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HISTOLOGICAL EFFECT OF *ASCOCOTYLE TENUICOLLIS* (DIGENEA: HETEROPHYIDAE) METACERCARIAL INFECTION ON THE HEART OF *FUNDULUS HETEROCLITUS* (TELEOSTEI: CYPRINODONTIDAE)

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ABSTRACT

A total of 40 mummichogs (*Fundulus heteroclitus*) from 3 localities in the Massachusetts area and 9 mummichogs from Georgetown, South Carolina were examined for the presence of *Ascocotyle* metacercariae. All metacercariae were located in the bulbus arteriosus of the heart. In Massachusetts area study, all fish from New Bedford Harbor were infected (mean intensity = 168; n = 25). Four out of five fish from West Island were infected (mean intensity = 2), and only 1 out of 10 fish from Slocums River Basin was infected (intensity = 1). None of the fish from Georgetown were infected. All metacercariae were identified as those of *Ascocotyle tenuicollis*. To determine if any significant damage to host tissue resulted from metacercarial infection, histological sections of infected hearts were examined and compared with those of normal (uninfected) hearts. Extensive examination of the bulbus arteriosus and the ventricle revealed no significant damage to infected heart tissues. Examination of areas near the bulbo-ventricular junction, however, revealed areas of elongated nuclei and decreased width of muscle fibers, suggesting stretching of the cells. No evidence of tearing or any other damage was identified. Further examination of infected tissues with the electron microscope revealed similar results. This research suggests that *Ascocotyle* infection has a minimal morphological effect on the mummichog heart.

INTRODUCTION

Heterophyid trematodes are a large family of microscopic pear-shaped flukes that exist throughout the world. Those of the genus *Ascocotyle* are particularly abundant on the American continents as parasites of fish-eating birds and mammals (Scholz et al., 1997). Serving as definitive hosts, these birds and mammals harbor the adult parasites in their intestines. Eggs produced by the mature parasites are passed out with feces initiating a series of stages in a complex life cycle. As defecation occurs in a marsh or estuary, certain snails ingest the eggs and become first intermediate hosts to the parasite. These eggs develop into sporocysts containing many rediae. Within the rediae are cercariae, which eventually leave the snail. The cercaria is a free-swimming larval stage that may become caught in the respiratory current of a fish, the second intermediate host to the parasite. Within the gills, *Ascocotyle* cercariae enter the blood stream by penetration of the vascular tissue and encyst to form metacercariae in the fish host. A myriad of locations may house the metacercariae including the gills, heart, mesenteries, muscles, intestine, or stomach wall (Scholz et al., 2001). The metacercariae continue development into adult flukes in the intestine of the final host – a piscivorous bird or mammal.

Often, the location of metacercariae is specific for each species of *Ascocotyle*, providing a feature that is useful in species identification. Arrangement of the spines on the oral sucker as well as certain characteristics of internal morphology distinguish between species. Releasing the juvenile parasites from their cysts for examination of spination patterns provides the basis for identification. Since spination patterns are difficult to observe, experimental infection of potential definitive hosts is sometimes necessary to produce adults for identification (Scholz et al., 1997). Cyst walls of the metacercariae may also provide clues for identification. Careful examination may reveal differences in the thickness of the cyst wall that can distinguish between *Ascocotyle* species (Ostrowski de Nunez, 2001).

