastewater may enhance adult mosquito populations by increasing the amount of resources available to mosquito larvae. Second, the presence of emergent vegetation and large areas of continuous standing water provide developmental sites and habitat for mosquitoes that might not otherwise exist

during certain times of the year in arid regions (Walton 2002). 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).

The Prado Wetlands are located 7 km northwest of Corona, CA (33.9°N, 117.9°W) near the borders of Riverside, Orange and San Bernardino counties. The Prado Wetlands are a 186-ha constructed treatment wetland system consisting of 47 marshes/ponds. The Orange County Water District owns and operates the wetland complex, and in conjunction with additional land, the Prado basin totals 870 ha situated behind the Prado Dam.

This wetland complex was designed to reduce nitrate-nitrogen levels in river water and receives approximately one-half of the flow ($1.7\text{-}2.3\text{ m}^3\text{ s}^{-1}$) from the Santa Ana River. The Santa Ana River is the main source of recharge for the Orange County groundwater basin, and the lower reaches consist primarily of tertiary-treated wastewater from upstream discharges. The wetland system removes approximately 1.8×10^4 kg of nitrate per month from the wastewater from these sources, and during the summer months the Prado Wetlands reduce nitrate concentrations from 10 mg liter^{-1} to less than 1 mg liter^{-1} (OCWD 2008). The wetlands began operation in July 1992, and presently approximately $1.7\text{ m}^3\text{ s}^{-1}$ of Santa Ana River water enters the wetland complex (OCWD 2008).

In addition to wastewater treatment and water quality improvements, the wetland complex is managed to provide habitat for riparian and wetland species,

(e.g. the endangered least Bell's vireo, *Vireo bellii pusillus* (Coues)), for recreation and educational purposes, as well as for flood control and water conservation. The Orange County Water District is also hopeful that the Prado Wetlands, along with providing much needed riparian and wetland habitat in southern California for avian species, can be used as part of a native fish restoration program.

Currently a large number of exotic fish species can be found inhabiting the Santa Ana River system, which has led to declines in the native fish populations. Brown et al. (2005) collected 17 different fish species at various sites within the watershed, of those only 4 species (31%) were native while greater than 66% of all fish collected were non-natives.

Populations of native fish have been declining in the southwestern U.S. for decades (Miller 1961; Minckley and Deacon 1968; Moyle 2002; Clarkson et al. 2005). Several factors including diversion of natural water flows for human consumption, habitat degradation, population growth, and introduction of invasive species have contributed to declines of these populations (Marchetti et al. 2004). Early declines in native fish populations were attributed to habitat destruction and alteration. During the last few decades the presence of non-native fishes in these watersheds precludes or negates the presumed benefits from habitat restoration and protection efforts (Clarkson et al. 2005; Mueller 2005). The presence of exotic fishes in these systems is now the most consequential factor

preventing recovery of imperiled native species in the Southwest (Meffe 1985; Minckley et al. 2003; Schooley and Marsh 2007; Minckley and Marsh 2009).

Mosquitofish

One of the exotic fish species that has been introduced, and has subsequently caused significant impacts to the natural ecology of the Santa Ana River system, is the western mosquitofish, *Gambusia affinis* (Baird and Girard) (Moyle 2002). The western mosquitofish, *G. affinis*, is native to the Gulf Slope drainage of North America, from central Indiana and southern Illinois to eastern Mexico, and from western Mobile Bay, Alabama to Texas and into eastern Mexico. The name mosquitofish arose from its reputation of eating mosquito larvae.

Mosquitofish are omnivorous, opportunistic feeders, with diets including both plant and animal matter. *Gambusia* primarily is a surface feeder, but foraging is not restricted to the hyponeustic zone of the water column. In the wild, the diet of *Gambusia* consists of an extremely wide array of different food types and may vary considerably from one habitat type to another (Rees 1958; Miura et al. 1979; Blanco et al. 2004; Pyke 2005). Animal components of the diet include insects, small crustaceans, rotifers, snails and even spiders. Plant material, such as algae, is a less important component of the diet but supplements intake of animal matter (Crivelli and Boy 1987; Arthington 1989; Arthington and Marshall 1999; Garcia-Berthou 1999; Pyke 2005).

Stomach content analysis of wild *Gambusia* showed that it often feeds on mosquito larvae, however, mosquitoes constitute only a small proportion of the diet overall (Sokolov and Chvaliova 1936; Washino 1968; Harrington and Harrington 1982). Nonetheless, occasionally the diets of mosquitofish have been found to consist predominantly of mosquito larvae (Harrington and Harrington 1961; Morton et al. 1988; Lounibos and Frank 1994). Comparisons of the stomach contents of wild *Gambusia*, with samples of available prey, have shown biases toward one type of food versus another, and these biases have generally been interpreted as feeding preferences (Hess and Tarzwell 1942; Farley 1980). Some studies have shown that *Gambusia* shows a preference towards chironomid and ceratopogonid larvae versus other types of invertebrate prey (Hess and Tarzwell 1942; Farley 1980), or one tribe of mosquito (*Culicini*) versus another (*Anopheles*) (Hess and Tarzwell 1942). More recent studies have indicated that *Gambusia* appear to have no effect on chironomid abundances in natural systems (Bay and Anderson 1966; Peck and Walton 2008; Walton et al. 2012). The size of preferred prey increases directly with the size of the *Gambusia*. Adult mosquitofish prey on mosquito larvae and other aquatic insects more so than do *Gambusia* fry (Pyke 2005; Walton et al. 2011).

Gambusia is extremely hardy and capable of surviving a broad range of environmental conditions (Swanson et al. 1996). Mosquitofish can tolerate a wider application range of certain pesticides used against mosquitoes, as well as other herbicides, in comparison to other native fishes and aquatic biota (Walton

2007; Lloyd and Arthington 2010). *Gambusia* also exhibits an extremely high reproductive capacity. Females bear live young, which eliminates the need for spawning sites. These characteristics, along with a preference for habitats where mosquito larvae are present, omnivorous, and ease of culture have all contributed to the “success” of *G. affinis* as a mosquito control agent (Moyle 2002). In the 1960s, the United States and Australia began to restrict the stocking and release of *Gambusia* into native watersheds due to their invasive nature (Wilson 1960; Hubbs and Deacon 1964; Pyke 2008). Pyke (2008) dubbed *Gambusia* the “plague minnow” due to its highly invasive nature and the inconclusive evidence presented to date as to the true effectiveness of the fish for purposes of larval mosquito control.

All of these issues cause problems for vector control districts and mosquito abatement programs. Vector control districts are tasked with keeping mosquito populations at low levels in order to prevent the spread of mosquito-transmitted diseases to human populations (OCVCD 2008), but are left with few, if any viable alternatives to the use of *G. affinis* for mosquito control in ponds, lakes and streams. Large-scale chemical control of mosquitoes is not readily accepted in the United States as well as many other areas of the world. Additionally chemical approaches to control can be cost-prohibitive in many areas (Chavasse and Yap 1997). Clearly additional research on the efficacy of fish predators to control mosquito populations is needed.

Arroyo chub

A native fish that has been demonstrated to be a potential alternative to the use of *G. affinis* in habitats connected to waters of the U.S. is the arroyo chub, *Gila orcutti* (Eigenmann and Eigenmann). This species is endemic to southern California watersheds and has been shown to be effective at controlling larval mosquito populations in manmade systems (Van Dam and Walton 2007).

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 Game and qualifies as a “Threatened Species” within its native range. The arroyo chub has been shown to hybridize readily with two other species endemic to California; the Mohave tui chub (*Siphateles bicolor mohavensis*) and the California loach (*Lavinia symmetricus*) (Hubbs and Miller 1943; Greenfield and Greenfield 1972; Greenfield and Deckert 1973).

The arroyo chub is a relatively small minnow, in the family Cyprinidae, which can reach lengths of 120 mm, but typically is 70-100 mm standard length (Moyle 2002; Fig. 1). Males can be distinguished from females by their larger fins, and during the breeding season, by a prominent patch of tubercles on the upper surface of the pectoral fin (Tres 1992). Both sexes have fairly large eyes in relation to the body, small mouths and a chunky body.

Arroyo chubs are fractional spawners breeding almost continuously from February to August; although, most spawning takes place during peak breeding season in June and July (Moyle 2002). Adults are ready to spawn at one year of age (Tres 1992). Most spawning occurs in water that ranges from 14-22 °C, and is calm, such as pools, or along the edges of streams and water bodies. During spawning, males actively chase females, rubbing their snouts on the female's pelvic fin region. This induces the female to release her eggs, which can be fertilized by one or more males (Tres 1992). The eggs are demersal and the fry hatch within four days, in water temperatures of 24°C. Over the next 3-4 months, the fry spend their time in the water column, inhabiting calmer waters and usually among vegetation or flooded cover (Moyle et al. 1995; Moyle 2002). Arroyo chubs rarely exceed four years of age (Tres 1992).

Laboratory studies have shown the arroyo chub to be omnivorous, feeding on insects, algae and small crustaceans. Greenfield and Deckert (1973) found that 60-80% of the stomach contents consisted of algae. Arroyo chubs are also known to feed on nematodes infesting the roots of a floating water fern (*Azolla*) (Moyle 1976).

Gila orcutti is endemic to watersheds within southern California, specifically, the Los Angeles, San Gabriel, San Luis Rey, Santa Margarita and Santa Ana river systems, as well as Malibu and San Juan creeks (Wells and Diana 1975; Fig. 2). *G. orcutti* have been introduced, and populations have been successfully established, in the Santa Ynez, Santa Maria, Cuyama, and Mojave

river systems as well as smaller coastal streams such as Arroyo Grande Creek and Chorro Creek in San Luis Obispo County (Miller 1968; Moyle 1976; Moyle et al. 1995).

Gila orcutti has been extirpated from much of its native range and is abundant only in the upper Santa Margarita River and its tributary De Luz Creek, San Juan Creek (San Juan Creek drainage), part of Trabuco Creek, Malibu Creek (Swift et al. 1993; Moyle et al. 1995) and the west fork of the upper San Gabriel River (Cal. Dept. Fish and Game, unpub. data). Populations of *G. orcutti* occur, but at much lower numbers in Pacoima Creek above the Pacoima Reservoir, Big Tujunga Canyon, the Sepulveda Flood Control Basin, Los Angeles River drainage, and the middle stretches of the Santa Ana River tributaries, between Riverside and the Orange County line (Swift et al. 1993).

It is difficult to ascertain the actual numbers of *G. orcutti* within these watersheds and presently they are common at only four locations within their native range: Trabuco Creek below O'Neill Park and San Juan Creek; upper Santa Margarita River and its tributary, De Luz Creek; West fork San Gabriel River below Cogswell Reservoir; and Malibu Creek (Swift et al. 1993, Moyle et al. 1995). *Gila orcutti* has become scarce in its native range because the low-gradient streams, which is preferred habitat, have largely disappeared due to urban development (Swift et al. 1993). This has been compounded by the introduction of several sport and non-native fishes to watersheds within southern California (Moyle et al. 1995). Green sunfish, *Lepomis cyanellus* (Rafinesque)

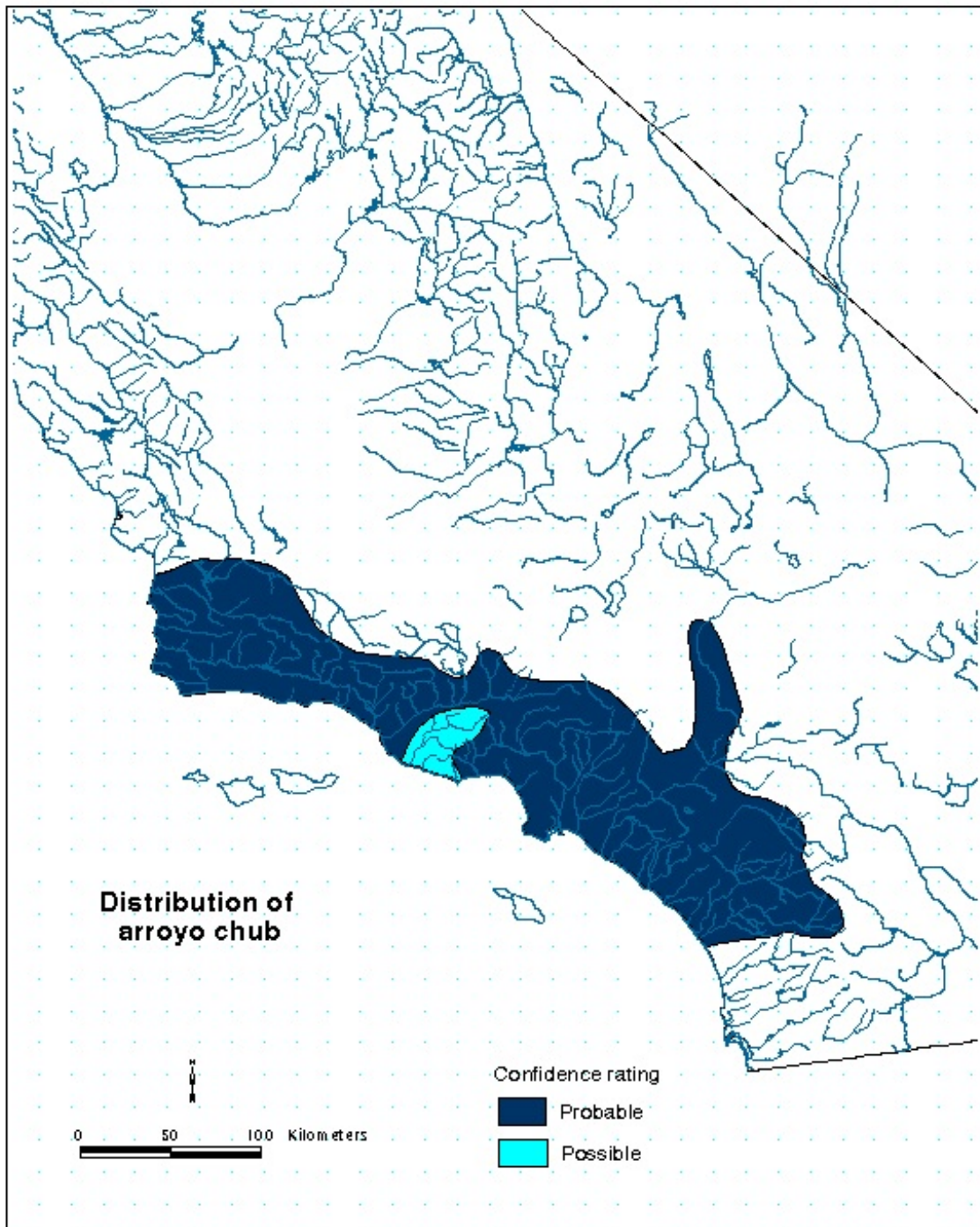
and largemouth bass, *Micropterus salmoides* (Lacepède) were introduced throughout the state for angling purposes, and adults are piscivorous (Baltz and Moyle 1993). The statewide introduction of mosquitofish, *G. affinis*, for mosquito control has also contributed to the declines of native fish populations in California (Moyle 1995; Moyle et al. 1995).

The objectives of this part of my thesis research were to evaluate (1) invertebrate community structure across a range of arroyo chub, *G. orcutti*, stocking densities in cage mesocosms, and (2) the suitability of a riverine constructed wetland as habitat for the arroyo chub, *G. orcutti*, population in the Santa Ana River.

Fig. 1. Adult female Arroyo chub, *Gila orcutti*. Photo: Adena Why



Fig. 2. Range map of arroyo chub, *Gila orcutti*. Source: Peter Moyle:
<http://ice.ucdavis.edu/aquadiv/fishcovs/fishmaps.html>



Responses of egg-laying mosquitoes to chemicals released by fish

Prey organisms have evolved numerous strategies for avoiding predation and lessening their contact with predators (Edmunds 1974; Endler 1986). These adaptations can be morphological (e.g. spines, horns, armor), physiological (e.g. toxins, venom), related to life history strategies, or behavioral (e.g. fleeing, hiding, threat display) (Kats and Dill 1998). Many antipredator tactics have energetic costs associated with them that divert energy away from reproduction (Ferrari et al. 2008). Mitigation of an individual's energy loss through prolonged periods of foraging might cause an increase of vulnerability to predators (Lima and Dill 1990; Ferrari et al. 2010).

Given the costs associated with the maintenance of defenses, it is plausible to assume that prey species will evolve reliable mechanisms to alert them to the presence of a potential predator. These species-specific signals can be visual, chemical, or tactile. Thresholds (e.g. a specific stimulus or a specified combination of different stimuli) may need to be triggered in order for a prey species to react to a "threat" (Lima and Dill 1990; Ferrari et al. 2010), for it becomes energetically costly to respond to a "false alarm" (Ferrari et al. 2010).

