TREMATODE PREVALENCE AS AN INDICATOR FOR GALVESTON MARSH HEALTH

An Undergraduate Research Thesis

by

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ABSTRACT

Trematode Diversity and Prevalence as Indicators for Galveston Marsh Health. (May 2014)

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Digenean trematodes have complex life histories involving several hosts and life stages. They are sensitive to biotic and abiotic factors such as metal pollutants and extreme pH and temperature, possibly making them valuable bioindicators for natural and anthropogenic disturbances. To test this, trematode diversity in the plicate horn snail, *Cerithidia pliculosa*, from four different marsh systems around Galveston, including relatively pristine marshes, marshes heavily impacted by garbage and recently restored marshes, was evaluated to determine whether trematodes can be used locally as a bioindicator for marsh health. The snails from the marsh near East Lagoon a marsh with a high incidence of anthropogenic garbage, had a 92% infection rate with 7 of the 8 species found in this study while the marsh along Highway 45, an old restored marsh, only had a 7% infection rate with 2 of the 8 species found. The third marsh, located near Sportsman's Road in West Galveston Bay had 3 species of parasites with a 33% infection rate. The fourth marsh was excluded from further analysis, as not enough host snails could be collected. The differences in trematode prevalence could be attributed to a number of factors such as recreational fishing activities, pollution, host density and physical properties of the marshes. Further studies are necessary to accurately determine whether trematode diversity in Galveston is a factor of natural or anthropogenic affects.

DEDICATION

I would like to dedicate this to my parents and my fiancé. Without their love and support, I would not have come so far.

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CHAPTER I

INTRODUCTION

Digenean trematodes are parasitic flatworms with complex life cycles that are generalized in Figure 1 (Huspeni et al., 2005). The ciliated swimming stage, miracidium, hatches from an egg in the final host's excrement and swims until finding a molluscan intermediate host where it transforms into either a sporocyst or redia (Schell, 1970). In the sporocyst, germinal cells and flame cells are multiplying and can either result in a daughter sporocyst stage or a redia. From the redia, the cercaria, the tailed swimming stage, emerges to search for a second intermediate host. The second intermediate host can be a range of organisms including crustaceans, fish, mollusks, cnidarians, polychaetes, and turbellarians (Huspeni et al. 2005). Mollusks, primarily gastropods, are a common intermediate host of choice. Because of this close association, Cribb et al. (2001) suggested that trematodes were originally parasites of mollusks and that any other intermediate and final hosts were due to host switching. The intermediate host, along with the established parasites, is eaten by a final host: generally birds, mammals, and fishes (Huspeni et al. 2005). The parasites then reproduce sexually, and the eggs get excreted through the final host's feces, starting the cycle again. This life cycle is highly variable among species of trematodes and stages can be skipped, added, and formed in different hosts as well as different numbers of hosts (Schell, 1970).

Due to the complexity of the trematodes' life cycle and their sensitivity to pollutants, they have begun to emerge as useful bioindicators of free-living diversity (Huspeni et al. 2004). The final host is the source of infectious trematodes, and therefore, trematode abundance is a direct reflection of final host abundance (Huspeni et al. 2005; Smith, 2001). Also, since final hosts will be around areas with a greater abundance of prey, an increase in trematodes reflects prey abundance as well (Huspeni et al. 2005). Trematodes have also been found to be sensitive to heavy metals, toxins, and environmental stressors such as extreme pH, and temperature change (Pietrock and Marcogliese, 2003; Siddall and des Clers, 1994). Due to this sensitivity and requirement of several species as hosts, high abundance of trematodes should reveal a healthy, productive system.

