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

by

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ABSTRACT

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

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 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 of host fish 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* (and Esch 1983) and Topeka shiners *Notropis tristis* (Jessica Koehle, University of Minnesota, personal communication). Experimental infections of Asian tapeworm caused significant reduction in length, weight, and survival 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 chub *G. bicolor mohavensis*. Endemic to the Mojave River system of southern California, Mohave tui chub hybridized with introduced arroyo chub *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 remaining 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, California (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 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 et al. 2004).

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

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

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. All work was conducted under U.S. Fish and Wildlife Service recovery permit TE086593-0. Following is a summary of the most important findings.

METHODS FOR SPAWNING THE ENDANGERED MOHAVE TUI CHUB IN AQUARIA

Temperature and photoperiod manipulations resulted in Mohave tui chub spawning in 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 tanks reached ambient temperature (20-23°C), I found eggs in tanks. 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 eggs hatched in about 4 days, and reached swim-up after less than 24 hours after hatching.

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

About 400 offspring were used in subsequent growth and survival assessing the impacts of experimental infection of Asian tapeworm. 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 growth of Mohave tui chubs. Significant disordinal interactions occurred between food ration and mosquitofish, and Asian tapeworm and mosquitofish (Appendix B, Table 1, Figure 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 increase in standard length when mosquitofish were present (Appendix B, Figure 3; Tukey-HSD, $q = 2.76$, $\alpha = 0.05$). Mohave tui chub with mosquitofish also grew larger than Mohave tui chub without mosquitofish at all levels of Asian tapeworm, but fish exposed to Asian tapeworm were smaller than control fish only when mosquitofish were present (Appendix B Figure 4; Tukey HSD, $q = 2.76$, $\alpha = 0.05$). I found similar, but less significant patterns for weight gain in Mohave tui chubs (Appendix B, Table 2, Figure 5)

No treatment factor significantly lowered Mohave tui chub survival (Appendix B Table 3, Figure 6). No fish still alive at day 96 were infected. One tapeworm was observed floating in an experimental tank, and I found one out of nine fish exposed to Asian tapeworm, but not used in the experiment, infected with Asian tapeworm.

Mohave tui chub are more opportunistic feeders than are predatory mosquitofish, and better at foraging in aquaria. Even with daily cleaning there was food and detritus left in all tanks. An explanation for the disordinal interaction of food ration and mosquitofish is Mohave tui chub are able to consume much more than 2% biomass daily when mosquitofish are present, essentially making high and low food rations the same in tanks with mosquitofish. These results suggest if Mohave tui chubs reach a size that mosquitofish are unable to prey upon them, mosquitofish are not a large threat.

Low infection prevalence and disordinal interactions confuse the effect of Asian tapeworm on Mohave tui chub. At least some fish were infected at the midpoint of the experiment. Tapeworms may have matured and passed out of fish during the experiment. Disordinal 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 did 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 chub on 4% biomass food ration (Hansen et al. 2006) found similar results for survival, but survival of bonytail chub infected with Asian tapeworm was reduced at 2% and 1.5% biomass ration. Personal observation of highly infected Topeka shiners and arroyo chub (>30 tapeworms per fish) held in captivity with low mortality support these conclusions. Asian tapeworm may affect Mohave tui chubs differently in the field, but this study and others produced no patterns of very high mortality.

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

I found significant differences in prevalence of between periods of sampling for Asian tapeworm ($X^2 = 83.8$, $df = 6$, $P < 0.001$). Increasing water temperature was associated with increasing infection prevalence of Asian tapeworm (Figure 1, $F_{1,6} = 7.6$ $P = 0.040$). I found similar positive relationships between increasing mean abundance of Asian tapeworm and water temperature (Appendix C, Figure 1, $F_{1,6} = 8.53$ $P = 0.043$) and increasing mean intensity and water temperature (Appendix C, Figure 1, $F_{1,6} = 9.25$ $P = 0.038$). Mean prevalence of Asian tapeworm infection ranged from 0.00 to 0.62 percent (Appendix C, Figure 2), while mean abundance and mean intensity ranged from 0 to 21 and 0 to 33 tapeworms per fish, respectively (Appendix C, Table 1.). Increasing total length of fish was associated with increasing infection intensity (Spearman’s rho correlation, $r_s = 0.21$, $P = 0.055$).

As water temperature increases, prevalence, abundance, and intensity of Asian tapeworm infection also increases. The highest prevalence and intensity coincided with the warmest water temperature, although the population dynamics of the copepod intermediate host and detectability of Asian tapeworm in cold water also probably play a role in fluctuating estimates of prevalence, abundance, and intensity. Handling, transport, and marking of fish, or any activities that 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 winter and early spring, when the stressor of Asian tapeworm is at a minimum. Additional research should be conducted on the effect of high temperature on Asian tapeworm infections on Mohave tui chub and other warm desert 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, when temperature was held at about 9°C for 30 days, and raised to 21°C over an 8-week period, and when artificial plants were provided as spawning substrate. Three spawning events produced over 1,700 larval fish.

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 *Gila 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 as endangered 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 efforts to maintain and proliferate the species. Vicker (1973) made several unsuccessful attempts to induce spawning in the laboratory and to collect fertile eggs from Lake Tuendae, and no other captive breeding has been attempted.

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). 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.

My objective was to develop photoperiod and temperature manipulation methods to spawn and rear Mohave tui chub in captivity without the use of hormones.

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 (U.S. Fish and Wildlife Service 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 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 1,830 mm) fitted with a custom-built recirculating biofilter and filled with well water (pH = 8.0). 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 of the tank 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, 1,220 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. During the temperature manipulation, I used a photoperiod of 10 h light:14 h dark. After the water temperature was 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. 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 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 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, 1 month after the tanks reached 20-23°C, I found eggs in tanks. 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, eggs hatched in about 4 days, and reached swim-up less than 24 hours 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 during 1 year of captivity. Also, no new eggs appeared in tanks after artificial plants were removed. Whether the plants served as a cue for spawning or simply provided cover to prevent 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. The remaining fish were transported back to Mojave National Preserve and used to assess habitat suitability for future populations.