A wide variety of organisms including fish, amphibians, mammals, reptiles, a bird, and numerous invertebrates have evolved chemosensory mechanisms for detecting predators (Weldon 1990; Chivers and Smith 1998; Kats and Dill 1998, Ferrari et al. 2010). Common responses to the detection of predator chemicals include marked changes in the rate of movement (Williams and Moore 1985;

Mathis et al. 1993; Ode and Wissinger 1993; Chivers et al. 1996; Huryn and Chivers 1999); reduced courtship behaviors (Stein 1979); reduced or altered foraging (Chivers et al. 2000; Hazlett 2003; McCormick and Larson 2007; Ferrari et al. 2010); and predator avoidance behaviors (Kerfoot and Sih 1987; Lima and Dill 1990; Ferrari et al. 2010).

A review of the literature by Kats and Dill (1998) found only 16 studies that provided evidence of chemically mediated detection of predation by insects. Almost all of these studies involved the larval/nymphal stages of aquatic species responding to aquatic predators. Ferrari et al. (2010) included another five studies, which looked at the role chemicals play in anti-predator behaviors of immature stages of aquatic insects. Many aquatic insects have complex life cycles that involve several instars and variable growth periods in the nymphal/larval stage, thus leaving the organisms vulnerable to prolonged periods of predation.

Not only direct effects, such as predation (Kerfoot and Sih 1987, Wellborn et al. 1996), but indirect effects between organisms in different trophic levels, or within the same trophic level, are important selective agents leading to patterns in community structure of aquatic ecosystems (Blaustein and Chase 2007). Prey organisms that have evolved the ability to detect predators via chemical cues are often influenced by chemicals excreted from the predator's diet. In studies, mayfly and damselfly nymphs were more likely to respond to chemical

exudates from fish, if the fish had recently consumed conspecific prey (Chivers et al. 1996; Huryn and Chivers 1999).

However, the detection of predators by aquatic insects is not restricted to the immature stages; adults looking for oviposition sites also respond to predator cues. While searching for oviposition sites, female insects will most likely encounter a range of microhabitats over which survival of offspring varies. Several components of habitat quality could influence offspring survival, such as the density of competitors, seasonal duration (e.g. vernal or temporary pools), overall productivity of the habitat (e.g. available food resources) and the risk of predation present (Angelon and Petranka 2002). Natural selection should then favor ovipositing females that can assess habitat quality and choose microhabitats that would maximize offspring survival. Gravid female mosquitoes use a combination of cues from the environment, including physical, biological and chemical, to select oviposition sites (Benzon and Apperson 1988; Bentley and Day 1989; Isoe and Millar 1995).

The factors affecting oviposition site choice by mosquitoes have been investigated extensively since the 1970s (Ikeshoji and Mulla 1970; Osgood and Kempester 1971). In the decades since, chemicals emitted by a variety of organisms: plants, bacteria, copepods, insects and amphibians have been studied to determine their effects on ovipositional site selection (Kramer and Mulla 1979; Chesson 1984; Bentley and Day 1989; Petranka and Fakhoury 1991; Blaustein and Kotler 1993; Isoe et al. 1995; Mokany and Shine 2003).

Chemical cues produced by conspecific mosquitoes attacked by predaceous insects, as well as other predators, have been shown to reduce oviposition rates in some mosquito species (Sih et al. 1985; Blaustein 1999). Several studies have shown a reduction in oviposition rate by female mosquitoes into habitats containing hemipteran predators (*Anisops*: Eitam et al. 2002 and *Notonecta*: Chesson 1984; Kiflawi et al. 2003; Blaustein et al. 2005).

Silberbush and Blaustein (2008) tested the ovipositional response of *Culiseta longiareolata* Macquart in the field, to chemicals produced by the predatory backswimmer *Notonecta maculata* Fabricius. Female mosquitoes had no direct contact with the *Notonecta*-conditioned water, so any change in oviposition rate was attributed to predator-released volatile compounds released from artificial pools. Significantly more females oviposited in controls, leading the authors to conclude that the chemical compounds contained in the *Notonecta*-conditioned water were volatile and acting as deterrents to oviposition.

It is only recently that investigators have begun looking at the semiochemical(s) produced by fish, specifically kairomones, and how they affect site selection during oviposition by female mosquitoes (Ritchie and Laidlaw-Bell 1994; Angelon and Petranka 2002; Van Dam and Walton 2008; Pamplona et al. 2009; Walton et al. 2009). Ritchie and Laidlaw-Bell (1994) found that the abundance of *Aedes taeniorhynchus* (Wiedemann) eggs in soil adjacent to pools containing fish was lower than in soil adjacent to pools lacking fish, but did not determine if physical, visual or chemical detection of the fish led to the decrease

in oviposition rate. Pamplona et al. (2009) tested the oviposition preference of *Aedes aegypti* Linnaeus in containers containing fighting fish, *Betta splendens* Regan, and guppies, *Poecilia reticulata* Peters, compared to controls. They observed a decrease in numbers of eggs laid in containers containing *B. splendens* but detected no decrease in oviposition rate in containers that had *P. reticulata* or controls. However since the fish were physically present in the experimental pools, and could be visually detected by the mosquitoes, it cannot be concluded whether or not the observed difference in ovipositional activity is due solely to chemical cues.

Petranka and Fakoury (1991) found that mosquitoes and phantom midges reduced oviposition rates in experimental pools that contained caged sunfish, *Lepomis macrochirus* Rafinesque, which were not visible to ovipositing females. However, they did not directly test oviposition rates, but used larval counts as the measure of ovipositional activity. Angelon and Petranka (2002) found a three-fold decrease in ovipositional activity by members of the *Culex pipiens* complex, as determined by larval counts, in pools containing water conditioned with chemicals released by mosquitofish, *G. affinis* as compared to control pools. Ovipositional rate was not measured and other factors, such as the seeding of pools with invertebrates and microorganisms, could have contributed to the differences seen in larval counts.

The larval stages of mosquitoes are found in a variety of aquatic habitats ranging in size from small containers (i.e., phytotelmata, treeholes, tires and

jars), water-filled depressions (i.e., hoofprints and tire ruts), to the edges of large water bodies, (e.g., lakes, ponds), and within emergent vegetation in wetlands (Walton et al. 2009). However, many mosquito species are found only within particular habitats. Habitat preferences are also linked to other factors, such as level of organic enrichment in the water, and hydrological permanence (i.e., floodwaters or ephemeral habitats versus permanent lakes and pools; Laird 1988).

Culex tarsalis Coquillett, the western encephalitis mosquito, frequently lays egg rafts in vegetation that has been inundated with water, such as sumps, rice fields and wetlands (Bohart and Washino 1978); this coincides with areas that often contain fish and other invertebrate predators. Van Dam and Walton (2008) found that *Cx. tarsalis* responds strongly to the presence of fish-associated chemicals in oviposition sites. On average, four times as many egg rafts were laid on control water when compared with water conditioned with fish in the laboratory.

In field trials, *Culex quinquefasciatus* Say did not differentiate between small oviposition sites containing water conditioned with mosquitofish and controls, while *Cx. tarsalis* egg rafts were rarely collected from these small oviposition sites. The number of egg rafts laid by *Cx. tarsalis* in large mesocosms containing caged mosquitofish decreased by 84% relative to mesocosms lacking fish. However, *Cx. quinquefasciatus* egg rafts were not collected from the larger mesocosms during the experiment (Walton et al. 2009).

Culex quinquefasciatus prefers to lay eggs in habitats that lack fish (e.g. small size, semipermanence, high levels of organic enrichment, hypoxic conditions), and presumably does not respond strongly to fish kairomones but uses other cues during selection of oviposition sites. *Culex tarsalis*, which routinely oviposits in comparatively large water bodies, may have evolved the ability to detect the presence of predatory fish.

To date, no studies have been conducted looking at the ovipositional responses of a single mosquito species across aquatic organisms in different guilds. The objectives of the study were to determine if semiochemicals emitted by organisms in three different aquatic guilds affected the oviposition behavior of *Cx. tarsalis* in the laboratory. Specifically, experiments were conducted comparing the ovipositional responses of *Cx. tarsalis* across multiple species of fish in different feeding guilds: predatory/larvivorous vs. herbivorous/algivorous. Experiments were also conducted looking at the ovipositional responses of female mosquitoes to predatory aquatic insects: two species of adult dytiscid beetles and nymphs of a libellulid dragonfly.

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Chapter One

Survival of the Arroyo Chub (*Gila orcutti*) and Effects on the Invertebrate Community in a Southern California Treatment Wetland

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 of 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 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). Mosquitofish are extremely tolerant of a wide range of environmental conditions (Swanson et al. 1996) and have a high reproductive potential relative to most other fish species (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 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, to 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 chub typically inhabits pools and runs of headwater creeks and small to medium-sized rivers (Fishbase 2011), and has been maintained successfully in rearing ponds (Van Dam and Walton 2007), but its ability to proliferate in riverine wetlands is unknown.

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

Methods

Study site. The experiment was carried out at the Prado Wetlands in Riverside County, California (Fig. 1). The 186-ha wetlands are located 7 km northwest of Corona, CA (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 ($1.7\text{-}2.3\text{ m}^3 \cdot \text{s}^{-1}$) of the Santa Ana River. A 0.9-ha wetland was used for this experiment (Fig. 2). 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 (Fig. 2).

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 (Fig. 2). 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 (Fig. 2). 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, *S. californicus*, 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 \pm SD: 66.65 \pm 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 (\pm SD) wet weight and mean standard length of the 40 *G. orcutti* stocked into the cages were 4.28 \pm 1.3 g per fish and 58.8 \pm 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.

Funnel activity traps were used to sample macroinvertebrate and microinvertebrate fauna leaving the benthos. Funnel traps were constructed by inserting and affixing the top 1/3rd 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 25X-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.

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 Smilauer 2002). 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 was 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 (\pm 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 (Fig. 3A), and 58.59 ± 8.77 mm (Fig. 3B), respectively. The exponent for relationship between length and weight of the stocked fish exceeds 3 (Fig. 3C), 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. 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 ($\text{NH}_4\text{-N}$), nitrate-nitrogen ($\text{NO}_3\text{-N}$), nitrite-nitrogen ($\text{NO}_2\text{-N}$) and phosphate (PO_4) concentrations were determined colorimetrically (Hach DR 5000 spectrophotometer; Hach Company, Loveland CO) using TNT test kits ($\text{NH}_3 = \text{TNT } 830$, $\text{NO}_3 = \text{TNT } 835$, $\text{NO}_2 = \text{TNT } 839$, $\text{PO}_4 = \text{TNT } 844$; 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 \times 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).

Taxa were spilt into the following groups: macroinvertebrate and microinvertebrate, 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: $F_{4,6} = 4.84$, $P = 0.24$; Figs. 4A and 4B) across the three fish stocking densities.

The first principal component was associated with changes in taxon abundance across the experiment. Chironomidae, *Callibaetis*, Ephydriidae 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 and in dip samples collected on the last date was negatively associated with axis 1 (Date1: $r = 0.649$; Date 5: $r = -0.458$; Fig. 5A). 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 (Fig. 5A).

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. 6A and 6B).

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 abundance of ostracods and amphipods increased towards the end of the experiment (Fig. 5B). A higher abundance of Cladocera, Copepoda, Mollusca, Chironomidae and Coenagrionidae was 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.

Wetland. Minnow traps deployed in the wetland collected a diverse range of taxa (Fig. 7), most notable were *G. affinis*, *L. cyanellus* and *Xenopus*. Arroyo chubs were not collected over the course of the 5-week cage experiment. Week 1 counts of invertebrates had the highest overall numbers, but this was due to the high number of leeches collected. 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. 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.

Ceratopogonidae, Oligochaeta and copepods were inversely related with cladocerans in the wetland along axis 1 (Fig. 8). The second canonical axis was positively associated with the presence of Gastrotricha and Muscidae and negatively correlated with copepods, ephydriids and mayflies in the wetland. The first PCA axis accounted for 41% of the total variability in the species data, with the second axis explaining an additional 18.9% of the total variability in species data present in the model.

The diversity and abundance of taxa collected during the experiment was most closely associated with the particular date the sample was taken, as well as the type of sample collected, dip or funnel trap, along axis 1. Axis 2 was correlated with water quality variables in the wetland (Table 4). The environmental variables explained 54.2% of the variability in the species data along axis 1, with the second axis explaining an additional 25.2% of the variability.

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 5). *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.

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. Prior to the start of the cage experiment, vegetation management was carried out, leading to a temporary decrease in the dissolved oxygen level and a slight rise in circumneutral pH as decomposition rates increased within the wetland (Table 4). Water quality remained consistently high in the wetland throughout the duration of the experiment and therefore should not have affected the overall health of the *G. orcutti* population (Table 6).

Discussion

Arroyo chub 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. 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 Cladocera 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.

However, 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); 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. 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 nektonic invertebrates and on invertebrates residing near the water surface. The changes detected in the composition of the invertebrate community can be attributed to the physiochemical 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 5 *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 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 out of the wetland at the end of the experiment, with 86% of the individuals being mosquitofish and another 12% comprised of green sunfish. While only a small number 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 raises 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 Game 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 to try and prevent both the reintroduction of invasive species and extirpation of the chub will be needed.

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.

Riparian 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 as well. Arroyo chub 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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Table 1. Macroinvertebrate and microinvertebrate taxa collected in the cage mesocosms in the Prado Wetlands during 2009 and 2010.

Macroinvertebrate/ Microinvertebrate	Taxa Collected in Dip/Funnel Trap Samples	Common/Rare*
Macroinvertebrate	Aeshnidae	Rare
	Amphipod	Common
	<i>Anopheles</i>	Rare
	<i>Callibaetis</i>	Common
	Coenagrionidae	Common
	Ceratopogonidae	Common
	Chironomidae	Common
	<i>Culex</i>	Rare
	Ephydriidae	Rare
	Gastrotricha	Common
	Hebriidae	Rare
	Hymenoptera	Rare
	Leech	Common
	Libellulidae	Rare
	Mollusca	Common
	Muscidae	Common
	Oligochaeta	Common
	Platyhelminthes	Common
	Ram's Horn snail	Rare
	Veliidae	Rare
Microinvertebrate	Cladocera	Very Common
	Copepoda	Very Common
	Ostracoda	Common

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

Table 2. MANOVA results for invertebrates in dip samples

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		

Table 3. MANOVA results for invertebrates in funnel trap samples

Source	df	MS	F	Pr>F
Between Subject Effects				
Stocking Density	2	5489448.8	0.17	0.8462
Error	9	3226845.9		
Within Subject Effects				
Taxon	5	11344618.8	3.22	<0.0001
Taxon*Stocking Density	10	4562108	0.14	0.99
Error (Taxon)	45	31667168		
Week	4	11344618.8	3.22	0.05
Week*Stocking Density	8	3061203.8	0.87	0.52
Error (Week)	36	3526305.5		
Taxon*Week	20	11272377.3	3.15	0.054
Taxon*Week* Stocking Density	40	3012815.3	0.84	0.53
Error (Taxon*Week)	180	3574277.0		

Table 4. Water quality in the study wetland from October through November 2009.

Water Quality Variable	
Dissolved oxygen [DO]	$3.4 \pm 0.7 \text{ mg L}^{-1*}$
Specific conductance	$1.07 \pm 0.02 \text{ mS/cm}^{-1}$
Temperature	$11.7 \pm 1.2 \text{ }^\circ\text{C}$
pH	7.47 ± 0.12
NO ₃ -N	$3.1 \pm 2.6 \text{ mg L}^{-1}$
NO ₂ -N	$0.09 \pm 0.04 \text{ mg L}^{-1}$
NH ₄ -N	$0.01 \pm 0.001 \text{ mg L}^{-1}$
PO ₄ ⁻³	$2.76 \pm 0.3 \text{ mg L}^{-1}$

*Mean \pm SD (N = 3)

Table 5. Fish collected from the test wetland at the end of the experiment in late summer 2010.

Species	Total # Collected	Length Mean \pm S.D. (minimum, maximum) mm	Weight Mean \pm S.D. (min.,max) g
<i>Cyprinus carpio</i>	23	360.4 \pm 94.9 (105, 480)	4.51 \pm 938.94 (35.6, 3175.15)
<i>Ameiurus melas</i>	40	54 \pm 9.43 (34, 74)	4.1 \pm 2.03 (0.7, 9.2)
<i>Gambusia affinis</i>	3184	23.46 \pm 6.10* (7, 35)	0.49 \pm 0.56* (0.1, 2.9)
<i>Lepomis cyanellus</i>	432	69 \pm 32.48 (21, 160)	18.58 \pm 23.92 (0.4, 127.3)
<i>Micropterus salmoides</i>	8	214.63 \pm 62.8 (72, 275)	311.61 \pm 198.4 (9.0, 680.39)
<i>Menidia beryllina</i>	1	61	1.8
<i>Ameriurus natalis</i>	1	171	90.9
Total Weight of Fish Collected			52,079.69 g (114 lbs.)

* N = 39

Table 6. Water quality in the study wetland from June through November 2009.