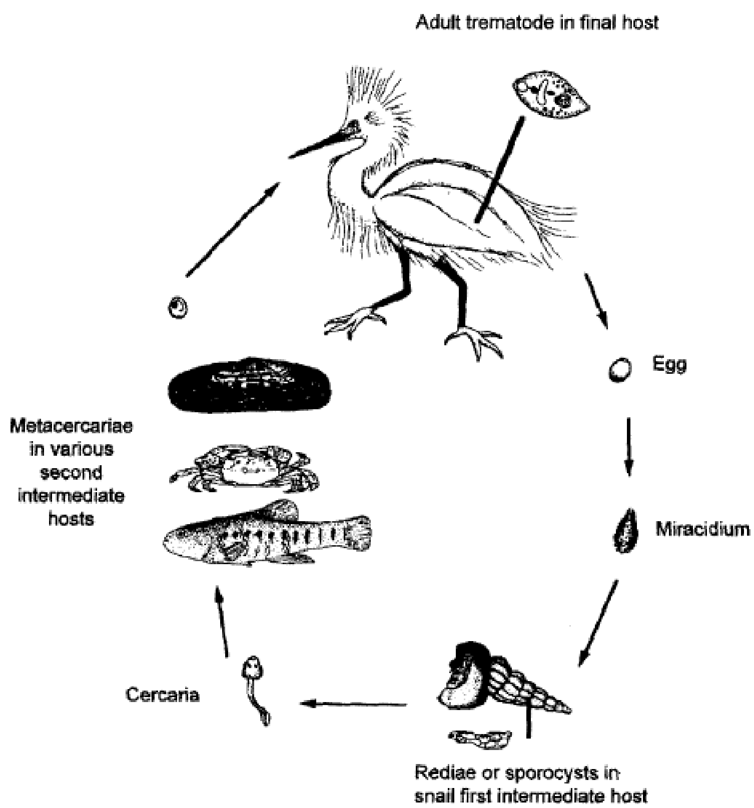

Figure 1. Digenean Trematode Life Cycle as generalized by Huspeni et al. (2005**).** The life cycle is specialized for each species of trematode and may not be necessarily well represented by this one depiction.

Marshes are an important part of the local fisheries as a nursery ground (Boesch and Turner, 1984) and Galveston marshes in particular are home to the commercially important white shrimp, brown shrimp, and blue crab, so their health is important to maintain (Minello and Webb, 1997). It would be helpful, therefore, to develop an inexpensive and accurate indicator of marsh health to monitor Galveston marshes.

For this project, trematode diversity and prevalence in the plicate horn snail, *Cerithidia pliculosa*, was compared among different marsh systems around Galveston, including relatively pristine marshes, marshes heavily impacted by garbage and other pollutants and recently restored marshes, to evaluate whether trematodes can be used locally as a bioindicator for marsh health.

CHAPTER II

METHODS

Study area

Marsh 1, Figure 2A, is south of I-45 and opens into Jones Bay. In this paper it is referenced to as I-45 for brevity, but the marsh is known as the John M. O'Quinn Estuarial Corridor and is part of the Scenic Galveston Preserve Complex. According to Scenic Galveston, it is an important migratory stop for shorebirds and a wintering habitat for grassland birds (http://www.gcbo.org/html/galveston.pdf). Construction on the marsh began in July of 1998, making it about 15 years old, according to the Galveston Bay Information Center (http://gbic.tamug.edu/Loc_pif.ASP?pif=SGX-01). The habitat had several *Spartina* patches and the mud was highly aerated compared to the other marshes in this study. Marsh 2, Figure 2B, was restored in 2007, making it about 7 years old, in order to protect the Isla Del Sol shoreline and benefit a few commercially important species (NOAA CRP Project #5007; http://www.gulfmex.org/archive/crp/5007.html). This marsh had very few patches and a gradual slope. Marsh 3, Figure 2C, at the East End Lagoon receives a lot of fishing, birding, and port traffic but has never been restored or cleaned. There are very few to no *Spartina* patches and it has a very gradual slope and therefore long tide. The Marsh near Sportsman Rd, Marsh 4, Figure 2D, is relatively pristine with a little traffic from fishermen and residents. The Galveston Bay Foundation created a breakwater using reef balls in order to protect the marsh's quickly eroding shoreline (http://galvbay.org/conservation_wetlands_sportsman.html).