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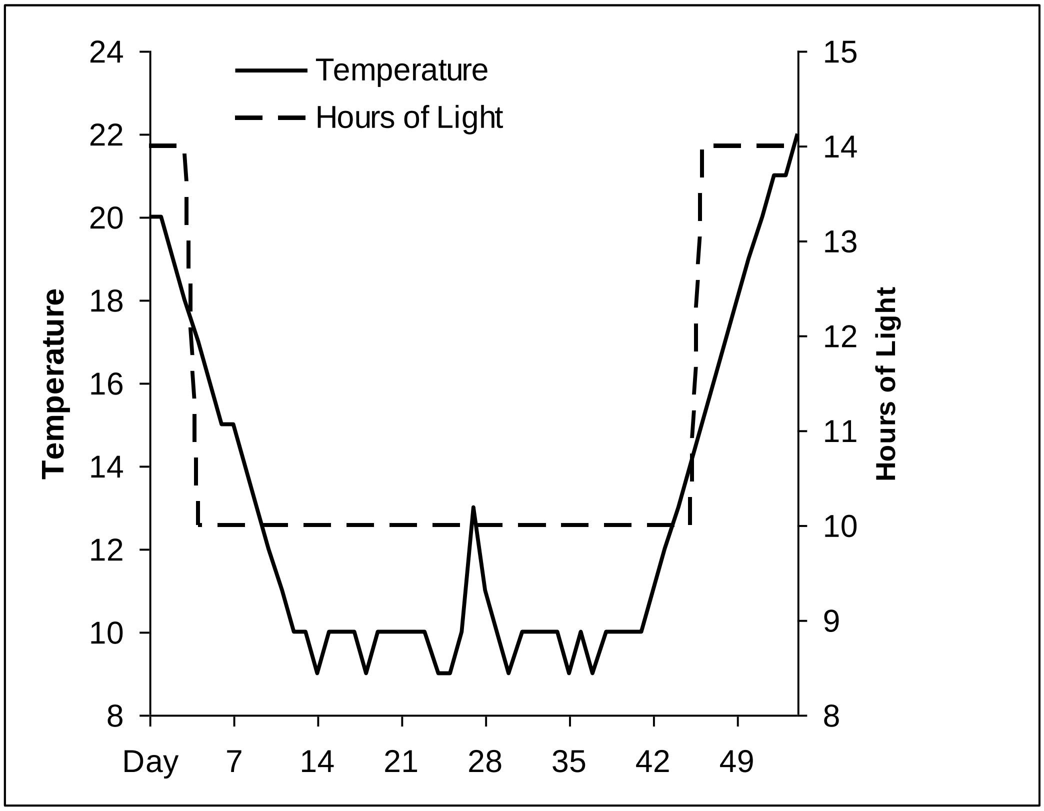

Figure 1 - Photoperiod (hours of light) and temperature (C°) regimen for laboratory spawning of Mohave tui chub. Temperature recorded daily at 0800.

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 fish parasite not native to the United States. Asian tapeworm was probably brought to the United States through shipments of grass carp *Ctenopharyngodon idella* and has spread through baitfish. Mohave tui chubs *Gila bicolor mohavensis* are federally endangered 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 discovered in the lake. Captive Mohave tui chub produced enough fish to experimentally infect fish with Asian tapeworm (Archdeacon, unpublished data). After exposure to Asian tapeworm, 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, exposed or unexposed to mosquitofish, and fed 5% or 2% biomass daily ration. Presence of mosquitofish increased growth of fish, and disordinal interactions occurred between exposure to mosquitofish and food ration, and exposure to mosquitofish and exposure to Asian tapeworm. 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 fish with mosquitofish, but there was no difference between exposed and unexposed fish 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 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 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 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 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 lowered survival at low food rations (Hansen et al. 2006). Roundtail chubs *Gila robusta* infected with Asian tapeworm showed a significant difference in total length compared to uninfected chubs, and a negative 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 *Gila bicolor mohavensis*. Endemic to the Mojave River system of southern California, Mohave tui chub hybridized with introduced arroyo chubs *Gila 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 remaining 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 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 studies have shown negative impacts of mosquitofish on native southwestern fishes through competition and predation (Deacon et al. 1964, Meffe et al. 1983, Meffe 1985, Courtenay and Meffe 1989, Mills et al. 2004).

I experimentally infected young Mohave tui chub with Asian tapeworm in a laboratory setting, and exposed them to high and low food rations, and to presence and absence of mosquitofish. The objective was to evaluate the impact of Asian tapeworm on growth and survival of Mohave tui chub, identify how presence or absence of mosquitofish alters the effect of Asian tapeworm on growth of Mohave tui chub, and provide recommendations for prioritizing perceived threats to their survival.

Methods

My experiment depended on obtaining Asian tapeworm, copepods, mosquitofish, and Mohave tui chub to complete the life cycle of Asian tapeworm and conduct the experiment. Below I discuss how I obtained the components of the experiment, infected Mohave tui chub, and monitored growth and survival of the exposed and unexposed Mohave tui chub. Throughout this description, I used conventions recommended by Margolis et al. (1982) and Bush et al. (1997) for 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, and used a pipette to transfer single copepods into smaller drops of water until I could no longer see any other organisms. I transferred the copepod to a small plastic container, added spring water, and inoculated each container with a wheat seed and *Paramecium caudatum*. When eggs hatched, I poured four or five containers into a large wading pool, inoculated the pool with wheat seeds and *P. 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-L fiberglass tanks. I filled 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 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 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 gravid tapeworms with tanned eggs in the posterior proglittids. I rinsed each tapeworm with 0.6% saline to remove debris, then placed the tapeworm in 50-mm diameter petri dish filled with spring water. Tapeworms often expel tanned eggs, when eggs would not expel, I used needles to tease out any remaining eggs from the proglottids. I repeated the process with more tapeworms until the bottoms of eight petri dishes were mostly covered with eggs.