Water Quality Variable	
Dissolved oxygen [DO]	$4.3 \pm 1.91 \text{ mg L}^{-1*}$
Specific conductance	$1.03 \pm 0.11 \text{ mS/cm}^{-1}$
Temperature	$19.3 \pm 4.45 \text{ }^\circ\text{C}$
pH	7.2 ± 0.25
NO ₃ -N	$1.4 \pm 2.0 \text{ mg L}^{-1}$
NO ₂ -N	$0.4 \pm 0.7 \text{ mg L}^{-1}$
NH ₄ -N	$0.2 \pm 0.3 \text{ mg L}^{-1}$
PO ₄ ⁻³	$2.6 \pm 1.5 \text{ mg L}^{-1}$

*Mean \pm SD ($N = 18$)

Figure 1. Schematic of the Prado Wetlands, Norco CA. Location of the test wetland is indicated by shading.

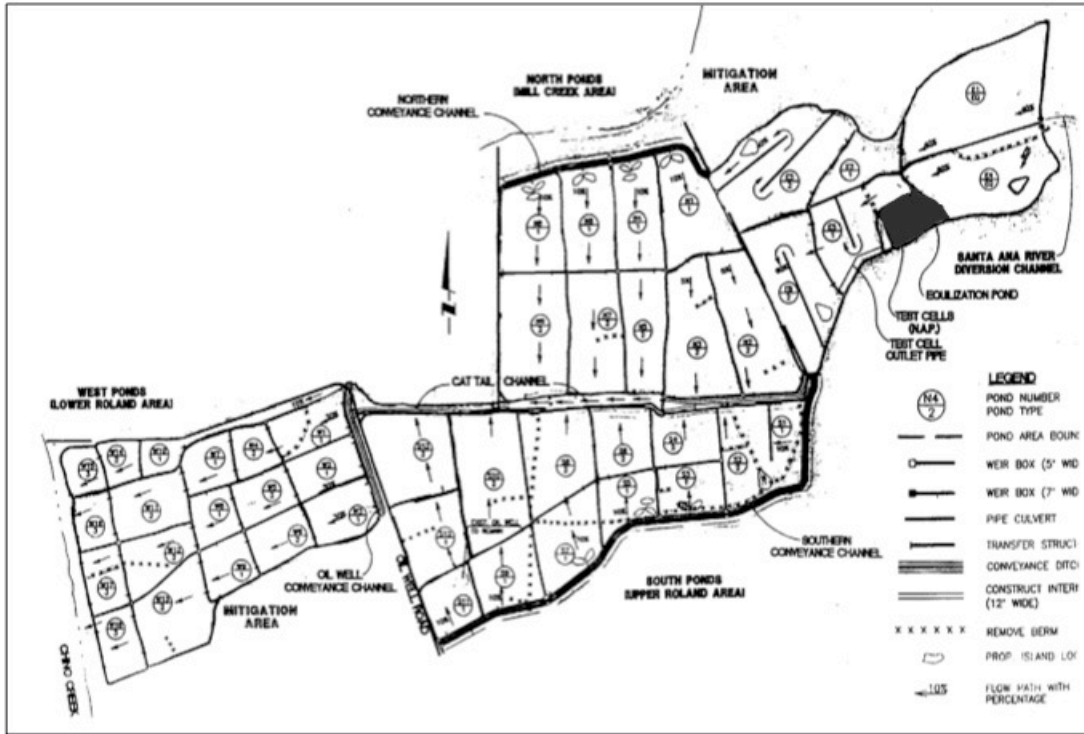


Figure 2. An aerial view of the test wetland.



Figure 3. Relative abundance of (A) wet weight and (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.

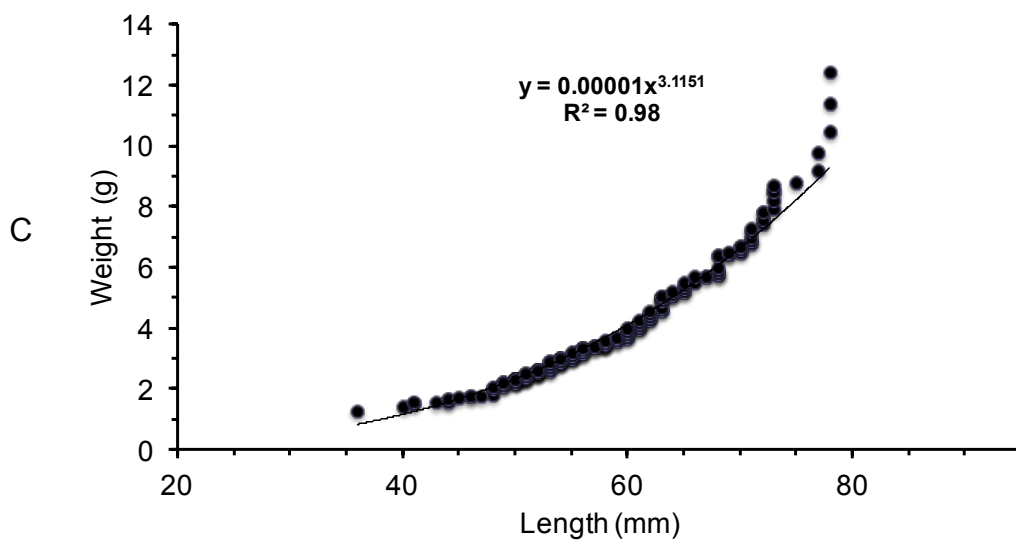
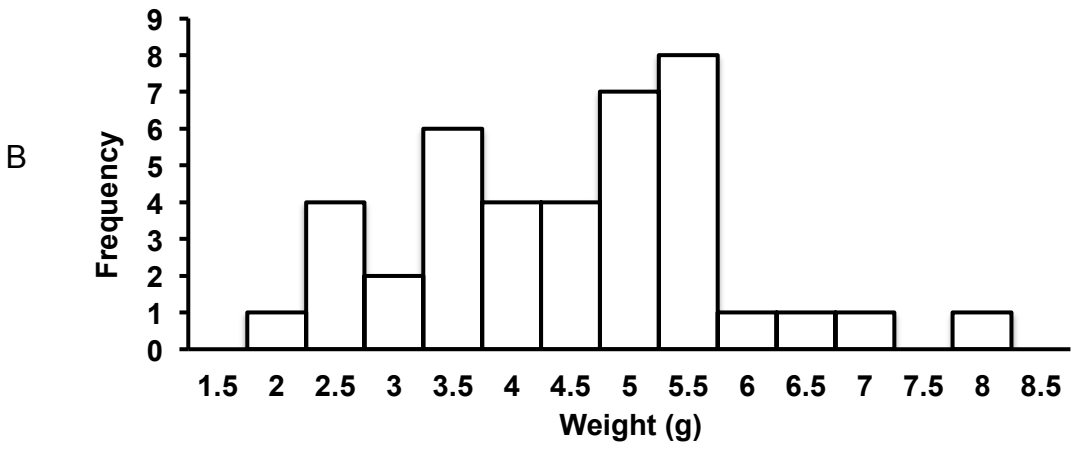
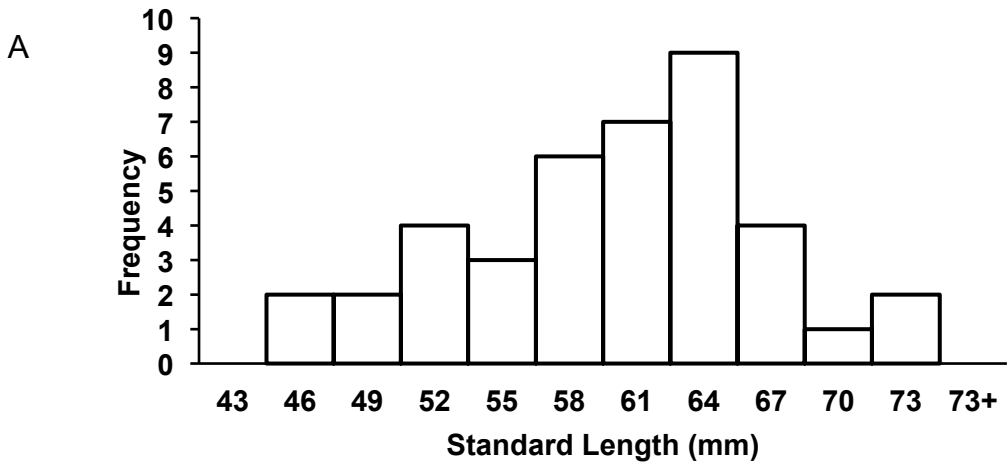


Figure 4. Mean (\pm SE) abundance of (A) macroinvertebrates and (B) microinvertebrates collected in dip samples from three fish stocking densities.

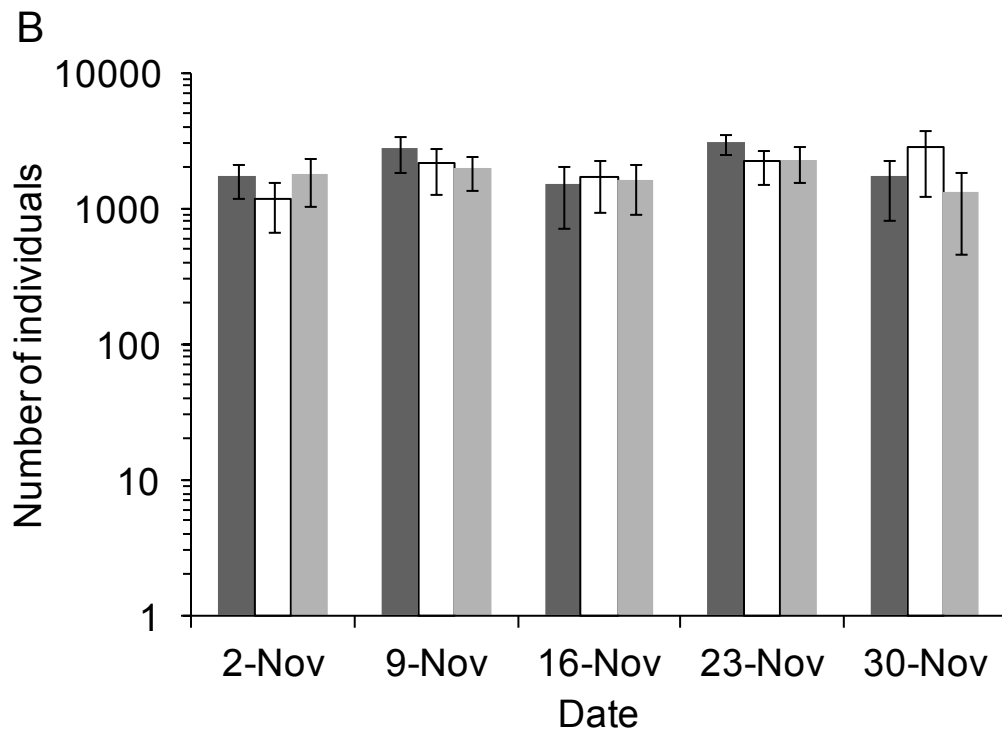
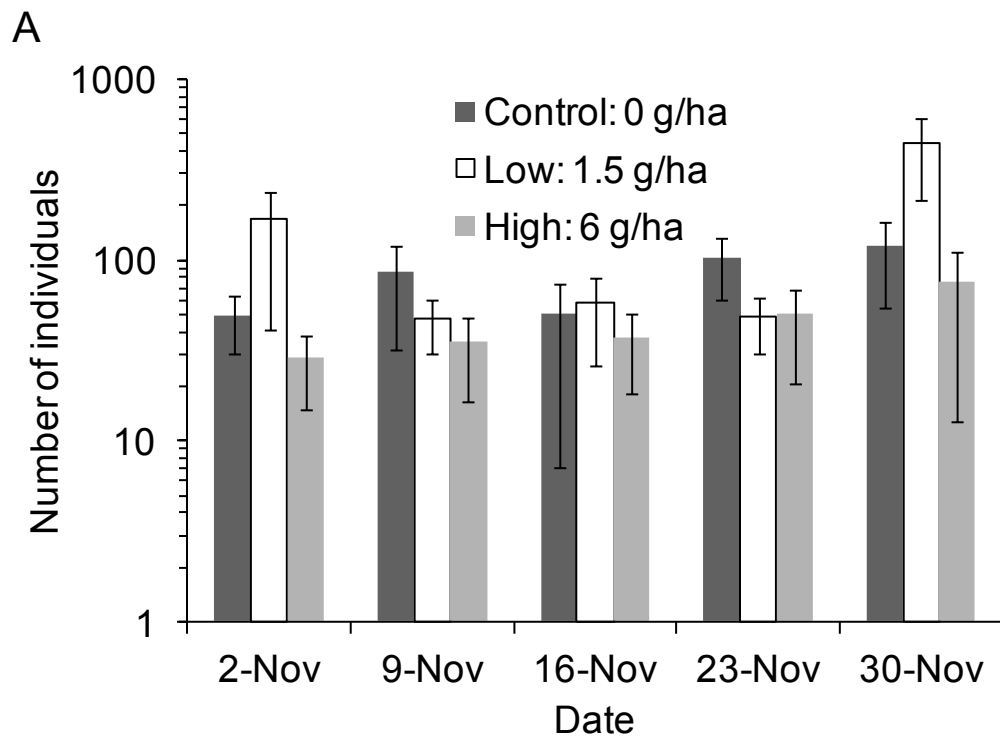
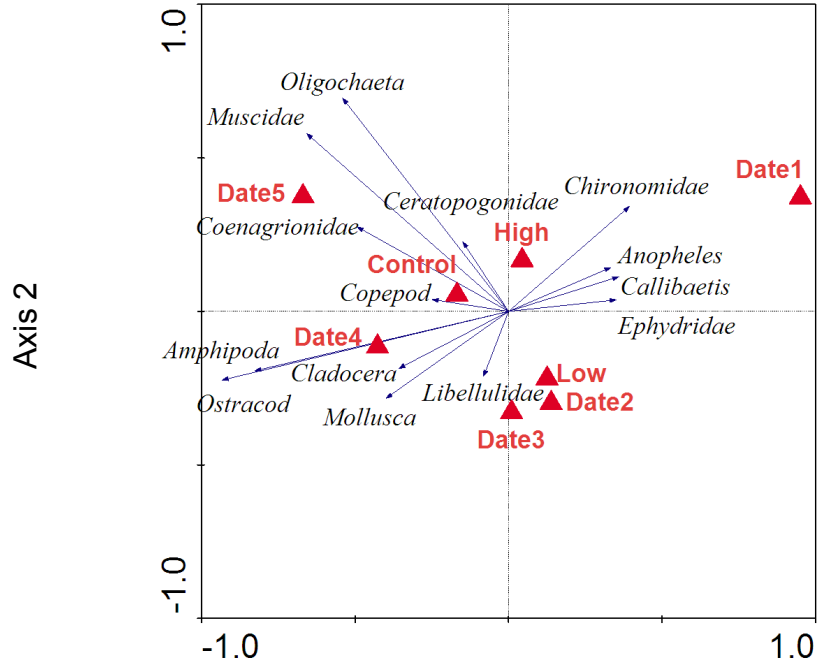


Figure 5 . Ordination (PCA) diagrams illustrating the variation in the abundances of invertebrate taxa in (A) dip samples and (B) funnel traps from three arroyo chub stocking densities. The centroids for the arroyo chub treatments (High, Low and Control) and the sampling dates (Date 1 – 5) are indicated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

A.



B.

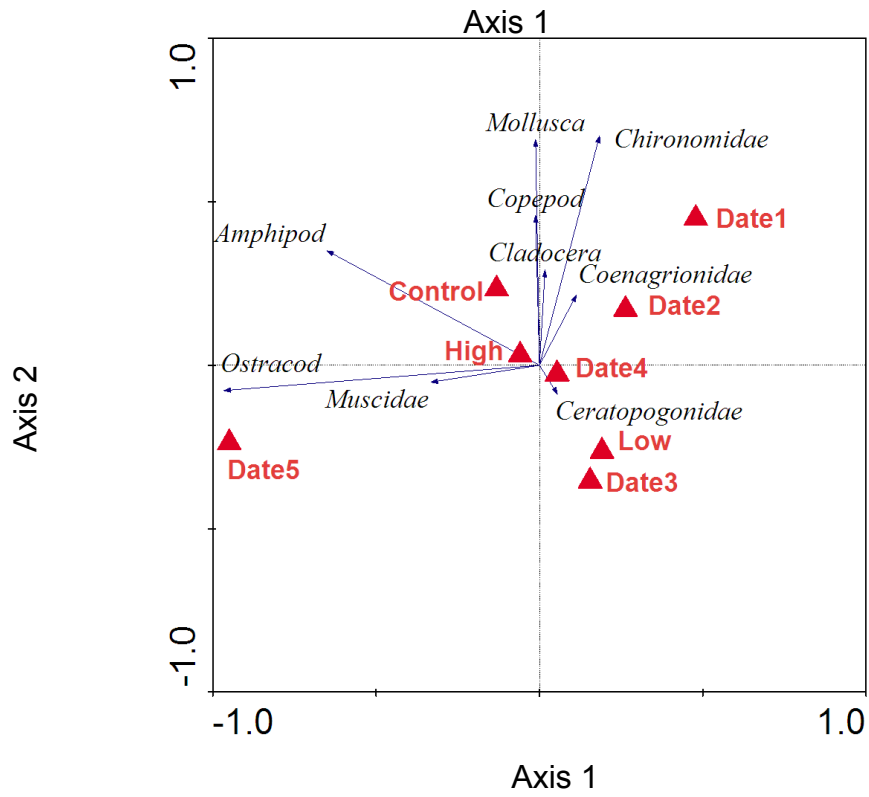


Figure 6. Mean (\pm SE) abundance of (A) macroinvertebrates and (B) microinvertebrates collected in funnel trap samples from three fish stocking densities.

