

**Parasite component community of Gulf killifish, *Fundulus grandis*, in an oiled Louisiana saltmarsh**

by

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A thesis submitted to the Graduate Faculty of  
Auburn University  
in partial fulfillment of the  
requirements for the Degree of  
Master of Science

Auburn, Alabama  
August 3<sup>rd</sup> 2013

Keywords: biodiversity, component community, ectoparasite, endoparasite,  
Gulf of Mexico, oil spill

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## Abstract

Fish parasites comprise a large portion of marine biodiversity but so far have been underutilized as Gulf of Mexico bioindicators. Shifts in parasite diversity, prevalence, and intensity resulting from the 2010 BP *Deepwater Horizon* Oil Spill (DHOS) could indicate spill-related changes to water quality, abundances and immunological health of free-living organisms, or the Gulf of Mexico food web. Ectoparasites with direct life cycles (no intermediate host or food-web mediated transmission) may be sentinels for acute spill effects, as they are typically small, have high surface area to volume ratios, and remain immersed in seawater. Endoparasites with indirect life cycles (intermediate host[s] required) involving food-web mediated transmission may be sentinels for detecting chronic spill effects, as they reside in a host where they are less vulnerable to toxins and have larvae requiring predator/prey transmission. To test these ideas, one must have a species-level understanding of the parasite component community in a geographic area. Chapter 1 enumerates the parasite component community of Gulf killifish, *Fundulus grandis* (Cyprinodontiformes: Fundulidae) in Barataria Bay, LA. Using those data as a critical baseline and for the units of analysis, Chapter 2 statistically tests for differences in the structure of the parasite component community across 4 oiled sites and 4 reference (non-oiled) sites in Barataria Bay. Regarding the survey and inventory of parasites, the parasite component community includes 44 species (31 endoparasites; 13 ectoparasites) infecting 23 fish tissues. Of these parasite species, 10 are putatively new to science, 24 constitute new host records to *F. grandis*, and nearly all, 42 of 44 (95%), are putatively new locality records in LA. Regarding the

use of those taxonomic units to test hypotheses concerning ecosystem functioning, no significant differences were detected between prevalence (aggregated) of ectoparasites (Monogenoidea, Hirudinida, Copepoda, Branchiura, Isopoda) and endoparasites (Myxozoa, Digenea, Cestoda, Nematoda, Acanthocephala) in oiled and non-oiled sites nor in mean intensity and prevalence (aggregated) of ectoparasites and endoparasites in oiled and non-oiled sites. However, significant differences were detected in species prevalence of acanthocephalans (19.6% in non-oiled sites vs. 2.9% in oiled sites), copepods (21.7% vs. 34.2%), and branchiurans (13.8% vs. 28.3). Seasonal effects were statistically detected in myxozoans (highest intensity of infection during May 2011) and digeneans (highest intensity in August 2011), and those seasonal patterns were not significantly different between oiled and non-oiled sites. Digenean metacercariae infecting gill and heart, monogenoideans infecting skin, and nematodes infecting body cavity each have a significantly higher log mean intensity in oiled sites. Species richness of ectoparasites and endoparasites was not significantly different between oiled and non-oiled sites and across seasons. Condition factor of *F. grandis* was not significantly different between oiled and non-oiled sites and across seasons.

## Acknowledgements

I thank my major professor, Dr. Ash Bullard (Assistant Professor, Auburn University, Department of Fisheries, Auburn, AL), for his encouragement and support and for enthusiastically sharing his knowledge and expertise with me. I thank my committee members, Dr. Cova Arias (Associate Professor, Auburn University, Department of Fisheries, Auburn, AL), and Dr. Chris Anderson (Assistant Professor, Auburn University, Department of Forestry and Wildlife, Auburn, AL) for providing comments concerning my graduate career at Auburn and resulting thesis. This work has been funded by grants from the Marine Environmental Science Consortium of Alabama, Gulf of Mexico Research Institute, and National Science Foundation.

I thank Dr. George Benz (Professor, Middle Tennessee State University, Department of Biology, Murfreesboro, TN) and Mr. Eric Salmon (MSc student, Middle Tennessee State University, Department of Biology, Murfreesboro, TN) for their assistance with field collections. I thank Myron Fischer (Director, Grand Isle Marine Laboratory, LA Department of Wildlife and Fisheries, Grand Isle, LA) and Clint Edds (Biologist, Grand Isle Marine Laboratory, LA Department of Wildlife and Fisheries, Grand Isle, LA) for providing logistical support for field collections.

I thank Andrew Austin, Elizabeth Cameron, Elliot Broder, Raquel Patella, and Stephanie Cox for assisting me with fish necropsies and data collection at Auburn University. I thank my lab mates, Matthew Womble and Andrew McElwain for providing me with transportation during a critical time of need that resulted in the completion of this thesis, and also Raphael Orelis-

Ribiero for his assistance with field collections. I thank Maria Ruiz (mom), Fernando Ruiz (dad), David and Dominick Ruiz (brothers), and Cameron Ruiz (sister), for their love and support.

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## Chapter 1

### **BIODIVERSITY SURVEY AND INVENTORY OF METAZOAN PARASITES OF *FUNDULUS GRANDIS*, BAIRD AND GIRARD, 1853, (CYPRINODONTIFORMES: FUNDULIDAE) IN BARATARIA BAY, LOUISIANA**

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## **1.1. Abstract**

No systematic survey of the parasite community infecting Gulf killifish, *Fundulus grandis*, has been published to date. Herein, I present a biodiversity survey and inventory of the parasite component community that infects *F. grandis* in Barataria Bay, LA. Parasites were collected from 480 individual *F. grandis* and identified from four seasonal collection events in order to capture as much parasite biodiversity as possible, while also accounting for seasonal fluctuations in parasite biodiversity. The parasite component community of *F. grandis* documented from Barataria Bay consisted of 44 species (31 endoparasites; 13 ectoparasites) that infected 23 fish tissues. Of these parasite species, 10 are putatively new to science and are not presently named in the primary literature, 24 constitute new host records to *F. grandis*, and nearly all, 42 of 44 (95%), are putatively new locality records for Louisiana, north-central Gulf of Mexico west of Mobile Bay. The present study documents how poorly understood the parasite component community of *F. grandis* was previous to the 2010 BP *Deepwater Horizon* Oil Spill. In addition to enhancing our knowledge of the parasite biodiversity that exploits *F. grandis*, the present data comprise a critical baseline for future studies that document potential changes to the parasite community in Barataria Bay and, by extension, the Gulf of Mexico food web subsequent to an anthropogenic disturbance.

## **1.2. Introduction**

### **1.2.1. Barataria Bay and the 2010 BP *Deepwater Horizon* Oil Spill**

My study employs a systematic survey of the parasite component community that infects Gulf killifish, *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in Barataria Bay, LA in order to test ecosystem functioning (food-web health) after the 2010 BP *Deepwater Horizon* Oil Spill (DHOS). Approximately 206 million gallons (Ramseur and

Hagerty, 2013) of light-sweet crude oil flowed from Canyon Block 252 of the Macondo Well from April 20<sup>th</sup> to July 15<sup>th</sup> 2010 (Liu et al., 2011). Media coverage of oil-laden and purportedly diseased wildlife exacerbated concerns that Gulf of Mexico resources were negatively affected by the spill, which impacted the socioeconomics of coastal communities (Safford, 2012) and prompted immediate action by state and federal agencies and scientists along the northern Gulf of Mexico. In Louisiana, Bay Jimmy, the eastern portion of Barataria Bay, was one of the most heavily oiled regions of the Gulf of Mexico after the DHOS (Lin and Mendelsohn, 2012) (see Chapter 1.5.1.) while the western portion, Hackberry Bay, was relatively untouched by surface oil. The unique distribution of surface oil in Barataria Bay has allowed researchers to use this region of the Gulf of Mexico as a center for ecological hypothesis testing after the DHOS.

The dominant species of marshgrass in Barataria Bay is smooth cordgrass, *Spartina alterniflora* Loisel, (Plantae: Poaceae) followed by needlegrass rush, *Juncus roemerianus* Scheele, (Plantae: Juncaceae) (see Lin and Mendelsohn, 2012). Recent data have been published by Lin and Mendelsohn (2012) showing that oil persisted in Bay Jimmy sediments seven months after oil hit the shoreline and caused acute mortality (kill-backs) of both marshgrass species in heavily oiled sites compared to reference sites in Hackberry Bay. However, the majority of mortality was documented on fringing marsh that also showed signs of recovery after seven months, i.e., increased growth/shoot biomass. Another study, also utilizing oiled sites in Bay Jimmy and reference sites in Hackberry Bay, similarly documented acute mortality and resilience of *S. alterniflora* and reported but did not quantify mortality of invertebrates including snails and oysters (Silliman et al., 2012). (Noteworthy but beyond the scope of this thesis is that Silliman et al. [2012] showed that the loss of saltmarsh habitat was not only a result of oiled conditions but was often irreversibly exacerbated by the effects of

subsidence and substrate erosion resulting from channelization of the Mississippi River Delta). McCall and Pennings (2012), however, showed that abundances of a common marsh snail, *Littoraria* Griffith and Pidgeon, 1834, (Gastropoda: Littorinidae) in heavily oiled sites in Barataria Bay were not significantly different from those in reference sites. The results of these studies are significant with regard to the ecology and subsequent health of *F. grandis*, which utilizes the saltmarsh habitat for its entire life history and is sympatric with other organisms like snails that are all part of the Gulf of Mexico food web.

### **1.2.2. The Gulf killifish, *Fundulus grandis***

The Gulf killifish is an abundant and non-vagile fish species that is a year-round resident of saltmarshes in the Gulf of Mexico (Lotrich, 1975; Subrahmanyam and Coultas, 1980; Rozas and Reed, 1993; Rozas and Zimmerman, 2000; Teo and Able, 2003). The species is commercially valued as bait for artisanal fishers, and is a keystone species that serves as an important food item for piscivorous birds and fishes that utilize saltmarsh habitats (Tatum et al., 1982). Eco-toxicological studies have been conducted using a cognate species, *Fundulus heteroclitus* (Linnaeus, 1776), (Cyprinodontiformes: Fundulidae) on the east coast of the United States. *F. heteroclitus* has been shown to serve as a model vertebrate sentinel in eco-toxicological studies (Burnett, et al., 2007; Van and Nacci, 2008). A single study documenting the putative genomic and physiological effects of the DHOS has been published for *F. grandis* in Barataria Bay (Whitehead, 2010). This study concluded that divergent gene expression was predictive of hydrocarbon exposure and indicated physiological and reproductive impairment in *F. grandis*.

