ECOLOGICAL COMPLEXITY OF NON-NATIVE SPECIES IMPACTS IN DESERT AQUATIC SYSTEMS

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Henkanaththegedara, Sujan Maduranga, Ph.D., Environmental & Conservation Sciences Graduate Program, North Dakota State University, March 2012. Ecological complexity of non-native species impacts in desert aquatic systems. Major Professor: Dr. Craig A. Stockwell.

Without an adequate understanding of complex interactions between native and non-native species, management of invasive species can result in unforeseen detrimental impacts. I used both field mesocosm experiments and laboratory predation trials to study reciprocal species interactions between the endangered Mohave tui chub (*Siphateles bicolor mohavensis*) and invasive western mosquitofish (*Gambusia affinis*). I also examined the impacts of both fish species on the aquatic invertebrate communities in desert springs.

I show a case of intraguild predation (IGP) as a mechanism facilitating co-persistence of endangered tui chub with invasive mosquitofish using field mesocosm experiments. This case of IGP appears to be size structured with adult Mohave tui chub preying on adult (and juvenile) mosquitofish, and adult mosquitofish preying on tui chub eggs and/or larvae. These results collectively suggest size structured IGP between these two fish species as the mechanism for co-persistence. In light of these findings, managers may consider habitats currently harboring mosquitofish as possible refuge sites for Mohave tui chub, an option previously un-available.

I conducted laboratory predation trials to assess the role of predator gape-limitation in the context of IGP between these two fish species. I explored sex specific differences in gape-size limitation in mosquitofish, because mosquitofish are highly dimorphic. Larval tui chubs had lower survival in the presence of female mosquitofish than in the presence of males. Reciprocally, adult tui chubs preyed upon adult mosquitofish causing a lower survival for male mosquitofish compared to female survival. These results combined with vulnerability modeling show that IGP in this system is size structured based on gape-size limitation.

In addition to complex reciprocal interactions, recently established fish populations may impact unique invertebrate communities. Mesocosm experiments with sympatric and allopatric populations of tui chub and mosquitofish showed changes of invertebrate community structures mainly due to population declines and local extirpations of invertebrates, presumably due to fish predation. These results may suggest important conservation implications of invasive fish as well as protected fish transplants into fishless desert springs.

Overall my research emphasizes the importance of considering complex ecological interactions between native and non-native species in management of invasive and protected fish species as well as unique invertebrate communities in fishless springs in desert ecosystems.

ACKNOWLEDGEMENTS

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DEDICATION

THIS DISSERTATION IS DEDICATED TO

MY PARENTS, WALTER AND THILAKA HENKANATHTHEGEDARA

MY IN-LAWS, KIRI BANDA AND CHANDRA HERATH

MY WIFE, BODINI HERATH

AND

OUR CHILDREN, MIHIN AND SANUTHI HENKANATHTHEGEDARA

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CHAPTER 1. GENERAL INTRODUCTION

Introduced species, together with habitat loss, play a major role in population declines and biodiversity loss (Diamond 1989; Wilcove et al. 1998; Mack et al. 2000). For example, the establishment of non-native fish species have been associated with the population declines of a variety of native aquatic organisms (Deacon et al. 1964; Courtenay and Meffe 1989; Kats and Ferrer 2003; Stockwell and Henkanaththegedara 2011). Hundreds of *exotic* fish species have been introduced to the United States from various countries since the early European colonization (Courtenay and Stauffer 1984; Moyle 1986; Gido and Brown 1999; Figure 1.1). A majority of these fish introductions were deliberate introductions for reasons such as sport fishing, farming for food, aquarium trade, and bio-control programs (Courtenay and Stauffer 1984; Fuller et al. 1999). In addition, non-native species were unintentionally introduced via the release of ship ballast water (Ricciardi and Maclsaac 2000) and accidental escapes from aquaculture facilities (Courtenay and Stauffer 1984). Furthermore, many native fish species have been introduced beyond their native historic range (Benson 2000) and these *non-native* species are also posing threats to native aquatic species (Figure 1.1).

Although native to the United States, mosquitofish (*Gambusia affinis* and *G. holbrooki*) have been widely introduced to other parts of the United States and and many other countries to control mosquito-borne diseases (Krumholz 1948; Pyke 2008). Despite its presumed mosquito control ability, detrimental impacts of mosquitofish on other aquatic organisms are well documented (Courtenay and Meffe 1989; Pyke 2008; Stockwell and Henkanaththegedara 2011). Unfortunately, both species have become invasive, and western mosquitofish (*G. affinis*) was recently listed among the “worst 100 invasive species” posing significant threats to native aquatic organisms (Lowe et al. 2000) typically via competition and/or predation (Hurlbert et al. 1972; Courtenay and Meffe 1989). For an example, mosquitofish preyed on zooplankton and aquatic macro-invertebrates reducing their densities (Hurlbert et al. 1972; Angeler et al. 2002). Mosquitofish also caused reduced growth and survival of fish species (Meffe 1985; Mills et al. 2004; Rogowski and Stockwell 2006) and amphibians (Kats and Ferrer 2003).

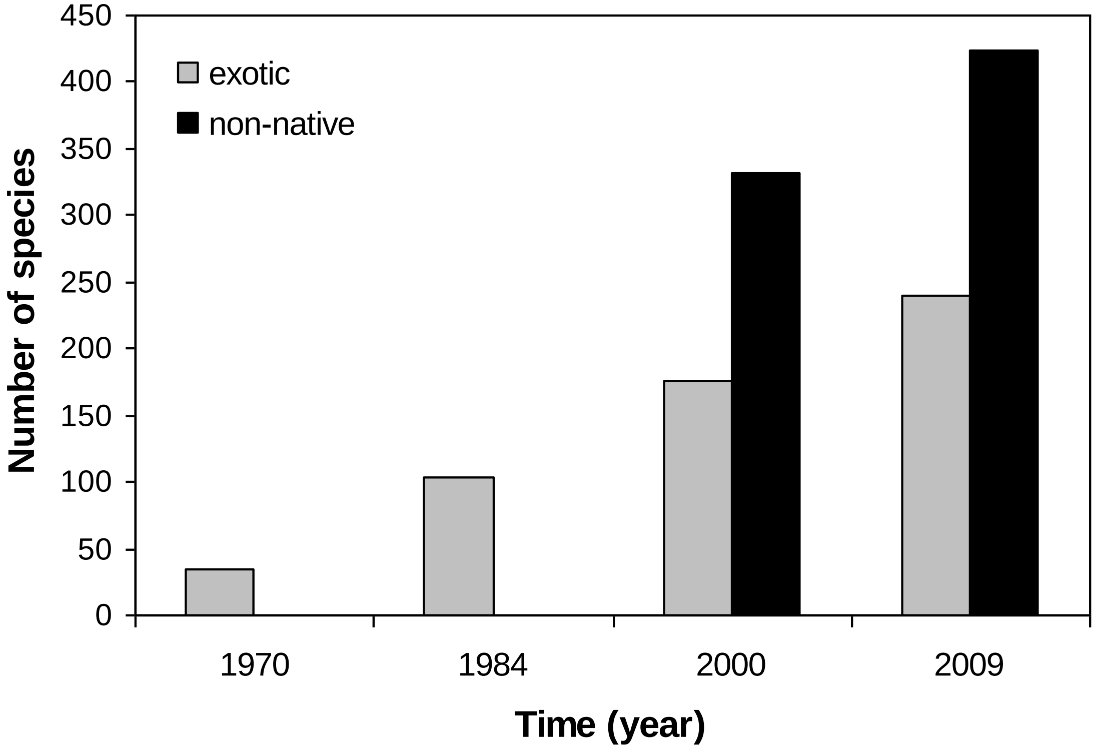

Figure 1.1. Fish species introductions to the United States (Sources: Lachner et al. 1970; Courtenay et al. 1984; Benson 2000 and USGS-NAS 2009). Grey bars represent the exotic fish species which were introduced from the areas *beyond* geographical boundaries of the United States and the black bars represent the non-native species introduced *within* the geographic boundaries of the United States.

Western mosquitofish was first noted in Lake Tuendae, one of the five populations of endangered Mohave tui chub (*Siphateles bicolor mohavensis*), in 2001 as a result of an unknown, illegal introduction (Steve Parmenter Personal Communication). These observations coincided with Lake Tuendae shifting to a turbid state in the fall of 2002 (Hughson and Woo 2004; Rob Fulton Personal Communication). These observations led managers to hold a workshop to revisit the Mohave tui chub recovery plan (U.S. Fish and Wildlife Service 1984) and to develop management recommendations for future management action plans (Hughson and Woo 2004). Two such important management recommendations resulted from this workshop are;

1. Identification and reduction of threats to extant and future populations
2. Research to understand Mohave tui chub ecology and habitat requirements

In order to assist with above recommendations I evaluated the potential impacts of western mosquitofish on both Mohave tui chub and associated invertebrate communities using field mesocosm experiments and laboratory predation trials. In addition, I studied the life history and population dynamics of four Mohave tui chub populations.

Ecological complexity of non-native species impacts

The negative impacts of non-native species on native species and ecosystems have been a major focus of the contemporary ecology, since the publication of “The Ecology of Invasions by Animals and Plants” by Charles Elton (Elton 1958). Accelerated human mediated dispersion of organisms beyond natural background levels has led to the homogenization of biota throughout the world (Elton 1958; Lodge 1993a; McKinney and Lockwood 2001). The emerging sub-discipline of invasion biology has focused on the negative impacts of invaders on native species or ecosystems and also evaluated the biotic characteristics that facilitate invasion of new habitats, as well as the characteristics that pre-dispose certain ecosystems more prone to invasions (Elton 1958; Baker and Stebbins 1965; Lodge 1993b; Sax et al. 2005; Davis 2009).

Invaded ecosystems with multiple established non-native organisms have become the norm rather than the exception (Bull and Courchamp 2009). In most ecosystems, invasive species often assume the ecological role of some native species, which in turn creates complex interactions among native and invading species (Zavaleta et al. 2001). Therefore, control or eradication of invasive species can result in unforeseen detrimental impacts (Zavaleta et al. 2001; Bull and Courchamp 2009). For example, removal of an invasive predator which co-occurs with invasive and native prey could result mesopredator release, resulting an increased impact on native species of conservation priority (Karl and Best 1982; Fitzgerald 1988). Similarly, eradication of invasive herbivores in order to restore native flora could promote the spread of an invasive plant (Kessler 2001). Therefore it is critical to understand the complex ecological interactions between native and invasive organisms before management actions are taken which may inadvertently harm native species (Courchamp et al. 2003; Bull and Courchamp 2009).

Managers are particular focused on containing the invasion of particular species that have been reputed to negatively impact native species. Such is the case with mosquitofish (*G. affinis* and *G. holbrooki*) which have negatively impacted native aquatic invertebrates, fish and amphibians mainly by predation and competition (Courtenay and Meffe 1989; Pyke 2008; Stockwell and Henkanaththegedara 2011). Although, mosquitofish predation on various developmental stages of native fish is well established (Meffe 1985; Belk and Lydeard 1994; Mills et al. 2004; Rogowski and Stockwell 2006), little work has been conducted to evaluate any potential impacts by native species on invasive mosquitofish, which could in theory lead to co-persistence of native species with non-natives. I used both field mesocosm experiments and laboratory predation trials to study reciprocal species interactions between endangered desert fish, Mohave tui chub and invasive western mosquitofish, and impacts of recently established fish populations on aquatic invertebrates (see Chapters 2, 3 and 4).

The initial research focused only on the impacts of western mosquitofish on Mohave tui chub, ignoring the possible effects of tui chub on mosquitofish (Stockwell and Henkanaththegedara 2011). This work led to an unexpected observation that tui chubs apparently prey on adult mosquitofish, leading to the work reported in chapter 2. Chapter 2 reports the results of a mesocosm experiment designed to evaluate the intraguild predation (IGP) between native Mohave tui chub and non-native western mosquitofish.

In order to further explore the size-structured IGP between tui chubs and mosquitofish, laboratory experiments were designed with predation trials using various developmental stages of Mohave tui chub and western mosquitofish. Because mosquitofish are sexually dimorphic, sex specific differences in gape-size limitation in mosquitofish were tested. The results of these experiments, together with vulnerability modeling allowed me to evaluate the role of predator gape-limitation in IGP, which is reported in chapter 3.

Additionally, I explored the impacts of recently established fish populations on aquatic invertebrates in desert springs, which is another aspect of the complex interactions between native and non-native species in desert ecosystems. Both Mohave tui chub and western mosquitofish are non-native to desert springs which harbor unique and endemic invertebrate communities. I explored the potential impacts of native fish transplants and introduction of invasive fish on aquatic invertebrate community structure in fishless desert water bodies, using filed mesocosm experiments, leading to the work reported in chapter 4.

Concurrent with this experimental work, my research also involved considerable descriptive work to better characterize life history and population dynamics of the four Mohave tui chub populations at Lake Tuendae, MC Spring, Camp Cady and China Lake. I have summarized these results in subsequent appendices together with additional descriptive work on habitat characteristics of Lake Tuendae and MC Spring.

Natural history of Mohave tui chub

Almost every isolated or partially isolated drainage system in California, Nevada and Oregon supports at least one distinctive form of tui chub (Moyle 2002). These populations are classified as sub-species of *Siphateles bicolor* species complex, as is the case with the Mohave tui chub (*S. b. mohavensis*; Figure 1.2). However, others have suggested that Mohave tui chub is sufficiently distinct to warrant specific status (Moyle 2002; Harris 1991). Although Snyder (1918) originally described Mohave tui chub from the Mojave River as a valid species, Miller (1973) lumped it with *Siphateles bicolor* species complex and downgraded its taxonomic status to a subspecies. However there are multiple lines of morphological and molecular evidence to support the uniqueness of Mohave tui chub as a valid species (Uyeno 1966; Harris 1994; May et al. 1997; Figure 1.2). Uyeno (1966) found two osteological characters that distinguish Mohave tui chub from *S. b. obesus*. In addition, May et al. (1997) reported a fixed differences between Mohave tui chub compared to other tui chubs (Lahontan and Owens basins) at one allozyme locus (Galactosaminidase) and an AFLP allele were reported (May et al. 1997). Furthermore, Harris (1994) found six nucleotide positions of mt-DNA (cytochrome b) were autapomorphies for Mohave tui chub and phylogenetic analysis revealed Mohave tui chub forms the sister species to all remaining tui chubs.

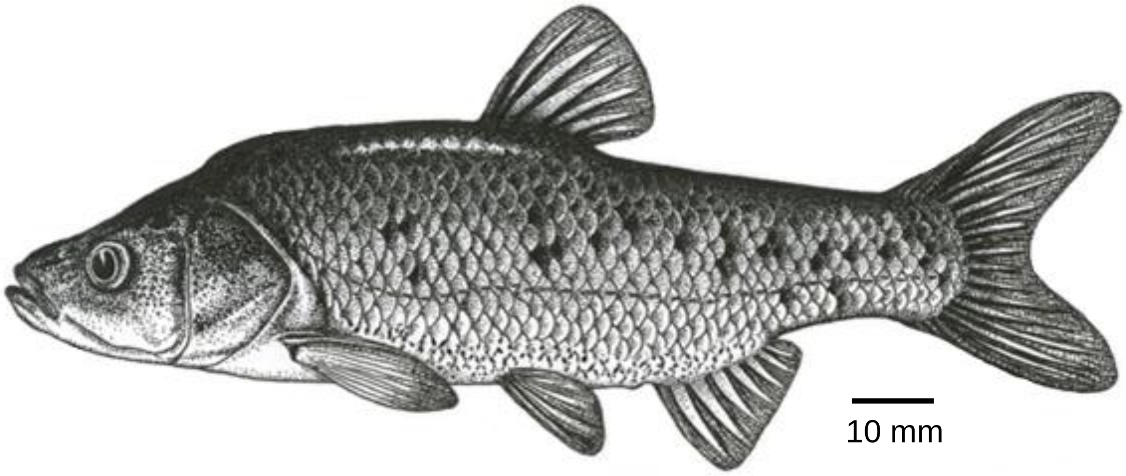

Figure 1.2. Mohave tui chub (*Siphateles bicolor mohavensis)*; an adult male from Lake Tuendae (Illustrated by Sujan Henkanaththegedara).

The Mohave tui chub is a California state and federally protected endangered minnow (Family Cyprinidae) which is endemic to the Mojave River drainage in southern California (U.S. Fish and Wildlife Service 1984). Once, Mohave tui chubs occurred in the deep pools and slow moving areas of the main-stream Mojave River (Snyder 1918). However, the river populations extirpated by late 1960s (Miller 1969), due to a combination of threats, including presumed hybridization with introduced arroyo chub (*Gila orcutti*), a severe flash flood in 1938 (Hubbs and Miller 1943), impacts of introduced brown bullhead (*Ameiurus nebulosus*), and habitat modification and degradation (Thompson 1929). A relictual population was discovered near Soda Dry Lake (Miller 1938), which approximates historic descriptions of the original spring-fed pool located at the site of Lake Tuendae (Thompson 1929; Miller 1938) before expanding it to create the lake in early 1940s (Turner and Liu 1976).

Mohave tui chub populations were subsequently established by extensive translocation efforts in the 1960s and 1970s (Miller 1968; St. Amant and Sasaki 1971). Fish were introduced from Lake Tuendae to a variety of sites, but only three populations persisted at the following habitats: (1) Bud's Pond at Camp Cady State Wildlife Area, (2) Seep system in China Lake Naval Air Weapons Station, Ridgecrest, and (3) Deppe Pond/Tui Slough system at Lewis Center for Academic Excellence, Victorville established in October, 2008 (Figure 1.3 and 1.4).

Life history of Mohave tui chub

The ecology and life history characteristics of Mohave tui chub are poorly documented. Vicker (1973) studied some aspects of life history of Mohave tui chub (*S. b. mohavensis*) focusing on age and growth, diet, and reproductive biology of Lake Tuendae population. In addition, Taylor and McGriff (1985) reported age and growth of tui chubs inhabited Lake Tuendae and Three-bats pond (extirpated) and Feldmeth et al. (1985) reported some preliminary studies on reproduction, diet, parasites and environmental tolerance of tui chubs from China Lake. However, none of these studies compared the life history variation within and among the various Mohave tui chub populations. Therefore, I conducted a comparative assessment of the four extant populations of Mohave tui chubs focusing on age structure, reproductive biology, population dynamics and diet (see Appendix A).

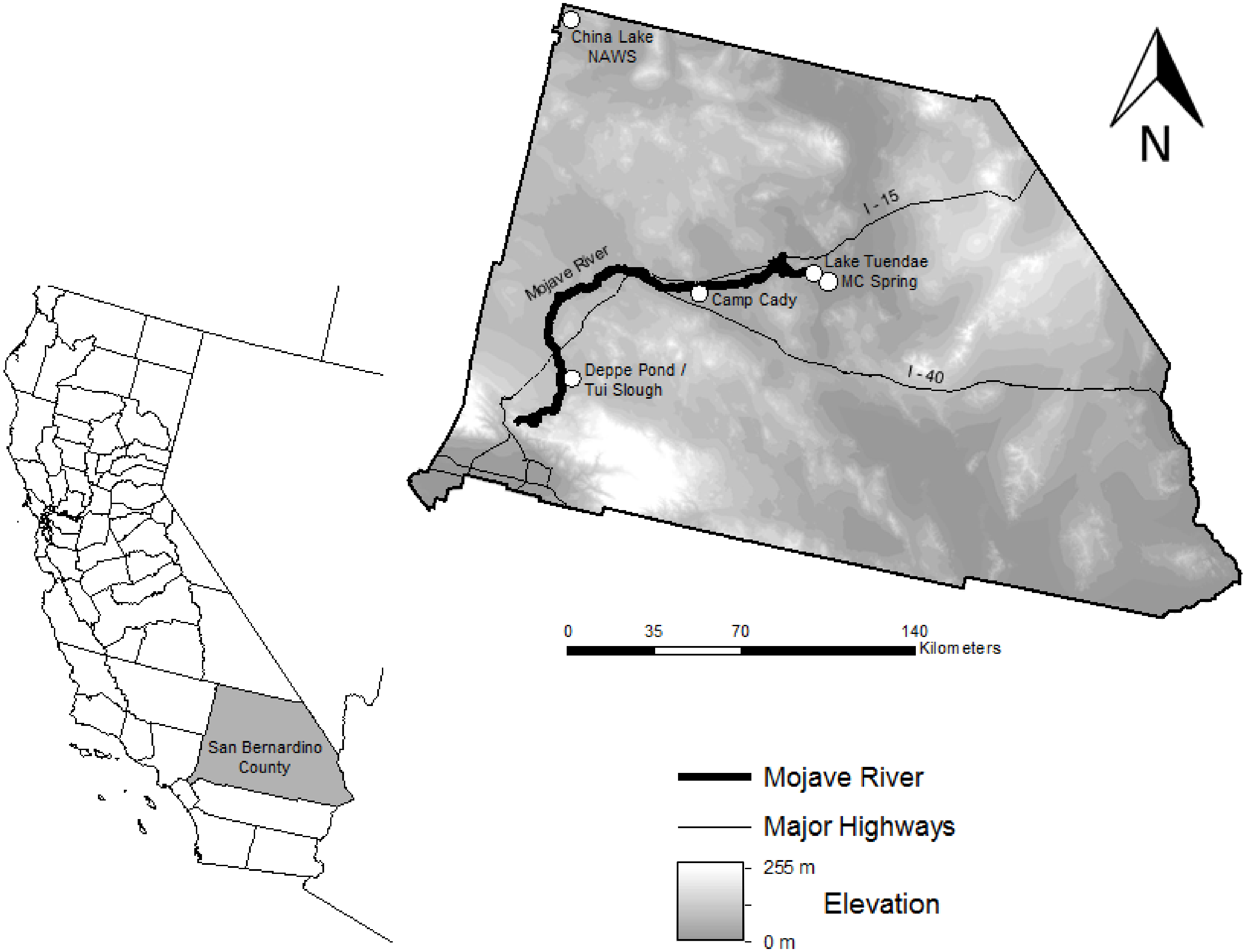

Figure 1.3. Current distribution of Mohave tui chub (*Siphateles bicolor mohavensis*) within San Bernardino County, California (Map by Justin Fisher).

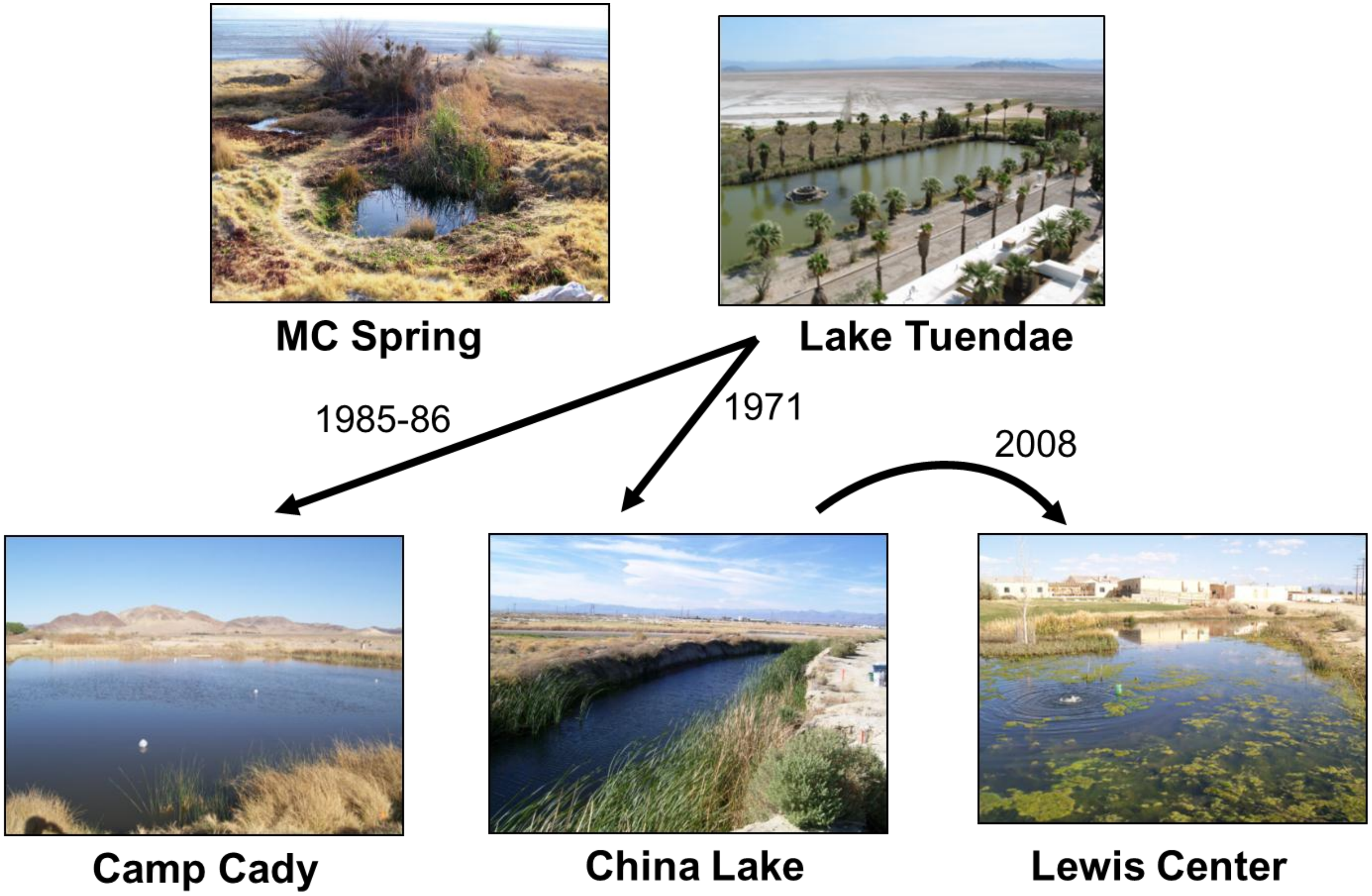

Figure 1.4. Extant populations of Mohave tui chub (*Siphateles bicolor mohavensis*) and its translocation history (Photos: Sujan Henkanaththegedara).

Population dynamics of Mohave tui chub

Although Mohave tui chub recovery plan (U.S. Fish and Wildlife Service 1984) warrants annual population census, there is no active, continuous, long-term population monitoring program. Sporadic work suggests that there is a considerable seasonal variation in Mohave tui chub population size (Vicker 1973; Taylor 1982; Garron 2006). A lack of methodological consistency among these studies prohibits direct comparison of population size and draws useful conclusions about population dynamic with confidence. Conjunction with California Department of Fish and Game, I have developed protocols to conduct annual population estimates of Mohave tui chub for Lake Tuandae and MC Spring. Population estimates were conducted at Lake Tuendae and Camp Cady using mark-recapture methods from 2007-2009 and at MC Spring using depletion method from 2006-2009. Additional sampling was conducted at North Channel segment of China Lake population in 2008 (see Appendix B).

Habitat ecology of Mohave tui chub

A sound understanding of habitat quality and requirements is an essential component of the management of endangered species (Noss et al. 1997). In fact, all existing Mohave tui chub occupy in highly altered habitats and one historic population (i.e. Three-bats pond) was extirpated presumably due to poor water quality (U.S. Fish and Wildlife service 1984; Hughson and Woo 2004). Therefore, the Mohave tui chub recovery plan (U.S. Fish and Wildlife service 1984) and management action plan (Hughson and Woo 2004) call for frequent water quality analysis in all Mohave tui chub habitats. Archbold (1994) developed a habitat evaluation scheme for Mohave tui chubs focusing on water quality parameters to evaluate existing habitat quality and to assess the habitat suitability for future refugia. However, this study did not include zooplankton community structure, which is very important in understanding trophic effects and nutrient cycling in the system (Carpenter et al. 1985, 1987). Therefore, I studied physico-chemical characteristics of water of Mohave tui chub habitats focusing on Lake Tuendae and MC Spring (see Appendix C).

Organization of Dissertation

My dissertation is intended provide experimental evidence to ecological complexity of non-native species impacts on native species using western mosquitofish and Mohave tui chub as model organisms. It also provides key information components and guidelines required for the recovery and down-listing of endangered Mohave tui chub. Information and guidelines provided are focusing on two major aspects of Mohave tui chub recovery plan and management action plan; 1) assessment of potential impacts of western mosquitofish on Mohave tui chub, and 2) Mohave tui chub life history descriptions and ecology (U.S. Fish and Wildlife Service 1984; Hughson and Woo 2004).

The dissertation is composed of five chapters including this general introduction and last chapter on general conclusions. The second chapter was formatted for submission to the journal *Conservation Biology* and focuses on reciprocal predation between invasive western mosquitofish (*G. affinis*) and endangered Mohave tui chub (*S. b. mohavensis*) based on field mesocosm experiments. Chapter three which was written for submission to the journal *Oecologia* examines the role of predator gape-limitation in intraguild predation between these two fish species. Chapter four which was written for submission to the journal *Diversity and Distributions* focuses on the impacts of recently established fish populations on endemic spring invertebrates. I concluded my dissertation with chapter five discussing the implications of my research findings in complex ecological interactions between non-native and native organisms, in broader sense, and the conservation management of endangered Mohave tui chub, in narrow sense. In addition I included four appendices on life history, population monitoring and habitat monitoring of endangered Mohave tui chub. This novel information may fill the information gaps in the particular areas and may help planning conservation actions towards the down-listing and delisting of this endangered fish species.

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CHAPTER 2. BEYOND THE DOGMA OF NON-NATIVE SPECIES: RECIPROCAL PREDATION MEDIATES CO-EXISTENCE OF NATIVE AND NON-NATIVE FISH

Abstract

Dogmatic views of non-native species as universally “bad” can constrain research objectives and options available to conservation practitioners. However, challenging such dogma can be important for understanding the circumstances under which native species may co-persist with non-native species which in turn can provide important insights on how best to manage altered systems. Here, I report a case of intraguild predation (IGP) as a mechanism facilitating co-persistence of an endangered species (Mohave tui chub, *Siphateles bicolor mohavensis*) with a non-native invasive species (western mosquitofish, *Gambusia affinis*). I established experimental sympatric and allopatric populations of Mohave tui chub and western mosquitofish to evaluate reciprocal trophic interactions between these two fish species. Mosquitofish had a significant negative effect on Mohave tui chub recruitment ($W = 142$; $P < 0.01$). Reciprocally, tui chubs had a significant negative effect on survival of adult mosquitofish ($W=155.0$; $P<0.001$) and juvenile production ($W = 48.5$; $P < 0.001$). Both male mosquitofish and small female mosquitofish had reduced survival in sympatry with tui chubs. Additionally, sympatric female mosquitofish survivors were significantly larger than female mosquitofish survivors in the allopatric populations, suggesting that large females were less vulnerable to predation by Mohave tui chub. These results collectively show size-structured IGP between these two fish species, probably due to gape-size limitation. Thus, IGP is an apparent mechanism facilitating co-persistence of these species. These findings provide more management options allowing managers to allocate resources to other threats and also will allow

managers to consider habitats currently harboring mosquitofish as possible refuge sites for Mohave tui chub, an option previously un-available. My findings also provide a cautionary tale on how negative perceptions of non-native species can constrain research and management options.

Introduction

Conservation biology is inherently a normative science whereby human values can and should affect scientific process (Soule 1991; Lindenmayer and Hunter 2010). However, perceptions may constrain the questions conservation biologists ask as well as approaches taken to answer those questions. For example, dogmatic views of non-native species as universally "bad" may constrain research objectives, interpretation of scientific experiments, and most importantly constrain options available to conservation practitioners. Unfortunately, such constraints may be very costly given the clear reality that most ecosystems already harbor or will eventually harbor non-native species (Zavaleta et al. 2001; Sax et al. 2005; Bull and Courchamp 2009).