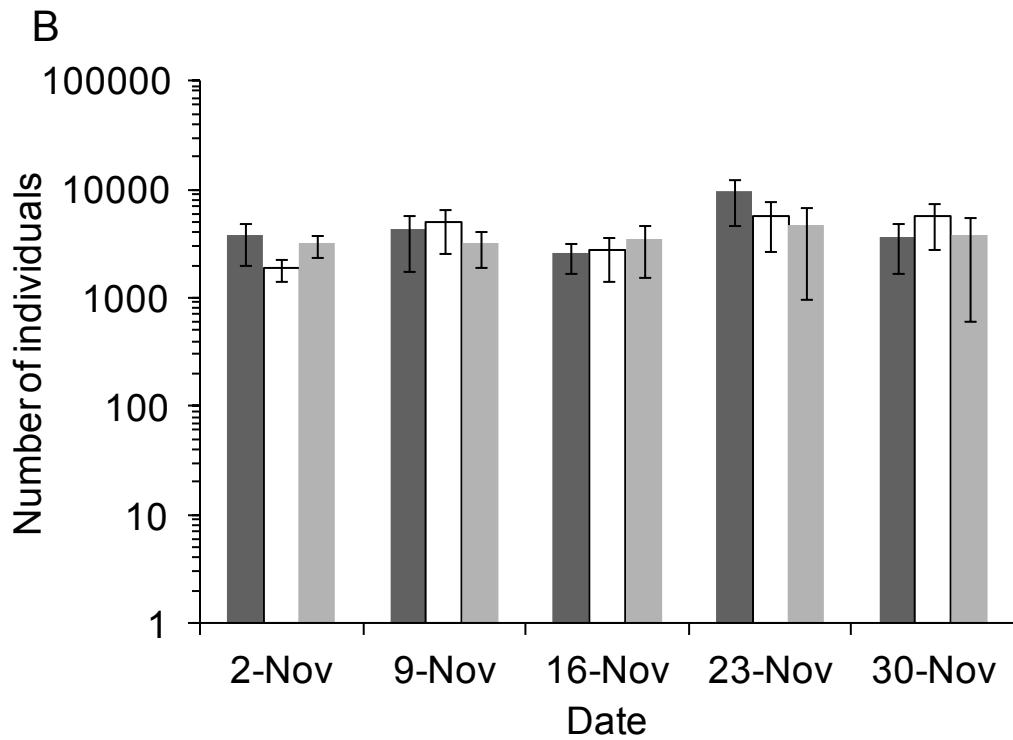
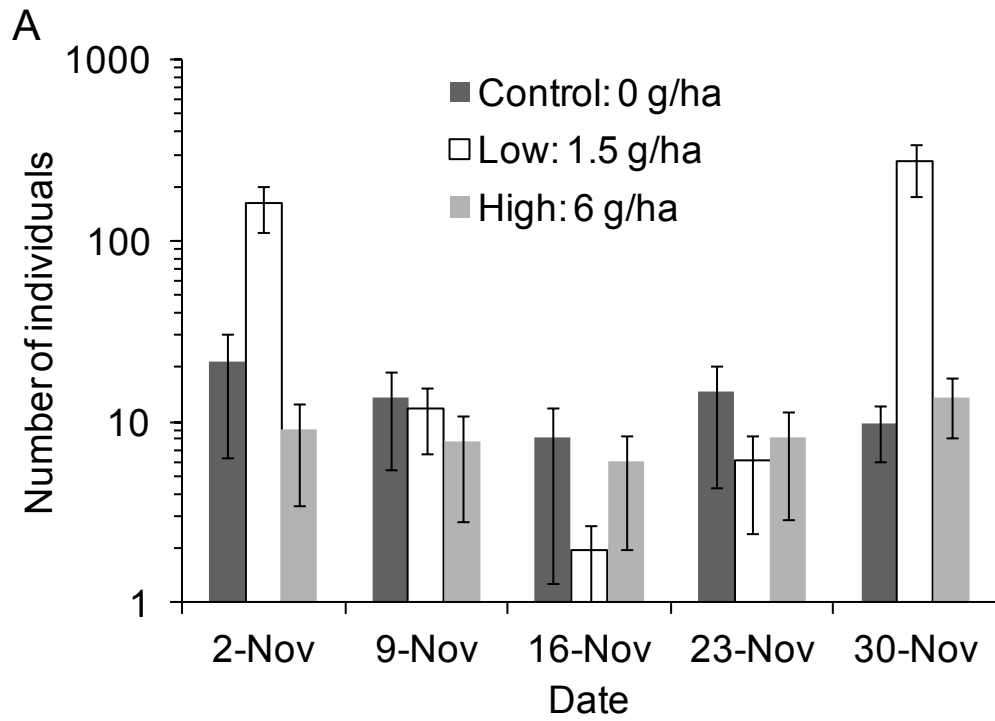


Figure 7. Abundances of taxa collected in minnow traps in the test wetland.

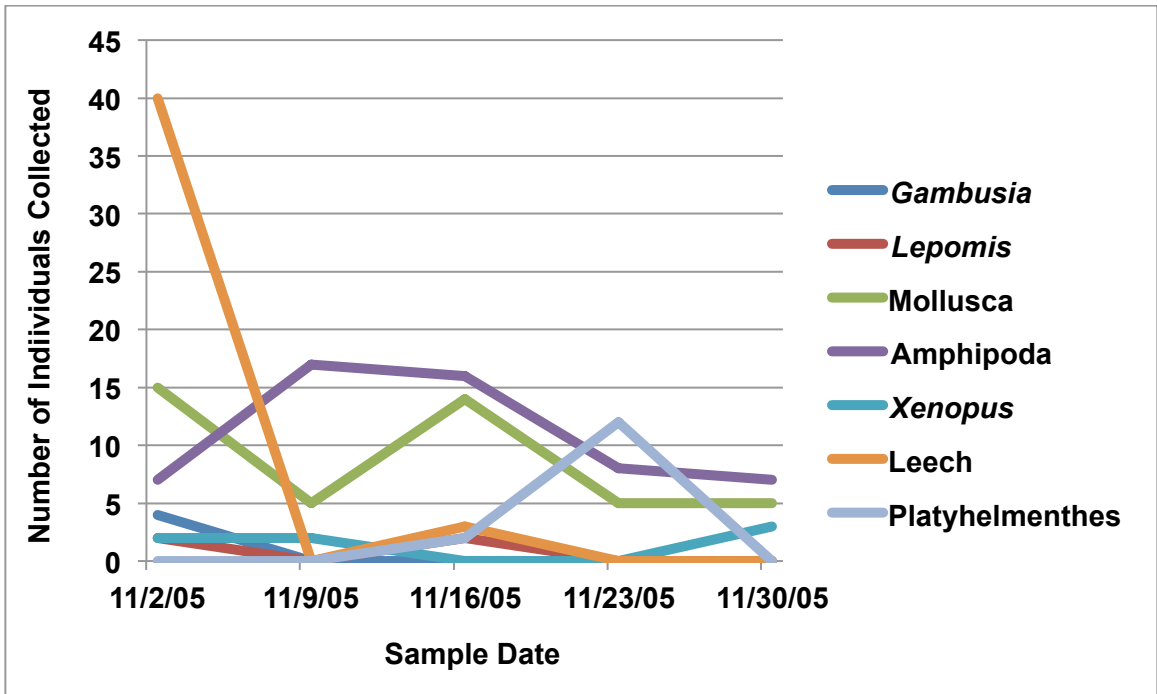
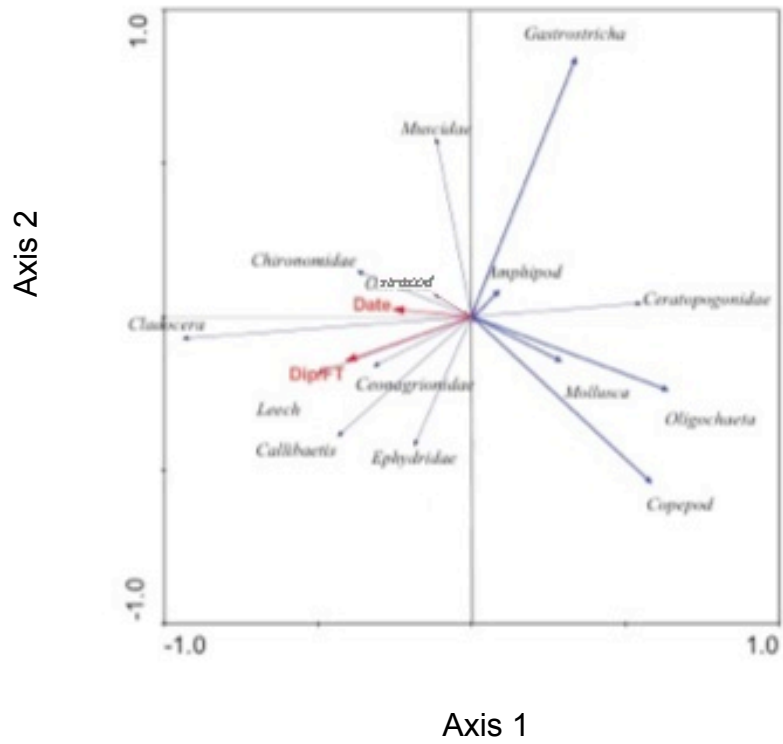


Figure 8. Ordination (PCA) diagram illustrating the variation in the abundances of invertebrate taxa in dipper (Dip) and funnel traps (FT) collections from the test wetland. Date is sample date during the five-week cage study.



Chapter Two

Ovipositional behavior of *Culex tarsalis* to aquatic organisms in three guilds

Insects detect potential predators by tactile, visual or chemical cues (Angelon and Petranka 2002). Prey organisms that have evolved the ability to detect predators via chemical cues are often influenced by chemicals excreted from the predator's diet. Mayfly and damselfly nymphs were more likely to respond to chemical exudates from fish if the fish had recently consumed conspecific prey (Chivers et al. 1996; Huryn and Chivers 1999).

However, the detection of predators by aquatic insects is not restricted to the immature stages. Adults searching for oviposition sites also respond to predator cues. Gravid female insects will likely encounter a range of microhabitats over which survival of their offspring will vary. Natural selection should favor females that can assess habitat quality and choose microhabitats that would maximize offspring survival. Gravid female mosquitoes use a combination of cues in the environment, including physical, biological and chemical, to select oviposition sites (Benzon and Apperson 1988; Bentley and Day 1989; Isoe and Millar 1995). Chemicals emitted by a variety of organisms, including plants, bacteria, copepods, insects and amphibians, have been studied to determine their effects on ovipositional site selection by female mosquitoes (Ikeshoji and Mulla 1970; Osgood and Kempester 1971, Kramer and Mulla 1979;

Chesson 1984; Petranka and Fakhoury 1991; Blaustein and Kotler 1993; Isoe et al. 1995; Mokany and Shine 2003).

Several studies have shown a reduction in oviposition rate by female mosquitoes into habitats containing semiochemicals associated with predators. Egg-laying by mosquitoes was reduced in response to chemicals released by hemipteran predators (*Anisops*: Eitam et al. 2002; *Notonecta*: Chesson 1984; Kiflawi et al. 2003; Blaustein et al. 2005; Silberbush and Blaustein 2008). It is only recently that investigators have begun looking at the semiochemical(s) produced by fish, and how they affect site selection by female mosquitoes (Ritchie and Laidlaw-Bell 1994; Angelon and Petranka 2002; Van Dam and Walton 2008; Pamplona et al. 2009; Walton et al. 2009). To date, no studies have been conducted looking at the ovipositional responses of a single mosquito species to semiochemicals produced by aquatic organisms in different guilds.

The present study investigated the effect of water conditioned with semiochemicals from three guilds (predaceous fish, herbivorous fish, predatory insects) on oviposition site selection by the western encephalitis mosquito, *Culex tarsalis* Coquillett. The effects of semiochemicals produced by two predatory/larvivorous fishes (arroyo chub, *Gila orcutti* (Eigenmann and Eigenmann), and the fathead minnow, *Pimephales promelas* Rafinesque), one herbivorous/algivorous fish [Mozambique/California hybrid tilapia, *Oreochromis mossambicus* x *O. urolepis hornorum* (Peters)] and three predatory aquatic insects (nymphal dragonflies [*Sympetrum tarnetrum* (Hagen),

Odonata:Libellulidae; adult beetles (Coleoptera: Dytiscidae; *Thermonectus bascilaris* (Harris), and *Cybister* sp.) on oviposition site selection of *Cx. tarsalis* were studied in laboratory behavioral assays.

Methods

Mosquito Colony and Rearing

Culex tarsalis adults were reared from a colony derived from wild individuals collected at the Eastern Municipal Water District's demonstration constructed treatment wetland (San Jacinto, CA) in 2001. *Culex tarsalis* larvae were reared in enamel pans under standard laboratory conditions (27°C, 16:8 h light:dark [LD] cycle with 1 hour dusk/dawn periods) and fed *ad libitum* on a mixture of ground rodent chow and Brewer's yeast (3:1, v:v). Pupae were collected into 300-mL cups (Solo Cup Co., Chicago IL) and placed into emergence cages. The adult mosquitoes were allowed to feed *ad libitum* on a 10% sucrose and water mixture, as well as a cup containing 3-4 raisins that had been soaked in water and sprinkled with 5 mL of granulated sugar. Once each week, female mosquitoes were fed overnight on a 2-5 day old restrained chick.

Within 18 h of a bloodmeal, 30 blood-fed female *Cx. tarsalis* were aspirated into cages measuring 30 x 30 x 30 cm (Model # 1450B; Bioquip Products, Rancho Dominguez, CA). Females were determined to have successfully blood-

fed by the appearance of blood in the abdomen – the abdomen is clearly dark and distended. Females were given a 10% sucrose and water mixture and allowed to feed *ad libitum*.

Mosquito Oviposition Experiments

Females in each cage were presented with 2 white 300-mL wax-lined cups (Solo Cup Co., Chicago IL), containing either 150 mL of fish-conditioned water or the control. The cups were placed into the cages at 16:30 hr, 30 min before the 1– h dusk period in the L:D cycle began. The placement of the cups within each replicate cage was randomized between the two positions. The cups were removed from the cages no sooner than 24 h the following day and number of egg rafts in each cup counted. Each female could lay a single egg raft during a trial and oviposition by the females in each cage was monitored over three successive nights. At the end of each trial, the number of egg rafts in each replicate was pooled across dates to obtain an overall number of rafts for each treatment. For each trial, no fewer than three replicate cages were used.

Trials were run independently for each predator or herbivore species to ensure that any volatiles emanating from the exudate-laden water would not cause cross contamination.

Fish-conditioned Water

A 3-day protocol was used to make fish-conditioned water. During the first 24 h, adult fish were allowed to acclimate and feed *ad libitum* on Tetra Pond flaked fish food (Spectrum Brands Inc., Melle, Germany) in a 5-gallon plastic bucket (Home Depot, Atlanta, GA) or an aquarium containing 10 L of tap water that had been continuously oxygenated with a standard aquarium pump and aged for 24 h. During the second 24 h period the fish were moved to a new bucket or aquarium containing 10 L of aged tap water and allowed to empty their guts. On day 3, the fish were moved to a new container containing 10 L of aged tap water. The fish were removed after 24 h and the fish-conditioned water was used to test ovipositional responses of the mosquitoes.

The control treatment consisted of 10 L of tap water that had been aged, using an aerator for 24 h.

Arroyo Chub Trials

Arroyo chub [*Gila orcutti* (Eigenmann and Eigenmann)] adults were collected from a captive population held at the UCR Aquatic Research Facility, Riverside, CA using minnow traps baited with a dog food kibble. Captured fish were transported to the laboratory in covered buckets with supplemental aeration provided by a battery-powered pump. Five adult arroyo chub were used for each trial. After the laboratory trial, fish were returned to the holding pond following acclimation to conditions at the field site.

