EFFECTS OF ASIAN TAPEWORM, MOSQUITOFISH, AND FOOD RATION ON MOHAVE TUI CHUB GROWTH AND SURVIVAL

by

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I am also deeply indebted to Jessica Koehle (University of Minnesota) and Scott Campbell (Kansas Biological Survey) for providing Asian tapeworm eggs for the laboratory portion of this experiment.

Many volunteers helped with field and laboratory sampling on this project, and I would specifically like to thank S. Jason Kline (Arizona Game and Fish Department), Alison Iles, Erica Sontz, and Shannon Grubbs (University of Arizona), and Sean Tackley. I am also very thankful for laboratory assistance from Andrea Francis.

Finally, I thank my advisor, Scott Bonar, for providing me with the opportunity to study desert fishes, and the rest of my committee, Bill Matter and Peter Reinthal for help with experimental design and manuscript review.

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ABSTRACT

Asian tapeworm *Bothriocephalus acheilognathi*, a non-native fish parasite, is potentially dangerous to native desert fishes. Mohave tui chubs *Gila bicolor mohavensis* are federally-listed fish native to the Mojave basin in southern California. Asian tapeworm was discovered in Mohave tui chubs in Lake Tuendae, near Baker, California, in 2001, about the same time western mosquitofish *Gambusia affinis* were also discovered there. Temperature and photoperiod manipulations stimulated Mohave tui chub to spawn in the laboratory and offspring were infected with Asian tapeworm. I examined Asian tapeworm population dynamics at Lake Tuendae. Mosquitofish presence increased chub growth, and Asian tapeworm significantly reduced growth in tanks with mosquitofish. There were no significant differences in survival between groups. Mohave tui chubs had high Asian tapeworm infection prevalence and intensity at higher water temperatures, and increasing total length was associated with increasing intensity.

INTRODUCTION

Introductions of non-native fishes have contributed to extinctions, extirpations, and declines of native fishes in the southwestern United States (Miller 1961). Non-native fish can be predators or competitors of native species, but they can also be the source of harmful non-native pathogens and parasites, including the Asian tapeworm *Bothriocephalus acheilognathi* (Heckmann et al. 1986, Heckmann et al. 1987, Heckmann et al. 1993). Asian tapeworm is a cestode native to the Amur River Basin, China (Dove and Fletcher 2000). This parasite spends part of its life cycle in an intermediate copepod host then inhabits the intestine of a freshwater fish host. Most commonly associated with cyprinid fishes, Asian tapeworm was widely introduced through translocations of common carp *Cyprinus carpio* and grass carp *Ctenopharyngodon idella* (Bauer et al. 1973, Dove and Fletcher 2000). It is now found on all continents except Antarctica (Hoffman 2000).

Asian tapeworm first appeared in the southwestern United States in 1979, when it was discovered in infected cyprinids from the Virgin River of Utah, Nevada, and Arizona, and was probably introduced to the Southwest through cyprinid baitfish (Heckmann et al. 1986, Heckmann et al. 1987, Heckmann et al. 1993). Asian tapeworm has spread rapidly, and it is now found in lakes and rivers throughout the region (Brouder and Hoffnagle 1997, Clarkson et al. 1997, Steve Parmenter, California Department of Fish and Game, personal communication). When parasites are numerous, marked enlargement of the abdomen can occur with severe hemorrhagic enteritis and intestinal blockage, often resulting in host fatalities (Hoole and Nisan 1994).

The effects of the Asian tapeworm on wild fish populations, especially in the Southwest, are poorly understood. Mortality of infected fish approached 90% in some Russian ponds (Bauer et al. 1973). Asian tapeworm infection was associated with reduced survival in western mosquitofish *Gambusia affinis* (Granath and Esch 1983) and in Topeka shiners *Notropis tristis* (Jessica Koehle, University of Minnesota, personal communication). Experimental infections of Asian tapeworm caused significant reduction in length and weight of bonytail chub *Gila cypha*, and negatively affected health indices (Hansen et al. 2006) Roundtail chubs *G. robusta* infected with Asian tapeworm showed a significant difference in total length compared to uninfected chubs, and a positive correlation between infection intensity and total length of fish (Brouder 1999). The parasite possibly contributed to declines of endangered Virgin River woundfin *Plagopterus argentissimus* (Deacon 1988). Little is known about impacts on potentially more vulnerable small cyprinids in warm water streams.

Lake Tuendae, an artificial lake near Zzyzx, California, harbors one of the few remaining populations of Mohave tui chubs. Endemic to the Mojave River system of southern California, Mohave tui chub hybridized with introduced arroyo chubs *G. orcutti* and by 1967 were completely eliminated from the main river (Hubbs and Miller 1943, Miller 1968). The Mohave tui chub was listed as endangered by the U.S. Department of the Interior on October 13, 1970, and the only existing natural population occurs at Soda Spring, Zzyzx, which was apparently colonized by Mohave tui chub when the Mojave River flooded. Additional populations have been established, with varying degrees of success (St. Amant and Sasaki 1971, Hoover and St. Amant 1983), and today only three populations are recognized: Zzyzx near Baker, California, Camp Cady near Harvard Road in Barstow, California, and Lark Seep on the China Lake Naval Weapons Center (Hughson and Woo 2004). Asian tapeworm was detected in the Lake Tuendae population in January 2001, nearly the same time western mosquitofish were discovered there (Steve Parmenter, personal communication).

Western mosquitofish are native to the southeastern United States, but range as far north as Indiana and Illinois, and as far west as the Rio Grande basin in New Mexico (Fuller et al.1999). Mosquitofish were stocked indiscriminately (and without regard to strain) because of their supposed abilities as mosquito-control agents, and are now established in nearly all 50 states (Fuller et al.1999). Numerous published studies have shown mosquitofish to have negative impacts on native southwestern fishes through competition and predation (Deacon et al. 1964, Meffe 1985, Meffe et al. 1983, Courtenay and Meffe 1989, Mills 2004).

My specific objectives for Appendix A were to develop methods to spawn and rear Mohave tui chub in the laboratory to use in subsequent growth and survival experiments described in Appendix B. The specific objectives for the second part (Appendix B) were to examine the effects food ration, mosquitofish presence, and Asian tapeworm exposure, and any interactions, had on Mohave tui chub growth and survival. The specific objectives in Appendix C were to monitor Asian tapeworm population dynamics at Lake Tuendae, California, in Mohave tui chubs. I used non-lethal detection methods, and examined the relationship between temperature detection of Asian tapeworm.

The overarching goal of the project was to determine the impacts mosquitofish and Asian tapeworm have on Mohave tui chub growth and survival, and monitor changes to determine when Mohave tui chub have the highest Asian tapeworm infection prevalence and the highest intensity. This information will help managers determine if Asian tapeworm or mosquitofish are threats to Mohave tui chub survival, and predict when fish are most vulnerable to additional stressor.

PRESENT STUDY

The methods, results, and discussions of this study are presented in three manuscripts appended to this thesis. Each paper discusses the methods and findings associated with my research on Mohave tui chubs, Asian tapeworm, and western mosquitofish in the field at Lake Tuendae, California, and under laboratory conditions. Each paper will be submitted for publication in peer-reviewed journals. Following is a summary of the most important findings in these papers.

METHODS FOR SPAWNING THE ENDANGERED MOHAVE TUI CHUB IN AQUARIA

Temperature and photoperiod manipulations resulted in Mohave tui chub spawning into artificial plants. As water was warmed to 15° C, I noticed increased activity among fish. Many fish developed a reddish tinge on the bases of the paired fins, and fish were often seen “milling” about the artificial plants. One month after the tanks reached ambient temperature (20-23°C), I found eggs in the tank. Three spawns occurred within 2 weeks, but the total number of eggs was difficult to estimate because they were hard to see in the artificial plants. The first spawn yielded 166 larval fish, and the latter two spawns yielded over 800 larval fish each. At 20-23° C it took about 4 days for eggs to hatch, and less than 24 hours to reach swim-up after hatching.

Ten fish kept under similar conditions with access to spawning plants but not subjected to temperature cycling or photoperiod manipulation did not spawn after 1 year of captivity. No new eggs appeared in the tanks after the artificial plants were removed. Whether the plants served as a cue for spawning or simply provided cover to prevent the eggs from being eaten immediately is unknown.

About 400 offspring were used in subsequent growth and survival assessing the impacts of experimental infection by Asian tapeworm *Bothriocephalus acheilognathi*. The remaining fish were transported back to Mojave National Preserve and used to assess habitat suitability for future populations.

EFFECTS OF ASIAN TAPEWORM ON GROWTH AND SURVIVAL OF MOHAVE TUI CHUB

Food ration, mosquitofish, and Asian tapeworm exposure had significant effects on the standard length gain of Mohave tui chubs. Significant non-additive interactions occurred between food ration and mosquitofish, and Asian tapeworm and mosquitofish (Appendix B Table 1). Mohave tui chubs with mosquitofish grew larger than Mohave tui chubs without mosquitofish at all levels of food ration; however, food ration had no effect on standard length gain when mosquitofish were present (Appendix B Figure 1, Tukey-

HSD, q = 2.76, α = 0.05). Mohave tui chub with mosquitofish also grew larger than Mohave tui chub without mosquitofish at all levels of Asian tapeworm, but Asian tapeworm reduced Mohave tui chub growth only when mosquitofish were present (Appendix B Figure 1, Tukey HSD, q = 2.76, α = 0.05).

No treatment factor significantly lowered Mohave tui chub survival (Appendix B Table 7). No fish remaining alive at day 96 were infected. One tapeworm was observed floating in an experimental tank, and I found one out of nine extra fish infected with Asian tapeworm.

Mohave tui chub are more opportunistic than predatory mosquitofish and better at foraging, and even with daily cleaning there was food and detritus left in the tanks. An unfortunate flaw in the design, and explanation for the non-additive food ration*mosquitofish interaction term, is that when mosquitofish are present Mohave tui chub able consume much more than 2% biomass daily, essentially making high and low food rations the same in tanks with mosquitofish. Field experiments are needed, but these results suggest that once Mohave tui chubs reach a size that mosquitofish are unable to prey upon them, they are of little threat when habitat refuges are available.

Low infection prevalence and non-additive interactions confuse the effect Asian tapeworm has on Mohave tui chub. At least some fish were infected at the midpoint of the experiment. The tapeworms may have matured and passed during the experiment. The non-additive interaction between tapeworm and mosquitofish was probably caused by low infection prevalence and randomization that did not result in even distribution of infected fish among groups.