Since *F. grandis* and *F. heteroclitus* share a recent ancestry and have similar physiologies and life history (Weisburg and Lotrich, 1982; Able and Hata, 1984; Rozas and Laselle, 1990; Kneib, 1997; Nordlie, 2006; Whitehead, 2010), *F. grandis* can serve as a model vertebrate sentinel for

ecosystem health in the Gulf of Mexico. Furthermore, *F. heteroclitus* apparently harbors a rich parasite community compared to what has been reported to infect *F. grandis* in the Gulf of Mexico (Harris and Vogelbein, 2006). Previous to this study, the parasites that exploit *F. grandis* were poorly known.

### **1.2.3. Parasite component community characterization**

A “parasite component community” is all parasite species that infect a host population (Bush et al., 1997). The taxonomic identity and respective abundances of all parasite species are required to sufficiently characterize a parasite component community. A parasite component community is distinct from “species richness”, which has a broader definition as the total number of parasite species in a sample (Bush et al., 1997). Parasite component communities can be further characterized by recording intensity of infection, which is the number of individuals of a parasite species infecting a single host (Margolis et al., 1982; Bush et al., 1997). “Mean intensity” is the average number of a parasite species divided by the number of infected hosts (Bush et al., 1997). Mean intensity is a parasitological measurement that demonstrates abundance of a parasite species in a sample of infected hosts only; this measurement does not inform how often a parasite infects a conspecific host population.

Prevalence is the number of individual hosts within a susceptible population infected by a parasite species divided by the host sample size (Bush et al., 1997). Prevalence tells us what percentage of a host population is infected by a parasite species; this is a proportion of infected to non-infected hosts and measures how often a parasite species infects a host population.

A parasite component community is also distinct from but is linked to parasite “biodiversity”, which is a concept used by ecologists to describe community composition in terms of the number and “types” of species that are present in an ecosystem (Bush et al., 1997). With regard to

parasite life cycles, there are two “types” of parasites that can comprise a component community: parasites having direct life cycles (requiring a single host to produce offspring; typically ectoparasites that are transmitted in the water column or by direct contact between hosts) and those having indirect life cycles (requiring multiple hosts to produce offspring; typically endoparasites that are trophically transmitted). In this study, parasite types were identified based on morphology and also delineated based on sites of infection, which is indirectly related to life cycle and thereby facilitated identification of species that comprised the parasite component community of *F. grandis* in Barataria Bay, LA.

### **1.3. Justification**

The proper taxonomic identification and parasite community characterization (Chapter 1) is the foundation of all subsequent ecological hypotheses concerning the DHOS (Chapter 2). The parasite component community of *F. grandis* in the Gulf of Mexico, in addition Barataria Bay, has yet to be systematically surveyed such that the result is a species-specific taxonomic listing of parasites.

### **1.4. Objective and hypothesis**

*Objective I:* Survey (over 12-months) the metazoan parasite component community of *Fundulus grandis* in Barataria Bay, Louisiana.

*Hypothesis I:* The parasite component community has been completely characterized in the published literature, i.e., no novel infections are present nor will be detected.

### **1.5. Materials and methods**

#### **1.5.1. Site selection**

Barataria Bay is located 170 km W/NW of the MC-252 Macondo well. As previously stated, the eastern portion, Bay Jimmy, was heavily oiled by the DHOS while the western portion,

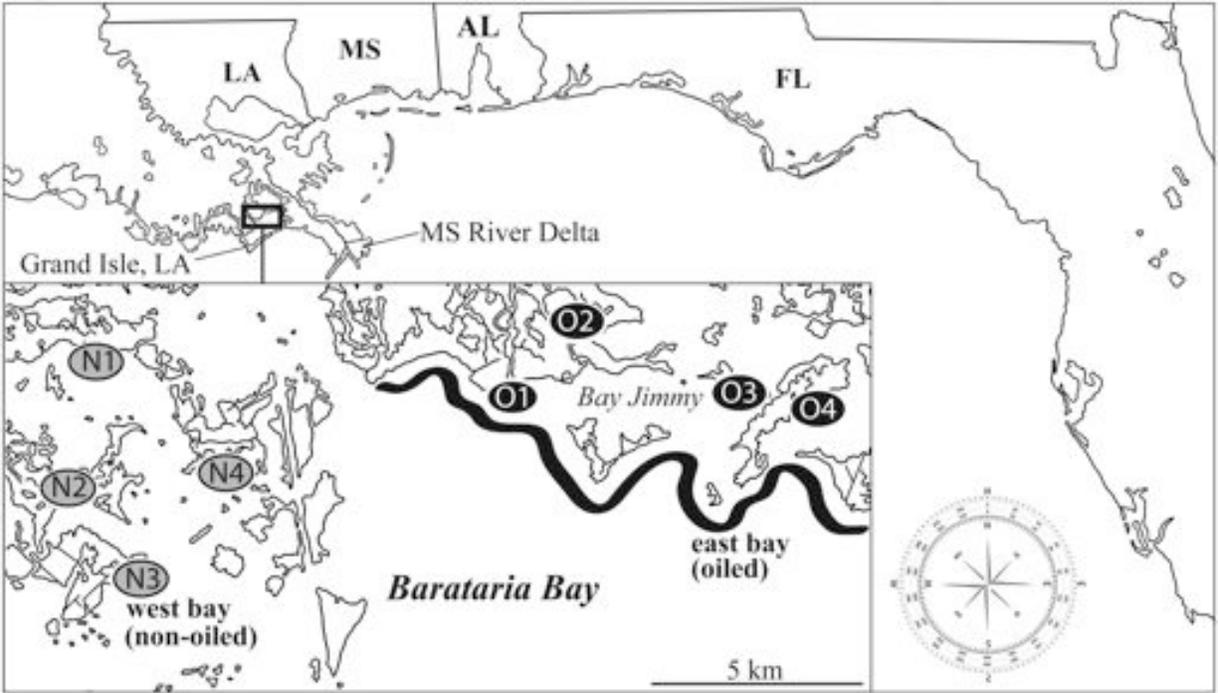
Northern Hackberry Bay, was relatively untouched by surface oil. Observations of the presence of surface oil in Barataria Bay have been continually monitored by Louisiana Department of Wildlife and Fisheries and logged in the government regulated public web-page known as the *Geospatial Platform*) (<http://www.geoplatform.gov/gulfresponse/>). Based on these data, four BP *Deepwater Horizon* oiled sites (O1: Wilkonson Canal, 29°27'33.1"N / 89°57'03.9"W; O2: Wilkonson Bay, 29°28'09.2"N / 89°56'17.6"W; O3: Wilkonson Bayou, 29°27'16.0"N / 89°53'50.6"W; O4: Northern Bay Jimmy, 29°27'28.0"N / 89°53'27.3"W) and four non-oiled reference sites (Northern Hackberry Bay, N1: 29°27'56.2"N / 90°02'07.9"W; N2: 29°26'17.5"N / 90°02'30.8"W; N3: 29°25'35.8"N / 90°01'54.0"W; N4: 29°26'35.7"N / 90°00'20.9"W) were designated for the collection of *F. grandis* in Barataria Bay (Plate 1). Clint Edds, Fisheries Biologist of LDWF, aided with navigation to and between sites.

To further validate sites, three collaborative collections (20–21 Aug 2011; 28–29 Oct 2011; 9–10 May 2012), made possible by GoMRI-RFPIII funding, permitted the sampling of sediment that was subsequently analyzed for polyaromatic hydrocarbon (PAH) concentrations in one heavily oiled (O4) and one non-oiled (N1) reference site. Dr. Mike Unger (Associate Professor, Virginia Institute of Marine Science, Gloucester Point, VA) obtained preliminary results using gas chromatography-mass spectrometry analysis and showed a significantly higher PAH concentration in the O4-heavily oiled site. These data are unpublished and were obtained by my advisor, Dr. Bullard, through correspondence with Dr. Unger.

### **1.5.2. Fish collection**

100 individuals of *F. grandis* were captured per site over four collection events (n = 3,200) (Collection event I: 16–19 Oct 2010; Collection event II: 25–27 Feb 2011; Collection event III: 10–11 May 2011; Collection event IV: 20–21 Aug 2011) using up to 15 baited minnow traps

**PLATE 1.** Oiled (O1–O4) and non-oiled (N1–N4) study sites in Barataria Bay, Louisiana.



(per site) that were set closely apposed to the edge of marsh grass dominated by *S. alterniflora*. Traps were set in 1-hr intervals at approximate depths of 12–22 cm. Catch from each site was separated into aerated and site-labeled buckets before transporting to the Grand Isle Marine Laboratory (LDWF) for subsequent tissue fixation.

### **1.5.3. Tissue fixation**

Each fish was abdominally injected with 10% formalin and submersed in a whirl-pak filled with 10% formalin and labeled with the collection site and collection date before subsequent necropsy at the Aquatic Parasitology Laboratory in Auburn, Alabama.

### **1.5.4. Laboratory Necropsy**

Fifteen fish per site per collection event (480 total fish, 240 oiled and 240 non-oiled) were randomly selected (blindly hand-drawn from a shaken bucket) for necropsy and parasite collection. The wet weight (g), standard length (mm), and total length (mm) were recorded for each fish. A host number corresponding to the collection site and collection event was assigned to each fish.

A stereo dissecting microscope (Meiji RZ 3288) fitted with a digital camera and a fiber-optic light source was used to examine external infection sites (buccal cavity, eye, fins, gill filaments, skin) and internal sites (body cavity, brain, fat, fin rays, gall bladder, gill lamellae, gonad, heart, intestine, kidney, liver, mesentery, peritoneum, pseudobranch, somatic muscle, stomach, swim bladder, and urinary bladder) for the presence of major metazoan ectoparasite (Monogenoidea, Copepoda, Branchiura, and Hirudinida) and endoparasite (Myxozoa, Digenea, Acanthocephala, Cestoda, and Nematoda) groups. Major parasite groups were identified based on external morphology and life history information found in Roberts and Janovy (2005) (see Chapter 1.5.6. below).

To facilitate parasite species identifications, infections were photographed *in situ* before parasites were excised from tissues, wet-mounted, and photographed at high magnification using a compound light microscope (Leica DM 2500) fitted with a digital camera and equipped with differential interference contrast (DIC) optics. Parasites and infected tissues were collected and stored in cryovials filled with 10% neutral buffered formalin for species identification. All collected parasites and infected tissues were assigned a unique accession number that corresponded with the host number and collection event. Intensity data for each parasite and sites of infection were recorded on a standardized data sheet, photocopied, and inventoried in a host-parasite matrix (Microsoft Excel).