Several species of fish serve as second intermediate hosts for *Ascocotyle* species, such as *Cyprinodon* variegates (Coleman, 1993), *Cnesterodon decemmaculatus, Jenynsia lineata, Gambusia affinis* (Ostrowski de Nunez, 2001), *Bramocharax caballeroik, Poecilia catemaconis,* and *Poeciliopsis catemaco* (Scholz et al., 2001). The fish intermediate hosts utilized in this study are the mummichogs, *Fundulus heteroclitus.* The mummichog is an estuarine fish that tolerates a wide range of salinity, temperature, and even low levels of oxygen. Several species of parasites representing different taxonomic groups have been reported in mummichogs collected from various localities (Barse, 1998; Dickinson, Threlfall, 1975; Hirshfield et al., 1983; Marcogliese, 1995; Weisberg et al., 1986). Although not valued as commercial or sport fishes, mummichogs are important in food chains and in transmission of parasites. They are a likely source of infection for sea birds because these fish are benthic feeders and are frequently consumed by birds (Weisberg et al., 1986). Mummichogs become infected when *Ascocotyle* cercariae penetrate the gills. These parasites are then carried by the circulatory system to the heart and lodge in the bulbus arteriosus where they remain encysted until a bird or mammalian host eats the fish.

Fish hosting *Ascocotyle* species offer no obvious signs of disease. According to Armitage (2000a), pathogenicity and host response to *Ascocotyle* infection is rarely found in either the fish or snail host, although blockage of blood flow may occur occasionally. The most visible sign of host reaction is production of a fibrous collagen capsule around the cyst (Armitage, 2000b). However, studies conducted by Coleman (1993) suggest that *Ascocotyle* infection in the bulbus arteriosus of the sheepshead minnow, *Cyprinodon variegatus*, causes subtle harm to the fish resulting in decreased swimming performance. In intense infections, the cysts act as barriers to blood flow resulting in increased ventricle size as well as increased oxygen demand as the heart is overburdened. Coleman and Travis (1998) further asserted that the entire host population is affected as *Ascocotyle* infection results in increased mortality during winter. As the water temperature cools, lessened blood circulation adds even more stress to the infected fish heart. Fish dependence on stored energy during the winter months also may harm the infected fish. Those stores are exhausted more quickly by heavily parasitized fish due to their higher metabolic rates (Coleman and Travis, 1998). These obstacles decrease the number of winter survivors as the fish become susceptible to predators or starvation. The purpose of this research is to determine if *Ascocotyle* infection inflicts any pathological effects on the mummichog heart morphology.

METHODS AND MATERIALS

Live, infected mummichogs were obtained from New Bedford Harbor and West Island, in Massachusetts. Uninfected fish were collected from Slocums River Basin in Massachusetts and from Georgetown, South Carolina for comparison. The live fish were euthanized and dissected

immediately. The hearts were removed; the diameter of the ventricle and bulbus arteriosus measured, and the prevalence and intensity of infection were determined.

Some live metacercariae were isolated, measured, and forced open to release the juvenile parasites. This was accomplished by placing a metacercaria in a small drop of saline on a glass slide. A coverslip was placed on top and a slight pressure was applied to it to cause the cyst to rupture. The live juvenile that emerged from the ruptured cyst was then observed for species identification.

For histological studies, the hearts were fixed in 10% buffered neutral formalin. Routine histological processing of the hearts were performed at the Spartanburg Pathology Consultants where the tissues were dehydrated through ascending series of alcohols and xylene, embedded in paraffin, and sectioned at 4 microns thick. The tissue sections were stained with Hematoxylin, counterstained with Eosin, mounted with Sub X mounting media, and examined with a light microscope. Some hearts were fixed in 2.5% glutaraldehyde in Sorensen's Phosphate Buffer (pH 7.2) and sent to the Electron Microscope Facilities at the University of South Carolina School of Medicine for ultrastructural study.

RESULTS

A total of 49 fish hearts were examined for the presence of metacercariae. All fish collected from New Bedford Harbor were infected with metacercaria. The intensity of infection ranged from 32 to 462 metacercariae in a single bulbus arteriosus, with a mean intensity of 168 (Table 1). Four out of five fish from West Island were infected, and only 1 out of 10 fish collected from Slocums River Basin was infected. However, in this infected fish, only 1 metacercaria was found in the heart. No metacercariae were found in any of the fish collected from Georgetown, South Carolina.

