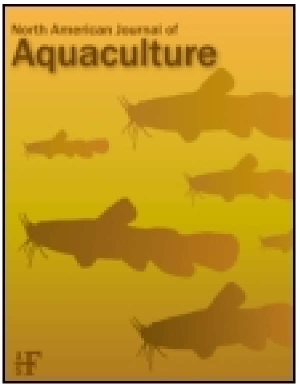

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[Article]

Effects of Praziquantel on Eggs of the Asian Tapeworm *Bothriocephalus acheilognathi*

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Abstract.—Praziquantel, an anthelmintic, is commonly used to control the Asian fish tapeworm *Bothriocephalus acheilognathi* in grass carp *Ctenopharyngodon idella* and baitfish. We treated 50 individuals from three different cyprinid fish species with praziquantel at 6 mg/L, a dose higher than the minimum recommended for complete Asian tapeworm removal in 24 h but much less than the concentration that would have ill effects on the fish. Praziquantel killed hundreds of adult tapeworms, but many ruptured and released eggs. We observed that the eggs released from Asian tapeworms treated with praziquantel were viable and produced thousands of coracidia over several days. We warn fishery managers that even if fish receive the typically recommended 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, the surfaces of equipment, or treatment personnel.

The Asian tapeworm *Bothriocephalus acheilognathi* is a parasite native to Asia. It is responsible for mortalities of fishes, particularly cyprinids, in its native range. Asian tapeworm has spread rapidly to 102 host species in 14 families and 7 orders of freshwater fishes worldwide (Salgado-Maldonado and Pineda-López 2003) and is found on all continents except Antarctica (Korting 1975; Boomker et al. 1980; Andrews et al. 1981; Heckmann et al. 1987; Font and Tate 1994; Dove et al. 1997). It is believed that Asian tapeworm entered the United States through introductions of grass carp *Ctenopharyngodon idella* in the 1960s (Stevenson 1965; Hoffman 1976). Asian tapeworm is a generalist whose intermediate hosts are cyclopoid copepods and final hosts are a wide range of fishes (Salgado-Maldonado and Pineda-López 2003) including many endangered species (e.g., *Gila* and *Poeciliopsis* spp.). Asian tapeworm was once thought to be limited to warm waters, but the presence of reproducing populations of this parasite in northern U.S. and Canadian fishes (Choudhury et al. 2006) suggests it can live in a wide variety of temperatures.

The lifecycle of Asian tapeworm occurs in four stages: egg, coracidia, procercoid, and adult. Adult worms shed eggs that are evacuated in the feces from the final host, usually a cyprinid fish. Eggs settle to the sediment and hatch over 3–5 d. Actual development time is dependent on water temperature; the warmer the water, the faster the development. Free-swimming coracidia hatch from the eggs. Coracidia are round, have three pairs of embryonic hooks, and are covered in cilia used to swim up into the water column. When the coracidia is swallowed by the intermediate host, a cyclopoid copepod, it perforates the intestine and develops into a procercoid. Procercoids are oblong and their posterior is differentiated into a cercomere with three 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, which is dependent on water temperature (Bauer et al. 1973; Korting 1975). It is thought that the procercoid can actually alter the behavior of a copepod host, causing it to swim erratically near the surface, where it will be more likely to be preyed upon by the final fish host. If a fish digests a copepod, the procercoid attaches to the intestine of the fish with the embryonic hooks. The procercoid then transforms into a tapeworm and within 20–25 d the worm develops into an adult and begins producing eggs. Again, the rate of development is temperature dependent. The entire lifecycle can be completed in 18 d if temperatures are optimal (Bauer et al. 1973).

Asian tapeworms have caused up to 90% mortality in grass carp in newly infected ponds (Bauer et al. 1973) and accelerated mortality of bonytail *Gila elegans* under low food conditions in the laboratory (Hansen et al. 2006). They have reduced growth in roundtail chub *G. robusta* in the Little Colorado River (Brouder 1999) and bonytail under laboratory conditions (Hansen et al. 2006). This, combined with its

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wide geographic range, suggests it may threaten native fish conservation. Concerns that Asian tapeworm will spread farther (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 2007; Mitchell and Darwish 2009). Praziquantel offers an advantage over other control methods because it allows the removal of Asian tapeworm without harming the host fish.

When conducting other experiments, we used praziquantel to remove Asian tapeworm from three species of infected cyprinids, including one that was a candidate for listing with the U.S. Endangered Species Act. We observed that adult Asian tapeworms were shed during the treatment, but eggs from the tapeworms subsequently hatched and produced tens of thousands of viable coracidia. Our goal was to report this observation to warn workers who use praziquantel during the transport of fish, and encourage studies that examine the issue in more detail, with the eventual goal of developing treatment protocols that consider both adult tapeworm and immature forms.