Under captive conditions, Asian tapeworm does not appear to directly reduce survival in Mohave tui chub, and reduces growth only slightly, especially when compared to the effects of mosquitofish and food ration. Studies on bonytail (Hansen et al. 2006) found similar results for survival, and personal observation of highly infected Topeka shiners (>30 tapeworms per fish) support these conclusions. Asian tapeworm may affect Mohave tui chubs differently in the field, but this study and others produced no patterns of extreme mortality.

ASIAN TAPEWORM DYNAMICS IN MOHAVE TUI CHUB IN LAKE TUENDAE, CALIFORNIA

I found significant differences in prevalence between sampling periods (X^2=83.8, *df*=5, *P*<0.001). Increasing water temperature was associated with increasing infection prevalence (Figure 1, $F_{1,4}$ = 9.31 *P* = 0.038). I found similar positive relationships between increasing mean abundance and water temperature (Appendix C, Figure 1, $F_{1,4}$ = 8.53 *P* = 0.043) and increasing mean intensity and water temperature (Appendix C, Figure 1, $F_{1,4}$ = 9.25 *P* = 0.038).Mean prevalence ranged from 0.00 to 0.62 (Appendix C, Figure 2), while mean abundance and mean intensity ranged from 0 to 21 and 0 to 33, respectively (Appendix C, Table 1.). Additionally, increasing total length of fish was associated with increasing infection intensity (Spearman's rho correlation, r_s = 0.21, *P* = 0.055).

The warmer the water, the more severe the prevalence and the more intense the infection becomes. The highest prevalence and mean intensity coincided with the warmest sampling period, although the population dynamics of the copepod intermediate host and detectibility in cold water also probably play a role in fluctuating prevalence, abundance, and intensity. Handling, transport, marking, or any activities that may add to the stressors already present in summer when water temperatures are high and infection prevalence and intensity peak should be avoided. Such activities should be carried out during the winter and early spring, when the stresses associated with Asian tapeworm are at a minimum. Additional research should be conducted on high temperature and Asian tapeworm interactions with Mohave tui chub and other warm desert region fishes.

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APPENDIX A:

METHODS FOR SPAWNING THE ENDANGERED MOHAVE TUI CHUB IN AQUARIA

Methods for spawning the endangered Mohave tui chub in aquaria

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Abstract

The Mohave tui chub *Gila bicolor mohavensis* is a federally listed fish not previously spawned in captivity. Laboratory spawning can be important for recovery efforts by reducing collection of wild fish for translocations, providing individuals for experimental studies, and ensuring survival of the species. Mohave tui chub successfully spawned under a photoperiod of 14 h light and 10 h dark, lowering the temperature to about 9°C for 30 days, then raising to 21°C over an 8-week period, and providing artificial plants as spawning substrate. No hormones were used; three spawning events produced over 1,700 larval fish used in subsequent growth and survival studies.

Introduction

Laboratory spawning of endangered fishes can be important for recovery efforts, allow accurate observations of early life-history traits, and reduce collection of wild fish for experimental studies and translocations (Buyanak and Mohr 1981; Rakes et al. 1999). Many common and endangered fishes, including endangered cyprinids (Cyprinidae), have been spawned in the laboratory (Buyanak and Mohr 1981; Hamman 1982a, 1982b; Kaya 1991; Brandt et al. 1993; Rakes et al 1999). Mohave tui chub *Gila bicolor mohavensis* (Snyder 1918) is the only native fish in the Mojave River basin. Populations declined after the 1930s, when competition occurred with arroyo chubs *G. orcutti* (Hubbs and Miller 1943), which were believed to have been introduced into the headwaters by anglers. Mohave tui chubs were eliminated from the Mojave River system by the late 1960s, and existed only at one isolated pool in Mojave National Preserve at Zzyzx Mineral Springs, California (Miller 1968). Mohave tui chubs were federally listed in 1970. Recovery efforts involved transplantations of fish to establish new populations, and in spite of many attempts, the U.S Fish and Wildlife Service recognizes only three populations in springs in southern California—one at Lark Seep on the China Lake Naval Weapons Center, one at Camp Cady near Harvard Road in Barstow, and one at Zzyzx in Mojave National preserve (Hoover and St. Amant 1983; Hughson and Woo 2004). Captive breeding would contribute to the proliferation and maintenance efforts for the species. Vicker (1973) made several unsuccessful attempts to induce spawning in the laboratory and to collect fertile eggs from Lake Tuendae. I know of no other captive breeding attempts. My objective was to develop methods to spawn Mohave tui chub in captivity without the use of hormones. Here I describe methods used to successfully spawn Mohave tui chubs in laboratory aquaria.

Methods

Fish collection and husbandry. I used minnow traps to collect 25 adult Mohave tui chub from Lake Tuendae, a spring located at Zzyzx, California (Mojave National Preserve), in August 2005 (US Fish and Wildlife recovery permit TE086593-0). Fish ranged in size from 120 to 220 mm TL at the time of collection. I followed guidelines in Widmer et al. (2005) to transport fish to holding facilities in Tucson, Arizona, and to treat them for external parasites. In addition, I placed the fish in a praziquantel bath (6 mg/L for 24 h) to remove Asian tapeworm *Bothriocephalus acheilognathi* and other internal parasites. I housed 15 fish, after 2 months of acclimation to aquaria, in a 476-L acrylic tank (510 mm x 510 mm x 1830 mm) fitted with a custom-built recirculating biofilter and filled with well water (pH = 8.0, GH = 6°). I pumped water to the filter with an in-line pump (Aquatic Ecosystems Model 5, maximum flow rate 1,900 L/h). Water was sprayed over nylon batting, trickled through approximately 12 L of Coralife Bio-balls$^{©}$, and finally filtered through a 1-cm thick layer of activated carbon. To stabilize the filter, I placed 15 cm of pea-sized aquarium gravel in the bottom to balance the weight of the water in the top. Water exited the bio-filter by gravity, passed through an in-line chiller (Prime Chiller model #2626), and returned to the tank through a 5-cm PVC “T” to diffuse the return flow. The water level in the tank was maintained at 450 mm, the same height water was returned to the tank from the chiller. I used two 40-watt fluorescent lights (placed 30 cm above water level) on electronic timers to control the light cycle. Bloodworms and pellet food (Aquatic Ecosystems ZP1) were fed once each per day, *ad libitum*. Waste was removed daily, and routine water changes and cleaning were performed as needed to maintain ammonia and nitrate levels at 0 ppm. At least once per month, I replaced 10% of the tank water with clean well water.

I covered the outside bottom, back, and sides with 5-mm foam insulation. I placed pea-sized gravel substrate on the bottom of half the tank, and the other half was left bare. I placed three pottery shards in the tank to provide cover. I used two 40-watt, 1220 mm fluorescent light bulbs for room lighting, and electronic timers to provide specific photoperiods.

Temperature and photoperiod manipulations. To induce spawning, I lowered the water temperature in the tank by 1° C per day to about 9° C to simulate natural conditions in Lake Tuendae. Mohave tui chub are inactive during the coldest months (Vicker 1973), and the average minimum water temperature during January is about 8° C. In Lake Tuendae, Mohave tui chub spawn as early as February, and peak spawning occurs when the water warms to 18° C in mid-March (Vicker 1973). During the temperature manipulation, I used a photoperiod of 10 h light: 14 h dark. After the water temperature was less than 10°C, I held it constant for 30 days. After 30 days, I allowed the tank to warm up 1°C per day to reach ambient air temperature (20-22°C) and I adjusted the photoperiod to 14 h light: 10 h dark when the tank reached 15°C (Figure 1).

Spawning substrate. Tui chubs spawn over vegetation, and Kimsey (1954) observed that the eggs of the Eagle Lake tui chubs that fall into the substrate do not develop. I attached two artificial plants (Fancy Plants Giants© asparagus fern) to a plastic grate and placed them in the tank with a large rock to prevent the grate from floating. Plants provided a substrate for egg attachment, as well as additional cover, and could easily be removed. For the second and third spawns, unglazed ceramic tiles were placed under the grate to capture eggs that did not adhere to the plants. I transferred the artificial plants and tiles containing eggs to 76-L rearing tanks after spawning occurred. Eggs were incubated at ambient temperature (20-23°C). Larval fish were fed appropriately-sized commercial larval fish food (Aquatic Ecosystems LD100, LD150, LD250 and ZP3).

Results and Discussion

As water was warmed to 15° C, I noticed increased activity among fish. Many fish developed a reddish tinge on the bases of the paired fins, and fish were often seen "milling" about the artificial plants. These observations are in agreement with previous studies of tui chub spawning in the wild (Kimsey 1954; Vicker 1973). On 6 February 2006, one month after the tanks reached ambient temperature (20-23°C), I found eggs in the tank. I suspect spawning occurs at night, because I checked tanks daily and never witnessed spawning; eggs were always first noticed in the morning. Three spawns occurred within 2 weeks, but the total number of eggs was difficult to estimate because they were hard to see in the artificial plants. The first spawn yielded 166 larval fish, and the latter two spawns yielded over 800 larval fish each. At 20-23° C it took about 4 days for eggs to hatch, and less than 24 hours to reach swim-up after hatching.

Fish acted nervous when first brought into captivity. Adding substrate and artificial plants calmed the fish. Temperature cycling and photoperiod may be important cues for spawning. Ten fish kept under similar conditions with access to spawning plants but not subjected to temperature cycling or photoperiod manipulation did not spawn after 1 year of captivity. Also, no new eggs appeared in the tanks after the artificial plants were removed. Whether the plants served as a cue for spawning or simply provided cover to prevent the eggs from being eaten immediately is unknown.

About 400 offspring were used in subsequent growth and survival assessing the impacts of experimental infection by Asian tapeworm *Bothriocephalus acheilognathi*. The remaining fish were transported back to Mojave National Preserve and used to assess habitat suitability for future populations.