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 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 copepod behavior was altered. I examined 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, pers. com.).

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 (chosen to minimize bias introduced with missing fins in some treatment groups) 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. 1978, Hanson and Leggett 1986, Roudebush and Taylor 1987, Werner 1992), density was held constant, food ration varied, and differences in temperature between tanks were randomly distributed. 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 38-L tank, then randomly assigned the remaining fish to another 38-L tank. I chose to split fish from within a single, exposed 6.1-L container, in order to maintain constant densities of both fish and copepods throughout all treatment groups during Asian tapeworm exposure. I randomly assigned high and low food rations to each 38-L 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 total fish biomass (ZP3, Aquatic Ecosystems) twice per day, at 0800 and at 1200. Low-ration fish were fed 1% of total 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 (e.g. missing fins, emaciation). I did not replace Mohave tui chubs that died after day 2, but I replaced mosquitofish that died to keep mosquitofish density constant. I examined dead fish to determine if they had Asian tapeworm. On day 96, I used an MS-222 overdose to euthanize all remaining fish, measured standard length and weight, and examined fish 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. Randomization ensured that initial expected differences in standard length and weight between treatment groups was zero. I plotted significant interactions and used Tukey-HSD post-hoc analysis on significant interactions that were disordinal to analyze the difference between cells.

To determine if there was an effect of Asian tapeworm exposure, food ration, or presence of mosquitofish on survival, I used the arcsine square-root transformed data of 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 test the effect of Asian tapeworm, food ration, mosquitofish, and all interactions of mosquitofish and Asian tapeworm on survival of Mohave tui chub.

Results

Growth. Food ration, mosquitofish, and Asian tapeworm exposure had significant effects on the standard length gain of Mohave tui chub (Table 1). Statistically significant disordinal interactions occurred between food ration and mosquitofish, and Asian tapeworm and mosquitofish (Figure 1). Mohave tui chub 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, α = 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 of nine extra fish infected with Asian tapeworm at day 48.

Discussion

Total density of fish in a tank can affect fish growth even when per capita food is held constant (Smith et al. 1978). I held six fish in every tank to avoid confusing effects of competitive interactions with effects of density. Other studies on interactions of mosquitofish with desert fishes have used treatments with differing total densities of fish (Mills et al. 2004). Growth of least chub was reduced at high densities of mosquitofish, but it is important to make comparisons at the same density of fish (i.e. compare growth of 110 least chub to growth of 10 least chub held with 100 mosquitofish, not 10 least chub compared to 10 least chub held with 100 mosquitofish. Although I have limited the scope of inference to fish in captivity fed only one type of food, I found that Mohave tui chub grew more in the presence of similar-sized mosquitofish. Mohave tui chub are more opportunistic than predatory mosquitofish and were observed foraging in tanks much more frequently than mosquitofish than when held alone (personal observation). Even with daily cleaning there was food and detritus left in tanks, and provides a potential explanation for the disordinal food ration and mosquitofish interaction term. When mosquitofish are present, Mohave tui chub are able to feed more effectively than when only Mohave tui chub are present, essentially making high and low food rations the same in tanks with mosquitofish. At the time of dissection, many Mohave tui chub from all treatment groups had food in their gut, in spite of not being fed for over 24 h prior to dissection (personal observation). However, in a pilot-study no cover was provided for fish, allowing thorough cleaning, mosquitofish were so aggressive that Mohave tui chub survival was significantly reduced (Archdeacon, unpublished data). These data suggest that once Mohave tui chubs reach a size that mosquitofish are unable to prey upon them, mosquitofish are of little threat when habitat refuges are available. Additionally, mosquitofish may provide additional resources for growth of Mohave tui chub, I observed mosquitofish in the gut of Mohave tui chub on multiple occasions in the field.

Reports of 90% mortality of carp in culture situations due to Asian tapeworm (Bauer 1973) are simply anecdotal. These reports lack causal inference about the mechanism that resulted in a fish kill, and also lack supporting details to rule out any other cause of death. 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 reduces growth, and higher intensities are associated with smaller fish (Brouder 1999) within a cohort, but data across several age classes suggest that large 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, Topeka shiners and arroyo chub held infected with high prevalence and intensity of Asian tapeworm had low mortality when held in aquaria (Archdeacon, unpublished data). 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. 2007, 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 (personal observation).

I did not find Asian tapeworm in the fish at the end of the experiment. This could have been caused by: (1) incorrect infection methods, (2) small Mohave tui chub or other laboratory characteristics prevented infection, or (3) Asian tapeworm life cycle was completed and tapeworms were evacuated from the fish.

I do not feel that there were procedural errors in these methods that would have prevented infection of the fish. I followed procedures of Hansen et al. (2006) successful infection of bonytail chub exactly, except that copepods were fed to fish during a one day period versus a three day period, and this experiment was run over 96 days instead of 180 days. I hired as a consultant one of the co-authors on Hansen’s experiment to advise me on infection techniques. I clearly saw production of viable Asian tapeworm coracidia and positively identified procercoids within infected copepods. This was confirmed independently when I had other University of Arizona biologists conducting Asian tapeworm studies examine the copepods and I sent photographs of infected copepods for review by a parasitologist (Anindo Choudhury, St. Norbert's College, personal communication).