Examination of all external infection sites preceded examination of internal sites. All fins were removed from the body and examined individually. To better inspect the buccal cavity, each operculum including all components of the syncranium was removed to expose the entire branchial basket. All 8 gill arches were excised as a single unit and the gill lamellae, filaments, and arches were grossly examined for ectoparasites before isolating the second gill arch for quantification of Myxozoa, Digenea (metacercariae), and Monogenoidea.

The viscera of each fish was grossly examined for the presence of endoparasites by first exposing the body cavity from the sinistral side of the fish. The visceral mass was removed and all organs were excised and examined separately from the digestive system, mesentery, and fat. Using a scalpel, a thin section of somatic muscle was sampled from the dextral side of the body. When infections were not grossly conspicuous, entire liver, head kidney, gonad, pseudobranch, gill, spleen, somatic muscle, and brain were compressed and examined using glass plates (0.64 cm thickness) and a dissecting microscope. The heart, including the bulbous arteriosus, was removed from the pericardial sac and the epicardium and compact myocardium were extensively

searched for endoparasites including blood flukes. Gall bladder, urinary bladder, swim bladder, and peritoneum of the body cavity were also excised and examined separately.

#### **1.5.5. Parasite specimen selection and preparation**

Inventoried parasites were sorted using Microsoft Excel according to major metazoan parasite groups and sites of infection. At least 3 representative specimens of each major metazoan parasite group from 23 sites of infection were selected across collection events from both oiled and non-oiled reference sites for identification. Parasite specimens were prepared for subsequent identification as follows: Myxozoan plasmodia were cleaned of host tissue, rinsed in deionized water, dehydrated with a graded ethanol-series, cleared in clove oil, and whole-mounted in Canada balsam. Digenean metacercariae were cleaned of host tissue, excysted, and rinsed in deionized water before wet-mounting. Specimens of Monogenoidea were rinsed in deionized water, cleared, and whole-mounted under slight cover-slip pressure directly in Gray and Wess medium from 10% nbf without dehydration. Cestode larvae were cleaned of host tissues, excysted, and rinsed in deionized water before wet-mounting. Nematode larvae were cleaned and rinsed of encapsulations in deionized before wet-mounting. Acanthocephalans were cleaned of host tissue before wet-mounting under slight coverslip pressure. Hirudinida, Copepoda, Branchiura, and Isopoda were rinsed in deionized water before wet-mounting.

#### **1.5.6. Parasite identification**

Parasites were identified to the lowest taxonomic level by comparing representative specimens to published species descriptions of parasites that infect species of *Fundulus* (Hahn, 1915, 1917a, 1917b; Davis, 1917; Kudo, 1918, 1920; Van Cleave, 1947; Chandler, 1935; Bond, 1937, 1938; Mueller, 1937; Bangham, 1940; Fantham, et al., 1940; Meglitsch, 1947; Martin, 1950; Martin, 1953; Hargis, 1955; Rigdon and Hendricks, 1955; Bullock, 1957; Sogandares-

Bernal and Lumsden, 1963; Sparks, 1960; Bullock, 1966; Mizelle and Kritsky, 1967; Abbott, 1968; Golvan, 1969; Rogers, 1969; Roberts, 1970; Williams and Rogers, 1971; Schmidt, 1973; Billeter, 1974; Kinsella and Heard, 1974; Williams and Gaines, 1974; Williams, 1980; Wiles, 1975; Fusco and Overstreet, 1978; Williams, 1980; Murith and Beverley-Burton, 1985; Overstreet et al., 1985; Kabata, 1986; Dyková et al., 1994; Billeter, et al., 2000; Akaishi et al., 2004; Eiras, Molnár, and Lu, 2005; King and Cone, 2009). Key diagnostic characters that matched those described in published literature were documented in order to demonstrate each putative species. This is distinct from a taxonomic description, which requires replicated morphometrics of all external and internal anatomical structures that are used to diagnose a genus plus a description of features that are unique to the species. A taxonomic description also requires that redescribed or newly described species are deposited as museum type material. Ideally, redescribed or newly described species are compared to museum type material of all species within the genus. These collective requirements for taxonomy result in a differential diagnosis that is often termed “Remarks” in published species descriptions. I did not deposit specimens and did not compare any specimens identified in this thesis to museum type material, thus, an in-depth differential diagnosis of species is beyond the scope of this thesis.

Alternatively, I provided remarks that diagnose each putative species and highlighted those species that are putatively new to science or are putatively new host or locality records. Species new to science are those parasite specimens that could not be matched with any other species description and may represent undiagnosed and unnamed species. Parasite specimens with three or more apparent morphological or morphometric differences not comparable to published species descriptions were also designated as putatively new species. Some species herein have been designated with the genus followed by “cf.” followed by the specific epithet. This means,

“compare to” and corresponds to a species description that closely matches the specimen, but the specimen bears one or two features that the taxonomic authority does not mention.

The major metazoan parasite groups infecting *F. grandis* were of the following phyla: Myxozoa (cnidarian endoparasites; see Siddall et al., 1995), Platyhelminthes including Digenea (endoparasitic flatworms), Monogenoidea (ectoparasitic flatworms), Cestoda (tapeworms), Phylum Nematoda (roundworms), Phylum Acanthocephala (spiny-headed worms), Phylum Annelida (leeches), and Phylum Arthropoda including Copepoda, Branchiura, Isopoda (ectoparasitic crustaceans). Myxozoan spores were identified based on spore shape, number of filament coils in polar capsules, presence or absence of a vacuole or dense bodies within the sporoplasm, and external spore features. Spores were bio-illustrated at high magnification using a compound light microscope equipped with a drawing tube (10 ocular units, 100X objective, 2X magnifier, DIC). Digenea were identified using body shape and morphology of oral suckers. Photomicrographs of isolated cysts, excysted whole-body specimens, and armed or unarmed oral suckers facilitated digenean identifications. Current digenean taxonomy is primarily based on adult specimens and thus the identification of most digeneans to a named species was not possible. Monogenoidea were identified according to the morphology of calcified structures associated with the haptor (posterior attachment organ). Haptors of each species were bio-illustrated using a compound light microscope equipped with DIC and a drawing tube. Cestode larvae were identified according to external morphology of the scolex (anterior attachment organ). Host records and sites of infection mostly facilitated nematode larvae identifications. Since key morphological characters for fish cestodes and nematodes are mainly described from adult specimens in the primary literature, the identification of cestode and nematode larvae to the level of species was not possible in this study. Acanthocephala were identified according to

morphology of the proboscis (anterior attachment organ). This feature was bio-illustrated using a compound light microscope equipped with a drawing tube. Hirudinida were identified based on body shape and morphology of caudal and oral suckers. Copepoda were identified by studying the morphology of second antennae. Branchiura were identified based on body shape and morphology of the base of the second maxilla (ventral). Bio-illustrations of copepod second antennae and branchiuran second maxillae were facilitated with a compound light microscope equipped with a drawing tube. A single isopod specimen was identified according to dimensions of thoracic segments.

#### **1.5.7. Reporting of taxonomic authorities and putatively new species**

Wherever discussed in this thesis and as outlined in the “International Code of Zoological Nomenclature” the taxonomic authority of parasites will follow immediately after the italicized genus and specific epithet; e.g., *Fundulotrema prolongis* (Hargis, 1955) Kritsky and Thatcher, 1977, (Monogenoidea: Gyrodactylidae). The latter is an example of a species that was described by Hargis (1955) as a species of *Gyrodactylus*, but was then reassigned to *Fundulotrema* by Kritsky and Thatcher (1977). Similarly, the taxonomic authority of fish hosts will follow immediately after the italicized genus and specific epithet; e.g., *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae). Some host species will bear taxonomic authorities that are enclosed in parentheses, which signifies that the species was originally described under another genus and the original authority and year of publication have not been determined from the literature (see Nelson et al., 2004); e.g. *Fundulus heteroclitus* (Linnaeus, 1766), (Cyprinodontiformes: Fundulidae). For both parasites and fishes, the taxonomic authority will follow the species when first mentioned in the text in each chapter of this thesis. To mitigate confusion in the text, I have included the word “see” following any literature reference that is not

a taxonomic authority; e.g., *Kudoa* cf. *funduli* (see Dyková et al., 1994).

Wherever discussed or demonstrated in this thesis, putatively new species are denoted with “n. sp.” following the taxon (putative genus). Parasites that could not be identified to the level of species and may represent species that have already been described are denoted by “sp.” followed by a sequential number if multiple species were identified to the same taxonomic level (e.g. Bucephalidae sp. 1, Bucephalidae sp. 2, etc.).

## **1.6. Results**

Diagnostic characters, sites of infection, and literature references regarding the taxonomy of 7 species of Myxozoa, 15 species of Digenea, 5 species of Monogenoidea, 2 species of Cestoda, 4 species of Nematoda, 3 species of Acanthocephala, 2 species of Hirudinida, 3 species of Copepoda, 2 species of Branchiura, and 1 species of Isopoda are presented below (Table 1).

### **1.6.1. Myxozoa**

#### **Multivalvuida**

##### **Kudoidae**

###### ***Kudoa* Meglitsch, 1947**

###### ***Kudoa* cf. *funduli* (Hahn, 1915) Meglitsch, 1947 (Plate 2)**

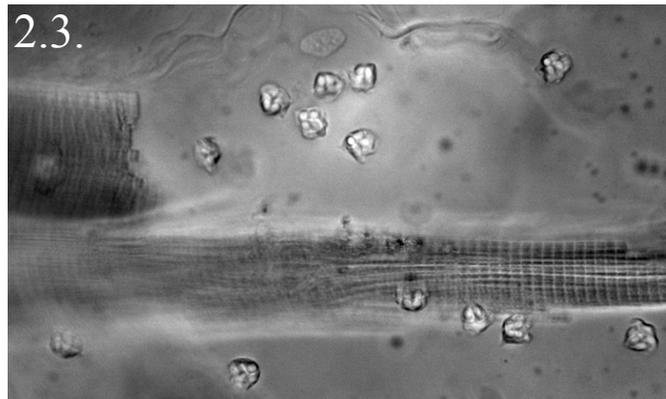
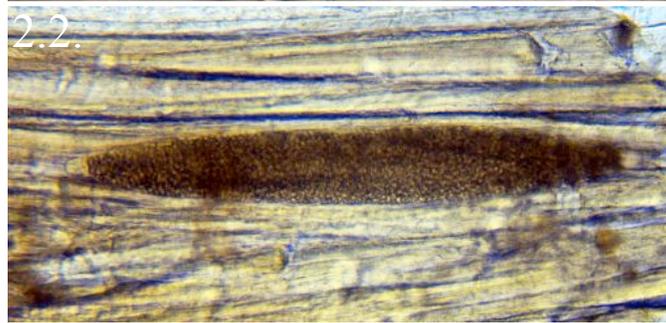
SITE(S) OF INFECTION: Somatic musculature of flank (Fig. 2.1, 2.2).