A recent debate has challenged the conventional dogmatic view of non-native species as universally "bad" (Davis et al. 2011; Lerdau and Wickham 2011; Lockwood et al. 2011; Schlaepfer et al. 2011; Simberloff 2011) and has created an opportunity to re-evaluate how conservation biologists study and manage non-native species. One area of fruitful exploration is for scientists to study the interactions among native and non-native species (Courchamp and Caut 2006; Roemer et al. 2002). Because ecological interactions among native and non-native species are often complex (Karl and Best 1982; Fitzgerald 1988; Bull and Courchamp 2009; Ehrenfeld 2010), control or eradication of invasive species can have unexpected impacts on conservation target species (Courchamp and Caut 2006; Bull and Courchamp 2009). For example, removal of feral cats (*Felis catus*) released non-native rats (*Rattus* spp.) from cat predation, and thus increased rat predation on native bird species (Karl and Best 1982; Fitzgerald 1988). Similarly, Roemer et al. (2002) showed that removal of non-native pigs could indirectly increase predation pressure on the endangered Channel Island fox (*Urocyon littoralis*) by golden eagles (*Aquila chrysaetos*).

A mechanism that may facilitate co-persistence of native and non-native species is intraguild predation (IGP), whereby individuals of a single species act as prey, competitors, and/or predators, depending on their age and size (Polis et al. 1989; Polis and Holt 1992). Intraguild predation has been reported in a variety of systems (Polis et al. 1989; Arim and Marquet 2004) and IGP is likely to occur in systems involving native and non-native species, which interact via reciprocal predation (Bampfylde and Lewis 2007). For instance, introduced alewives (*Alosa pseudoharengus*) preyed on the larvae of native walleye (*Sander vitreus*; Brooking et al. 1998), and adult walleyes preyed on alewives (Schneider and Leach 1977).

This theoretical framework is likely to be important in cases where non-native species play key roles as predators and/or competitors. Such is the case for invasive western mosquitofish (*Gambusia affinis*), which has been labeled as one of the world's "worst 100 invasive species" (Lowe et al. 2000) due to its impacts on native fishes, amphibians and invertebrates via predation and competition (Pyke 2008). Two pioneering papers (Deacon et al. 1964; Minckley and Deacon 1968) identified western mosquitofish as one of the major threats imperiling native desert fish of the south-western United States. A series of subsequent publications presented experimental data (Hurlbert et al. 1972; Meffe 1985; Mills et al. 2004; Rogowski and Stockwell 2006) and anecdotal evidence (Arthington 1984; Galat and Robertson 1992) further cementing a negative perception of mosquitofish as reflected in their numerous derogatory monikers; “the fish destroyer”, “damnbusia” and “plague-minnow” (Myers 1965; McCullough 1998; Pyke 2008).

Notably, this perception has affected the motivation for previous research and management actions including work conducted by my own research group (Rogowski and Stockwell 2006; Stockwell and Henkanaththegedara 2011). The motivation for the current study was to evaluate the impacts of non-native western mosquitofish on the federally listed Endangered Mohave tui chub (*Siphateles bicolor mohavensis*). The Mohave tui chub recovery plan lists mosquitofish presence as a threat to recovery, but little data were available to support this claim.

My initial approach to this problem was to design a “removal” experiment to simulate how Mohave tui chub would perform if mosquitofish were eradicated. Thus, I consciously employed an explicit one-tailed design that included Mohave tui chub both in allopatry and sympatry with western mosquitofish. I reasoned that an additional treatment including mosquitofish in allopatry would have been a misallocation of critical experimental resources. This dogmatically influenced design did not allow me to test the effects of Mohave tui chub on mosquitofish (Stockwell and Henkanaththegedara 2011). Much to my surprise, I found un-controlled evidence of such an effect at the conclusion of the experiment when I observed very low survivorship of mosquitofish adults – effectively my treatment died! Here, I report the results of a subsequent mesocosm experiment designed to evaluate IGP between invasive western mosquitofish and Endangered Mohave tui chub. I also discuss the importance of this experiment in understanding complex interactions between native and non-native taxa.

Methods

Experimental Setup

Thirty large circular mesocosms with a diameter of 1.8 m were employed to host experimental sympatric and allopatric populations of Mohave tui chub and western mosquitofish (Figure 2.1). Mesocosms have been effectively used to evaluate the interactions between native and invasive species under semi-natural conditions (Belk and Lydeard 1994; Rincon et al. 2002; Rogowski and Stockwell 2006). Mesocosms were deployed adjacent to Lake Tuendae (Desert Studies Center, Zzyzx, CA) and filled with lake water filtered through 1.18 mm mesh to exclude larval fish. Mesocosms were filled to a depth of 55 cm (volume ~1.20 m^3) to mimic the typical depth of habitats where mosquitofish co-occur with Mohave tui chub. Each tank was provided with a constant aeration system, 3 linear meters of plastic "plants" to provide cover for fish larvae/juveniles and poultry fence to exclude avian predators.

The thirty mesocosms were randomly assigned to one of three treatments each with 10 replicates: allopatric Mohave tui chubs (MTC); allopatric western mosquitofish (WMF); and Mohave tui chubs sympatric with western mosquitofish (MTC + WMF). Tanks were stocked with adult Mohave tui chubs and mosquitofish captured from Lake Tuendae using minnow traps and hand nets. Prior to stocking, tui chubs were anesthetized (MS-222 100 mg/L) and measured for total length (nearest 1 mm) and mass (nearest 0.1 g). Mosquitofish were not measured due to initial mortality associated with handling stress. Mesocosms receiving Mohave tui chubs (MTC and MTC+WMF) were each stocked with 8 adult Mohave tui chubs of typical size (80-120 mm). Mesocosms receiving mosquitofish (WMF and MTC+WMF) were each stocked with 25 male and 50 female mosquitofish. Relative densities of the two species and the sex ratio of mosquitofish were chosen to reflect relative densities and sex ratios in Lake Tuendae. An introduced population of the Saratoga Springs pupfish (*Cyprinodon nevadensis nevadensis*) inhabits Lake Tuendae, but this species has at exceptionally low densities, not affording us the opportunity to evaluate its role in the fish community.

Mohave tui chub and western mosquitofish at Lake Tuendae are members of the same feeding guild (88% food niche overlap, Morisita's index; Morisita 1959; Appendix A). Thus to limit competition, fish were fed ground pelleted fish food at a ration of 4% of stocked fish biomass per day. This feeding ration was based on our experience from laboratory rearing of both species and our previous mesocosm experiments (Stockwell and Henkanaththegedara 2011). The biomass of mosquitofish was determined based on a weight of 75 randomly picked (25 males and 50 females) Lake Tuendae mosquitofish.

Mesocosm sampling

The mesocosm experiment was initiated 8 March 2009, prior to the active breeding season of both fish species, and continued until 12 May 2009 when mesocosm temperatures reached 32 ^{o}C (upper thermal tolerance for Mohave tui chubs is 33.5-36.2 ^{o}C; McClanahan et al. 1986). All mesocosms were inspected daily for water level, fish mortality, and aeration. Water level in each mesocosm was maintained by adding well water every 2-3 days. Physico-chemical parameters of all mesocosms were tested biweekly. Water temperature, salinity, and dissolved oxygen were tested using a YSI 85 meter. Turbidity was measured with a Micro TPI portable turbidimeter and pH was measured using a Hanna pH-EC-TDS meter. Ammonia contents were tested once in each tank during the first 2 weeks using Jungle Quick Dip ammonia test kits and subsequently tested in 10 randomly selected tanks biweekly.

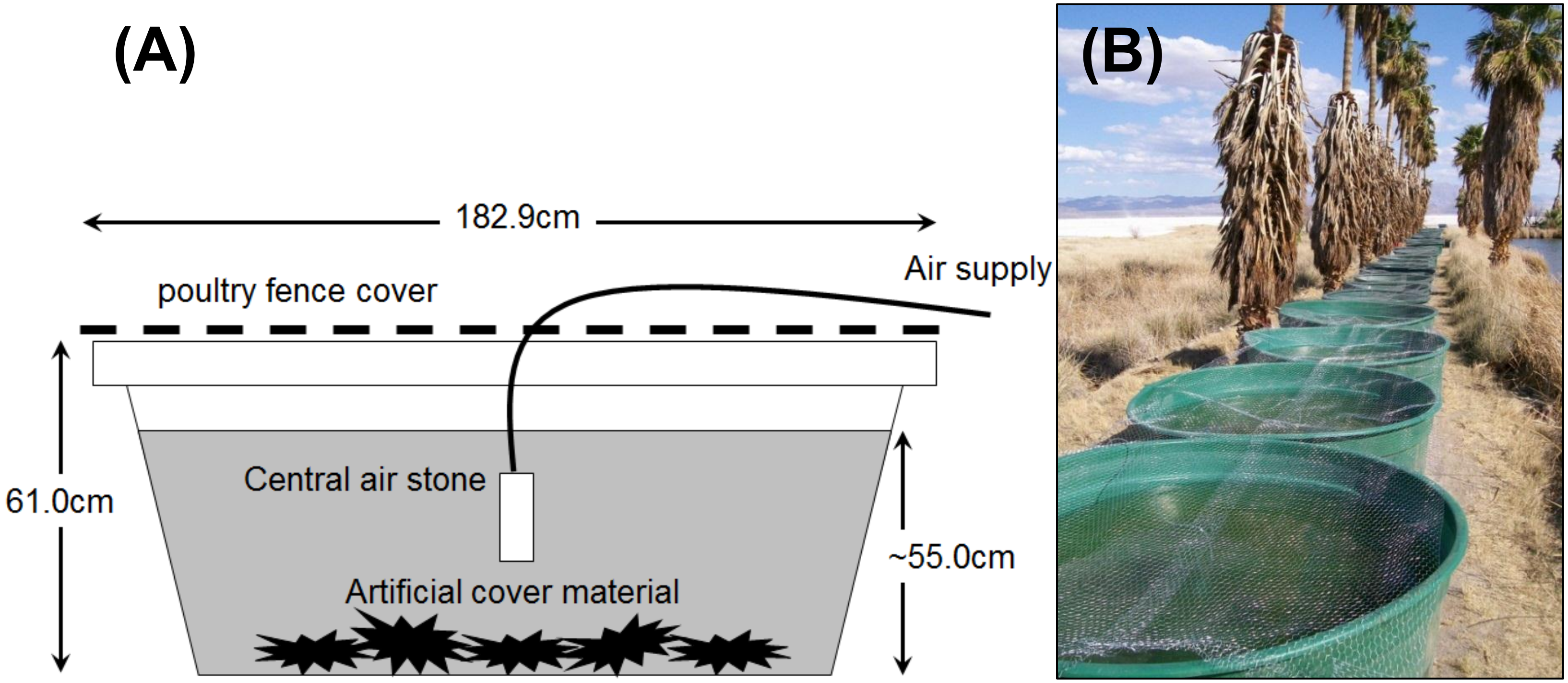

Figure 2.1. Components of 320 gallon tank set up used for mesocosm experiment; (A) a schematic cross section of a tank; (B) the tank set up in the field (Photo: Sujan Henkanaththegedara).

At the conclusion of the experiment, all surviving fish including larval stages, were collected and enumerated by seining each tank 5 times (Rogowski and Stockwell 2006) and then filtering all tank water through a fine mesh net. All mosquitofish were counted, euthanized with a lethal dose of MS-222 (500 mg/L) and preserved in 10% formalin. All Mohave tui chub adults and larvae were counted. A sub-sample of Mohave tui chub larvae (up to 30 from each tank) were retained as vouchers and remaining larvae and all adult tui chubs were released to Lake Tuendae.

Fish measurements

To evaluate size selective predation, the size of mosquitofish which survived to the end of the experiment were measured. Wet mass of formalin fixed individual mosquitofish were measured (nearest 0.1 mg; Denver Instrument Company; model A-250), as well as total length, and body depth (at the base of pelvic fins) (nearest 0.01 mm).

Data analysis

There were no significant differences of tui chub size between the MTC and MTC+WMF treatments in terms of initial wet biomass ($F_{1,159} = 0.00$; $P > 0.05$), initial average total length ($F_{1,159} = 0.08$; $P > 0.05$) and average gape size ($F_{1,159} = 0.08$; $P > 0.05$). Thus, those parameters were not utilized as covariates for subsequent analysis.

To evaluate population level treatment effects, I considered mesocosms as the unit of replication for fish population sizes. To characterize population level responses, I examined the following response variables: (1) Mohave tui chub larvae, (2) male mosquitofish, (3) female mosquitofish, (4) juvenile mosquitofish, (5) per capita production of mosquitofish juveniles (juveniles/adult female) and (6) per capita production of tui chub larvae (larvae/adults). Because the western mosquitofish is sexually dimorphic, male and female population sizes were examined separately for this species. The treatment effects on population sizes were tested using the 2-tail, 2-sample Wilcoxon test (NPAR1WAY Procedure) with a normal approximation for both mosquitofish and Mohave tui chub responses (SAS v. 9.2; SAS Institute 2009).

Individual mosquitofish were treated as replicates when analyzing mean treatment differences of mosquitofish body size. Hence, tanks were nested within treatments to capture tank effects. Separate ANOVA mixed effects models (GLIMMIX Procedure) were ran for each morphometric trait considering treatments as a fixed factor and tanks as a random factor (SAS v. 9.2; SAS Institute 2009). Because, unbalanced designs can result in unreliable p-values for nested ANOVA (Hurlbert 1984; Quinn and Keough 2002), I also used 2-sample Wilcoxon test on the replicate means (mesocosm means) to test for treatment effects on mosquitofish total length, body depth and body mass (SAS v. 9.2; SAS Institute 2009).

Results

Mesocosm conditions

Most physico-chemical characteristics of mesocosms did not significantly vary among treatments but all varied across time (Table 2.1). Dissolved oxygen varied significantly among treatments but, continuous aeration of mesocosms kept the oxygen levels above the desired minimum levels for fish (5 mg/L; Davis 1975). In addition, the significant variations of pH among treatments and significant interactions with time were well within the recorded variation of pH in tui chub habitats (Henkanaththegedara and Stockwell Personnel Observations). Mesocosm water temperature gradually increased from 17.85 ^{o}C (SE ± 0.18) in mid-March to 30.55 ^{o}C (SE ± 0.03) in early May. Salinity increased from 2.39 ppt (SE ± 0.01) to 4.24 ppt (SE ± 0.02) and turbidity of the mesocosms also increased due to growth of algae from 4.31 NTU

(SE ± 0.18) to 11.83 NTU (SE ± 0.40). Water pH slightly increased towards the end of the experiment, but remained within the acceptable range for these fish species.

Table 2.1. ANOVA summary table for water quality variations in mesocosms.

Source	df	MS	F-value	p-value
Temperature				
Treatment	2	0.43	0.62	0.36
Time	4	745.24	1068.30	<0.0001
Treatment*Time	8	0.18	0.26	0.90
Error	135	0.70		
Dissolved Oxygen				
Treatment	2	3.98	9.73	<0.001
Time	4	16.62	40.65	<0.0001
Treatment*Time	8	0.30	0.73	0.19
Error	135	0.41		
Salinity				
Treatment	2	0.00	0.03	0.93
Time	4	14.84	1618.10	<0.0001
Treatment*Time	8	0.01	0.83	0.09
Error	135	0.01		
Turbidity				
Treatment	2	2.20	0.49	0.38
Time	4	400.85	89.79	<0.0001
Treatment*Time	8	1.69	0.38	0.65
Error	135	4.46		
pH				
Treatment	2	0.05	3.89	<0.05
Time	4	0.83	66.42	<0.0001
Treatment*Time	8	0.02	1.55	<0.05
Error	135	0.01		

Fish survival

The mesocosms provided a suitable fish habitat as indicated by high survival and reproduction of both species in allopatry. In fact, adult Mohave tui chub had 100% survival in 9 of 10 sympatric tanks and all 10 allopatric tanks. Two tui chubs died within one sympatric tank, which did not produce any tui chub offspring. Mosquitofish presence had a significant negative impact on Mohave tui chub recruitment ($W = 142.0$; $P < 0.01$). Tui chub populations sympatric with mosquitofish had low tui chub larval production (mean 5.4 ± SE 3.4 larvae/mesocosm; Figure 2.2-a), with 6 sympatric mesocosms producing no tui chub larvae. By contrast, allopatric Mohave tui chub produced 33.8 (SE ± 7.6) larvae/ mesocosm with only a single mesocosm producing no tui chub larvae. After controlling for adult survival, we observed a negative effect of mosquitofish on per capita tui chub larval production with only 0.70 ± 0.43 larvae/adult/mesocosm for sympatric tanks compared to 4.22 ± 0.95 larvae/adult/mesocosm for allopatric tanks ($W = 141$; $P < 0.01$; Figure 2.2-b).

Mosquitofish population size was significantly lower ($W = 155.0$; $P < 0.001$) in the presence of tui chubs (22.1 ± 4.0 mosquitofish/mesocosm) compared to allopatric mosquitofish populations (157.2 ± 26.9 mosquitofish/mesocosm). Notably, mosquitofish were extirpated from one of the sympatric tanks. Male mosquitofish survival was significantly lower ($W = 155.0$; $P < 0.001$; Figure 2.3-a) in the presence of tui chubs (0.8 ± 0.5) compared to male survival in allopatric mosquitofish populations (22.5 ± 1.3). Female mosquitofish survival was also significantly reduced in the presence of Mohave tui chubs (17.2 ± 3.6), compared to female mosquitofish survival from allopatric populations (52.5 ± 0.9; $W = 155.0$; $P < 0.001$; Figure 2.3-b). In addition, the survival of sympatric mosquitofish was sex-biased, with exceptionally low male survival (3%) relative to female survival (34%).

Tui chub presence had a significant effect on mosquitofish juvenile production. Notably, juveniles were not produced in 6 of the 10 sympatric tanks, but one of these 6 tanks also experienced loss of adults as well. Excluding this latter tank, mosquitofish productivity was significantly lower for the sympatric tanks (4.6 ± 2.9 juveniles/mesocosm) compared to the allopatric tanks (82.2 ± 26.4 juveniles/mesocosm; W = 48.5, $P < 0.001$; Figure 2.3-c). After controlling for adult survival, sympatric mosquitofish populations had significantly lower per capita larval production (0.8 ± 0.5 juveniles/female/mesocosm) compared to allopatric mosquitofish populations (1.5 ± 0.5 juveniles/female/mesocosm; W = 63.0; $P < 0.05$; Figure 2.3-d).

Mosquitofish Size

Tui chubs apparently preyed on small adult mosquitofish in sympatry. Male mosquitofish, which are notably smaller than female mosquitofish, had high survival in allopatry (90% survival), but extremely low survival when sympatric with Mohave tui chub (3% survival; Figure 2.3-a). Furthermore, female mosquitofish from sympatric populations had significantly larger body mass (0.97 g ± 0.02) than female mosquitofish from allopatric mosquitofish populations (0.80 g ± 0.01; $F_{1,17} = 10.18$; $P < 0.01$; Figure 2.4-a), suggesting that larger females were less vulnerable to Mohave tui chub predation.

Female mosquitofish from sympatric populations were significantly longer (40.45 mm ± 0.32) compared to females from allopatric populations (36.82 mm ± 0.18; $F_{1,17} = 26.59$; $P < 0.0001$; Figure 2.4-b). However, female mosquitofish body depth did not significantly differ between two groups ($F_{1,17} = 2.00$; $P > 0.05$) (Figure 2.4-c). Non-parametric analyses also showed that sympatric female mosquitofish were significantly larger than allopatric females in terms of total length (W = 133, P < 0.001) and body mass (W = 120.5, P < 0.05), but not body depth (W = 105, P > 0.05).

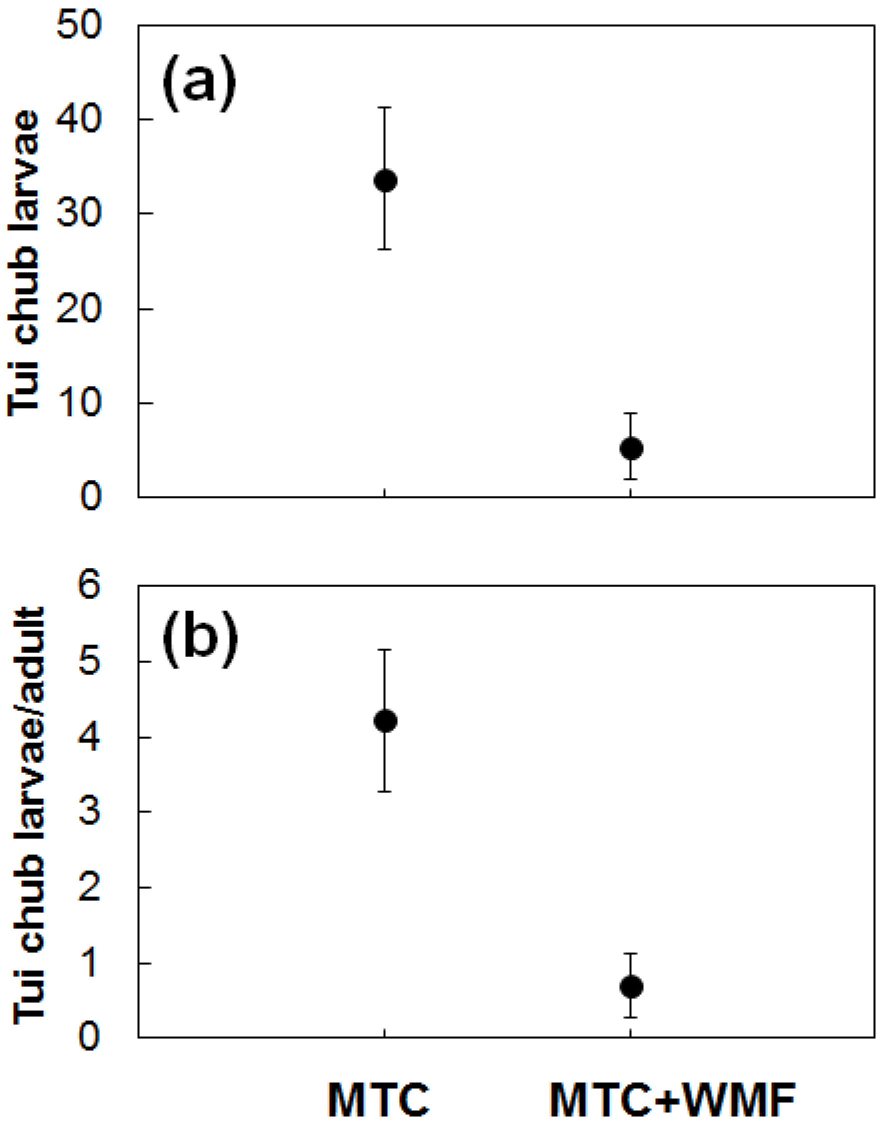

Figure 2.2. Mohave tui chub (*Siphateles bicolor mohanvensis*) total (a) and per capita (b) recruitment in the presence (sympatric; MTC + WMF) and absence (allopatric; MTC) of mosquitofish (*Gambusia affinis*) is shown. Error bars represent 1 ± SE.

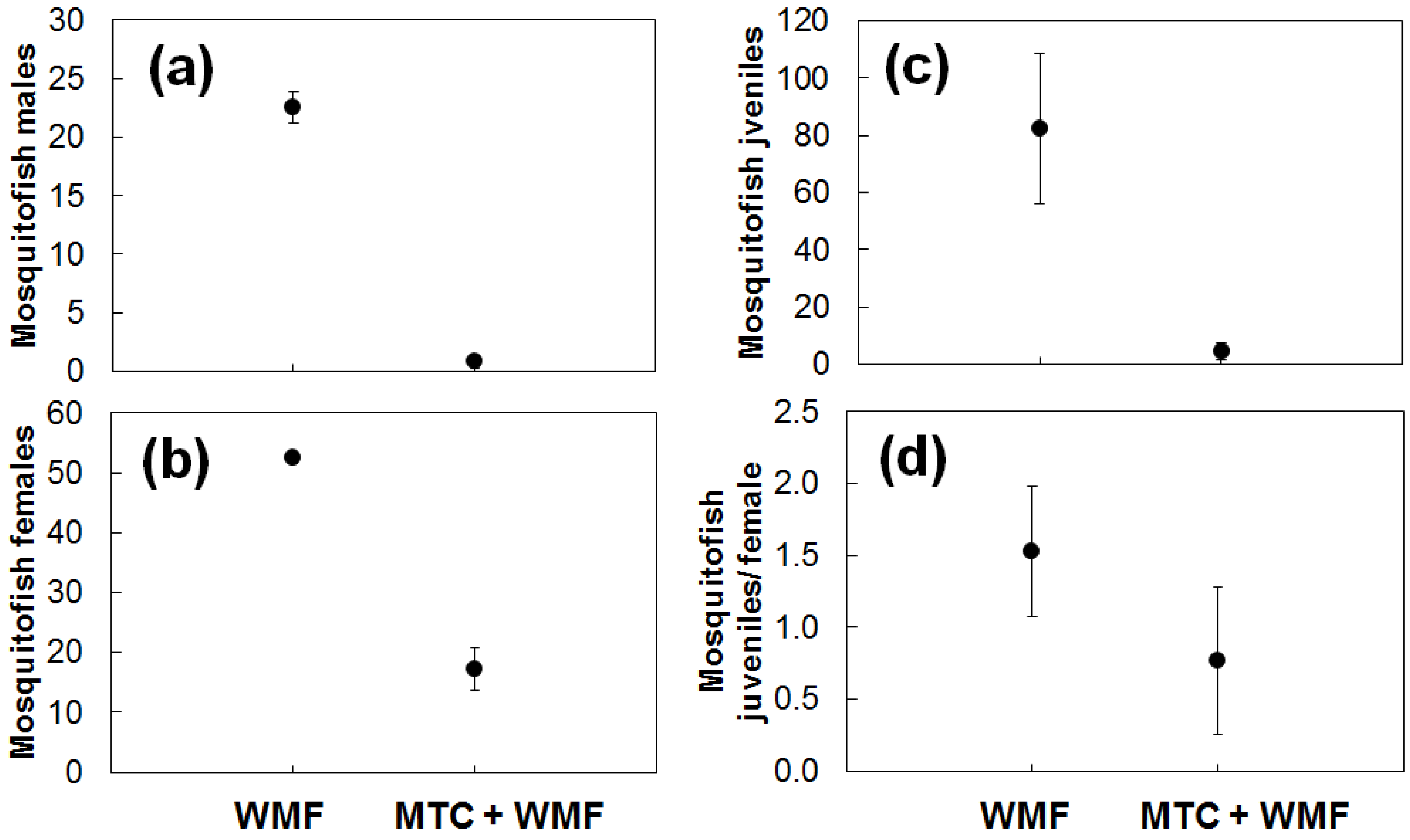

Figure 2.3. Average mosquitofish (*Siphateles bicolor mohanvensis*) survival for adult male (a), adult female (b), juveniles (c), and per capita mosquitofish (*Gambusia affinis*) recruitment (d) in the presence (sympatric; MTC + WMF) and absence (allopatric; WMF) of Mohave tui chubs. Error bars represent 1 ± SE.

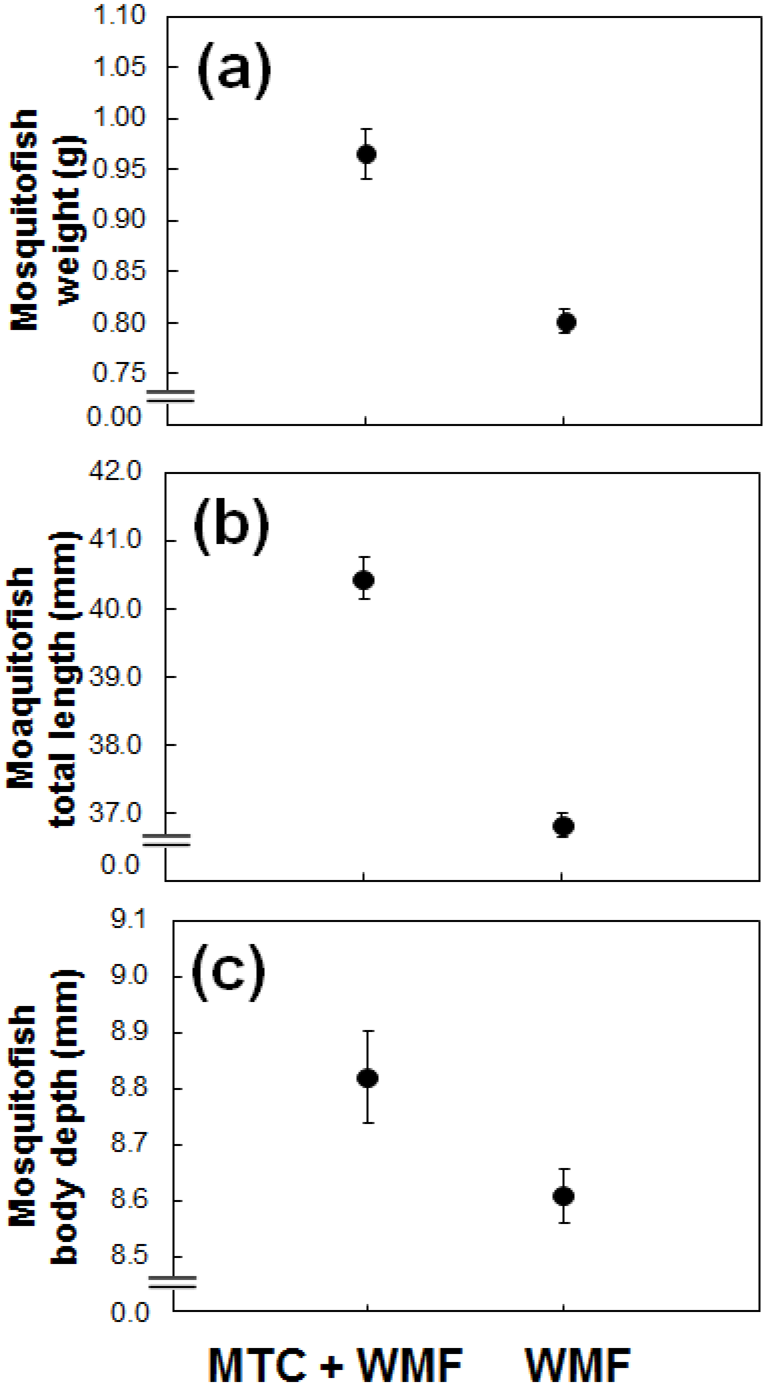

Figure 2.4. The size of surviving female mosquitofish (*Gambusia affinis*) in the presence (sympatric; MTC + WMF) and absence (allopatric; WMF) of Mohave tui chubs (*Siphateles bicolor mohanvensis*) in terms of (a) body mass, and (b) total length), and (c) body depth. Error bars represent 1 ± SE.

Discussion

This study provides experimental evidence for IGP between non-native western mosquitofish and the protected Mohave tui chub. As expected, mosquitofish had significant impacts on Mohave tui chub recruitment by reducing the larval survival in sympatric mesocosms. Reduced recruitment was probably due to mosquitofish predation on tui chub eggs and/or larvae, rather than competition, because food was provided. Further, it is noteworthy that mosquitofish preyed on tui chub larvae during laboratory trials (Henkanaththegedara and Stockwell unpublished data). Other workers have reported mosquitofish predation as the primary mechanism of mosquitofish impact on native fishes (Belk and Lydeard 1994; Rincon et al. 2002; Mills et al. 2004). Therefore, we suspect mosquitofish predation on tui chub eggs and/or larvae as the primary cause for the reduction of tui chub recruitment in sympatric tanks.