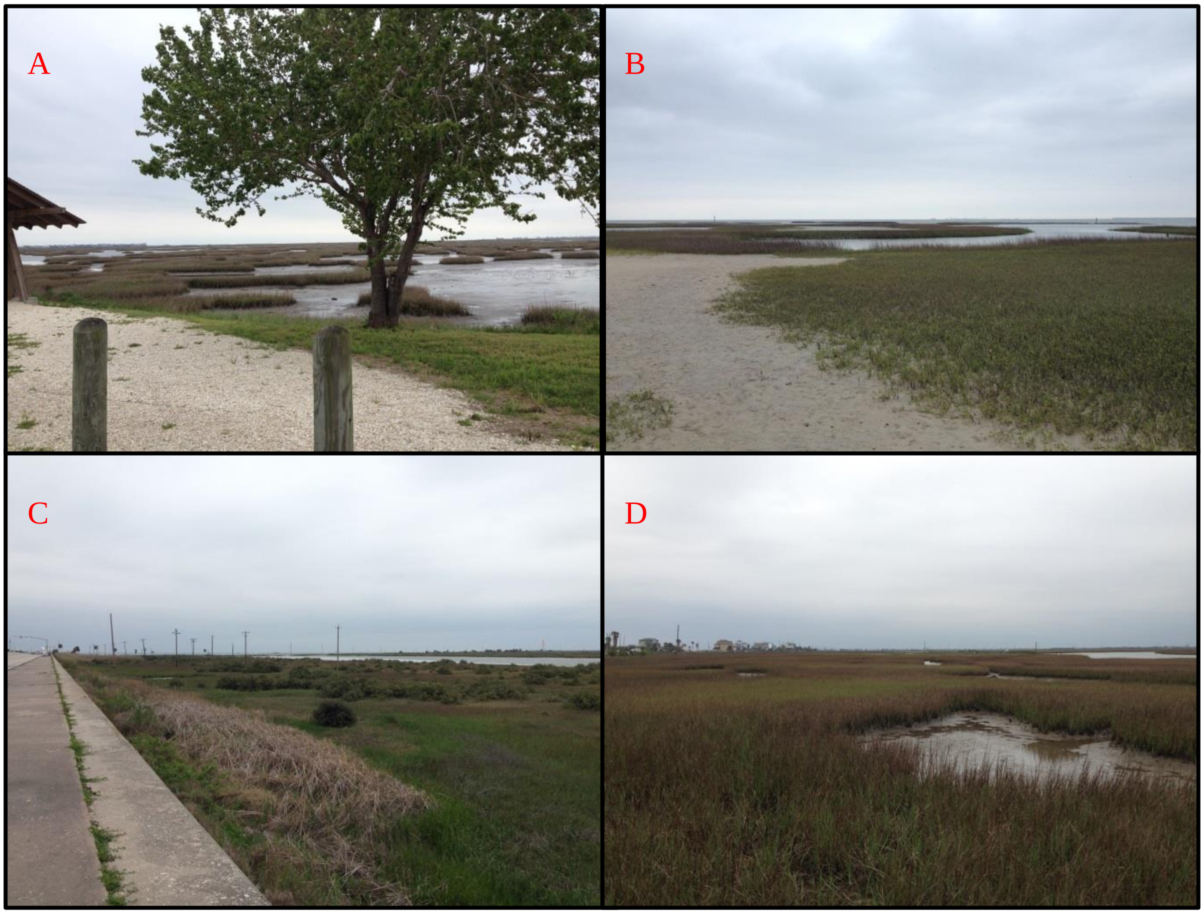

Figure 2. Pictures of the four marsh sites. A) I-45 with the Highway parallel with the parking lot and picnic area. B) Isla Del Sol marsh and the houses would be at the left and out to the right of the picture. C) East Lagoon marsh. Unfortunately, the oil spill caused the lagoon to be inaccessible and is back further where the telephone poles are located. However, this shows that the lagoon is heavily impacted by the port traffic. D) Sportsman Rd marsh with a line of houses to the left. The reef balls are located behind the photographer.