DIAGNOSTIC CHARACTER(S): Spore masses elongate. Spores quadrate with anterior and posterior ends slightly pointed in sutural view, with 4 polar capsules. Polar capsules with unknown number of filament coils (Fig. 2.3, 2.4).

REFERENCE(S): Meglitsch, 1947; Meglitsch et al., 1948; Dykova et al., 1994; Moran et al., 1999; Akaishi et al., 2004; Blaylock et al., 2004.

#### **Bivalvuida**

**PLATE 2. Figures 2.1–2.4.** *Kudoa cf. funduli* (Hahn, 1915) Meglitsch, 1947 (Myxozoa, Multivalvuvida, Kudoidae) infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. **Fig. 2.1.** Low magnification view of elongated spore mass encysted in somatic muscle (75X). **Fig. 2.2.** High magnification view of spore mass (10 ocular units, 20X objective). **Fig. 2.3.** Wet-mounted quadrate spores from ruptured spore mass (10 ocular units, 100X oil immersion objective, DIC). **Fig. 2.4.** High magnification view of wet-mounted spores (10 ocular units, 100X oil immersion objective, 2X magnifier, DIC).



## **Myxobolidae**

### ***Myxobolus* Bütschli, 1882**

#### ***Myxobolus* n. sp. 1 (Plate 3)**

SITE(S) OF INFECTION: Gill lamellae (Fig. 3.1).

DIAGNOSTIC CHARACTER(S): Spores pyriform, rarely with caudal process, with 3–6 posterior-sutural bumps, variably with 2 posterior ridges. Polar capsules with 7–8 filament coils (Fig. 3.2).

REFERENCE(S): Hahn, 1915; Kudo, 1920; Bond, 1938; Fantham et al., 1940; Landsberg and Lom, 1991.

#### ***Myxobolus* n. sp. 2 (Plate 3)**

SITE(S) OF INFECTION: Skin beneath scales (Fig. 3.3).

DIAGNOSTIC CHARACTER(S): Spores pyriform with 3 posterior-sutural bumps. Polar capsules with 5 filament coils. Sporoplasm with dense bodies at posterior ends of polar capsules (Fig. 3.4).

REFERENCE(S): Hahn, 1915; Kudo, 1920; Bond, 1938; Fantham et al., 1940; Landsberg and Lom, 1991.

#### ***Myxobolus* n. sp. 3 (Plate 3)**

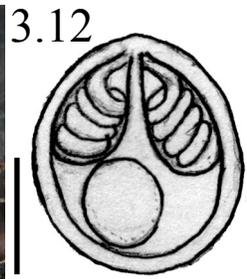
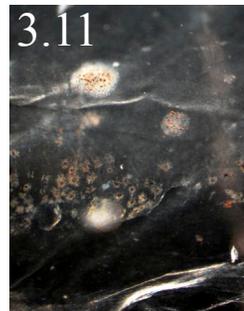
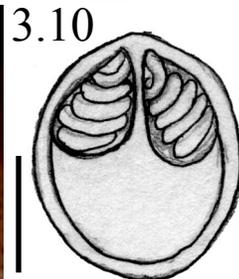
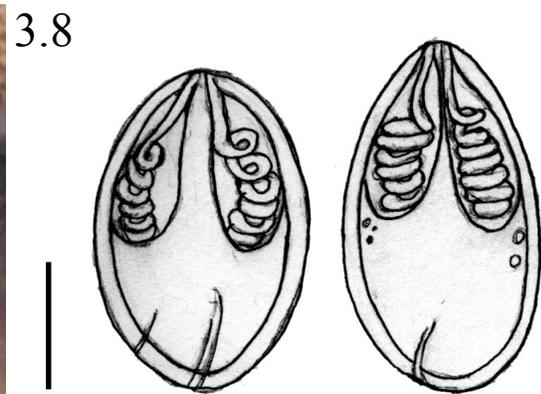
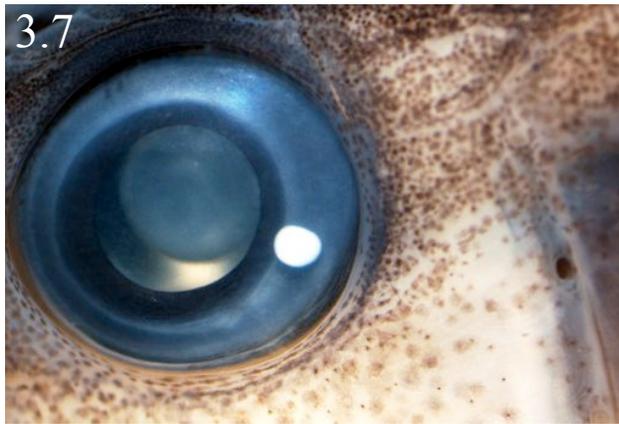
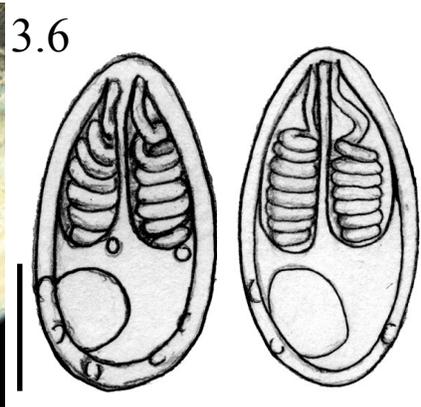
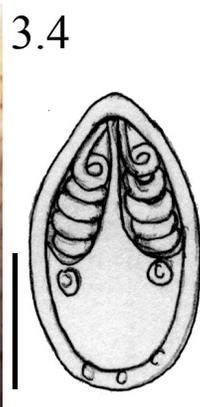
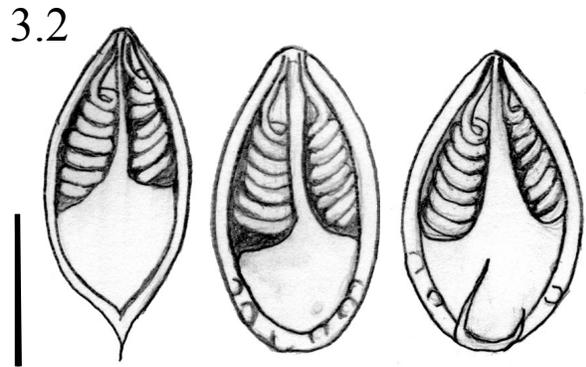
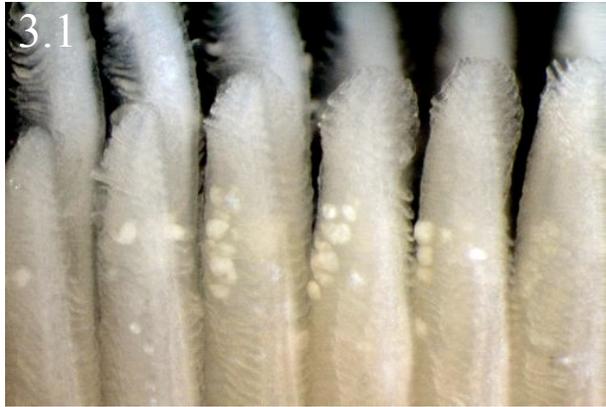
SITE(S) OF INFECTION: Branchiostegal skin (Fig. 3.5).

DIAGNOSTIC CHARACTER(S): Spores pyriform. Polar capsules with 7–8 filament coils. Sporoplasm with conspicuous vacuole and 2 dense bodies (Fig. 3.6).

REFERENCE(S): Hahn, 1915; Kudo, 1920; Bond, 1938; Fantham, Porter, and Richardson, 1940; Landsberg and Lom, 1991.

#### ***Myxobolus* n. sp. 4 (Plate 3)**

**PLATE 3. Figures 3.1–3.12.** *Myxobolus* spp. (Myxozoa: Bivalvuvida: Myxobolidae) infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. All scale bars equivalent to 5  $\mu$ m. **Fig. 3.1.** Low magnification view of *Myxobolus* sp. 1 spore masses in gill lamellae (20X). **Fig. 3.2.** *Myxobolus* sp. 1 spore types (n=3). **Fig. 3.3.** Low magnification view of *Myxobolus* sp. 2 spore masses in skin beneath scales (7.5X). **Fig. 3.4.** *Myxobolus* sp. 2 spore. **Fig. 3.5.** Low magnification view of *Myxobolus* sp. 3 spore masses in branchiostegal skin (15X). **Fig. 3.6.** *Myxobolus* sp. 3 spore types (n=2). **Fig. 3.7.** Low magnification view of *Myxobolus* sp. 4 in skin of eye (15X). **Fig. 3.8.** *Myxobolus* sp. 4 spore types (n=2). **Fig. 3.9.** Low magnification view of *Myxobolus* sp. 5 embedded at base of pectoral fin (7.5X). **Fig. 3.10.** *Myxobolus* sp. 5 spore. **Fig. 3.11.** Low magnification view of *Myxobolus* sp. 6 in swim bladder (25X). **Fig. 3.12.** *Myxobolus* sp. 6 spore.



SITE(S) OF INFECTION: Skin of eye (Fig 3.7).

DIAGNOSTIC CHARACTER(S): Spores ovoid or pyriform with 1–2 posterior ridges. Polar capsules with 6–7 filament coils (Fig. 3.8).

REFERENCE(S): Hahn, 1915; Kudo, 1920; Bond, 1938; Fantham et al., 1940; Landsberg and Lom, 1991.

***Myxobolus n. sp. 5* (Plate 3)**

SITE(S) OF INFECTION: Base of pectoral fins (Fig. 3.9).

DIAGNOSTIC CHARACTER(S): Spore masses robust and spherical. Spores sub-circular. Polar capsules with 5 filament coils. Sporoplasm without vacuole (Fig. 3.10).

REFERENCE(S): Hahn, 1915; Kudo, 1920; Bond, 1938; Fantham et al., 1940; Landsberg and Lom, 1991.

***Myxobolus n. sp. 6* (Plate 3)**

SITE(S) OF INFECTION: Swim bladder (Fig. 3.11).