Table 1. Prevalence and Intensity of *Ascocotyle* Infection

	New Bedford	West Island	Slocums River Basin	Georgetown
Number of fish hosts	25	5	10	9
Number of hosts infected	25	4	1	0
Parasite Prevalence	100%	80%	10%	0%
Parasite Intensity (Min, Max)	168 (32, 462)	2 (1,6)	1	--
Mean fish length (mm)	78.1	67.6	76.2	54.3

When fresh, the more heavily infected whole hearts were usually easy to identify. The round metacercarial cysts were clearly visible through the transparent heart wall and the bulbus arteriosus bulges (Figure 1). However, lightly infected hearts (with very few metacercariae) were indistinguishable from uninfected hearts until they are dissected open and searched for metacercariae. The metacercariae are usually held in clusters by a connective tissue (Figure 2 and Figure 3).

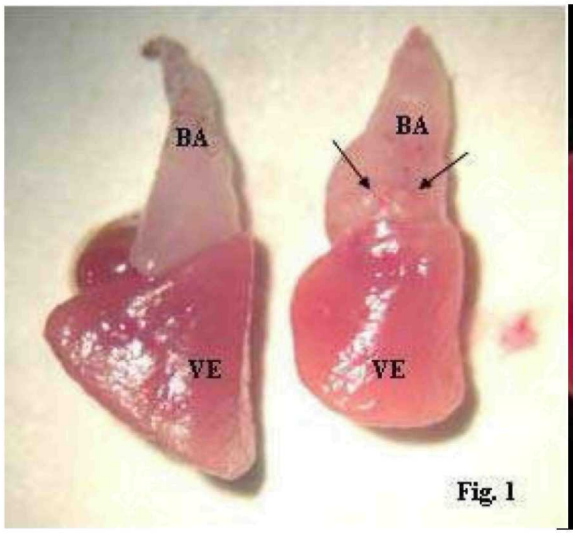


Figure 1. Infected heart (right) of mummichog with bulging bulbus arteriosus (BA) and irregular shaped ventricle (VE). Note round metacercarial cyst (arrow) within the bulbus arteriosus. The heart on the left is uninfected.

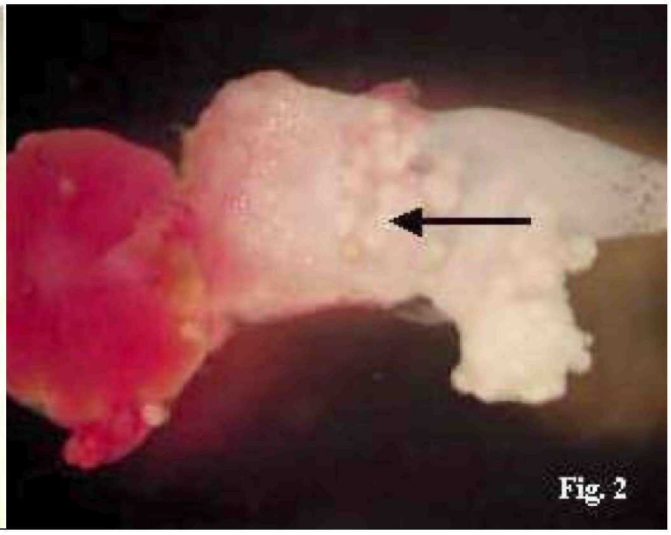


Figure 2. Bulbus arteriosus dissected open to show clusters of metacercaria (arrow).

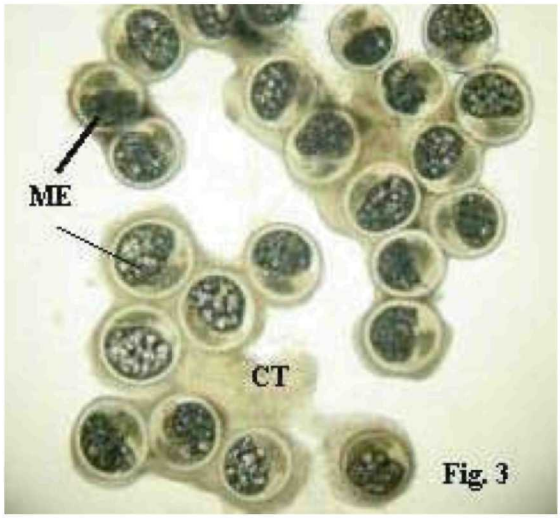


Figure 3. Metacercariae (ME) isolated from the bulbus arteriosus are surrounded by connective tissue (CT). 40X.