Surprisingly, tui chub presence also significantly impacted mosquitofish populations. As expected, allopatric mosquitofish populations increased rapidly with a two-fold increase in total population size in less than 10 weeks. By contrast, tui chubs caused mosquitofish populations to decrease by approximately 70%, with complete extirpation in one mesocosm. These population effects were due to reduced adult survival and reduced larval production. In fact, sympatric mosquitofish did not produce offspring in 60% of the tanks, and overall mosquitofish juvenile production was substantially reduced in the presence of tui chubs. Decreased mosquitofish juvenile production may be partly due to fewer females contributing juveniles; however, after controlling for adult female survival, the per capita mosquitofish recruitment was still reduced by 50% for sympatric populations.

Collectively, our results provide evidence for IGP between endangered Mohave tui chub and non-native western mosquitofish. This case of IGP appears to be size structured with adult

Mohave tui chub preying on adult (and juvenile) mosquitofish, and adult mosquitofish preying on tui chub eggs and/or larvae. Mohave tui chub predation on adult mosquitofish is apparently gape-limited, as has been reported for other systems with piscivorous fish species (Hambright et al. 1991; Nilsson and Bronmark 2000). Larger mosquitofish females apparently avoided predation by Mohave tui chub, which is consistent with gape-size limited tui chub predation. Low male survival is also consistent with the gape size limitation hypothesis as male mosquitofish are much smaller than females. Overall, size-structured IGP may facilitate co-existence of native and non-native species. In fact, IGP has been shown to be very important in determining structure and stability for a variety of communities (Arim and Marquet 2004). We suggest that IGP plays a role in co-persistence of Mohave tui chub and western mosquitofish. In fact, mosquitofish and tui chubs have been co-existed for at least 27 years in China Lake (U.S. Fish and Wildlife Service 1984) and 9 years in Lake Tuendae (Steve Parmenter Personnel Communication).

In addition to IGP, other mechanisms such as niche partitioning may play an important role in promoting co-persistence of Mohave tui chub with mosquitofish in the wild. For instance, spatial and temporal habitat partitioning may limit impacts of western mosquitofish on native fishes (Barrier and Hicks 1994; Ling 2000; Ayala et al. 2007). Spatial and temporal niche partitioning between adult mosquitofish and tui chubs in Lake Tuendae has yet to be quantified.

Exploring the mechanisms that may permit co-persistence was not an original objective for this project. In fact, my initial work (Stockwell and Henkanaththegedara 2011) was influenced by the prevailing dogma that mosquitofish constitute an important threat to many native aquatic species (Courtenay and Meffe 1989; Pyke 2008). In a more general sense, this dogmatic view has influenced conservation planning with mosquitofish identified as a threat for the recovery of a variety of listed fish and amphibian species. I searched the Endangered Species Recovery Plans Database (U.S. Fish and Wildlife Service 2011) and found that western mosquitofish (*G. affinis*) was identified as a limiting factor for 18% of amphibian (n=17) and 25% of fish (n=110) species and populations. For example, western mosquitofish (*G. affinis*) have been identified as a limiting factor in recovery plans for 6 of 7 Cyprinodontids (pupfish), 3 of 4 Goodeids (poolfish and springfish), all 6 species of Poeciliids (mosquitofish and topminnows) and both Ranid frogs (U.S. Fish and Wildlife Service 2011). However, evidence demonstrating that mosquitofish limit recovery has been largely anecdotal with very limited experimental work. Therefore I argue that additional work is warranted to verify if mosquitofish presence truly constrains recovery of such protected species.

The dogmatic views of mosquitofish as a universally “bad” further constrain available management options. The Mohave tui chub recovery plan calls establishing 6 geographically isolated, self-sustaining populations for down-listing the status of this species from *Endangered* to *Threatened* (U.S. Fish and Wildlife Service 1984). When my work started, there were 4 populations that counted as 3 geographically isolated populations toward the down-listing goal of 6 established populations, thus seeking suitable refuge sites has been a high priority for tui chub recovery. It is noteworthy that my research suggests mosquitofish presence may not necessarily limit the suitability of a site for colonization by Mohave tui chub. Indeed, a forth population of Mohave tui chub was recently established at a site inhabited by mosquitofish (i.e. Deppe Pond/tui slough system; M. Huffine, personnel communication).

These findings support an emerging view calling for a more nuanced and sophisticated evaluation of non-native species (Davis et al. 2011; Prévot-Julliard et al. 2011; Schlaepfer et al. 2011). Understanding the complex interactions among native and non-native species in the whole-ecosystem context may help conservation practitioners identify novel management options.

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CHAPTER 3. THE ROLE OF GAPE-LIMITATION IN INTRAGUILD PREDATION BETWEEN NATIVE AND NON-NATIVE FISH

Abstract

Intraguild predation (IGP) is a mechanism that may facilitate the co-persistence of native species with non-native invasive species. We conducted laboratory predation trials to assess the role of predator gape-limitation in the context of IGP between the endangered Mohave tui chubs (*Siphateles bicolor mohavensis*) and invasive western mosquitofish (*Gambusia affinis*). I explored sex specific differences in gape-size limitation in mosquitofish, because female mosquitofish have notably larger body depths and gape-sizes than male mosquitofish. Larval tui chubs had significantly lower ($\chi^2 = 74.74$; $P < 0.001$) survival in the presence of female mosquitofish (10.0%) than in the presence of male mosquitofish (73.3%). Reciprocally, adult tui chubs preyed upon adult mosquitofish causing a significantly lower ($\chi^2 = 11.33$; $P < 0.001$) survival for male mosquitofish (60%) compared to female mosquitofish survival (96.7%). The average depth/gape ratios for consumed fish were 0.91 (± 0.03; N = 12) and 0.72 (± 0.04; N = 27), for tui chub and mosquitofish predators, respectively. Vulnerability modeling revealed that mosquitofish with a body depth less than 4.4 mm and a larval tui chub with a body depth less than 1.8 mm were completely vulnerable to predation by Mohave tui chub and mosquitofish, respectively. My results suggest that IGP in this study system is size structured based on gape-size limitation and may have some conservation implications for the recovery of endangered Mohave tui chub.

Introduction

Predation is one of the most widespread ecological interactions shaping community structure (Sih et al. 1985; Diehl 1992; Post et al. 2008) as well as life history evolution of interacting organisms (Reznick et al. 1990; Stibor 1992; Ingram et al. In Press). However, the relative roles of predator and prey are not static and may change with individuals of a single species acting as both predator and/or prey depending on their age and size (Polis et al. 1989; Polis and Holt 1992). Such *intraguild predation* (IGP) is a widespread phenomenon in nature (Polis and Holt 1992; Arim and Marquet 2004); nevertheless, few studies have evaluated how IGP may affect the co-persistence of native and non-native species (Taniguchi et al. 2002; Arim and Marquet 2004).

In theory, IGP may facilitate invasion dynamics, but IGP may also facilitate the co-persistence of native species with non-natives. Taniguchi et al. (2002) showed IGP provided a competitive advantage for non-native, stream-dwelling rainbow trout (*Oncorhynchus mykiss*) over native, anadromous Masu salmon (*O. masou*), facilitating rainbow trout invasion in Japanese streams. By contrast, size structured IGP may facilitate co-persistence of endangered Mohave tui chub (*Siphateles bicolor mohavensis*) with non-native western mosquitofish (*Gambusia affinis*; Stockwell and Henkanaththegedara 2011; Henkanaththegedara and Stockwell unpublished data). In both case studies, IGP appeared to be size-structured based on predator gape-size limitation.

The vulnerability of prey to potential predation is often limited by the gape-size of predators relative to their prey (Hambright 1991; Nilsson and Bronmark 2000; Magnhagen and Heibo 2001; Webb and Shine 1993). For example, Magnhagen and Heibo (2001) reported a positive correlation between gape-size of northern pike (*Exos lucius*) and body depth of its piscine prey. When gape-size is a limiting factor, predation risk is reduced as the prey grows and body depth approaches and ultimately exceeds the predator's maximum gape-size (Hambright et al. 1991; Nilsson et al. 1995). Therefore, predator gape-size and prey body depth may have important implications in a system where predation is structured based on relative body sizes of predator and prey.

We reported a case of IGP between endangered Mohave tui chub and non-native western mosquitofish, where tui chubs preyed on adult and juvenile mosquitofish while mosquitofish preyed on eggs and larvae of tui chubs (Henkanaththegedara and Stockwell unpublished data). Two lines of evidence suggest that IGP between these two species is structured by gape-size limitation. First, male mosquitofish, which is the smaller sex of this dimorphic species, had very low survival (3%) compared to female mosquitofish (34%) in the presence of Mohave tui chub. Second, female mosquitofish that survived in the presence of adult tui chubs were relatively large. Reciprocally, mosquitofish preyed upon Mohave tui chub larvae, which is also likely to be gape-limited (Henkanaththegedara and Stockwell unpublished data; also see Mills et al. 2004).

Here, we report the results of a series of laboratory predation trials designed explicitly to assess gape-limitation in the context of IGP between Mohave tui chub and western mosquitofish. We also present a prey vulnerability model (Hambright et al. 1991) which allows us evaluate how gape-size limited predation affects each fish population.

Methods

Laboratory predation experiments

Predation on adult western mosquitofish was assessed by using adult Mohave tui chubs (sexually monomorphic) as candidate predators. I tested both adult male and female mosquitofish as candidate prey because mosquitofish are sexually dimorphic. Mohave tui chubs were collected from Lake Tuendae, MC Spring, and Camp Cady (all in southern California) in November 2009 and transported to North Dakota State University (NDSU). Western mosquitofish were collected from Deppe Pond/Tui slough system at the Lewis Center for Academic Excellence in Apple Valley, CA and transported to NDSU.

Sixty 37.8 L glass aquaria were used as experimental chambers. Three vertical sides of each aquarium were covered with black plastic sheets to avoid any visual interference among tanks. Aquaria were continuously aerated by a centrally suspended aerator in each tank. A full spectrum light source was placed 35 cm above each tank and a light cycle of 16 hrs light / 8 hrs dark was used.

This experimental design provided 30 replicates, using either male mosquitofish or female mosquitofish allowing an assessment of tui chub predation on mosquitofish by sex. Mohave tui chub predators were measured for total length (nearest 1 mm) and gape-size (nearest 0.01 mm). Tui chub gape-size was measured from the ventral side (tui chubs have a sub-terminal mouth) as the linear distance between posterior limit of maxilla with mouth fully closed. Mosquitofish were measured for total length and body depth (nearest 0.01 mm). Female mosquitofish body depths were measured at the base of pelvic fins while male mosquitofish body depths were measured at the deepest point of the gonopodial base (Figure 3.1). Measured mosquitofish were kept in individual containers prior to introduction into aquaria. Mohave tui chubs assigned between the two treatments did not significantly differ in total length ($t = -1.05$; $P > 0.05$) and gape-size ($t = -1.06$; $P > 0.05$). As expected, female mosquitofish were significantly larger in total length ($t = -12.74$; $P < 0.001$) and gape-size ($t = -11.40$; $P < 0.001$) compared to male mosquitofish (Table 3.1).

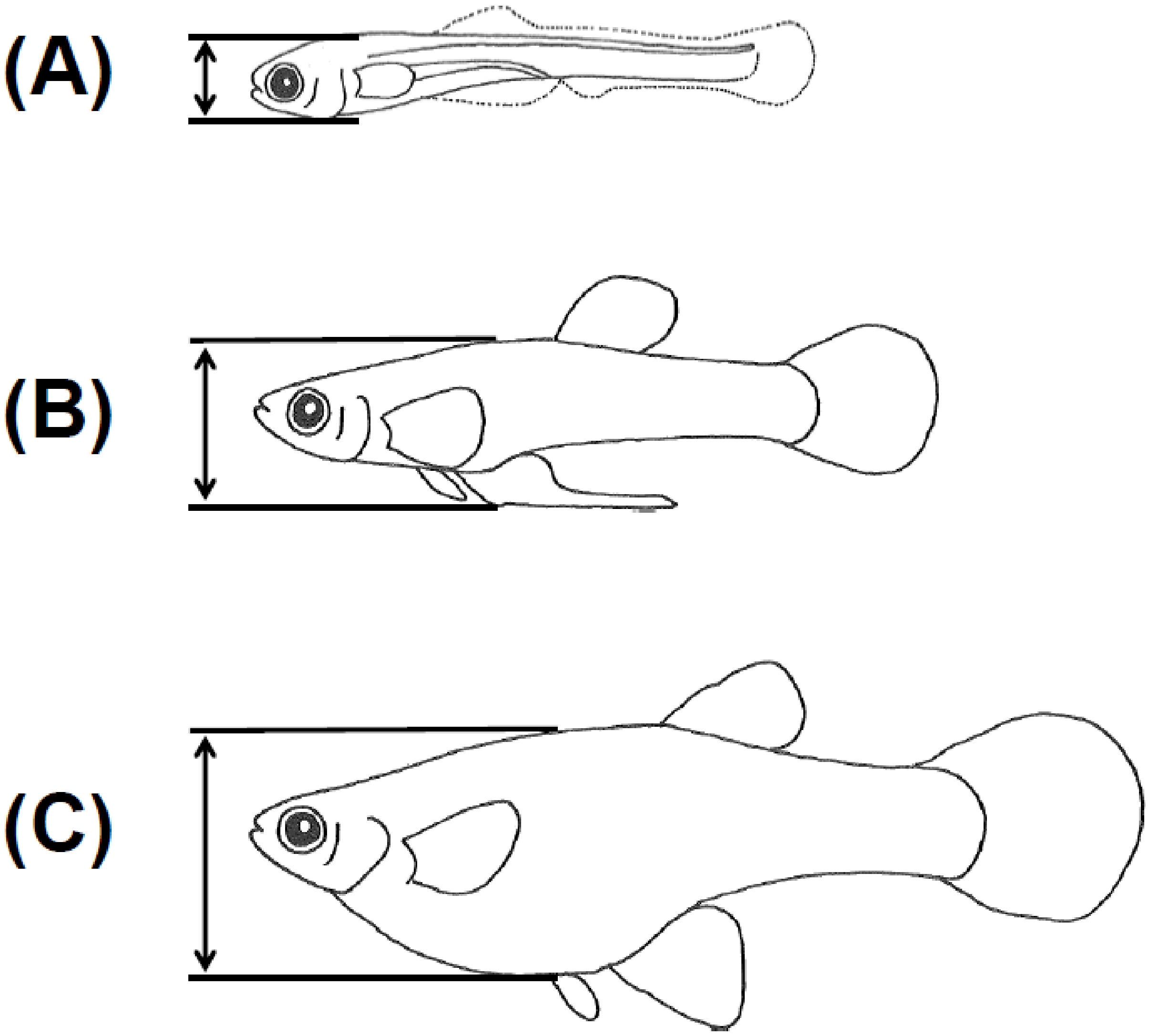

Figure 3.1. Body depth measurements of a Mohave tui chub larvae (*Siphateles bicolor mohavensis*) (A), a mature male (B) and a pregnant female (C) of western mosquitofish (*Gambusia affinis*; Illustrated by Sujan Henkanaththegedara).

Table 3.1. Size of Mohave tui chub (*Siphateles bicolor mohavensis*) and western mosquitofish (*Gambusia affinis*) utilized for predation experiments.

Experiment / Measurement	Prey Treatment		N	t-value	p-value
	♂ mosquitofish	♀ mosquitofish			
Mohave tui chubs as predators					
Adult Mohave tui chub					
Total length (mm)	101.70 (± 2.68)	105.50 (± 2.43)	30	-1.049	> 0.05
Gape-size (mm)	6.24 (± 0.20)	6.54 (± 0.20)	30	-1.057	> 0.05
Total length (mm)	30.00 (± 0.29)	40.72 (± 0.79)	30	-12.742	< 0.001
Body depth (mm)	6.22 (± 0.06)	8.31 (± 0.17)	30	-11.404	< 0.001
Mosquitofish as predators					
Adult western mosquitofish					
Total length (mm)	26.02 (± 0.66)	38.12 (± 0.88)	30	-11.013	< 0.001
Gape-size (mm)	2.00 (± 0.04)	3.43 (± 0.09)	30	-14.590	< 0.001
Larval Mohave tui chub					
Total length (mm)	13.78 (± 0.48)	14.67 (± 0.54)	30	-1.243	> 0.05
Estimated body depth (mm)	2.30 (± 0.09)	2.47 (± 0.10)	30	-1.243	> 0.05

For each predation trial, an adult tui chub was deprived of food for 24h prior to being placed in a randomly selected aquarium. After a 4hr acclimation period, a single mosquitofish was introduced into each aquarium. Survival was monitored every 3h over the 72h test period and time to death (TTD) was recorded for each mosquitofish.

Mosquitofish predation on larval tui chubs was assessed by using either adult male or adult female mosquitofish as candidate predators and Mohave tui chub larvae as candidate prey (Table 3.1). For these trials, mosquitofish were obtained from a commercial stock from Arizona (Arizona Aquatic Gardens; www.azgardens.com). Mohave tui chub larvae were provided by Mojave National Preserve, California (Debra Hughson Personnel Communication).

Small 4.7 L opaque plastic containers were used as experimental chambers. Chambers were not aerated due to the short experimental time period and low fish density. This experiment consisted of two predator treatments, male and female mosquitofish, with 30 replicates each. In addition, I included a control of Mohave tui chub larvae maintained in the absence of mosquitofish (n = 28), to account for any mortality of tui chub larvae due to handling stress.

Mosquitofish predators were measured for total length and gape-size (nearest 0.01 mm). Mosquitofish gape-size was measured dorsally due to superior nature of their mouths. Again, male mosquitofish were significantly smaller in total length ($t = -11.01$; $P < 0.001$) and gape-size ($t = -14.59$; $P < 0.001$) compared to female mosquitofish (Table 3.1).

Tui chub larvae were measured for total length (nearest 0.01mm) using digital calipers, while resting on a watch glass filled with a small amount of water. Subsequently, tui chub larvae were monitored for at least 3h following measurements to assess any handling associated mortality. Body depths were difficult to measure on live tui chub larvae. Therefore, I measured a sample of Mohave tui chub larvae voucher specimens to derive a regression formula of body depth on total length (body depth = 0.1986 x (total length) – 0.4251; $r^2 = 0.96$; N = 148). Mohave tui chub larval body depth was measured at the middle of the head (Figure 3.1). The average total length of Mohave tui chub larvae exposed to mosquitofish predation did not significantly differ between male and female mosquitofish treatments ($t = -1.24$; $P > 0.05$; Table 3.1).

Adult mosquitofish were deprived of food for 24h prior to being placed in a randomly selected experimental chamber. After the 4h acclimatization period, a single tui chub larva was introduced into each experimental chamber and its survival was monitored every 15 minutes over a 4h test period and TTD was recorded for each tui chub larva.

Statistical analysis

I conducted all statistical analyses using R statistical software program Version 2.11.0 (R Development Core Team 2010). Package *survival* was utilized to analyze prey survival (Therneau and Lumley 2009). This package uses the *Surv( )* function to simultaneously evaluate TTD and the censoring information (0=live; 1=dead; Maindonald and Braun 2010). Survival functions were estimated with Kaplan-Meier survival estimate (*survfit* function) using TTD data. Hazard functions for treatment groups were tested using Cox proportional hazards model (*coxph* function).

The ratio between prey body depth and predator gape width (here after depth/gape ratio) was utilized to assess gape-size limitation. In theory, predators could not consume prey larger than their gape-size (Hambright et al. 1991); hence the depth/gape ratio should be ≤ 1.0 for prey consumed and >1.0 for survivors. I ran separate one-sample, 2-tail t-tests (*t.test* function) with depth/gape ratios using a null hypothesis of $\mu = 1$ to detect any significant deviations from 1 (when prey body depth = predator gape-size) after testing data for normality with a Shapiro-Wilk normality test (*shapiro.test* function). Prey which were presumably killed by the predator but not consumed were excluded from these analyses (mosquitofish as predator, N=5; tui chubs as predator, N=1).

Vulnerability modeling

Hambright et al.'s (1991) vulnerability model assumes that predator gape-size and prey body depth are the critical factors which determine the prey size ingested by a predator. Relative vulnerability of prey (V) to predation was estimated as a function of prey body depth (d) and the frequency of predators' gape-size (W) in the predator population.

$$V_d = 1 - \sum_{w=0}^{d} W$$

Prey with body depths larger than the gape-size of the largest individual of predator population were considered to be unavailable for predation (i.e., V=0). However, prey with body depths smaller or equal to the gape-size of the smallest individual of the predator population were considered to be completely vulnerable to all the predators in the community (i.e., V=1). The prey with intermediate body depths are vulnerable to a proportion of the predator population depending on the body depth ($0 < V < 1$).

Relative vulnerabilities of adult mosquitofish and larval tui chubs were estimated using the cumulative gape-size frequency distributions for the Lake Tuendae populations of adult Mohave tui chub and adult mosquitofish respectively (Table 3.2). Corresponding total lengths of mosquitofish were estimated using two regression formulas relating body depth to total length based on fish collected from Lake Tuendae in March-May 2009 (females: total length = (body depth + 3.0746) / 0.3017; $r^2 = 0.85$; N = 90, males: total length = (body depth + 0.2775) / 0.2123; $r^2 = 0.76$; N = 79). Adult chubs were collected in 2008 from Lake Tuendae, Camp Cady, China Lake (n=30 each) and MC Spring (n=24). Mosquitofish were collected from Lake Tuendae in

2009 (n=149). The gape-size of tui chubs and mosquitofish were measured as previously described.

Table 3.2. Size of Mohave tui chub (*Siphateles bicolor mohavensis*) and western mosquitofish (*Gambusia affinis*) utilized for vulnerability modeling.

Species/measurement (mm)		N	Average (±SE)	Range
Mohave tui chub				
Adults	TL	114	107.19 (± 2.11)	62.71 - 150.85
	Gape	114	7.92 (± 0.17)	4.34 - 12.62
Larvae	TL	178	15.77 (± 0.52)	6.19 - 44.67
	Depth	178	2.70 (± 0.10)	3.59 - 10.30
Western mosquitofish				
Females	TL	90	32.30 (± 0.56)	21.63 - 45.38
	Gape	90	2.76 (± 0.05)	1.74 - 4.16
	Depth	90	6.67 (± 0.18)	3.59 - 10.30
Males	TL	59	25.30 (± 0.25)	20.54 - 29.20
	Gape	59	1.83 (± 0.03)	1.19 - 2.46
	Depth	59	5.06 (± 0.07)	3.77 - 6.01

Results

Laboratory predation experiments

Survival in the presence of tui chubs was significantly lower (χ^2= 11.33; d.f. = 1; P<0.001) for male mosquitofish (60.0%; 95% CI: 44.8-80.4%) than for female mosquitofish (96.7%; 95% CI: 90.4-100.0%; Figure 3.2-A) The average depth/gape ratio for male mosquitofish consumed by tui chub predators (0.91 ± 0.03; N=12) was significantly lower than 1.0 (t = -3.13; d.f. = 11; p = 0.0096), whereas the depth/gape ratio for male mosquitofish survivors (1.11 ± 0.05; N = 18) was significantly greater than 1.0 (t = 2.22; d.f. = 17; p = 0.406). Furthermore, 29 out of 30 female mosquitofish survived the experiment (1 killed but not consumed) and their average depth/gape ratio (1.31 ± 0.06) was significantly higher than 1.0 (t = 5.14; d.f. = 28; p = 1.86 e-5). Because of small sample size, we did not test the depth/gape ratio for the single non-surviving female mosquitofish.

Tui chub larval survival in the absence of mosquitofish was 100%, suggesting that handling stress was limited. Mohave tui chub larval survival was significantly lower (χ^2=74.74; d.f. = 1; P<0.001; Figure 3.2-B) in the presence of female mosquitofish (10.0%; 95% CI: 3.4-29.3%), than in the presence of male mosquitofish (73.3%; 95% CI: 59.1-91.0%). The average depth/gape ratio for tui chub larval prey consumed by female mosquitofish (0.72 ± 0.04; N=27) was significantly less than 1.0 (t = -6.5; d.f. = 26; p = 6.84 e-7). The depth/gape ratio was not tested for the small sample of tui chub larvae that survived in the presence of female mosquitofish. The average depth/gape ration for the tui chub larvae survived with male mosquitofish predators (1.22 ± 0.06; N = 22) was significantly higher than 1.0 (t = 3.58; d.f. = 21; p = 0.0018). The depth/gape ratio was not tested for the small sample of tui chub larvae that were consumed by male mosquitofish.

Vulnerability modeling

Vulnerability modeling revealed that mosquitofish with a body depth less than 4.4 mm (total length, female: 24.8 mm, male: 22.0 mm) were completely vulnerable to tui chub predation. However, with increasing size, vulnerability of mosquitofish to tui chub predation decreased to zero with a body depth greater than 12.8 mm (total length, female: 52.6). The body depth of male mosquitofish never reaches 12.8 mm, indicating that the entire Lake Tuendae male mosquitofish population is vulnerable to tui chub predation. Furthermore, the size distribution for the Lake Tuendae mosquitofish population shows that male mosquitofish have a higher vulnerability to tui chub predation compared to female mosquitofish. The size frequency distribution of mosquitofish from Lake Tuendae suggests that all mosquitofish have some vulnerability to tui chub predation. However, female mosquitofish population has comparatively lower vulnerability to tui chub predation compared to male mosquitofish, due to relatively large body size (Figure 3.3-A).

Tui chub larvae with body depths less than 1.8 mm (total length: 11.2 mm) were fully vulnerable to predation by all size classes of adult female mosquitofish. With tui chub larval growth, their vulnerability to female mosquitofish predation decreases. The vulnerability to predation reaches zero when tui chub larvae reach a body depth greater than 4.2 mm (total length: 23.3 mm) providing a complete size-refuge from female mosquitofish predation. Tui chub larvae with body depths less than 1.2 mm (total length = 8.2 mm) were fully vulnerable to male mosquitofish predation; however vulnerability of tui chub larvae to predation by adult male mosquitofish reached zero at a body depth of 2.6 mm (total length = 15.2 mm; Figure 3.3-B).

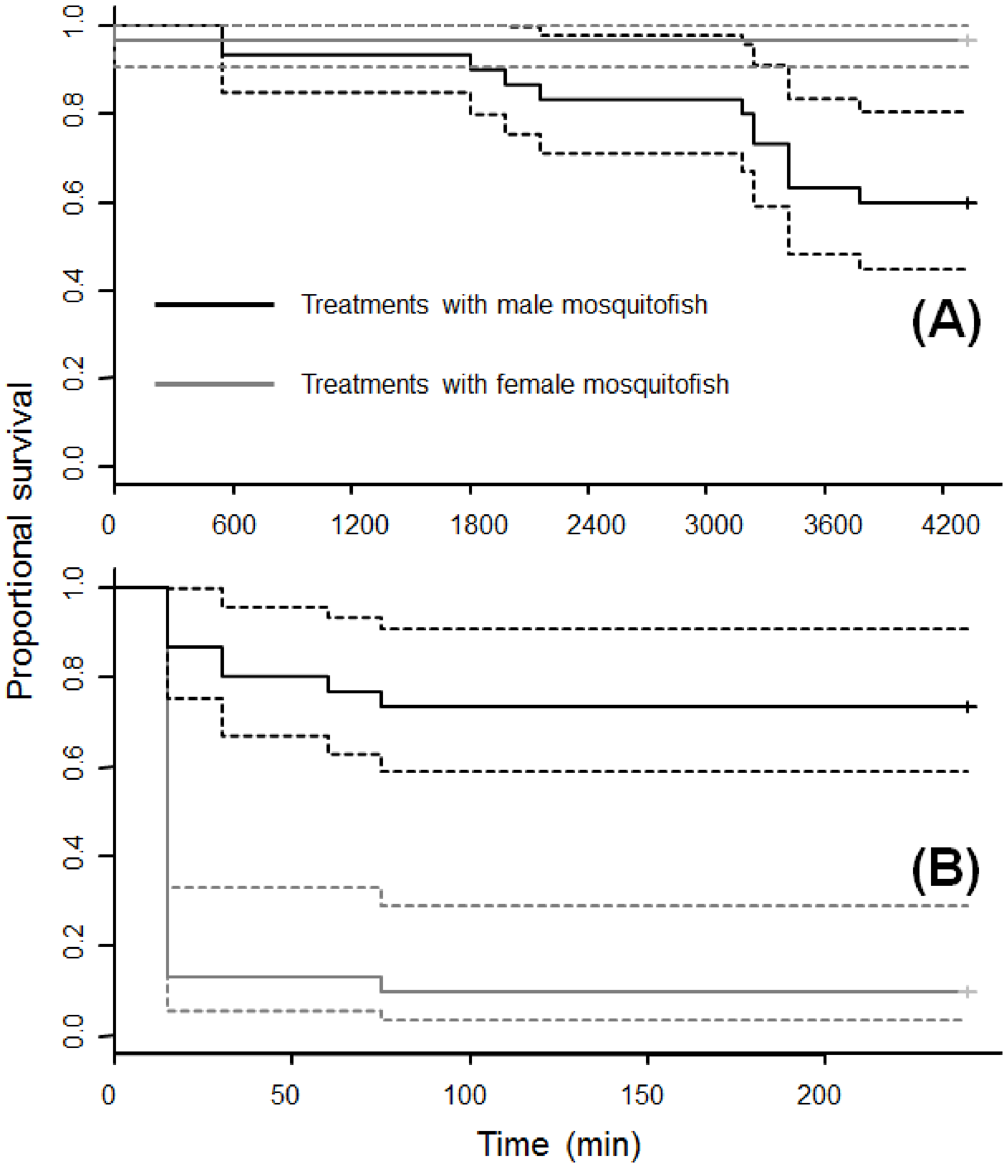

Figure 3.2. Kaplan-Meier estimates of proportional survival of prey during predation trials for (A) adult western mosquitofish (*Gambusia affinis*) prey with adult Mohave tui chub (*Siphateles bicolor mohavensis*) as predators and (B) Mohave tui chub larval prey with adult western mosquitofish as predator. Solid lines indicate Kaplan-Meier proportional survival function and dashed lines indicate 95% confidence intervals. Black and grey lines indicate the treatments with male and female mosquitofish respectively.