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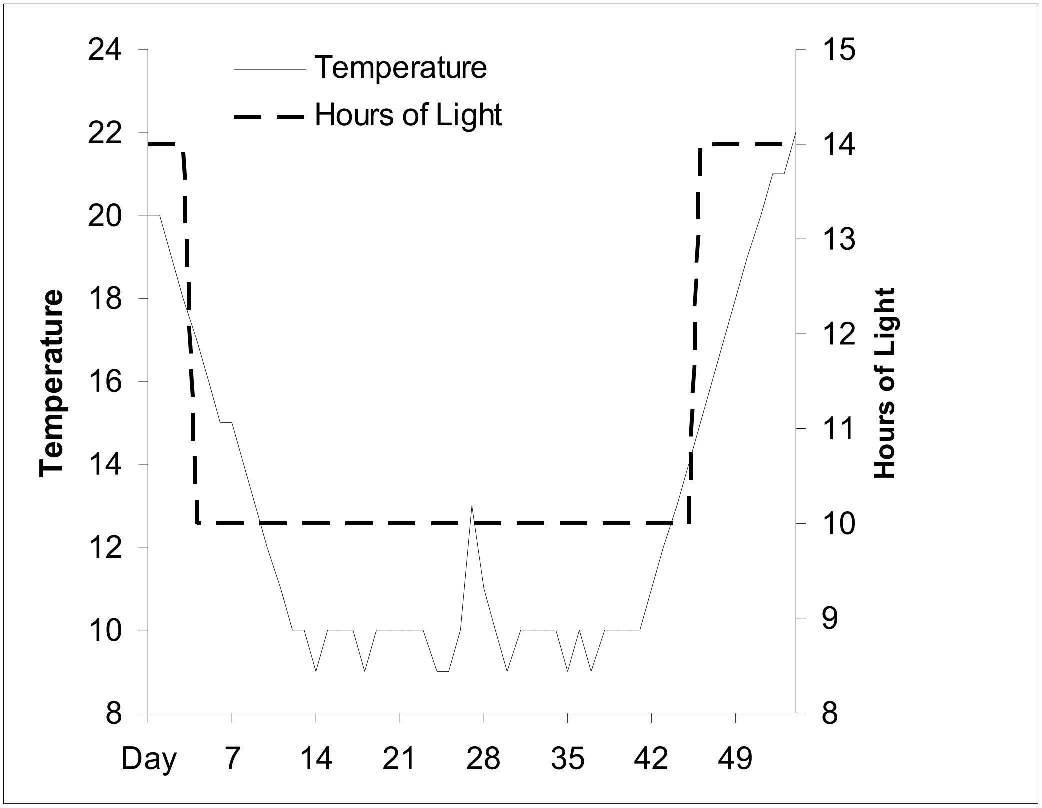
Figure captions

Figure 1.—Photoperiod (hours of light) and temperature (temperature °C) regimen for laboratory spawning of Mohave tui chub. Temperature recorded at 0800 daily.

Figure 1


Temperature
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APPENDIX B:

EFFECTS OF ASIAN TAPEWORM ON GROWTH AND SURVIVAL OF MOHAVE TUI CHUB

Effects of Asian tapeworm on growth and survival of Mohave tui chub

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Abstract

Asian tapeworm *Bothriocephalus acheilognathi* is a potentially dangerous non-native fish parasite. Asian tapeworm was brought to the United States through shipments of grass carp *Ctenopharyngodon idella* and has been spread through baitfish. Mohave tui chubs *Gila bicolor mohavensis* are federally-listed fish native to the Mojave basin in southern California. Asian tapeworm was discovered in Mohave tui chubs in Lake Tuendae, near Baker, California, in 2001, about the same time western mosquitofish *Gambusia affinis* were also discovered in the lake. I spawned Mohave tui chub in the laboratory to produce enough fish to experimentally infect with Asian tapeworm. After exposure, standard length, weight, and survival of Mohave tui chubs were recorded every two weeks for 16 weeks. Mohave tui chubs were either exposed or unexposed to Asian tapeworm, with or without mosquitofish, and on 5% or 2% biomass daily food ration. I found that mosquitofish presence increased chub growth, and non-additive interactions occurred between mosquitofish and food ration, and mosquitofish and Asian tapeworm exposure. Food ration had no effect on Mohave tui chubs with mosquitofish present, but higher food ration increased growth in tanks without mosquitofish. Asian tapeworm significantly reduced growth in tanks with mosquitofish, but there was no difference between exposed and unexposed tanks without mosquitofish. There were no significant differences in survival between groups. The final infection prevalence was 0%, but there was evidence that at least some of the fish were infected.

Introduction

Introductions of non-native fishes have contributed to extinctions, extirpations, and declines of native fishes in the southwestern United States (Miller 1961). Non-native fish can be predators or competitors of native species, but they can also be the source of harmful non-native pathogens and parasites, including the Asian tapeworm *Bothriocephalus acheilognathi* (Heckmann et al. 1986, Heckmann et al. 1987, Heckmann et al. 1993). Asian tapeworm is a cestode native to the Amur River Basin, China (Dove and Fletcher 2000). This parasite spends part of its life cycle in an intermediate copepod host then inhabits the intestine of a freshwater fish host. Most commonly associated with cyprinid fishes, Asian tapeworm was widely introduced through translocations of common carp *Cyprinus carpio* and grass carp *Ctenopharyngodon idella* (Bauer et al. 1973, Dove and Fletcher 2000). It is now found on all continents except Antarctica (Hoffman 2000).

Asian tapeworm first appeared in the southwestern United States in 1979, when it was discovered in infected cyprinids from the Virgin River of Utah, Nevada, and Arizona, and was probably introduced to the Southwest through cyprinid baitfish (Heckmann et al. 1986, Heckmann et al. 1987, Heckmann et al. 1993). Asian tapeworm has spread rapidly, and it is now found in lakes and rivers throughout the region (Brouder and Hoffnagle 1997, Clarkson et al. 1997, Steve Parmenter, California Department of Fish and Game, personal communication).

When parasites are numerous, marked enlargement of the abdomen can occur with severe hemorrhagic enteritis and intestinal blockage, often resulting in host fatalities

(Hoole and Nisan 1994). The effects of Asian tapeworm on wild fish populations, especially in the Southwest, are poorly understood. Mortality of infected fish approached 90% in some Russian ponds (Bauer et al. 1973). Asian tapeworm infection was associated with reduced survival in western mosquitofish *Gambusia affinis* (Granath and Esch 1983) and in Topeka shiners *Notropis tristis* (Jessica Koehle, University of Minnesota, personal communication). Experimental infections of Asian tapeworm caused significant reduction in length and weight of bonytail chub *Gila cypha*, and negatively affected health indices (Hansen et al. 2006) Roundtail chubs *G. robusta* infected with Asian tapeworm showed a significant difference in total length compared to uninfected chubs, and a positive correlation between infection intensity and length of fish (Brouder 1999). The parasite possibly contributed to declines of endangered Virgin River woundfin *Plagopterus argentissimus* (Deacon 1988). Little is known about impacts on potentially more vulnerable small cyprinids in warm water streams.

Lake Tuendae, an artificial lake near Zzyzx, California, harbors one of the few remaining populations of Mohave tui chub *G. bicolor mohavensis*. Endemic to the Mojave River system of southern California, Mohave tui chub hybridized with introduced arroyo chubs *G. orcutti* and by 1967 were completely eliminated from the main river (Hubbs and Miller 1943, Miller 1968). The Mohave tui chub was listed as endangered by the U.S. Department of the Interior on October 13, 1970, and the only existing natural population occurs at Soda Spring, Zzyzx, which was apparently colonized by Mohave tui chub when the Mojave River flooded. Additional populations have been established, with varying degrees of success (St. Amant and Sasaki 1971, Hoover and St. Amant

1983), and today only three populations are recognized: Zzyzx near Baker, California, Camp Cady near Harvard Road in Barstow, and Lark Seep on the China Lake Naval Weapons Center (Hughson and Woo 2004). Asian tapeworm was detected in the Lake Tuendae population in January 2001, nearly the same time western mosquitofish were discovered there (Steve Parmenter, California Department of Fish and Game, personal communication).

Western mosquitofish are native to the southeastern United States, but range as far north as Indiana and Illinois, and as far west as the Rio Grande basin in New Mexico (Fuller et al.1999). Mosquitofish were stocked indiscriminately (and without regard to strain) because of their supposed abilities as mosquito-control agents, and are now established in nearly all 50 states (Fuller et al.1999). Numerous published studies have shown mosquitofish to have negative impacts on native southwestern fishes through competition and predation (Deacon et al. 1964, Meffe et al. 1983, Meffe 1985, Courtenay and Meffe 1989, Mills 2004).

I experimentally infected young Mohave tui chub in a laboratory setting, and exposed them to high and low food rations, and to mosquitofish: the overarching objective was to determine the impact of Asian tapeworm on growth and survival of Mohave tui chub, and provide recommendations for prioritizing perceived threats to survival.

Methods

Terminology. I used terms and conventions recommended by Margolis et al. (1982) and Bush et al. (1997) to avoid misuse of parasitological terms.

Animal Husbandry

Copepods. I collected copepods *Cyclops vernalis* from San Bernardino National Wildlife Refuge in southeastern Arizona. I used methods developed by J. Rey to produce pure cultures of copepods (University of Florida, personal communication, available at http://fmel.ifas.ufl.edu/culture.htm). I isolated single females, readily identified by external eggs, by using a pipette to transfer single copepods into smaller drops of water until I could no longer see any other organisms. Next, I transferred the copepod to a small plastic container, added spring water, and inoculated each container with a wheat seed and *Paramecium caudatum*. When the eggs hatched, I poured four or five containers into a large wading pool, inoculated the pool with wheat seeds and *Paramecium caudatum*, and covered each pool to prevent contamination. Within several weeks I was able to produce high densities of pure copepods.

Western mosquitofish. I collected several hundred mosquitofish from Lake Tuendae, Mojave National Preserve, California. I housed them in multiple 1,100-liter fiberglass tanks. I filled the tanks with well water and added artificial plants for cover. I added a pinch of flake food daily, and moved young fish to other tanks as needed when crowding was evident.

Mohave tui chub. I used temperature and photoperiod manipulations described in Appendix A to induce Mohave tui chub to spawn (USFWS permit recovery permit

TE086593-0). I housed all vertebrates in accordance with an approved Institutional Animal Care and Use Committee protocol.

Experimental Infection

Copepod exposure. I received 125 Topeka shiners *Notropis tristis* from an experimental hatchery population known to be heavily infected with Asian tapeworm (Jessica Koehle, University of Minnesota, and Scott Campbell, Kansas Biological Survey, personal communication). I dissected the shiners and removed any gravid tapeworms with tanned eggs in the posterior proglittids. I rinsed each tapeworm with 0.6% saline to remove any debris, then placed the tapeworm in 50-mm diameter petri dish filled with spring water. Tapeworms often expel tanned eggs, when it did not I used needles to tease out any remaining eggs from the proglottids. I repeated the process with more tapeworms until the petri dish bottom was mostly covered with eggs, and continued to do so until I filled eight petri dishes.