Gastropoda collections

A total of 38 specimens of *Cerithidea pliculosa* were collected from four localities along Galveston Island, seen in Figure 3, generally around the perimeters of marsh grass. They were collected in bowls with a few centimeters of their habitat marsh water and saved without preservatives in order to keep the trematodes within the specimens alive.

Figure 3. Four marshes around Galveston Island where *Cerithidea pliculosa* were collected. Marsh 1 was restored several years ago, Marsh 2 is recently restored, Marsh 3 has been heavily impacted by garbage and pollutants, and Marsh 4 is relatively pristine.

Trematoda collections

After measuring their length, snail shells were broken and removed in order to reveal the snail's underlying tissue. Trematodes generally infect the gonadal region of the snail, but can be found in other tissues, such as the heart and kidney (Huspeni et al. 2005). Specimens from each infected region were collected through pipette and isolated to be viewed with light microscopy and occasionally Hitachi TM 3000 scanning electron microscopy to be morphologically identified using S.C. Schell (1970) and unpublished data by Wardle (1987) and Childs (2009). The identified specimens were then immediately transferred to 10µL Extract-N-Amp solution for DNA extraction in order to confirm the morphological identity. Prevalence was calculated by finding the proportion of snails infected by trematodes.

CHAPTER III

RESULTS

Trematode infections had the highest occurrence with about 92% of snails infected at the most impacted site near East Lagoon, seen in Figure 4. The old restored marsh near I-45 had the lowest prevalence of trematode infections showing only a 7% infection rate. Marsh 2, the recently restored site, was not included in analyses due to the difficulty of finding *Cerithidia pliculosa*. Only a single live snail (infected) was found in the area; however, the presence of *C. pliculosa* shells inhabited by hermit crabs indicates that the snails are at least occasionally present.