Tilapia Trials

Juvenile tilapia [California Mozambique/California hybrid tilapia] were reared from eggs and maintained as a laboratory stock population at the University of California, Riverside. In trial 1, ten *Oreochromis* juveniles were placed into an aquarium with 10 L of aged tap water for 24 h to make the fish-conditioned water. In trials 2-5, five *Oreochromis* in 10 L of aged tap water was used to make fish-conditioned water.

Fathead Minnow Trials

A laboratory stock population of fathead minnows kept at the University of California, Riverside was used to produce fish-conditioned water. Five adult fish per 10 L of aged tap water was used to make the fish-conditioned water in an aquarium.

Insect-conditioned Water

'Insect-conditioned water' was made in an equivalent way to the fish-conditioned water described above. However, the ratio of the number of insects to water volume was varied (see below) between trials in order to test differing concentrations of chemicals on ovipositional site selection by female mosquitoes.

Libellulid Trials

Libellulid nymphs (*Sympetrum corruptum* (Hagen)) were collected from a pond at the University of California, Riverside (UCR) Aquatic Research Facility, Riverside, CA. The density of dragonfly nymphs was either 0.5 nymph/L (trial 1) or 1 nymph/L (trial 2). The wet weight of nymphs was (mean \pm SD) 0.52 ± 0.1 g.

Thermonectus Trials

Thermonectus bascularis (Harris) adults were collected from the Valley Sanitary District's constructed treatment wetland in Indio, CA at the beginning of August 2009. Ten adult *Thermonectus* in 10 L of aged tap water were used to test ovipositional responses in trials 1 through 3. Thirty adult *Thermonectus* in 5 L of aged tap water were used in trials 4 through 6. This was done so that the average wet weight (mean \pm SD = 0.35 ± 0.02 g; average length = 1 cm) of the *Thermonectus* was equivalent to the average wet weight of the libellulid nymphs used in the previous trials.

In trials 5 and 6, a third 300-mL wax-lined cup (Solo Cup Co., Chicago IL), containing 150 mL of aged tap water and either 1 (trial 5) or 2 (trial 6) live *Thermonectus* was placed into the experimental cages. This third treatment tested whether female *Cx. tarsalis* would be deterred from ovipositing in an area that contained the chemicals and other cues (visual, physical disturbance) of a dytiscid beetle.

Cybister Trials

Cybister sp. adults (mean \pm SD = 1.80 g \pm 0.12 g; average length = 2 cm) were collected from the Valley Sanitary District constructed treatment wetland in Indio, CA at the beginning of August 2009. Ten adult *Cybister* in 10 L of aged tap water were used to test ovipositional responses in the four trials. Pairwise comparisons were made in trials 1 and 2. In trials 3 and 4, a third 300-mL wax-lined cup (Solo Cup Co., Chicago IL), containing 150 mL of aged tap water and 1 live *Cybister* beetle was placed into the experimental cages to test the additional effect of visual and physical cues provided by the large predatory beetle.

Statistical Analyses

Data were analyzed using SAS (Version 9.2; SAS Institute Inc., Cary, NC). The number of egg rafts/treatment in each cage was summed across all the days of a given trial. A paired *t*-test was performed on the total number of egg rafts in each treatment among replicate cages for those experiments with only a binary choice. A Replicated Goodness of Fit test (*G*-test), as well as a standard ANOVA, were carried out to compare the pattern of distribution of oviposition choice in the trials with three treatments (e.g. control, beetle exudate-laden water, a cup with a live beetle) among the replicate cages.

An ovipositional activity index (OAI) was calculated by cage across dates where the activity index was given as: $(NT-NS)/(NT+NS)$; NT is the number of

egg rafts laid in the test cups and NS is the number of egg rafts laid in the control cups (Kramer and Mulla 1979). This index varies between -1 and +1 so that -1 indicates complete preference for oviposition in control cups, +1 represents a complete preference for treatment cups (exudate-laden water), and 0 represents no preference between the two treatments. OAI was only calculated for those experiments run as binary choice trials.

Results

Predatory/Larvivorous Fish Guild

Arroyo Chub

The western encephalitis mosquito responded strongly to the presence of chub-associated chemicals in oviposition sites ($t = -6.97$, d.f. = 3, $P < 0.0061$; Fig. 1 and Table 1). On average, five times (mean \pm standard error [SE]: 5.0 ± 0.9 , $n = 4$) as many egg rafts were laid on control water compared with water that contained chub kairomones. Less than 20% of females on average laid egg rafts during the one-day trial; nevertheless, a statistically significant difference in oviposition rate between the two treatments can be seen. The OAI results (OAI = -0.89) concurred with the paired t-test results, indicating that female *Cx. tarsalis* showed a preference toward oviposition in control cups.

Fathead Minnow

Water conditioned with *P. promelas* did not deter egg laying by *Cx. tarsalis* in four of the five trials (Fig. 1 and Table 1). *Culex tarsalis* responded strongly to the presence of minnow-associated chemicals in oviposition sites in only one trial (Trial 5: $t = -3.75$, d.f. = 7, $P < 0.0072$). The OAI results for Trial 5 (OAI = -0.28) were in accordance with the results from the paired-t test. The OAI for the remaining four trials were close to 0 or slightly negative, indicating that *Cx. tarsalis* was not deterred from ovipositing in sites that contained minnow-conditioned water (OAI; Trial 1 = -0.04, Trial 2 = 0.09, Trial 3 = 0.01, Trial 4 = -0.13).

Herbivorous/Algivorous Fish Guild

Tilapia

The western encephalitis mosquito did not respond to the presence of Tilapia-associated chemicals in oviposition sites (Fig. 1). On a per cage basis, the number of egg rafts laid on fish-conditioned water did not differ significantly from the number laid in control cups (Table 1). The OAI for all the trials had values very close to zero (OAI; Trial 1 = 0.22, Trial 2 = -0.12, Trial 3 = 0.09, Trial 4 = 0.04, Trial 5 = -0.07), indicating *Cx. tarsalis* either showed a slight preference for ovipositing in control cups or no preference for a particular treatment during the oviposition trials.

Predatory Insect Guild

Libellulid nymphs

The western encephalitis mosquito was not deterred from ovipositing in water that had been conditioned with dragonfly nymphs (Fig. 2). No statistical difference between treatments was detected in either trial (Trial 1: $t = -0.52$, d.f. = 3, $P = 0.641$; Trial 2: $t = 1.27$, d.f. = 7, $P = 0.246$).

The OAI also indicated that there was no difference in ovipositional responses among the different trials. Female *Cx. tarsalis* showed no preference between either the dragonfly-conditioned or control water (mean \pm SD: Trial 1: OAI = -0.08 ± 0.25 ; Trial 2: OAI = 0.14 ± 0.23).

Thermonectus

Egg-laying *Cx. tarsalis* females did not distinguish between oviposition cups containing water conditioned with *Thermonectus* semiochemicals and aged tap water (Fig. 2 and Table 2); however, this result was not consistent across the four trials. For trials 1, 2 and 4, female *Cx. tarsalis* showed no ovipositional preference between the treatments (Trial 1: $t = 1.06$, d.f. = 6, $P > 0.328$; Trial 2: $t = -1.68$, d.f. = 2, $P > 0.235$; Trial 4: $t = -0.59$, d.f. = 4, $P > 0.587$). The OAI also indicated that *Cx. tarsalis* only slightly preferred control cups in trial 1 (OAI = 0.14) and trial 2 (OAI = 0.07), and the opposite preference was seen in trial 4,

where females slightly preferred ovipositing in semiochemical-laden water (Trial 4: OAI = -0.07).

In trial 3, the number of egg rafts laid by *Cx. tarsalis* females on *Thermonectus*-conditioned water was significantly fewer than was laid on aged tap water ($t = -2.72$, d.f. = 8, $P < 0.026$). The OAI also indicates a slight avoidance of oviposition by female *Cx. tarsalis* on exudate-laden water (OAI = 0.17) in trial 3.

Culex tarsalis females were deterred from ovipositing in cups that contained one live beetle when compared to cups with aged tap water or *Thermonectus*-conditioned water alone (Fig. 3a). When differences among treatments were analyzed using ANOVA (trial 5: $F = 13.41$; d.f = 2, 11; $P < 0.0020$), female mosquitoes were significantly deterred from ovipositing in cups that contained a live beetle (ANOVA : $F = 13.41$; d.f = 2, 11, $P < 0.0015$), but no significant difference was detected between the beetle-conditioned water and the control cups (ANOVA : $F = 13.41$; d.f = 2, 11, $P < 0.0685$). It is unlikely to obtain the distribution of egg rafts laid in the different treatments by chance ($G_T = 21.063$, $P < 0.01$ level). While the heterogeneity seen within each cage of the trial was not significant ($G_H = 6.715$), the overall pooled G was significant indicating that there was a deviation from the expected ratios seen in the oviposition responses ($G_P = 14.348$, $P < 0.001$ level).

Oviposition of egg-laying *Cx. tarsalis* females into cups containing two live beetles differed significantly from the other treatments: control water vs. water

with 2 live beetles (ANOVA: $F = 9.84$; d.f = 2, 17; $P < 0.0015$; exudate-laden water vs. water with 2 live beetles: (ANOVA: $F = 9.84$; (d.f = 2, 17; $P < 0.036$). Oviposition did not differ significantly between the control cups and the beetle-conditioned-water (ANOVA: $F = 9.84$; d.f = 2, 17; $P < 0.27$).

As for trial 5, the heterogeneity seen between the trials was not significant ($G_H = 8.522$) but the overall pooled G was significant ($G_P = 15.836$, $P < 0.001$ level), indicating there was a deviation from the expected ratios seen in the oviposition responses. Egg rafts were not distributed randomly among the treatments ($G_T = 21.03$, $P < 0.05$ level).

Cybister

Female *Cx. tarsalis* did not respond to the presence of *Cybister*-conditioned water when compared to control cups (Fig. 2). In both trials, slightly more egg rafts were laid on the conditioned water than on the control; however, oviposition rates on the two treatments did not differ significantly (Trial 1: $t = 1.41$, d.f. = 3, $P < 0.065$; Trial 2: $t = 1.65$, d.f. = 7, $P = 0.143$). The OAI indicated that a slight preference for the test cups existed, but overall this preference was negligible (OAI: Trial 1 = 0.24 and Trial 2 = 0.20).

Egg-laying *Cx. tarsalis* females were deterred from ovipositing in cups that contained one live beetle when compared to aged tap water or water conditioned with *Cybister* (Fig. 4). When differences between treatments were analyzed using ANOVA (Trial 3: $F = 13.57$; d.f = 2, 14; $P < 0.0008$), female mosquitoes

were significantly deterred from ovipositing in cups that contained a live beetle when compared to conditioned water (ANOVA: $F = 13.57$; d.f = 2, 14; $P < 0.0009$), but no significant difference was detected between the conditioned water and the control cups (ANOVA: $F = 13.57$; d.f = 2, 14; $P < 0.588$). A significant difference was also detected between the control cups and the cups containing a live beetle (ANOVA: $F = 13.57$; d.f = 2, 14; $P < 0.0053$).

It is unlikely to have obtained the numbers of egg rafts laid in the different treatments by chance ($G_T = 55.80$, $P < 0.001$ level). While the heterogeneity seen within each cage of the trial was not significant ($G_H = 14.358$), the overall pooled G was significant indicating that there was a deviation from the expected ratios seen in the oviposition responses ($G_P = 41.444$, $P < 0.001$ level).

Culex tarsalis females were deterred from ovipositing in cups that contained one live beetle when compared to aged tap water or water conditioned with *Cybister* (Fig. 4b). Female mosquitoes were significantly deterred from ovipositing in cups that contained a live beetle (Trial 4: ANOVA: $F = 7.66$; d.f = 2,26; $P < 0.0027$). No difference was detected between the beetle-conditioned water and the control cups (ANOVA: $F = 7.66$; d.f = 2,26; $P < 0.689$). However a significant difference was seen between the beetle-conditioned water and the cups containing 1 live beetle (ANOVA: $F = 7.66$; d.f = 2,26; $P < 0.0029$), as well as the control cups vs. the cups containing one 1 live beetle (ANOVA: $F = 7.66$; d.f = 2, 26; $P < 0.0207$).

It is unlikely to obtain the distribution of egg rafts laid in the different treatments by chance ($G_T = 99.49$, $P < 0.001$ level). Significant heterogeneity was seen between each replicate ($G_H = 59.47$ at the $P < 0.001$ level), indicating that there was a non-uniform departure from the expected ratios. The overall pooled G was highly significant indicating that there was a deviation from the expected ratios seen in the oviposition responses ($G_P = 39.76$ at the $P < 0.001$ level).

Discussion

Our experiments indicate that the response of *Cx. tarsalis* egg-laying females to semiochemicals released by potential predators during oviposition site selection is correlated with the probability of larvae coming into contact with a given taxa in natural oviposition sites, as well as the likelihood of the predator to actively prey on mosquito larvae. The selection of oviposition sites by female mosquitoes can greatly influence the individual fitness of their offspring as well as the population dynamics and structure of the aquatic community (Blaustein 1999; Spencer et al. 2002; Blaustein et al. 2004). The presence of a wide variety of predator semiochemicals in aquatic environments has been shown to benefit prey species survival (Petranka and Hayes 1998; Von Elert and Pohnert 2000; Binkley and Resetarits 2003; Blaustein et al. 2004), perhaps in part as a result of the ease in which the aquatic environment can transmit chemical messages (Dodson et al. 1994; Wisenden 2000; Blaustein et al. 2004).

Mosquitoes have been shown to use chemical and non-chemical cues detected in and around aquatic environments (Bentley and Day 1989; McCall and Kelly 2002; Blaustein et al. 2004) to avoid ovipositing in sites containing invertebrate predators (Tietze and Mulla 1991; Stav et al. 2000; Blaustein et al. 2004). Chemical cues are thought to be more valuable than non-chemical cues for predator detection and selection of oviposition sites because of the often murky water and nocturnal conditions into which mosquitoes oviposit (Blaustein et al. 2004)

Culex tarsalis lays its egg rafts in a wide variety of habitats (Bohart and Washino 1978), many of which often contain predatory fish and invertebrates. The ability of a female mosquito to detect the presence of fish, and other insects, in a given body of water can be important in the determination of larval survival, but the ability to discern predatory/larvivorous from non-larvivorous species may be even more important. Predatory insects may also be able to colonize habitats that cannot sustain a larger organism such as a fish, (e.g. water tank of a bromeliad), therefore the ability of a female mosquito to assess the suitability of an oviposition site, while at the same time determining if the species already present could potentially be detrimental to her offspring, would be an advantageous evolutionary trait.

Our studies indicate that female *Cx. tarsalis* are not deterred from ovipositing on water conditioned by taxa that do not pose a great risk to her offspring. *Cx. tarsalis* were not deterred from ovipositing in cups that contained

libellulid- or *Cybister*-conditioned water. Libellulids are predaceous in the nymphal stage and can primarily be found hidden in the benthos, among vegetation and sediment, camouflaged and lying in wait to ambush their prey (Merritt et al. 2008). Mosquito larvae spend most of their time at the surface of the water, in order to respire through their siphons, and are not commonly found inhabiting the benthic layer (Merritt et al. 2008). Mosquito larvae would therefore not be the main source of prey for libellulid nymphs and this may explain why female *Cx. tarsalis* were not deterred from ovipositing in cups containing libellulid-conditioned water.

Mosquito larvae can make up an important part of the diet of nymphal odonates (Merritt et al. 2008), however work done by Stav et al. (2000) showed similar results when they tested the ovipositional responses of female *Culiseta longiareolata* Macquart to *Anax imperator* Leach nymphs. No difference in oviposition rate was seen between the control treatment and treatments containing caged *Anax*. Fewer egg rafts were laid in treatments containing *Anax* nymphs that were freely moving about the oviposition sites, leading to the conclusion that mechanical or visual stimuli led to the decrease seen in oviposition rate.

Cybister beetles co-occur with *Cx. tarsalis* larvae (e.g. in wetlands) and are predaceous in all life stages. However, adult *Cybister* are much larger than mosquito larvae and mosquito larvae are most likely not within their preferred size range of prey. They are known to attack and feed on larger prey, such as

fish and tadpoles (Brigham et al. 1982), which may indicate why there was no difference in oviposition rate seen between the control cups and the *Cybister*-conditioned water.

Female *Cx. tarsalis* were only slightly deterred from ovipositing in water conditioned with *T. bacilaris*. This result was unexpected as mosquito larvae are within the preferred size range of prey for *Thermonectus* (Merritt et al. 2008) and it was thought that female *Cx. tarsalis* would react negatively to the presence of *Thermonectus* semiochemicals in oviposition sites.

However, oviposition sites that contained live beetles led to the most significant decrease in oviposition rate by *Cx. tarsalis* females when compared to semiochemical-conditioned water or control cups. Our results indicate that they seem to be responding to other cues from the beetles, such as physical or mechanical (e.g., vibrations on the surface of the water caused by the movement of the beetle(s)) and not solely to semiochemicals emitted by adult *Cybister* or *Thermonectus*. More research will need to be conducted looking at whether the cues leading to a change in oviposition behavior are mediated by visual and/or mechanical stimuli.