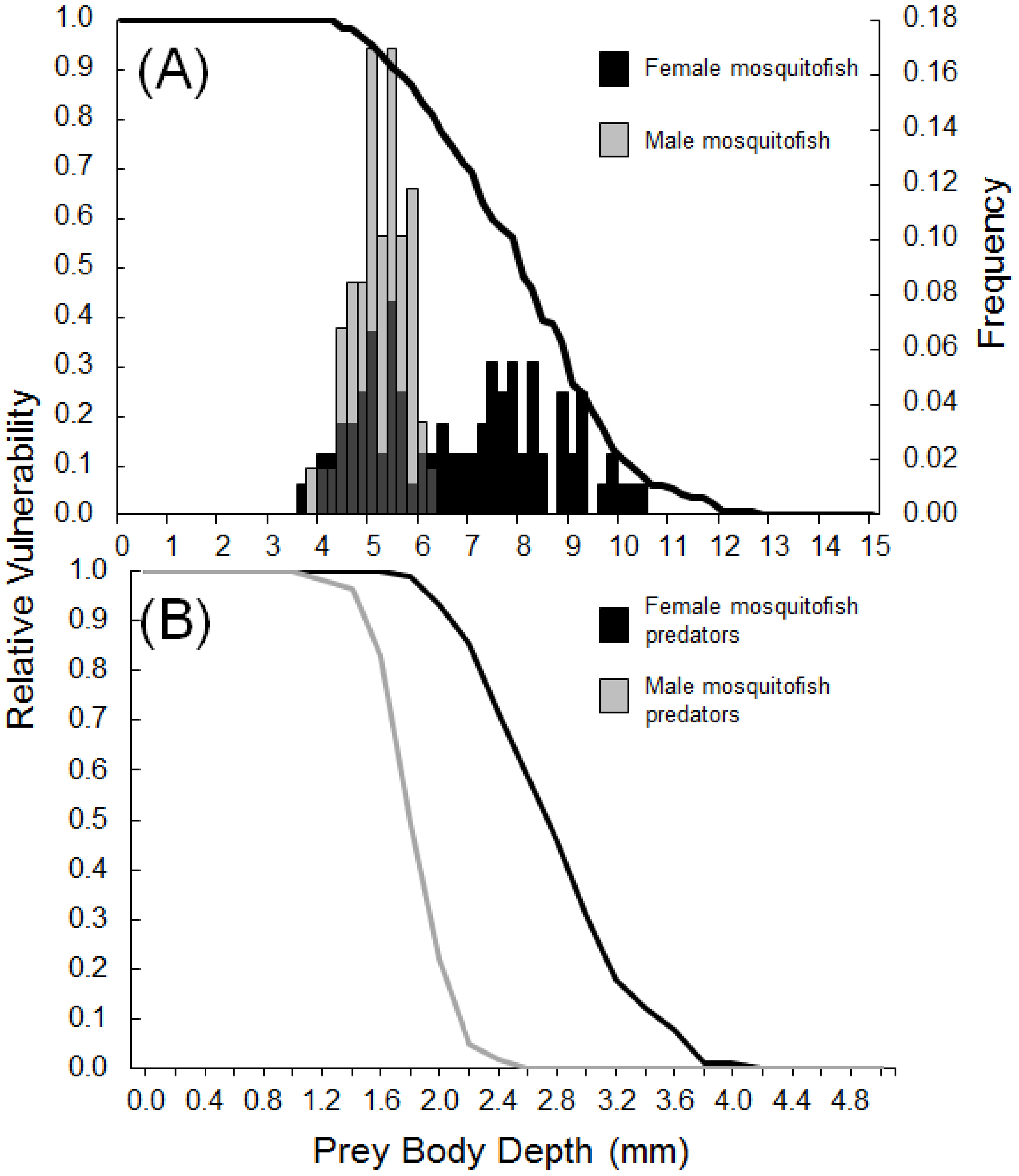

Figure 3.3. Relative vulnerability (lines) of (A) mosquitofish (*Gambusia affinis*) prey under adult Mohave tui chub (*Siphateles bicolor mohavensis*) predation and (B) Mohave tui chub larval prey under adult mosquitofish predation. Frequency distribution of body depths of mosquitofish (A) are indicated with vertical bars and were constructed based on both male (n = 79) and female (n = 90) mosquitofish collected from Lake Tuendae in March-May 2009. The dark gray bars represent the portion of female mosquitofish size distribution overlapped with male mosquitofish size distribution.

Discussion

This study provided experimental evidence that IGP between invasive western mosquitofish and native Mohave tui chub is gape-size limited. Correlated with their smaller size, male mosquitofish had higher vulnerability to tui chub predation than female mosquitofish. Similar results were obtained from two mesocosm experiments, where tui chubs caused low survival rates for male mosquitofish compared to female mosquitofish (Stockwell and Henkanaththegedara 2011; Henkanaththegedara and Stockwell unpublished data). Furthermore, gape-size limitation is indicated by the larger depth/gape ratio of surviving male mosquitofish compared to non-survivors.

The differential predation by male and female mosquitofish on tui chub larvae was also consistent with the gape-size limitation hypothesis. Adult female mosquitofish reduced tui chub larval survival to 10% whereas male mosquitofish only reduced tui chub larval survival to 73%. Two lines of evidence suggest that mosquitofish predation on tui chub larvae was gape-limited. First, tui chub larval survival was notably lower in the presence of female mosquitofish (larger gapes) than in the presence of male mosquitofish (smaller gapes). Second, the depth/gape ratio was significantly smaller than 1.0 for tui chub larvae consumed by female mosquitofish, whereas the few survivors all had relatively larger body depths. The gape-size limitation of mosquitofish predation on larvae of native minnows was also reported where mosquitofish co-occur with native least chub (*Iotichthys phlegethontis*). The survival of large young-of-the year (YOY) least chub was greater than that of smaller YOY least chub (Mills et al. 2004).

Vulnerability modeling showed that gape-size limitation is likely to have important differential effects on survival of male mosquitofish at Lake Tuendae. Male mosquitofish are more vulnerable to predation due to their relatively smaller body sizes compared to females.

Because male mosquitofish growth after sexual maturation is exceptionally slow (Hughes 1986), they are vulnerable to predation throughout their entire lifespan. By contrast, female mosquitofish have indeterminate growth (Hughes 1986), and thus can escape predation risk as they grow. These differential effects may have important implications for life history evolution of mosquitofish. For example, relatively higher predation pressure on adult male mosquitofish may lead to maturation at smaller sizes (Reznick et al. 1990).

All newly hatched tui chub larvae (total length = 6.56 ± 0.04 mm; N = 30) are completely vulnerable to both adult male and female mosquitofish predation. Tui chub larvae may reach a complete size refuge from male and female mosquitofish when they reach a total length of 14.2 mm and 21.3 mm, respectively. However, the vulnerability model proposed by Hambright et al. (1991) is exceptionally liberal, because it assumes complete vulnerability to predation, if prey body depth is less that predator gape-size, i.e. depth/gape ratios ≤1.0. Additionally, depth/gape ratio for consumed prey ranged from 0.7 (mosquitofish predators) to 0.9 (tui chub predators) in our experiments, which would reduce proportion of population vulnerable to predation (also see Truemper and Lauer 2005).

These findings provide an important caveat to the dogmatic view of mosquitofish as a threat whenever they invade. Mosquitofish predation on eggs and/or larvae of native fish has been widely reported as a major threat to the existence of native fish (Meffe 1985; Mills et al. 2004; Rogowski and Stockwell 2006; Pyke 2008). However, it is important to note that many previous studies involved native species with relatively small body sizes, compared to the size of mosquitofish. To further evaluate if mosquitofish impacts are limited to fishes with relatively smaller body sizes, I conducted a literature review. I searched Google scholar using the following keywords; mosquitofish, *Gambusia*, impacts, native fish.

In 13 of 17 case studies, mosquitofish had negative impacts on small bodied native fish species (generally less than 65 mm; Table 3.3). By contrast, the remaining four studies included larger bodied fish species and showed co-existence of both species presumably due to reciprocal predation (Blaustein 1991; this study) or habitat partitioning (Barrier and Hicks 1994) despite mosquitofish predation on larval/egg of the native species. For example, mosquitofish co-existed with black mudfish *Neochanna diversus* (grows up to 110 mm), despite mosquitofish predation on their larvae (Barrier and Hicks 1994; Ling 2004).

Size structured IGP has been suggested as an important mechanism allowing co-existence of various interacting predatory communities (Polis et al 1989; Holt and Polis 1997). In this case, IGP interactions may explain the continued, long-term persistence of native Mohave tui chub with invasive mosquitofish (Henkanaththegedara and Stockwell unpublished data). In fact, Mohave tui chub has co-persisted with western mosquitofish up to 9 years at Lake Tuendae (Steve Parmenter Personal Communication) and up to 27 years at China Lake (U.S. Fish and Wildlife Service 1984). Our results suggest that a better understanding of trophic interactions may shed light on the mechanism(s) that facilitate the persistence of native species in the presence of invasive species.

Table 3.3. Size-dependent impacts of western mosquitofish, *Gambusia affinis* and eastern mosquitofish, *G. holbrooki* (indicated with †) on native fish species. Approach code: ME = mesocosm experiments, FO = field observations, LE = laboratory experiments, FE = field experiments, FS = field surveys.

	Species impacted	Mosquitofish interaction(s) with native fish species	Negative Impact	Maximum Body size (mm)	Approach	Reference
1	Dwarf livebearer† *Heterandria formosa*	Significant negative effect of population growth	Yes	36	ME	Lydeard & Belk 1993
2	Dwarf livebearer† *H. formosa*	Size selective predation on small individuals	Yes	36	ME	Belk & Lydeard 1994
3	White Sands pupfish *Cyprinodon tularosa*	Significant impact on population size and biomass	Yes	50	ME	Rogowski & Stockwell 2006
4	Spanish toothcarps† *Aphanius iberus* and *Valencia hispanica*	Heavy predation on juveniles	Yes	52*	ME	Rincon et al. 2002
5	Big Bend gambusia Gambusia gagei	Endangerment of local populations	Yes	54	FO	Minkley & Deacon 1968
6	Pacific blue-eye† *Pseudomugil signifer*	Lack of recruitment and reduced growth of adults	Yes	56**	ME	Howe et al. 1997
7	Gila topminnow *Poeciliopsis occidentalis*	Rapid replacement from most of its native range	Yes	60	FO	Minkley & Deacon 1968
8	Sonoran topminnow *P. occidentalis*	Replacement in native range possibly by Predation on of juveniles	Yes	60	LE & FE	Meffe 1985
9	Sonoran topminnow *P. o. sonorensis*	Population decline by presumed mosquitofish predation	Yes	60	FO & FS	Galat & Robertson 1992
10	Least chub *Iotichthys phlegethontis*	Reduction of survival and growth rate of larva/juveniles by predation.	Yes	64	FE	Mills et al. 2004
11	Rainbowfish *Rhadinocentris ornatus*	Apparent displacement from native habitat	Yes	65**	FO	Arthington 1984

Table 3.3. (continued)

	Species impacted	Mosquitofish interaction(s) with native fish species	Negative Impact	Maximum body size (mm)	Approach	Reference
12	White River springfish *Crenichthys baileyi*	Population decline by presumed mosquitofish predation	Yes	90	FB & FS	Deacon et al. 1964
13	Barrens topminnow *Fundulus julisia.*	Reduced survival of larva/juveniles by predation , Injury risk to adults	Yes	94	LE	Laha and Mattingly 2007
14	Black mudfish *Neochanna diversus*	Predation of mudfish larvae Overall coexistence may be due to spatial / temporal habitat partitioning	Yes No	106*	LE & FO	Barrier and Hicks 1994
15	Mohave tui chub *Siphateles bicolor mohavensis*	Predation of tui chub larvae Predation of mosquitofish by adult tui chubs	Yes No	300*	ME & LE	Henkanaththegdara and Stockwell unpublished
16	Green sunfish *Lepomis cyanellus*	Predation of sunfish larvae Predation of mosquitofish by adult sunfish	Yes No	310	FE	Blaustein 1991
17	Largemouth bass *Micropterus salmoides*	Population reduction of mosquitofish due to predation	No	970	FE	Nowlin et al. 2006

* Specific maximum body size was extracted from the reference cited; **Pusey et al. 2004, other values according to Page and Burr 1991.

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CHAPTER 4. CONSERVATION TRANSLOCATIONS BENEFIT PROTECTED FISH, BUT MAY IMPACT ENDEMIC INVERTEBRATES

Abstract

Desert springs, which harbor diverse and endemic invertebrate assemblages, are often used as refuge habitats for protected fish species. Additionally, these springs may also be invaded by non-native invasive fish species. The impact of recently established fish populations on the unique invertebrate communities has not received much attention. I conducted a mesocosm experiment to assess the impact of both protected and invasive fish species on community structure of spring-dwelling invertebrates. Invertebrate communities were established in large mesocosms with one of four treatments: 1) fishless, 2) invasive western mosquitofish, *Gambusia affinis* 3) endangered Mohave tui chub, *Siphateles bicolor mohavensis* and 4) both fish species in sympatry. Final populations of invertebrates and fish were sampled, sorted, identified and counted after 67 days. Heterogeneity of the invertebrate communities and densities of major invertebrate taxa were compared among the four treatments. The diversity of invertebrate communities in experimental mesocosms was negatively impacted by the presence of fish. Further, model selection also showed a negative association between fish and invertebrates. Invertebrate community structures changed mainly due to population declines and local extirpations of invertebrates, presumably due to fish predation. In fish treatments, densities of Crustaceans and Chironomid larvae dropped and Cladocerans were virtually eliminated. An NMDS analysis showed a strong disassociation of the majority of invertebrate taxa among the different fish treatments. Native protected fish transplanted to fishless desert springs may also

have negative impacts on unique spring-dwelling invertebrate communities, in addition to widespread invasive fish species. Therefore, invertebrate communities should be surveyed when desert springs are evaluated as potential refuge habitats for protected fish species translocations.

Introduction

Desert springs function as “keystone” ecosystems playing a major role in evolutionary process and regional biodiversity (Stevens and Meretsky 2008). In arid regions of the southwestern United States, these patchily distributed springs harbor highly diverse faunal assemblages, especially fish, mollusks and aquatic insects (Soltz and Naiman 1978, Shepard 1993, Sada and Vinyard. 2002). Furthermore, desert springs host the highest number of endemic taxa in North America (Stevens and Meretsky 2008) making them one of the conservation priorities of the region (Williams et al. 1985; Shepard 1993).

Recent studies have uncovered unique, highly diverse and endemic macro-invertebrate assemblages from these desert springs (Hershler and Sada 1987, 2002; Shepard 1990; Polhemus and Polhemus 2002). Despite their ecological and evolutionary importance, most of these invertebrate taxa are poorly studied (Williams et al. 1985; Sada and Vinyard. 2002) and threatened with extinction risk due to anthropogenic impacts (Shepard 1993; Unmack and Minckley 2008). For an example, 13 new Cochliopid spring snails have been recently discovered from Chihuahua Desert; however, two of these species were extinct prior to formal description (Hershler et al. 2011). Major threats for desert springs and its fauna include water mining, habitat alterations, and introduction of non-native species (Williams et al. 1985; Shepard 1993; Unmack and Minckley 2008).

Many desert springs have been stocked with non-native fish (Pister 1974; Unmack and Minckley 2008) to control mosquito-borne diseases (western mosquitofish *Gambusia affinis*; Meffe 1985), promote sport fishing (large-mouth bass *Micropterus salmoides*; Soltz and Naiman 1978), as breeding ponds for aquarium fish (sailfin molly *Poecilia latipinna*; Deacon and Williams 1991) and more recently as refuge habitats for protected fish species (Hendrickson and Brooks 1991; Minckley 1995; Hendrickson and Minckley 1985). Collectively, many non-native fish introductions to desert springs have negatively impacted native fish populations, sometimes leading to their extirpations (Pister 1974; Moyle 1976; Soltz and Naiman 1978).

Virtually all work concerning fish impacts on invertebrate communities has focused on the effects of invasive mosquitofish (*Gambusia affinis* and *G. holbrooki*). The impact of invasive mosquitofish on invertebrate communities has been documented from many aquatic systems (Hurlbert et al. 1972; Englund 1999; Angeler et al. 2002; Pyke 2008; Stockwell and Henkanaththegedara 2011). Hurlbert et al. (1972) reported elimination of Cladocera and significantly reduced densities of rotifers, crustaceans and aquatic insects in experimental mesocosms as a result of predation by western mosquitofish (*G. affinis*). Further, *Linderiella occidentalis*, a fairy shrimp species, endemic to California vernal pools, had lower survival in experimental ponds sympatric with western mosquitofish compared to control ponds (Leyse et al. 2004). Despite this work, very little work has considered desert spring systems which have often been subjected to mosquitofish introductions to control mosquito-borne diseases (Pister 1974; Moyle 1976; Soltz and Naiman 1978).

In addition to non-native fish, protected native fish species have also been transplanted to desert springs as a widely practiced conservation management strategy (Hendrickson and Brooks 1991; Minckley 1995; Hendrickson and Minckley 1985). Twenty four endemic fish species in the Great Basin have been transplanted to aquatic systems within and outside of their historic range (Sada and Vinyard 2002). In fact, managers actively transplanted endangered Gila topminnow (*Poeciliopsis occidentalis occidentalis*) in to natural springs and ciénegas during vigorous reintroductions in the 1960s and 1970s (Hendrickson and Minckley 1985; Hendrickson and Brooks 1991). Likewise, endangered Mohave tui chub (*S. b. mohavensis*; Miller 1968; Hoover and St. Amant 1983) and Pahrump poolfish (*Empetrichthys latos latos*; Shawn Goodchild Personal Communication) were transplanted to fishless springs as a strategy to increase the security of these fish species. Such conservation transplants have successfully reduced extinction probability for many protected fish species (Minckley 1995; Johnson and Hubbs 1989; Hendrickson and Brooks 1991), but these activities may have unexpected negative impacts on spring-dwelling native invertebrates. In addition to spring habitat modifications (e.g. damming to create pools) to create secure refuges for protected native fish (Shepard 1993; Hershler 1989), direct predation by newly established fish populations may have negative impacts on spring-dwelling invertebrate communities.

To my knowledge, the potential impact of fish on spring-dwelling invertebrate communities has never been quantified with controlled experiments. Previously, I used mesocosm experiments (Stockwell and Henkanaththegedara 2011, Henkanaththegedara and Stockwell unpublished data) to evaluate the interactions between the protected Mohave tui chub (*S. b. mohavensis*) and invasive western mosquitofish (*G. affinis*). Here I take advantage of this experiment to contrast the invertebrate communities of mesocosms with experimental fish populations to a series of fishless control mesocosms, established simultaneously. This experimental approach allowed us to gain valuable insights to the potential impacts of fish introductions on spring-dwelling invertebrate assemblages.

Methods

Study system

The Mohave tui chub is endemic to the Mojave River Drainage (Hubbs and Miller 1943) and it was restricted to deep pools of the main stream Mojave River and at least one land locked refuge population close to Soda Dry Lake (i.e. Zzyzx), the terminal sink of the Mojave River (Miller 1938). However, the river populations have extirpated by late 1960's (Miller 1969). A series of transplant attempts was initiated in early 1950's to reduce the extinction risk of the Mohave tui chub (Miller 1968; Hoover and St. Amant 1983). Nevertheless, only China Lake and Camp Cady populations survived to-date. As a result of its very restricted range and potential impacts of non-native species, the Mohave tui chub was listed as a federally endangered species (U.S. Fish and Wildlife Service 1984).

Zzyzx has several desert springs of which, two harbor populations of the endangered Mohave tui chub (*Siphateles bicolor mohavensis*). The larger tui chub population co-habits Lake Tuendae with the invasive western mosquitofish (*Gambusia affinis*) and a small population of Saratoga Springs pupfish (*Cyprinodon nevadensis nevadensis*). Lake Tuendae is a highly modified desert spring (Turner and Liu 1976) approximately 140 × 40 m in size with a maximum depth reaching about 2 m. The other Zzyzx tui chub population inhabits a relatively small spring called MC Spring (4 × 5 m and approximately 1 m deep), approximately 300 m south of Lake Tuendae.

Recently the interest in Mohave tui chub was sparked by the discovery of western mosquitofish at Lake Tuendae in 2001 (Steve Parmenter Personal Communication), leading managers to recommend research concerning the effects of mosquitofish on Mohave tui chub and to more aggressively pursue recovery of the Mohave tui chub (U.S. Fish and Wildlife Service 1984; Hughson and Woo 2004). The down-listing of the Mohave tui chub requires the establishment of additional populations, making an overall of 6 geographically isolated self-sustaining populations with 500 breeding fish for 5 years (U.S. Fish and Wildlife Service 1984). Nevertheless, potential aquatic habitats as refuges are limited in desert ecosystems and some have previously been invaded by mosquitofish. Recent findings that Mohave tui chub may be able to co-persist with mosquitofish (Stockwell and Henkanaththegedara 2011; Henkanaththegedara and Stockwell unpublished data), led managers to introduce tui chubs to Deppe Pond in 2008, a site inhabited by western mosquitofish (Matt Huffine and Steve Parmenter Personal Communications). With this recent transplant, Mohave tui chub currently occupies five habitats, and co-occurs with western mosquitofish in three of these habitats. However, two additional populations of Mohave tui chub need to be successfully established for the recovery.

Experimental setup

I conducted a mesocosm experiment mimicking the spring environment in large mesocosms. Mesocosms were deployed at the Desert Studies Center at Zzyzx, California from 8 March 2009 to 12 May 2009. Thirty five large circular mesocosms were employed to host experimental populations of aquatic invertebrates in the absence and presence of Mohave tui chub and/or western mosquitofish. At the onset of experiment, mesocosms were filled with water from Lake Tuendae to introduce invertebrate communities, but filtered through 1.18 mm mesh to exclude larval fish (may have excluded some macro-invertebrates too). Each tank was provided with a constant aeration system, equal amounts of plastic “plants” to provide cover and substrate, and poultry fence to exclude avian predators.

The mesocosms were randomly assigned to one of three fish treatments each with 10 replicates: allopatric Mohave tui chubs (MTC); allopatric western mosquitofish (WMF); and Mohave tui chubs sympatric with western mosquitofish (MTC + WMF). Lastly, the remaining five mesocosms were left fishless as controls. The thirty mesocosms hosting fish populations and two of the fishless mesocosms were established in tanks with a diameter of 1.8 m (volume ~ 1.2 m^3), but the three other fishless mesocosms were hosted in larger tanks with diameter of 2.5 m (volume ~ 2.6 m^3). Because this work focuses on invertebrate densities, the difference in volume was not expected to generate substantive effects. In fact, the final invertebrate densities between the large and small tanks, were not significantly different (Wilcoxon rank sum test; Table 1) indicating the lack of tank size effects on invertebrate densities.

Table 4.1. The final invertebrate densities in small (volume ~ 1.2 m^3) and large (volume ~ 2.6 m^3) tanks used for fishless control treatment.

Invertebrate Group	Average density (per L ± SE)		W	p-value
	Small tank (n=2)	Large tank (n=3)		
Rotifers	39682.0 (± 20918.0)	63033.3 (± 5068.9)	1.0	0.40
Crustacean nauplii	79.5 (± 2.5)	66.3 (± 3.8)	6.0	0.20
Cladocera	554 (± 58.0)	528.0 (± 42.4)	4.0	0.76
Ostracods	3.5 (± 3.5)	4.0 (± 0.58)	3.0	1.00
Calanoid copepods	41.0 (± 18.0)	50.3 (± 14.2)	2.0	0.80
Cyclopoid copepods	210.0 (± 102.0)	134.3 (± 56.3)	5.0	0.37
Chironomid larvae	6.0 (± 2.0)	3.0 (± 1.5)	4.5	0.55
Water mites	24.0 (± 4.0)	9.0 (± 3.1)	6.0	0.20

This design allowed us to evaluate the impacts of the invasive western mosquitofish (mimicking fish invasion) as well as endangered Mohave tui chub (mimicking fish transplants) on spring-dwelling invertebrate communities. In addition, the sympatric treatments allowed us to understand the combined effect of both invasive and native fish species on invertebrates, which represents the contemporary conditions of many desert springs.

Tanks were stocked with adult Mohave tui chubs and mosquitofish captured from Lake Tuendae using minnow traps and hand nets. Mesocosms receiving Mohave tui chubs (MTC and MTC+WMF) were each stocked with 8 adult Mohave tui chubs of typical size range (80-120 mm). Mesocosms receiving mosquitofish (WMF and MTC+WMF) were each stocked with 25 male and 50 female mosquitofish. Relative densities of the two species and the sex ratio of mosquitofish were chosen to reflect relative densities and sex ratios in Lake Tuendae. I did not include Saratoga Springs pupfish, because this species was at very low density when our experiments were conducted. Fish were fed ground pelleted fish food at a ration of 4% of stocked fish biomass per day.

Sampling and diversity analysis

Upon conclusion of the experiment, aquatic invertebrates were collected using a 2.0 L Van Dorn type horizontal water sampler. Two water samples, one close to the surface and the other close to the bottom, were collected from each mesocosm and filtered through a 65 µm mesh net funnel to recover invertebrates. The recovered material was fixed in 10% sugar-formalin (Lind 1985) and a drop of Rose-Bengal stain (stains animal proteins in red) was added to each vial before laboratory analysis to enhance the visibility. All surviving fish including larval stages were collected and enumerated by seining each tank 5 times and then filtering all tank water through a fine mesh net.

In the laboratory, samples were filtered through a 65 µm mesh net funnel again and the materials were suspended in 50 ml of water. Suspended invertebrates were sub-sampled (5.0 ml) five times and counted using a counting wheel under a stereo-microscope. Invertebrates were identified to major taxonomic levels using identification keys provided by Pennak (1989). I restricted our analysis only to the invertebrate taxa which had a cumulative sum of more than 2 individuals (i.e. Rotifera, Cladocera, Ostracoda, Calanoid copepods, Cyclopoid copepods, Chironomid larvae, Hydracarina and Crustacean nauplii) due to extreme rarity of such taxa and to avoid associated zero truncation problem where absence of a particular taxa (zeros) may constrain the analysis (Beals 1984).

Invertebrate densities were converted to number per liter before analyses. The diversity of invertebrate communities of different treatments was estimated with Shannon-Weiner Diversity Index (H’; Krebs 1999). Where, p_i = Proportion of species i in the community.

$$H' = -\sum_{i=1}^{s}(p_i)(\log_2 p_i)$$

Fish diet analysis

I examined diet for 30 adult Mohave tui chubs and 30 adult western mosquitofish which were collected simultaneously from Lake Tuendae in February 2008. The biomass of tui chub (nearest 0.1 g) and mosquitofish (nearest 0.0001 g) were obtained using digital scales and the entire gastrointestinal tract was extracted. Contents from the first 1/3 of the gut was removed, sorted, identified to the lowest possible taxa using standard keys and counted (Numerical Method; Windell 1968). This analysis was restricted to the same invertebrate taxa considered for the mesocosm experiments for comparative purposes and due to comparative rarity of other taxa in fish guts. However, amphipods were included for this analysis due to its high abundance in fish guts. Also Calanoid and Cyclopoid copepods were lumped together (Copepods). The percentage occurrence was estimated by estimating the percentage of all stomachs containing food in which each food category occurred (Wallace 1981). Percentage composition was estimated as the percentage of each food category contributed to the total number of food items in all stomachs by pooling data for all fish (Wallace 1981).

Statistical analysis

All statistical analyses were conducted using R statistical software program (Version 2.11.0; R Development Core Team 2010). For invertebrate densities and Shannon-Weiner diversity (H'), data were tested for normality with a Shapiro-Wilk normality test (*shapiro.test* function). Because data were not normally distributed, a Kruskal-Wallis rank sum test (*kruskal.test* function) was used to test for treatment effects. Separate tests were run for each invertebrate group using population density as the response variable and treatment as the explanatory variable. Post-hoc analyses were conducted by performing a Tukey HSD test (*TukeyHSD* function) on ranked data while maintaining a 0.05 experimental-wise error rate (Zar 2010).

Both number of invertebrates in each gut as well as weighted number of invertebrates were used for the analysis of fish diet. Weighted numbers were estimated to standardize the data by dividing number of invertebrates with fish biomass due to prominent size differences between these fish species. Number of invertebrates and weighted number of invertebrates in guts of tui chub and mosquitofish were compared non-parametrically using Wilcoxon rank sum test (*Wilcox.test* function) due to highly skewed nature of these data.

Nonmetric multidimensional scaling (NMDS) was performed to reduce the taxa matrix of eight to two dimensions. The best two-dimensional solution of 10,000 random starts was chosen and we generated the coefficient of determination (R^2) of the final simulated distance matrix which was regressed against the actual distance matrix to assess how much of the original distances were preserved in our solution. Stress ~ 0.20 was considered an adequate solution (McCune and Grace 2002). Because the difference between the most abundant taxa and least abundant taxa was a magnitude of four, the taxa abundances were relativized by the maximum to equalize the impact of individual taxa on the ordination. Final ordination was performed based upon a Bray Curtis distance measure. To show taxa relationships to modeled axes, Pearson Correlation Coefficients for each taxa against the NMDS axes were generated and showed these as directional vectors on the ordination plot with site scores relativized by their maximum.

The two NMDS axes scores were separately used in a multiple regression along with model selection using the small sample size corrected Akaikie Information Criteria (AICc; Burnham and Anderson 2002) to determine the best suite of treatment variables influencing the invertebrate community. Six models were determined a priori as follows: (1) null model featuring the simple average as the best predictor, (2) additive factorial model with levels including Mohave tui chubs and mosquitofish, (3) multiplicative factorial model as above but including an interaction between Mohave tui chubs and mosquitofish, (4) factorial design with "fish" or "no fish" as levels, (5) covariate model with total number of fish as a predictor without respect to species, and (6) a covariate model including numbers of Mohave tui chubs and mosquitofish further delimited into age categories: adult and larvae. The coefficient of determination was generated to determine goodness of fit for the best model on each axis. Statistical analyses were conducted in R using packages "vegan" and "labdsv".

Results

The diversity of invertebrate communities in experimental mesocosms was impacted by the presence of fish. The mean heterogeneity (H') of invertebrate taxa showed significant differences among treatments (X^2_3=25.56; $p < 0.001$). Post-hoc pairwise comparisons showed significantly low invertebrate diversity in sympatric fish treatment and allopatric mosquitofish treatment compared to both allopatric tui chub treatment and fishless control. This indicates a clear reduction of invertebrates in the treatments with mosquitofish compared to both treatments with tui chubs and fishless control (Figure 4.1).

Invertebrate community structures also changed mainly due to population declines and local extirpations of invertebrates, presumably due to fish predation. Treatments with fish had lower densities of invertebrates compared to fishless treatment except for rotifers and water mites (Figure 4.2). The density of mature forms and nauplii of Crustaceans (except Ostracods) and Chironomid larvae showed significant differences among treatments (Figure 4.2). For example, smaller bodied-mobile crustaceans (Cladocera and Crustacean nauplii) had low densities within mosquitofish treatments. Larger bodies-mobile crustaceans (Calanoid and Cyclopoid copepods) had lower densities in the presence of fish, but the density of these invertebrates did not differ among the three fish treatments. The lowest density of Chironomid larvae was reported with allopatric tui chub treatment which is marginally significant ($p = 0.060$) compared to fishless control (Figure 4.2). The densities of Rotifers, Ostracods and Hydracarina water mites had no significant differences among treatments (Figure 4.2).

NMDS achieved a two-dimensional solution for invertebrate community assemblages with a Stress = 0.21 (Figure 4.3). We identify 3 distinct clusters for the allopatric tui chub, allopatric mosquitofish and fishless treatments. The sympatric fish mesocoms containing both tui chubs and mosquitofish are mostly in the upper quadrants but overlap considerably between the allopatric fish treatments as expected (Figure 4.3). All correlation vectors for invertebrate taxa with the exception of rotifers pointed into the lower two quadrants and were strongly associated with experimental units with no fish.

Model selection indicated that the best predictors for the second axis was “fish/no fish” model (Multiple Regression R^2adj = 0.75) such that there were more invertebrates where fish did not occur (Table 4.2). The best model selected for the first axis was the multiplicative model that included an interaction between Mohave tui chub and mosquitofish (Multiple Regression R^2_{adj} = 0.57; Table 4.2). Thus, there were fewer invertebrates overall (i.e., second axis result) in experimental units containing fish, but the invertebrates that were found in these samples tended to clump into two major groups. Benthic invertebrates, including Chironomid larvae and water mites were negatively associated with the allopatric tui chub treatments. Crustaceans in the left lower corner of the plot were negatively associated with mosquitofish treatments. Plots with both fish were harder to distinguish based on invertebrate community, suggesting that when both fish species are present they have an effect of increasing diversity; given any measure of diversity that includes evenness.