Consequently, either small Mohave tui chubs may be unsuitable hosts for Asian tapeworm growth and maturation, there were environmental variables that prevented infection, or Asian tapeworm life cycle was completed in the laboratory and tapeworms passed out of the fish. Determining whether high intensity Asian tapeworm infections kill small Mohave tui chubs or whether intraspecific among between tapeworms within the gut produces the pattern seen in Lake Tuendae is an important question for future research.

Low infection prevalence and disordinal interactions confuse the effect of Asian tapeworm on Mohave tui chub. At least some fish were infected at the midpoint of the experiment. Tapeworms may have matured and passed out of the fish 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 an even distribution of infected fish among groups. One interpretation is that exposure to Asian tapeworm only has an effect when mosquitofish are present. More likely, in light of zero final infection percentage, is there were not enough infected fish in the mosquitofish-absent tanks to show a significant difference.

Studies involving multiple hosts and life-stages are very difficult. In this study, I needed enough fish of the correct size and enough copepods to infect these fish when a viable source of Asian tapeworm was discovered. Two previous experimental infections resulted in no infected fish by the end of the experiment (Jason Kline, Arizona Game and

Fish Department, personal communication, personal observation), 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.

Exposing Mohave tui chubs in this manner does not directly reduce survival, and reduces growth only slightly, especially when compared to the effects of mosquitofish and food ration. Studies on bonytail chub given adequate food (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. However, bonytail chub infected with Asian tapeworm showed increased mortality at low food ration (2% and 1.5%). Asian tapeworm may affect Mohave tui chubs differently in the field, but this study and others showed low mortality. It will be important 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, a result of tapeworm competition within the gut, or a result of colonizing an unsuitable host.

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Table 1 - ANOVA summary table of effects on growth (standard length) of Mohave tui chub over 96 days in aquaria.

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 of effects on growth (weight) of Mohave tui chub over 96 days in aquaria.

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 of effects on survival of Mohave tui chub over 96 days in aquaria.

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		

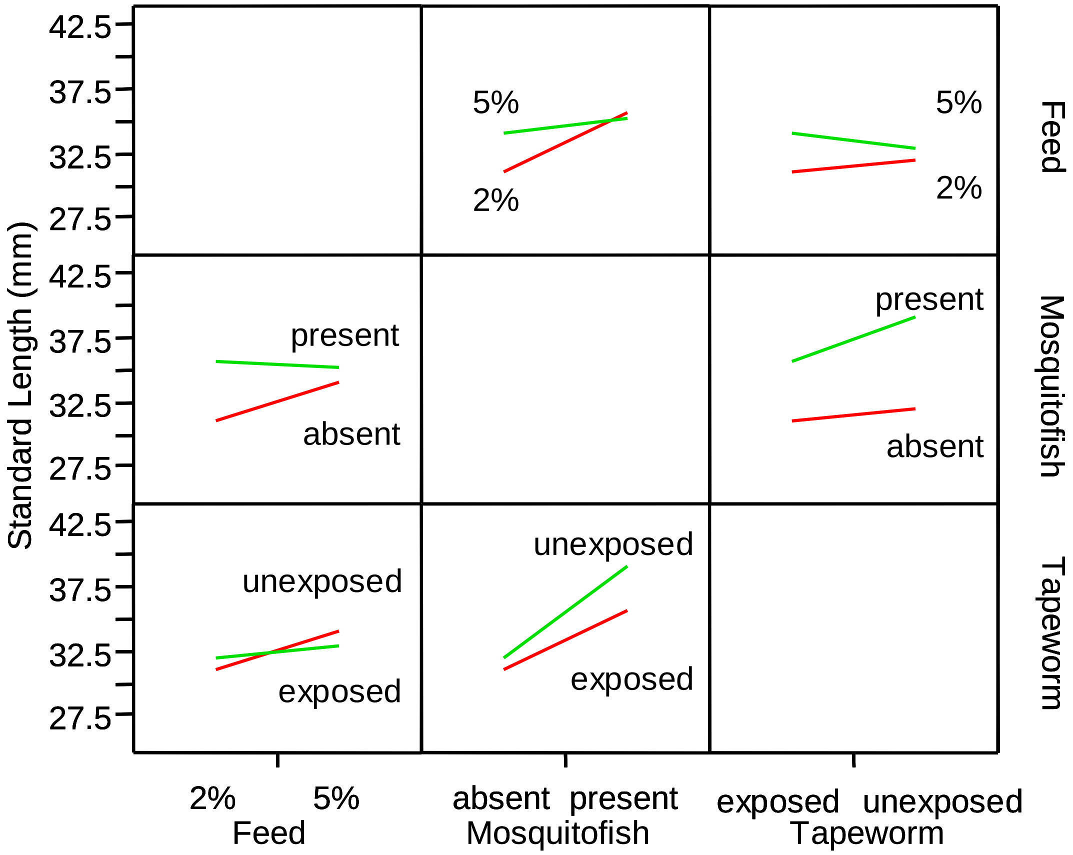

Figure 1 - Plots of 2-way interactions between treatment factors affecting Mohave tui chub growth over 96 days in aquaria.