The cysts measured an average of 304 micrometers in diameter and the cyst wall ranged from 10 to 20 micrometers thick. Examination of juveniles released from the ruptured cysts revealed oral sucker with 32 spines arranged in two rows of 16 each. This spination pattern, the characteristic pear shaped body of the juvenile (Figure 4), and the measurements of the cysts suggest that these metacercariae are those of *Ascocotyle tenuicollis* (Scholz et al., 1997; Ostrowski de Nunez, 2001).

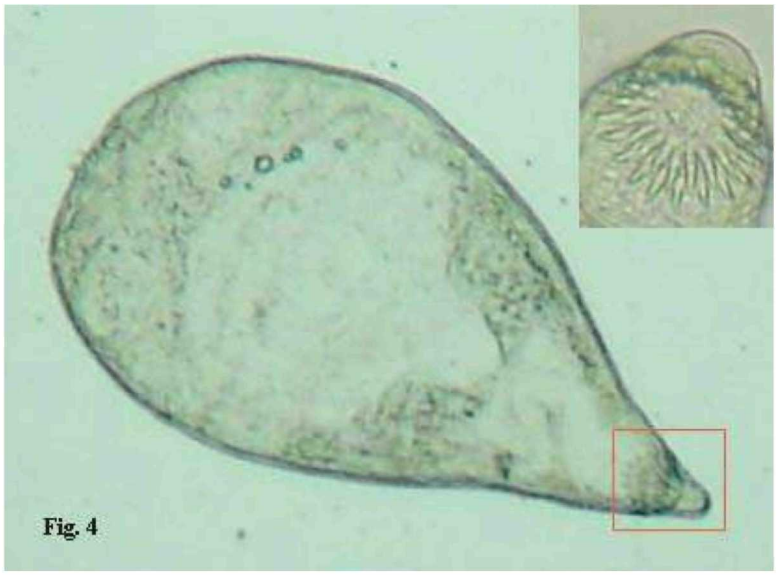
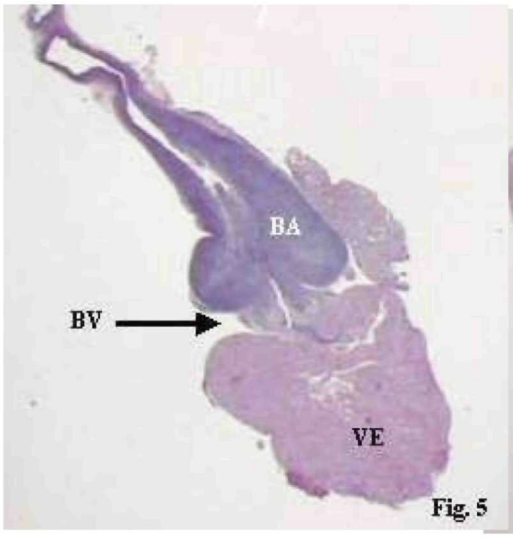


Figure 4. Juvenile *Ascocotyle tenuicollis* isolated from the cyst (100x). Inset. The anterior end of the juvenile showing oral sucker armed with spines (400x).

Longitudinal and cross sections of infected hearts revealed obvious distortions compared to the uninfected hearts. The metacercariae were clearly visible in the lumen of the bulbus arteriosus. The most obvious differences between infected and uninfected hearts were clearly observed in longitudinal sections. In uninfected hearts, the bulbo-ventricular junction is an indented area connecting the ventricle and bulbus (Figure 5). In infected hearts, this junction is obviously stretched, as there is much less of an external indention between ventricle and bulbus. The lumen of the infected bulbus is also much wider than that of the uninfected bulbus. Likewise, the bulbus wall is much thinner in the infected heart (Figure 6).