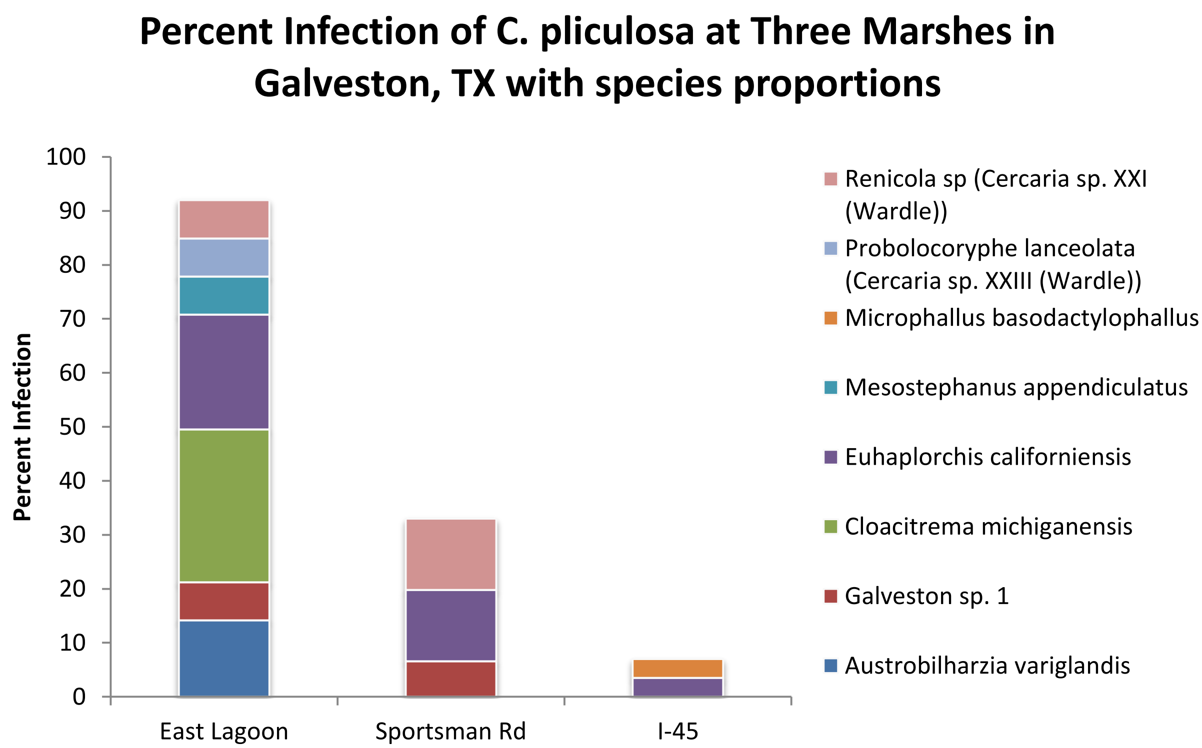

Figure 4. Percent infection of *C.pliculosa* at three localities in Galveston with parasite species composition. A total of 8 species were found, with Highway 45 having one unique species and East Lagoon having four unique species.

Accordingly, East Lagoon also had the most species, with Sportsman Rd. and Highway 45 following. Only one or two double trematode infections were found at each site, although several nematode-trematode double infections were found at East Lagoon and Sportsman Rd. A total of 8 species were found compared to a total of 12 reported species in Galveston (Wardle, 1974) and are introduced in Figure 4 and seen in Figure 5. An unusual species was found, noted as Galveston sp. 1, which did not have the typical cercaria form found in infected snails, but rather had an elongated shape with an extruding proboscis-like feature, Figure 6.

The hosts for the species found were mostly shore birds, although there were a few trematodes that infected humans: *A. variglandis* causes “swimmer’s itch” (Stunkard and Hinchcliffe, 1952) and *E. californiensis* is in the family Heterophyidae which has been shown to infect human intestines and release their eggs, causing blocks and lesions (summarized by Martin, 1950). One other important species, *M. basodactylophallus*, generally uses the commercially important blue crab as an intermediate host, but has also been noted to use snails (Heard and Overstreet, 1983).