I used the guidelines of Hansen et al. (2006) to infect copepods with Asian tapeworm. One week before exposing copepods to Asian tapeworm, I placed 50 copepods, avoiding females when possible, into each of 15 small plastic containers. I added only spring water, starving the copepods for 1 week prior to exposing them to coracidia. I examined Asian tapeworm eggs daily, and hatching began 2 days after dissection, the largest hatch occurring 3 days after dissection. On the third day, I poured all the petri dishes containing tapeworm eggs and emergent coracidia into one small dish. After mixing the water thoroughly to distribute coracidia throughout the sample, I examined six 10-µL aliquots under a dissecting scope to estimate the number of coracidia per 1-mL of water. I thoroughly stirred the bowl and pipetted enough water to transfer approximately 2,800 coracidia to each bowl of 50 copepods. I maintained copepods in small plastic bowls for 13 days. On day nine, I noticed the copepod behavior was altered. I examined the copepods and was able to see the developing procercoids. I checked each bowl of 50 copepods for infected individuals, and all contained at least one. Copepods were photographed on day 10 and had infective procercoids developing (Anindo Choudhury, St. Norbert's College, personal communication).

Fish exposure. I filled 28 Rubbermaid© 6.1-L plastic shoeboxes with water. I placed six Mohave tui chub, between 16 and 24 mm standard length (SL) in each container. To minimize any size biases, I placed only one fish in each container before adding a second fish, and so on. I randomly assigned one of six treatments to each 6.1-L box. I designated boxes in experimental tanks containing three Mohave tui chub and three western mosquitofish as "split" (described below). I replaced fish that died during exposure or on the first day of the experiment, which I assumed to be from handling stress and not due to Asian tapeworm infection. I starved fish for 36 h prior to exposure, and allowed fish 24 h to acclimate to small tanks. After the acclimation period, I poured one bowl of infected copepods into each of the 14 6.1-L boxes assigned Asian tapeworm exposure. I poured a similar amount of unexposed copepods into control boxes. I allowed fish 24 h to forage on copepods, and checked for uneaten copepods the following day.

Experimental design and allocation of fishes to tanks. I used a fully-crossed design with three treatment factors: Asian tapeworm (exposed/unexposed), mosquitofish (present/absent) food ration (2% biomass per day/5% biomass per day). Temperature, food availability, and fish density all affect fish growth (Smith et al. 1977, Hanson and Leggett 1985, Roudebush and Taylor 1987, Werner 1992). I calculated the biomass of fish in each tank to keep food ration constant, to avoid confounding density effects, I placed only six fish in every tank; either six Mohave tui chub or three Mohave tui chub and three mosquitofish. Temperature was maintained at ambient room temperature (20-25° C). Randomization of tank assignment ensured expected temperature differences between groups was zero.

I randomly assigned each group of fish to a 38-L glass tank. In 6.1-L boxes designated "split," I haphazardly netted three fish and randomly assigned a tank, then randomly assigned the remaining fish to a tank. I chose to split fish from within a single exposed 6.1-L box to maintain constant densities of both fish and copepods throughout all treatment groups. I then used randomly assigned high and low food rations to each tank. I used four complete replicates, with eight treatment combinations in each replicate for a total of 32 tanks.

I placed a 1-cm layer of pea-sized gravel on the bottom of each tank, and added two pieces of pottery and an artificial plant for cover. I used a re-circulating powerfilter on each tank (Aquaclear© 200) and siphoned waste daily, resulting in approximately a 10% daily water change for each tank.

Growth monitoring, maintenance, and terminal sampling. On day 00 (24 h after exposure to copepods), I used MS-222 to anesthetize each fish, and recorded standard length (nearest 1 mm) and weight (nearest 0.01 g). I calculated the biomass of fish in each tank, high-ration (5%) treatment fish were fed 2.5% of the fish biomass (ZP3, Aquatic Ecosystems) twice per day, at 0800 and at 1200. Low-ration fish were fed 1% of the fish biomass twice per day. On day 12 and approximately every 14 days thereafter, I measured weight and standard length of fish and recalculated food rations to reflect changes in growth. I also noted any missing fish (assumed dead) and recorded external condition of fish (missing fins, emaciation). I did not replace fish that died after day 02. I examined dead fish to determine if they had Asian tapeworm. On day 096, I used an MS-222 overdose to euthanize all remaining fish, measured length and weight process, and examined them to determine prevalence and intensity of Asian tapeworm.

Data analysis. I used program JMP IN 5.1© to perform multifactor ANOVA to compare overall mean growth (final – initial) for weight and standard length among groups, as well as mean final standard length and weight. Randomization ensured that initial expected differences in standard length and weight between treatment combination groups was zero. I plotted significant interactions and used Tukey-HSD post-hoc analysis on significant interactions that were non-additive to analyze the difference between cells.

To determine if there was an effect Asian tapeworm exposure, food ration, or presence of mosquitofish on survival, I used the arcsine square-root transformation on the final proportion of Mohave tui chub that survived, weighted by the initial number of Mohave tui chub in the tank (three or six). I used a multifactor ANOVA model to determine the effect of Asian tapeworm, food ration, mosquitofish, and all interactions of mosquitofish and Asian tapeworm on the survival of Mohave tui chub.

I summarized field data on Asian tapeworm presence, prevalence, and intensity collected during the experiment. The collection times, species, and methods were not standardized.

Results

Growth. Food ration, mosquitofish, and Asian tapeworm exposure had significant effects on the standard length gain of Mohave tui chubs (Table 1).   Statistically significant disordinal interactions occurred between food ration and mosquitofish, and Asian tapeworm and mosquitofish (Figure 1). Mohave tui chubs with mosquitofish present in the tank grew on average 4.3 mm longer (95% C.I. 3.1—5.4 mm) than Mohave tui chubs without mosquitofish , when accounting for the effects of food ration and Asian tapeworm infection (Figure 2). When mosquitofish were present the tanks, I found no significant differences between 5% and 2% food ration; however, when mosquitofish were absent, fish fed 5% biomass daily grew on average 2.5 mm longer (95% C.I. 0.3—4.7 mm) than fish fed 2% biomass daily when accounting for the effect of Asian tapeworm (Figure 3, Tukey HSD, $q = 2.76$, $\alpha = 0.05$).   Mohave tui chub exposed to Asian tapeworm were on average 2.4 mm less (95% C.I. 0.2—4.6 mm) than fish not exposed to Asian tapeworm when mosquitofish were present, but I found no significant differences between exposed and unexposed Mohave tui chub when mosquitofish were absent from tanks (Figure 4, Tukey HSD, $q = 2.76$, $\alpha = 0.05$).

I found similar, but less statistically significant relationships for means weights (Table 2, Figure 5).

Survival. Mohave tui chub survival ranged from 100% to 83% (Figure 6), but I found no significant differences in survival (Table 3).

Final infection rate. No fish remaining alive at day 96 were infected. One tapeworm was observed floating in an experimental tank on day 36, and I found one out of nine extra fish infected with Asian tapeworm at day 48 of the experiment.

Discussion

Total density of fish in a tank is an important variable, it affects fish growth even when per capita food is kept constant (Smith 1978). I kept six fish in every tank to avoid confusing competitive interaction effects with density effects, other studies on mosquitofish interactions with desert fishes have used treatments with differing total densities (Mills et al. 2004). Least chub growth was reduced at high densities of mosquitofish, but I believe it is important to make comparisons at the same density of fish, i.e. compare growth of 110 least chub to 10 least chub and 100 mosquitofish, not 10 least chub compared to 10 least chub with 100 mosquitofish. Although I have limited the scope to fish in captivity fed only one type of food, I found that Mohave tui chub grow better in the presence of similar-sized mosquitofish. Mohave tui chub are more opportunistic than predatory mosquitofish and better at foraging, and even with daily cleaning there was food and detritus left in the tanks. An unfortunate flaw in the design, and explanation for the non-additive food ration*mosquitofish interaction term, is that when mosquitofish are present Mohave tui chub able consume much more than 2% biomass daily, essentially making high and low food rations the same in tanks with mosquitofish. During the dissection, many Mohave tui chub had food in their gut, in spite of not being fed for over 24 hours prior to dissection. However, when no cover is provided for fish in the tank, enabling thorough cleaning, mosquitofish are so aggressive that Mohave tui chub survival is reduced significantly and differing density effects prevent valid growth analysis. Field experiments are needed, but these data suggest that once Mohave tui chubs reach a size that mosquitofish are unable to prey upon them, they are of little threat when habitat refuges are available. Mosquitofish may be beneficial to Mohave tui chub growth, I observed mosquitofish in the gut of Mohave tui chub on multiple occasions in the field.

Reports of 90% mortality in culture situations (Bauer 1973) are unsubstantiated claims and without references. These reports lack causal inference about the mechanism that resulted in a fish kill. Parasites alone do not cause simultaneous fish deaths, but Asian tapeworm is associated with reduced life-span in mosquitofish (Granath and Esch 1983) and Topeka shiners (Jessica Koehle, University of Minnesota, personal communication). Asian tapeworm does reduce growth, and higher intensities are associated with smaller fish (Brouder 1999) within a cohort, but data across several age classes suggest that larger Mohave tui chub are associated with higher infection intensities (Appendix C). High intensity infections might kill small fish, and only fish with low intensity infections remain. However, personal observations of Topeka shiners and arroyo chub *Gila orcutti* with high prevalence and intensity of Asian tapeworm did not present any patterns of mortality. From an evolutionary standpoint, it would be maladaptive for Asian tapeworm to kill its host through a high-intensity infection.

Evacuated tapeworms do not necessarily die; they may be eaten by another, more suitable host (Hansen et al. 2006, Ward *in press*) where they can reach maturity. I noted goldfish *Carassius auratus*, golden shiners *Notemigonus crysoleucus*, Yaqui chub *Gila pupurea*, and Mohave tui chub readily ate tapeworms dropped into the tank and acquired post-cyclic infection. These data suggest that lack of infection at the conclusion of the experiment is not a procedural error, instead small Mohave tui chubs may be unsuitable hosts for Asian tapeworm growth and maturation. Determining whether high intensity Asian tapeworm infections kill small Mohave tui chubs or intraspecific competition between tapeworms within the gut produces the pattern seen in Lake Tuendae is an important question for future research.