DIAGNOSTIC CHARACTER(S): Spore masses diffusely rounded in tissue. Spores sub-circular. Polar capsules with 4 coils. Sporoplasm with conspicuous vacuole (Fig. 3.12).

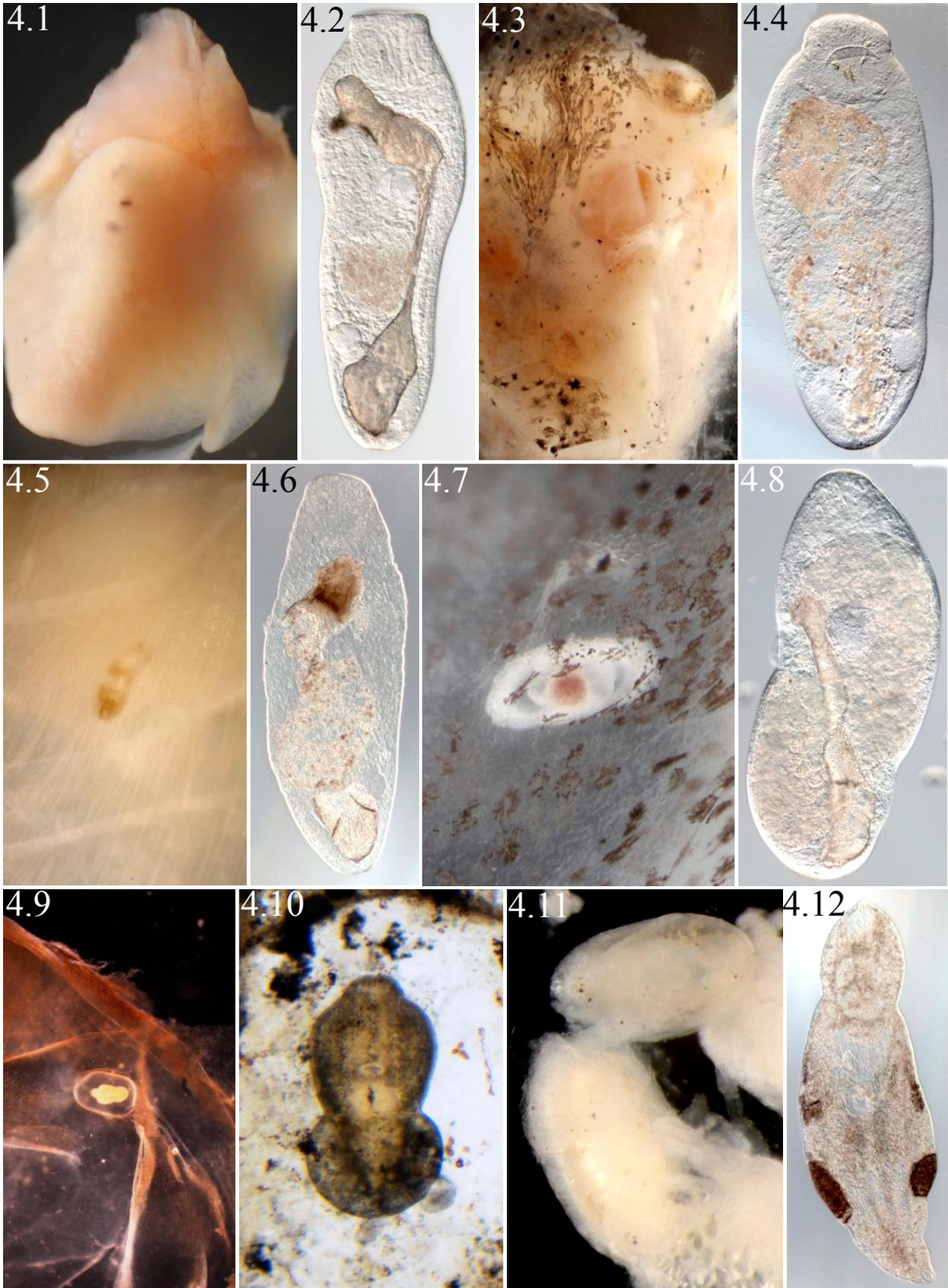
REFERENCE(S): Hahn, 1915; Kudo, 1920; Bond, 1938; Fantham et al., 1940; Landsberg and Lom, 1991.

### **1.6.2. Platyhelminthes**

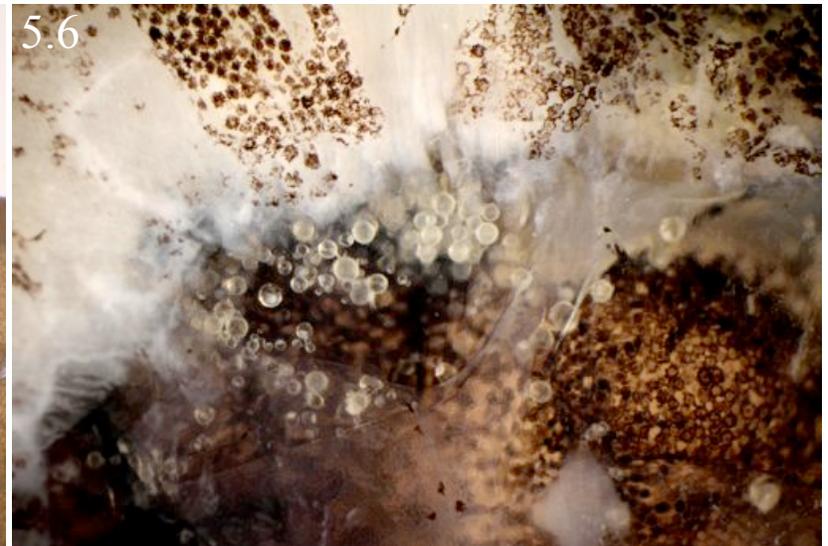
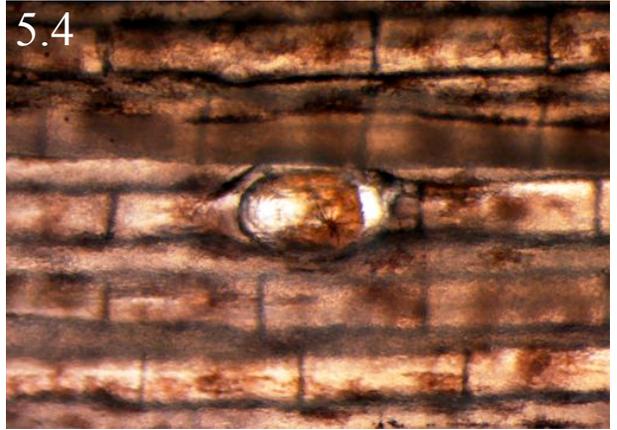
#### **Digenea**

The majority of species within this group were metacercariae that utilize *F. grandis* as a second intermediate host. Species in this group infected a variety of tissue sites (Plates 4, 5). Two adult species of Digenea infected the stomach and swim bladder of *F. grandis*. These two species were not identified to any other taxonomic level other than Digenea. Diagnostic

**PLATE 4. Figures 4.1–4.12.** Non-heterophyid metacercariae (Platyhelminthes: Digenea) encysted in *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. **Fig. 4.1.** Bucephalidae sp. 1 metacercariae infecting ventricle myocardium (20X). **Fig. 4.2.** Bucephalidae sp. 1 excysted from ventricle myocardium (10 ocular units, 10X objective, DIC). **Fig. 4.3.** Bucephalidae sp. 2 metacercariae infecting head kidney (50X). **Fig. 4.4.** Bucephalidae sp. 2 excysted from head kidney (10 ocular units, 10X objective, DIC). **Fig. 4.5.** Bucephalidae sp. 3 metacercaria infecting somatic muscle of flank (20X). **Fig. 4.6.** Bucephalidae sp. 3 excysted from head kidney (10 ocular units, 10X objective, DIC). **Fig. 4.7.** Bucephalidae sp. 4 metacercariae infecting lumen of swim bladder (75X). **Fig. 4.8.** Bucephalidae sp. 4 excysted from swim bladder (10 ocular units, 10X objective, DIC). **Fig. 4.9.** Diplostomidae sp. metacercariae infecting swim bladder (7.5X). **Fig. 4.10.** High magnification view of Diplostomidae sp. 1 metacercaria excysted from swim bladder (50X). **Fig. 4.11.** Strigeidae sp. infecting fat (30X). **Fig. 4.12.** Strigeidae sp. excysted from fat (50X).



**PLATE 5. Figures 5.1–5.6.** Heterophyidae metacercariae (Platyhelminthes: Digenea) encysted in tissues of *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. **Fig. 5.1.** *Ascocotyle diminuta* Stunkard and Haviland, 1924 infecting gill (75X). **Fig. 5.2.** *Ascocotyle* sp. infecting pseudobranch (75X). **Fig. 5.3.** Heterophyidae sp. infecting serosa of intestine (75X). **Fig. 5.4.** Heterophyidae sp. infecting fin ray (75X). **Fig. 5.5.** *Ascocotyle tenuicolis* Price, 1935 infecting bulbous arteriosus (75X). **Fig. 5.6.** *Euhaplorchis* sp. infecting neurocranium (75X).



characters included relatively large oral and ventral suckers. These species are not demonstrated as bio-illustrations or photomicrographs in this thesis. However, these are inventoried as part of the parasite component community (Digenea sp. 1 and Digenea sp. 2; see Table 1) of *F. grandis*.

### **Bucephalidae**

#### **Bucephalidae sp. 1 (Plate 4)**

SITE(S) OF INFECTION: Ventricle myocardium (Fig. 4.1).

DIAGNOSTIC CHARACTER(S): Oral sucker hemi-spherical with flattened anterior edge (Fig. 4.2).

REFERENCE(S): Hopkins, 1954; Kinsella and Heard, 1974; Stunkard, 1976; Curran and Overstreet, 2009.

#### **Bucephalidae sp. 2 (Plate 4)**

SITE(S) OF INFECTION: Head kidney (Fig. 4.3).

DIAGNOSTIC CHARACTER(S): Oral sucker inflated, well-demarcated, hemi-spherical, with rounded anterior edge (Fig. 4.4.).

REFERENCE(S): Hopkins, 1954; Kinsella and Heard, 1974; Stunkard, 1976; Curran and Overstreet, 2009.

#### **Bucephalidae sp. 3 (Plate 4)**

SITE(S) OF INFECTION: Somatic musculature (Fig. 4.5).

DIAGNOSTIC CHARACTER(S): Oral sucker uninflated, hemi-spherical, with rounded anterior edge (Fig. 4.6).

