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Factors Influencing Distribution of Introduced Asian Tapeworm and Effects on Selected Southwestern Fishes (Yaqui Topminnow and Yaqui Chub)

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Executive Summary

Yaqui chub *Gila purpurea* and Yaqui topminnow *Poeciliopsis occidentalis sonoriensis* are small fish native to the Rio Yaqui Basin in Arizona, and northern Mexico. Both are threatened by habitat loss, through ground water pumping and land use changes, and by introductions of non-native organisms including fish, amphibians, and parasites. San Bernardino National Wildlife Refuge (SBNWR), located in southeastern Arizona, is the only place both species occur together in the wild in the United States.

Asian tapeworm, *(Bothriocephalus acheilognathi)*, a parasite known to be pathogenic or able to impair growth in cyprinid fishes, is established in SBNWR. We conducted a laboratory experiment to measure the effects of Asian tapeworm on the growth and survival of these Yaqui fishes. In addition to what was required by the project, we report several aspects of the biology and management of Asian tapeworm and the Yaqui fishes not previously known. We discuss the methods we used to spawn and rear Yaqui chub and Yaqui topminnow in captivity, which will be useful in future conservation efforts. The antihelmenthic praziquantel (*Droncit*, Bayer) has been used to treat Asian tapeworm infections in captive fish. However, we show that eggs of Asian tapeworm can be viable following treatment. Finally we discuss and test the effectiveness of using praziquantel to treat entire pond systems. The major findings from our work are as follows:

- Yaqui chub were more affected by Asian tapeworm exposure than Yaqui topminnow. We found a significant difference between growth rates of Yaqui chub that were exposed to Asian tapeworm and those that were not. We did not find a difference between the growth rates of Yaqui topminnow, nor did we find a difference in the mortalities of between exposed and unexposed tanks. Infections of exposed fish were lighter than those reported previously in the literature. Tests with heavier infections will be necessary to test the upper levels of the effects of Asian tapeworm.

- We developed methods to successfully propagate and rear Yaqui chub and Yaqui topminnow. Almost nothing was known previously about the propagation of these species. These fish were produced for the Asian tapeworm experiments.
- We were most successful propagating Yaqui topminnow in four 556-L plastic wading pools containing cobble, gravel, artificial plants and a vertical mesh barrier impassible to larger fish to create refuge for offspring. Water in pools increased from 19° C as the ambient temperature increased. Yaqui topminnow produced young after 1 month when the temperature exceeded 21° C. Numbers of offspring were variable but reproduction was continuous after the temperature exceeded 21 C. On average, 7.4 offspring were captured each day from the four pools collectively.
- We spawned Yaqui chub in four 189-L glass aquaria stocked with 6-9 fish per tank. We chilled water to 17° C for 30 d and then increased the temperature to 21° C over 14 d. After the temperature was 21° C for 3 d, we covered the bottom of aquaria with glazed ceramic tiles and a raised plastic grid over the tiles to protect eggs from predation. Fish usually spawned at night, and we retrieved tiles containing eggs the following morning and placed them in incubation tanks. Yaqui chub eggs hatched in the next 5 d with a success rate of 83%.

- We treated several cyprinid fishes, both native and non-native to Arizona, with Praziquantel, an anthelmintic commonly used to control Asian tapeworm. At the recommended dosage, Praziquantel killed adult tapeworm, but some tapeworm ruptured and released eggs. Eggs released from Asian tapeworm treated with Praziquantel were viable and produced coracidia over several days. Fishery managers should be aware that even if fish receive a typical Praziquantel treatment regime and all adult tapeworms are killed, viable eggs and coracidia may be present in the holding water or attached to the skin of treated fish, surfaces of equipment, or to treatment personnel.
- We treated fish infected with Asian tapeworm in experimental tank microcosms simulating pond systems to help evaluate the effects of Praziquantel applied to ponds in the wild. Pond microcosms contained cyclopoid copepods, aquatic plants, sediment, and water from the San Bernardino system. Treatments were applied twice, 19 days apart.
- The treatment of Asian tapeworm with Praziquantel in the presence of the tapeworm's intermediate host, cyclopoid copepods, proved effective. There was no evidence of tapeworm recruitment in the treatment microcosms since no tapeworms were found; however, recruitment was occurring in the control microcosms.
- Although our results were encouraging, much further research is necessary before implementing the use of Praziquantel in the field. In particular, the chronic, physiological effects of Praziquantel exposure on all aquatic

organisms, the eco-toxicity of Praziquantel, and its effectiveness against other Asian tapeworm life-stages needs to be determined.

Acknowledgements

Funding for this project was provided by the Arizona Game and Fish Department Heritage Program, the National Fish and Wildlife Foundation, the U.S. Fish and Wildlife Service, the University of Arizona, and the U. S. Geological Survey.

We wish to give a special thanks to Andrea Francis, the technician responsible for the husbandry of the fish used in these experiments whose hard work allowed our success. Dr. Anindo Choudhury from St. Norbert's University was instrumental in assisting us with questions and procedures related to Asian tapeworm infections and biology. We also thank Dr. Bill Matter, University of Arizona; and Chris Cantrell and Don Mitchell of Arizona Game and Fish Department, whose reviews and comments greatly improved this manuscript, and Scott Hansen, Sean Tackley, Peter Reinthal, Joe and Valerie Austin, Dave Ward, Cori Carveth, William Radke, Andrew Shultz and Nina King for their assistance. Reference to trade names does not imply endorsement.

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General Introduction and Literature Review

Asian tapeworm *Bothriocephalus achelognathii* is a cestode parasite primarily affecting fish in the minnow family, Cyprinidae. Native to Eurasia, it was first described by Yamaguti in 1934 and has since spread to all continents except Antarctica (Korting 1975, Boomker 1980, Andrews 1981, Font 1994, Dove 1997). It is thought to have been introduced to the United States through stocking of its native hosts, carp *Cyprinius carpio* and grass carp *Ctenopharyngodon idella*, (Hoffman 2000, Stevenson 1965). The Asian tapeworm was introduced into the southwestern United States in shipments of baitfish. In Arizona, it was first found in the Virgin River (Heckmann 1987). In the current study, Asian tapeworm was found in the Verde, Gila, Salt, and Yaqui Rivers and their tributaries.

Asian tapeworm has the greatest effect on Cyprinid species of fish, which are the largest group of threatened and endangered fishes in the southwestern United States. Furthermore, reproduction and development of Asian tapeworm is maximized in warm waters (Granath & Esch 1983c). Most native cyprinids in the southwestern United States are small, and are living in small streams where water temperatures can be high. Thus, Asian tapeworm represents a large potential threat for native Southwestern fishes.

Usually little damage is associated with the presence of cestodes in the digestive tracts of fish (Hoffman 1999); however, the Asian tapeworm is an important exception. It has been responsible for mass mortalities of fish in both cultured and wild populations (Bauer 1973, Izyumova 1987). Bauer reported up to 90 % mortality in grass carp in newly infected ponds (1973) and the parasite reduces growth in roundtail chub in the Little Colorado River (Brouder 1999). Asian tapeworm causes severe intestinal blockage, and is known to burrow through intestinal walls and damage somatic and gonadal tissue, possibly killing the fish

(Heckman 2000). Hanson et al. (2005) found reduced growth and negative changes in health indices in bonytail chub *Gila elegans* infected with Asian tapeworm. Research on the effects of Asian tapeworm on other endangered species is necessary to assess threats to these species as well.

Of particular concern are infections of fishes found in the wetlands of the San Bernardino National Wildlife Refuge in southeastern Arizona, where Asian tapeworm was first identified in 1995 (King 1995). Set up specifically for fish, this refuge is the only place in the United States to host Yaqui chub *Gila purpurea*, Yaqui topminnow *Poeciliopsis occidentalis sonoriensis*, and Yaqui catfish *Ictalurus pricei*, in the wild, and one of the few areas where Beautiful shiner *Cyprinella formosa* are still found. All except Yaqui catfish are known hosts for Asian tapeworm. The Asian tapeworm infestation has the potential to complicate recovery efforts for these endangered species that are otherwise protected in the refuge. In a survey of the refuge in 2000, Asian tapeworm was found in fish from all wetlands except those where only Yaqui topminnow populations existed (N. King, USFWS, personal communication). All wetlands with Yaqui chub and/or beautiful shiners were infected with Asian tapeworm (King, 2000). It was also found that Yaqui chub were most susceptible to infection (53-95% prevalence) and Yaqui topminnow was least susceptible (0-17%; N. King, USFWS, personal communication).

The lifecycle of Asian tapeworm occurs in four stages: egg, coracidia, procercoid and adult (Fig 1). The egg and coracidia stages are free living in the water, the procercoid stage is within the intermediate host, a Cyclopoid copepod, and the final adult state is within the definitive host, usually a Cyprinid fish. Adult worms shed eggs from their gravid segments, or proglottids, and are evacuated from the fish’s digestive system. Eggs settle to the substrate and hatch into free-swimming coracidia over 2-10 d depending on temperature. Coracidia are spherical with 3 pairs of embryonic hooks and are covered in cilia, which they use to swim up into the water column. When the intermediate host, a Cyclopoid copepod, ingests the coracidia, it perforates the copepod body cavity and develops into a procercoid. Procercoids are oblong with the posterior end differentiated into a cercomere with 3 distinct pairs of embryonic hooks. At this stage, the procercoid is considered invasive and is able to infect the final host. It can take 4-11 d to complete this transformation, again dependent on water temperature (Bauer, et al. 1973). It is thought that the procercoid can actually alter the behavior of the copepod, causing it to swim erratically near the surface and more likely to be preyed upon by a fish. Cyprinid fish digest the copepod and the procercoid attaches to the intestine of the fish with the embryonic hooks. The procercoid then transforms into a tapeworm and within 20-25d the worm develops into an adult and begins producing eggs. The Asian tapeworm can live up to one year in the host, and produce tens of thousands of eggs.

Development of Asian tapeworm at all stages is temperature dependent (Granath & Esch 1983c). In both lab studies and field observations, as water temperature increases above 25°C in the spring, the majority of worms tend to be segmented or gravid and when temperatures decline below 25°C in the fall, most worms are non-segmented or immature (Granath & Esch 1983c). Laboratory studies showed no maturation occurred at 20ºC and almost 100% of the worms matured at 30ºC (Granath & Esch 1983c). Temperature also affects egg maturation and hatching, with both peaking at 30ºC (Granath & Esch 1983c), (Hanzelova & Zitnan 1987). The entire lifecycle of the Asian tapeworm can be completed in 18 d, if temperatures are optimal (Bauer, et al. 1973).