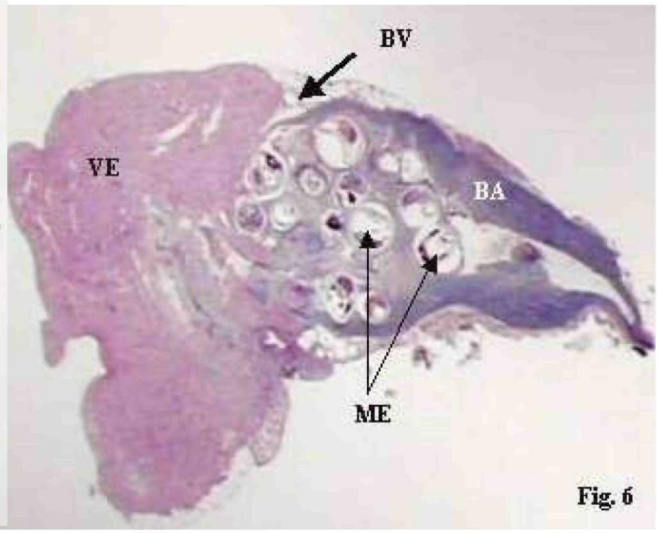


Figure 5. Longitudinal section of uninfected heart. (BA, bulbus arteriosus; BV, bulbo-ventricular junction, VE, ventricle). 40X.

Figure 6. Longitudinal section of infected heart showing metacercariae (ME) within the enlarged lumen of the bulbus arteriosus (BA). The wall of the bulbus arteriosus is thin as a result of stretching near the bulbo-ventricular junction (BV). 40X.

The muscle layer of the bulbus arteriosus consists of smooth muscle fibers, while that of the ventricle wall consists of cardiac muscle. At the junction between the bulbus arteriosus and ventricle (bulbo-ventricular junction), both smooth muscles and cardiac muscles were present.

Examination of infected hearts at 1000x magnifications revealed stretching of individual muscle fibers in areas of the ventricle close to the bulbo-ventricular junction and in areas of the bulbus arteriosus where cysts were most dense. Stretched muscle fibers appear smaller in width, and nuclei appear elongated in comparison to those of the uninfected hearts (Figures 7-10). Interestingly, no evidence of tearing or any other damage to stretched muscle fibers were identified.

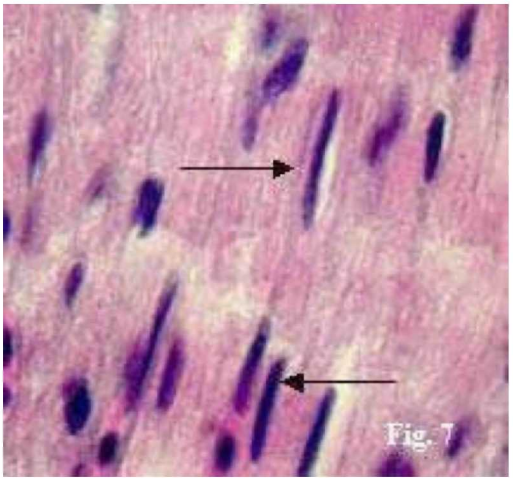


Figure 7. Longitudinal section of cardiac muscles near the bulbo-ventricular junction of infected heart. Note stretched cells with elongated nuclei (arrow). Striations are not clearly visible. 1,000X.