REFERENCE(S): Hopkins, 1954; Kinsella and Heard, 1974; Stunkard, 1976; Curran and Overstreet, 2009.

#### **Bucephalidae sp. 4 (Plate 4)**

SITE(S) OF INFECTION: Swim bladder (Fig. 4.7).

DIAGNOSTIC CHARACTER(S): Oral sucker nearly spherical with 4 papillae-like structures (Fig. 4.8).

REFERENCE(S): Hopkins, 1954; Kinsella and Heard, 1974; Stunkard, 1976; Curran and Overstreet, 2009.

### **Diplostomidae**

#### **Diplostomidae sp. (Plate 4)**

SITE(S) OF INFECTION: Swim bladder (Fig. 4.9).

DIAGNOSTIC CHARACTER(S): Metacercariae with bulbous posterior end (Fig. 4.10).

REFERENCE(S): Hoffman, 1958; Bullard and Overstreet, 2008.

### **Heterophyidae**

#### ***Ascocotyle* Looss, 1899**

#### ***Ascocotyle diminuta* Stunkard and Haviland, 1924 (Plate 5, 6)**

SITE(S) OF INFECTION: Gill lamellae (Fig. 5.1).

DIAGNOSTIC CHARACTER(S): Oral sucker with 16 oral spines plus two shorter dorsal accessory spines (Fig. 6.1).

REFERENCE(S): Stunkard and Haviland, 1924; Chandler, 1941; Martin, 1953; Sogandares

Bernal and Lumsden, 1963; Ostrowski de Núñez, 1993.

#### ***Ascocotyle tenuicolis* Price, 1935 (Plate 5, 6)**

SITE(S) OF INFECTION: Bulbous arteriosus (Fig. 5.5).

DIAGNOSTIC CHARACTER(S): Oral sucker with two rows of 16 spines (Fig. 6.2).

REFERENCE(S): Brock and Font, 2009; Scholz et al., 1995.

#### ***Ascocotyle* sp. (Plate 5, 6)**

**PLATE 6. Figures 6.1–6.6.** Heterophyidae metacercariae (Platyhelminthes, Digenea) infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. **Fig. 6.1.** *Ascocotyle diminuta* Stunkard and Haviland, 1924 excised from gill (10 ocular units, 40X objective, DIC). **Fig. 6.2.** *Ascocotyle tenuicolis* Price, 1935 excised from bulbous arteriosus (10 ocular units, 20X objective, DIC). **Fig. 6.3.** *Ascocotyle* sp. excised from pseudobranch (10 ocular units, 20X objective, DIC). **Fig. 6.4.** Oral spines of Heterophyidae sp. 1 from fin ray (10 ocular units, 40X objective, 2X magnifier, DIC). **Fig. 6.5.** Oral spines of Heterophyidae sp. 2 from fin ray (10 ocular units, 40X objective, 2X magnifier, DIC). **Fig. 6.6.** Oral spines of Heterophyidae sp. 3 from fin ray (10 ocular units, 40X objective, 2X magnifier, DIC).

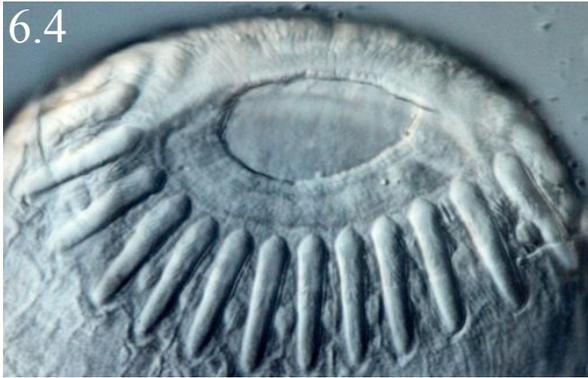
6.1



6.2



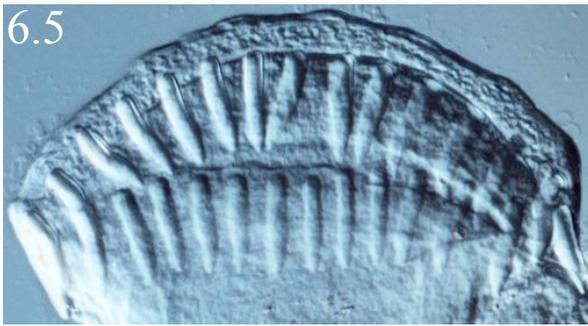
6.4



6.3



6.5



6.6



SITE(S) OF INFECTION: Pseudobranch (Fig. 5.2).

DIAGNOSTIC CHARACTER(S): Oral sucker with two rows of 18 spines (Fig. 6.3).

REFERENCE(S): Sogandares-Bernal and Bridgman, 1960; Sogandares-Bernal and Lumsden, 1963; Font et al., 1984.

***Euhaplorchis* Martin, 1950**

***Euhaplorchis* sp. (Plate 5)**

SITE(S) OF INFECTION: Neurocranium (Fig. 5.6).

DIAGNOSTIC CHARACTER(S): Metacercariae developing and with high intensity of infection (Fig. 5.6).

REFERENCE(S): Martin, 1950; Abbott, 1968; Bullard and Overstreet, 2008.

**Heterophyidae sp. 1 (Plate 5, 6)**

SITE(S) OF INFECTION: Fin rays (Fig. 5.4).

DIAGNOSTIC CHARACTER(S): Oral sucker with two rows of 24 spines (Fig. 6.4).

REFERENCE(S): None recovered.

**Heterophyidae sp. 2 (Plate 5, 6)**

SITE(S) OF INFECTION: Fin rays (Fig. 5.4).

DIAGNOSTIC CHARACTER(S): Oral sucker with one rows of 28 spines (Fig. 6.5).

REFERENCE(S): None recovered.

**Heterophyidae sp. 3 (Plate 5, 6)**

SITE(S) OF INFECTION: Fin rays (Fig. 5.4).

DIAGNOSTIC CHARACTER(S): Oral sucker with one row of 24 spines (Fig. 6.6).

REFERENCE(S): None recovered.

**Strigeidae**

**Strigeidae sp. (Plate 4)**

SITE(S) OF INFECTION (Fig. 4.11): Fat.

DIAGNOSTIC CHARACTER(S): Body with tapered anterior and posterior ends (Fig. 4.12).

REFERENCE(S): Bullard and Overstreet, 2008.

**Monogenoidea**

**Ancyrocephalidae**

***Salsuginus* Beverley-Burton, 1984**

***Salsuginus* n. sp.1 (Plate 7)**

SITE(S) OF INFECTION: Gill lamellae (Figs. 7.1, 7.3).

DIAGNOSTIC CHARACTER(S): Transverse dorsal and ventral bars highly arched. Hamuli sharply recurved (Fig. 7.2).

REFERENCE(S): Williams, 1980; Beverley-Burton, 1984; Murith and Beverley-Burton, 1985.

***Salsuginus* n. sp. 2 (Plate 7)**

SITE(S) OF INFECTION: Gill lamellae (Figs. 7.1, 7.3).

DIAGNOSTIC CHARACTER(S): Transverse ventral bar with deep ridges (Fig. 7.4).

REFERENCE(S): Williams, 1980; Beverley-Burton, 1984; Murith and Beverley-Burton, 1985.

**Gyrodactylidae**

***Gyrodactylus* Nordmann, 1832**

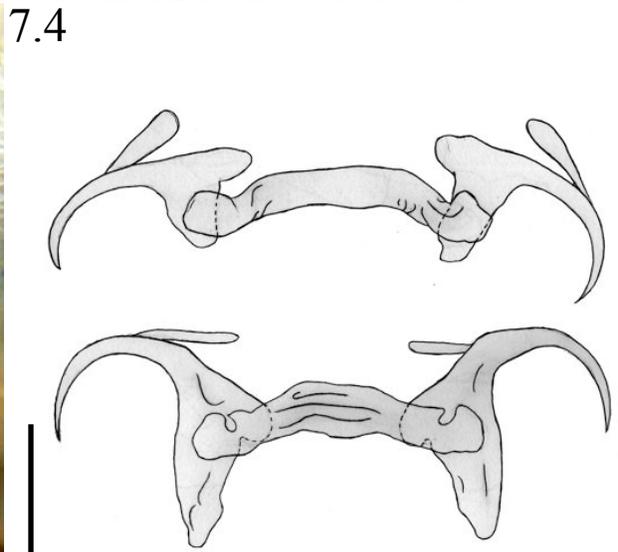
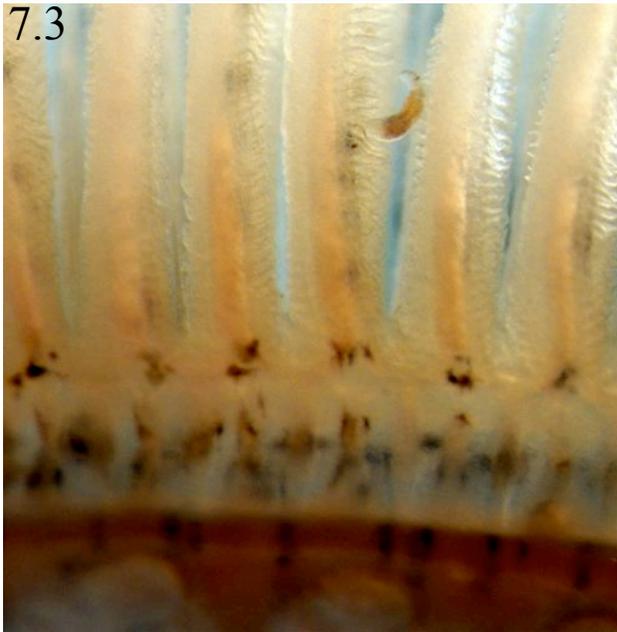
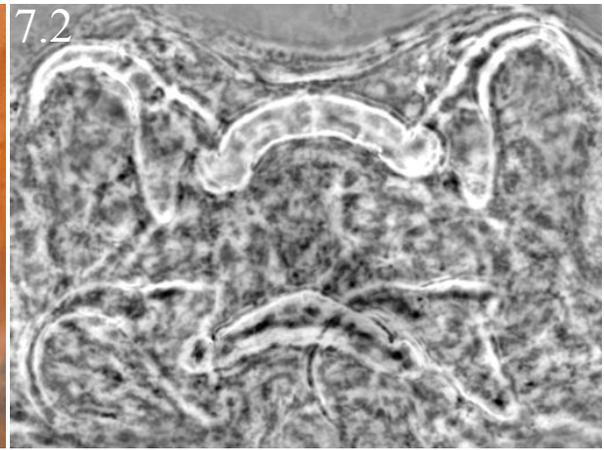
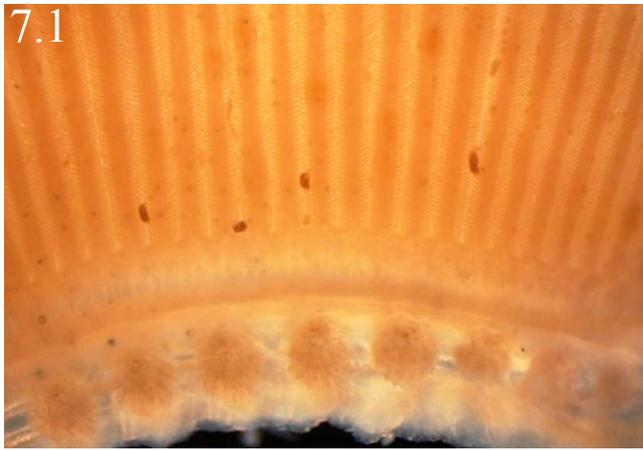
***Gyrodactylus stephanus* Mueller, 1937 (Plate 8)**

SITE(S) OF INFECTION: Buccal cavity (Fig. 8.1) and skin of body.