Exudates produced by predatory species have been shown to affect the larval and ovipositional behavior of other mosquito species, along with other nematoceran Diptera. Prey species' responses to semiochemicals can be quite different depending on the risk of predation, even within the same ecosystem. *Culiseta longiareolata* Macquart avoided ovipositing in pools that contained

notonectid kairomones (Eitam et al. 2002; Blaustein et al. 2004). However, chironomids did not show any ovipositional deterrence to the presence of notonectid kairomones, even though their larvae inhabit the same temporary pools as the *Culiseta* larvae. The difference seen in oviposition behavior may be related to the fact that chironomid larvae are primarily benthic in nature and are not heavily preyed upon by notonectids.

Chaoborus flavicans (Meigen), a lake-dwelling chaoborid midge, migrates to the benthic region from planktonic zones during the day to avoid predation in response to fish chemicals (Dawidowicz et al. 1990; Tjossem 1990). *Chaoborus* species that inhabit fishless lakes have not evolved the vertical migratory defensive mechanism in response to the presence of fish chemicals and thus cannot coexist with fish (Berendonk and O'Brien 1996). Even more interesting is that fact that, females of *Chaoborus* species found in fishless lakes will avoid ovipositing in water that contains fish chemicals (Berendonk 1999).

Culex tarsalis responded strongly to the presence of fish-associated chemicals in oviposition cups, but this response was not universal across all fish species tested. Of the two predatory/larvivorous fish species tested, *Cx. tarsalis* responded strongly to the presence of arroyo chub semiochemicals in oviposition sites. Even though some studies have shown the diet of arroyo chub to consist largely of plant material, they do readily eat invertebrates (Greenfield and Deckert 1973) and *Cx. tarsalis* responded strongly to the presence of chub-

semiochemicals in oviposition sites by exhibiting a marked decrease in the number of egg rafts laid.

Cx. tarsalis females were only weakly deterred from ovipositing in sites that contained fathead minnow-associated semiochemicals and the decrease seen in ovipositional activity was not consistent throughout the trials. The lack of response by female *Cx. tarsalis* to fathead minnow semiochemicals was surprising in light of the fact that fathead minnows are omnivorous, and studies have shown that while they feed mainly on detritus and algae, they do feed readily on invertebrates (Coyle 1929). The lack of a consistent response to the presence of fathead minnow-semiochemicals in oviposition sites during the experiment may be due to the fact that invertebrates do not compose the majority of their diet.

Culex tarsalis and fathead minnows have coevolved together throughout parts of North America (Page and Burr 1991; Reisen 2002) and fathead minnows are found in the same types of aquatic habitats in which *Cx. tarsalis* lays its eggs (Bohart and Washino 1978; Page and Burr 1991; Etnier and Starnes 1993). Fathead minnows are also related taxonomically to the arroyo chub indicating that the response of *Cx. tarsalis* to fish-semiochemicals in oviposition sites cannot be generalized at the family level and is more species-specific.

Culex tarsalis showed no response to the presence of Tilapia-conditioned water in oviposition sites. Tilapia feed mainly on vegetable and algal matter as adults but animal matter, such as from invertebrates, is found in the diets of

juveniles (Trewevas 1983). The lack of response of female *Cx. tarsalis* to Tilapia-conditioned water indicates that Tilapia are not perceived as a predatory threat. Tilapia spp. are native to Africa whereas *Cx. tarsalis* is native to North America (Reisen 2002; Costa-Pierce 2003) so the two organisms have not coevolved together, and this could be another reason why *Cx. tarsalis* showed no decrease in oviposition rate when presented with Tilapia-conditioned water.

The results from the fish-semiochemical trials suggest that the particular chemical compound(s) excreted by arroyo chub are not universal to all fish species and may not be widespread even among fish within the same taxonomic family. Work to identify the particular compound(s) produced by the fish, and their subsequent synthesis could then be studied to see if one particular compound, or a suite of compounds, is needed to elicit a response in *Cx. tarsalis*.

The attractiveness of several compounds to mosquitoes during oviposition have been investigated (Navarro-Silva et al. 2009), but until recently much less attention has been devoted to characterizing the chemical nature of compounds repellent to mosquitoes during oviposition. Hexadecyl pentanoate, tetradecyl heptanoate and tridecyl octanoate presented significant oviposition repellent activity in laboratory trials against *Aedes aegypti* L. and *Aedes albopictus* (Skuse) (Sharma et al. 2008). Nonanoic acid (C₉) was found to be a repellent against *Cx. quinquefasciatus* Say, *Cx. tarsalis* and *Aedes aegypti* L. in laboratory trials (Hwang et al. 1982) and several straight chain fatty acids were identified and shown to present repellent activity in lab trials with *Cx. quinquefasciatus*

(Hwang et al. 1984). Octanoic acid (C₈) and nonanoic acid (C₉) were tested in field trials and were shown to be repellent to ovipositing female *Cx tarsalis* and *Cx. peus* Speiser (Schultz et al. 1982). However work on the characterization of fish-kairomones has only just begun.

Von Elert and Loose (1995) found that the kairomone exuded by *Leucaspilus delineates* Heckel, a planktivorous fish, characterized as a nonolefinic low-molecular-weight anion of intermediate lipophilicity, induced a predator avoidance response in *Daphnia*, leading to a change in diel vertical migration patterns. They also compared the chemical structure of the kairomone released by *Leucaspilus delineatus* with those of two different cyprinid species (*Carassius carassius* Linnaeus and *Rutilus rutilus* Linnaeus), and no differences were found.

Studies focusing on the chemical nature of a kairomone produced by the fish *Alburnus alburnus* L. (Family Cyprinidae) have discovered that it is of low molecular weight (500 Da), non-volatile, anionic compound of medium polarity, extreme pH (0.8\pH\14.0) and temperature stability (-20°C \ T \ 120°C) and proteinase resistance (Loose et al. 1993; Von Elert and Loose 1995). Out of many possible functional groups (e.g. amino, carboxy, olefinic/ester bonds, sulphate or phosphate groups), hydroxyl groups are revealed as essential for biological activity of the kairomone (Von Elert and Loose 1995; Von Elert and Pohnert 2000). The kairomone is water-soluble and loses its activity under non-

sterile conditions due to microbial degradation (Loose et al. 1993) and the presence of planktonic bacterial processes (Beklioglu et al. 2007).

It has been suggested that enzymes originating from fish mucus-associated bacteria may initiate the production and breakdown of the polysaccharides found in the mucus, and this is what ultimately functions as the fish kairomone (Forward and Rittschof 1999). Seasonal shifts, as well as temperature, are also key factors that may influence the responsiveness of the given organism to the kairomone (Stibor and Lampert 2000; Lass and Spaak 2003; Beklioglu et al. 2007). Despite this and other work to date, the precise chemical structure, origin, persistence in the environment and seasonality of fish-associated semiochemicals still has not been fully characterized.

Our experiments investigated the oviposition behavior of *Cx. tarsalis* to semiochemicals released by organisms in different aquatic guilds and showed that the response of egg-laying female mosquitoes differed depending on the species tested. Further research is needed to determine whether other predatory invertebrates produce semiochemicals that affect the oviposition of *Cx. tarsalis* and the relative influence of chemical vs. visual and mechanical stimuli on mosquito oviposition behavior.

It remains to be determined whether the semiochemicals of larvivorous fish are derived from specific compound(s) associated with the fish themselves, or are produced by bacteria found in the associated fish-mucus. The ubiquity of compounds that deter mosquito oviposition among larvivorous fish requires

further study. Experiments conducted with other fish species native to Southern California, as well as species within the rest of *Cx. tarsalis* native range, will show if co-evolution of the two species is an important factor necessary for *Cx. tarsalis* to respond to the given kairomone. Results of such experiments will then need to be tested in the field to determine their biological relevance. Our studies indicate that the presence of larval predator semiochemicals in oviposition sites affects the ovipositional behavior of *Cx. tarsalis*, and the degree to which females respond is mediated by the perceived threat that the predator poses to her offspring.

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Tables:

Table 1. Paired t-test values for the Tilapia and Fathead minnow fish-semiochemical trials.

	Tilapia	df	Fathead Minnow	df	Arroyo chub	df
Trial 1	p-value = 0.1770	4	0.7417	9	0.0061	3
Trial 2	0.1908	5	0.5801	8		
Trial 3	0.5088	5	0.9449	8		
Trial 4	0.9385	4	0.2190	7		
Trial 5	0.8223	4	0.0072	7		

Table 2. Paired t-test and Oviposition Activity Index (OAI) values for *Thermonectus* Trials 1-4.

<i>Thermonectus</i> Trials 1-4				
Control vs. Exudate-laden water	T-value	df	P-value	OAI
Trial 1	1.06	6	0.3289	0.14
Trial 2	-1.68	2	0.2355	0.07
Trial 3	-2.72	8	0.0263	0.17
Trial 4	-0.59	4	0.5878	-0.07

Figures:

Fig. 1. Mean (\pm SE) difference in the number of egg rafts laid between treatments by *Culex tarsalis* (30 females/cage) in cups containing aged tap water or water conditioned with fish. A negative value indicates deterrence by female mosquitoes to oviposition on semiochemical-laden water. (n = minimum of 3 cages)

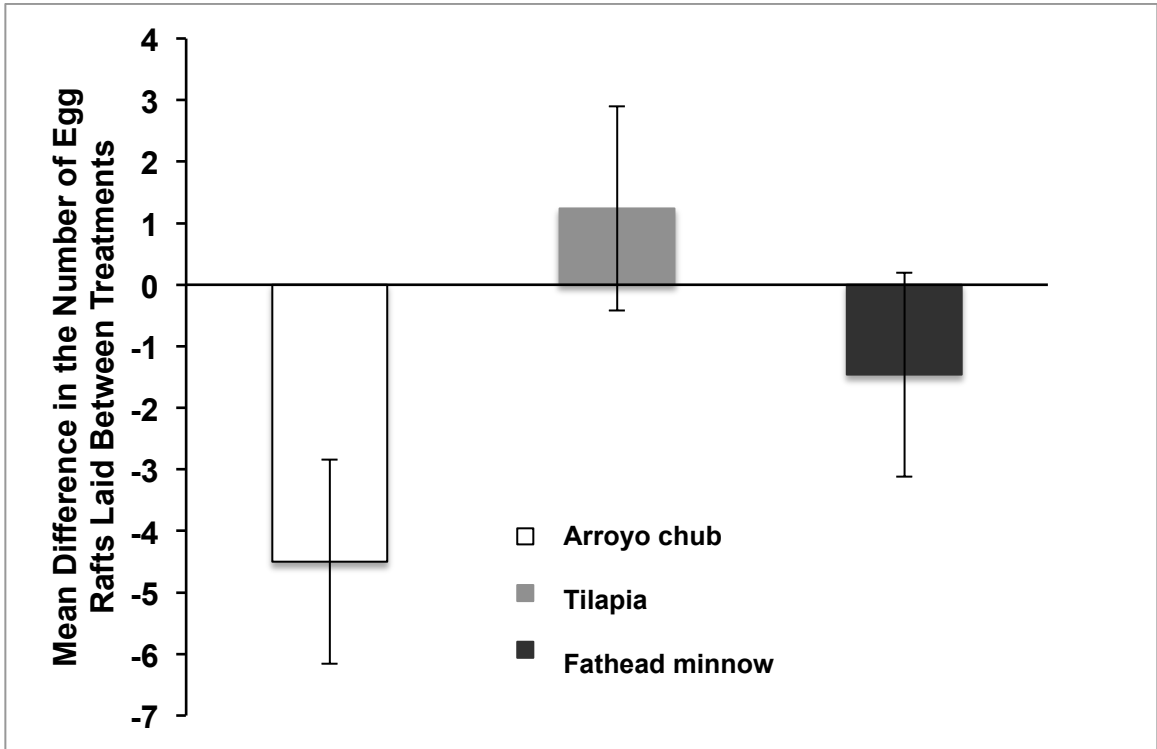


Fig. 2. Mean (\pm SE) difference in the number of egg rafts laid between treatments by *Culex tarsalis* (30 females/cage) in cups containing aged tap water or water conditioned with predatory aquatic insect semiochemicals. A negative value indicates deterrence by female mosquitoes to oviposition on semiochemical-laden water. (n = minimum of 3 cages)

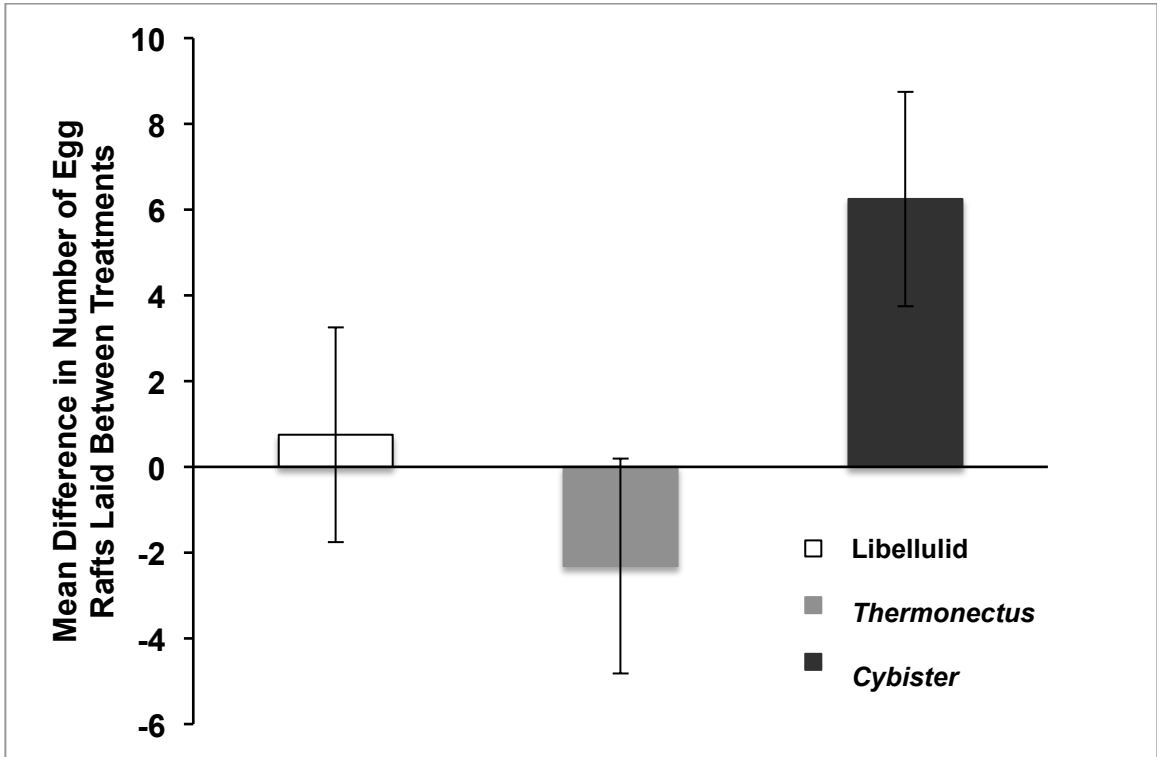
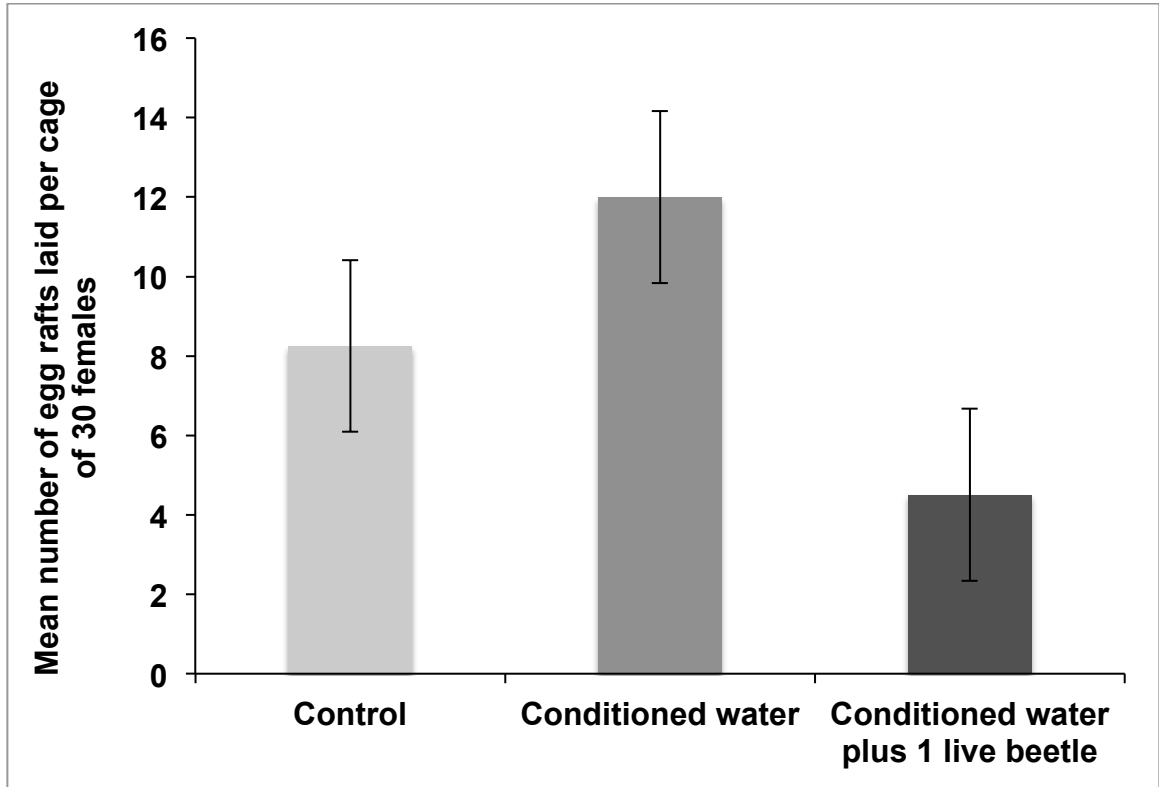