Table 4.2. Akaikie Information Criteria with the small sample size correction on six candidate models used to model scores from a two-dimensional NMDS ordination. Abbreviations are MF = mosquitofish, MTC = Mohave tui chub, Fish/No Fish = presence or absence of fish, Total Fish = counts of fish present in experimental tanks, and Age Structure = a four parameter model where MF and MTC are further divided into adults and larval fish with counts used as predictors.

		Axis 1		Axis 2	
Model	K	ΔAIC_c	w_c	ΔAIC_c	w_c
MF*MTC	5	0.00	0.97	2.42	0.23
MF+MTC	4	12.23	0.00	8.69	0.01
Fish/No Fish	3	21.41	0.00	0.00	0.76
Total Fish	3	26.45	0.00	18.69	0.00
Age Structure	6	7.13	0.03	9.07	0.01
Null	2	39.27	0.00	16.35	0.00

Fish diet analysis provided additional information to understand fish impacts on invertebrates. I did not find any completely empty guts in any of the fish examined. The percentage occurrence of invertebrates differed among invertebrate taxa (Figure 4.4-A). For example, Crustacean Nauplii were the least commonly observed taxa in both mosquitofish and tui chubs. By contrast, Chironomid larvae occurred in 90% of tui chubs and 83% of mosquitofish making it the most common food item in the guts of both fish species (Figure 4.4-A). I found significantly higher numbers of Ostracods (w = 589; p = 0.016), Chironomid larvae (w = 638.5; p 0.005) and Amphipods (w = 693; p < 0.001; Figure 4.4-B) in tui chubs compared to mosquitofish. By contrast, mosquitofish had significantly higher number of Cladocerans (w = 328; p = 0.04), Chironomid larvae (w = 163.5; p < 0.001) and Amphipods (w = 637; p < 0.01) after adjusting the numbers for fish biomass, indicating unparalleled impact of mosquitofish on invertebrates regardless of its smaller body size (Figure 4.4-C). Furthermore, percentage composition showed the importance of different invertebrate taxa as major food items. Collectively, Chironomid larvae represented more than 50% of the food items in both tui chubs and mosquitofish. Furthermore, mosquitofish had a higher affinity for Cladocera (18%) and tui chub for Copepods (34%) followed by Chironomid larvae (Figure 4.5).

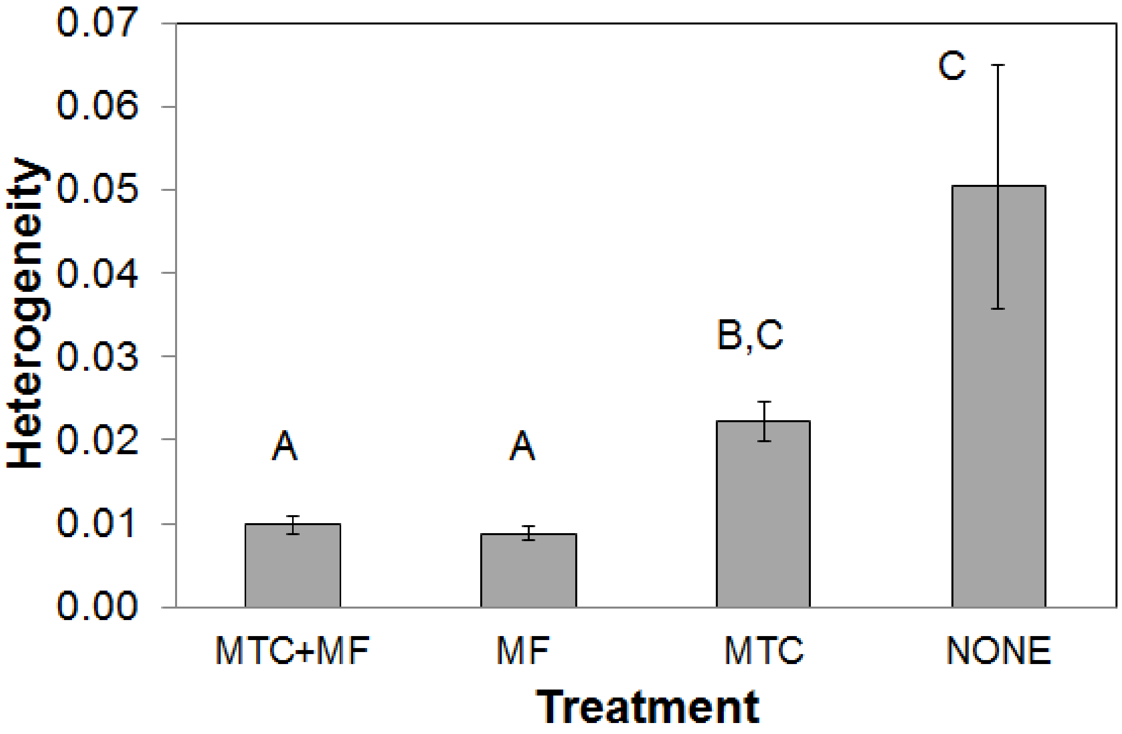

Figure 4.1. The mean heterogeneity (Shannon-Wiener Index, H') of aquatic invertebrate diversity was significantly differed among treatments (X^2_3=25.56; p < 0.001). Treatment codes: MTC+MF = sympatric fish, MF only = allopatric mosquitofish, MTC only = allopatric tui chubs, and NONE = fishless control. Error bars are one standard error. Bars with different letters are significantly different at p = 0.05.

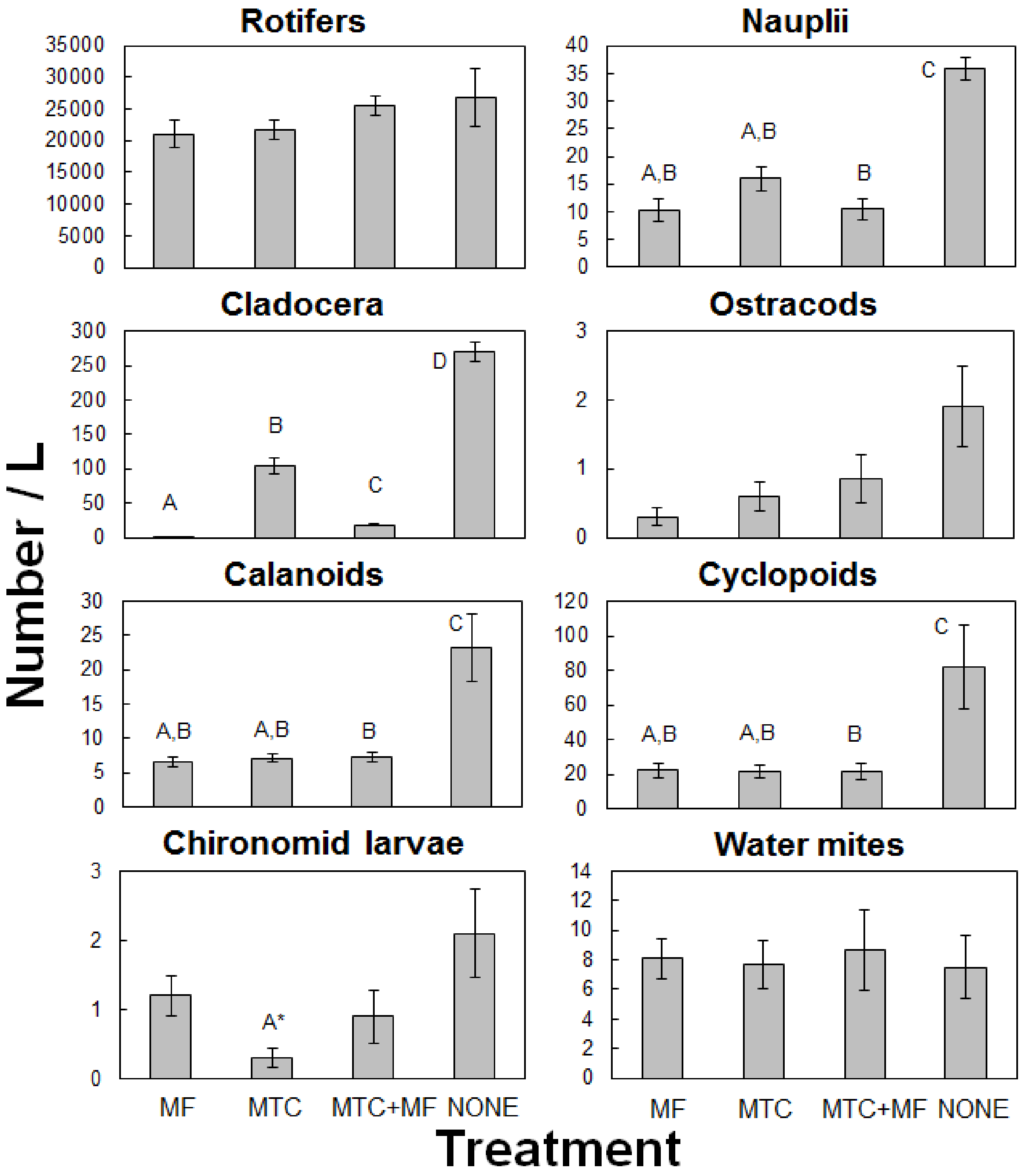

Figure 4.2. Differences in mean density of major invertebrate taxa among treatments. Significant differences of mean densities were observed for Nauplii ($X^2_3 = 14.75$; $p < 0.01$), Cladocera ($X^2_3 = 31.93$; $p < 0.05$), Calanoid copepods ($X^2_3 = 12.75$; $p < 0.01$), Cyclopid copepods ($X^2_3 = 10.61$; $p < 0.05$), and Chironomid larvae ($X^2_3 = 7.83$; $p < 0.05$). Treatment codes: MTC+MF = sympatric fish, MF only = allopatric mosquitofish, MTC only = allopatric tui chubs, and NONE = fishless control. Error bars are one standard error. Bars with different letters are significantly different at $p = 0.05$.

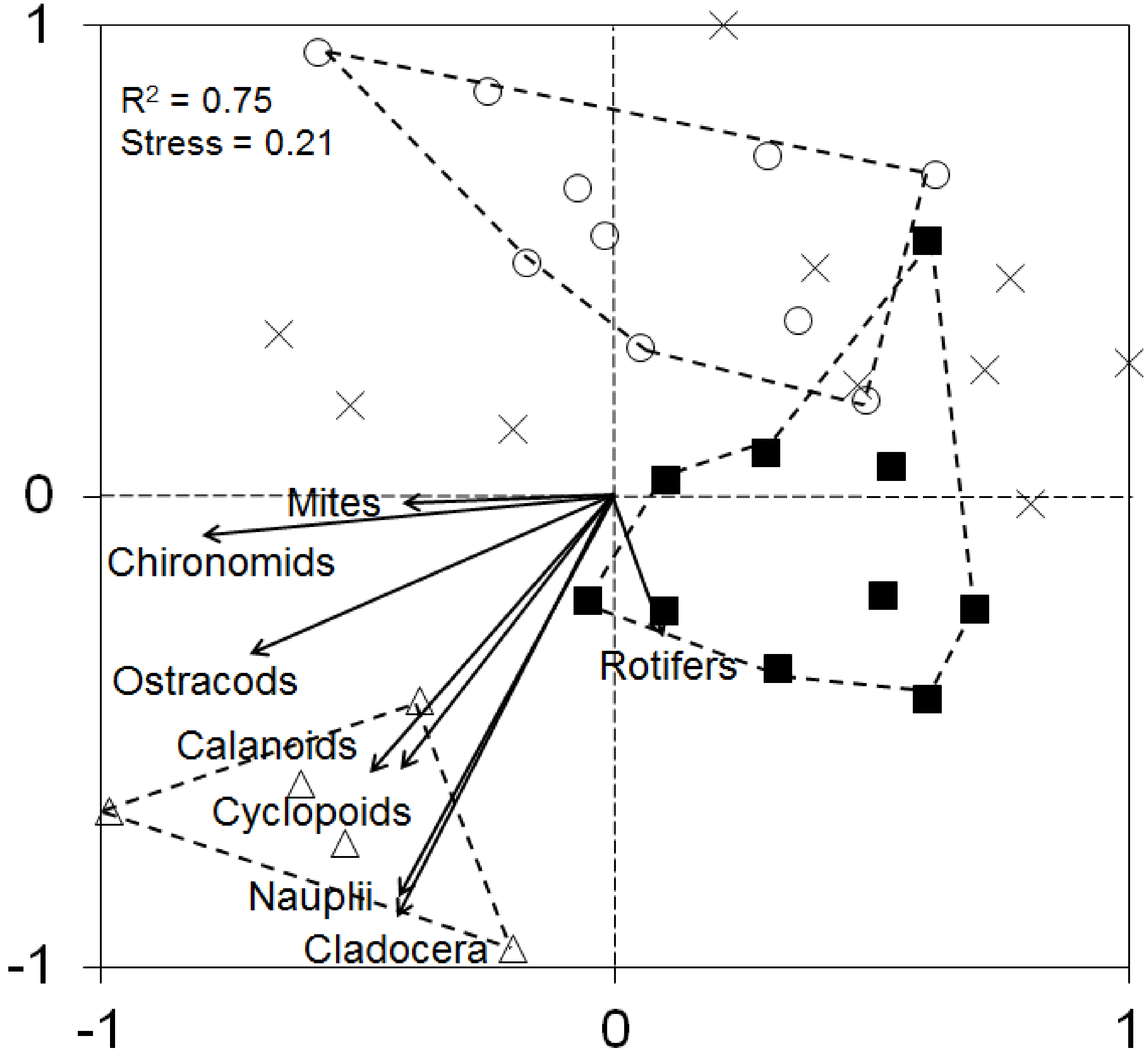

Figure 4.3. Nonmetric multidimensional scaling plot where points represent experimental units from treatments: Mohave tui chub only (■), mosquito fish only (○), both Mohave tui chub and mosquite fish (×), and no fish (△). Site scores were relativized by the maximum and vectors represent the Pearson Correlation Coefficients for each taxa included in the ordination. The goodness of fit measure (R^2) is for the relationship between the modeled distance matrix and the actual distance matrix. The NMDS was performed on maximum relativized taxa counts and used the Bray Curtis distance measure.

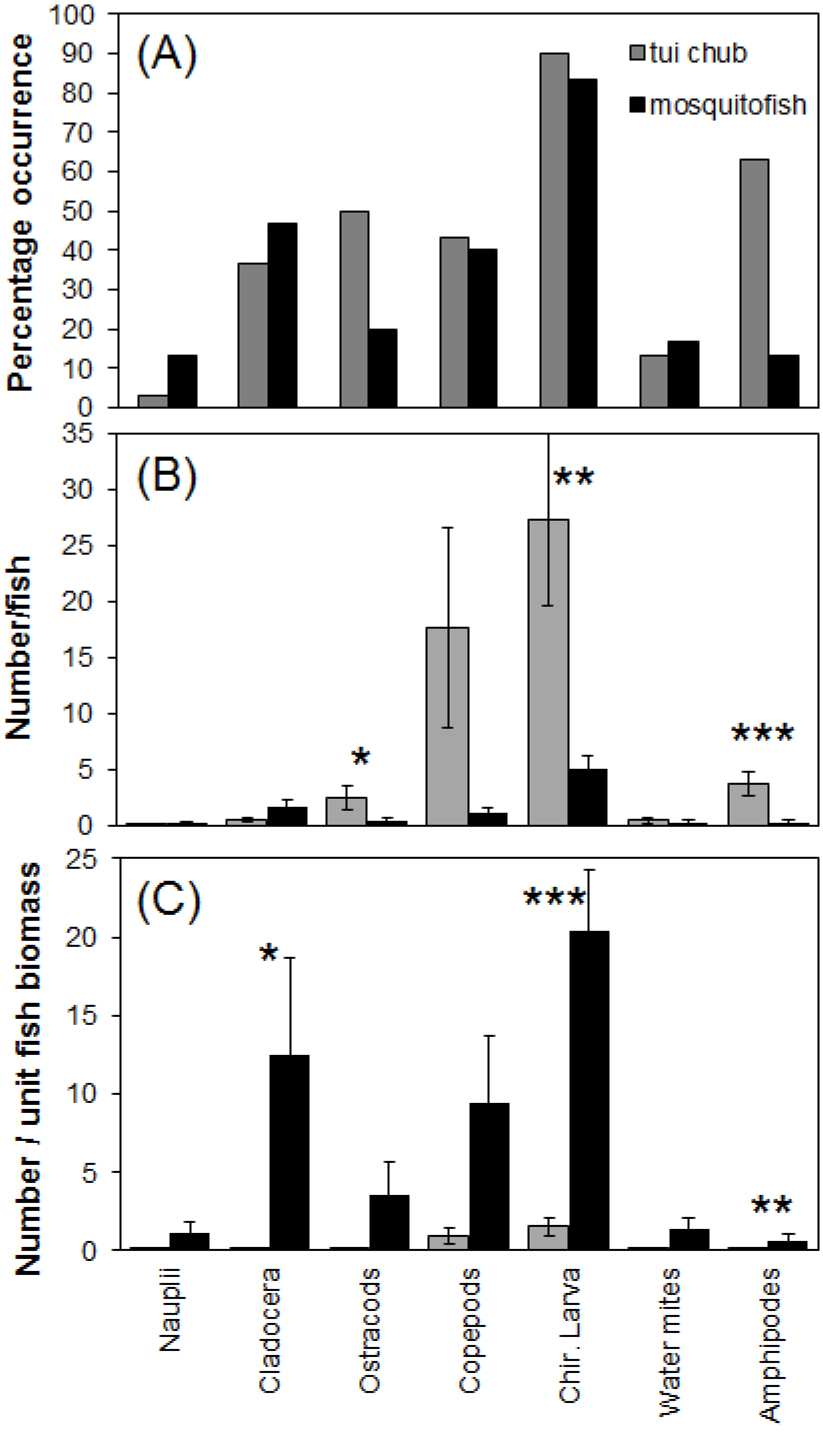

Figure 4.4. The distribution of various invertebrate food items in the guts of tui chub and mosquitofish (n = 30 each). Percentage occurrence (A), average number of invertebrates (B), and average number of invertebrates adjusted for fish biomass (C). Dark grey and black represent Mohave tui chub (*Siphateles bicolor mohavensis*) and western mosquitofish (*Gambusia affinis*), respectively. Error bars are one standard error. Significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

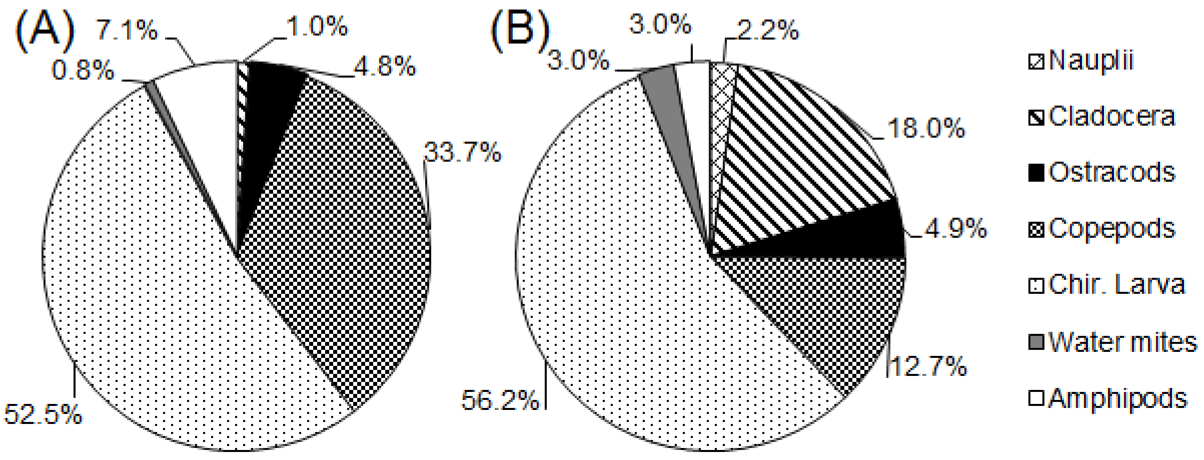

Figure 4.5. Percentage composition of various invertebrate food items in the guts of Mohave tui chub (A) and mosquitofish (B).

Discussion

This study provided experimental evidence for impacts of fish on spring-dwelling aquatic invertebrate communities, presumably due to predation. Specifically, both fish species reduced the diversity as well as the density of invertebrates, but mosquitofish impacts were more severe. The substantial impact of mosquitofish on invertebrate communities is consistent with reports from other systems (Hurlbert et al.1972; Englund 1999; Angeler et al. 2002; Leyse et al. 2004). In fact, diversity as a whole and density of some invertebrates (Crustacean Nauplii and Cladocera) were very low in both allopatric and sympatric mosquitofish treatments (WMF and MTC + WMF) compared to allopatric tui chub treatment (MTC).

Multivariate community analysis and model-selection also indicated the negative impacts of fish on invertebrate community composition. Fishless mesocosms were positively associated with the majority of invertebrate taxa, while mesocosms with fish were negatively associated with invertebrate density, indicating the negative impacts of fish on invertebrates. The scattered nature of sympatric treatments on the ordination plot may be explained by the varying number of surviving mosquitofish within the sympatric mesocosms (range; 0-39; Henkanaththegedara and Stockwell unpublished data). Model-selection also produced evidence for fish impacts on invertebrates indicating multiplicative factorial model (MTC*WMF) as the best suite of treatment variables influencing the invertebrate community. Overall, both univariate and multivariate tests supported the negative impacts of fish on aquatic invertebrates.

These results suggest the impacts of invasive mosquitofish as a very effective predator on aquatic invertebrates. The densities of Copepods, Crustacean nauplii and Chironomid larvae were greatly reduced in our experimental mesocosms by the presence of mosquitofish. Further, Cladocerans were extirpated in 80% of allopatric mosquitofish mesocosms, while they were not extirpated from any tanks in the other treatments. Fish diet analysis also showed the significant impact of mosquitofish on Cladocera, Chironomid larvae and Amphipods after adjusting for mosquitofish biomass. In fact, mosquitofish is a voracious carnivore fish (Pyke 2005, 2008) and similar impacts on invertebrate communities have been widely reported (Hurlbert et al. 1972; Hurlbert and Mulla 1981; Bence 1988; Angeler et al. 2002). Elimination of Cladocerns from mosquitofish treatments have previously been reported for many other systems (Hurlbert et al. 1972; Hurlbert and Mulla 1981; Angeler et al. 2002). For example, enclosure experiments by Angeler et al. (2002) reported absence of Cladocera in treatments with eastern mosquitofish (*G. holbrooki*) compared to controls. In addition, western mosquitofish eliminated *Daphnia pulex* and *Ceriodaphnia* sp. (Cladocera) populations and reduced *Diaptomus pallidus* (Calanoid copepod) and *Keratella quadrata* (Rotifera) populations in experimental ponds (Hurlbert and Mulla 1981).

This experiment also shows that Mohave tui chub can impact invertebrate communities. Tui chub presence reduced the densities of Cladocera, Copepods, Crustacean naulpii, and Chironomid larvae. The lowest Chironomid larval density was reported by allopatric Mohave tui chub treatment and extirpations of Chironomids occurred in 60% of allopatric Mohave tui chub mesocosms. This may reflect the selectivity in tui chub predation on Chironomids. In fact, diet analysis revealed that 90% of tui chubs in Lake Tuendae contained Chironomid larvae as a food item and Chironomids represented 52% of food composition (n = 30) , making it a major food item of tui chub diet. Overall, this suggests that native fish transplanted to fishless desert springs have a potential to harm its invertebrate communities.

Some invertebrate taxa such as Rotifers, Ostracods and Hydracarina water mites were not affected by the presence of fish. Previous studies have shown that Rotifers can easily escape fish predation as a result of comparatively very small body size (Brooks and Dodson 1965). In fact, we did not observe rotifers in the gut contents of any fish we examined. Additionally, controlled experiments have shown that Hydracarina water mites with a red body color were rejected by fish predators presumably due to distastefulness (Kerfoot 1982). In fact, majority of the water bugs recovered had red pigmentation. In the case of Ostracods, their densities were low in all treatments.

Regardless of the significant results of this experiment, I acknowledge three limitations of experimental setup. First, as previously mentioned, this experiment was originally designed to evaluate the interactions between protected and invasive fish species. The fishless tanks were not randomly assigned, but represented 5 available tanks that were maintained and monitored in the same fashion as the other tanks. However, it provided a unique opportunity for us to understand fish impacts on aquatic invertebrates. Although we used tanks with two sizes for fishless control treatment, the invertebrate densities did not differ between two tank sizes providing an adequate number of replicates for the fishless control treatment. Second, the spring source for invertebrates (i.e. Lake Tuendae) is not a fishless desert spring. Therefore, the fishless treatments actually provide insights to how invertebrate communities respond to removal of fish. Third, filtering of water to exclude fish larvae during the establishment of mesocosms may have limited the introduction of larger macro-invertebrates (e.g. Odonate nymphs, Amphipods) into the mesocosms. However, the water was similarly filtered for the fishless mesocosms and the invertebrate community at Lake Tuendae is dominated by smaller bodied taxa (~98%; Appendix C). Therefore, filtering of water may have not excluded any abundant invertebrate taxa from the mesocosms. Despite these limitations, our experiment clearly shows how fish presence may affect invertebrate community structure and that these effects may differ based on the particular fish species introduced.

Federal and state agencies focusing on public health (e.g. Vector Control Districts) as well as wildlife conservation (e.g. U.S. Fish and Wildlife Service) identify the fishless water bodies as prime locations for fish introductions due to utterly different reasons. Vector Control Districts actively spread mosquitofish to fishless water bodies as a biocontrol agent of mosquitoes hoping to control dangerous mosquito-borne diseases such as Malaria and West Nile (Downs 1991; Duryea et al. 1996; Ghosh and Dash 2007). On the other hand, conservation practitioners promote transplants as a very effective management strategy for the recovery of protected fish species (Williams et al. 1988; Hendrickson and Brooks 1991; Minckley 1995). The vulnerability of fishless desert springs to fish introductions effectively ignores the inherent ecological and evolutionary value of the unique invertebrate assemblages in these habitats.

My results take on more importance, when one considers that desert springs often harbor endemic species. Many such endemics have only recently been described (Hershler and Sada 1987, 2002; Shepard 1990; Polhemus and Polhemus 2002) and some were lost prior to formal description (Hershler et al. 2011). Thus, it is quite possible that fish introductions may impact endemic invertebrate species. Thus, fish transplants which are pursued to reduce extinction risk for the targeted fish taxon, may actually reduce global diversity. Therefore a precautionary approach would be to conduct invertebrate surveys before any fish introductions (see Leyse et al. 2004). This may allow managers to identify potential refuge habitats for protected fish transplants as well as appreciate the inherent biodiversity of fishless desert springs.

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CHAPTER 5. GENERAL CONCLUSIONS

Non-native species invasion is an unavoidable global crisis (Elton 1958; Sax et al. 2005; Davis 2011). In fact, invaded ecosystems are the norm rather than the exception in contemporary world (Bull and Courchamp 2009; Davis 2011). In most of the invaded systems, invasive species already took over the ecological role of some native species, establishing complex interactions between native and non-native species (Zavaleta et al. 2001; Bull and Courchamp 2009). One of the main reasons for the failure of invasive species management programs is lack of understanding of complex ecological interactions between native and non-native species in invaded systems (Bull and Courchamp 2009). Therefore it is worth exploring ecological interactions between native and non-native species and it may provide novel insights to invasive species management.

Invasive mosquitofish (*Gambusia affinis and G. holbrooki*) impacts on native aquatic organisms and ecosystems are widely reported and often detrimental (Pyke 2008; Stockwell and Henkanaththegedara 2011). However, in some instances, non-native mosquitofish co-persist with native fish species providing opportunities to explore underlying mechanisms. Such is the case with endangered desert fish, Mohave tui chub (*Siphateles bicolor mohavensis*), where this native minnow co-persist with western mosquitofish (*G. affinis*) in three of five refuge populations of tui chubs. Mesocosm experiments reported in chapter 2 revealed tui chub predation on adult and juvenile mosquitofish while mosquitofish predation on eggs and larvae of tui chubs. This case of intraguild predation between tui chubs and mosquitofish appeared size-structured, because male mosquitofish, which is the smaller sex of this dimorphic species, had very low survival compared to female mosquitofish. This experimental work provided experimental evidence for intraguild predation between native and non-native fish species. Thus, intraguild predation may be an important mechanism facilitating the co-persistence of these two fish species. Furthermore, these findings have led managers to consider habitats currently harboring mosquitofish as possible refuge sites for Mohave tui chub, an option previously unavailable

Previous mesocosm experiments provided some evidence for size-structured intraguild predation between tui chubs and mosquitofish. In chapter 3, I explored the role of predator gape-limitation in intraguild predation between tui chubs and mosquitofish. The differential predation by male and female mosquitofish on tui chub larvae and tui chub male-biased predation on mosquitofish were consistent with the gape-size limitation hypothesis. Further, a literature review showed mosquitofish impacts have been limited to fishes with relatively smaller body sizes. My results suggest that a better understanding of trophic interactions may shed light on the mechanism(s) that facilitate the persistence of native species in the presence of invasive species.

Another important aspect of ecological complexity of non-native species is associated with the impacts of recently established fish populations on unique aquatic invertebrate assemblages in fishless desert water bodies. Recent surveys showed extremely high diversity and endemism of desert spring invertebrate communities (Hershler and Sada 2002; Polhemus and Polhemus 2002). However, these fishless water bodies are often used as refuge habitats for protected fish species, but the impact of such management actions has not received much attention. In chapter 3, I explored the potential impacts of both protected (Mohave tui chub) and invasive fish (western mosquitofish) species on aquatic invertebrate community structure. The diversity of invertebrate communities in experimental mesocosms was negatively impacted by the presence of fish, presumably due to fish predation. My results suggested that both invasive fish species and protected fish species can impact the structure of invertebrate communities in desert springs. Therefore a precautionary approach would be to conduct invertebrate surveys before any fish introductions.

In summary, my research explored ecological complexity of non-native species impacts on native species. These findings provide some novel insights to the management of desert aquatic ecosystem. Better understanding of complex ecological interactions between native and non-native species may provide alternative management options (e.g. consider habitats with invasive species as refuges for protected species) allowing managers to allocate resources to other threats. Furthermore, managers can maximize overall biodiversity of a managed system by taking a more cautionary whole ecosystem approach to managing desert springs.

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APPENDIX A. LIFE HISTORY OF ENDANGERED MOHAVE TUI CHUB

Background

Life history and demographic data are very important in conservation management of endangered species. Nevertheless, these data are lacking or poorly documented for majority of protected taxa (Heppell 1998). Life history theory predicts trade-offs in energy allocation (growth, reproduction, maintenance) based on the differential survival of various developmental stages (Roff 1992). For example, the differences of life history traits such as offspring size and number, size and age at maturity, and reproductive allotment may vary depending on factors such as resource availability and/or predation pressure (Reznick et al. 1990; Roff 1992). The Mohave tui chub recovery plan (U.S. Fish and Wildlife Service 1984) and management action plan (Hughson and Woo 2002) accentuate the importance of obtaining demographic and life history information for all Mohave tui chub populations to better inform conservation management of this endangered species. However, the life history of endangered Mohave tui chub (*Siphateles bicolor mohavensis*) has been poorly studied with most previous work limited to only the Lake Tuendae population (Vicker 1973). I studied life history of this endangered fish species and here provide data on age structure, reproductive biology and the diet of four of five extant populations of Mohave tui chub.