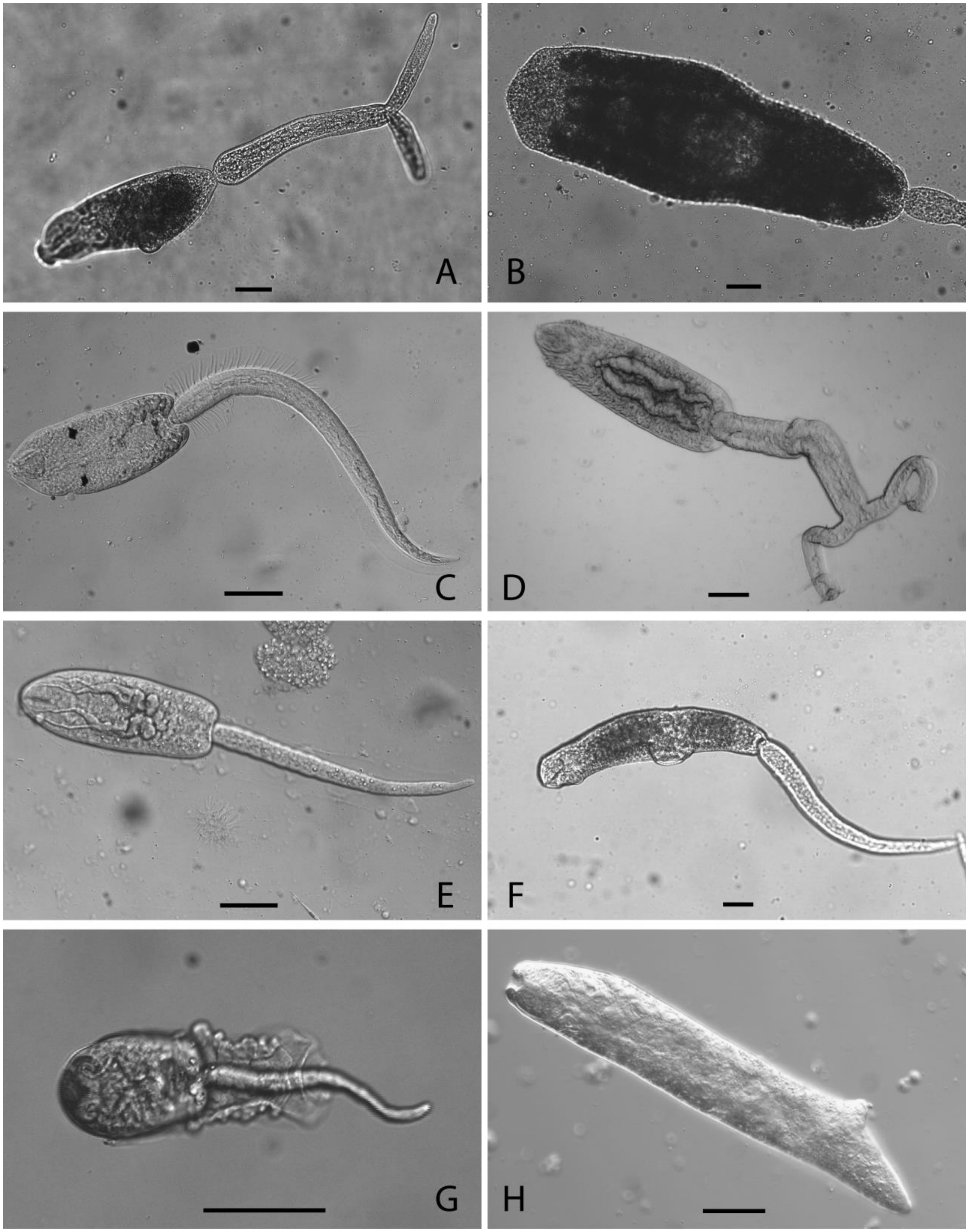

Figure 5. The 7 confirmed species of Trematoda and 1 netmatode found in Galveston, TX. Each scale bar is 50μm. A) *Austrobilharzia variglandis* B) *Cloacitrema michiganensis* C) *Euhaplorchis Californensis* D) *Mesostephanus appendiculatus* E) *Microphallus basodactylophallus* F) *Renicola* sp (Cercaria sp. XXI (Wardle)) G) *Probolocoryphe lanceolata* (Cercaria sp. XXIII (Wardle)) H) Nematode

Figure 6. Galveston Species 1 with a slightly protruding proboscis-like feature (where the black arrow points). These specimens were larger than most species of trematodes.

Table 1. Galveston trematode species and their common intermediate and final hosts.

Species	Intermediate Host	Final Host	Reference
Austrobilharzia variglandis	Marine Snails	Shore Birds and human skin	Grodhaus & Keh, 1958; Stunkard and Hinchcliffe, 1952
Galveston sp. 1	unknown	unknown	
Cloacitrema michiganensis	Marine Snails	Shore Birds	LeFlore, Bass & Martin, 1985
Euhaplorchis californiensis	Fish, Frogs, Marine Snails	Shore Birds and Human Blood vessels	Martin, 1950; Africa, 1938
Mesostephanus appendiculatus	Marine Snails	Shore Birds and occasionally Dogs and Cats	Martin, 1961
Microphallus basodactylophallus	Blue Crab	Racoon	Heard and Overstreet, 1983
Probolocoryphe lanceolata (Cercaria sp. XXIII (Wardle))	Marine Snails	Shore Birds	Smith, 2001
Renicola sp.	Marine Snails	Shore Birds	Stunkard, 1964