Fig. 3. Mean numbers (\pm SE) of egg rafts laid per cage of *Culex tarsalis* (30 females/cage) in cups containing aged tap water (control), *Thermonectus*-conditioned water or *Thermonectus*-conditioned water plus live adult beetles (a: 1 beetle, $n = 4$ cages; b: 2 beetles, $n = 6$ cages).

a



b

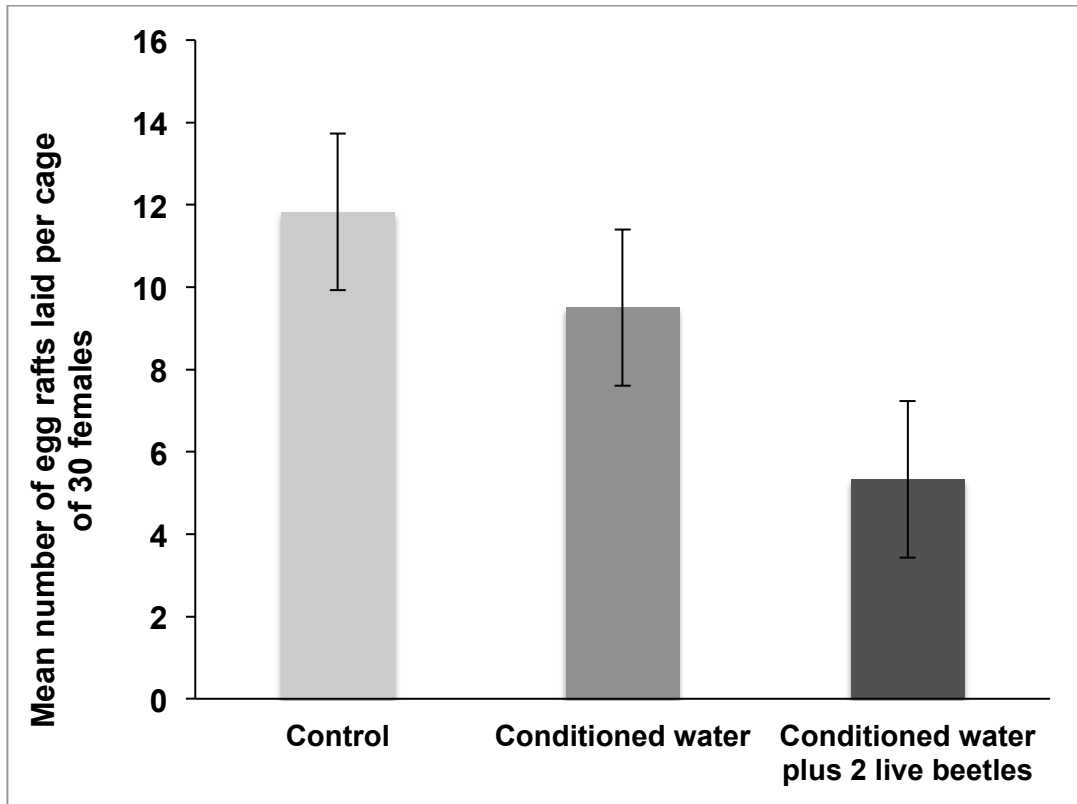
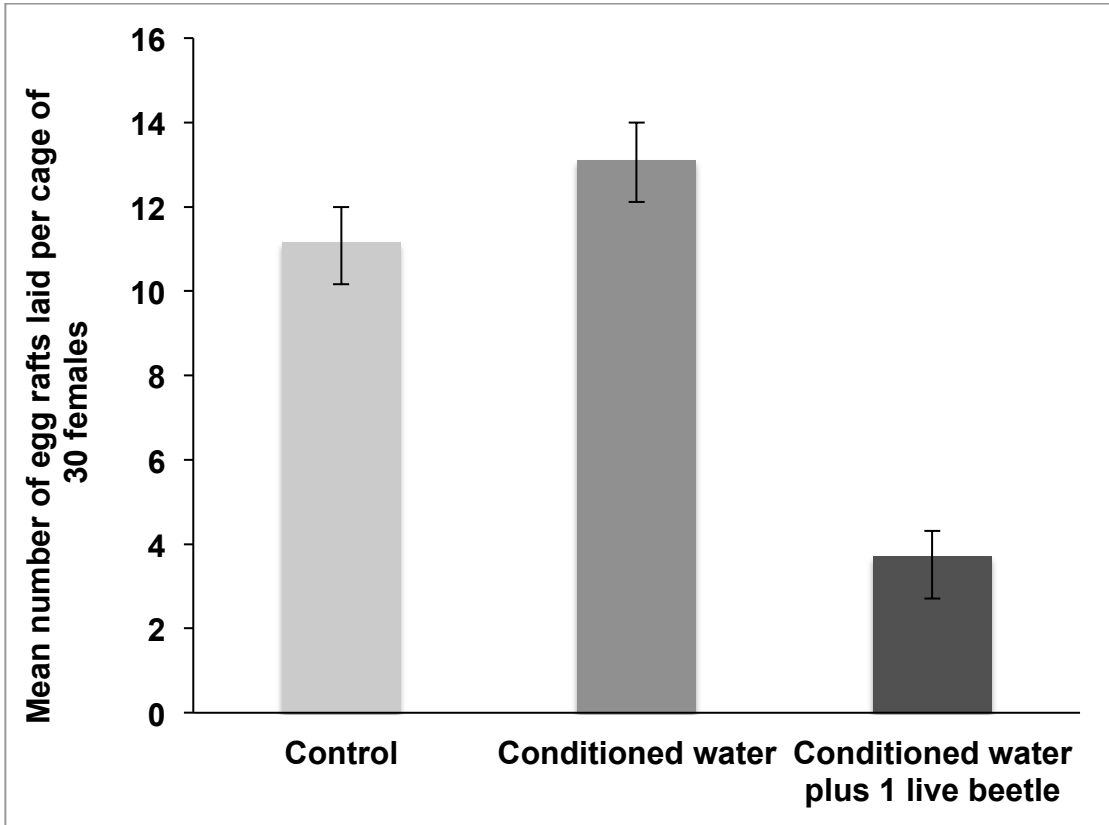


Fig. 4. Mean numbers of egg rafts laid per cage of *Culex tarsalis* (30 females/cage, Trial 1: $n=4$ cages, Trial 2: $n=8$) in cups containing *Cybister* semiochemicals and a treatment with 1 live beetle present.



Appendices

Appendix A - Chapter 1

A1 - Data on Arroyo chub stocked into the wetland.

Species	Standard Length (mm)	Weight (g)	Male/Female	Location
<i>G. orcutti</i>	44	1.3	F	Pond
<i>G. orcutti</i>	46	1.41	F	Pond
<i>G. orcutti</i>	36	1.54	F	Pond
<i>G. orcutti</i>	43	1.56	F	Pond
<i>G. orcutti</i>	41	1.6	F	Pond
<i>G. orcutti</i>	44	1.6	F	Pond
<i>G. orcutti</i>	47	1.69	F	Pond
<i>G. orcutti</i>	41	1.7	F	Pond
<i>G. orcutti</i>	46	1.72	F	Pond
<i>G. orcutti</i>	50	1.78	M	Pond
<i>G. orcutti</i>	48	1.8	M	Pond
<i>G. orcutti</i>	44	1.8	M	Pond
<i>G. orcutti</i>	51	1.85	M	Pond
<i>G. orcutti</i>	45	2	F	Pond
<i>G. orcutti</i>	40	2	J	Pond
<i>G. orcutti</i>	50	2.05	M	Pond
<i>G. orcutti</i>	50	2.1	F	Pond
<i>G. orcutti</i>	50	2.18	F	Pond
<i>G. orcutti</i>	52	2.18	F	Pond
<i>G. orcutti</i>	50	2.2	M	Pond
<i>G. orcutti</i>	49	2.2	F	Pond
<i>G. orcutti</i>	50	2.2	F	Pond
<i>G. orcutti</i>	49	2.21	F	Pond
<i>G. orcutti</i>	48	2.3	M	Pond
<i>G. orcutti</i>	50	2.3	F	Pond
<i>G. orcutti</i>	55	2.3	F	Pond
<i>G. orcutti</i>	50	2.3	M	Pond
<i>G. orcutti</i>	51	2.3	F	Pond
<i>G. orcutti</i>	51	2.3	F	Pond
<i>G. orcutti</i>	48	2.3	F	Pond
<i>G. orcutti</i>	54	2.3	M	Pond
<i>G. orcutti</i>	54	2.33	M	Pond
<i>G. orcutti</i>	52	2.33	M	Pond
<i>G. orcutti</i>	52	2.34	M	Pond
<i>G. orcutti</i>	49	2.36	F	Pond

<i>G. orcutti</i>	49	2.4	F	Pond
<i>G. orcutti</i>	51	2.4	F	Pond
<i>G. orcutti</i>	48	2.4	F	Pond
<i>G. orcutti</i>	50	2.4	F	Pond
<i>G. orcutti</i>	53	2.4	M	Pond
<i>G. orcutti</i>	53	2.4	F	Pond
<i>G. orcutti</i>	53	2.4	M	Pond
<i>G. orcutti</i>	52	2.48	F	Pond
<i>G. orcutti</i>	51	2.5	F	Pond
<i>G. orcutti</i>	54	2.53	F	Pond
<i>G. orcutti</i>	53	2.55	M	Pond
<i>G. orcutti</i>	54	2.55	F	Pond
<i>G. orcutti</i>	55	2.58	F	Pond
<i>G. orcutti</i>	55	2.58	F	Pond
<i>G. orcutti</i>	53	2.58	M	Pond
<i>G. orcutti</i>	52	2.6	F	Pond
<i>G. orcutti</i>	54	2.6	F	Pond
<i>G. orcutti</i>	52	2.6	F	Pond
<i>G. orcutti</i>	53	2.6	F	Pond
<i>G. orcutti</i>	49	2.6	F	Pond
<i>G. orcutti</i>	52	2.6	F	Pond
<i>G. orcutti</i>	51	2.6	F	Pond
<i>G. orcutti</i>	54	2.6	M	Pond
<i>G. orcutti</i>	54	2.65	F	Pond
<i>G. orcutti</i>	54	2.7	F	Pond
<i>G. orcutti</i>	51	2.7	F	Pond
<i>G. orcutti</i>	49	2.8	F	Pond
<i>G. orcutti</i>	58	2.8	F	Pond
<i>G. orcutti</i>	50	2.8	F	Pond
<i>G. orcutti</i>	52	2.9	M	Pond
<i>G. orcutti</i>	55	2.9	F	Pond
<i>G. orcutti</i>	50	2.9	F	Pond
<i>G. orcutti</i>	53	2.9	M	Pond
<i>G. orcutti</i>	51	2.9	M	Pond
<i>G. orcutti</i>	57	2.91	F	Pond
<i>G. orcutti</i>	56	2.91	F	Pond
<i>G. orcutti</i>	54	2.92	M	Pond
<i>G. orcutti</i>	51	2.93	M	Pond
<i>G. orcutti</i>	51	3	F	Pond
<i>G. orcutti</i>	55	3	F	Pond
<i>G. orcutti</i>	56	3	F	Pond
<i>G. orcutti</i>	56	3.01	F	Pond
<i>G. orcutti</i>	61	3.02	F	Pond

<i>G. orcutti</i>	57	3.02	F	Pond
<i>G. orcutti</i>	51	3.1	F	Pond
<i>G. orcutti</i>	50	3.1	F	Pond
<i>G. orcutti</i>	52	3.1	M	Pond
<i>G. orcutti</i>	61	3.1	M	Pond
<i>G. orcutti</i>	55	3.1	M	Pond
<i>G. orcutti</i>	52	3.1	F	Pond
<i>G. orcutti</i>	54	3.1	F	Pond
<i>G. orcutti</i>	54	3.1	F	Pond
<i>G. orcutti</i>	55	3.1	F	Pond
<i>G. orcutti</i>	52	3.2	F	Pond
<i>G. orcutti</i>	56	3.2	F	Pond
<i>G. orcutti</i>	56	3.2	F	Pond
<i>G. orcutti</i>	54	3.2	F	Pond
<i>G. orcutti</i>	55	3.2	F	Pond
<i>G. orcutti</i>	58	3.23	F	Pond
<i>G. orcutti</i>	55	3.3	F	Pond
<i>G. orcutti</i>	59	3.33	F	Pond
<i>G. orcutti</i>	60	3.34	F	Pond
<i>G. orcutti</i>	55	3.35	F	Pond
<i>G. orcutti</i>	58	3.37	M	Pond
<i>G. orcutti</i>	56	3.39	F	Pond
<i>G. orcutti</i>	60	3.4	M	Pond
<i>G. orcutti</i>	58	3.4	F	Pond
<i>G. orcutti</i>	62	3.42	M	Pond
<i>G. orcutti</i>	55	3.46	F	Pond
<i>G. orcutti</i>	55	3.5	F	Pond
<i>G. orcutti</i>	53	3.5	F	Pond
<i>G. orcutti</i>	56	3.5	F	Pond
<i>G. orcutti</i>	58	3.5	F	Pond
<i>G. orcutti</i>	58	3.5	M	Pond
<i>G. orcutti</i>	58	3.5	M	Pond
<i>G. orcutti</i>	56	3.5	M	Pond
<i>G. orcutti</i>	61	3.5	M	Pond
<i>G. orcutti</i>	62	3.55	M	Pond
<i>G. orcutti</i>	58	3.6	F	Pond
<i>G. orcutti</i>	59	3.6	F	Pond
<i>G. orcutti</i>	58	3.6	F	Pond
<i>G. orcutti</i>	57	3.65	F	Pond
<i>G. orcutti</i>	57	3.7	F	Pond
<i>G. orcutti</i>	53	3.7	F	Pond
<i>G. orcutti</i>	62	3.72	M	Pond
<i>G. orcutti</i>	62	3.8	M	Pond

<i>G. orcutti</i>	60	3.8	F	Pond
<i>G. orcutti</i>	56	3.8	M	Pond
<i>G. orcutti</i>	61	3.85	F	Pond
<i>G. orcutti</i>	59	3.9	M	Pond
<i>G. orcutti</i>	58	3.9	F	Pond
<i>G. orcutti</i>	65	3.97	M	Pond
<i>G. orcutti</i>	63	4	M	Pond
<i>G. orcutti</i>	60	4	F	Pond
<i>G. orcutti</i>	63	4.05	F	Pond
<i>G. orcutti</i>	60	4.1	F	Pond
<i>G. orcutti</i>	60	4.1	M	Pond
<i>G. orcutti</i>	58	4.1	F	Pond
<i>G. orcutti</i>	61	4.15	F	Pond
<i>G. orcutti</i>	62	4.17	M	Pond
<i>G. orcutti</i>	60	4.2	F	Pond
<i>G. orcutti</i>	59	4.2	M	Pond
<i>G. orcutti</i>	61	4.2	F	Pond
<i>G. orcutti</i>	63	4.3	M	Pond
<i>G. orcutti</i>	63	4.3	M	Pond
<i>G. orcutti</i>	62	4.33	M	Pond
<i>G. orcutti</i>	64	4.36	F	Pond
<i>G. orcutti</i>	61	4.4	F	Pond
<i>G. orcutti</i>	64	4.4	F	Pond
<i>G. orcutti</i>	60	4.5	M	Pond
<i>G. orcutti</i>	62	4.5	M	Pond
<i>G. orcutti</i>	60	4.6	M	Pond
<i>G. orcutti</i>	61	4.6	F	Pond
<i>G. orcutti</i>	65	4.61	M	Pond
<i>G. orcutti</i>	64	4.66	M	Pond
<i>G. orcutti</i>	63	4.9	F	Pond
<i>G. orcutti</i>	65	4.9	M	Pond
<i>G. orcutti</i>	60	5	F	Pond
<i>G. orcutti</i>	63	5	F	Pond
<i>G. orcutti</i>	63	5	F	Pond
<i>G. orcutti</i>	71	5.05	F	Pond
<i>G. orcutti</i>	66	5.1	F	Pond
<i>G. orcutti</i>	65	5.1	F	Pond
<i>G. orcutti</i>	69	5.1	F	Pond
<i>G. orcutti</i>	62	5.1	F	Pond
<i>G. orcutti</i>	61	5.2	M	Pond
<i>G. orcutti</i>	64	5.2	M	Pond
<i>G. orcutti</i>	67	5.23	F	Pond
<i>G. orcutti</i>	63	5.3	F	Pond

