# Ameliorating The Effects Of The Digenetic Trematode, *Bolbophorus damnificus* On The Channel Catfish Industry

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## Abstract:

In the middle to late 1990s, an emerging digenetic trematode problem was recognized in the channel catfish industry. Morphologic and molecular analysis identified the culprit as *Bolbophorus damnificus*. The American White Pelican, *Pelecanus erythrorhynchos* serves as the definitive host, while the marsh rams-horn snail (*Planorbella trivolvis*) is the first intermediate host. Diagnosis of an infection involves identifying the metacercariae in the subcutaneous tissues. Infestations can be life threatening to catfish fingerlings but the proximate cause of mortality has not been ascertained. They may also predispose fingerlings to secondary infections. Larger fish do not usually succumb to the infestation, but have poor production thus posing significant economic losses. Current methods of control (biological and chemical) have centered on eradication of the snail host. Chemical control includes shoreline treatments using copper sulfate or hydrated lime. This information is the result of multi-institutional and multidisciplinary efforts that have been expended to identify and explore the pathobiology of the parasite as well as its intermediate and final hosts.

**Key words:** digenetic trematode, channel catfish, *Bolbophorus damnificus*

## Introduction

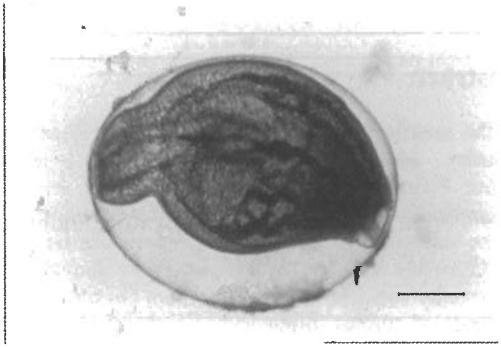
Digenetic trematode infections are not uncommon in aquaculture. In channel catfish, *Ictalurus punctatus,* these infestations are usually due to *Clinostomum* sp., which commonly encysts in the superficial dermis or subepithelium of the fins and gills, or *Diplostomum spathaceum,* which encysts in the lens of the eyes (Hoffman 1999, Terhune et al. 2003, Overstreet and Curran 2004). Overstreet and Curran (2004) also reported infestation by *Bursacetabulus pelecanus*, which encysted in the central nervous system (brain, spinal cord, optic nerve) and the eye; *Austrodiplostomum compactum,* which was found in the vitreous humor of the eye; and *Hysteromorpha* cf. *triloba,* which encysted in the deep musculature around the vertebral column. In most instances, infestations were not life threatening and relatively innocuous, except in cases of heavy infestations by *Clinostomum* sp., which can pose problems at processing due to the unsightly lesions (Terhune et al. 2003). However, in 1997, an emerging digenetic trematode problem was recognized in the channel catfish industry in Louisiana (Venable 1998, Hawke and Camus 1998). This trematode was tentatively identified as the prodiplostomid, *Bolbophorus confusus*, Krause 1914. The parasite sequentially infects the American white pelican (*Pelecanus erythrorhynchos)* as the definitive host, the rams-horn snail (*Planobella trivolvis)* as a first intermediate host, and multiple fish species as the second intermediate host (Fox 1965). Clinical presentations, including gross and microscopic lesions that were associated with this parasite, were reported at the 6th Biennial Fish Diagnosticians Meeting in 1998 by Drs. John Hawke, Al Camus and Drew Mitchell. Metacercariae were found encysted with occasional hemorrhage around the caudal peduncle. Fish often had exophthalmia and pendulous abdomens containing clear ascites. Histologically, there was renal tubular necrosis and granulomatous inflammation associated with the fibrous capsule that surrounded the encysted metacercariae. This parasite had been seen in the Mississippi Delta prior to these reports, but the biological significance of these infestations was not recognized at that time. Later in 1998, complaints of infestations by this parasite were received by the Fish Diagnostic Laboratory in Stoneville (Mississippi) from channel catfish producers in Mississippi.

## Initial Investigations

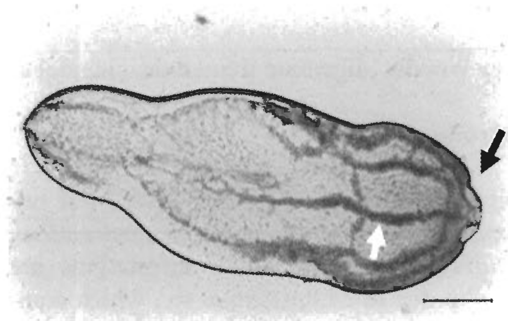
Diagnosis was based on the identification of characteristic metacercariae in the dermis and superficial musculature of channel catfish. Metacercariae were surrounded by a host fibrous capsule and by its own thin, parasitic capsule (Figure 1a and 1b). They also had prominent lateral pseudosuckers and anastamozing reserve excretory channels. Utilizing these characteristics, the prevalence of the parasite throughout the industry was investigated. Blocks of ponds or whole farms were surveyed utilizing physical examination of at least 30 fish per pond for the presence of the metacercariae. An examination of 821 ponds on 32 farms revealed a widespread problem (32% of ponds examined were affected). This surveillance was conducted following preliminary research at the Fish Diagnostic Laboratory confirming the lethality of infestations by this parasite.