Snail lengths show a similar, but slight, trend to infections, with the significantly (p = 0.00) longest snails existing at the East Lagoon marsh and the smallest snails living in the Sportsman Rd. and I-45 marshes, although this is difficult to see with standard deviation, Figure 7. The longer snails were more likely to have an infection, Figure 8, due either to a longer life span (Sousa, 1983) or to gigantism caused by parasitism (Mouritsen and Jensen, 1994). This emphasizes the relationship of East Lagoon's longest snail lengths and higher infection rate. Surprisingly, the longest snails only had 1 infection, while average snails had up to 2 infections.

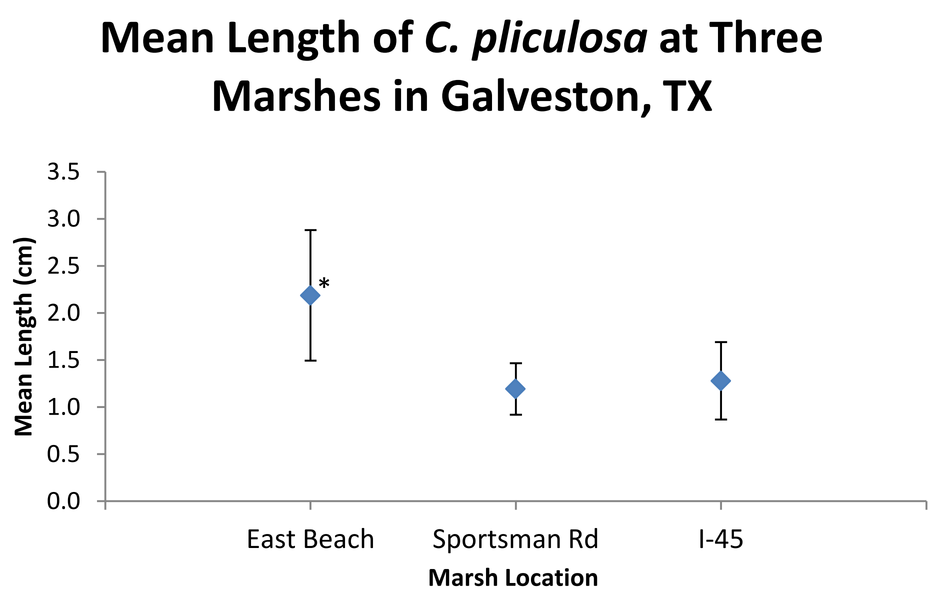

Figure 7. Average shell length of *Cerithidea pliculosa* at the three Galveston marshes shown with Standard Deviation. The star indicates significant difference (East Beach p=0.00, Sportsman Rd and Highway 45 p=0.54).

