Determining the First Intermediate Host to Parasites *Glossocercus caribaensis* and *Cyclustera ibisae* that are found in *Fundulus heteroclitus* in the North Inlet-Winyah Bay National Estuarine Research Reserve

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Fundulus heteroclitus serves as a host to many parasites, providing a model system to investigate questions regarding evolution and ecology of host-parasite interactions in natural populations. Previous research from the North Inlet Estuary at Belle W. Baruch shows that *F. heteroclitus* serve as second intermediate hosts to cestodes, *Glossocercus caribaensis* and *Cyclustera ibisae*. Finding larval parasites in the first intermediate host will complete gaps in the knowledge about these parasites' life cycles. In the experiment, 27 fish were collected from the North Inlet Estuary and were dissected and examined for parasites around the gastrointestinal tract. Parasites collected were sent to parasitologist, Dr. Anindo Choudhury, the associate editor for the *Journal of Parasitology*, for identification. The parasites were positively identified as *G. caribaensis* and *C. ibisae*, indicating that the cestodes were still present in the community. Then, possible first intermediate hosts were collected from the North Inlet Estuary; these specimens included grass shrimp, snails, clams, and fiddler crabs. These possible hosts were dissected and examined for early stage tapeworms (oncospheres). Parasites found were also sent to Dr. Choudhury to be identified. Several different species of parasites were found in dissection of first intermediate hosts. Parasites identified were isopods, trematodes, and a possible cestode. The finding of a possible cestode in only one host, a grass shrimp, out of seventy dissected, led us to the conclusion that grass shrimp are most likely an accidental host to the tapeworms.

Introduction

Fundulus heteroclitus, also known as the mummichog, is found along the Atlantic coast of North America in coastal waters. *F. heteroclitus* are often used as laboratory species due to their adaptability to environmental changes, such as salinity, oxygen levels, temperature, and pollution in the environment.[1] They are food sources for larger fish, wading birds, and sea birds. *F. heteroclitus* also serve as hosts to many parasites providing model systems to investigate questions regarding evolution and ecology of host-parasite interactions in natural populations.[1] Previous research has found that *F. heteroclitus* in the North Inlet Estuary at Belle W. Baruch Marine Sanctuary serve as second intermediate hosts to parasites, specifically the larval tapeworms *Glossocercus caribaensis and Cyclustera ibisae*.[2]

As an intermediate host, *F. heteroclitus* serves as a temporary environment to complete the parasite's life cycle.[3] The complete life cycles of *Glossocercus caribaensis* and *Cyclustera ibisae* are unknown. Mestacestodes, a larval stage of *G. caribaensis* and *C. ibisae* have been found in mummichogs from the North Inlet Estuary.[2] Adults of *C. ibisae* and *G. caribaensis* belong within the cestode family Dilepididae, which is a group of tapeworms found to infect piscivorous birds. [2] These fish-eating birds are the definitive host to the parasites. Within the definitive hosts, the parasite is able to become an adult worm, attain sexual maturity and reproduce.[3] Adult tapeworm eggs are shed in the feces of the bird definitive host.[3] The first intermediate host is an organism that eats the tapeworm eggs. Development of the parasite occurs in this host, and the parasite becomes a stage known as an oncosphere. [3] *F. heteroclitus* then eats an infected first intermediate host and becomes infected itself. The fish is now known as a second intermediate host, and within the fish, the parasite develops into the metacestode stage. A bird then eats *F. heteroclitus* and the tapeworm becomes an adult, and the life cycle is complete.

A recent review by Scholz and Choudhury indicates the current number of life cycle studies of fish and parasites since the 1960s and 1970s is negligible.[6] Also, the current number of papers on observations of parasitic life cycles in fish has declined, except for studies on myxozoans. Neither experimental studies nor field research on life cycles and transmission patterns of fish parasites are being carried out in any significant way.[6] Thus, more studies are needed. Finally, as Burnett et al. report, the presence of parasites in *Fundulus* has been used to indicate the presence of definitive hosts in an area as indicators of changes in the food web, and as bioindicators of ecosystem health.[1] Thus, this research project is important in providing information on fish-parasite dynamics in a local ecosystem.