Within the definitive host *B. acheilognathi* shows three distinct developmental stages: (1) non-segmented worms, which are immature and were assumed by Marcogliese & Esch (1989) to be recently recruited (< 9 days old); (2) segmented worms with adult scolices but immature proglottids; and (3) gravid, or egg producing worms (Granath & Esch 1983c). Ambient temperature appears to have a direct influence on growth and maturation of *B. acheilognathi*. In both lab studies and field observations, as water temperature increase above 25°C in the spring, the majority of worms tend to be segmented or gravid and when temperatures decline below 25°C in the fall, most worms were non-segmented or immature (Granath & Esch 1983c). Densities of tapeworms tend to decrease in the spring, which is though to be initially due to intraspecific exploitative competition between tapeworms for space or nutrients as they grow and become gravid, and mortality of heavily infected hosts (Granath & Esch 1983b). Later, in the summer, the decline in the number of gravid worms has been thought to be due to death of the over wintering cohort of tapeworms, post-spawning death of the host species and recruitment of uninfected fry (Riggs et al. 1987).

Many studies have noted distinct seasonal patterns in *B. acheilognathi* abundance and prevalence (Granath & Esch 1983c, a, Heckmann et al. 1986); however, it appears that these patterns are not simply seasonally driven, but are the result of complex interactions between environmental conditions and intermediate and definitive host populations (Riggs & Esch 1987, Marcogliese & Esch 1989, Clarkson et al. 1997, Choudhury et al. 2004). Furthermore, seasonal patterns have been shown to vary from year to year (Marcogliese & Esch 1989) and from region to region (Choudhury et al. 2004). For example, in a cooling reservoir in North Carolina, Granath & Esch (1983c, 1983a) noted that largest infrapopulations (all individual tapeworms in an individual host) of *B. acheilognathi* occurred during early winter and lowest infrapopulatons occurred during mid to late summer. This rise in infection in early winter was later thought not to be associated with a corresponding increase in reproduction of adult worms, but to an emergence of 4th instar copepodites (an intermediate host) which became infected in the spring (Riggs & Esch 1987). A later study at the same reservoir, found that both prevalence and abundance was highest in the summer and that this seasonal shift correlated with a shift in the copepod community composition (Marcogliese & Esch 1989).

Even though *B. acheilognathi* infects many species and several orders of fish (Dove & Fletcher 2000), the maintenance of local suprapopulations (all individuals of Asian tapeworm in all stages of development in an ecosystem) is usually dependent on “required hosts”, in which prevalence and abundance of gravid tapeworms are greatest (Riggs et al. 1987). In Australia, *B. acheilognathi* fails to mature properly in native fishes (gravid worms are rare), so the local suprapopulations are maintained by non-native carp (Dove & Fletcher 2000). Choudhury et al. (2004) recovered *B. acheilognathi* from all species of fish in the Little Colorado River; however, it was most abundant in cyprinids. Riggs et al. (1987) found that the host species was a very significant influence on tapeworm fecundity, with gravid worms regularly found in the cyprinids, red shiner (*Cyprinella lutrensis*) and fathead minnow (*Pimephales promelas*) , but rarely in mosquitofish (*Gambusia affinis*), a cyprinodontiform. According to Riggs et al. (1987), mosquitofish are not known to support gravid individuals of any cestode and thus may have an intestinal environment that is unsuited for harboring tapeworms. Furthermore, it was noted that the growth of even a few Asian tapeworms to more than 40-50 proglottids long was usually fatal to mosquitofish.

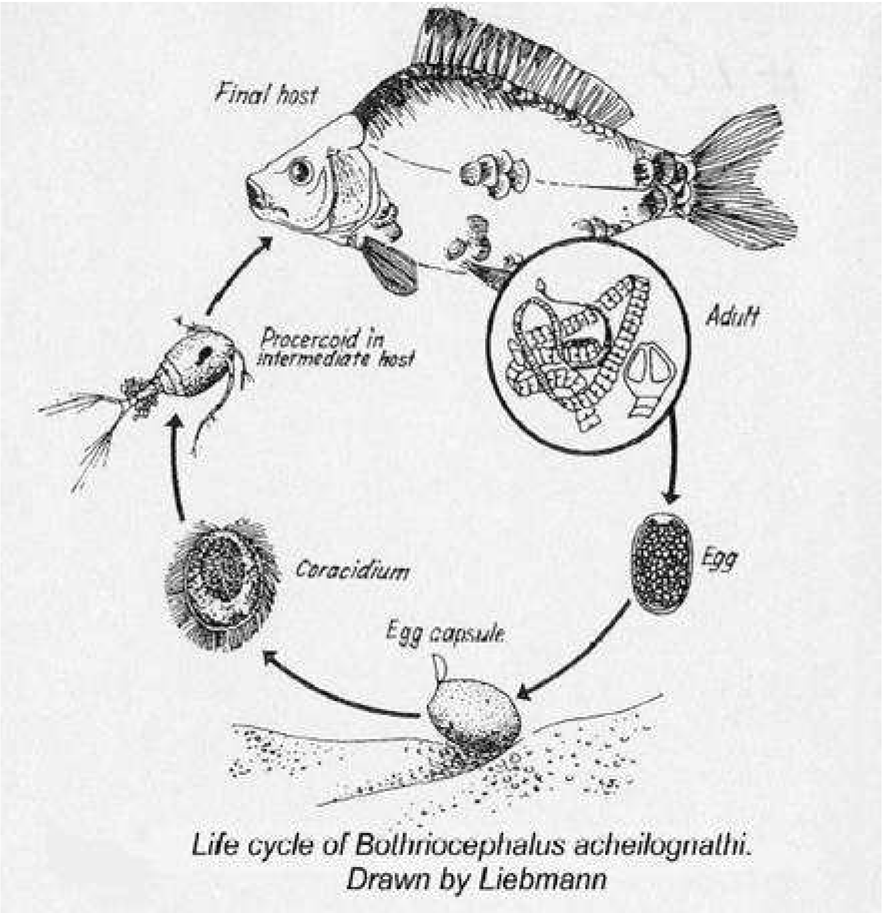

Figure 1: Life cycle of the Asian fish tapeworm from Liebmann.

Objectives

We examined the effects of Asian tapeworm infection on the growth and mortality of Yaqui chub and Yaqui topminnow by comparing the growth and survival rates of fish exposed to Asian tapeworm to fish that were not (Chapter 1). We report on our findings on how praziquantel, an antihelmintic drug lethal to tapeworms affects tapeworm eggs (Chapter 2). We also assess the usefulness of this drug for treating small pond systems (Chapters 3). Finally, we report on the methods we developed to successfully spawn and rear Yaqui chub and Yaqui topminnow in the laboratory (Chapter 4).

Chapter 1: Effects of Asian Tapeworm on Yaqui Chub and Yaqui Topminnow

ABSTRACT

Asian tapeworm *Bothriocephalus acheilognathi*, a parasite known to be pathogenic to cyprinid fishes, is established in San Bernardino National Wildlife Refuge, southeastern Arizona, USA. The San Bernardino refuge is home to endangered Yaqui chub *Gila purpurea*, and Yaqui topminnow *Poeciliopsis occidentalis sonoreinsis*, and our experiment measured the effects of Asian tapeworm on the growth and mortality of these species. Our experiment employed forty 75.8-L aquaria, with 10 different treatments, each having 4 replicates. We stocked fish at 2 single species densities and 1 mixed species density to see if competition has an impact on infection effects. We found a significant difference between growth rates of Yaqui chub that were exposed to Asian tapeworm and those that were not. We did not find a difference between the growth rates of Yaqui topminnow, nor did we find a difference in the mortalities of between exposed and unexposed tanks.

INTRODUCTION

Effects of Asian tapeworm vary from up to 90 % mortality in grass carp in newly infected ponds (Bauer, Musselius & Strelkov 1973) to reductions in growth in roundtail chub in the Little Colorado River (Brouder 1999). *B. acheilognathi* causes severe intestinal blockage, and is know to burrow through the intestine and damage somatic and gonadal tissue, sometimes killing the fish (Heckman 2000).

In the current study we researched the effects of Asian tapeworm on two endangered species of the San Bernardino National Wildlife Refuge, in Southeastern Arizona, where Asian tapeworm was first identified in 1995 (King 1995). Knowing the effects of Asian tapeworm on Yaqui chub *Gila purpurea* and Yaqui topminnow *Poeciliopsis occidentalis* *sonoriensis* is critical to successful management of these species. We experimentally tested the hypothesis that Asian tapeworm negatively affects the growth and mortality of Yaqui chub and Yaqui topminnow under laboratory conditions.

METHODS

Animal Culture

Copepods

We collected copepods *Cyclopoid vernalis* from the San Bernardino National Wildlife Refuge with a 153-µ plankton tow. Copepods were sorted under a binocular microscope (Ziess Stemi-2000) and gravid females were isolated in small plastic bowls filled with spring water and fed paramecium. Bowls were aerated with a pipette every other day, and after 2 weeks, copepods were transferred into 400-L wading pools filled with well water and stocked with paramecium. We covered pools with plastic to maintain pure cultures of copepods and aerated the water with an air pump (Rena model 400). We stocked each pool with 5 bowls of copepod cultures. We harvested copepods from pools with a 153-µ mesh net and then transferred them into 250-mL glass culture dishes, stocking 75 copepods/dish. (http://fmel.ifas.ufl.edu/culture.htm)

Yaqui Topminnow and Yaqui Chub

These fishes were bred in the laboratory using the techniques presented in detail in Chapter 4.