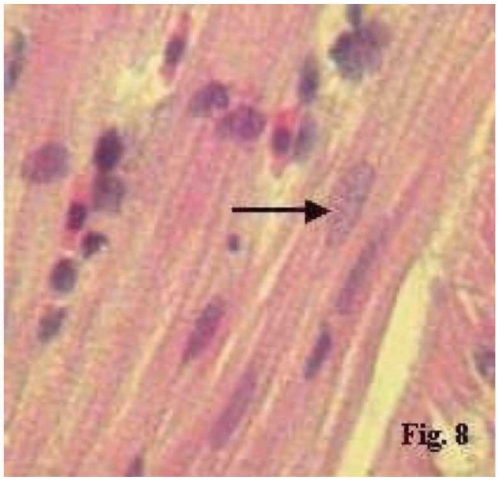


Figure 8. Longitudinal section of cardiac muscles near the bulbo ventricular junction of uninfected heart. Note normal shape of nucleus (arrow) and clearly visible striations. 1,000X.

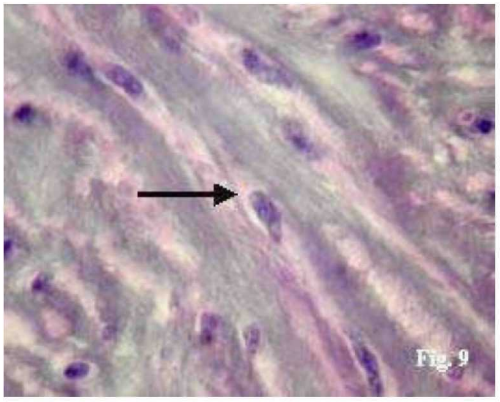


Figure 9. Longitudinal section of uninfected bulbus arteriosus. Note normal spindle shape of smooth muscle fibers (arrow). 1,000X.

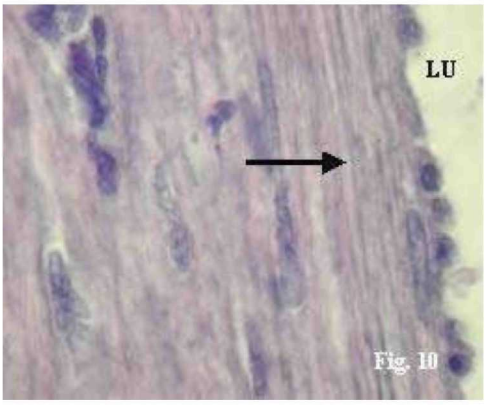


Figure 10. Longitudinal section of infected bulbus arteriosus. Note stretched smooth muscle fibers (arrow) near the lumen (LU). 1,000X.

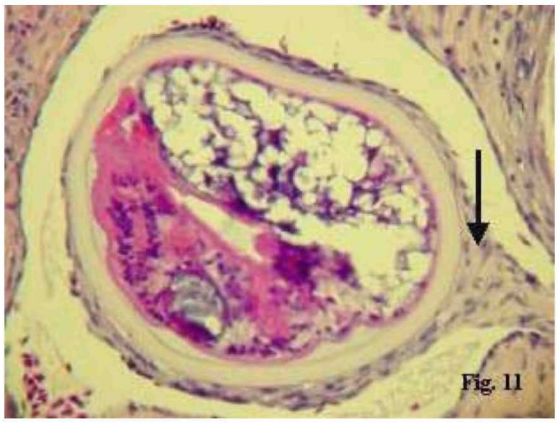


Figure 11. Section through a metacercaria within bulbus arteriosus showing proliferation of host cells around the cyst (arrow). 450X.

Host response to the presence of the cysts in both longitudinal and cross-sections was clearly evident. Connective tissue, apparently caused by proliferation of cells and material secreted by the host surrounded the cysts. In certain areas, it appeared that formation of connective tissue started on one side of the cyst (Figure 11, arrow) and worked its way around the outside of the cyst.

Ultrastructural studies of infected hearts revealed no evidence of damage to muscle fibers of ventricle or bulbus arteriosus and further confirms the histological results. Electron microscopic examinations of host tissue surrounding the cysts revealed abundance of large and small electron dense secretions (Figure 12) and collagen fibers in the adjacent cardiac tissue (Figure 13). These secretions were not observed in uninfected hearts.