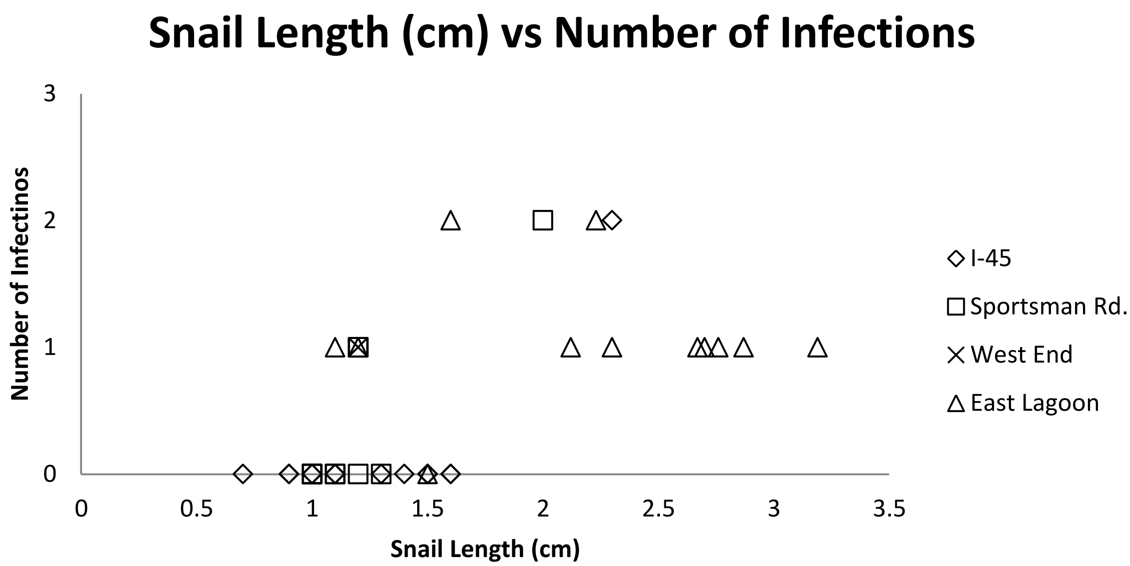

Figure 8. Each snail's length with correlating infections. The longest snails were all from East Lagoon. The one snail found at West End was included in the Data.

CHAPTER IV

DISCUSSION

A high trematode prevalence in an area, in theory, should be an indicator of a healthy marsh system due to their sensitivity to pollutants, environmental changes, and host dependency (Pietrock and Marcogliese, 2003; Siddall and des Clers, 1994; Huspeni et al. 2004); however, this was not obvious in Galveston marshes. The marsh that appeared the most polluted with trash, East Lagoon, had the highest prevalence of trematodes. This brings up an interesting "chicken vs. egg" question though: Is the high trematode prevalence due to a diverse system which would otherwise be healthy except for the anthropogenic traffic good fishing spots bring? Or is the discard and wastes from fishing activities bringing undesirable species such as rats and mice into the environment, also increasing trematode prevalence? *Mesostephanus appendiculatus* is a species that will inhabit dogs and cats as well as shore birds, giving evidence to the last question. However, the overwhelming majority of species inhabit shore birds, giving evidence to the latter. It is still rather difficult to determine host prevalence, though, due to the opportunistic behavior of trematodes, since mollusks are a common intermediate host of choice and any other intermediate and final hosts may be due to host switching (Cribb, et al., 2001). So did *M. basodactylophallus* inhabit a gastropod because there is a high blue crab population and *M. basodactylophallus* is abundant, or because there is a low blue crab population and the single *M. basodactylophallus* could not find a blue crab host and inhabited a snail instead? Population surveys of each of the tested marshes are needed to determine baseline correlations between trematodes and host densities before trematodes can be used further as an indicator species.

Another factor affecting trematode abundance could be gastropod predator abundance. Studies have noted crab predation on gastropods, even if they are not primarily gastropod predators (Sousa, 1993; Behrens and Boulding, 1998). East Lagoon had the significantly longest average shell lengths than any other marsh and had fewer notable crabs while Sportsman Rd marsh had a moderate number of crabs and I-45 had the most crabs present. Further studies would need to quantify the correlation before any significance can be placed on the observation, but since longer snails are more likely to have trematodes than smaller snails, crab populations that control snail sizes or densities will inadvertently control trematode populations. This may mean that in some marsh environments, crabs may be a better indicator species.

In conclusion, further studies are needed to determine usefulness of trematodes as bioindicators for marsh health in Galveston due to the many factors affecting their populations. An interesting study would be to measure trematode prevalence in a grid transect from the highest anthropogenically impacted zone at East Lagoon out to a less impacted area to get a gradient of occurrence and quantify the effect of human impact on trematode populations. Also, surveys of host populations would be important to determine host-trematode dynamics and whether high trematode populations are a direct and accurate indicator of communities. For now, trematodes are not useful bioindicators until further correlations can be determined about their presence in Galveston.

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