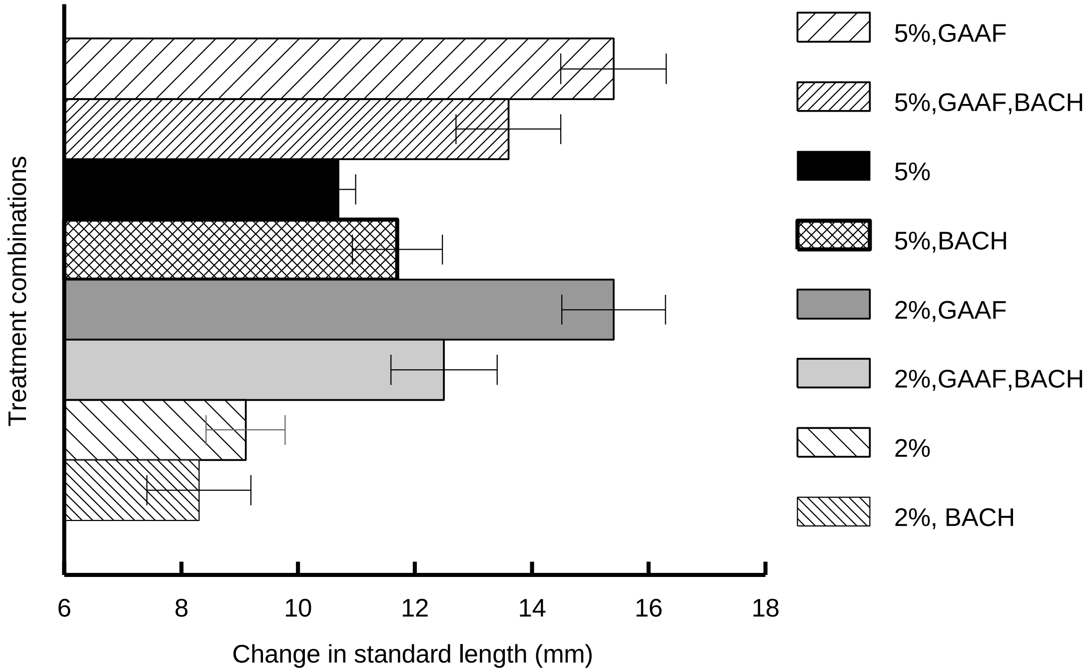

Figure 2 - Growth (mm) of Mohave tui chub in aquaria with treatment factors of 5% or 2% biomass daily food ration, exposed or unexposed to Asian tapeworm (BACH), and in the presence or absence of mosquitofish (GAAF). Error bars represent one standard error of the mean.

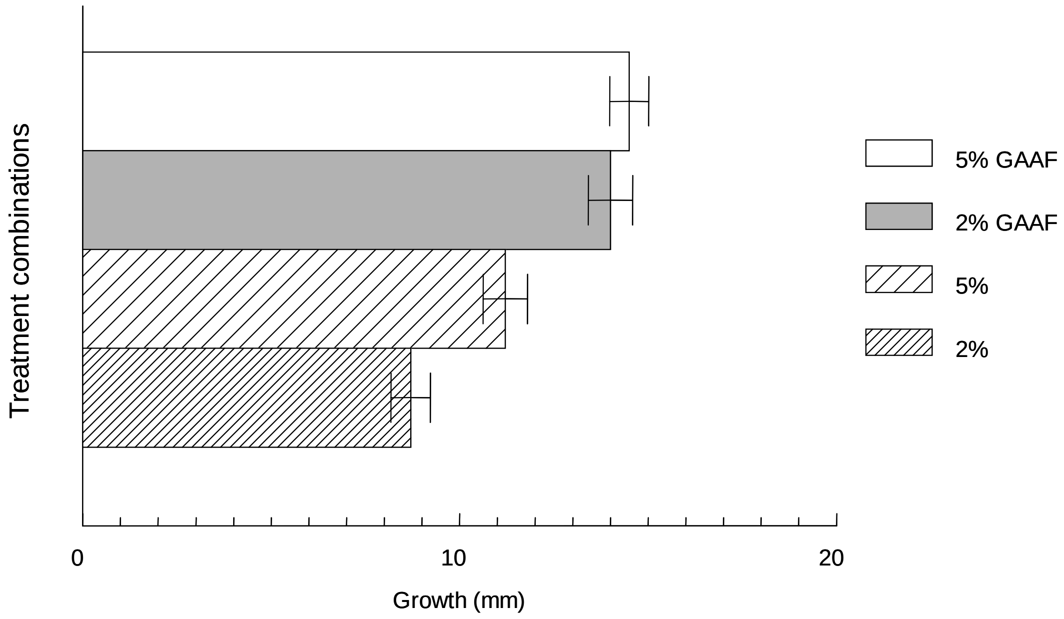

Figure 3 – Average growth (mm) of Mohave tui chub in aquaria over 96 days with treatment factors of 5% or 2% biomass daily food ration and in the presence or absence of mosquitofish (GAAF), when accounting for the effect of Asian tapeworm. Error bars represent one standard error of the mean.