<i>G. orcutti</i>	61	5.4	F	Pond
<i>G. orcutti</i>	63	5.5	F	Pond
<i>G. orcutti</i>	64	5.5	F	Pond
<i>G. orcutti</i>	68	5.69	F	Pond
<i>G. orcutti</i>	65	5.7	F	Pond
<i>G. orcutti</i>	61	5.7	F	Pond
<i>G. orcutti</i>	68	5.78	F	Pond
<i>G. orcutti</i>	68	5.8	M	Pond
<i>G. orcutti</i>	70	5.8	M	Pond
<i>G. orcutti</i>	71	5.9	M	Pond
<i>G. orcutti</i>	66	6	M	Pond
<i>G. orcutti</i>	71	6.4	F	Pond
<i>G. orcutti</i>	68	6.42	F	Pond
<i>G. orcutti</i>	69	6.43	F	Pond
<i>G. orcutti</i>	71	6.44	M	Pond
<i>G. orcutti</i>	71	6.5	F	Pond
<i>G. orcutti</i>	67	6.5	F	Pond
<i>G. orcutti</i>	68	6.6	M	Pond
<i>G. orcutti</i>	72	6.65	M	Pond
<i>G. orcutti</i>	68	6.7	F	Pond
<i>G. orcutti</i>	69	6.7	F	Pond
<i>G. orcutti</i>	70	6.8	M	Pond
<i>G. orcutti</i>	70	6.9	M	Pond
<i>G. orcutti</i>	70	6.9	F	Pond
<i>G. orcutti</i>	70	7	M	Pond
<i>G. orcutti</i>	72	7.17	F	Pond
<i>G. orcutti</i>	71	7.3	M	Pond
<i>G. orcutti</i>	73	7.44	M	Pond
<i>G. orcutti</i>	77	7.6	M	Pond
<i>G. orcutti</i>	72	7.6	F	Pond
<i>G. orcutti</i>	72	7.8	M	Pond
<i>G. orcutti</i>	72	7.8	F	Pond
<i>G. orcutti</i>	73	7.9	F	Pond
<i>G. orcutti</i>	75	8.2	M	Pond
<i>G. orcutti</i>	73	8.5	F	Pond
<i>G. orcutti</i>	73	8.5	M	Pond
<i>G. orcutti</i>	78	8.7	M	Pond
<i>G. orcutti</i>	77	8.8	F	Pond
<i>G. orcutti</i>	73	9.2	M	Pond
<i>G. orcutti</i>	78	9.8	F	Pond
<i>G. orcutti</i>	78	10.5	F	Pond
<i>G. orcutti</i>	80	11.4	F	Pond
<i>G. orcutti</i>	94	12.45	M	Pond

<i>G. orcutti</i>	52	no weight	F	Pond
<i>G. orcutti</i>	68	no weight	F	Pond
<i>Total</i>	209 Fish			

A2 – Data on Arroyo chub stocked into the enclosures/exclosures.

Species	Length	Weight	Cage
Arroyo chub	50.0mm	2.3g	1
Arroyo chub	44.0mm	1.9g	1
Arroyo chub	62.0mm	5.3g	1
Arroyo chub	44.0mm	2.8g	1
Arroyo chub	65.0mm	5.3g	1
Arroyo chub	53.0mm	3.2g	1
Arroyo chub	68.0mm	6.3g	1
Arroyo chub	62.0mm	4.6g	1
Arroyo chub	73.0mm	7.6g	2
Arroyo chub	58.0mm	4.1g	2
Arroyo chub	61.0mm	4.7g	4
Arroyo chub	63.0mm	5.0g	4
Arroyo chub	71.0mm	6.8g	5
Arroyo chub	60.0mm	4.8g	5
Arroyo chub	63.0mm	5.5g	5
Arroyo chub	62.0mm	5.3g	5
Arroyo chub	48.0mm	2.1g	5
Arroyo chub	63.0mm	5.2g	5
Arroyo chub	60.0mm	4.7g	5
Arroyo chub	64.0mm	5.2g	5
Arroyo chub	58.0mm	4.4g	7
Arroyo chub	60.0mm	3.9g	7
Arroyo chub	60.0mm	3.8g	8
Arroyo chub	67.0mm	3.7g	8
Arroyo chub	61.0mm	4.9g	9
Arroyo chub	54.0mm	3.3g	9
Arroyo chub	56.0mm	3.3g	9
Arroyo chub	62.0mm	5.2g	9
Arroyo chub	66.0mm	5.5g	9
Arroyo chub	50.0mm	3.6g	9
Arroyo chub	55.0mm	4.2g	9
Arroyo chub	62.0mm	4.3g	9
Arroyo chub	56.0mm	3.5g	12
Arroyo chub	56.0mm	3.2g	12
Arroyo chub	51.0mm	2.2g	12
Arroyo chub	67.0mm	5.9g	12
Arroyo chub	61.0mm	5.0g	12
Arroyo chub	48.0mm	2.6g	12
Arroyo chub	51.0mm	2.3g	12
Arroyo chub	58.0mm	3.5g	12
Average	57.4mm	4.17g	

A3 – Standard lengths and wet weights of the fish collected from a 0.9-ha wetland at the Prado Wetlands, Norco, CA at 16 months after inundation.

Species	# of Individuals	Length (mm)	Weight (g)
Carp	1	250	48.8
Carp	1	105	35.6
Carp	1	260	2 lbs
Carp	1	400	5 lbs
Carp	1	390	6 lbs
Carp	1	390	5 lbs
Carp	1	430	5.5 lbs
Carp	1	420	6.5 lbs
Carp	1	370	4.5 lbs
Carp	1	480	7 lbs
Carp	1	400	5 lbs
Carp	1	400	5 lbs
Carp	1	360	4 lbs
Carp	1	113	47.2
Carp	1	440	6 lbs
Carp	1	390	5.5 lbs
Carp	1	400	6.5 lbs
Carp	1	370	5 lbs
Carp	1	400	5 lbs
Carp	1	340	4 lbs
Carp	1	390	5 lbs
Carp	1	450	7 lbs
Carp	1	340	4 lbs
Catfish	1	64	5.5
Catfish	1	53	2.6
Catfish	1	65	5
Catfish	1	52	3.5
Catfish	1	56	4.3
Catfish	1	70	9.2
Catfish	1	52	3.4
Catfish	1	48	2.3
Catfish	1	61	7.6
Catfish	1	50	3.4
Catfish	1	52	3.1
Catfish	1	62	6.3
Catfish	1	40	0.7
Catfish	1	51	2.6
Catfish	1	44	3.1

Catfish	1	50	3.5
Catfish	1	61	6
Catfish	1	50	3.2
Catfish	1	57	3.9
Catfish	1	56	4.7
Catfish	1	50	3.9
Catfish	1	68	8.2
Catfish	1	60	6
Catfish	1	52	3.6
Catfish	1	55	3.9
Catfish	1	59	1.4
Catfish	1	68	7
Catfish	1	74	8.2
Catfish	1	65	5.8
Catfish	1	40	1.9
Catfish	1	56	3.8
Catfish	1	37	1.4
Catfish	1	42	1.7
Catfish	1	61	4.9
Catfish	1	61	4.2
Catfish	1	44	2.7
Catfish	1	47	3.4
Catfish	1	50	3.2
Catfish	1	34	1.2
Catfish	1	43	3.8
Gambusia	445	*	*
Gambusia	1	30	0.4
Gambusia	1	26	0.4
Gambusia	1	31	0.4
Gambusia	1	30	0.5
Gambusia	1	35	0.8
Gambusia	1	25	0.8
Gambusia	1	29	0.3
Gambusia	1	34	0.7
Gambusia	1	29	0.3
Gambusia	1	18	*
Gambusia	1	21	*
Gambusia	1	22	*
Gambusia	1	31	0.6
Gambusia	1	35	0.3

Gambusia	1	23	0.3
Gambusia	1	22	0.2
Gambusia	1	27	0.3
Gambusia	1	18	*
Gambusia	1	16	*
Gambusia	1	18	*
Gambusia	1	16	*
Gambusia	1	18	*
Gambusia	1	24	0.2
Gambusia	1	24	0.2
Gambusia	1	20	0.1
Gambusia	1	25	0.3
Gambusia	1	20	*
Gambusia	1	16	*
Gambusia	1	21	*
Gambusia	1	19	*
Gambusia	1	16	*
Gambusia	1	28	0.4
Gambusia	1	19	*
Gambusia	1	7	*
Gambusia	1	27	0.2
Gambusia	1	23	*
Gambusia	1	25	0.3
Gambusia	1	20	*
Gambusia	1	27	0.3
Gambusia	5	*	*
Gambusia	1	*	2.9
Gambusia	22	*	*
Gambusia	20	*	*
Gambusia	134	*	*
Gambusia	760	*	*
Gambusia	397	*	*
Gambusia	42	*	*
Gambusia	1319	*	*
Lepomis	1	38	1.7
Lepomis	1	87	22.9
Lepomis	1	74	11.7
Lepomis	1	21	0.4
Lepomis	1	70	10.2
Lepomis	1	105	51.9

Lepomis	1	38	2
Lepomis	1	100	30
Lepomis	1	85	19.8
Lepomis	1	44	2.8
Lepomis	1	88	25
Lepomis	1	90	24.4
Lepomis	1	90	20.8
Lepomis	1	90	26.1
Lepomis	1	40	1.7
Lepomis	1	47	3
Lepomis	1	93	26.1
Lepomis	1	42	2.1
Lepomis	1	92	27
Lepomis	1	42	2.4
Lepomis	1	39	1.6
Lepomis	1	43	2.5
Lepomis	1	47	3
Lepomis	1	42	2.5
Lepomis	1	48	3.5
Lepomis	1	38	1.9
Lepomis	1	40	2.2
Lepomis	1	39	1.5
Lepomis	1	39	2
Lepomis	1	38	1.2
Lepomis	1	35	0.7
Lepomis	1	43	2.1
Lepomis	1	41	2.4
Lepomis	1	38	1.8
Lepomis	1	37	1.6
Lepomis	1	38	2
Lepomis	1	45	3.3
Lepomis	1	39	2
Lepomis	1	43	2.6
Lepomis	1	41	2.1
Lepomis	1	39	2.3
Lepomis	1	37	1.2
Lepomis	1	39	1.9
Lepomis	1	40	2.2
Lepomis	1	37	1.4
Lepomis	1	51	6.6

Lepomis	1	47	3
Lepomis	1	37	2.1
Lepomis	1	35	1.4
Lepomis	1	88	19.2
Lepomis	1	38	2.1
Lepomis	1	131	72.3
Lepomis	1	160	127.3
Lepomis	1	139	94.1
Lepomis	1	97	31.3
Lepomis	1	70	13.4
Lepomis	1	92	18.5
Lepomis	1	47	3.7
Lepomis	1	48	4.2
Lepomis	1	45	3
Lepomis	1	50	4.5
Lepomis	1	98	30.5
Lepomis	1	42	2.6
Lepomis	1	40	2.2
Lepomis	1	37	1.5
Lepomis	1	66	8.7
Lepomis	1	48	3.1
Lepomis	1	37	1.8
Lepomis	1	38	1.8
Lepomis	1	92	23.1
Lepomis	40	*	*
Lepomis	1	134	80.4
Lepomis	1	95	30.7
Lepomis	1	87	18.7
Lepomis	1	43	2.9
Lepomis	1	128	65.6
Lepomis	1	134	71.6
Lepomis	1	102	32.3
Lepomis	1	107	37.5
Lepomis	1	115	56.8
Lepomis	1	96	27.9
Lepomis	1	73	13.9
Lepomis	1	79	15.2
Lepomis	1	124	65.1
Lepomis	1	67	11.9
Lepomis	1	70	10.1

Lepomis	1	67	10
Lepomis	1	114	47.5
Lepomis	1	118	50.1
Lepomis	1	92	24.3
Lepomis	1	70	11.4
Lepomis	1	52	4.6
Lepomis	1	67	9.6
Lepomis	1	71	8.5
Lepomis	1	47	2.6
Lepomis	1	57	5
Lepomis	1	45	2.3
Lepomis	1	43	2.5
Lepomis	1	74	14.6
Lepomis	1	85	20.1
Lepomis	1	82	20.1
Lepomis	1	92	27.2
Lepomis	1	97	32.9
Lepomis	1	84	17.4
Lepomis	1	34	0.9
Lepomis	1	78	14.7
Lepomis	1	94	26
Lepomis	1	45	3.1
Lepomis	1	38	1.9
Lepomis	1	47	3
Lepomis	1	43	2.5
Lepomis	1	40	1.9
Lepomis	1	127	60.2
Lepomis	1	139	93.3
Lepomis	1	90	25.3
Lepomis	1	73	14.4
Lepomis	1	41	3
Lepomis	1	34	1.2
Lepomis	1	29	1
Lepomis	1	32	1.1
Lepomis	1	35	1.8
Lepomis	1	34	1.7
Lepomis	1	35	1.7
Lepomis	1	102	33.9
Lepomis	1	98	30
Lepomis	1	40	6.7

Lepomis	1	65	9.4
Lepomis	1	90	23
Lepomis	1	132	77.9
Lepomis	1	133	80.4
Lepomis	1	102	35.8
Lepomis	1	95	30.1
Lepomis	1	89	19.9
Lepomis	1	87	21.3
Lepomis	1	106	35.8
Lepomis	1	46	2.8
Lepomis	1	97	27.1
Lepomis	1	68	11.4
Lepomis	1	39	2.1
Lepomis	1	120	62.1
Lepomis	1	132	70.2
Lepomis	1	137	81
Lepomis	2	*	1.8
Lepomis	14	*	28.8
Lepomis	14	*	40.9
Lepomis	2	*	6.7
Lepomis	58	*	*
Lepomis	161	*	*
Lg M Bass	1	250	1 lb
Lg M Bass	1	275	1.5 lbs
Lg M Bass	1	230	302.4
Lg M Bass	1	210	246.5
Lg M Bass	1	210	203
Lg M Bass	1	72	9
Lg M Bass	1	260	374
Lg M Bass	1	210	224
Silverside	1	61	1.8
Yellow catfish	1	171	90.9

A4 – Counts of all the other taxa collected at the end of the experiment.

Species	# of Individuals
Bullfrog tadpole	5
<i>Xenopus</i>	6
Crayfish	67
Total	78

Appendix B

Chapter 2

B1 - Paired t-test and Oviposition Activity Index (OAI) values for *Thermonectus* Trials 1-4.

Thermonectes Trials 1-4				
Control vs. Exudate-laden water	T-value	df	P-value	OAI
Trial 1	1.06	6	0.3289	0.1413
Trial 2	-1.68	2	0.2355	0.0717
Trial 3	-2.72	8	0.0263	0.1727
Trial 4	-0.59	4	0.5878	-0.0669

B2: G-test values for *Thermonectus* Trial 5

Tests		df	G
<i>Thermonectus</i> Trial 5			
Pooled		2	14.348
Heterogeneity		6	6.715
Total		8	21.063

B3: G-test values for *Thermonectus* Trial 6.

Tests		df	G
<i>Thermonectus</i> Trial 6			
Pooled		2	15.836
Heterogeneity		10	8.522
Total		12	21.03

B4: Paired t-test and Oviposition Activity Index (OAI) values for *Cybister* Trials 1 & 2.

Cybister Trials 1 & 2	t-value	df	P-value	OAI
Trial 1	1.41	3	0.0649	0.2408
Trial 2	1.65	7	0.1431	0.200

B5: G-test values for *Cybister* Trial 3.

G-Tests <i>Cybister</i> Trial 3		df	G
Pooled		2	41.444
Heterogeneity		8	14.358
Total		10	55.802

B6: G-test values for *Cybister* Trial 4.

G-Tests <i>Cybister</i> Trial 4		df	G
Pooled		2	39.758
Heterogeneity		16	59.469
Total		18	99.493

B7: Paired t-test values for Libellulid, *Cybister* and *Thermonectus* trials — Control vs. Exudate-laden water.

	Libellulid	df	Cybister	df	Thermonectus	df
Trial 1	p-value = 0.6408	3	0.0649	3	0.3289	6
Trial 2	0.2457	7	0.1431	7	0.2355	2
Trial 3					0.0263	8
Trial 4					0.5878	4