Asian Tapeworm

We treated several cyprinid fishes with praziquantel, including 20 headwater chub *Gila nigra* and 10 roundtail chub *Gila robusta,* to collect gravid Asian tapeworm. We treated a 1,892-L pool with Praziquantel at 6 mg/L, dissolved in 10 mL of 70% isopropyl alcohol and aerated the pool with a Rena 400 air pump (Mitchell 2004, Ward 2005). We held individual fish in 3-L plastic containers anchored to the bottom of the pool with rocks or sandbags. We painted the bottom of each 3-L container black, to see worms more easily, and covered the top of containers with screen to allow treated water to flow through the container but retain any expelled worms. We held fish in the treatment pool for 24 h and checked each container for expelled worms. We selected gravid adult worms and placed them in petri dishes (60 mm x 15 mm) filled with spring water. We examined gravid worms under a binocular microscope (Zeiss Stemi-2000) and teased eggs out of worms by tearing apart segments and expelling eggs into the petri dish (A. Choudhury, St. Norbert College, personal communication). We covered the petri dishes and held them at room temperature (approximately 22° C). We checked dishes daily for coracidia, the first life stage, and poured coracidia off into 250-mL glass culture dishes holding copepods awaiting infection. We then refilled petri dishes with spring water and returned them to incubation.

Experiment

Infection

We stocked 15 glass culture dishes (250 mL) with 75 copepods. We starved copepods for 24 h and fed them coracidia for 2-3 days as they hatched from harvested eggs. The copepods were held in culture dishes for 15 days to allow the coracidia to develop into procercoids, the intermediate stage of Asian tapeworm. Fish randomly chosen for infection were placed in 19-L aquaria for infection. Fish were starved for 24 h and the water level was drawn down to 1/3 full and the powerfilter shut off. We poured culture dishes containing the infected copepods into the infection tanks. We allowed fish to feed on infected copepods for 12 h, then water level was returned to full and, after 24h, we restarted the powerfilter.

Treatment assignment

We placed all Yaqui chub into one 111-L Rubbermaid tote and all Yaqui topminnow in an identical tote for treatment assignment. We used a random lottery to assign forty 76-L tanks a treatment and stocking rate. We used 10 stocking combinations as shown in Table 1, each with four replicates. The tanks were filled with well water treated with Amquel®. Each tank had its own powerfilter (Aquaclear 200) to filter water and provide aeration. We used a Rena® (model 400) air pump to provide aeration via a 10-cm airstone; each air pump supplied 4 tanks. We stocked fish according to each tank assignment and began the experiment.

Table 1: Treatment combinations in 76 L tanks for Asian tapeworm experiments.. There were four replicates per treatment.

Exposed	Not Exposed
12 Yaqui chub	12 Yaqui chub
6 Yaqui chub	6 Yaqui chub
12 Yaqui topminnow	12 Yaqui topminnow
6 Yaqui topminnow	6 Yaqui topminnow
6 Yaqui chub & 6 Yaqui topminnow	6 Yaqui chub & 6 Yaqui topminnow

Husbandry

We fed fish at 4% of their biomass in each tank per day; 2% at 07:00, and 2% at 11:00. All waste and excess food vacuumed out at 17:00 with a Python® aquarium cleaner resulting in a 10% water change per day. We fed Yaqui chub pellet (AES® finfish starter) and the Yaqui topminnow flake feeds (AES® tropical fish flakes). Temperature was regulated by the HVAC of the laboratory and fluctuated between 68-71 degrees over the duration of the experiment.

Growth Monitoring

On day 0 and every two weeks subsequently, we anesthetized fish with MS-222 (1:10,000) and recorded the total length (mm) and weight (.01gram). At 124 d post exposure, fish were euthanized with an overdose of MS-222. We recorded final weights and lengths and necropsied the fish.

Data Analysis

We used multifactor ANOVA for analysis completed with the SAS® JMP program. We compared each species independently of each other. Data from tanks containing both species were analyzed by species, not by tank. Growth was measured by subtracting first measurements from final measurements. We measured mortality by counting the number of individuals that died per tank.

RESULTS

Animal Culture

Copepods

The isolation techniques proved successful for culturing copepods. Copepods readily reproduced and within 1 month, several thousand copepods were visible in each pool.

Yaqui topminnow and Yaqui chub

Culture techniques for both species of Yaqui fishes were highly successful. We produced enough fish for the experiment, plus surplus. See detailed results presented in Chapter 4.

Asian tapeworm

Eggs harvested from the Praziquantel treatment proved viable. Eggs began hatching the next day and coracidia, the first life stage, emerged over the next few days.

Effects of Asian Tapeworm on Survival and Growth

We found convincing evidence that Yaqui chub not exposed to Asian tapeworm increased in weight more than Yaqui chub that were exposed (Multifactor ANOVA Partial $F_{1, 24} = 12.79$, $P = 0.0017$)(Figure 2).

We found moderate evidence Yaqui chub not exposed to Asian tapeworm grew longer than Yaqui chub exposed (Multifactor ANOVA Partial $F_{1, 24} = 5.79$, $P = 0.024$)(Figure 3).

Yaqui topminnow showed no evidence of a difference between exposed and non-exposed treatments for weight (Multifactor ANOVA Partial $F_{1, 24} = 0.003$, $P = 0.95$)(Figure 4) or for length (Multifactor ANOVA Partial $F_{1, 24} = 0.089$, $P = 0.77$)(Figure 5).

We found no difference in mortalities between exposed and unexposed Yaqui chub tanks (Multifactor ANOVA Partial $F_{1, 24} = 1.31$, $P = 0.26$). The same was true for exposed and unexposed Yaqui topminnow (Multifactor ANOVA Partial $F_{1, 24} = 0.335$, $P = 0.57$).

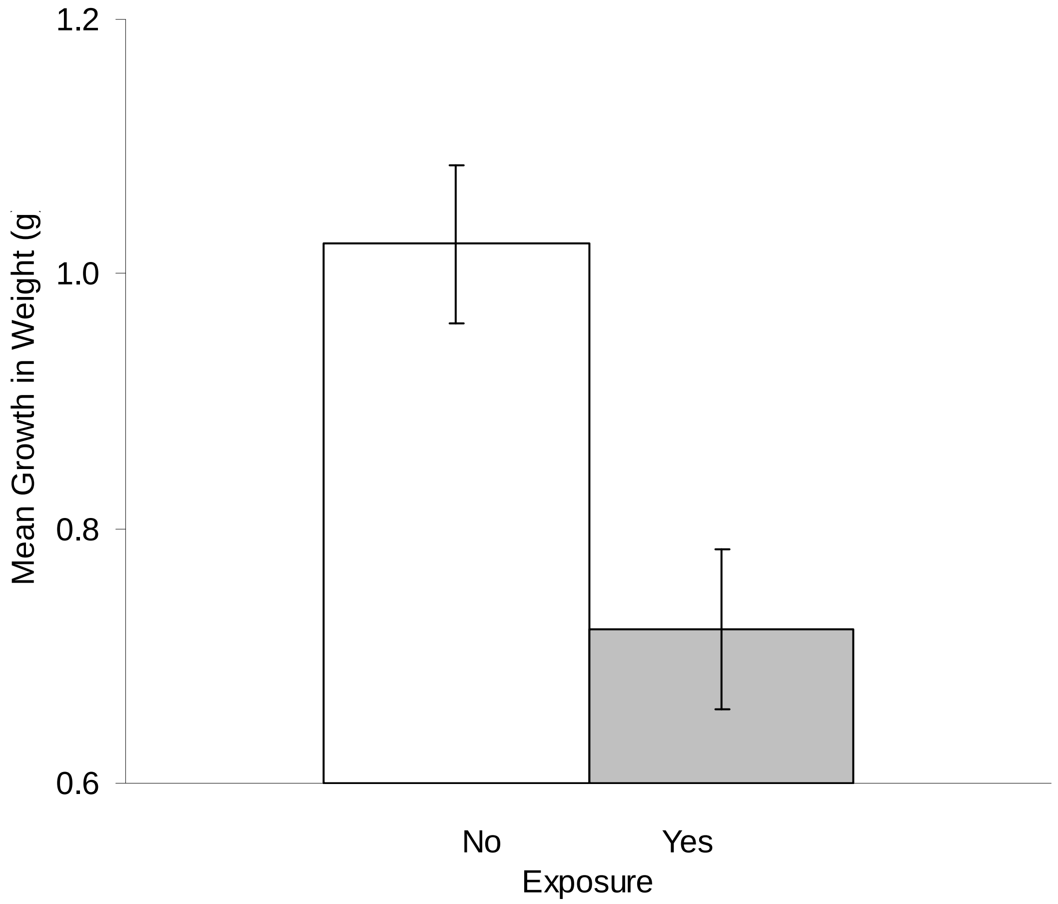

Figure 2: Yaqui chub growth measured as the difference in weight (g) between the start and finish of the experiment. The graph illustrates the significant difference in weight between exposed and unexposed Yaqui chub (Multifactor ANOVA Partial $F_{1,\,24} = 12.79$, $P = 0.0017$).