Methods

We collected a total of 50 individuals from three species of cyprinid fishes infected with Asian tapeworms in the southwestern United States (Table 1). One of these fishes, the headwater chub *G. nigra*, was a candidate for listing under the U.S. Endangered Species Act. All fish for this experiment were collected between April and November of 2005. Fish were treated for Asian tapeworm at the time of collection using the following protocol, and only one species was treated at a time. We treated a 1,892-L pool with praziquantel at 6 mg/L, dissolved in 10 mL of 70% isopropyl alcohol for 24 h. Water in the pool for all tests was from a local well, and had a pH of 7.9, alkalinity of 175 mg/L $CaCO_3$, total dissolved solids of 420 mg/L, nitrate as N of 1.2 mg/L, sodium of 60 mg/L, and ambient water temperature of 21–23°C. We held individual fish in 3-L plastic containers anchored to the bottom of the pool with rocks or sandbags, and aerated the pool with a Rena 400 air pump. We painted the bottom of each 3-L container black to see worms more easily, and covered the top of the containers with screens to allow treated water to flow through the containers 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 × 15 mm) filled with spring water. We examined gravid worms under a binocular microscope (Zeiss Stemi-2000) and teased eggs out of the worms by tearing apart segments and expelling eggs into the petri dish (Hansen et al. 2006). In each trial, we also placed entire worms into petri dishes, without tearing segments, to see whether coracidia would hatch from eggs not removed from worms. We covered the petri dishes and held them at room temperature (approximately 22°C) for up to 1 week. We checked dishes daily for coracidia, and poured coracidia off and into 250-mL glass culture dishes holding copepods awaiting infection. We then refilled the petri dishes with spring water and returned them to incubation.

Results and Discussion

Our observation was limited in scope and few factors were quantified. However, the primary result, that tens of thousands of live, apparently healthy coracidia were obtained, was unquestionable. We obtained viable coracidia from treated adult Asian tapeworms from all three fish species. Hundreds of adult Asian tapeworms were killed, yet eggs and coracidia that hatched from harvested eggs were not killed during a 24-h exposure to praziquantel at 6 mg/L.

Headwater chub and roundtail chub contained up to hundreds of Asian tapeworms per fish. Red shiners *Cyprinella lutrensis* contained up to 40 Asian tapeworms per fish. The percent of infected fish per species

TABLE 1.—Fish treated with praziquantel (6 mg/L) for infections with the Asian fish tapeworm *Bothriocephalus acheilognathi* and hatching of coracidia from eggs of adult worms killed during treatment. The occurrence of hatch was noted instead of the number of coracidia because the coracidia were too numerous to be counted.

Species	Collection site	Treatment date	Fish treated for tapeworms (*N*)	Infected (%)	Coracidia hatching
Roundtail chub	Aravaipa Creek, Arizona	Nov 2005	10	90	Yes
Headwater chub	Fossil Creek, Arizona	Oct 2005	20	100	Yes
Red shiner	Aravaipa Creek, Arizona	Apr 2005	20	15	Yes

ranged from 15% to 100% (Table 1). We dissected all red shiners after treatment and found no tapeworms, indicating that treatment had removed 100% of adult worms from the digestive tracts. We did not dissect headwater chub and roundtail chub after treatment because these species are candidates for listing as endangered, threatened, or species of concern, and living fish were needed for other experiments.

For each species, we were able to hatch eggs obtained from tapeworms expelled during praziquantel treatment (Table 1). Eggs were light tan spheres that adhered to the substrate. Free-swimming coracidia emerged for 4–6 d with the highest numbers evident on the second day. They were too numerous to quantify, but we estimated numbers in the hundreds or thousands. The hatching rate slowed after the third day. In one trial with headwater chub, we found swimming coracidia in water treated with praziquantel even 12 d after the collection of adult worms. It is possible these coracidia hatched over several days and probably did not survive for 12 d.

We used a praziquantel dosage higher than the minimum required to kill all adult Asian tapeworms in 24-h baths in other studies (1.5 mg/L at a fish density of 6 g/L water, Mitchell 2004; 1.5 mg/L at a fish density of 0.03 fish/L, Ward 2007; 0.75 mg/L at a fish density of 60 g/L; Mitchell and Darwish 2009), but much less than that shown to result in ill effects to treated fish (49.7–63.4 mg/L, range of concentrations corresponding to 24–96-h LC50 values for other cyprinids; lethargy was seen after 2 h at concentrations of 20–30 mg/L in 24-h trials; Mitchell and Hobbs 2007).

Future studies may benefit from additional replication, testing of varying treatment doses, and tests under a variety of water quality conditions. However, live, apparently healthy coracidia were produced from eggs released from dead or dying Asian tapeworms in all of our trials from all three fish species at treatment dosages effective for killing adult worms (Mitchell 2004; Ward 2007; Mitchell and Darwish 2009). Future studies will be needed to identify under what conditions the released living coracidia would develop into adult worms.

Our finding that praziquantel did not kill Asian tapeworm eggs is of concern because eggs can adhere to treated fish, equipment, or even persons conducting the treatment. Our finding suggests caution should be exercised when transporting fish that have been treated using praziquantel for Asian tapeworm infections. Pool (1985) found that adult *B. acheilognathi* contracted immediately when placed directly in a praziquantel solution 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 longer than 15 min. In mature proglottids, bursting led to the expulsion of eggs. In Pool's (1985) tests, where adult Asian tapeworms were placed directly in the praziquantel solution, these expelled eggs were also able to hatch.

Because eggs are expelled after exposure to praziquantel, and eggs are apparently viable, we recommend detailed studies be conducted to assess the risk of spreading infections via viable eggs and coracidia, and if needed, procedures developed to minimize the possibility of moving eggs and coracidia following praziquantel treatments. Until then, there are some procedures that managers who use praziquantel can conduct to minimize transfer of eggs and coracidia. At a minimum, thoroughly rinsing treated fish with freshwater before returning them to the water body and sanitizing, rinsing, and drying all gear would minimize the chances of survival and spread of eggs. Another option is to hold fish for longer than 16 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 temperature and development of the embryo in the egg, and coracidia do not typically survive more than 6 d (Bauer et al. 1973). A quarantine period exceeding 16 d would allow enough time for all eggs to hatch and resulting coracidia to die. If praziquantel is used to eradicate Asian tapeworms 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 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.

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