**Figure 1. a:** *Bolbophorus damnificus* metacercaria excised from a naturally infected fish. Note that the parasitic capsule encapsulating the metacer-cariae (Bar~250 μm); **b:** a metacercaria that was removed from the parasitic capsule. Black arrow points to one of the prominent lateral pseu-dosuckers and the white arrow to the anastamosing reserve excretory channels (Bar~250 μm).

1a.



1b.



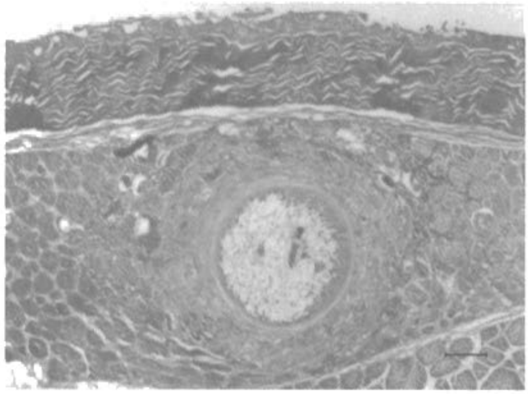
Rams-horn snails were collected from the banks of known positive ponds. The snails were placed in individual glass scintillation vials and incubated overnight at room temperature (~22°C). Only *Bolbophorus* type cercariae were collected (the snails shed at least four distinct varieties of cercariae), enumerated and used to infect channel catfish fingerlings (~10-30g). Catfish were exposed to 0, 100, 150, 200, 250 cercariae per fish for 30 min in 500 mL of well water, transferred to aquaria with flow through well water (27°C ± 2°C) and monitored for mortality. Mortalities were observed from day 10 to day 14 post-infection (PI) in fish exposed to 250 cercariae/fish. Fish that succumbed to the infection had multiple raised red papules on the whole body. Most fish had bilateral exophthalmia and pendulous abdomens caused by a clear ascitic fluid. Histologically, the changes were consistent with those described by Hawke and Camus (1998). Metacercariae were subdermal with mild to moderate mono-nuclear inflammation and hemorrhage surrounding the parasitic capsule (Figure 2). There was mild renal tubular necrosis, splenic congestion and lymphoid depletion, but these were only seen in lethally infested fish. Metacercariae were rarely seen in branchial tissue, liver or head kidney.

Basic research was then carried out to identify the parasite and its definitive host, characterize the pathobiology of the parasite and gain a better understanding of the biology of the intermediate host. Applied research, based on information obtained from the above projects, was then utilized to provide a management scheme for the producers.

## Basic research

Overstreet et al. (2002) determined that this trematode was not *Bolbophorus confusus*, but it was actually a novel species, *B. damnificus*. They also identified another sympatric unnamed species, *Bolbophorus* sp. Overstreet et al. (2002) confirmed their findings utilizing experimental infections with nestling and de-wormed adult pelicans, in conjunction with molecular analysis. In addition to molecular differences, these two species had larger eggs (*B. damnificus* at 100-112 µm, *Bolbophorus*. sp. at 123-129 µm) than *B. confusus* (90-102µm).

**Figure 2.** Section of the lateral body wall of a channel catfish with an encysted *B. damnificus* metacercaria within the superficial muscle demonstrating the concentric arrangement of inflamematory cells and fibrous connective tissue surrounding the metacercaria. (H & E; Bar ~100 µm).



Levy et al. (2002) utilized morphologic and molecular analysis of the 18S rDNA genes to determine that these were indeed two distinct species and developed species specific polymerase chain reaction (PCR) assays to identify and differentiate these species. *Bolbophorus* sp. type II is the proposed name of the second trematode.

Overstreet et al. (2002) also investigated the possibility that multiple bird species served as definitive hosts. Besides the American white pelican (through which successful life cycle studies were completed), several double-crested cormorants (*Phalacrocorax auritus*), neotropic cormorants (*P. brasilianus*), great blue herons (*Ardea herodias*), great egrets (*Ardea alba*), snowy egrets (*Egretta thula*), little blue herons (*E. caerulea*), tricolored herons (*E. tricolor*), black-crowned night-herons (*Nycoticorax nycticorax*) and yellow-crowned night herons (*Nyctanassa violacea*) were examined, but were negative for *B. damnificus* and other *Bolbophorus* species. These birds were collected adjacent to catfish ponds and they were presumed to have fed on infected catfish and defecated in the ponds. Dronen et al. (1999) reported finding *B. confusus* from 1 of 4 brown pelican from the Gulf coast of Texas; however, Courtney and Forrester (1974) did not find the trematode in 113 brown pelicans collected from Florida and Louisiana. Based on examination of archived specimens of the reddish egret *(E. rufescens)* collected by Conti et al. (1986), Overstreet et al. (2002) concluded the trematode had been misidentified based on the lack of a genital bulb, a characteristic diagnostic feature of the *Bolbophorus* genus.