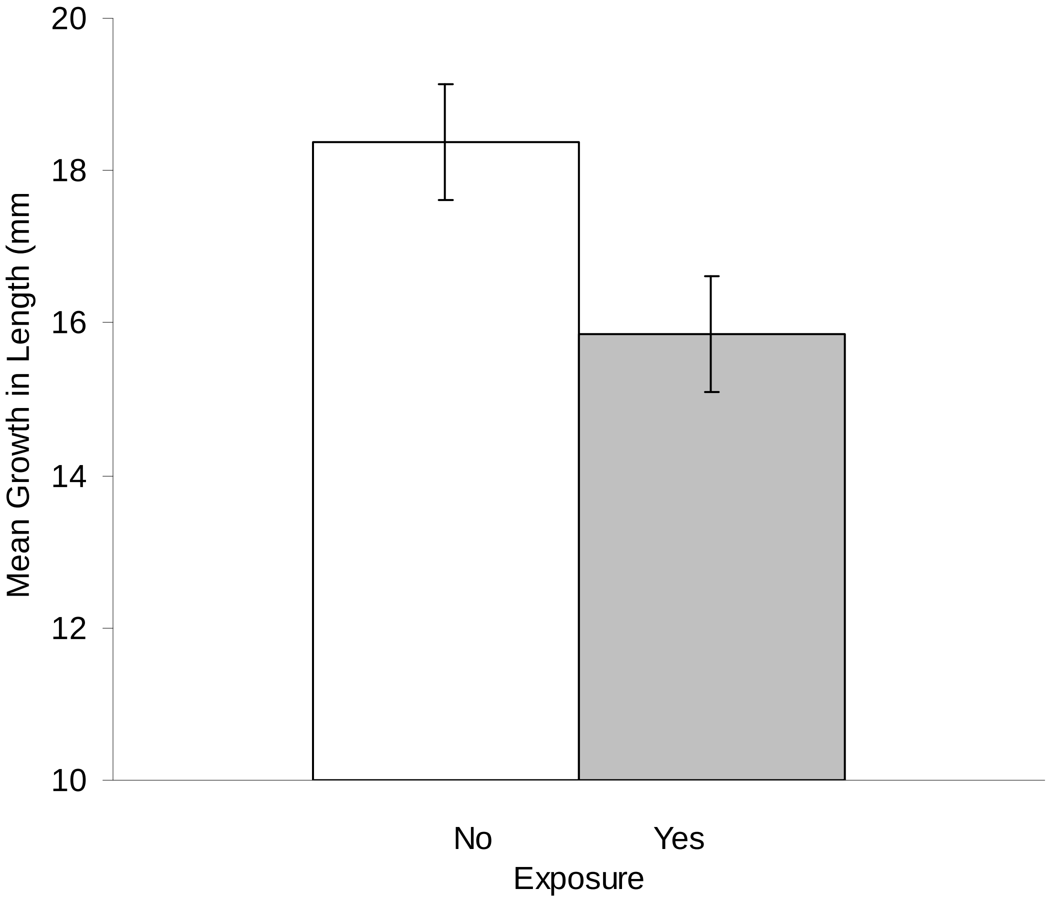

Figure 3: Yaqui chub growth measured as the difference in total length (mm) between the start and finish of the experiment. The graph illustrates the moderately significant difference I length between exposed and unexposed Yaqui chub (Multifactor ANOVA Partial $F_{1,24}$ = 5.79, P = 0.024).

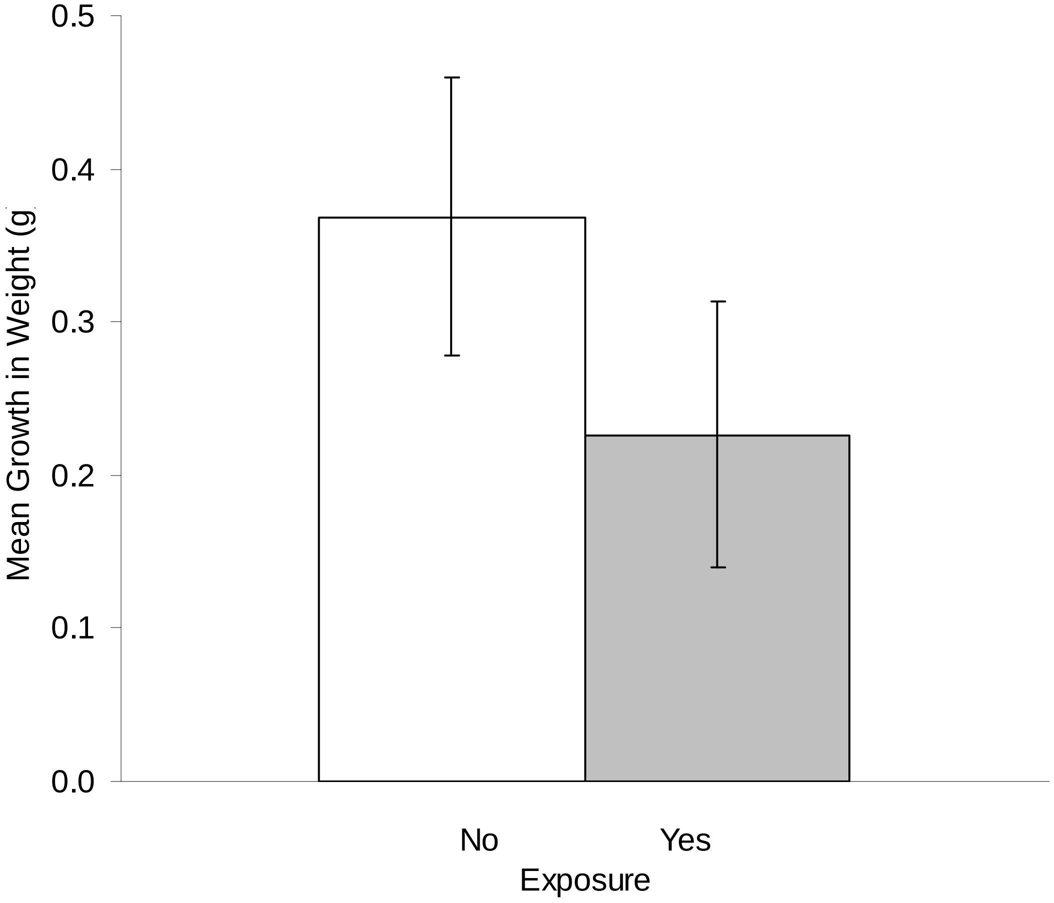

Figure 4: Yaqui topminnow growth measured as the difference in weight (g) between the start and finish of the experiment. The graph illustrates that there is no significant difference in weight between exposed and unexposed Yaqui topminnow (Multifactor ANOVA Partial $F_{1, 24} = 0.003$, $P = 0.95$).

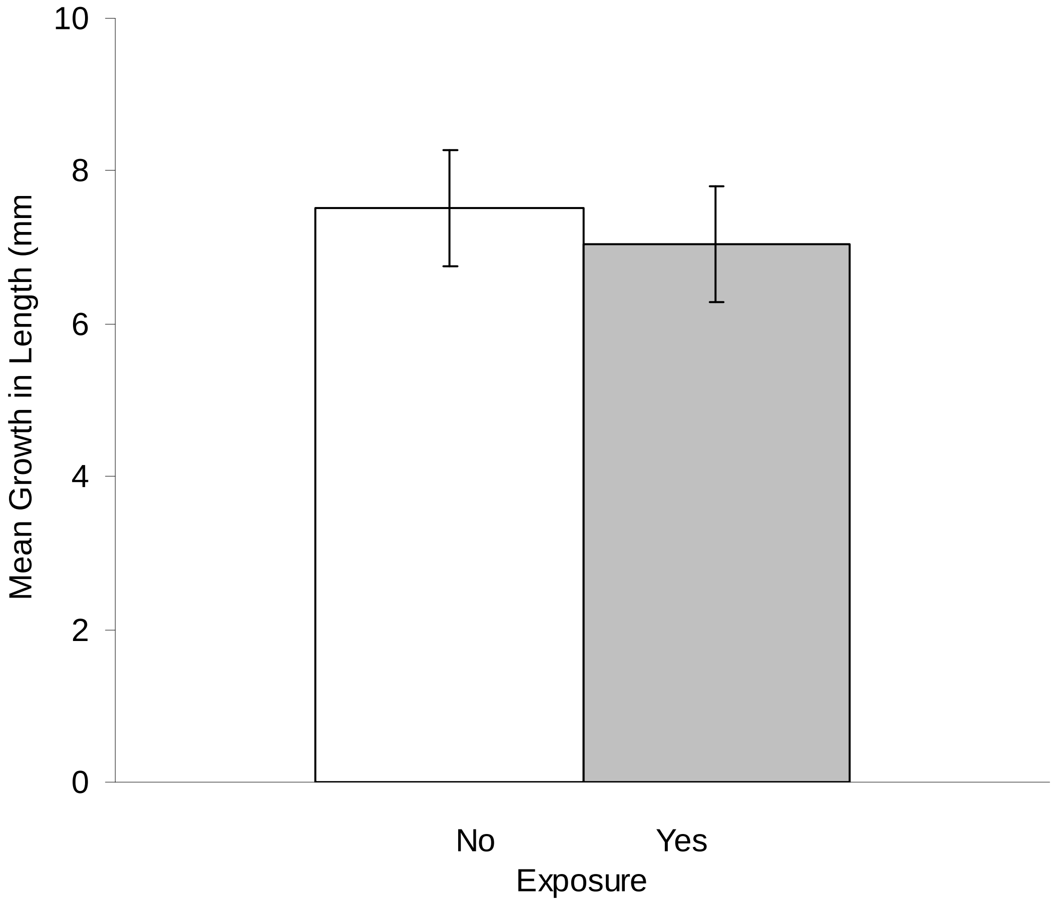

Figure 5: Yaqui topminnow growth measured as the difference in total length (mm) between the start and finish of the experiment. The graph illustrates that there is no significant difference in length between exposed and unexposed Yaqui topminnow (Multifactor ANOVA Partial $F_{1,\,24} = 0.089$, $P = 0.77$).

DISCUSSION

Animal Culture

Pure cultures of copepods proved difficult to maintain. We tried several methods before finding the isolation technique, which was then quite successful. It is crucial to be certain the isolated female is rinsed to be certain no other organisms are in the culture. We tried feeding a commercial diet (Roti-Rich®), with poor results, it was necessary to feed live paramecium to maintain copepod cultures.

After trying several times to obtain gravid adult tapeworms by dissecting thousands of small nonnative fish including red shiner *Cyprinella lutrensis* , fathead minnow *Pimephales promelas*, and mosquitofish *Gambusia affinis* we were unable to obtain enough gravid worms for the experiment. We believe these species may be too small to harbor tapeworm large enough to contain many gravid segments. We used roundtail chub *Gila robusta* from Aravaipa creek and headwater chub *Gila nigra* from Fossil creek as a source of gravid tapeworm. Because these fish grow larger and have the ability to harbor many gravid worms, we suggest that they or other large cyprinids be used as hosts in similar experiments.

It appears as though there is a seasonal effect on the Asian tapeworm lifecycle. We rarely found gravid adult worms in the summer months in Arizona, but frequently found immature worms. In fall, we found fewer worms per fish, but gravid adults were present. Further studies would be beneficial to determine the seasonal dynamics of Asian tapeworm.

Effects of Asian Tapeworm on Survival and Growth

At the end of the experiment, we checked all fish for remaining Asian tapeworm and found none. However, the following evidence strongly suggests that the results obtained were due to infection by Asian tapeworm. We saw highly significant differences in growth among exposed and non-exposed fish. All fish in both exposed and non-exposed groups were handled in the same manner. We held all factors constant and all experimental tanks were kept in the same room. We randomly assigned all fish to treatments and randomly assigned treatments to tanks. Both groups were fed identical feeding regimes including copepods, and prepared foods. The only difference between the treatments was exposure or non-exposure of copepods to coracidia from Asian tapeworm eggs.