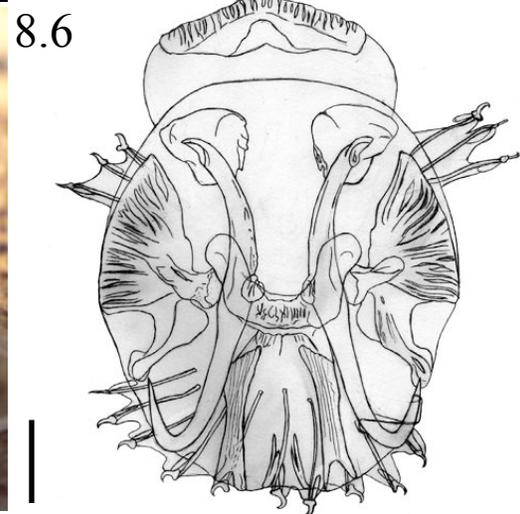
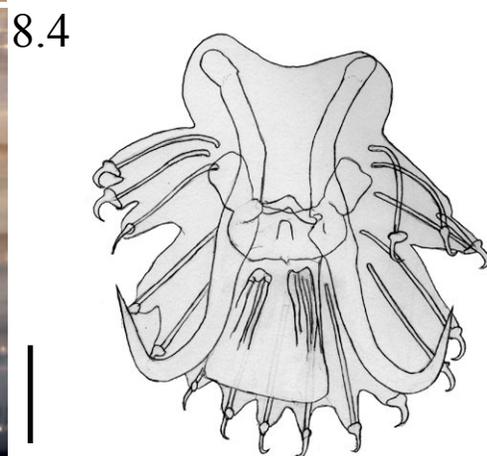
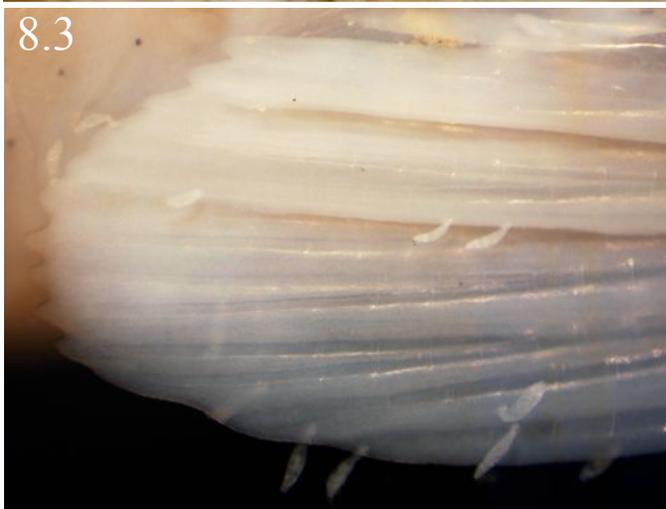
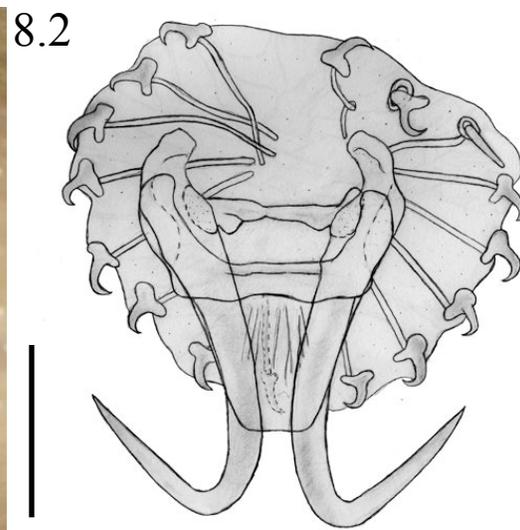
DIAGNOSTIC CHARACTER(S): Haptor with sixteen marginal hooks and single pair of hamuli with ventro-mesial knobs articulating with ventral bar (Fig. 8.2).

REFERENCE(S): Mueller, 1937; Hargis, 1955; Mizelle and Kritsky, 1967 (key to North

**PLATE 7. Figures 7.1–7.4.** *Salsuginus* spp. (Platyhelminthes: Monogeneoidea: Ancyrocephalidae) infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. **Fig. 7.1.** Low magnification view of *Salsuginus* infecting gill lamellae (25X). **Fig. 7.2.** Light micrograph of *Salsuginus* sp. 1 haptor with transverse bars and hamuli. **Fig. 7.3.** Higher magnification view of *Salsuginus* infecting gill lamellae (50X). **Fig. 7.4.** *Salsuginus* sp. 2 transverse bars and hamuli. Scale bar equal to 15  $\mu\text{m}$ .



**PLATE 8. Figures 8.1–8.6.** Gyrodactylidae (Platyhelminthes: Monogenoidea) infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. All scale bars equal 20  $\mu\text{m}$ . **Fig. 8.1.** *Gyrodactylus stephanus* Mueller, 1937 infecting buccal cavity (15X). **Fig. 8.2.** Haptor of *G. stephanus*. **Fig. 8.3.** *Fundulotrema* cf. *prolongis* (Hargis, 1955) Kritsky and Thatcher, 1977 infecting skin of fins (30X). **Fig. 8.4.** Haptor of *F. cf. prolongis*. **Fig. 8.5.** *Swingleus polyclithroides* Rogers, 1969 infecting skin of eye (75X). **Fig. 8.6.** Haptor of *S. polyclithroides*.



American species of *Gyrodactylus*); Hendrix, 1994; King and Cone, 2009.

***Fundulotrema* Kritsky and Thatcher, 1977**

***Fundulotrema* cf. *prolongis* (Hargis, 1955) Kritsky and Thatcher, 1977**

(Plate 8)

SITE(S) OF INFECTION: Skin of buccal cavity (Fig. 8.2) and fins (Fig. 8.3).

DIAGNOSTIC CHARACTER(S): Haptor with sixteen marginal hooks and single pair of relatively long hamuli supported by dorsal and ventral bars and lacking lateral wing-like bars (Fig. 8.4).

REFERENCE(S): Kritsky and Thatcher, 1977 (generic diagnosis); Cone and Odense, 1988 (emended generic diagnosis); Beverley-Burton, 1984; Hendrix, 1994.

***Swingleus* Rogers, 1969**

***Swingleus polyclithroides* Rogers, 1969 (Plate 8)**

SITE(S) OF INFECTION: Skin of eye (Fig. 8.3), body, and fins (Fig. 8.5).

DIAGNOSTIC CHARACTER(S): Haptor with sixteen marginal hooks and pair of relatively long hamuli supported by ventral bar with shield, with lateral wing-like bars, and without dorsal bar (Fig. 8.6).

REFERENCE(S): Rogers, 1969 (generic diagnosis); Hendrix, 1994; Hoffman, 1999.

**Cestoda**

**Gryporhynchidae**

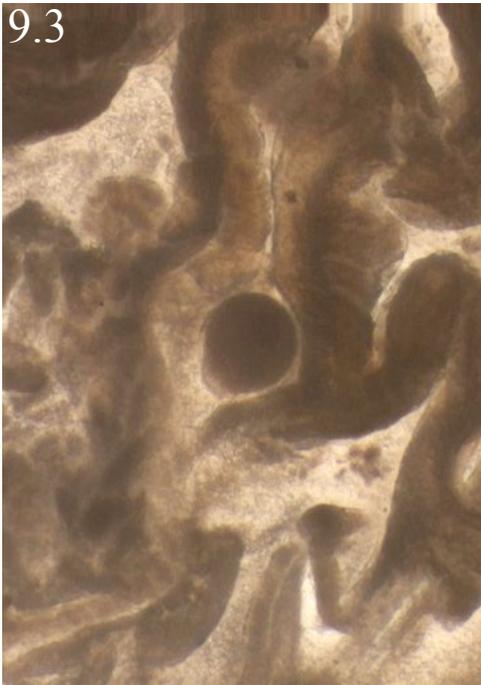
**Gryporhynchidae sp. 1 (Plate 9)**

SITE(S) OF INFECTION: Body cavity (Fig. 9.1), gonad, liver, mesentery.

DIAGNOSTIC CHARACTER(S): Metacestode with armed scolex (Fig. 9.2).

REFERENCE(S): Chandler, 1935; Scholz and Salgado-Maldonado, 2001; Scholz et al. 2002;

**PLATE 9. Figures 9.1–9.4.** Larval cestodes (Platyhelminthes: Cestoda) infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. **Fig. 9.1.** Gryporhynchidae sp. metacestode larvae infecting serosa of testes (25X). **Fig. 9.2.** Excysted Gryporhynchidae sp. metacestode larva (40X). **Fig. 9.3.** Proteocephalidae sp. pleurocercoid infecting mucosa of stomach (75X). **Fig. 9.4.** Heat-killed Proteocephalidae sp. (pleurocercoid) larva (10 ocular units, 20X objective).



Scholz et al., 2004; Scholz and Harris, 2006.

### **Proteocephalidae**

#### **Proteocephalidae sp. 1 (Plate 9)**

SITES OF INFECTION: Mucosa of stomach (Fig. 9.3).

DIAGNOSTIC CHARACTER(S): Pleurocercoid with 4 unarmed suckers and apical organ (Fig. 9.4).

REFERENCE(S): Freze, 1969; Hoffman, 1999.