The impact of the American white pelican on the trematode problem was summarized by King (2005). The eastern metapopulation of American white pelican (the North American Continental Divide separates the American white pelican into two relatively distinct metapopulations) has adapted to take advantage of channel catfish aquaculture in the southeastern United States. This metapopulation breeds mainly in the Northern Great Plains and migrates along the Mississippi River, wintering in the lower Mississippi River Valley and along the Gulf of Mexico. However, a subset of non-breeding birds has been found to remain in Arkansas, Louisiana and Mississippi during the summer. This metapopulation has increased 18-fold since 1985, exploiting the abundant and readily available food source. American white pelicans that forage on catfish ponds spend less time foraging (4% versus 28% of their day) and less time loafing (97% versus 72%) than those that forage in other habitats. This difference may be due to more efficient capture of fish in the relatively shallow, heavily stocked catfish fish ponds. Because these birds are both diurnal and nocturnal foragers, 24-h harassment patrols are necessary to reduce their impacts. They have also appeared to break into smaller flocks of 1-50 birds that spread throughout the farm when feeding (prior to 1995 they were usually in flocks with ≥300 individuals) making harassment and dispersal more difficult.

The biology of *Planorbella trivolvis* in the laboratory, as well as its population dynamics in commercial ponds, was investigated (George 2008). The latter was done on four separate ponds in the Mississippi Delta for more than two years. Significant findings included the number of days post infection until cercaria were shed (23 d), the number of cercaria shed per day (~1400 cercaria/day), the number of days infected snails shed cercaria (at least 21 d), the year-round prevalence of snails and snail eggs, the time when snails are first seen in the ponds (in May), the number of eggs laid by a single snail/month (600 eggs/month), the time to reproductive maturity for snails (2 months), and the age when snails can be infected by the trematode (2 months old). These findings indicated that relatively few infected snails could cause significant infestations and confirmed preliminary findings that in most affected ponds a relatively small population of snails were affected (~10%). Also, most snails are found on the vegetation close to the pond bank, although smaller numbers may be found a distance from the shore, which complicates the effectiveness of shoreline-based treatments.

Utilizing molecular methods, Yost (2008) confirmed the life cycle of *B. damnificus* and examined the pathology associated with this parasite. She also proved that the snail *Biomphalaria havaensis*, which also inhabits catfish ponds, could serve as an intermediate host for *B. damnificus* (Yost et al. 2009). The PCR confirmed that *B. damnificus* cercariae were capable of infecting fingerlings at doses of 25, 50, 100 and 200 cercariae/fish (the previous work was most likely a mixed infection of *B. damnificus* and *B.* sp. type II). Fish were exposed individually for 2-h, rinsed and placed in flow through systems. Mortalities first appeared at the 200 cercariae/fish dose on day 5 PI and ranged from 20-100% by day 6 PI. Microscopic findings included a progressive buildup of the mild, mononuclear inflammatory infiltrate and hemorrhage, formation of the fibrous capsule around the developing metacercariae, splenic lymphoid depletion and decreased vacuolation of the liver. The lymphoid depletion may be a partial explanation of the increased susceptibility of *Bolbophorus* infected catfish to secondary bacterial infections (Labrie et al. 2004).

## Applied Research

Apart from dispersing the American white pelicans, control of infestations have focused on biological and chemical control of the rams-horn snail (Terhune et al. 2003). Black carp (*Mylopharyngodon piceus*) or redear sunfish (*Lepomis microlophus*) have also been used as biological controls. Unfortunately, black carp are nonindigenous species and redear sunfish have a gape size that is too small for larger rams horn-snails. Shoreline treatments using copper sulfate and hydrated lime have been developed (Terhune et al. 2003). Although other chemicals have been evaluated (Mischke et al. 2005), these two treatments have proven to be most effective and have been refined to allow for water temperatures and chemistries (Mitchell and Hobbs 2003, Mitchell et al. 2007). A whole pond copper sulfate treatment has also been evaluated, but this treatment poses a risk of killing fish (Wise et al. 2006).

A recent publication by Wise et al. (2008) described the economic impact that *B. damnificus* infections can have on commercial farms. Sixty-four ponds from one farm were evaluated for the prevalence of the infection. The ponds were characterized as negative, light (1-33% infection rate), moderate (34-66% infection rate) or severe (≥ 67% infection rate). Production was reduced by 14, 35 and 40% for light, moderate and severe infections, respectively. Actual feed costs and estimated catfish receipts were used in a generalized enterprise budget format to calculate income over variable costs and net returns. A light infection rate category resulted in a 61% reduction of net returns, while moderate and severely infected populations resulted in negative net returns. These figures provided clear indications of the economic losses that *B. damnificus* infestations can inflict.

## Conclusions

Current diagnostic submissions indicate that *B. damnificus* infestations still occur, although at a low prevalence. Current and future research will focus on more precise means of diagnosing possible infections utilizing quantitative PCR to detect *B. damnificus* cercariae in pond water. This will allow for a more cost efficient use of pond shoreline treatments only when the infective cercaria stage is present. Current methods rely on diagnosis of the metacercariae in the fish, which may be from the previous year's infection.

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