So what could have accounted for the absence of Asian tapeworm at the conclusion of the experiment? Possibly, the aquaria provided no opportunity for re-infection and the worms dropped out of the hosts. Asian tapeworm can drop out of the host in just 1 month, if the conditions are not favorable (Anindo Choudhury, St. Norbert's College, personal communication). We fed commercial pellets and flakes and kept fish in sterile tanks, conditions that may have been unfavorable to Asian tapeworm. Our infection technique has never been tried before. Methods from previous experiments involve dissecting host fish to harvest tapeworms, but since we were working with native threatened and endangered fish, we used praziquantel to remove worms to avoid harming the fish. There may be an effect on Asian tapeworm eggs harvested in this manner. We recommend harvesting Asian tapeworm from fish without praziquantel treatment, to verify if there is an effect.

Nevertheless, the infection in our tanks was probably light. We recommend further studies be conducted to try infect fishes with a heavier load of Asian tapeworm, to test the effects of a more pronounced infection.

We are not surprised that the Yaqui chub showed a greater effect when exposed to Asian tapeworm than the Yaqui topminnow. Typically, cyprinids are more susceptible than poeciliids to Asian tapeworm infections. They have larger and longer gastrointestinal (GI) tracts, which give them greater ability to harbor the parasite. Yaqui topminnow have a short, narrow GI tract that is not conducive to tapeworm infection. King (2000) found the percentage of Yaqui topminnow surveyed at SBNWR infected (0 – 17% per survey) was lower than Yaqui chub (53-95%). Ponds only stocked with Yaqui topminnow in SBNWR had no Asian tapeworm, while all ponds that contained Yaqui chub had Asian tapeworm (King 2000).

In summary, even a light infection of Asian tapeworm will significantly affect growth in Yaqui chub. Yaqui topminnow seems little affected by the parasite. Mortality of exposed fish in our tests was not significant. Tests with heavier infections would be necessary to define the upper range of effects of Asian tapeworm on these fishes.

Chapter 2: Precautions for Praziquantel Use for Control of Asian Tapeworm

ABSTRACT

Praziquantel, an anthelmintic, is commonly used to control Asian tapeworm *Bothriocephalus acheilognathi* in grass carp *Ctenopharyngodon idella* and baitfish during transport. It is also used for controlling Asian tapeworm in small ponds. We treated several cyprinid fishes, both native and non-native to Arizona, with praziquantel. At the recommended dosage, Praziquantel killed adult tapeworm, but some tapeworm ruptured and released eggs. Eggs released from Asian tapeworm treated with praziquantel were viable and produced coracidia over several days. Fishery managers should be aware that even if fish receive a typical praziquantel treatment regime and all adult tapeworms are killed, viable eggs and coracidia may be present in the holding water or attached to the skin of treated fish, surfaces of equipment, or to treatment personnel.

INTRODUCTION

The wide distribution of Asian tapeworm across the Southwest, the fact that it infects all Cyprinid fishes and that it does well in warm water make it a serious threat to native fish conservation in Arizona. Concerns that Asian tapeworm will spread further (Choudhury et al. 2006) have made it one of the most regulated warmwater-fish parasites in the United States (Mitchell 2004). These concerns have prompted studies into methods of control and treatment for Asian tapeworm, including the use of anthelmintics such as praziquantel (Droncit®), developed by Bayer Corporation for treatment of tapeworm infestation in humans and animals (Andrews et al. 1983). Fisheries managers and aquaculturists commonly use praziquantel to treat Asian tapeworm infections in fish (Mitchell 2004, Ward 2005). Praziquantel offers an advantage because it allows the removal of Asian tapeworm without harming host fish. We used praziquantel to remove Asian tapeworm from a variety of infected fish, both native and not native to Arizona. We report on the incidence of hatching of eggs produced by adult Asian tapeworm shed during praziquantel treatment.

METHODS

We treated several cyprinid fishes with praziquantel; 20 headwater chub *Gila nigra*, 10 roundtail chub *Gila robusta*, 20 Yaqui chub *Gila* purpurea, 110 Mojave tui chub *Gila bicolor mohavensis* and 20 red shiner *Cyprinella lutrensis* to collect gravid Asian tapeworm. We treated a 1,892-L pool with praziquantel at 6 mg/L, dissolved in 10 mL of 70% isopropyl alcohol and aerated the pool with a Rena 400 air pump (Mitchell 2004, Ward 2005). We held individual fish in 3-L plastic containers anchored to the bottom of the pool with rocks or sandbags. We painted the bottom of each 3-L container black, to see worms more easily, and covered the top of containers with screen to allow treated water to flow through the container but retain any expelled worms. We held fish in the treatment pool for 24 h and checked each container for expelled worms. We selected gravid adult worms and placed them in petri dishes (60 mm x 15 mm) filled with spring water. We examined gravid worms under a binocular microscope (Zeiss Stemi-2000) and teased eggs out of worms by tearing apart segments and expelling eggs into the petri dish (A. Choudhury, St. Norbert College, personal communication). In each trial, we also placed entire worms into petri dishes, without tearing segments, to see if coracidia would hatch even if eggs were not removed from worms. We covered the petri dishes and held them at room temperature (approximately 22° C). We checked dishes daily for coracidia, and poured coracidia off into 250-mL glass culture dishes holding copepods awaiting infection. We then refilled petri dishes with spring water and returned them to incubation.

RESULTS

Although adult Asian tapeworms were killed, eggs and coracidia that hatched from harvested eggs were not killed during treatments at recommended dosages. In every trial, we were able to hatch eggs obtained from worms expelled during praziquantel treatment. Free-swimming coracidia emerged for 4-6 d, with the highest numbers evident on the second day. The hatching rate slowed after the third day. In one trial with *G. nigra*, we found swimming coracidia in water treated with praziquantel even 12 d after collection of adult worms. It is possible these coracidia hatched over the course of several days and probably did not survive for 12 d. Each trial with intact worms produced viable coracidia, potentially from eggs released from dead or dying tapeworms. We saw no ill effects on any of the fish treated with praziquantel.

DISCUSSION

Expelled Asian tapeworm eggs were not killed by praziquantel. Eggs developed into active coracidia, and we assume, that the coracidia could successfully develop into adult Asian tapeworm, although further research would be required to confirm this. Finding praziquantel did not kill Asian tapeworm eggs is of concern because eggs can adhere to treated fish, equipment, or even persons conducting the treatment. Pool (1985) found that adult *B. acheilognathi* contracted immediately when placed in praziquantel and suffered considerable tegumental damage, especially to the neck region. Vacuolization and "bubbling" occurred in the tegument at praziquantel concentrations of 1 mg/L and "bubbles" burst when exposed for greater than 15 min. In mature proglottids, bursting led to the expulsion of eggs. Because eggs are expelled after exposure to praziquantel, we recommend managers using praziquantel to control the spread of Asian tapeworm to conduct procedures that minimize transfer of eggs and coracidia. At minimum, thoroughly rinsing treated fish with fresh water before returning them to the water body and sanitizing, rinsing, and drying all gear would minimize the chances of survival and spread of eggs. We recommend holding fish for 15 d in a quarantine tank, free of copepod intermediate hosts, before returning fish to a water body. Viable eggs hatch within 2-10 d, depending on development of the embryo in the egg, and coracidia do not typically survive more than 72 h (A. Choudhury, St. Norbert's College, personal communication). A 15-d quarantine would allow enough time for all eggs to hatch and resulting coracidia to die. If praziquantel is used to eradicate Asian tapeworm from small systems, such as ornamental ponds, it may be necessary to treat multiple times, because there will be eggs in the substrate, coracidia in the water, and procercoids in infected copepods, even after treatment of the fish. Development of methods to ensure mortality of all life stages of Asian tapeworm or effective interruption of the life cycle will be important to protect uninfected waters.

Chapter 3: Use of Praziquantel to Control Asian Tapeworm in Pond Microcosms

ABSTRACT

The anthelmintic drug, praziquantel, does not kill the eggs, coracidia, or procercoid life stages of Asian tapeworm, making it ineffective at treating fish in systems containing the intermediate host. In microcosms containing the intermediate host, copepods, we tested the effectiveness of using a second application of praziquantel to kill tapeworms that may develop from eggs released during the first treatment. There was no evidence of recruitment in microcosms treated twice for Asian tapeworm. Tapeworm prevalence and intensity in the control tanks remained the same throughout the experiment.

INTRODUCTION

Praziquantel (PZQ; Droncit®, Bayer Corporation) has been used for decades to treat many species of captive fish for Asian tapeworm. Originally developed as an anthelminthic treatment of trematodes and tapeworms in humans and animals (Andrews et al. 1983), PZQ kills a wide range of helminth parasites but fish, amphibians, reptiles, birds and mammals tolerate it well Andrews & Riley 1982). Most recent studies on the effectiveness of PZQ for fish add it directly to the water (Mitchell 2004, Ward 2005). These simple bath treatments are effective, expose all fish to the treatment and avoid handling stress. These studies have shown that with adequate dosage and duration of treatment 100% of Asian tapeworms can be eliminated from captive fish in a safe manner (Mitchell 2004, Ward 2006). In fact, many state agricultural and food departments have mandatory tapeworm eradication procedures using PZQ for the intrastate and interstate movement of live fish (eg. UDAF 1997).

Although no extended studies have been performed, Mitchell (2004) noted no immediate side effects on grass carp after a 72 h exposure to 10 mg/L concentration of PZQ.

No mortality or side effects were observed from PZQ treatments of 36 mg/L of native bonytail chub (Ward 2005). However, in acute toxicity tests on fry of the African sharptooth catfish *Clarias gariepinus*, Obiekezie and Okafor (1995) determined the 24 h lethal concentration of PZQ for 50% of test fish (LC50) to be 13.4 mg/L.

The existing protocols for treating fish using PZQ have only been applied to captive fish in tanks where fish are held for 24 hours in the treatment water and the water is exchanged with clean water before the fish are returned to their natural habitat. The exchange of water is a necessary step because treatment with PZQ does not kill the eggs in the expelled proglottids of the tapeworm (see Chapter 2). If treated in their natural habitat in the presence of the copepod intermediate host, the fish may be re-infected. Because of this, treating an entire pond with PZQ may fail. Existing methods for treating ponds involve removing the fish, treating them in captivity, draining and drying the pond, disinfecting with calcium hydroxide or exposing to winter conditions to destroy the parasite eggs and the intermediate host, and then restocking the pond with tapeworm-free fish (UDAF 1997). These methods are costly and time-consuming, particularly when there are several ponds that are infected. Hoffman (1976) recommended these methods before the availability of PZQ.