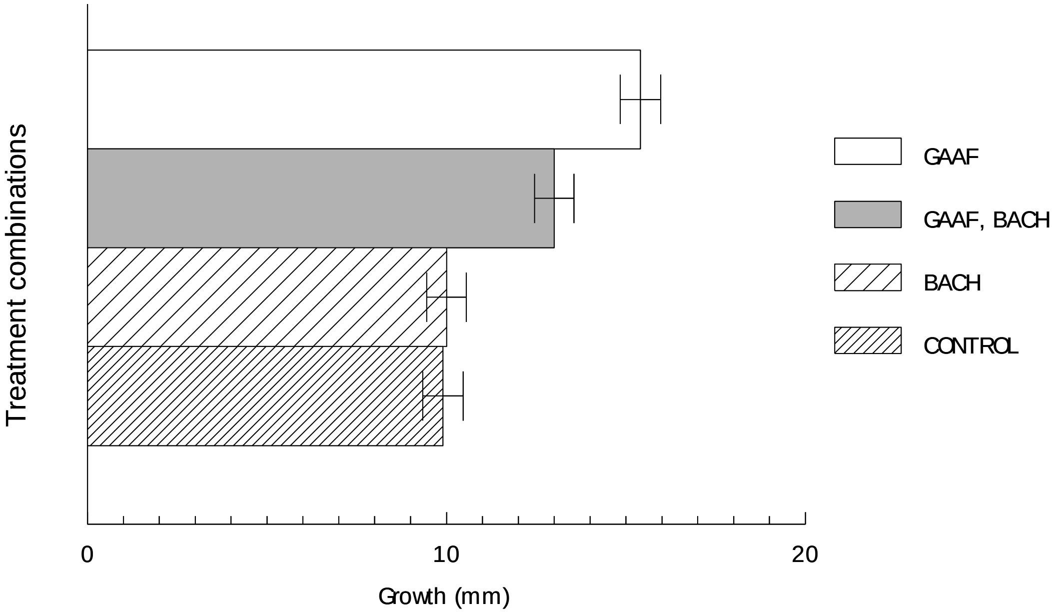

Figure 4 - Average growth (mm) of Mohave tui chub in aquaria over 96 days with treatment factors of exposed or unexposed to Asian tapeworm (BACH) and presence or absence of mosquitofish (GAAF), when accounting for the effect of food ration. Error bars represent one standard error of the mean.

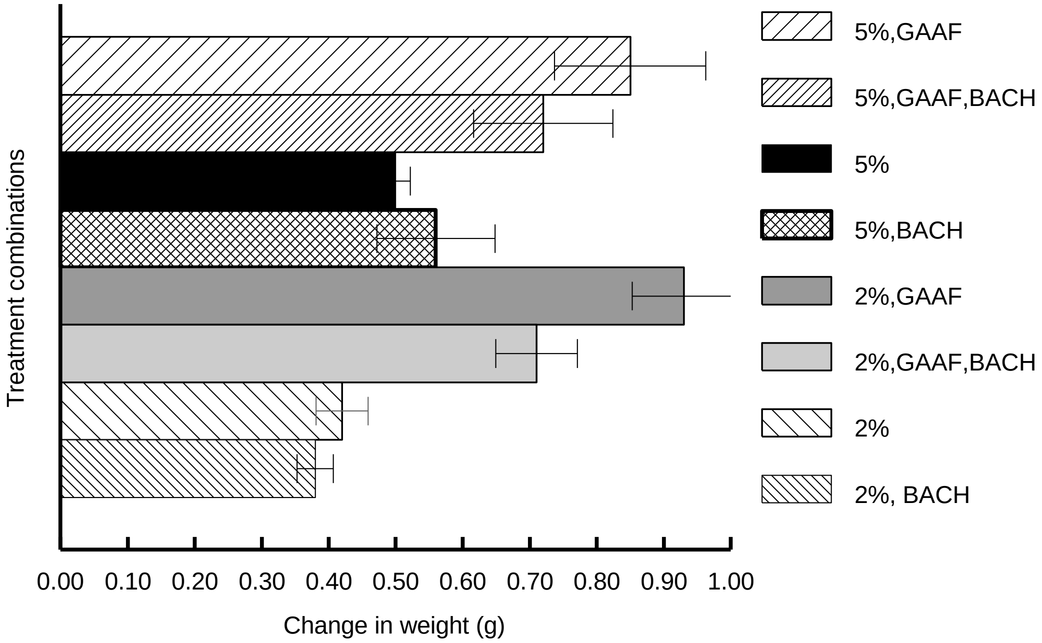

Figure 5 - Growth (g) of Mohave tui chub in aquaria with treatment factors of 5% or 2% biomass daily food ration, exposed or unexposed to Asian tapeworm (BACH), and in the presence or absence of mosquitofish (GAAF). Error bars represent one standard error of the mean.

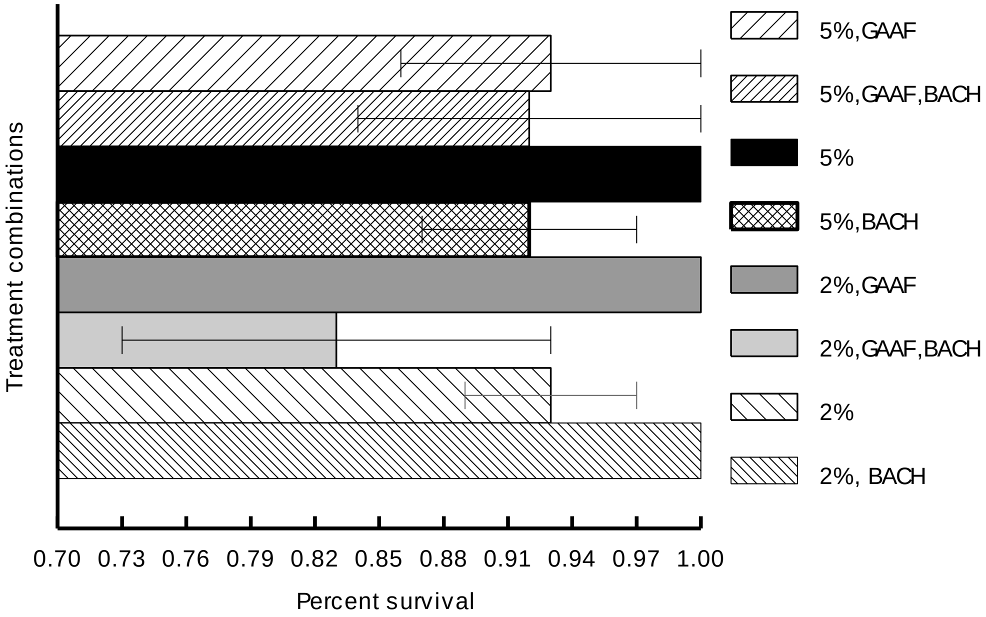

Figure 6 – Percent survival of Mohave tui chub in aquaria with treatment factors of 5% or 2% biomass daily food ration, exposed or unexposed to Asian tapeworm (BACH), and in the presence or absence of mosquitofish (GAAF). Error bars represent one standard error of the mean.