Low infection prevalence and non-additive interactions confuse the effect Asian tapeworm has on Mohave tui chub. At least some fish were infected at the midpoint of the experiment. The tapeworms may have matured and passed during the experiment. The non-additive interaction between tapeworm and mosquitofish was probably caused by low infection prevalence and randomization that did not result in even distribution of infected fish among groups. One interpretation is that exposure Asian tapeworm only has an effect when mosquitofish are present. More likely, in light of zero percent final infection rate, is that there were not enough infected fish in the mosquitofish-absent tanks to show a significant difference.

Studies involving multiple hosts and life-stages on living organisms are very difficult. In this study, I needed enough fish of the correct size and enough copepods to infect the fish when a viable source of Asian tapeworm was discovered. Two previous experimental infections produced similar results at the end of the experiment, verifying the difficulties associated with a mass experimental infection. The last attempt produced many infected copepods, some which were photographed. Not all of the copepods may have had infective procercoids, which may have caused low infection prevalence. Replication of this study or similar studies should be planned well in advance, and a viable source of Asian tapeworm eggs should be located as early as possible.

I feel this study shows, at least under captive conditions, Asian tapeworm does not directly reduce survival in Mohave tui chub, and reduces growth only slightly, especially when compared to the effects of mosquitofish and food ration. Studies on bonytail (Hansen et al. 2006) found similar results for survival, and personal observation (TPA) of highly infected Topeka shiners (>30 tapeworms per fish) support these conclusions. Asian tapeworm may affect Mohave tui chubs differently in the field, but this study and others produced no patterns of extreme mortality. Important future research is to determine whether the pattern of increasing intensity of Asian tapeworm infection with increasing total length of Mohave tui chub in Lake Tuendae is a result of small fish dying, or a result of tapeworm competition within the gut of an unsuitable host.

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Table Captions

Table 1-Effect tests of food ration, mosquitofish, and Asian tapeworm treatment on Mohave tui chub change in standard length over 96 days from univariate multi-factor ANOVA.

Table 2-Effect tests of food ration, mosquitofish, and Asian tapeworm treatment on Mohave tui chub final length over 96 days from univariate multi-factor ANOVA.

Table 3- Final mean standard length estimates of food ration, mosquitofish, and Asian tapeworm treatment on Mohave tui chub final standard length over 96 days.

Table 4-Effect tests of food ration, mosquitofish, and Asian tapeworm treatment on Mohave tui chub weight gain over 96 days from univariate multi-factor ANOVA.

Table 5-Effect tests of food ration, mosquitofish, and Asian tapeworm treatment on Mohave tui chub final weight over 96 days from univariate multi-factor ANOVA.

Table 6- Mean final weight estimates of food ration, mosquitofish, and Asian tapeworm treatment on Mohave tui chub final weight over 96 days.

Table 7-Effect tests of food ration, mosquitofish, and Asian tapeworm treatment on Mohave tui chub survival over 96 days from univariate multi-factor ANOVA.

Table 8- Means estimates of food ration, mosquitofish, and Asian tapeworm treatment on Mohave tui chub survival over 96 days from univariate multi-factor ANOVA.

Table 1 - ANOVA summary table for standard length gain

Source	*df*	SS	F-value	*P*
Model	7	205.58	11.80	<0.0001
Feed	1	17.46	7.01	0.0141
Mosquitofish	1	141.15	56.59	<0.0001
Tapeworm	1	9.62	3.86	0.0610
Feed*Mosquitofish	1	7.47	3.00	0.0961
Feed*Tapeworm	1	4.29	1.72	0.2019
Mosquitofish*Tapeworm	1	12.40	4.98	0.0352
Mosquitofish*Tapeworm*Feed	1	0.25	0.100	0.7535
Error	24	59.76		
Total	31	265.34		

Table 2 - ANOVA summary table for weight gain

Source	*df*	SS	F-value	*P*
Model	7	1.11	7.25	<0.0001
Feed	1	0.02	0.85	0.37
Mosquitofish	1	0.89	40.39	<0.0001
Tapeworm	1	0.06	2.57	0.12
Feed*Mosquitofish	1	0.05	2.27	0.14
Feed*Tapeworm	1	0.02	0.76	0.39
Mosquitofish*Tapeworm	1	0.06	2.77	0.11
Mosquitofish*Tapeworm*Feed	1	0.00	0.00	.96
Error	24	0.53		
Total	31	1.65		

Table 3 - ANOVA summary table for survival

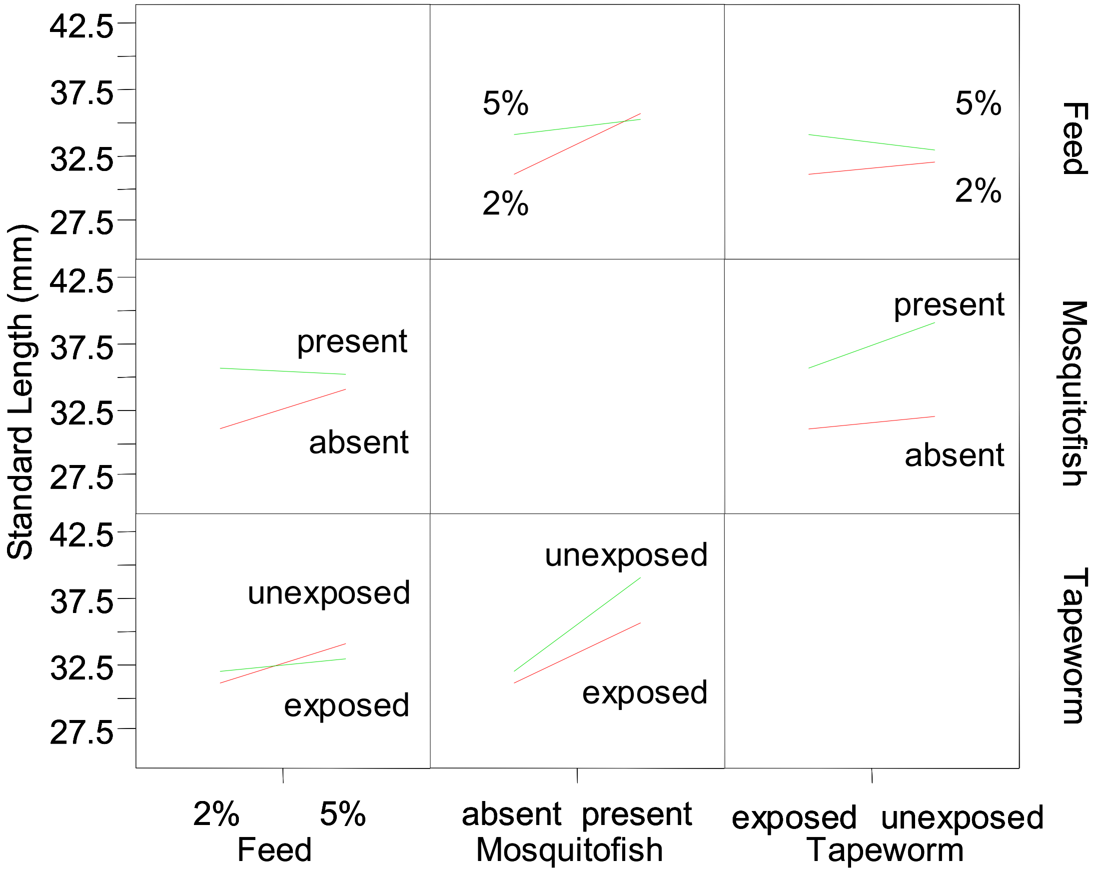
Source	*df*	SS	F-value	*P*
Model	7	0.09	0.86	0.55
Feed	1	0.00	0.00	1.0
Mosquitofish	1	0.01	0.94	0.34
Tapeworm	1	0.02	1.35	0.26
Feed*Mosquitofish	1	0.00	0.04	0.85
Feed*Tapeworm	1	0.00	0.00	0.99
Mosquitofish*Tapeworm	1	0.01	0.94	0.34
Mosquitofish*Tapeworm*Feed	1	0.04	3.03	0.09
Error	24	0.34		
Total	31	0.43		