Our objective is to determine if it is feasible to treat a system containing both the intermediate copepod and fish hosts for Asian tapeworm with multiple doses of PZQ. An initial treatment would kill any adult tapeworms and the following treatment would kill any tapeworms that develop from the eggs released after the first treatment, before they mature in the fish and start producing eggs themselves. Secondarily, we would like to determine if there is any long-term chronic effects of exposure of fish to PZQ.

METHODS

We used six microcosms, each consisting of a 1,098-liter (290-gallon), round, flat-bottomed fiberglass tanks to simulate natural pools. We equipped each with a trickling biofilter containing bioballs to circulate water and remove ammonia. Artificial macrophytes in each microcosm simulated natural vegetation and provide cover for fish and copepods. Sand and gravel provided habitat for benthic phases of the copepod life history. Water temperature depended on the moderated greenhouse temperature, and varied according to season. To simulate natural ponds, we inoculated each microcosm with water samples obtained from ponds at San Bernardino National Wildlife Refuge, Arizona. We maintained the microcosms for two months prior to fish and copepod stocking to make sure they were operating properly.

To ensure that Asian tapeworm was capable of completing its life cycle, we stocked cultures of the known intermediate copepod host, *Cyclops vernalis*, in all tanks. We added copepods to each tank every other day for the duration of the experiment to guarantee their continued presence. On the days when copepods were not added to the tanks, we fed the fish a supplemental diet of high-quality pellet food to alleviate predation on the copepods.

We chose to use red shiner *Cyprinella lutrensis* as the Asian tapeworm host in the microcosms. Red shiners are known hosts for Asian tapeworm, they are non-native, numerous, and ubiquitous in southern Arizona and many populations are already infected with Asian tapeworm. Using a hand seine, we caught ~400 red shiners from the confluence of the Verde River and the Salt River, Arizona. We measured the total length of the red shiners before randomly assigning them to the six microcosms and a seventh group, which was dissected at the start of the experiment to assess the initial infection rate. It is important that fish were randomly assigned to tanks because smaller fish tend to show higher rates of

Asian tapeworm infection in the wild (Brouder 1999). Fish were left in the microcosms for 1 week before the first PZQ dose. Any fish mortalities that occurred before the first PZQ dose were replaced with additional fish.

We checked water temperature and water quality weekly. Any fish mortalities were measured for total length (mm), and examined for Asian tapeworm and other diseases or parasites. The experiment was conducted at the end of the summer when greenhouse temperatures were above 20ºC, because Asian tapeworm eggs develop slowly under 20ºC, delaying their maturation into coracidia (Hanzelova & Zitnan 1987). Below 20ºC, tapeworms also take longer to develop into segmented worms (Oskinis 1994). At warmer temperatures, maturation of the adult tapeworm in fish and egg maturation and hatching are stimulated, and recruitment of coracidia by copepods is enhanced (Granath & Esch 1983a, Hanzelova & Zitnan 1987). Thus, performing the experiment at temperatures > 20^{o}C ensures that no "hibernating" tapeworm eggs will endure the treatments. However, because development of eggs, procercoids and juvenile tapeworms is enhanced in warm water, a repeated dose is necessary to prevent interim egg production.

We randomly chose three of the tanks and added PZQ (Prazipond^{TM} by AquaScience®) at a concentration of 2.5mg/L. After 19 days, we repeated the treatment to kill any tapeworms that might have developed from the eggs released after the first dose. We based the time interval of 19 days between treatments from previous studies on the rate of tapeworm development in warm water (~25ºC; Odkinis 1994). Because PZQ is only slightly soluble in water, we first dissolved it in 5ml of 70% isopropyl alcohol before being added to the tanks. The other three tanks served as controls and were not treated with PZQ. After the treatment cycle, we left the microcosms for 2.5 months, giving time for any remaining tapeworms to reestablish. Finally, we sacrificed the fish, measured total length, and examined the intestines for Asian tapeworm. Following necropsy, we preserved the fish in 70% isopropyl alcohol solution.

To analyze the data, we first calculated the prevalence (the percent of fish that were infected) and intensity (the mean number of tapeworms per fish that were infected) of Asian tapeworm infection in each tank. We used ANOVA to determine if there was a significant difference between the control group and the treatment group in the prevalence and intensity of *B. acheilognathi* infection. ANOVA was also used to determine if there were any significant differences between the initial and final total lengths of the two groups.

RESULTS

There was no significant difference in initial red shiner total length among the six microcosms or the seventh group that was assessed for the initial tapeworm infection rates in the original population (Table 2; ANOVA $F_{2, 4} = 2.60$, $P = 0.19$). After 3 months in the PZQ water at the end of the experiment, there was still no significant difference in mean total length between the treatment and control groups (Table 2; ANOVA $F_{1, 4} = 0.20$, $P = 0.68$).

There was a highly significant difference between control and treatment microcosms in the percent prevalence of Asian tapeworm (Table 2; ANOVA $F_{1, 4} = 27$, $P = 0.01$).

There were no significant differences in percent prevalence (Table 2; ANOVA $F_{1, 2} = 0.33$, $P = 0.62$) or mean intensity (Table 2; ANOVA $F_{1, 2} = 0.29$, $P = 0.65$) of Asian tapeworm infection between the control groups at the end of the experiment and the group dissected initially to determine the initial infection rates.

Although there was a temperature gradient across the greenhouse where the microcosms were held, there was no significant difference in water temperature between the treatment and control groups since the treatments were randomly assigned (Table 2; ANOVA $F_{1,4} = 0.96$, $P = 0.38$).

All fish survived the experiment except for 16 mortalities in one of the treatment tanks. These mortalities were detected early in the experiment and occurred at the same time as an increase in ammonia in this one tank alone. We subsequently added oxidizing bacteria to all tanks to help break down ammonia and nitrite (Cycle^{TM} by NUTRIFIN®) and no further mortality was detected.

DISCUSSION

The treatment of Asian tapeworm with PZQ in the presence of the tapeworm's intermediate host, cyclopoid copepods, proved effective. There was no evidence of tapeworm recruitment in the treatment tanks since no tapeworms were found; however, recruitment was occurring in the control tanks. The following evidence led us to this conclusion: Tapeworm prevalence and intensity in the control tanks remained the same throughout the experiment. If recruitment was not occurring we would have expected the prevalence and intensity to decline due to the natural maturity and senescence of the adult tapeworms. Furthermore, during the final dissection, we noted many immature, non-segmented tapeworms that were little more than just scolexes. Marcogliese & Esch (1989) considered such small tapeworms to be recently recruited (within the last 9 days).

Although our results are encouraging, much further research is necessary before implementing the use of PZQ in the field. In particular, the chronic, physiological effects of PZQ exposure on all aquatic organisms, the eco-toxicity of PZQ, and its effectiveness against other Asian tapeworm life-stages needs to be determined. Any negative effects of the PZQ treatment would need to be weighed against the negative effects of not treating at all.

No long-term effects on the red shiners in the present study were noted and no deaths could be attributed to the PZQ treatment; however, we recommend that a more thorough examination of the chronic, physiological effects of PZQ on all life-stages of all fishes to be treated. Although a few studies have looked at the pharmacokinetics of PZQ in orally administered fish (Rogstad et al. 1993, Tubbs & Tingle 2006), no studies on the bioavailability and tissue concentration of PZQ have been done for bath treated fish. This is particularly important for bath treatments in which the water is not exchanged, as the fish would be exposed to the drug until it degrades or sediments out of the water column.

Very little is known about the degradation products of PZQ, but they are not likely to be hazardous (Andrews et al. 1983). No studies have been performed on the eco-toxicity of PZQ or its rate of biodegradation. Because of the broad-spectrum activity of PZQ against many parasitic trematodes and cestodes (Andrews et al. 1983), we advise caution when administering it to natural ecosystems, as it is likely that it would affect native Platyhelminths.

We also recommend testing the effectiveness of PZQ on other life-stages of Asian tapeworm. Moser et al. (1986) found that the activity of procercoids of the parasitic trematode, *Nybelinia sp.*, in its intermediate host, the marine copepod *Tigriopus californicus,* was not affected by PZQ. This is important because copepods can go into diapause during unfavorable conditions, yet remain infected by Asian tapeworm procercoids (Riggs & Esch 1987). If the procercoid stage of Asian tapeworm is not susceptible to PZQ, it may persist through the treatment period and infect fish after the copepods emerge from diapause.

Finally, the minimum effective dose would need to be determined before using PZQ in the field, since we only tested its effectiveness at 2.5 mg/L. Although Ward (2005) found a few live tapeworms after treating bonytail chub *Gila elegans* for 24 h at 36 mg/L, such a high dose may not be necessary if the water is not exchanged and the fish are exposed until the PZQ degrades or sediments out of the water column.

Table 2: Summary of the prevalence and mean intensity of infection of Asian tapeworm (95% confidence interval) in all tank microcosms as well as the group dissected to assess the initial prevalence and intensity. The average water temperature for the duration of the experiment and the initial and final total lengths of the fish are listed as well.