The overall goal of this project was to determine the first intermediate host of *G. caribaensis* and *C. ibisae* by first, collecting *F. heteroclitus* from the North Inlet Estuary and determining if the fish were still infected with the tapeworm metacestodes, and second, to collect organisms that *F. heteroclitus* is known to eat and examine them for early stage (oncosphere) parasites. *F. heteroclitus* is known to feed near marsh grasses. As omnivores, their diet includes plant matter, mollusks, crustaceans and insect larvae.[4] Results generated could provide knowledge not only about the unknown life cycle of the parasites, but also could contribute to knowledge about the health of the ecosystem as a whole.

Methods

Fish Collection

Fundulus heteroclitus were collected from the North Inlet Estuary at Belle W. Baruch using baited minnow traps. Twenty-seven fish were collected and characteristics of fish were recorded such as weight, length, and sex. Using a dissecting microscope, the abdominal cavity of fish was dissected to search for parasites, and the GI tracts were examined to determine what the fish were eating. Parasites found in fish were preserved using 10% neutral buffered formalin (NBF), and were photographed using an iPhone with an iDuOptics LabCam.

Invertebrate collection

After fish collection, invertebrates and plant matter were collected from the North Inlet Estuary. Invertebrates, such as grass shrimp, snails, fiddler crabs, and clams were collected using nets and a yabby pump. These organisms were examined and dissected using a dissecting microscope. Parasites found in invertebrates were also photographed as previously mentioned and preserved using 10% NBF.

Parasite Identification

Parasite samples were then transferred to 70% ethanol and sent to Dr. Anindo Choudhury, associate editor for the *Journal of Parasitology*, for identification.

Results

Parasites in fish

Ninety-two percent of *F. heteroclitus* were infected with parasites. Parasitic tapeworms (metacestodes) were positively identified as *Cyclustera ibisae* and *Glossocercus caribaensis* (Figure 1).

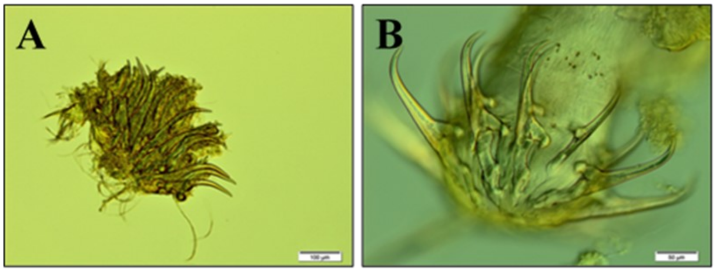

Figure 1. Anterior end with hooks (scolex) used to identify the tapeworms, *Cyclustera ibisae* (A) and *Glossocercus caribaensis* (B), found in *F. heteroclitus* from the North Inlet Estuary. Photos by Dr. Anindo Choudhury.

Contents of *F. heteroclitus* Gastrointestinal Tracts

The gastrointestinal tracts of the fish were dissected and observed to see what *F. heteroclitus* was eating in order to get an idea of possible first intermediate hosts of the parasites. Remnants of *Spartina* grass and plant matter, claws of fiddler crabs, and exoskeletons of shrimp were the predominant organisms found inside GI tracts of the fish.

Possible Invertebrate First Intermediate Hosts

Grass shrimp, snails, fiddler crabs, and clams were examined for early stage larval tapeworms. Early stage parasites found from grass shrimp, snails and clams included larval trematodes. There were no parasites found in fiddler crabs. The parasitic isopod, *Probopyrus pandalicola,* and a possible cestode larvae (oncosphere) (Figure 2) were also found in the grass shrimp, *Palaemonetes*. *Spartina* grass was also examined, but no parasites were found associated with this marsh grass.