Fish sampling

Mohave tui chubs were sampled from Lake Tuendae, MC Spring, Camp Cady, and China Lake in 2008 for life history descriptions (age structure, reproductive biology and fish diet). Up to 30 Mohave tui chubs from each population were collected using baited minnow traps (Table A.1). At China Lake, 10 individuals each from G1 Channel, North Channel and George Channel were collected. Furthermore, 30 additional fish were collected from Camp Cady in 2007 for preliminary work on fish aging. All fish were sacrificed using a lethal dosage of MS-222 (500mg/L) and fixed in 10% formalin. Mohave tui chubs were measured for total length (to the nearest 0.01 mm) and wet mass (to the nearest 0.1 g).

Table A.1. Size of Mohave tui chubs (*Siphateles bicolor mohavensis*) used for life history descriptions in 2008. SE represents one standard error.

Population	N	Date collected	Total length (mm)		Wet mass (g)	
			Mean (± SE)	Range	Mean (± SE)	Range
MC Spring	25	4/25-26/08	78.90 (± 2.64)	62.71 - 111.05	6.45 (± 0.86)	2.5 - 20.9
Lake Tuendae	30	2/13/2008	124.20 (± 2.33)	95.00 - 143.00	25.18 (± 1.38)	9.7 - 37.9
Camp Cady	30	4/3/2008	109.19 (± 3.84)	68.39 - 150.85	17.06 (± 2.04)	4.6 - 46.9
China Lake	30	4/22-24/08	110.80 (± 2.97)	83.87 - 142.09	19.51 (± 1.54)	7.4 - 38.2

Fish age structure

Scales, vertebrae, otoliths, and opercula from 30 Mohave tui chubs collected in 2007 from Camp Cady (TL: 89-130 mm) were extracted for preliminary examination. Scale impressions were prepared on acetate slides using an Ann Arbor Roller Press. Vertebrae were air dried and connective tissues were removed as much as possible. Otoliths were mounted on glass slides and prepared for inspection using 320 and 600 grit sand paper to obtain a smooth cross section through the focus. Opercula were boiled for 1 minute and connective tissues were removed (Crain and Corcoran 2000). For 2008 samples, only opercula (Figure A.1) were used for aging tui chubs (Table A.1). Two independent readers read all structures and any discrepancies were resolved by joint examination.

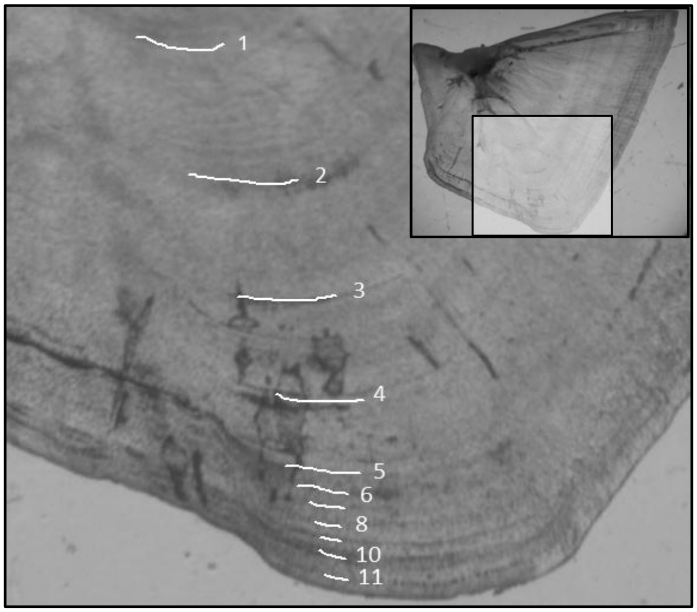

Figure A.1. Annual growth rings visible on an operculum of Mohave tui chub (*Siphateles bicolor mohavensis*). Insert shows the full operculum with the magnified area highlighted (Photo: Sujan Henkanaththegedara).

We assumed that otoliths would provide more accurate age estimates because they have been previously validated for other fish species (Beamish and McFarlane 1987; Quist et al. 2007). For Camp Cady fish (collected in 2007), otolith age estimates varied from 5-16 years, where scale age estimates varied only from 1-4 years for the same fish. Therefore, scale age underestimates the fish age by 2-14 years, compared to otolith age. Additionally, Vertebrae age over- or underestimates the age by 4-8 years compared to otolith age. However, opercula age closely matched with the otolith age (Figure A.2). Other workers have also reported scales to underestimate fish ages (Beamish and McFarlane 1987; Scoppettone 1988). Furthermore, Eagle Lake tui chub (*Siphateles bicolor spp*.), a closely related subspecies to Mohave tui chub, was aged up to 35 years by opercula readings while maximum age determined by scales was only 8 years (Crain and Corcoran 2000).

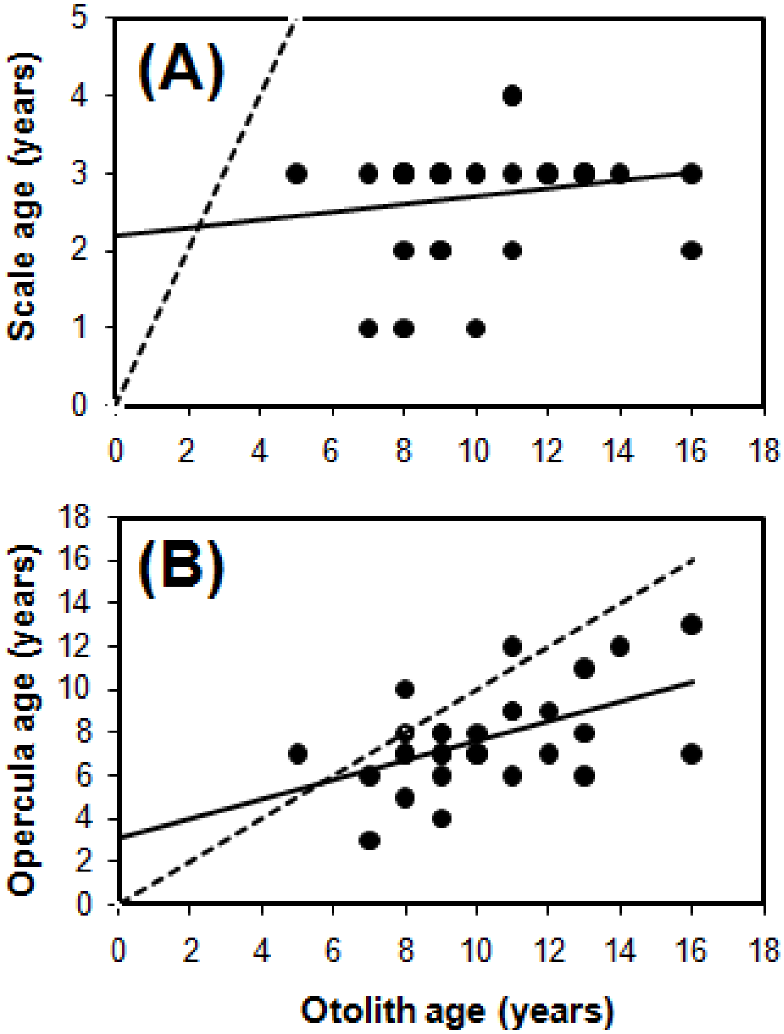

Figure A.2. Age bias plot of Mohave tui chub (*Siphateles bicolor mohavensis*) for ages determined from scales (A) and opercula (B) compared to otolith ages. Dotted line indicates the theoretical 1:1 agreement line of age estimates.

Based on the 2007 Camp Cady analysis, I examined fish age using opercula for four populations of Mohave tui chub collected in 2008. China Lake fish lack or had only poorly defined annual rings on opercula suggesting a stable environment. Therefore, we could not age China Lake fish. The age distribution varied among MC Spring, Lake Tuendae and Camp Cady, collectively representing age classes from 1 – 18 years. MC Spring fish appear to be short-lived (1-8 years) compared to Lake Tuendae and Camp Cady fish. Camp Cady fish ages varied from 3-13 years, while Lake Tuendae fish ages varied from 8-14 years (Figure A.3).

Previous work on Lake Tuendae tui chubs based on fish scales reported fish age classes only up to 4 years (Vicker 1973; Taylor and McGriff 1985). However, my results suggest that Mohave tui chub is a long-lived desert fish species and question the validity of the ages derived from scales (Vicker 1973; Taylor and McGriff 1985).

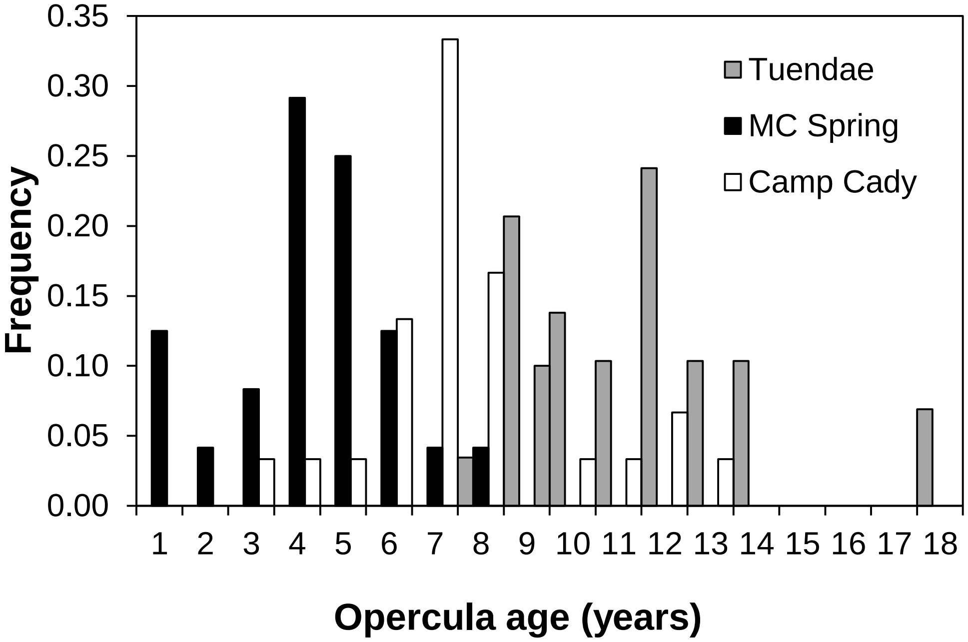

Figure A.3. Frequency of Mohave tui chub (*Siphateles bicolor mohavensis*) age based on opercula from Lake Tuendae (N = 29), MC Spring (N = 24) and Camp Cady (N = 30).

Reproductive biology

Preserved tui chub specimens (Table A.1) were carefully inspected for secondary sexual characteristics to establish any sexual dimorphism before dissections. The gonads were extracted and excess moisture was removed using paper towels and weighed to the nearest 0.0001 g (Denver Instrument Company; model A-250). The gonado-somatic index (GSI) was estimated using following formula for each fish in order to assess variation in reproductive allotment within and among populations (Cailliet et al. 1986).

$$\text{Gonado-somatic index (GSI)} = (\text{gonad weight}/\ \text{body weight}) \times 100$$

Fecundity of mature females was estimated by gravimetric method (Cailliet et al. 1986). A subsample of up to 100 eggs was separated and weighed to the nearest 0.0001 g (Denver Instrument Company; model A-250). The fecundity was estimated using the following formula.

$$\text{Fecundity} = \frac{\text{weight of the subsample}}{\text{number of eggs in the subsample}} \times \text{total gonad weight}$$

Ten separate mature eggs from each mature female were randomly selected and measured for the egg diameter to the nearest 0.01 mm under a 10X dissection microscope. The same 10 eggs were placed on a paper towel to remove excess moisture and weighed to the nearest 0.0001 g (Denver Instrument Company; model A-250) and the typical egg mass was estimated by calculating the average of ten eggs.

Differences of GSI of Mohave tui chubs were non-parametrically compared between habitats using Kruskal-Wallis rank sum test (function *kruskal.test*), using GSI as the response variable and habitat as the predictor variable. The fecundity was regressed against the total length of females and typical egg mass to understand the relationship between these factors (R version 2.11.0; R Development Core Team 2010).

The sex ratio of Mohave tui chub populations represented approximately 1 female: 2 males except for Lake Tuendae where the sex ratio was 2 females: 1 male. Sexually mature males possessed small, light-colored tubercles mainly on head, dorsum and pectoral fins (Figure A.4). In addition, some large males (approximately > 200 mm) possessed tubercles all over the body including pelvic and anal fins. A distinct hump was developed in sexually mature males, separating the head from the rest of the body. Rarely, I observed very large females (approximately > 200 mm) with faint tubercles on the head. Therefore tubercle production may suggest the individual is a male, unless the fish is very large.

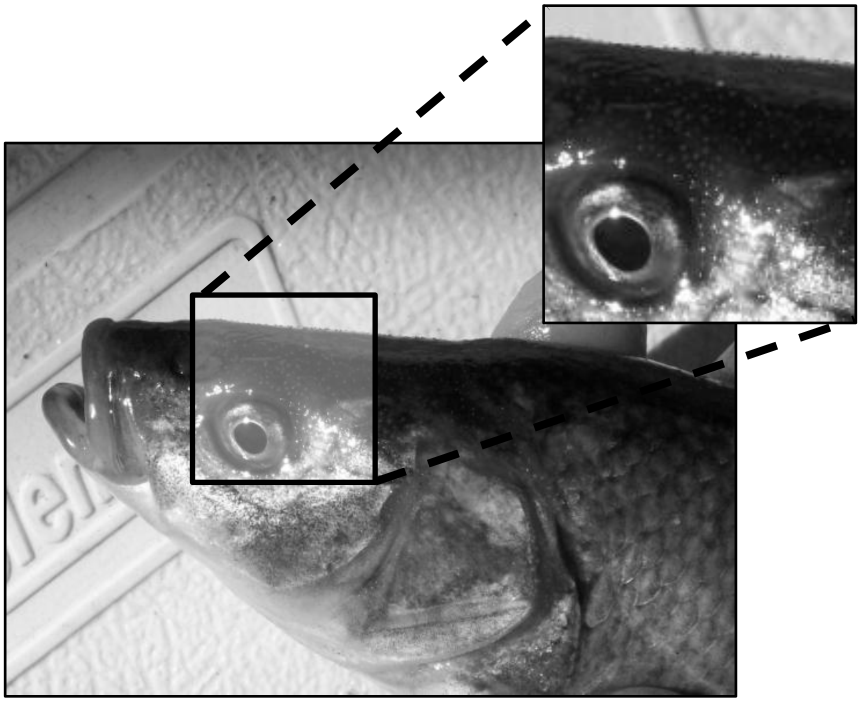

Figure A.4. Tubercle formation on an adult male Mohave tui chub (*Siphateles bicolor mohavensis*) from Lake Tuendae. Also note the dorsal hump (Photo: Sujan Henkanaththegedara).

The average GSI did not vary significantly across habitats ($X^2_3 = 5.5759$; $p = 0.1342$; Figure A.5). The fecundity of female tui chubs exponentially increased ($y = 27.5e^{0.0437x}$; $R^2 = 0.68$) with the total length (Figure A.6-A) and exponentially decreased ($y = 11325e^{-2716x}$; $R^2 = 0.65$) with typical egg mass (Figure A.6-B). The lowest average fecundity was reported from MC Spring (1161 ± 748), while the highest average fecundity was reported from Lake Tuendae (8200 ± 1064). However, the largest eggs were produced by MC Spring fish and the smallest by Lake Tuendae fish (Figure A.6-B). The fecundity of fish sampled by Vicker (1973) from Lake Tuendae ranged from 3393 – 8964 with an average of 5255. In addition, he reported a larger female tui chub (standard length: 215 mm) with an estimated fecundity of about 50,000 eggs (Vicker 1973).

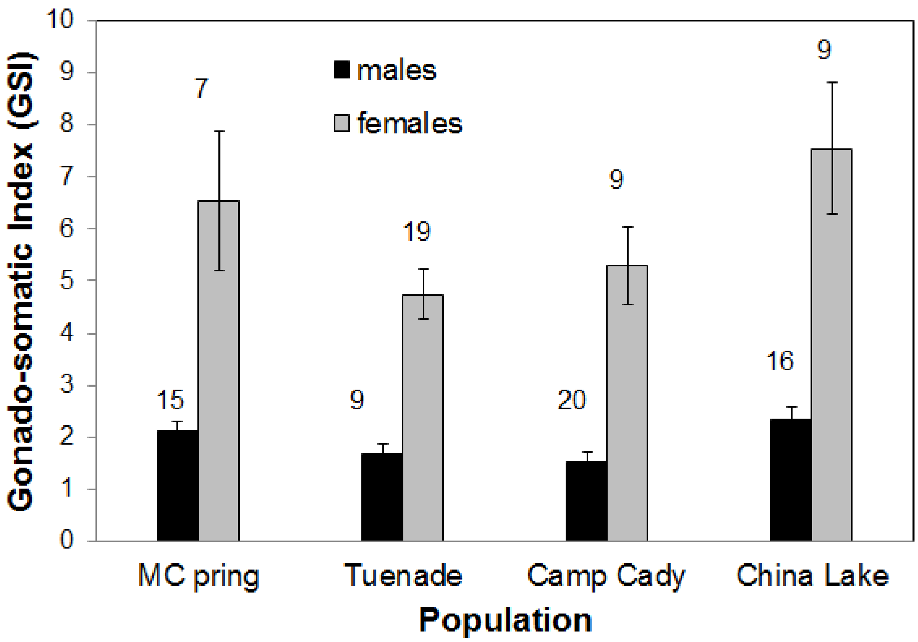

Figure A.5. Variations of Gonado-somatic Index (GSI) of Mohave tui chubs (*Siphateles bicolor mohavensis*) across sex and habitats. Error bars represent ± 1 SE Numbers above bars represent the sample size for each category.

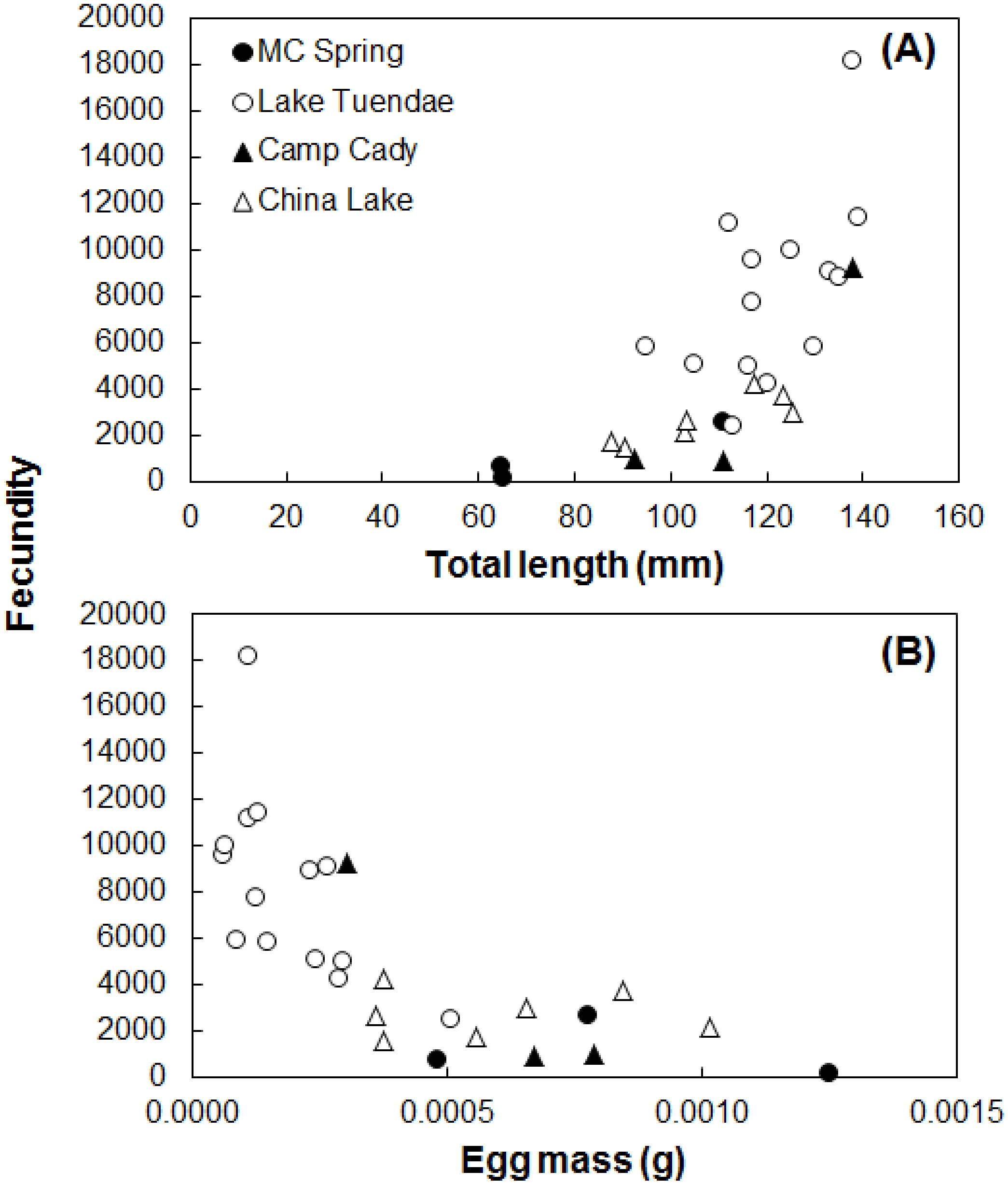

Figure A.6. Correlations of fecundity with body size (A) and typical egg mass (B) of female Mohave tui chub (*Siphateles bicolor mohavensis*). Different symbols represent different populations of tui chub.

Fish diet

The same tui chub specimens dissected for age structures were used for diet analysis (Table A.1). Additionally, at Lake Tuendae western mosquitofish (*Gambusia affinis*) and tui chubs were collected at the same time allowing me to evaluate diet overlap. Gut contents from the first 1/3 of the gut was removed, sorted, identified to the lowest possible taxa using standard keys and counted (Numerical Method; Windell 1968). Mohave tui chub diet was described using several standard indices. Percentage occurrence of different food items was described as the percentage of all stomachs containing food in which each food category occurred (Wallace 1981). Percentage composition was estimated as the percentage of each food category contributed to the total number of food items in pool stomachs across all individuals (Wallace 1981).

In addition, the heterogeneity of food items consumed was estimated with Shannon-Weiner Diversity Index (H') (Krebs 1999) and the number of equally common species in the diet (N_1) was estimated using H' (McArther 1965). This measure is recognized as the best heterogeneity measure due to its sensitivity to the abundance of rare species in the community (Peet 1974). I excluded insect parts and plant matter because they were hard to quantify.

$$H' = -\sum_{i=1}^{s} (p_i)(\log_2 p_i)$$

$$N_1 = e^{H'}$$

Where,

P_i = proportion of individuals using resource i

e = 2.71828 (base of natural logs)

Evenness of the food items consumed was estimated using Simpson's index of evenness

($E_{1/D}$). First it is required to estimate Simpson's diversity index (D) in order to estimate $E_{1/D}$.

$$D = \sum p_i^2$$

$$E_{1/D} = \frac{1/\hat{D}}{S}$$

Where,

Pi = Proportion of species I in the community

s = Number of species in the sample

Food niche overlap between Mohave tui chub and mosquitofish at Lake Tuendae was estimated using 5 different niche overlap indices with 16 resource states assuming equal abundance of all resource states (Krebs 1999). Use of multiple niche overlap indices is recommended, because different indices can result in different values for food niche overlap for the same data set (Wallace 1981). A software program accompanied by Krebs (version 7.1; 1999) was employed for estimates of heterogeneity, evenness and food niche overlap indices.

In general, Mohave tui chubs fed on invertebrates such as cladocerans, copepods, ostracods, chironomid larvae and amphipods. However, only a few recognizable food items were reported from Camp Cady compared to other sites. In addition, I found considerable amounts of unidentifiable insect parts and plant matter in tui chub guts from all four populations. Overall 15 identifiable food items were reported from all tui chub populations excluding unidentifiable insect parts and plant matter. Fourteen food items were reported for Lake Tuendae Mohave tui chubs followed by 8, 7 and 4 food items for tui chubs from MC Spring, China Lake and Camp Cady respectively. Percentage occurrence of various food items in tui chub guts was different between habitats (Figure A.7). Some invertebrate taxa were represented in all tui chub populations and some were restricted to one or a few populations. For example, cladocerans and chironomid larvae were present in tui chub guts from all four tui chub populations, while copepods and water mites were absent in Camp Cady tui chub guts (Figure A.7). Previous work on Lake Tuendae tui chub diet reported gyrinid larvae, chironomid larvae, and small tui chubs (Vicker 1973). In addition, tui chub diet studies from China Lake reported cladocerans, chironomids, amphipods, trichopterans, small tui chubs, and filamentous green algae (Feldmeth et al. 1985).

Also there were apparent differences of percentage composition of food items between habitats (Figure A.8). For example, cladocerans represented 92%, 34%, 3% and 1% in China Lake, MC Spring, Camp Cady and Lake Tuendae respectively. Fish eggs contributed for 50% of the food items in Camp Cady, 0.2% in Lake Tuendae and absent in two other populations. It is important to note that guts from Camp Cady tui chubs were 50% full of fish eggs. There are no other fish species in this habitat and this may reflect poor food availability for this dense population (Figure A.8).

Heterogeneity and evenness indices indicated the highest heterogeneity of food items at MC Spring. This may reflect the fair distribution of various food items in the diet of MC Spring tui chubs compared to dominance of other habitats by one food item. However, it accounts for comparatively higher number of equally common species and higher evenness, probably due to large amounts of cladocerans in the diet. The estimates for Lake Tuendae, Camp Cady and China Lake indicated a balance between heterogeneity and evenness, and China Lake indicated the least heterogeneity probably due to dominance of cladocerans in tui chub diet. Lake Tuendae and Camp Cady heterogeneity estimates (H’) indicate intermediate values probably due to dominance of the diet by chironomids and fish eggs respectively (Table A.2).

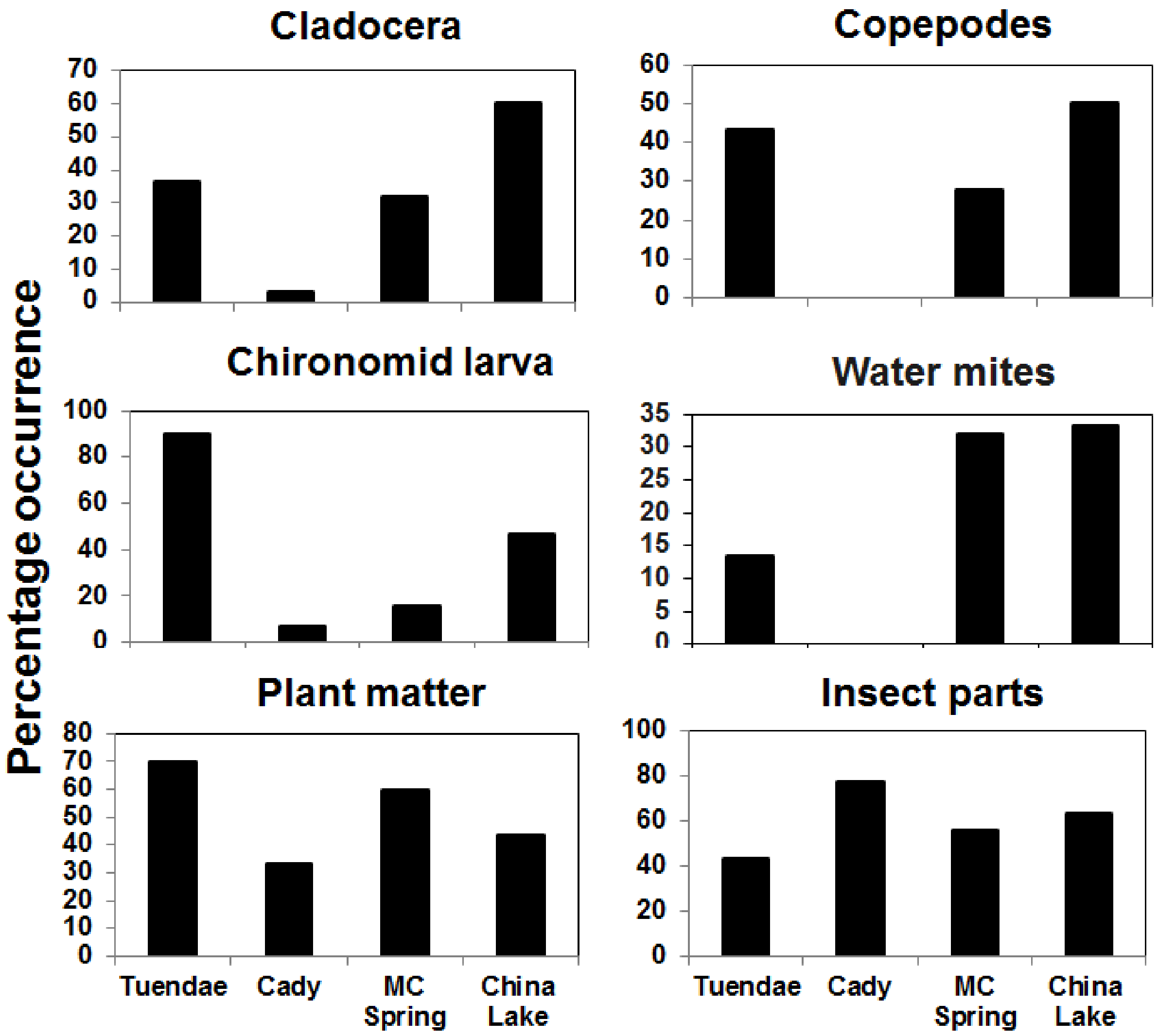

Figure A.7. Percentage occurrence of major food items in Mohave tui chub (*Siphateles bicolor mohavensis*) guts among four populations.