Tank	**Prevalence (%)**	**Mean Intensity**	**Water Temp (ºC)**	**Initial TL (mm)**	**Final TL (mm)**
Initial	14 (0.00-39.81)	2.14 (1.14-4.00)	NA	51.3 (50.1-52.5)	NA
Control	18 (11.20-24.80)	3.45 (1.05-5.86)	21.0 (19.2-22.7)	50.0 (49.1-50.9)	53.5 (50.7-56.3)
Treatment	0 (0-6.8)	NA	20.1 (18.4-21.9)	49.9 (49.0-50.8)	54.1 (51.4-56.9)

Chapter 4: Laboratory Spawning and Rearing of Yaqui Chub and Yaqui Topminnow

ABSTRACT

Development of methods to spawn and rear threatened and endangered fish is necessary for their conservation. We report methods to propagate and rear Yaqui chub and Yaqui topminnow, two endangered fishes from the Rio Yaqui basin of northern Sonora, Mexico and southeastern Arizona, USA. We held mature Yaqui topminnow (2 males and 6 females) in 556-L plastic wading pools housed in a greenhouse with an evaporative cooler. We added cobble, gravel, and artificial plants to pools and installed a vertical mesh barrier impassible to larger fish to create refuge for offspring. Water in pools increased from 19° C as the ambient temperature increased. Yaqui topminnow produced young after 1 month when the temperature exceeded 21° C. Numbers of offspring were variable but reproduction was continuous after the temperature exceeded 21 C. On average, 7.4 offspring were captured each day from the four pools collectively. We spawned Yaqui chub in four 189-L glass aquaria stocked with 6-9 fish per tank. We chilled water to 17° C for 30 d and then increased the temperature to 21° C over 14 d. After the temperature was 21° C for 3 d, we covered the bottom of aquaria with glazed ceramic tiles and a raised plastic grid over the tiles to protect eggs from predation. We found Yaqui chub to be broadcast spawners. They spawned at night, and we retrieved tiles containing eggs the following morning and placed them in incubation tanks. Yaqui chub eggs hatched in the next 5 d with a success rate of 83%. Both methods worked well and we recommend them to propagate these species.

INTRODUCTION

Less is known about laboratory breeding of threatened and endangered fishes than those raised for bait and sport. As of 1999, spawning modes were known only for 13 of 43 imperiled minnows in North America (Johnston 1999). Almost nothing is known about the spawning of Yaqui chub *Gila purpurea* and Yaqui topminnow *Poeciliopsis occidentalis sonoriensis,* two federally endangered fish endemic to the northern Rio Yaqui basin in Arizona and Mexico.

The Yaqui topminnow is a small poeciliid; males rarely grow larger than 25.0 mm TL and females rarely grow larger than 50.0 mm TL (Minckley 1973). The Yaqui topminnow is found in the Rio Yaqui basin only within the San Bernardino National Wildlife Refuge (SBNWR) in Arizona (AGFD 2001b). It was listed as federally endangered in 1967, and endangered by the state of Arizona in 1988 (AGFD 2001b). Males are distinguished from females by their gonadopodium and dark coloration exhibited in breeding season.

Artificial propagation may be required for survival of Yaqui chub (DeMarais and Minckley 1993). The Yaqui chub is a cyprinid that grows to 13.0 cm TL (Rinne and Minckley 1991). Its range in Arizona is restricted primarily to artesian ponds in the San Bernardino and Leslie Canyon National Wildlife Refuge, and El Coronado Ranch (Turkey Creek and ponds) in the extreme southeastern part of the state (AGFD 2001a). It was listed as federally endangered in 1984, and endangered by the state of Arizona in 1988 (AGFD 2001a).

Both species are threatened by loss of cienegas through groundwater pumping and land use changes; and by introductions of non-native species, including fish, frogs and parasites (Bagley 1991; DeMarais and Minckley 1993). Our objective was to develop laboratory methods to propagate these two endangered fishes to aid in conservation efforts.

METHODS

Yaqui Topminnow

We used unbaited minnow traps to collect Yaqui topminnow broodstock from the SBNWR (USFWS Permit #TE676811-0). Yaqui topminnow were transported to the laboratory in coolers fitted with a battery-operated aerator. A water conditioner (Stresscoat®) was added to the transport water. We held fish in recirculating 76-L aquaria filled with well water conditioned with Amquel plus® (Kordan Aquarium Products). Water was filtered with a power filter (Aquaclear™ 200) and tanks were aerated with a Rena (model 200) air pump and a 2.5-cm airstone. Yaqui topminnow were prophylactically treated for *Ichthyophthirius* on the second day with Quickcure® (Aquarium Products Inc.). The third day, we began feeding fish a combination of ¾ tropical flakes [Aquatic Ecosystems (AES®)] and ¼ frozen bloodworms (Hikari Aquatic Diets), both high in protein. Transport and acclimation methods were adapted from Widmer et al. (2005). Fish held in aquaria produced insufficient numbers of offspring, so we designed larger propagation pools. We used large plastic wading pools (556 L), each fitted with a 20-L bucket biofilter and recirculating pump (Rio 1100) as designed by Widmer et al. (2005) (Fig 1). We aerated pools with a Rena 400 air pump connected to 5-cm air stones. For structure, we added four plastic 20-cm plants (*Ceratophyllum sp*.) and five to seven pieces of cobble (20 cm diameter). We placed a plastic rigid panel with 0.32 cm mesh across one quarter of the pool to provide refuge for offspring produced. The mesh panel and plastic plants were anchored in place by 2-6 mm gravel piled along their bases. We stocked pools with adult fish (2 males and 5-7 females per pool). We housed the pools in a greenhouse with an evaporative cooler, held at approximately 25° C. We placed shade cloth over the greenhouse and stretched a 1.82 x 2.43 m tarp over the pools inside the greenhouse.

Yaqui Chub

We used hoop nets to capture adult Yaqui chub broodstock from Tennis Court Pond at the El Coronado Ranch in southeastern Arizona (USFWS Permit #TE676811-0). We used the same transport and acclimation methods used for Yaqui topminnow mentioned previously. We housed the fish in 189-L recirculating aquaria with two power filters (Aquaclear®500) and a Rena (model 400) air pump attached to two 2.5-cm airstones to aerate tanks. We stocked the tanks with 6-9 adult fish, sex was unknown as Yaqui chub are not sexual dimorphic unless in spawning condition. Male Yaqui chub exhibit a distinctive bluish sheen over their body when in mating condition (Minckley 1973). We fed fish a combination of ½ AES high protein finfish starter pellets® (50% protein) and ½ frozen bloodworms and mysis shrimp (Hikari) because a high protein diet is thought to aid in the propagation of *Gila* species (Andrew Schultz, University of Arizona, personal communication).

Yaqui chub exhibited nervous behavior when in bare aquaria, hitting corners and trying to leap from tanks. We added two terra cotta flowerpots with an access hole cut into each and anchored two artificial plants (*Ceratophyllum* sp.) to the pots for cover. Behavior and feeding of the fish improved; however; we observed no breeding behavior or coloration. During winter, we allowed tanks to cool to approximately 17° C for 30 d. We then added a 200-W tank heater (Ebo-jager) to each tank, and gradually increased the temperature to 21° C over 30 d. We lined the bottoms of tanks with 10 x 10 cm ceramic tile and covered the tiles with “egg-crate” light diffusers used for fluorescent lighting fixtures in drop ceilings (Andrew Schultz and Scott Bonar, University of Arizona, personal communication) (Fig. 2). The width of the spaces in the “egg-crate” diffusers (1.2 cm) was small enough to prevent predation by adult Yaqui chub on newly laid eggs on tiles. After fish spawned, we moved tiles containing eggs to an incubation tank. The incubation tank consisted of a 75-L aquarium (long model) with a plastic-coated metal rack to hold tiles vertically, and a 200-W heater (Ebo-jager) to hold the temperature at 21° C. We used an Aquaclear® 200 power filter fitted with a FilterMax II pre-filter (Aquarium Technologies Inc. Decatur, GA) to keep larval fish out of the filter and to provide water movement and filtration (Fig 3). Incubation tanks must be kept clean and eggs well aerated to inhibit growth of fungus on eggs. After swim-up, fry were moved to rearing tanks that were 38-L aquaria with a biofilter (Aquaclear 200) fitted with a FilterMax II pre-filter over the intake to protect fry from the filter. Fry are delicate and gently sucking them into a turkey baster was the best method of transporting fry from incubation tanks to rearing tanks. Even specially designed soft nets proved too stressful for fry. As Yaqui chub grew, we separated them into larger tanks with fewer fish.

We kept all fish in accordance with protocols approved by the University of Arizona’s Institutional Animal Care and Use Committee.

RESULTS

Yaqui topminnow

Once acclimated to propagation pools, Yaqui topminnow produced large numbers of offspring. Within 1 month, offspring were visible in the pools, often within the screened refuge. We netted young out daily and placed them in separate pools with the same filtration and aeration set-up. We fed the young crushed tropical flake food (AES®), a 45% protein diet. The numbers of young produced were highly variable, the number captured from four pools in one day ranged from 0 to 71 offspring (Table 1). We recorded a daily average from four pools of 7.4 offspring. It was not possible to see and remove all juvenile Yaqui topminnow every day, so some juveniles collected on a given day may have been produced on previous days.

Yaqui chub

Once Yaqui chub were acclimated to the spawning set-up, we placed tiles in the aquarium in the afternoon and Yaqui chub usually spawned by the following morning. Every spawn occurred between 22:00 and 06:00 the following morning. Eggs were an average of 1.76 mm diameter (n = 15) and adhered to the tiles. Yaqui chub produced an average of 378 eggs per spawn (range = 150-734 eggs per spawn) (Table 3). Fertilized eggs were a yellowish color and unfertilized eggs remained clear. Eggs hatched starting the fourth day and fry remained at the bottom of the tank for 24-48 h, while fry absorbed the yolk sac. Fry averaged 4.55 mm (n = 5, range = 4.1- 4.9 mm) on day 1and 6.5 mm (n = 10, range 6.0 - 6.9 mm) on day 3. We observed swim up fry after 5 d and we moved them to rearing tanks (38 L aquaria) after 7 d. The average hatch rate for seven spawns was 83.13 % (Table 1). We fed fry First Bites food (Hikari®) and frozen baby brine shrimp (Hikari®) for the first 10 days and increased the food size as the fry grew. After 10 d, we fed AES high protein finfish starter food, starting with the meal (0.4-0.6 mm) for approximately 1 week, then the #1 crumble (0.6-0.85 mm) for another 2 weeks, then #2 crumble (0.85-1.2 mm) for an additional 2 weeks. After this point, Yaqui chub were fed the pellet size (1.5 mm) food and frozen bloodworms (Hikari®). We raised fry to approximately 25 mm TL, the size needed for another experiment.

DISCUSSION

Yaqui Topminnow

Yaqui topminnows are livebearers, with the ability to store sperm and carry 2 broods totaling 6 to 49 young at once (Minckley 1973). We thought that fish with these characteristics would require only food, cover and the proper temperature to breed. However, we found that topminnow held in aquaria cannibalized most of the offspring produced, even with cover and surplus food provided. We believe conditions were too crowded in the 76-L aquaria so we designed the 556-L propagation pools and achieved desired results. It would be interesting to test if a maximum number of fish can be produced in a pool of a given size and if the number increases as the size of the pool increases.