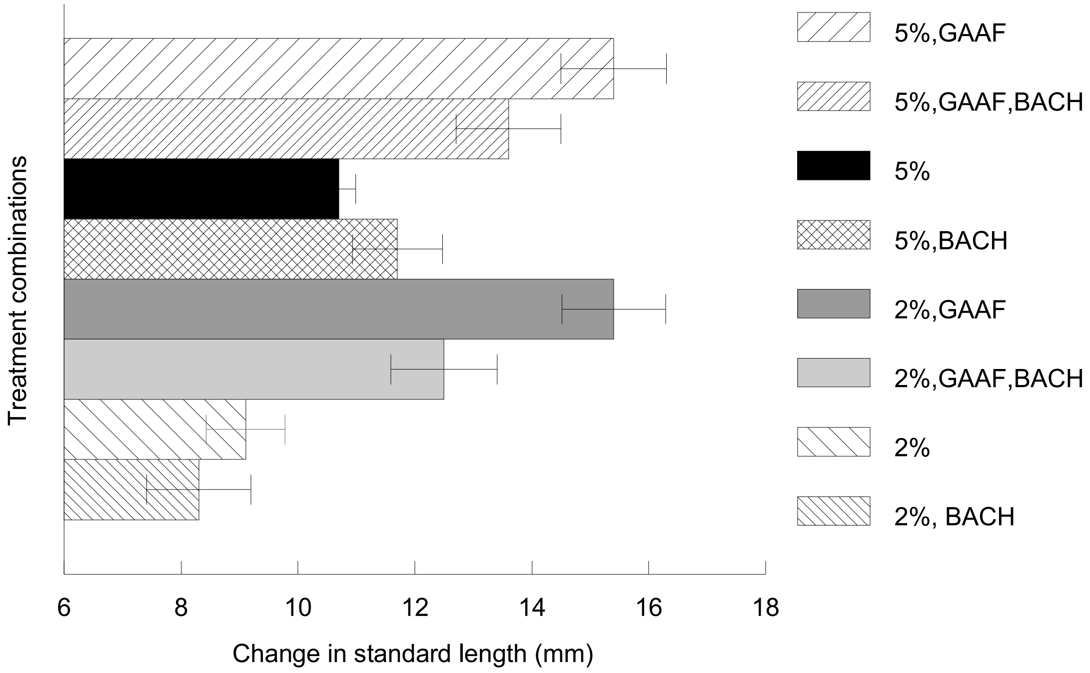
Figure Captions

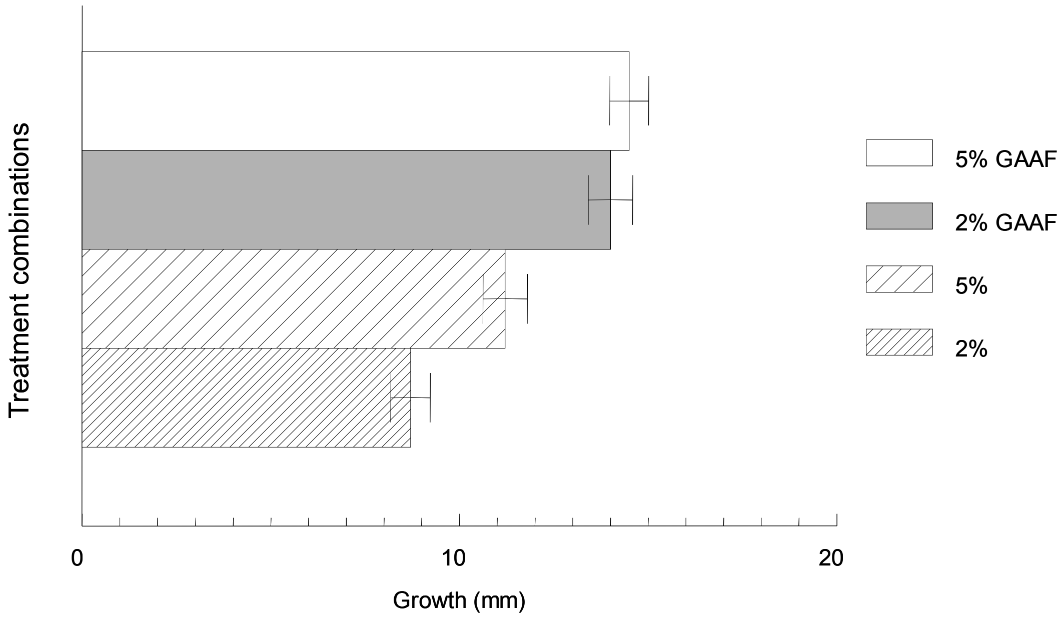
Figure 1-Standard length (mm) gain of Mohave tui chub over 96 days with treatment factors of 5% biomass daily food, 2% biomass daily food, mosquitofish (GAAF), and Asian tapeworm (BACH). Error bars represent one standard error of the mean.

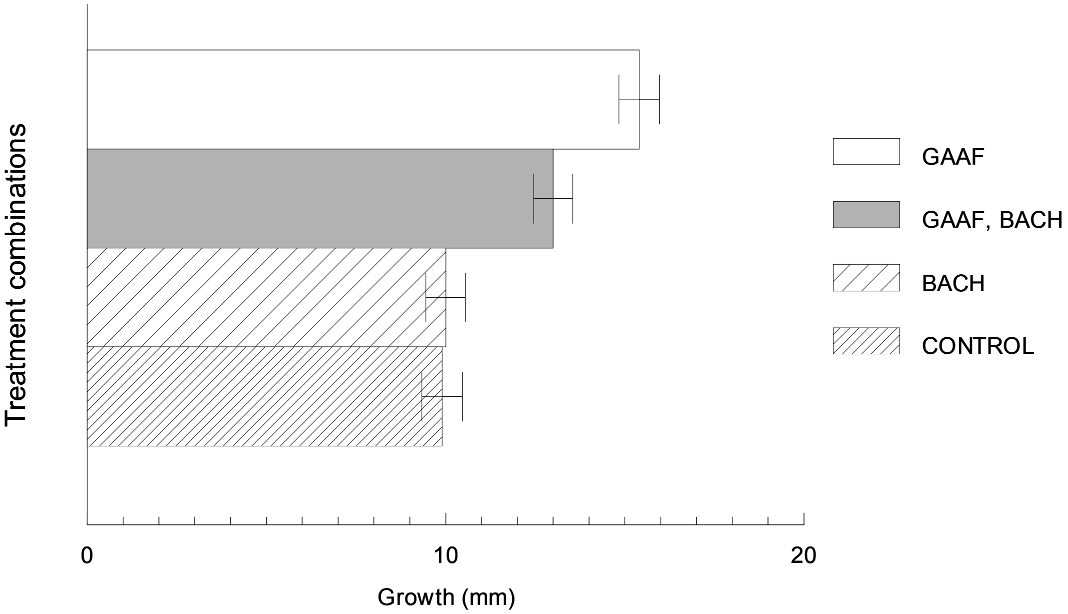
Figure 2-Effects of interactions between treatment factors on standard length of Mohave tui chub after 96 days.

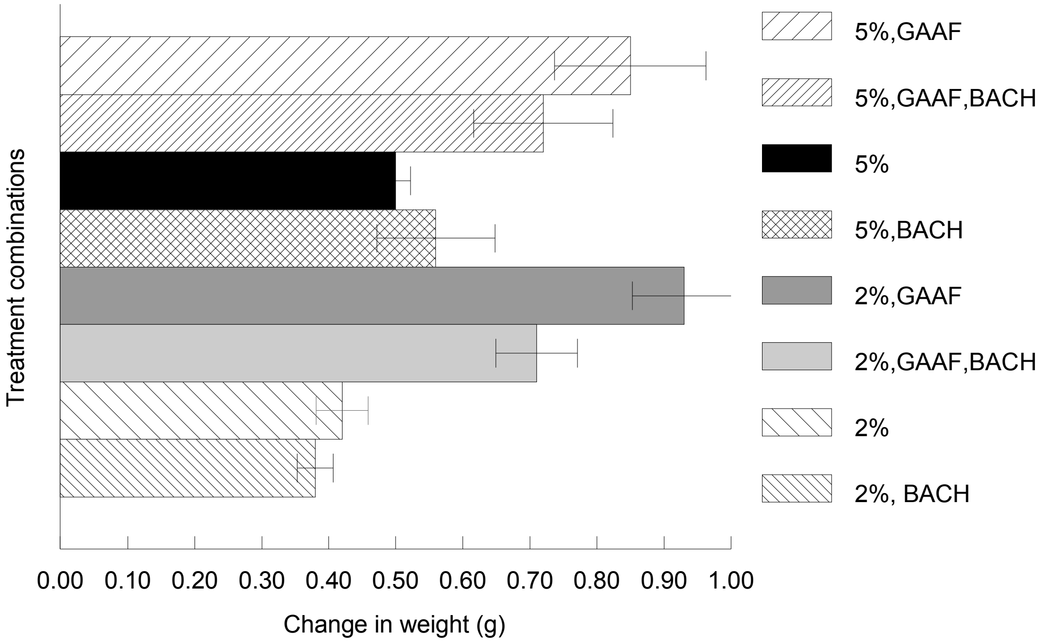
Figure 3- Weight (g) gain of Mohave tui chub over 96 days with treatment factors of 5% biomass daily food, 2% biomass daily food, mosquitofish (GAAF), and Asian tapeworm (BACH). Error bars represent one standard error of the mean.

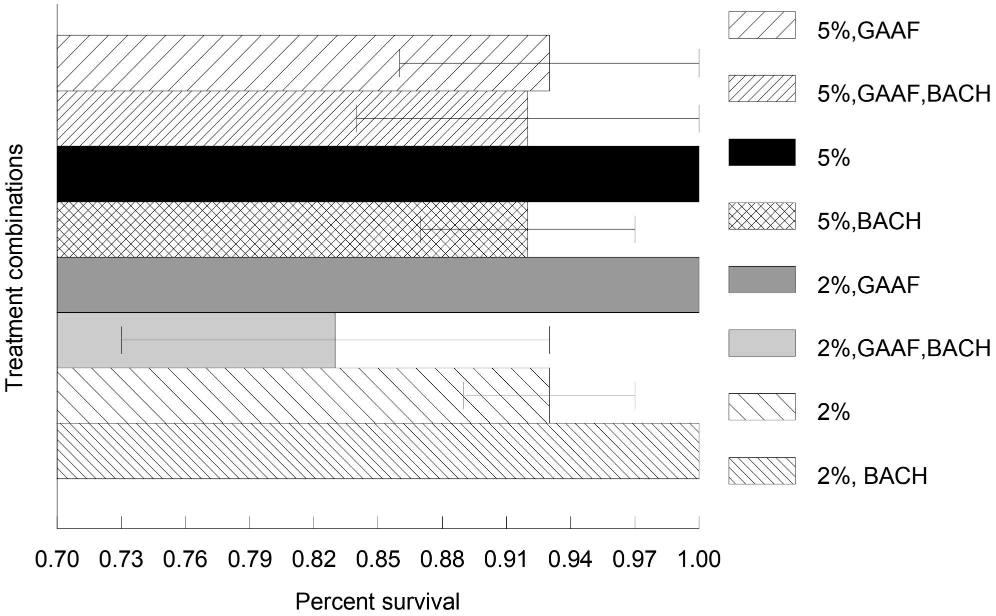

Standard Length (mm)
42.5
37.5
32.5
27.5
42.5
37.5
32.5
27.5
42.5
37.5
32.5
27.5
5%
2%
5%
2%
Feed
present
absent
present
absent
Mosquitofish
unexposed
exposed
unexposed
exposed
Tapeworm
2%
5%
Feed
absent
present
Mosquitofish
exposed
unexposed
Tapeworm


Treatment combinations
Change in standard length (mm)
6
8
10
12
14
16
18
5%,GAAF
5%,GAAF,BACH
5%
5%,BACH
2%,GAAF
2%,GAAF,BACH
2%
2%, BACH


Treatment combinations
5% GAAF
2% GAAF
5%
2%
0
10
20
Growth (mm)


Treatment combinations
GAAF
GAAF, BACH
BACH
CONTROL
0
10
20
Growth (mm)


Treatment combinations
Change in weight (g)
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
5%,GAAF
5%,GAAF,BACH
5%
5%,BACH
2%,GAAF
2%,GAAF,BACH
2%
2%, BACH



Treatment combinations
Percent survival
0.70
0.73
0.76
0.79
0.82
0.85
0.88
0.91
0.94
0.97
1.00
5%,GAAF
5%,GAAF,BACH
5%
5%,BACH
2%,GAAF
2%,GAAF,BACH
2%
2%, BACH

APPENDIX C:

ASIAN TAPEWORM DYNAMICS IN MOHAVE TUI CHUB IN LAKE TUENDAE, CALIFORNIA

Asian tapeworm dynamics in Mohave tui chub in Lake Tuendae, California

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Abstract

Asian tapeworm is a potentially dangerous introduced fish parasite found in many southwestern fishes in the family Cyprinidae. I used praziquantel as a non-lethal method to monitor the seasonal dynamics of Asian tapeworm populations in federally listed Mohave tui chub in Lake Tuendae, Zzyzx, California. I found a significant positive relationship between increasing water temperature and increasing prevalence, mean abundance, and mean intensity of Asian tapeworm infection. Non-detections were caused by small sample size and low infection rates, or not allowing sufficient gut evacuation time, although praziquantel is still effective even at low temperatures. Additional stressors to fish (handling, marking, transport, habitat modifications) should be avoided during summer months when water temperature and Asian tapeworm prevalence, mean intensity, and mean abundance peak.