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 and in many southwestern United States fishes in the family Cyprinidae. I monitored the seasonal dynamics of Asian tapeworm populations in federally endangered 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. Failures to detect Asian tapeworm were likely caused by small sample size and low infection rates, or insufficient time for gut evacuation in low temperature water, although praziquantel is still effective at killing Asian tapeworm even at low temperatures. Additional stressors to fish (e.g. handling, marking, transport, habitat modifications) should be avoided during summer months when water temperature and Asian tapeworm prevalence, intensity, and 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 et al. 1980, Andrews et al. 1981, Font and Tate 1994, Dove et al. 1997). The tapeworm was probably 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, pers. com.) when ten fish were accidentally killed and subsequently dissected.

Seasonal changes in mean abundance (total number of tapeworms divided by total number of potential hosts), mean intensity (total number of tapeworms divided by total number of infected hosts), and prevalence (percent of infected hosts) of Asian tapeworm are common (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 Asian tapeworm occurred during early winter and the smallest infrapopulations occurred during mid to late summer (Granath and Esch 1983a, 1983b). The rise in infection in early winter may 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 prevalence and abundance of Asian tapeworm were highest in 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, and the fact that it infects all cyprinid fishes, and does well in warm water make it a serious potential threat to conservation of native fish 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 anti-helminthics 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 to biologists because it eliminates Asian tapeworm without killing the host fish and tapeworms can be quantified without dissection of hosts (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 are likely to have the highest prevalence and intensity of Asian tapeworm infections, and eliminate additional stressors to fish such as handling. I monitored changes in tapeworm populations in Mohave tui chubs in Lake Tuendae, Zzyzx (Mojave National Preserve), California, where the water temperatures range from about 5°C in winter to over 30°C in summer. I tested 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, I use parasite terminology recommended by 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 (U.S. Fish and Wildlife Service 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 approximately 800 L of well water from the same source as the lake. I placed rocks as needed to prevent the boxes from floating. I added 4.8 g of praziquantel (6 mg/L) to a small amount of isopropyl alcohol to create a 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 were 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 prevalence of Asian tapeworm because freezing surface water prevented a praziquantel bath. In April 2007 I treated fish with praziquantel and subsequently dissected the same fish to evaluate spring population parameters and evaluate the effectiveness of praziquantel. I dissected only 30 fish on these dates.

Finally, I compiled data on prevalence and intensity at a variety of field sites in Arizona and southern California.

Laboratory temperature experiment. I treated Topeka shiners *Notropis tristis* known to be heavily infected (prevalence 100%, mean intensity >30) with 6.0 mg\L praziquantel solution in the laboratory. I placed 10 fish into a 76-L tank with 22° C water and 10 fish into another tank with 13° C water. The tanks were identical except for the water pump leading to the chiller on the cold-water tank. After 24 h I dissected half the fish in each tank and recorded presence or absence of Asian tapeworm. After 48 h I dissected the remaining fish and recorded presence and absence of Asian tapeworm.

Data analysis. I used Fisher's-exact test 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). I used linear regression to test 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 intervals, median intensity and distribution-free confidence intervals, 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 strength of association between total length of fish and number of tapeworms per fish.

Results

Field monitoring. I found significant differences in prevalence between sampling periods ($X^2 = 83.8$, $df = 6$, $P < 0.001$). Increasing water temperature was associated with increasing prevalence of infection (Figure 1, $F_{1,6} = 7.6$ $P = 0.040$). I found similar positive relationships between increasing mean abundance of Asian tapeworm and water temperature (Figure 1, $F_{1,6} = 8.53$ $P = 0.043$) and increasing mean intensity of Asian tapeworm and water temperature (Figure 1, $F_{1,6} = 9.25$ $P = 0.038$). Mean prevalence ranged from 0.00 to 0.62 (Figure 2), and 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 ($r_s = 0.21$, $P = 0.055$). In April 2007 neither method found any Asian tapeworm in Mohave tui chub.

Laboratory experiment – No tapeworms were found in the gut of any fish in the 22°C tank after either 24 or 48 h, but we found tapeworms in all fish after 24 and 48 h in the 13°C tank. However, we noted none of the tapeworms were alive even when found in the gut.

I found Asian tapeworm in six out of eight unique water bodies at least one time, and overall 22 of 38 samples had Asian tapeworm present. Mean intensity ranged from 0 to 38.1 tapeworms per fish. However, there is considerable variation in sample size, host species, and season between locations.

Discussion

Topeka shiners take longer to empty their guts in cold water, including any Asian tapeworms. Praziquantel is still effective at killing tapeworms even in low temperatures, but use as a non-lethal detection method may be limited in cold waters. Even after 48 h, fish still had many tapeworms within the gut, and we noted none floating in the tank. Failure to detect Asian tapeworm in two of four samples at the field site were in cold weather, when we waited only 24 hours. However, during dissection in January and April 2007, we also found no infected fish (*n* = 30 and 38, respectively). The initial detection of Asian tapeworm in Mohave tui chub occurred in January when ten out of ten fish were infected. We found Asian tapeworm on visits after failure to detect, and were likely due to a combination of low prevalence (see confidence intervals associated with sample sizes in Table 1) as well as insufficient time for the gut to evacuate. Praziquantel is still effective at killing tapeworms, but its usefulness as a monitoring tool in cold water may be limited to situations when more than 48 h are available for treatment. Sample size should always be kept in mind, as well as season when sampling for presence/absence of Asian tapeworm. Populations parameters vary greatly at the same location through time, and small sample sizes during periods of low prevalence could lead to incorrectly assuming Asian tapeworm is absent from a system (Table 2).

Prevalence and intensity of Asian tapeworm infection in Mohave tui chubs was higher in warmer water. The highest prevalence and mean intensity coincided with the warmest sampling period, although the population dynamics of the copepod intermediate host and detectability of Asian tapeworm in cold water also probably played a role in fluctuating prevalence, abundance, and intensity I observed. However, dissection during winter and spring revealed no infected fish.