Temperature seemed to play the greatest role in triggering spawning. In nature, Yaqui topminnow are thought to propagate from January through August (Minckley 1973), but our fish reproduced at 21° C in the laboratory, and did not reproduce in the greenhouse until the temperature exceeded 21° C. First breeding in the greenhouse occurred 1 month after fish were introduced. The delay in breeding may have been due to the stress of translocation and acclimation to captive conditions, but we feel it was lower temperatures in the greenhouse that delayed production of offspring. We recommend further research to find the precise breeding temperature requirements of Yaqui topminnow to make our pool method even more successful for producing Yaqui topminnow.

Yaqui Chub

Yaqui chub proved more difficult to induce to breed. There is little literature available relating to the mating of Yaqui chub or similar species. In preliminary trials, we altered several factors to induce spawning including changing light cycles, temperature, feed types and spawning substrate. Ultimately, temperature had the greatest effect on the spawning of the Yaqui chub. Until we manipulated the water temperature for 30 d, we observed no breeding behavior. We were able to mimic the method used by Schultz and Bonar (unpublished) at the University of Arizona to propagate Gila chub *Gila intermedia*, a closely related species. The variation in the numbers of eggs produced per spawn may have been due to variation in the number of females spawning. It was not possible to definitively distinguish males from females; therefore, the ratio of males to females was unknown. We kept light cycles at 12 h light:12 h dark and all other factors were held constant throughout the entire period. The terra cotta pots and plastic plants in the tanks were necessary so the Yaqui chub could hide in them and spawn over them. However, these structures did not seem to influence where fish spawned, as we found eggs throughout the bottom of the tank. We found Yaqui chub to be broadcast spawners, as many other species in the *Gila* clade are thought to be (Johnston 1999). The tiles were necessary as a substrate for adherence of eggs and the grating protected eggs from predation by adults. Yaqui chub will consume eggs, as demonstrated when a small individual swam under the grating and was seen eating eggs.

Fry should be fed three times a day, especially if they are kept in crowded conditions. Growth of fish was dependent on stocking density, feed rates, and temperature. We recommend use of the methods shown here to propagate and rear Yaqui chub and Yaqui topminnow in laboratory settings. These methods alone cannot ensure conservation of these species, but laboratory propagation may help secure individuals for future re-introductions of these Yaqui fishes within their native range.

Table 3: Number of Yaqui topminnow offspring removed from four 556 L tanks, June 2005. Each pool was stocked with 2 adult males and 5-7 adult females.

Date	Total # Offspring	Date	# Offspring	Date	# Offspring
6/1	9	6/10	9	6/19	0
6/2	8	6/11	16	6/20	0
6/3	2	6/12	43	6/21	6
6/4	11	6/13	16	6/22	71
6/5	18	6/14	13	6/23	40
6/6	23	6/15	15	6/24	0
6/7	13	6/16	20	6/25	44
6/8	7	6/17	8	6/27	33
6/9	8	6/18	0	6/29	36

Table 4: Production of Yaqui chub in laboratory aquaria, 2005. The average hatching success rate was 83.13%. Eggs began hatching after 4 days and took up to 6 days to hatch.

Date Tiles Laid	Spawn Date	Number of Eggs	Hatch Date	Hatch Percentage	# of Fry
03/21	03/22	387	03/26	82.4 %	319
04/06	04/07	150	04/12	89.3 %	134
04/19	04/20	195	04/25	86.2 %	168
05/11	05/15	619	05/19	76.1 %	471
06/14	06/17	734	06/20	79.0 %	581
06/22	06/23	169	06/27	84.6 %	143
10/03	10/04	392	10/09	84.3 %	329

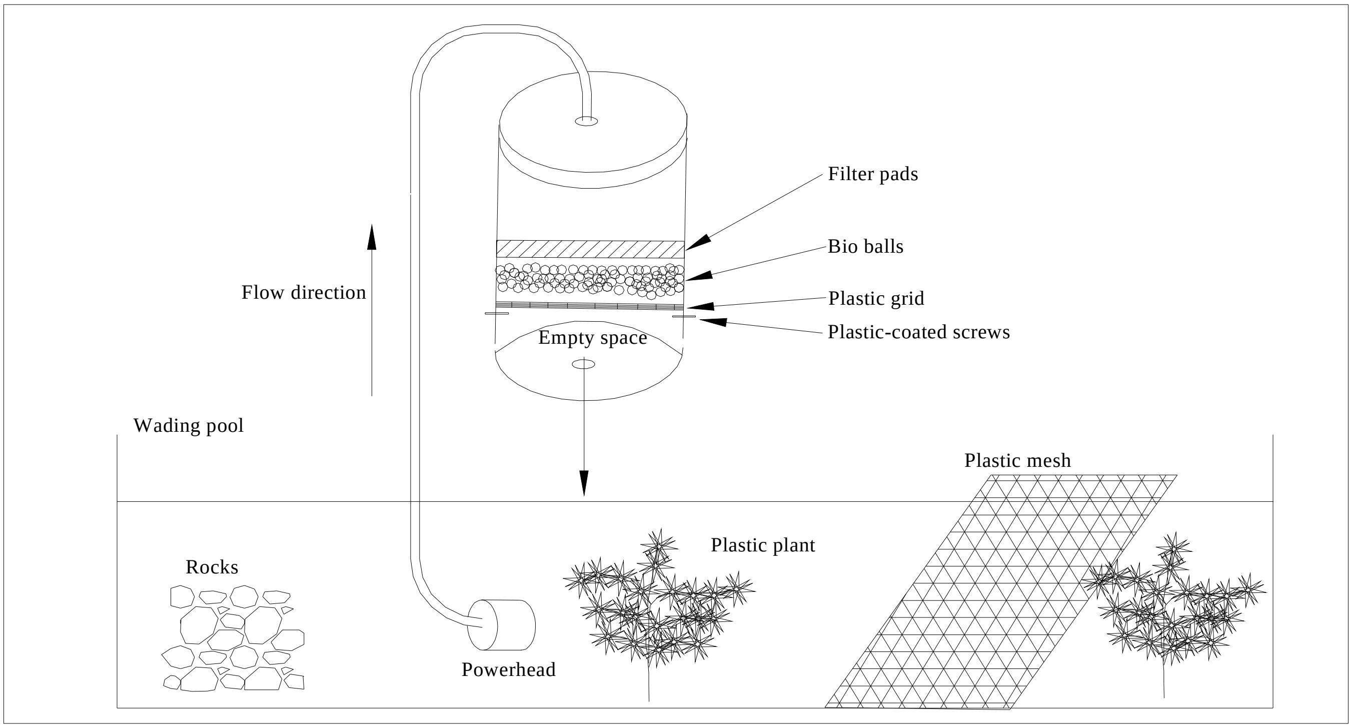

Figure 6: Yaqui topminnow propagation pool made from a 556-L plastic wading pool. The biofilter is homemade from a 20-L plastic bucket, a Rio® powerhead, bioballs and plastic grating. The plastic mesh is a 0.32 cm rigid net to provide refuge for offspring. The mesh and plants are held in place by gravel poured around the base and the top of the mesh is secured to the edge of the pool. The entire pool can be constructed for approximately $100.

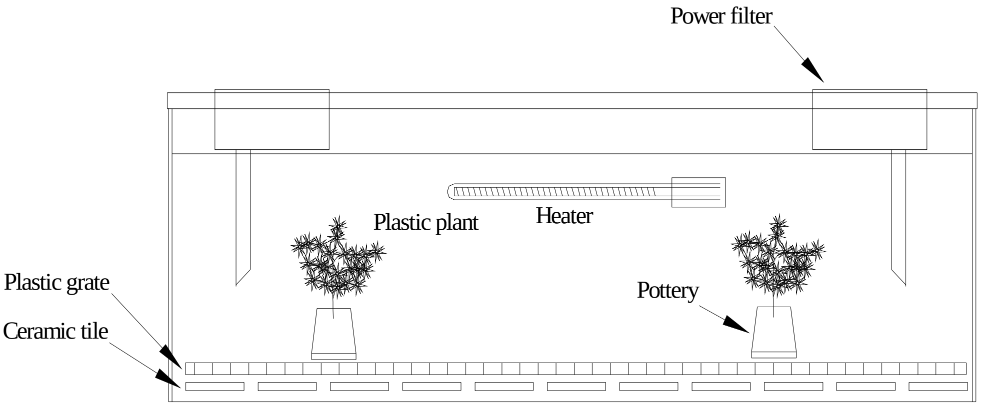

Figure 7: Propagation tank for Yaqui chub- The 189 L aquaria with bottom lined with 10cm x 10cm ceramic tiles, which are protected by a raised plastic “egg crate” grating. The pottery and plastic plants provide cover for the Yaqui chub.

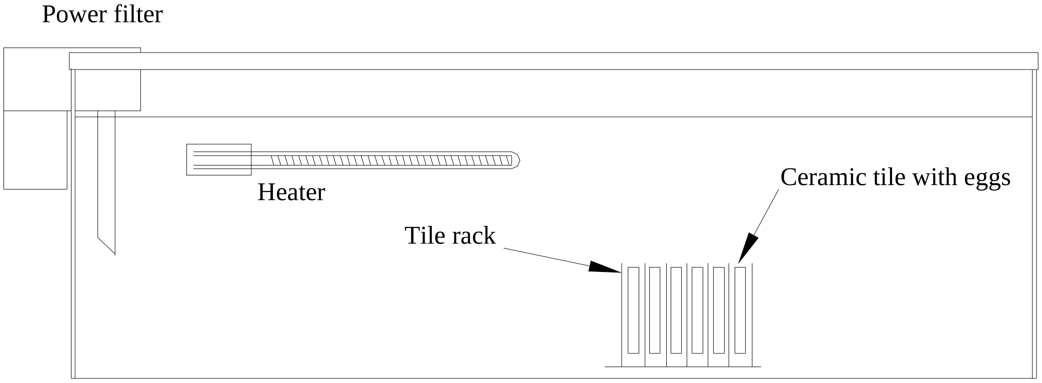

Figure 8: Incubation Tank**-** The tank is a 75 l long tank with a heater to maintain 21°C temperature. The tiles are placed in a rack to keep them horizontal; a powerfilter provides water movement and filtration.

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