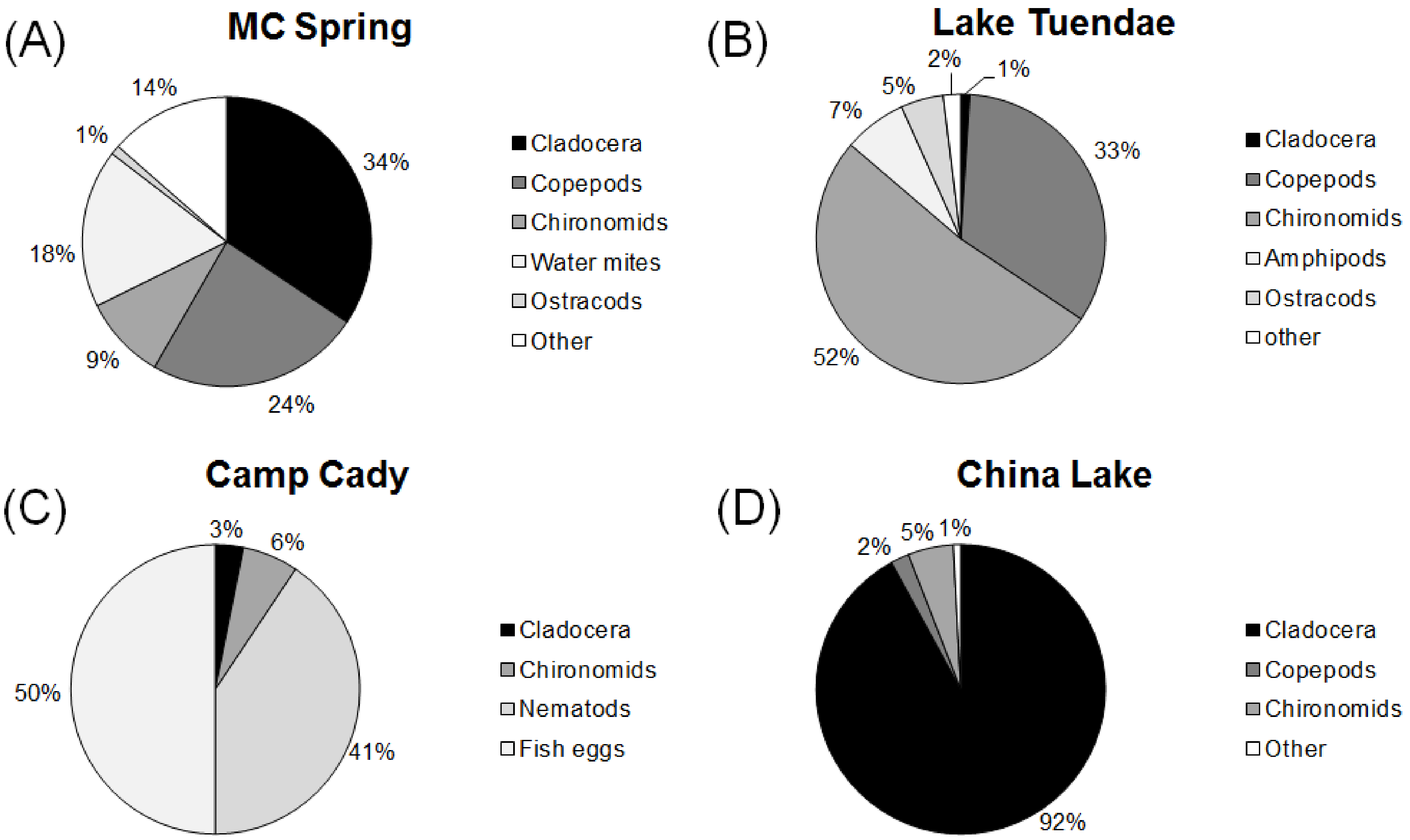

Figure A.8. Percentage composition of major food items in Mohave tui chub (*Siphateles bicolor mohavensis*) guts among four populations.

Table A.2. Heterogeneity and evenness of food items of Mohave tui chubs (*Siphateles bicolor mohavensis*) from four populations. Total N indicates the total number of individual food items in pooled gut samples (24 for MC Spring and 30 each for the rest) excluding unidentifiable insect parts and plant matter. Heterogeneity was measured in terms of Shannon-Weiner index (H') and number of equally common species (N_1) while evenness was measured using Simpson's measure of evenness ($E_{1/D}$).

Population	Number of food items	Total N	Heterogeneity		Evenness
			H'	N_1	$E_{1/D}$
MC Spring	8	96	2.423	5.36	0.559
Lake Tuendae	14	1578	1.727	3.31	0.184
Camp Cady	4	32	1.434	2.7	0.595
China Lake	7	4183	0.51	1.42	0.168

Five different indices estimated the food niche overlap between Mohave tui chub and western mosquitofish at Lake Tuendae in the range of 0.690 – 0.891. However, four of five indices estimated fairly close values except percent overlap measure (Table A.3). In general, Morisita's Index has been accepted as the best measure of niche overlap, due to zero bias at all sample sizes and when there are a large number of resources (Smith and Zaret 1982). Furthermore, food niche overlap over 0.6 (in 0-1 scale) is considered to be biologically significant and can provide circumstances for inter-specific competition for food (Zaret and Rand 1971; Mathur 1977).

Table A.3. Food niche overlap between Mohave tui chub (*Siphateles bicolor mohavensis*) and western mosquitofish (*Gambusia affinis*) at Lake Tuendae.

Food niche overlap index	Possible range	Niche overlap
Pianka's measure of overlap (Pianka 1973)	0 – 1	0.891
Percent overlap measure (Renkonen 1938)	0 – 100	68.999
Morisita's original index of overlap (Morisita 1959)	0 – 1	0.884
Simplified Morisita's index of overlap (Horn 1966)	0 – 1	0.880
Horn's index of overlap (Horn 1966)	0 – 1	0.823

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APPENDIX B. MONITORING THE CONSERVATION STATUS OF ENDANGERED MOHAVE TUI CHUB

Background

()The recovery plans for many endangered fishes, including Mohave tui chub (*Siphateles bicolor mohavensis*; U.S. Fish and Wildlife Service 1984), typically call for long-term population monitoring to provide critical biological data need to assess the effectiveness of management activities (Williams 1981;Minckley and Douglas 1989; Yoccoz et al. 2001; Campbell et al. 2002). However, in most cases rich data are unavailable, leaving managers to take a "best current data approach" (Johnson 1999), which utilizes existing data and uses the latest techniques to evaluate the efficiency of current management practices. However, management success is best achieved when a proper sampling design which targets specific management questions is used (Noble et al. 2010).

I have developed protocols for monitoring of extant Mohave tui chub populations, in collaboration with California Department of Fish and Game (Steve Parmenter Personal Communication), in order to understand population dynamics, population structure, and fish condition. These data can be used with existing molecular data (Chen 2006) to identify source populations for future transplants. Here I report the results of monitoring efforts of Mohave tui chub populations for past 5 years (2005-2009). Further, I attempted to place these results in the context of sparse historic information, future recovery goals and adaptive management strategies.

Methods

Mark-recapture methods

We estimated the size of breeding populations for Mohave tui chub in Lake Tuendae (2007-2009), Camp Cady (2007-2009) and the North Channel of China Lake (2008). We used multiple mark-multiple recapture methods, and marked only fish which were larger than 70 mm in total length (TL here after) and fish less than 70 mm in TL were excluded from population estimates allowing us to estimate the “breeding population” of Mohave tui chub. Fish were captured using either one or a combination of three types of traps, baited with bread (Table B.1 and B.2). Permanent trap stations were set along the banks of the habitat, and traps were deployed randomly at one of three distances from the bank (0 m, 10 m or 20 m from the bank) for each sampling session. In Camp Cady, traps were suspended just beneath the water surface using plastic buoyants to avoid possible lethal oxygen levels previously observed in deeper areas. Such lethal oxygen levels were only an issue at Camp Cady. The type and the number of traps were determined based on the habitat type and the availability of traps (Table B.1).

Traps were deployed approximately 3 hours after which they were recovered. The catch was removed from traps and placed in to an aerated live car. Subsequently fish were anesthetized using 50 mg/L of MS-222 before handling. Every unmarked fish was measured for TL (to the nearest 1 mm) and a subsample of fish up to 10 fish in each 10 mm size classes were measured for wet mass (to the nearest 0.1 g). All fish above 70 mm in TL were marked by clipping a small portion of one pelvic fin. Marked fish were returned to a well aerated live car until all fish were processed. Subsequently, marked fish were returned to the habitat at several random locations to maximize mixing of the population (Schnabel 1938). We repeated recapturing and marking new fish up to 8 times and recorded the numbers of marked fish, and unmarked fish. The time duration between two marking sessions varied from 12-24hrs depending on the habitat, available labor, and number of fish caught in the previous session (Tables B.1 and B.2).

Schumacher-Eschmeyer method (Schumacher and Eschmeyer 1943) was utilized to estimate the population size of Mohave tui chubs at Lake Tuendae, Camp Cady and China Lake. These populations met the spatially closed population criterion under the assumptions of Schumacher-Eschmeyer method. Additionally, we assumed that the population was demographically closed (zero births and deaths during the sampling period) and each sample was assumed to represent a random sample (Young and Young 1998). A software program by Krebs (version 7.1; 1999) was employed for all estimates. We accounted for an unexpected fish mortality event which occurred at Camp Cady in 2007 by adjusting the values accordingly.

Table B.1. Details of the Mohave tui chub sampling (*Siphateles bicolor mohavensis*): dates, methods, traps used and trapping sessions.

Population	Year	Dates	Method	Traps used			No. of	Inter-session
				Promar	16.5’ Minnow	24’ “Susan”	sessions	time interval
MC Spring	2007	1/22-24/2007	Depletion	0	25	1	2	~24 hrs
	2008	3/9/08-3/11/08	method	0	25	1	6	~12 hrs
	2009	3/07/09-3/09/09		0	25	1	6	~12 hrs
Lake Tuendae	2007	4/26/07-5/7/07	Mark-recapture	0	7	7	7	~24 hrs
	2008	3/12/08-3/27/08	method	0	0	18	6	~12 hrs
	2009	3/10/09-3/12/09		0	0	16	5	~12 hrs
Camp Cady	2007	5/10/07-5/12/07	Mark-recapture	20	0	0	3	~24 hrs
	2008	3/31/08-4/02/08	method	0	0	10	6	~12 hrs
	2009	4/30/09-5/05/09		0	0	16	5	~24 hrs
China Lake (North Channel)	2008	4/21/08-4/24/08	Mark-recapture method	0	0	18	8	~12 hrs

Table B.2. Details of the traps used to sample Mohave tui chub (*Siphateles bicolor mohavensis*).

Trap type	Shape	Length (cm)	Mesh type	Mesh size (mm)	Number of entrances	Entrance diameter (cm)
Promar TR-501	Cuboid	45.72	polyethylene netting	1.2	2	6.35
16.5” standard Minnow	Torpedo shaped	41.91	Steel mesh	6.35	2	2.54-5.08
24” custom-made (“Susan”)	Cylindrical	60.96	Steel mesh	6.35	2	5.08

Depletion methods

Due to small size of MC Spring (Volume ~20 m^3), I employed a depletion method (2007-2009) to estimate population size, consistent with previous censuses in 2005 and 2006 by California Department of Fish and Game (Steve Parmenter Personal Communication). Fish were captured using either one or a combination of two types of traps baited with bread (Tables B.1 and B.2). Every unmarked fish was anesthetized using 50 mg/L of MS-222 and measured for TL (to the nearest 1 mm) and wet mass (to the nearest 0.1 g). Processed fish were held in a 55 x 55 x 155 cm^3 live car, suspended in the habitat for up to three days. However, the effort was kept constant by deploying the same type and number of traps between sessions. We used absolute accumulated count of captured fish as the total population size, since regression models based on catch-per-unit-effort and accumulated catch (Leslie regression model; Leslie and Davis 1939) underestimated the population size in most cases (Table B.3).

Population density and structure

The population density of each population was estimated by dividing the estimated population size by the approximate volume of the habitat. Approximate volumes of habitats were estimated with surface dimensions and depth profile data collected using a 10 m x 20 m grid. North Channel information was provided by California Department of Fish and Game (Steve Parmenter Personal Communication). For Lake Tuendae, Camp Cady and China Lake, the population density estimates are limited to adult “breeding” population (fish > 70 mm in TL). For MC Spring, we estimated total population size and density using all fish caught during depletion sampling (above and below 70 mm). In addition, we included the tui chubs temporarily removed from Lake Tuendae for field experiments when estimating the population density for Lake Tuendae (Table B.3).

Fish TL data collected during population were used to analyze length frequency distributions. Length frequency histograms were visually examined for characteristics for a stable population (Neumann and Allen 2010). Young-Adult Ratio, the number of young per adult, was used to compare the relative reproductive success of MC Spring population (Reynolds and Babb 1978). We defined all tui chubs less than 50 mm in TL as young-of-the year (YOY) and all fish above 70 mm in TL as adult (Vicker 1973). Fish between 50 mm and 70 mm in TL were excluded to minimize any discrepancies of the developmental stage.

Fish condition

Fish condition, an estimate of individual fish health (Blackwell et al. 2000), was assessed using length-weight data collected during population estimates. Fulton's condition factor (K) was utilized as an index to compare the fish condition of Mohave tui chubs (Pope and Kruse 2010). This index may provide a relative measurement of fish health (Anderson and Neumann 1996).

$$K = (\text{weight}/\ \text{length}^3) \times 10{,}000$$

Assuming isometric growth of tui chubs, K = 1.0 indicates an average health while deviations indicate either poor health (K < 1.0) or good health (K > 1.0; Anderson and Neumann 1996).

Results and Discussion

MC Spring

MC Spring is a small spring-like habitat (volume ~20 m^3) located near the shore line of Soda Dry Lake. Some researchers and managers argue that MC Spring is the presumptive stock for all existing Mohave tui chubs (Hughson and Woo 2004; Chen 2006; Steve Parmenter Personal Communication). However, the first report of Mohave tui chub in MC Spring was reported by Vicker (1973) and further mentioned that fish was introduced from Lake Tuendae by the tenants of the property.

The depletion sampling method allowed us to census the MC Spring population. Total population size of Mohave tui chubs declined from 618 in 2005 to 255 in 2008, and "recovered" to 495 in 2009 (Table B.3). Length frequency distribution of a fish population reflects interactions of reproduction rate, recruitment, growth and mortality (Neumann and Allen 2010). In this case, the histograms revealed that the extreme fluctuations of the population size were due to fluctuations of recruitment success. MC Spring population had extremely low recruitment success from 2006-2008. However, fish in 20-40 mm size class were abundant in 2005 and 2009 (Figure B.1). Young-Adult Ratio of the MC Spring population also reflects the limited recruitment from 2006 to 2008 compared to 2005 and 2009 (Figure B.2).

The MC Spring supports a relatively high density of fish (6.17 – 14.95 individuals/m^3) compared to historic data. In contrast to my sampling, Soltz (1978) trapped only 3 individuals in August 1977 with 3 minnow traps submerged for 11 hours. The historic highest population of 58 fish (95% confidence intervals: 42-102) was reported in April 1981 from a yearlong mark-recapture study (Taylor 1982).

Table B.3. Estimated total population size of Mohave tui chubs (*Siphateles bicolor mohavensis*) for MC Spring (Leslie regression model: Leslie and Davis 1939) and breeding population sizes for Lake Tuendae, Camp Cady-Bud's Pond and China Lake-North Channel (Schumacher-Eschmeyer method; Schumacher and Eschmeyer 1943). The number of Lake Tuendae fish removed from the population for field experiments is shown in the last column.

Population	Year	Number marked/ captured	Estimated population size	Lower 95% C.I.	Upper 95% C.I.	Fish in Mesocosms
MC Spring	2005†	618	591.2	537.7	664.4	-
	2006†	357	424.1	**	**	-
	2007	369	345.5	**	**	-
	2008	255	269.8	183.3	1134.5	-
	2009	496	482.6	463.8	503.3	-
Lake Tuendae	2007	1049	1233.7	1119.8	1373.5	200
	2008	2568	3201	2887.3	3591.2	160
	2009	2741	4277.9	3581.6	5310.3	160
Camp Cady	2007	1488	3409.7	2813.7	4326.1	-
	2008	3897	5095.3	4813.5	5412.2	-
	2009	4273	5782.4	5420.7	6195.7	-
China Lake (North Channel)	2008	504	1687.9	1055.4	4213.1	-

** Sample size is too small to calculate the confidence intervals.

† MC Spring 2005 and 2006 data courtesy of Steve Parmenter, California Department of Fish and Game.

Table B.4: TL (mm) of Mohave tui chub (*Siphateles bicolor mohavensis*) sampled across 4 habitats and 5 years. Sample size depicts total population size for MC Spring and number of fish handled during mark-recapture sessions for other habitats.

Population	Year	Sample size	Mean (+/-SE)	Median	Range
MC Spring	2005†	618	52.45 (+/-0.74)	48	20 - 170
	2006†	356	56.76 (+/-0.70)	53	38 - 156
	2007	369	62.61 (+/-0.75)	59	29 - 175
	2008	255	70.34 (+/-0.62)	69	47 - 148
	2009	495	57.84 (+/-0.68)	53	40 - 156
Lake Tuendae	2007	821	110.43 (+/-0.96)	104	53 - 292
	2008	3033	101.05 (+/-0.46)	96	46 - 272
	2009	3651	88.78 (+/-0.34)	83	55 - 309
Camp Cady	2007	1099	106.37 (+/-0.34)	106	61 - 202
	2008	4515	93.89 (+/-0.24)	95	95 - 161
	2009	4589	95.23 (+/-0.20)	95	57 - 230
China Lake (North Channel)	2008	755	139.07 (+/-1.83)	138	44 - 258

† MC Spring 2005 and 2006 data courtesy of Steve Parmenter, California Department of Fish and Game.

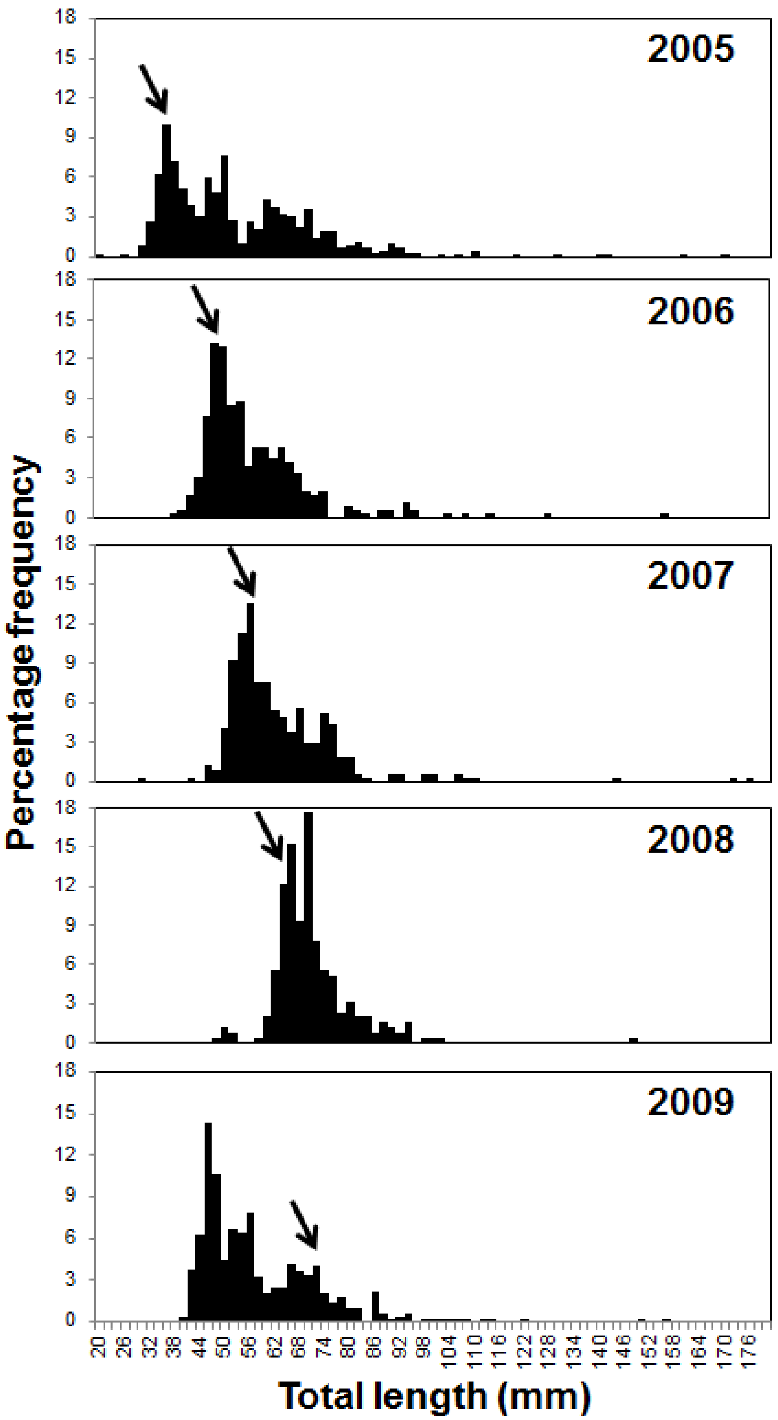

Figure B.1: Percentage length frequency distribution of MC Spring Mohave tui chub (*Siphateles bicolor mohavensis*) population sampled during 2005-2009. Arrows indicate 2005 YOY cohort. MC Spring 2005 and 2006 data courtesy of Steve Parmenter, California Department of Fish and Game.

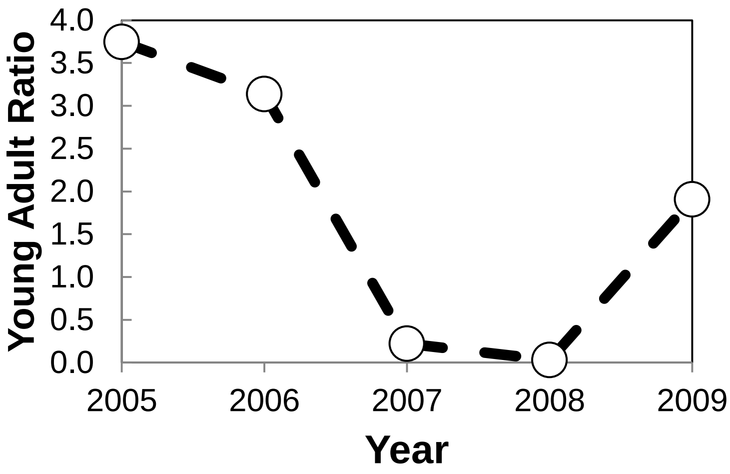

Figure B.2: Fluctuations of Young-Adult Ratio (YAR) of Mohave tui chub (*Siphateles bicolor mohavensis*) at MC Spring from 2005-2009. MC Spring 2005 and 2006 data courtesy of Steve Parmenter, California Department of Fish and Game.

Table B.5. Population density of Mohave tui chub (*Siphateles bicolor mohavensis*) for MC Spring, Lake Tuendae, Camp Cady-Bud's Pond, and China Lake-North Channel. Density and confidence intervals C.I. were estimated by dividing the total/estimated population size by the approximate volume of the habitat.

Population	Year	Estimated population density (individuals/m^3)	Lower 95% C.I. (individuals/m^3)	Upper 95% C.I. (individuals/m^3)
MC Spring	2005†	14.95	-	-
	2006†	8.64	-	-
	2007	8.93	-	-
	2008	6.17	-	-
	2009	12.00	-	-
Lake Tuendae	2007	0.24	0.22	0.26
	2008	0.56	0.51	0.62
	2009	0.74	0.62	0.91
Camp Cady	2007	1.43	1.18	1.81
	2008	2.14	2.02	2.27
	2009	2.43	2.27	2.60
China Lake (North Channel)	2008	0.88	0.55	2.19

† MC Spring 2005 and 2006 data courtesy of Steve Parmenter, California Department of Fish and Game.

Fish condition was negatively correlated with MC Spring population density. The lowest average Fulton's condition factor was recorded for 2006 fish (0.896 ± 0.007) indicating "poor" fish condition. However, with decreasing population size, it increased to 1.013 (± 0.007) in 2008 suggesting "average" condition and dropped back to 0.915 (± 0.009) with increased population in 2009 (Table B.6).

Table B.6. Fulton's condition factor (C) of Mohave tui chub (*Siphateles bicolor mohavensis*) sampled across 4 habitats and 4 years. MC Spring values are based on the data collected from the total population while values for other populations are based on the data collected for "breeding" population (fish > 70 mm TL).

Population	Year	Sample size	Mean (+/-SE)	Median	Range
MC Spring	2006†	355	0.896 (+/-0.007)	0.886	0.359-1.469
	2007	369	0.922 (+/-0.006)	0.911	0.540-1.429
	2008	255	1.013 (+/-0.007)	1.004	0.669-1.391
	2009	495	0.915 (+/-0.009)	0.904	0.235-2.113
Lake Tuendae	2007	151	1.183 (+/-0.008)	1.176	0.922-1.634
	2008	172	1.107 (+/-0.013)	1.139	0.482-1.565
	2009	142	1.186 (+/-0.013)	1.160	0.810-2.101
Camp Cady	2007	92	0.949 (+/-0.011)	0.941	0.718-1.282
	2008	114	1.091 (+/-0.013)	1.139	0.482-1.565
	2009	94	1.186 (+/-0.013)	1.160	0.714-1.762
China Lake (North Channel)	2008	192	1.240 (+/-0.017)	1.220	0.797-2.890

† MC Spring 2005 and 2006 data courtesy of Steve Parmenter, California Department of Fish and Game.

MC Spring population recovery in 2009 after a steady decline since 2005 may be due to several management actions conducted in collaboration with California Department of Fish and Game. From 2007 to 2009, managers removed the largest individuals from the population in order to reduce variance in reproductive success and any cannibalistic impacts. Additionally, more than 8 large individuals of predaceous diving beetles (family Dytiscidae) were removed from the MC Spring during the 2008 population census. Artificial cover material was also deployed along the periphery of the habitat in April 2008 (Figure B.3) to provide cover for developing larvae. These management actions appear to have reduced predation pressure and increased larval survival by providing protective cover during the early developmental stages (Figure B.1).

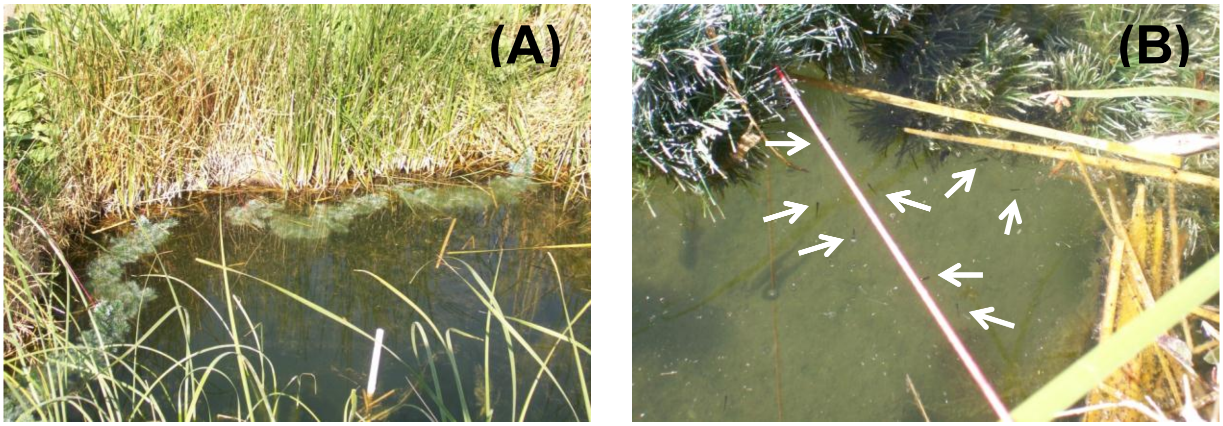

Figure B.3. Artificial cover suspended along the periphery of MC Spring (A) may have helped the survival of developing Mohave tui chub (*Siphateles bicolor mohavensis*) larvae and subsequent recruitment (B). Arrows indicate developing chub larvae (Photos: Sujan Henkanaththegedara).

Lake Tuendae

Historic reports (Thompson 1929; Miller 1938) discuss a large spring-fed pool located at the approximate location of present-day Lake Tuendae. Furthermore, some early works on the fish in Lake Tuendae describe the occurrence of “minnows” in the original spring-fed pool in as early as 1907 (Miller 1938) and enlargement of the original spring pool to create Lake Tuendae in early 1940s (Vicker 1973; Turner and Liu 1976). Lake Tuendae tui chubs have been used as the source population for new refuge populations since early 1950s (Miller 1968; Hoover and St.Amant 1983).

The estimated population of “breeding” tui chubs (fish > 70 mm in TL) at Lake Tuendae varied from 1434 fish in 2007 to 4438 fish in 2009 (Table B.3). The initial low population size may reflect the natural variation of the population size. However, these values are well within the previous population estimates for this population. The earliest estimate of 5500-6000 total population size in March-October in 1973 was an estimate based on visual observations and collections (Vicker 1973). Soltz (1978) reported a “breeding” population size of 2744 fish in July 1977. A yearlong study conducted by Taylor (1982) reported a lowest total population size of 1450 fish in February 1982 and a highest of 5678 fish in August 1981, providing clues for seasonal fluctuations of population size. Garron (2006) reported an April breeding population size of 2241 and 3354 in 2004 and 2005, respectively (Table B.7 for more details).

Length frequency histograms suggested a consistent recruitment for tui chubs in Lake Tuendae (Figure B.4). The average fish size in 2007 (TL = 110.43 ± 0.96 mm) dropped with increasing population size in 2009 (88.78 ± 0.34 mm) (Table B.4). Fulton’s condition factor for Lake Tuendae stood above 1.1 for the entire sampling period of this study, indicating “good” fish condition (Table B.6). Overall this information on Lake Tuendae tui chub population suggests a relatively robust population despite of the occurrence of invasive western mosquitofish (*Gambusia affinis*). Recent population genetic work also confirmed Lake Tuendae population to have relatively higher genetic variability (Chen 2006).

Camp Cady

Historically there were two man-made earthen ponds (east and west) at Camp Cady which supported two Mohave tui chub populations. The west pond (also called Bud's Pond) tui chub population was established by transplanting 10 and 55 fish from Lake Tuendae in 1986 and 1987, respectively. The east pond was established by transplanting 59 fish from Lake Tuendae in 1987 and augmented by introducing nearly 5000 young-of-the year fish from west pond in 1988. However, east pond tui chubs were temporary transferred to "fire" pond, leaving only one surviving Mohave tui chub population in Camp Cady. Eventually, 1769 Mohave tui chubs (originally from east pond) were introduced to west pond in 1992 (Steve Parmenter Personal Communication). Recently in 2008, nearly 600 captively propagated Mohave tui chubs were introduced to west pond (Steve Parmenter Personal Communication).

This is the first study to report information on population size and structure of Camp Cady Mohave tui chub population. Camp Cady breeding tui chub population ranged from 3410 fish in 2007 to 5782 fish in 2009. This indicates a net increase of the breeding population towards 2009 (Table B.3). Recruitment of this population may not consistent due to lack of smaller size classes and unimodal nature of the length-frequency histogram for 2007 and unusual peaks in 2008 histogram (Figure B.5). These shifts may reflect inconsistent recruitment and introduction of about 600 captively bred tui chubs in 2008 by California Department of Fish and Game. These fish were derived from a captive population and were temporally housed in "fire" pond prior to their introduction to west pond.

In general, a stable decline of smaller to larger size classes in length-frequency histograms may suggest a “balanced” population (Anderson and Neumann 1996) with continuous recruitment and stable structure. Any deviations from this general pattern may warrant “unbalanced” nature of a population. Therefore, 2009 length-frequency histogram may suggest a partial recovery of the population status. Parallel to the fluctuations of the population size, the structure of the population also changed with comparatively larger fish in 2007, compared to 2008 and 2009 (Table B.4). In addition, 2009 fish indicated “good” condition compared to 2007 fish and 2008 fish, which indicated “poor” and “average” fish conditions, respectively (Table B.6).

Bud’s Pond (i.e. west pond) population of tui chubs were originally founded with 10 fish in 1986 and 55 fish in 1987 from Lake Tuendae. This low number of founders may have posed a severe genetic bottleneck effect in this population (Meffe 1986; Allendorf 1986). In fact, Chen (2006) showed low genetic diversity of this population compared to Lake Tuendae and China Lake. The lower genetic diversity combined with “unstable” demographics of this tui chub population makes this population less suitable as a source population for future recovery efforts.

Table B.7. A comparison of population estimates of Mohave tui chub (*Siphateles bicolor mohavensis*) conducted at Lake Tuendae.