Introduction

Asian tapeworm *Bothriocephalus acheilognathi*, a cestode that primarily affects fish in the family Cyprinidae, has spread to all continents except Antarctica (Korting 1975, Boomker 1980, Andrews 1981, Font 1994, Dove 1997). The tapeworm was introduced to the United States through stocking of common carp *Cyprinus carpio* and grass carp *Ctenopharyngodon idella* (Hoffman 2000). Asian tapeworm was most likely introduced into the southwestern United States in shipments of baitfish, and was first found in federally-listed Mohave tui chub *Gila bicolor mohavensis* in Lake Tuendae at the Zzyzx Mineral Springs resort, California, in January 2001 (Steve Parmenter, Personal Communication) when ten fish were accidentally killed and subsequently dissected.

Seasonal changes in abundance and prevalence occur in Asian tapeworm populations (Granath and Esch 1983a, 1983b, Heckmann et al. 1986), but the patterns are not simply seasonally driven, they are the result of interactions between seasons and intermediate and definitive host populations (Riggs and Esch 1987, Marcogliese and Esch 1989, Clarkson et al. 1997, Choudhury et al. 2004). Seasonal patterns vary from year to year (Marcogliese and Esch 1989) and from region to region (Choudhury et al. 2004). In North Carolina, the largest infrapopulations (all the individuals of a species in an individual host) of *B. acheilognathi* occurred during early winter and the smallest infrapopulations occurred during mid to late summer (Granath and Esch 1983a, 1983c). The rise in infection in early winter was thought to be due to an emergence of 4th instar copepodites (an intermediate host) which became infected in the spring (Riggs and Esch 1987). A later study at the same reservoir found that both prevalence and abundance of Asian tapeworm were highest in the summer and this seasonal shift correlated with a shift in the copepod community composition (Marcogliese and Esch 1989).

The wide distribution of Asian tapeworm across the Southwest, the fact that it infects all cyprinid fishes, and does well in warm water make it a serious potential threat to native fish conservation in the Southwest. Concerns that Asian tapeworm will spread further (Choudhury et al. 2006) have made it one of the most regulated warm-water fish parasites in the United States (Mitchell 2004). These concerns have prompted studies into methods of control and treatment for Asian tapeworm, such as the use of antihelminthics such as praziquantel (Droncit®), developed for treatment of tapeworm infections in humans and animals (Andrews et al. 1983).

Fisheries managers and aquaculturists use praziquantel to treat Asian tapeworm infections in fish (Mitchell 2004, Ward *in press*). Praziquantel offers an advantage because it eliminates Asian tapeworm without killing the host fish and tapeworms can be quantified without dissection (Ward *in press*). The effectiveness of praziquantel in cold water (<15°C) has not been evaluated. Low temperatures may make praziquantel ineffective, or increase the time to detection by decreasing the gut evacuation rate (Specziár 2002) to greater than 48 h.

Understanding the seasonal fluctuations in tapeworm populations will help managers decide when fish have the highest prevalence and mean intensity of Asian tapeworm infections, and eliminate additional stressors such as handling, transport, or habitat modification. I used praziquantel as a non-lethal method to monitor changes in tapeworm populations in Mohave tui chubs *Gila bicolor mohavensis* in Lake Tuendae, Zzyzx (Mojave National Preserve), California, where the water temperatures range from about 5°C in winter to over 30°C in the summer. I examined the relationship between Asian tapeworm populations and temperature, discuss possible causes for non-detection, and make recommendations for future research and management.

Methods

To avoid confusion, parasite terminology was taken from recommendations by the Bush et al. (1997) and Margolis et al. (1982). I used statistical tests and measures of central tendency (described below) recommended as appropriate for highly skewed parasite populations (Rozsa et al. 2000).

Field monitoring. I modified methods used to quantify Asian tapeworm infections in bonytail chub (Ward *in press*). I trapped approximately 50 Mohave tui chub from Lake Tuendae during each sampling trip in October 2005, February, May, August, and November 2006, January and April 2007 (US Fish and Wildlife recovery permit TE086593-0). When more than 50 chubs were captured, I haphazardly selected individuals for treatment, and placed a single fish into a 6.1-L plastic Rubbermaid© shoebox painted black. I covered each box with nylon screen secured with a rubber band, and placed each box into a collapsible circular pool containing ~800 liters of well water from the same source as the lake. I placed rocks as needed to prevent the boxes from them floating. I added 4.8g of praziquantel (6mg/L) to a small amount of isopropyl alcohol to create a dissolved solution, and then added it to the pool. I monitored the fish for 24 h for signs of stress, while keeping the water aerated with air pumps. After 24 h I removed the boxes from the circular pool. I measured the total length (mm) of each fish, recorded the number of tapeworms in the box, and released each fish. Temperature data was obtained from a data logger, and I recorded the temperature at 1100 the day the fish were caught.

In January 2007, I dissected fish to determine winter prevalence because freezing surface water prevented a praziquantel bath. I was restricted to dissecting only 30 fish on this date.

Data analysis. I used Fisher's-exact test was used to compare prevalence of Asian tapeworm and Mood's median test to compare median intensities of Asian tapeworm between sampling dates (Rozsa et al. 2000), and used linear regression to examine the relationship between infection prevalence and temperature, weighted by sample size of each sampling period. Within each sampling period, I used program Quantitative Parasitology 3.0 to calculate prevalence and Stern-Wald confidence intervals, mean intensity and bootstrap confidence interval, median intensity and distribution-free confidence interval, and mean abundance and bootstrap confidence intervals (Bush et al. 1997; Rozsa et al. 2000). I used Spearman's rho correlation (r_s) to measure of strength of association between total length and number of tapeworms per fish.

Results

I found significant differences in prevalence between sampling periods (X^2=83.8, *df*=5, P<0.001). Increasing water temperature was associated with increasing infection prevalence (Figure 1, $F_{1,4} = 9.31$ $P = 0.038$). I found similar positive relationships between increasing mean abundance and water temperature (Figure 1, $F_{1,4} = 8.53$ $P = 0.043$) and increasing mean intensity and water temperature (Figure 1, $F_{1,4} = 9.25$ $P = 0.038$).Mean prevalence ranged from 0.00 to 0.62 (Figure 2), while mean abundance and mean intensity ranged from 0 to 21 and 0 to 33, respectively (Table 1.) Larger fish were associated with more intense Asian tapeworm infections ($r_s = 0.21$, $P = 0.055$)

Discussion

The warmer the water, the more severe the prevalence and the more intense the infection becomes. The highest prevalence and mean intensity coincided with the warmest sampling period, although the population dynamics of the copepod intermediate host and detectibility in cold water also probably play a role in fluctuating prevalence, abundance, and intensity. The critical thermal maximum for Mohave tui chub is about 35°C (McClanahan et al. 1986), and August water temperatures in Lake Tuendae are regularly over 30°C. Additional summer stressors include low dissolved oxygen. In a temperature experiment, highly infected Topeka shiners had significantly lower survival than uninfected Topeka shiners at all temperatures except the warmest group at 35°C, but also had decreasing infection prevalence with increasing temperature (J. Koehle, personal communication).

Larger fish were associated with more intense infections, in direct contrast to roundtail chub (Brouder 1999), and Asian tapeworm caused reduced growth in bonytail chub (Hansen 2006). However, I compared infection intensity across age groups, both roundtail chub and bonytail were all from the same cohort and exposed to the same environmental conditions. Fish that are infected do grow less, but smaller fish are associated with lower intensity infections. Two explanation could result in this pattern, one being small, intensely infected fish die quickly and are eliminated from the population. Killing its host would not be adaptive for Asian tapeworm. Instead, small Mohave tui chubs may be poor hosts for Asian tapeworm. Larger fish are associated with more intense infections because their gut is more suitable to sustain a large population of Asian tapeworm than can reproduce. Laboratory observations of Topeka shiners

Notropis tristis and arroyo chubs *Gila bicolor* provide anecdotal evidence that Asian tapeworm does not kill small fish (personal observation). Experimental research is needed to establish whether or not high-intensity Asian tapeworm infections kill small fish, or if intra-specific competition among tapeworms in the gut limits infection intensity in small fish, resulting in the association between smaller fish and lower intensity infections. Post-cyclic transmission is possible (Hansen et al. 2007), and tapeworms can be removed from the gut and fed to another fish to establish infection in a new host (Ward *in press*, personal observation). It would be evolutionarily advantageous for a tapeworm to be evacuated from the gut of a small fish and possibly be ingested by a more suitable host than to remain in an unsuitable host where reproduction is not possible.

Handling, transport, marking, or any activities that may add to the stressors already present in summer when water temperatures are high and infection prevalence and intensity peak should be avoided. Such activities should be carried out during the winter and early spring, when the stresses associated with Asian tapeworm are at a minimum. Additional research should be conducted on high temperature and Asian tapeworm interactions with Mohave tui chub and other warm desert region fishes.

Acknowledgements

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Table Captions

Table 4-Summary statistics of Asian tapeworm infection in Mohave tui chubs in Lake Tuendae, California. Parentheses indicate 95% confidence interval. Sample size too small to calculate confidence intervals.