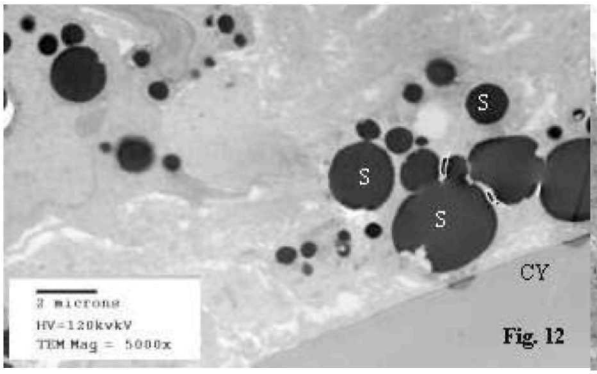


Figure 12. Electron micrograph showing host cell secretions (S) surrounding the cyst (CY). 5,000X.

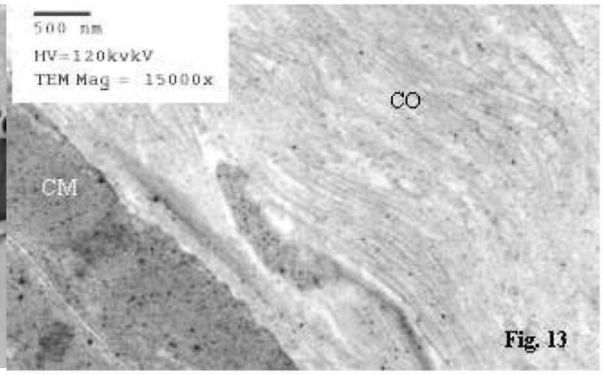


Figure 13. Electron micrograph showing host collagenous secretions (CO) adjacent to the cardiac muscle (CM) of infected heart. 15,000X.

DISCUSSION

Except for the visible stretching of muscle fibers, the presence of *Ascocotyle* metacercariae in the bulbus arteriosus of the fish revealed no areas of significant damage to the heart. In ultrastructural studies of the bulbus arteriosus of different types of fishes (Icardo et al., 2000) it was reported that in general, cells of the middle layers of the bulbus arteriosus have characteristic flexibility from elastin, and the outer layer has collagen to provide strength. These features may account for the ability of the mummichog heart to resist damage from *Ascocotyle* metacercariae. In this study of the infected heart tissue, it is apparent that the presence of cysts has minimal effects on the heart morphology of the intermediate fish hosts. It should be noted however, that

infected mummichogs (from New Bedford Harbor) that have been placed in non-aerated containers for several hours during transport appeared to have higher mortality rate than the uninfected ones (from Slocums River and Georgetown). The presence of parasites may have affected the physiological conditions of the fish.

Other interesting features of infected heart morphology were the abundance of connective tissues surrounding the metacercariae. The abundance of cells visible in the lumen of the infected mummichog bulbus may result from proliferation of endocardial epithelium, which lines the innermost layer of bulbus arteriosus in teleosts. These endothelial cells contain vacuoles capable of secretion functions (Icardo et al., 2000). This may account for the abundant electron dense secretions observed in the ultrastructural study. However, further studies are necessary to identify the histochemical nature of these secretions.

The host reaction to the presence of parasites appears to be limited to fibrous (collagenous) secretions, which could be due to the mechanical pressure exerted by the cyst wall to the surrounding host tissue (Stein, Lumsden, 1971). The minimal response of the host obviously is advantageous to the parasites, which are dependent on the fish intermediate host to be transmitted to their final hosts where they can continue development into adults (Stein, Lumsden, 1971). In addition, stretching of the cardiac and smooth muscles may limit the pumping ability of the heart to distribute oxygenated blood throughout the body. This may result in sluggish movement of the fish, which then becomes readily available for consumption by their piscivorous predators.

ACKNOWLEDGEMENTS

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