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, University of Minnesota, personal communication).

Larger fish were associated with higher intensity infections, in direct contrast to roundtail chub *Gila robusta* (Brouder 1999). However, I compared infection intensity across age groups, roundtail chub were all from the same cohort and exposed to the same environmental conditions. Fish that are infected grow less, but smaller fish are associated with lower intensity infections. Several explanations could account for this pattern. Small, intensely infected fish may die quickly and be eliminated from the population. However, killing a host would not be adaptive for Asian tapeworm, and I found small fish very hard to infect (Appendix B). 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 data are needed to establish whether or not high-intensity Asian tapeworm infections kill small fish, or if small Mohave tui chubs are poor hosts, 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 unlikely.

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 stresses associated with Asian tapeworm are at a minimum. Additional research should be conducted on interaction between high temperature and Mohave tui chub infected with Asian tapeworm and other warm-desert fishes.

Acknowledgements

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Table 1 - Summary statistics of Asian tapeworm infection in Mohave tui chubs in Lake Tuendae, California. Parentheses indicate 95% confidence interval. *Indicates dissection used to collect data.

Date	Mean prevalence	Mean abundance (tapeworms)	Mean intensity (tapeworms)	*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
APR07*	0	0	0	38	19.0

Table 4

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-07	Lake Tuendae, CA	Mojave NP	30	0.00	0.0
Mohave tui chub	1-Apr-07	Lake Tuendae, CA	Mojave NP	38	0.00	0.0

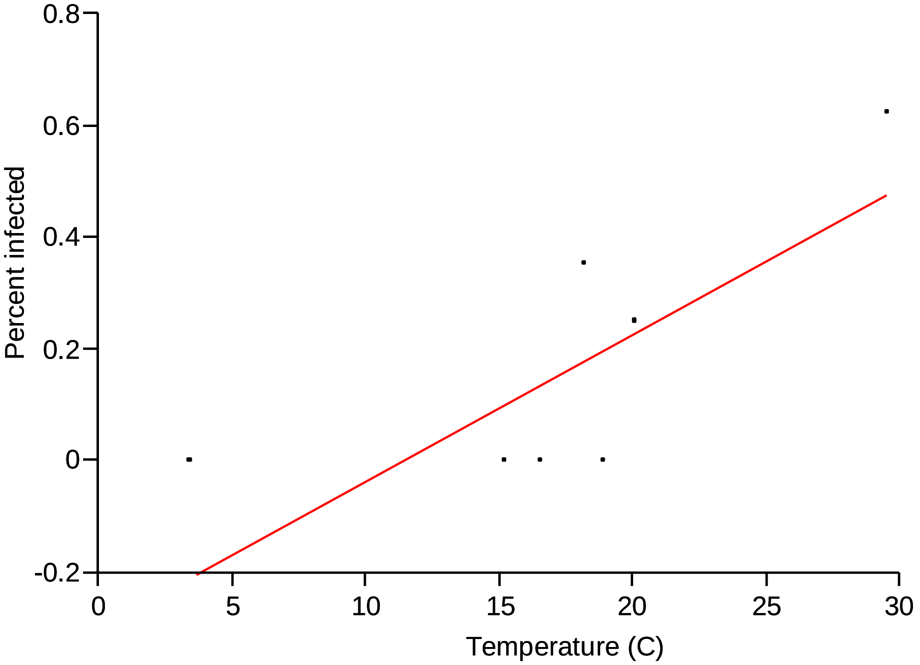

Figure 1 - Percentage of infected fish vs. the water temperature at 1100 in Mohave tui chub in Lake Tuendae, California.

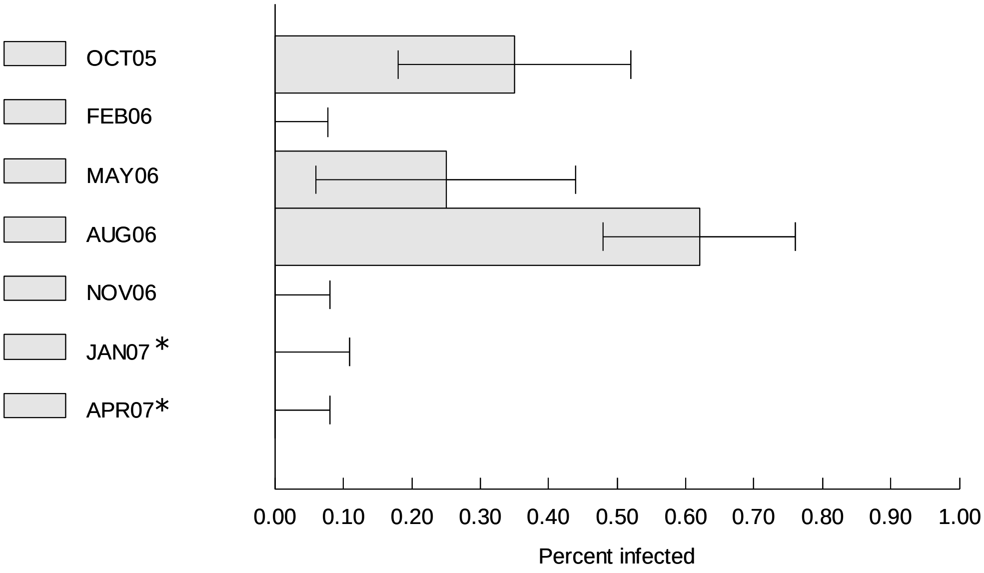

Figure 2 - Mean number of infected Mohave tui chub by sampling period found in Lake Tuendae, California. Bars represent 95% C.I. *Indicates dissection.