Time	Method	Representative population	Population size	lower 95% CI	upper 95% CI	Reference
Mar-Oct-73	Visual estimate	Total population	5500-6000	-	-	Vicker 1973
Jul-77[a]	Mark recapture	Fish larger than 60 mm SL	2744	2135	3525	Soltz 1978
Jul-77[b]	(Peterson method)		1538	1091	2257	
Apr-81	Mark recapture	Fish larger than 38 mm SL	2782	1862	5093	Taylor 1982
Jun-81	(Schnabel method)		4130	3424	5203	
Aug-81			5678	4303	8327	
Oct-81			5588	4314	7929	
Dec-81			2272	1855	2931	
Feb-82			1450	1251	1725	
Apr-04	Mark recapture	Fish larger than 70 mm TL	2241	2090	2416	Garron 2006
Oct-04	(Schnabel method)		3708	3539	3894	
Apr-05			3354	3213	3509	
Apr-07	Mark recapture	Fish larger than 70 mm TL	1234	1120	1374	This study
Mar-08	(Schnabel method)		3201	2887	3591	
Mar-09			4278	3582	5310	

a- Fish were trapped using umbrella net and minnow traps.

b- Fish were trapped using only umbrella net.

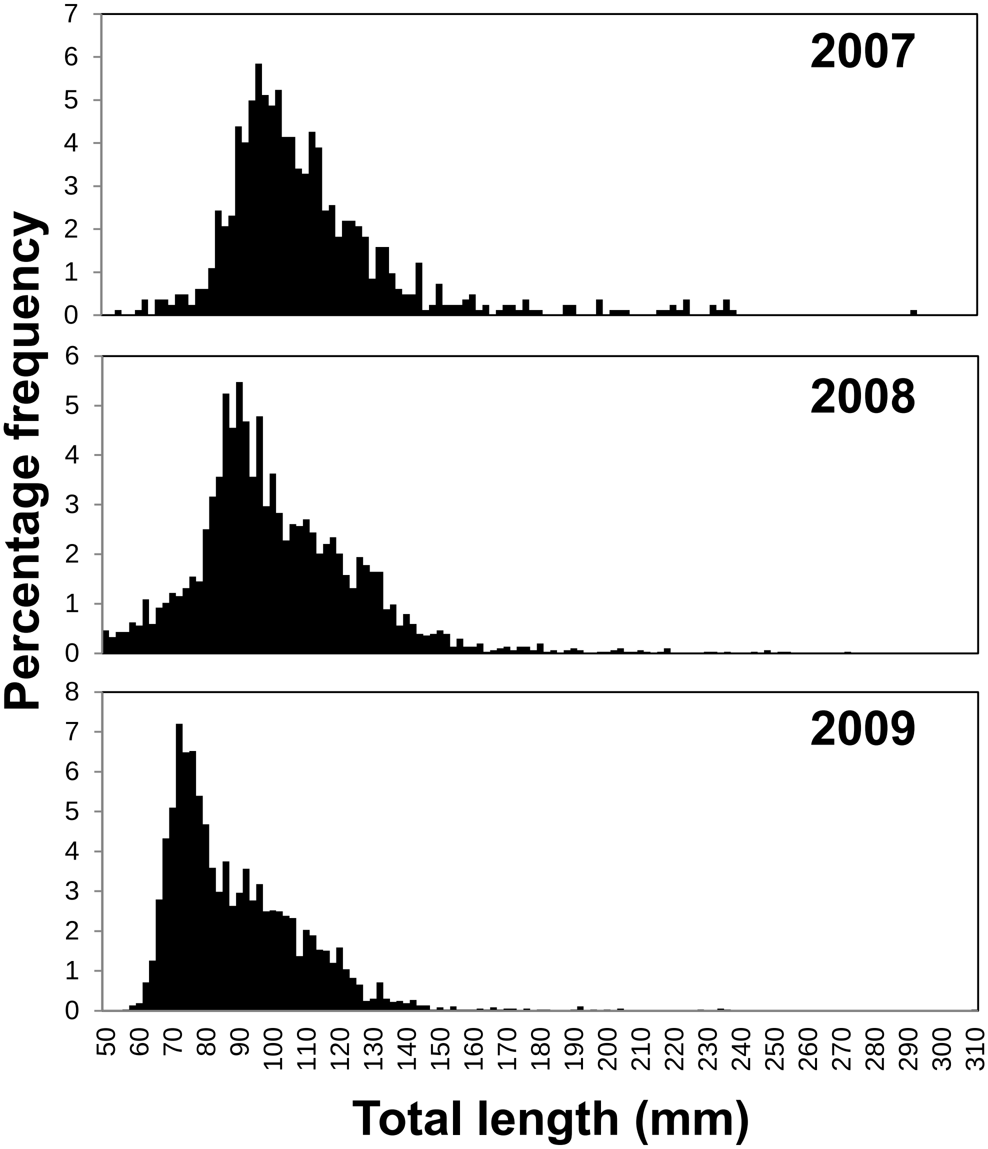

Figure B.4: Percentage length frequency distribution of Lake Tuendae Mohave tui chub (*Siphateles bicolor mohavensis*) population sampled during 2007-2009.

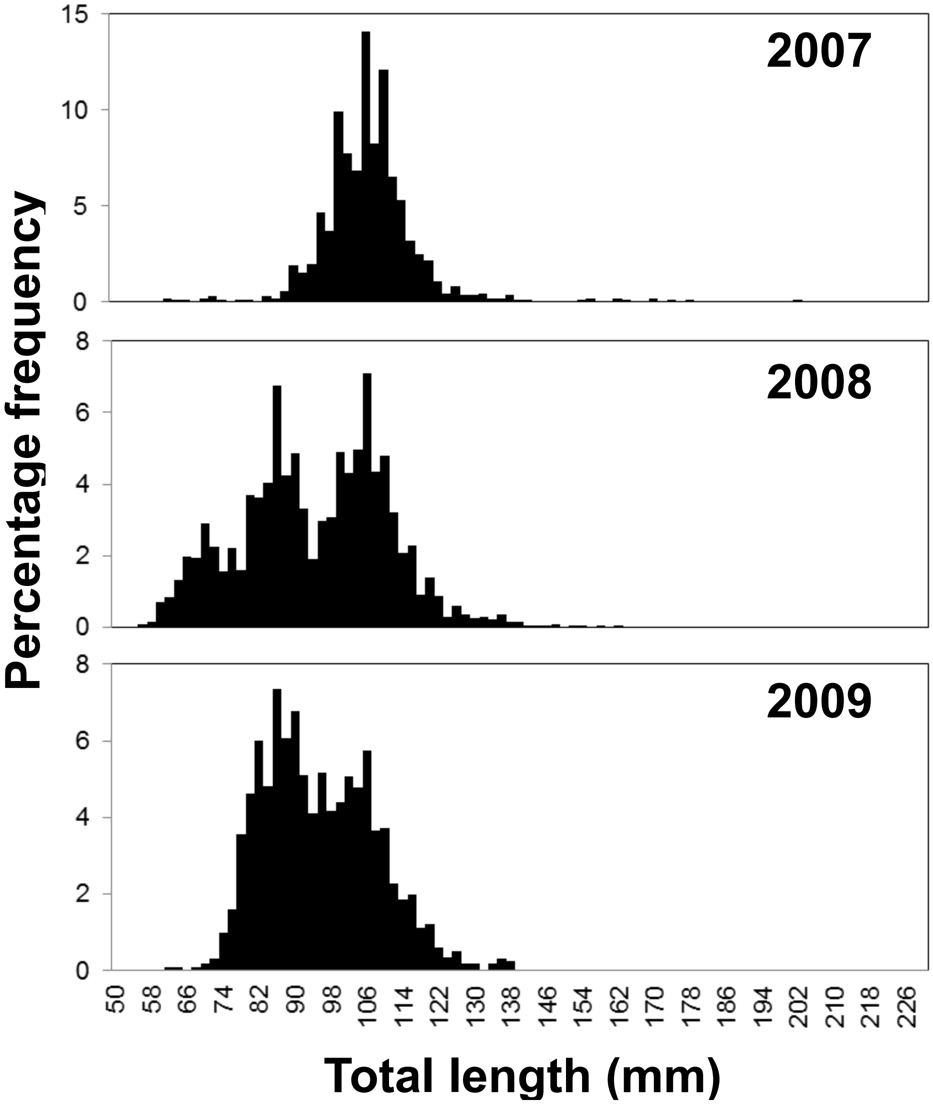

Figure B.5: Percentage length frequency distribution of Camp Cady Mohave tui chub (*Siphateles bicolor mohavensis*) population sampled during 2007-2009.

China Lake

China Lake Mohave tui chub population was established by transplanting 400 and 75 Mohave tui chubs from Lake Tuendae to Lark Seep in 1971 and 1976, respectively (Hoover and St. Amant 1983). Subsequently, Mohave tui chubs have colonized extensive channel and seep system (Feldmeth et al. 1985), making China Lake, the largest extant refuge population of Mohave tui chub (Susan Williams and Anna-Marie Easley Personal Communications). Currently, tui chubs occupy four major areas within China Lake seep system: 1) G1 Channel, 2) North Channel, 3) George Channel and 4) Lark Seep (Susan Williams Personal Communication).

At China Lake, I was able to sample only the North Channel habitat in 2008 due to security reasons. The breeding tui chub population size for North Channel was 1688 in 2008 (Table B.3) with relatively higher average Fulton’s condition factor (1.240 ± 0.017). Length-frequency histogram for 2008 indicated healthy recruitment as well as a considerable number of large individuals indicating a “healthy” population (Figure B.6). A mark-recapture survey for this sub-population conducted in October 2002 estimated 1400 fish (95% confidence intervals: 817 – 1983; Susan Williams Personal Communications). Our results for population size may suggest a growth of the population since 2002. However, the average fish size remains more or less the same (TL of 139.07 mm in 2008 vs. 140.97 mm in 2002). Despite the limited information from our study combined with recent population genetic works (Chen 2006), it appears that this population is sufficiently “healthy” to consider as a source of future translocations. In fact, California Department of Fish and Game recently established a new Mohave tui chub population at Deppe Pond, Victorville by transplanting 400 young-of-the-year tui chubs from China Lake (Steve Parmenter Personal Communication).

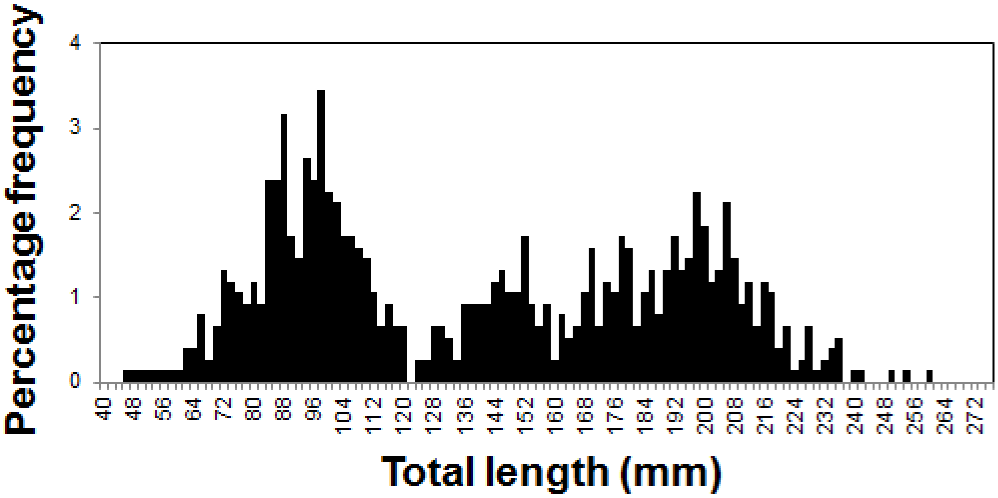

Figure B.6: Percentage length frequency distribution of China Lake-North Channel Mohave tui chub (*Siphateles bicolor mohavensis*) population sampled during 2008.

Management implications

This study is the first comparative assessment on population dynamics, structure and fish condition of all established Mohave tui chub populations. These results may allow comparison of some aspects of demographic and biological properties of refuge populations of endangered Mohave tui chub while providing an opportunity to assess the past management efforts. Collectively our results suggest that Lake Tuendae and North Channel segment of China Lake population are comparatively more robust in terms of population size, population structure, and fish condition. However, the temporal stability of the North Channel population is inconclusive due to lack of data. MC Spring population appears “unstable” at the moment in terms of extreme population density compared to historic data, wide range of population fluctuations, and “poor” fish condition, possibly due to density dependent effects (Smith 1981; Dunham and Vinyard 1997). Furthermore, MC Spring stability may highly depends on keen conservation management practices.

The population structure of Bud's Pond (i.e. west pond) population at Camp Cady appears to be "unstable", possibly due to inconsistent recruitment and some evidence for density dependent effects. Based on this study and recent population genetic work (Chen 2006), it is advisable to employ Lake Tuendae and China Lake as source populations for future transplants. Camp Cady should be avoided regardless of the fish abundance and ease of capture due to "poor health" of the population and limited genetic variability.

This study showed the importance of collecting baseline population information for this protected fish species. These data are also useful for assessing management actions such as the intensive management of MC Spring. More importantly this study enhances the knowledge base of demography, biology and population dynamics of endangered Mohave tui chub, and in turn these data can be useful for making informed management decisions in conservation management of this endangered desert fish. These data also produced some insights for the biology and management of other fish occupying small habitats.

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APPENDIX C. WATER QUALITY DATA

Table C.1. MC Spring water quality data for 2009.

Transect	Depth	Date	Temperature (C^0)	DO[a] (mg/L)	Salinity (ppt)	Specific Conductivity (µS)	Turbidity (NTU)	pH	Secchi depth (cm)	Depth (cm)
H	Surface	3/18/2009	23	8.91	1.7	3233	3.39	7.9	80	80
H	Bottom	3/18/2009	22.5	6.32	1.7	3225	3.41	7.9	80	80
H	Surface	4/18/2009	24.4	7.57	1.7	3556	5.04	7.9	80	80
H	Bottom	4/18/2009	23.7	7.57	1.7	3200	5.19	7.9	80	80
H	Surface	5/13/2009	26.3	10.58	1.7	3280	2.41	8.3	79	79
H	Bottom	5/13/2009	26.3	10.52	1.7	3261	1.41	8.3	79	79
I	Surface	3/18/2009	22.6	8.63	1.7	3217	1.85	7.9	80	80
I	Bottom	3/18/2009	22.5	8.61	1.7	3222	3.72	7.9	80	80
I	Surface	4/18/2009	23.8	8.09	1.7	3217	1.64	7.9	76	76
I	Bottom	4/18/2009	23.7	8.13	1.7	3219	3.51	7.9	76	76
I	Surface	5/13/2009	25.8	10.44	1.7	3222	6.06	8.3	73	73
I	Bottom	5/13/2009	25.8	9.41	1.7	3218	1.95	8.3	73	73
J	Surface	3/18/2009	22.6	8.32	1.7	3215	0.58	7.9	75	75
J	Bottom	3/18/2009	22.6	8.23	1.7	3216	1.29	7.9	75	75
J	Surface	4/18/2009	23.8	8.42	1.7	3143	1.24	7.9	76	76
J	Bottom	4/18/2009	23.1	9.28	1.7	3204	2.97	7.9	76	76
J	Surface	5/13/2009	25.8	9.79	1.7	3244	2.48	8.2	78	78
J	Bottom	5/13/2009	25.8	9.39	1.7	3221	0.82	8.2	78	78
K	Surface	3/18/2009	22.7	8.43	1.7	3219	1.05	7.9	75	75
K	Bottom	3/18/2009	22.7	8.13	1.7	3218	1.39	7.9	75	75
K	Surface	4/18/2009	23.8	8.64	1.7	3220	2.06	7.9	75	75
K	Bottom	4/18/2009	23.4	8.37	1.7	3140	2.15	7.9	75	75
K	Surface	5/13/2009	26	9.84	1.7	3237	1.61	8.2	75	75
K	Bottom	5/13/2009	25.6	9.98	1.7	3233	0.93	8.2	75	75

Table C.2. Lake Tuendae water quality data for 2009.

Transect	Depth	Date	Temperature (C^0)	DO[a] (mg/L)	Salinity (ppt)	Specific Conductivity (µS)	Turbidity (NTU)	pH	Secchi depth (cm)	Depth (cm)
1A	Surface	3/18/09	18.0	11.74	2.3	4254	4.53	8.3	80	111
1A	Bottom	3/18/09	16.5	11.80	2.3	4238	6.85	8.3	80	111
2A	Surface	3/18/09	17.9	11.60	2.3	4245	5.07	8.7	90	135
2A	Bottom	3/18/09	16.5	11.64	2.3	4246	9.68	8.7	90	135
3A	Surface	3/18/09	17.4	11.43	2.3	4255	6.10	8.7	70	140
3A	Bottom	3/18/09	16.4	11.66	2.3	4243	7.84	8.7	70	110
4A	Surface	3/18/09	17.8	11.01	2.3	3860	5.47	8.6		59
4A	Bottom	3/18/09	17.2	8.65	2.3	4234	6.72	8.6	59	59
1B	Surface	3/18/09	18.2	11.90	2.3	4256	5.76	8.3	80	145
1B	Bottom	3/18/09	16.7	11.89	2.3	4245	6.33	8.3	80	145
2B	Surface	3/18/09	18.3	12.07	2.3	4251	5.87	8.3	81	140
2B	Bottom	3/18/09	17.0	11.69	2.3	4248	5.43	8.3	81	140
3B	Surface	3/18/09	18.6	11.68	2.3	4262	5.28	8.3	80	115
3B	Bottom	3/18/09	16.6	12.80	2.3	4246	5.34	8.3	80	115
4B	Surface	3/18/09	19.5	13.40	2.3	4280	5.72	8.3		22
1C	Surface	3/18/09	20.2	11.84	2.3	4280	5.16	8.3	80	100
1C	Bottom	3/18/09	17.1	12.86	2.3	4239	6.53	8.3	80	100
2C	Surface	3/18/09	19.9	12.00	2.3	4264	4.33	8.3	80	101
2C	Bottom	3/18/09	17.1	13.32	2.3	4243	5.85	8.3	80	101
3C	Surface	3/18/09	19.3	12.16	2.3	4272	4.91	8.3	79	79
3C	Bottom	3/18/09	17.0	13.50	2.3	4242	6.93	8.3	79	79
4C	Surface	3/18/09	19.4	12.76	2.3	4260	4.66	8.3		44
4C	Bottom	3/18/09	shallow	shallow	shallow	shallow	shallow	shallow	shallow	shallow
1D	Surface	3/18/09	20.4	12.10	2.3	4245	4.42	8.2		79
1D	Bottom	3/18/09	16.8	14.60	2.3	4228	4.42	8.2	79	79

[a] Dissolved oxygen content

shallow: water depth was too low to measure the water quality data.

Table C.2. (Continued).

Transect	Depth	Date	Temperature (C^0)	DO[a] (mg/L)	Salinity (ppt)	Specific Conductivity (µS)	Turbidity (NTU)	pH	Secchi depth (cm)	Depth (cm)
2D	Surface	3/18/09	19.4	12.84	2.3	4240	5.46	8.2		30
2D	Bottom	3/18/09	shallow	shallow	shallow	shallow	shallow	shallow	shallow	shallow
3D	Surface	3/18/09	19.4	12.65	2.3	4241		8.2		26
3D	Bottom	3/18/09	shallow	shallow	shallow	shallow	shallow	shallow	shallow	shallow
4D	Surface	3/18/09	20.1	13.52	2.3	4245	4.85	8.3		31
4D	Bottom	3/18/09	shallow	shallow	shallow	shallow	shallow	shallow	shallow	shallow
1E	Surface	3/18/09	20.2	12.15	2.3	4274	4.79	8.2	75	200
1E	Bottom	3/18/09	17.0	12.28	2.3	4231	6.69	8.2	75	200
2E	Surface	3/18/09	20.2	12.17	2.3	4243	4.85	8.2	75	182
2E	Bottom	3/18/09	17.1	12.69	2.3	4227	8.13	8.2	75	182
3E	Surface	3/18/09	19.8	11.90	2.3	4265	5.38	8.2	90	175
3E	Bottom	3/18/09	17.2	12.65	2.3	4225	5.61	8.2	90	175
4E	Surface	3/18/09	20.3	12.21	2.3	4276	4.58	8.3	80	170
4E	Bottom	3/18/09	17.6	12.96	2.3	4217	7.68	8.3	80	170
1F	Surface	3/18/09	21.1	11.97	2.3	4261	5.22	8.5	90	150
1F	Bottom	3/18/09	17.2	13.05	2.3	4236	7.50	8.5	90	150
2F	Surface	3/18/09	21.0	11.91	2.3	4266	4.34	8.6	80	190
2F	Bottom	3/18/09	16.9	12.09	2.3	4230	5.25	8.6	80	190
3F	Surface	3/18/09	21.5	11.89	2.3	4287	4.03	8.2	80	165
3F	Bottom	3/18/09	17.0	13.53	2.3	4227	5.77	8.2	80	165
4F	Surface	3/18/09	21.2	12.07	2.3	4268	5.59	8.6	80	140
4F	Bottom	3/18/09	17.1	14.22	2.3	4230		8.6	80	140
2G	Surface	3/18/09	21.7	11.50	2.3	4281	5.74	8.6	80	181
3G	Surface	3/18/09	21.3	11.84	2.3	4270	5.69	8.2	87	140
3G	Bottom	3/18/09	17.0	12.13	2.3	4225	6.18	8.2	87	140

[a] Dissolved oxygen content

shallow: water depth was too low to measure the water quality data.

Table C.2. (Continued).

Transect	Depth	Date	Temperature (C^0)	DO[a] (mg/L)	Salinity (ppt)	Specific Conductivity (µS)	Turbidity (NTU)	pH	Secchi depth (cm)	Depth (cm)
1A	Surface	4/26/09	20.7	12.73	2.3	4396	9.40	8.9		65
1A	Bottom	4/26/09	20.6	11.20	2.3	4334	14.79			
2A	Surface	4/26/09	20.6	11.75	2.4	4400	11.29	8.9	78	130
2A	Bottom	4/26/09	18.3	12.89	2.3	4380	13.37			
3A	Surface	4/26/09	21.1	11.80	2.4	4405	11.10	8.9	90	130
3A	Bottom	4/26/09	18.4	13.25	2.4	4378	11.33			
4A	Surface	4/26/09	21.7	12.17	2.3	4420	10.65	8.9		50
4A	Bottom	4/26/09	21.1	11.77	2.3	4338	13.52			
1B	Surface	4/26/09	21.4	12.60	2.4	4425	9.25	8.9		62
1B	Bottom	4/26/09	18.8	13.07	2.4	4396	11.55			
2B	Surface	4/26/09	20.9	12.52	2.4	4418	9.62	9	70	140
2B	Bottom	4/26/09	18.9	12.50	2.4	4394	10.32			
3B	Surface	4/26/09	20.7	12.44	2.4	4400	10.96	8.9	80	110
3B	Bottom	4/26/09	18.4	14.18	2.3	4382	20.96			
4B	Surface	4/26/09	20.8	13.08	2.3	4407	11.06	9		15
1C	Surface	4/26/09	21.2	12.65	2.4	4423	9.47	8.9	70	95
1C	Bottom	4/26/09	18.7	14.97	2.4	4389	11.72			
2C	Surface	4/26/09	21.4	12.74	2.4	4429	9.64	8.9	75	103
2C	Bottom	4/26/09	18.7	14.27	2.4	3870	12.74			
3C	Surface	4/26/09	21.5	12.78	2.4	4422	9.22	8.9	70	90
3C	Bottom	4/26/09	18.8	14.85	2.4	4399	10.80			
4C	Surface	4/26/09	21.6	13.67	2.3	4422	9.13	8.9		40
4C	Bottom	4/26/09	21.4	11.43	2.3	4323	11.62			
1D	Surface	4/26/09	22.0	13.23	2.3	4429	11.64	8.9	80	109
1D	Bottom	4/26/09	18.5	15.99	2.3	4386	10.49			

[a] Dissolved oxygen content

shallow: water depth was too low to measure the water quality data.

Table C.2. (Continued).

Transect	Depth	Date	Temperature (C^0)	DO[a] (mg/L)	Salinity (ppt)	Specific Conductivity (µS)	Turbidity (NTU)	pH	Secchi depth (cm)	Depth (cm)
2D	Surface	4/26/09	21.7	12.52	2.4	4412	9.00	8.9		
2D	Bottom	4/26/09	18.1	13.34	2.4	4377	10.78			
3D	Surface	4/26/09	21.5	13.92	2.3	4400	9.41	9		25
3D	Bottom	4/26/09	shallow	shallow	shallow	shallow	shallow	shallow	shallow	shallow
4D	Surface	4/26/09	22.5	14.29	2.4	4410	8.03	9		20
4D	Bottom	4/26/09	shallow	shallow	shallow	shallow	shallow	shallow	shallow	shallow
1E	Surface	4/26/09	21.8	12.90	2.4	4412	8.18	8.9	70	190
1E	Bottom	4/26/09	18.3	12.55	2.4	4381	13.74			
2E	Surface	4/26/09	21.3	12.02	2.4	4420	9.30	8.9	85	182
2E	Bottom	4/26/09	18.1	13.00	2.4	4376	11.20			
3E	Surface	4/26/09	21.1	12.01	2.3	4419	10.17	8.9	75	180
3E	Bottom	4/26/09	18.2	13.60	2.3	4371	11.17			
4E	Surface	4/26/09	20.6	12.36	2.4	4409	9.87	8.9	80	170
4E	Bottom	4/26/09	18.3	14.22	2.4	4362	11.52			
1F	Surface	4/26/09	22.0	12.11	2.3	4428	8.95	8.9	75	132
1F	Bottom	4/26/09	18.6	13.27	2.3	4376	9.50			
2F	Surface	4/26/09	21.0	12.55	2.3	4420	10.23	8.9	81	175
3F	Surface	4/26/09	20.9	12.47	2.4	4059	10.09	8.9	80	160
3F	Bottom	4/26/09	18.3	13.47	2.4	3806	11.85			
4F	Surface	4/26/09	20.9	12.44	2.3	4402	9.99	8.9	80	162
4F	Bottom	4/26/09	18.3	13.42	2.3	4367	9.81			
2G	Surface	4/26/09	21.4	12.63	2.3	4422	9.64	8.9	75	163
2G	Bottom	4/26/09	18.5	11.86	2.3	4370	8.75			
3G	Surface	4/26/09	21.2	12.69	2.3	4400	8.94	8.9	70	140
3G	Bottom	4/26/09	18.3	12.17	2.3	4375	11.36			

[a] Dissolved oxygen content

shallow: water depth was too low to measure the water quality data.

Table C.2. (Continued).

Transect	Depth	Date	Temperature (C^0)	DO[a] (mg/L)	Salinity (ppt)	Specific Conductivity (µS)	Turbidity (NTU)	pH	Secchi depth (cm)	Depth (cm)
1A	Surface	5/13/09	26.3	12.34	2.4	4585	11.26	9.3	55	108
1A	Bottom	5/13/09	26.3	12.34	2.4	4585	21.57			
2A	Surface	5/13/09	26.5	12.93	2.4	4602	13.10	9.1	53	145
2A	Bottom	5/13/09	26.5	12.93	2.4	4602	15.20			
3A	Surface	5/13/09	26.1	12.24	2.4	4590	14.15	9.1	54	142
3A	Bottom	5/13/09	26.1	12.24	2.4	4590	13.70			
4A	Surface	5/13/09	27.1	12.20	2.4	4600	16.33	9.2	54	54
4A	Bottom	5/13/09	27.1	12.20	2.4	4600	31.57			
1B	Surface	5/13/09	27.1	11.27	2.4	4604	12.85	9.1	55	90
1B	Bottom	5/13/09	27.1	11.27	2.4	4604	12.85			
2B	Surface	5/13/09	26.9	12.39	2.4	4598	11.82	9.2	55	162
2B	Bottom	5/13/09	26.9	12.39	2.4	4598	10.68			
3B	Surface	5/13/09	27.0	12.35	2.4	4604	11.25	9.2	52	125
4B	Surface	5/13/09	27.3	15.54	2.4	4602	18.65	9.2	39	39
4B	Bottom	5/13/09	27.3	15.54	2.4	4602	18.65			
1C	Surface	5/13/09	27.5	11.02	2.4	4591	10.03	9.1	58	58
1C	Bottom	5/13/09	27.5	11.02	2.4	4591	10.03			
2C	Surface	5/13/09	27.2	12.25	2.4	4607	11.83	9.1	52	115
2C	Bottom	5/13/09	27.2	12.25	2.4	4607	11.87			
3C	Surface	5/13/09	27.4	11.76	2.4	4533	8.81	9.1	58	98
3C	Bottom	5/13/09	27.4	11.76	2.4	4528	30.15			
4C	Surface	5/13/09	27.4	12.98	2.4	4587	20.92	9.1	33	33
4C	Bottom	5/13/09	27.4	12.98	2.4	4498	20.92			
1D	Surface	5/13/09	27.3	12.08	2.4	4771	10.85	9	52	110
1D	Bottom	5/13/09	27.3	12.08	2.4	4671	10.86			

[a] Dissolved oxygen content

shallow: water depth was too low to measure the water quality data.

Table C.2. (Continued).

Transect	Depth	Date	Temperature (C^0)	DO[a] (mg/L)	Salinity (ppt)	Specific Conductivity (µS)	Turbidity (NTU)	pH	Secchi depth (cm)	Depth (cm)
2D	Surface	5/13/09	27.4	10.46	2.4	4589	11.77	8.9	56	85
2D	Bottom	5/13/09	27.4	10.46	2.4	4509	12.77			
3D	Surface	5/13/09	27.5	10.82	2.4	4584	16.69	9.1	33	33
3D	Bottom	5/13/09	27.5	10.82	2.4	4567	16.69			
4D	Surface	5/13/09	27.4	11.08	2.4	4572	10.38	9	35	35
4D	Bottom	5/13/09	27.4	11.08	2.4	4598	10.38			
1E	Surface	5/13/09	27.9	10.76	2.4	4609	10.89	9	56	188
1E	Bottom	5/13/09	27.9	10.76	2.4	4613	10.73			
2E	Surface	5/13/09	27.8	11.62	2.4	4589	10.86	9	54	187
2E	Bottom	5/13/09	27.8	11.62	2.4	4521	13.11			
3E	Surface	5/13/09	27.6	10.65	2.4	4583	8.30	9.1	58	189
3E	Bottom	5/13/09	27.6	10.65	2.4	4556	9.87			
4E	Surface	5/13/09	27.5	10.21	2.4	4546	10.41	9	55	178
4E	Bottom	5/13/09	27.5	10.21	2.4	4544	11.64			
1F	Surface	5/13/09	28.1	9.85	2.4	4609	10.61	9	51	135
1F	Bottom	5/13/09	28.1	9.85	2.4	4623	16.92			
2F	Surface	5/13/09	28.2	10.70	2.4	4609	8.86	9	58	200
3F	Surface	5/13/09	28.0	10.76	2.4	4577	8.75	9	58	168
3F	Bottom	5/13/09	28.0	10.76	2.4	4577	9.78			
4F	Surface	5/13/09	27.8	9.92	2.4	4584	9.70	9	55	129
4F	Bottom	5/13/09	27.8	9.92	2.4	4584	8.26			
2G	Surface	5/13/09	28.1	10.51	2.4	4605	10.87	9.1	58	175
2G	Bottom	5/13/09	28.1	10.51	2.4	4605	8.88			
3G	Surface	5/13/09	28.0	10.88	2.4	4600	9.97	9.1	59	180
3G	Bottom	5/13/09	28.0	10.88	2.4	4600	8.26			

[a] Dissolved oxygen content

shallow: water depth was too low to measure the water quality data.