Table 1

Date	Mean prevalence	Mean abundance	Mean intensity	*n*	Temperature
OCT05	0.35(0.19—0.55)	3(1—7)	8(3—17)	31	18.2
FEB06	0	0	0	49	15.3
MAY06	0.25(0.05—0.57)	0.5(0.1—1.2)	2(1—3)	12	20.1
AUG06	0.62(0.47—0.76)	21(8—54)	33(13—81)	45	29.8
NOV06	0	0	0	40	16.6
JAN07	0	0	0	30	3.5

Species	Date	Water body	Location	N	Prevalence	Mean Intensity
Fathead minnow	28-Apr-05	San Pedro River	Hereford Bridge	7	0.57	2.5
Red shiner	2-May-05	Sonoita Creek	Below Patagonia Lake	7	0.00	0.0
Fathead minnow	3-May-05	San Pedro River	Hereford Bridge	3	0.00	0.0
Red shiner	10-May-05	Gila River	Safford	37	0.08	1.0
Fathead minnow	10-May-05	Gila River	Safford	11	0.00	0.0
Red shiner	21-May-05	Bonita Creek	Confluence of Gila	15	0.00	0.0
Red shiner	26-May-05	Aravaipa Creek	Fish barrier	21	0.14	4.0
Red shiner	9-Jun-05	Aravaipa Creek	Fish barrier	51	0.16	9.7
Red shiner	23-Jun-05	Aravaipa Creek	Fish barrier	45	0.29	5.4
Red shiner	30-Jun-05	Aravaipa Creek	Fish barrier	30	0.30	5.9
Red shiner	30-May-05	Verde River, AZ	River mile 26	6	0.40	2.0
Red shiner	31-May-05	Verde River, AZ	River mile 27.5	4	0.00	0.0
Red shiner	31-May-05	Verde River, AZ	River mile 30	1	0.00	0.0
Red shiner	2-Jun-05	Verde River, AZ	River mile 44	8	0.00	0.0
Red shiner	10-Jun-05	Verde River, AZ	River mile 61	5	0.00	0.0
Red shiner	11-Jun-05	Verde River, AZ	River mile 62	26	0.00	0.0
Red shiner	11-Jun-05	Verde River, AZ	River mile 65	5	0.00	0.0
Red shiner	12-Jun-05	Verde River, AZ	River mile 68	5	0.25	1.0
Red shiner	13-Jun-05	Verde River, AZ	River mile 72	1	0.00	0.0
Red shiner	13-Jun-05	Verde River, AZ	River mile 77	1	0.00	0.0
Red shiner	22-Jun-05	Verde River, AZ	River mile 89.5	33	0.33	1.8
Red shiner	23-Jun-05	Verde River, AZ	River mile 92	32	0.10	1.7
Red shiner	23-Jun-05	Verde River, AZ	River mile 93.5	33	0.17	1.0
Red shiner	23-Jun-05	Verde River, AZ	River mile 95	26	0.17	1.0
Red shiner	24-Jun-05	Verde River, AZ	River mile 96	40	0.22	2.6
Red shiner	24-Jun-05	Verde River, AZ	River mile 99	45	0.12	1.5
Red shiner	25-Jun-05	Verde River, AZ	River mile 103	35	0.29	1.2
Red shiner	25-Jun-05	Verde River, AZ	River mile 104.5	30	0.23	1.6
Arroyo chub	15-Aug-06	Mojave River, CA	Afton Canyon	30	1.00	14.1
Arroyo chub	14-Nov-06	Mojave River, CA	Afton Canyon	24	0.88	38.1
Mohave tui chub	19-Oct-05	Lake Tuendae, CA	Mojave NP	31	0.35	8.0
Mohave tui chub	25-Feb-06	Lake Tuendae, CA	Mojave NP	49	0.00	0.0
Mohave tui chub	11-May-06	Lake Tuendae, CA	Mojave NP	12	0.25	2.0
Mohave tui chub	16-Aug-06	Lake Tuendae, CA	Mojave NP	45	0.62	33.0
Mohave tui chub	14-Nov-06	Lake Tuendae, CA	Mojave NP	40	0.00	0.0
Mohave tui chub	13-Jan-06	Lake Tuendae, CA	Mojave NP	30	0.00	0.0

Figure Captions

Figure 1-Percentage of infected fish vs. the water temperature at 1100 in Mohave tui chub in Lake Tuendae, California.

Figure 2-Mean number of infected Mohave tui chub by sampling period found in Lake Tuendae, California. Bars represent one standard error.

Figure 1

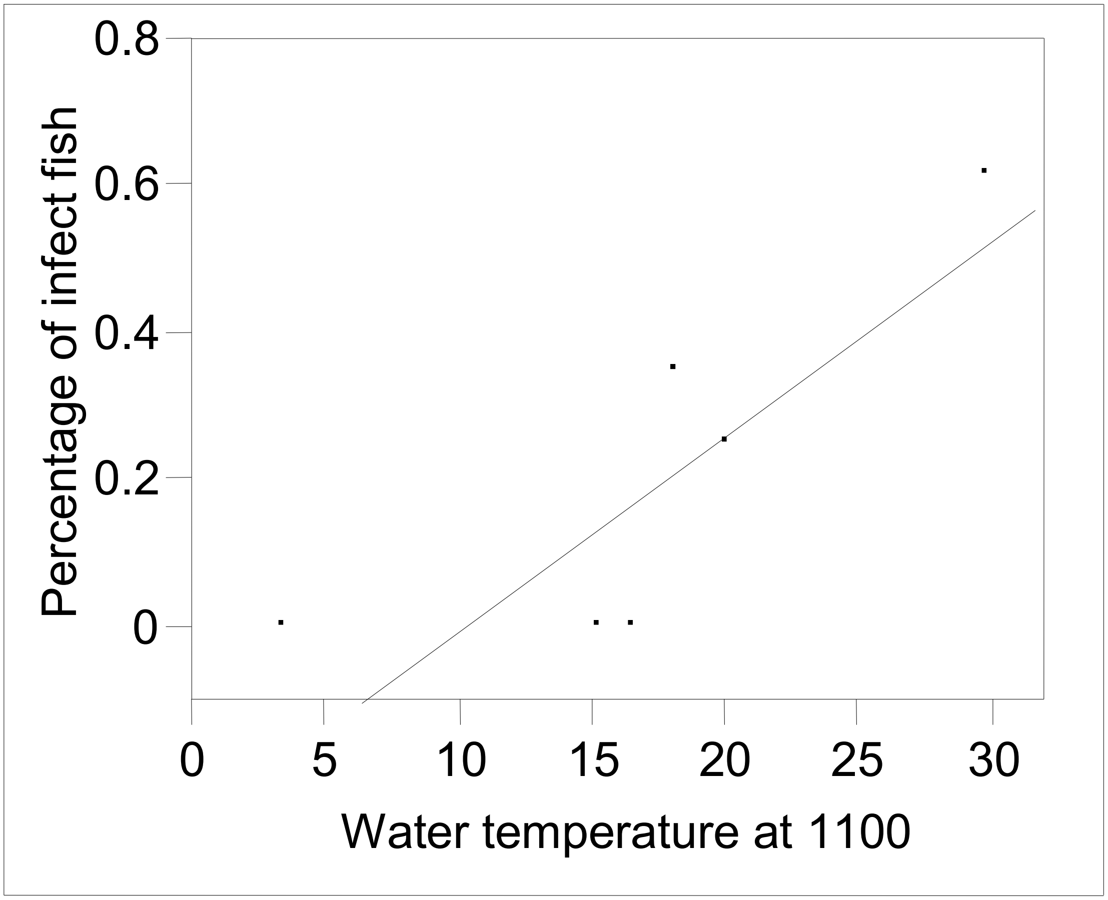
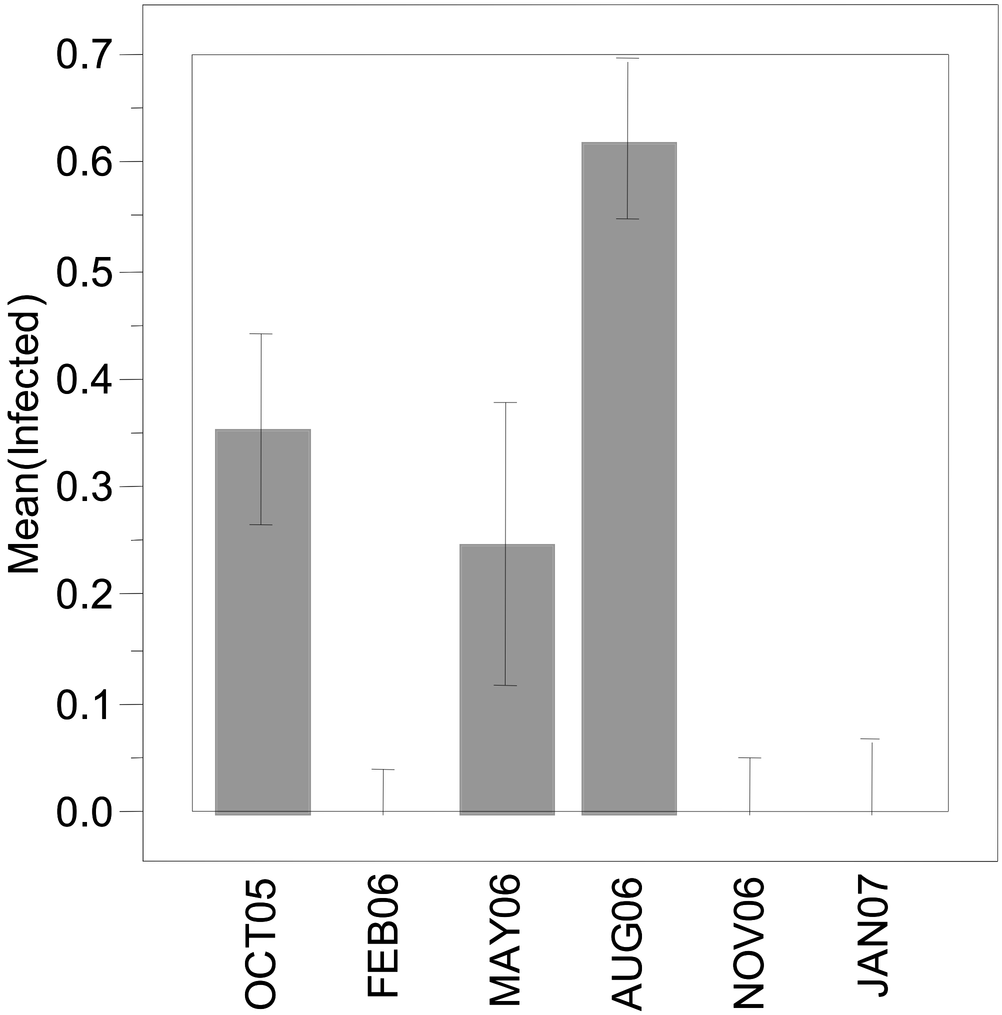

0.8
0.6
0.4
0.2
0
Percentage of infect fish
0
5
10
15
20
25
30
Water temperature at 1100

Figure 2



Mean(Infected)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
OCT05
FEB06
MAY06
AUG06
NOV06
JAN07