Discussion

The purpose of this research was to determine the first intermediate host of the parasitic tapeworms, *Glossocercus caribaensis* and *Cyclustera ibisae,* that have been found in the fish, *Fundulus heteroclitus* from the North Inlet Estuary at Belle W. Baruch Marine Research Reserve. Metacestodes of *Glossocercus caribaensis* and *Cyclustera ibisae* have previously been identified in infected *F. heteroclitus* from the North Inlet Estuary[2], but that study was completed 16 years ago, so confirmation that these tapeworms were still infecting the fish was important. This research showed that *G. caribaensis* and *C. ibisae* are currently infecting *F. heteroclitus* (Figure 1).

Searching for the early larval stage (oncosphere) of *C. ibisae* and *G. carabaensis* in possible first intermediate hosts proved challenging. First, *F. heteroclitus* is an omnivore known to eat many different organisms so we had to collect and dissect as many different prey organisms as we could in the areas we collected the fish.[4] What we collected was based on, but not limited to, what was observed in *F. heteroclitus* GI tracts. Second, the oncosphere stage of these tapeworms has not been recorded in the literature, so we did not know exactly what we were looking for in the first intermediate hosts. However, using Roberts et al. as a guide for comparison, we looked for small structures that had a defined outer envelope with hooks on the inside.[3]

After dissecting eighty-five possible first intermediate hosts, we found three different parasites. As seen in Table 1, the mollusks and fiddler crab did not appear to be an intermediate host for tapeworms. The possible oncosphere was found in a single grass shrimp of the genus *Palaemonetes*. We believe the grass shrimp to be an accidental host because over seventy grass shrimp were dissected, and only one shrimp had a possible oncosphere of a tapeworm. If grass shrimp are true first intermediate hosts, we suspect the prevalence of infection in the shrimp would be higher. After dissection of over 80 possible first intermediate hosts, we only found one possible oncosphere. Unfortunately, this oncosphere could not be positively identified with certainty because generally, two specimens are needed, one for morphological analysis and one for DNA analysis.

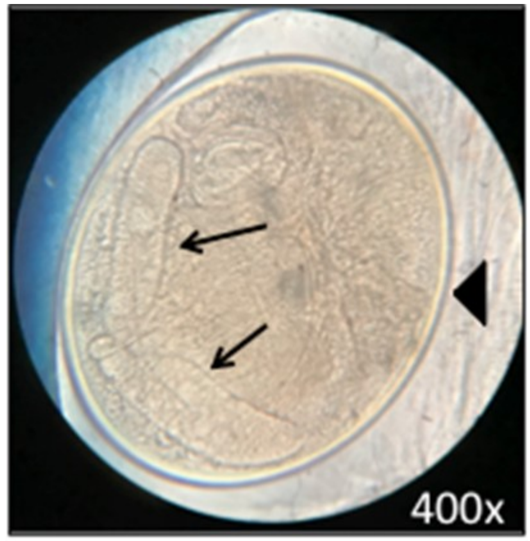

Figure 2. Possible larval cestode (oncosphere) found in the grass shrimp, *Palaemonetes.* Like oncospheres from other species of cestodes, there appears to be an outer envelope (arrowhead) and hooks (arrows). Photo by Emiley Masloski.

Table 1. Possible first intermediate host collection included grass shrimp, snails, fiddler crabs, clams, and *Spartina* grass.

Possible first IH	Number collected and dissected	Parasite(s) found
Grass shrimp, *Palaemonetes*	75	Larval trematodes *Probopyrus pandalicola* Possible oncosphere
Snail	5	Larval trematodes
Fiddler Crab	1	No findings
Clams	3	Larval trematodes
Spartina	1	No findings

In conclusion, while we were unable to definitively discover the first intermediate host(s) to *Glossocercus caribaensis* and *Cyclustera ibisae,* it was noted that grass shrimp could be a possible accidental host to these tapeworms. In the future, we would like to return to the North Inlet Estuary in order to collect different prey species, particularly marine oligochaetes, as these worms have been implicated in the life cycle of other closely related cestodes of piscivorous birds.[5] The North Inlet Estuary remains a relatively pristine and healthy marsh ecosystem on the coast of South Carolina, as indicated by the presence of numerous *F. heteroclitus* in the waters, as well as the birds that continue to spread *C. ibisae* and *G. carabaensis* as when these parasites were first reported almost two decades ago.

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