### **1.6.3. Nematoda**

Two adult forms are previously reported to infect *F. grandis*. These are *Capillaria cyprinodonticola* Huffman and Bullock, 1973 (in liver) and *Spirocamallanus cricotus* Fusco and Overstreet, 1978 (in intestine). During this study, only two specimens of adult nematodes were collected from the intestine of two individuals of *F. grandis*. These specimens have not been studied and require further taxonomic comparison with the aforementioned adult forms. Therefore, adult nematodes are not demonstrated in this thesis but are included as part of the parasite component community infecting *F. grandis*.

#### ***Eustrongylides Jägerskiöld, 1909* (Plate 10)**

SITE(S) OF INFECTION: Atrium of heart (Fig. 10.1), pericardium, body cavity, caudal kidney, liver (Fig. 10.2).

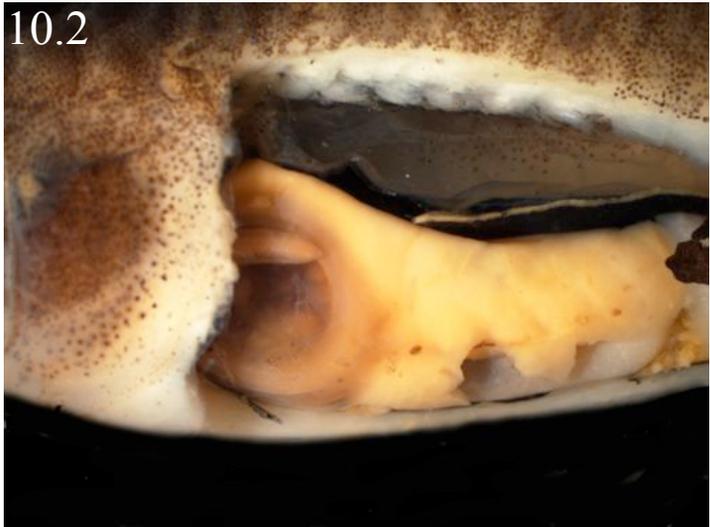
DIAGNOSTIC CHARACTER(S): Body relatively large, opaque, grossly visible with naked eye, red in color when alive.

REFERENCE(S): Lichtenfels and Pilitt, 1986; Hoffman, 1999; Overstreet, 2003.

### **Spiuridae**

#### **Spiuridae sp. (Plate 10)**

**PLATE 10. Figures 10.1–10.3.** Nematode larvae infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. **Fig. 10.1.** *Eustrongylides* sp. (L3) infecting atrium of heart (15X). **Fig. 10.2.** *Eustrongylides* sp. (L3) infecting liver (15X). **Fig. 10.3.** Spiuridae sp. (L3) excised from mucosa of digestive system (10 ocular units, 40X objective).



SITE(S) OF INFECTION: Mucosa of intestine.

DIAGNOSTIC CHARACTER(S): L3 with tapered posterior end (Fig. 10.3).

REFERENCE(S): Hoffman, 1999.

#### **1.6.4. Acanthocephala**

Two adult species of acanthocephala primarily infected the mucosa of the intestine of *F. grandis* and their key diagnostic features are demonstrated herein. An additional species infected the body cavity of *F. grandis* and was identified as an encysted cystacanth larva. This larval form was identified based on the presence of a thick cyst wall that surrounded retracted specimens. Proboscis hooks were visible through both the cyst and the tegument. Heat-killed specimens with a fully everted proboscis were not collected or extensively studied and photographs and drawings of this rare species are not demonstrated in this thesis. However, this species is included as part of the parasite component community of *F. grandis* (Table 1).

#### **Echinorhynchida**

##### **Illiosentidae**

##### ***Dollfusentis* Golvan, 1969**

##### ***Dollfusentis* cf. *chandleri* Golvan, 1969 (Plate 11)**

SITE(S) OF INFECTION: Mucosa of intestine (Fig. 11.1).

DIAGNOSTIC CHARACTER(S): Proboscis with 6 elongated hooks at base. Neck with cuticular spines (Fig. 11.2).

REFERENCE(S): Golvan, 1969.

#### **Neoechinorhynchida**

##### **Neoechinorhynchidae**

##### ***Octospiniferoides* Bullock, 1957**

**PLATE 11. Figures 11.1–11.4.** Acanthocephala infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. Scale bars equal to 50  $\mu\text{m}$ . **Fig.11.1.** *Dollfusentis* cf. *chandleri* Golvan, 1969 (Echinorhynchida; Illiosentidae) in sediment after scraping mucosa of intestine (25X). **Fig. 11.2.** Proboscis and neck armature of *D.* cf. *chandleri*. **Fig. 11.3.** *Octospiniferoides chandleri* Bullock, 1957 (Neoechinorhynchida: Neoechinorhynchidae) removed from mucosa of intestine (100X). **Fig. 11.4.** Proboscis armature of *O. chandleri*.

11.1



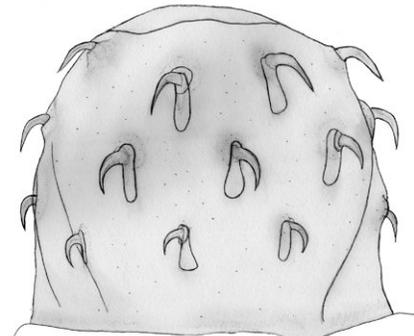
11.2



11.3



11.4



***Octospiniferoides chandleri* Bullock, 1957** (Plate 11)

SITE(S) OF INFECTION: Mucosa of intestine.

DIAGNOSTIC CHARACTER(S): Proboscis short and with 3 rows of 8 slender hooks (Figs. 11.3–11.4).

REFERENCE(S): Bullock, 1957; Bullock, 1966; Salgado-Maldonado et al., 1992; Salgado-Maldonado et al., 1997.

**1.6.5. Annelida**

**Hirudinida**

**Piscicolidae**

***Malmiana* Strand, 1942**

***Malmiana philotherma* Sawyer, Lawler, and Overstreet, 1975** (Plate 12)

SITE(S) OF INFECTION: Skin of fins (Fig. 12.1) and branchiostegal membrane.

DIAGNOSTIC CHARACTER(S): Body flattened. Caudal sucker large and well-demarcated from posterior end of body (urosome) (Fig. 12.2).

REFERENCE(S): Sawyer et al., 1975; Sawyer and Kinard 1980; Burreson and Kalman, 2006.

***Myzobdella* Leidy, 1851**

***Myzobdella lugubris* Leidy, 1851** (Plate 12)

SITE(S) OF INFECTION: Skin of fins and branchiostegal membrane (Fig. 12.3).

DIAGNOSTIC CHARACTER(S): Caudal sucker confluent with urosome (Fig. 12.4).

REFERENCE(S): Sawyer et al., 1975; Appy and Dadswell, 1980.

**1.6.6. Arthropoda**

**Copepoda**

Two species of adult copepods primarily infected the gill filaments of *F. grandis*. Another

**PLATE 12. Figures 12.1–12.4.** Hirudinida (Annelida: Piscicolidae) infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. **Fig. 12.1.** *Malmiana philotherma* Sawyer, Lawler, and Overstreet, 1975 infecting anal fin (40X). **Fig. 12.2.** Heat-killed *M. philotherma* from fin (30X). **Fig. 12.3.** *Myzobdella lugubris* Leidy, 1851 infecting pectoral fin (7.5X). **Fig. 12.4.** Heat-killed *M. lugubris* from fin (25X).



juvenile species, a copepodid of Caligidae (Cressey, 1991), infected the fins of *F. grandis*. This species was not identified to any other level other than family and is not demonstrated in this thesis. However, this species is included as part of the parasite component community of *F. grandis* (Table 1).

## **Ergasilidae**

### ***Ergasilus* Nordman, 1832**

#### ***Ergasilus* cf. *arthrosis* Roberts, 1969** (Plate 13)

SITE(S) OF INFECTION: Gill filaments (Fig. 13.1, 13.3).

DIAGNOSTIC CHARACTER(S): Second antennae with 4<sup>th</sup> segment 80% as long as 3<sup>rd</sup> segment (Fig. 13.2).

REFERENCES: Roberts, 1970; Kabata, 1986.

#### ***Ergasilus funduli* Krøyer, 1863** (Plate 13)

SITE(S) OF INFECTION: Gill filaments (Fig. 13.1, 13.3).

DIAGNOSTIC CHARACTER(S): Second antennae with inflated 2<sup>nd</sup> segment of second antenna and single tubercle on inner margin of 4<sup>th</sup> segment (Fig. 13.4).

REFERENCE(S): Roberts, 1970; Kabata, 1986.

## **Branchiura**

### **Argulidae**

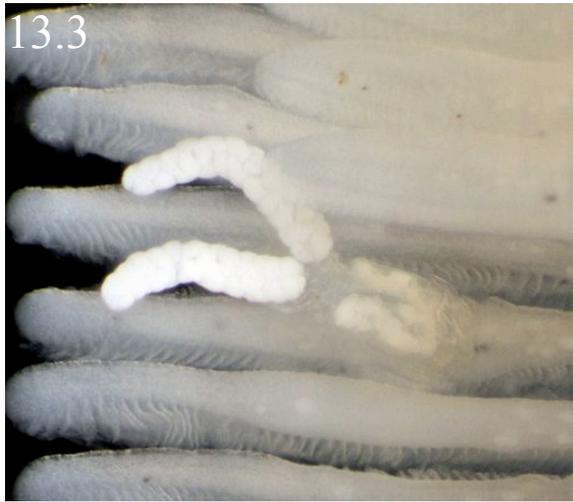
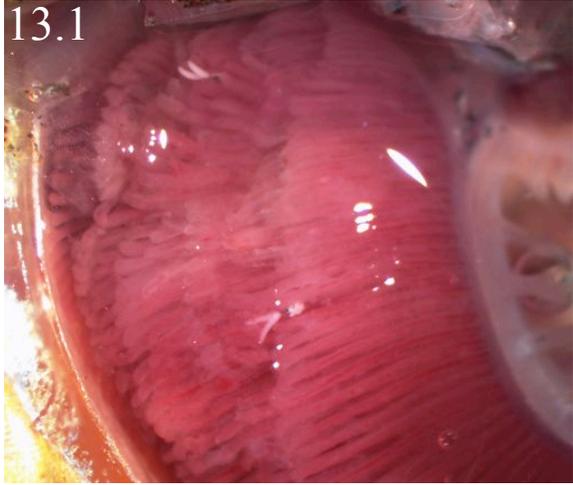
#### ***Argulus* Müller**

#### ***Argulus* n. sp. 1** (Plate 14)

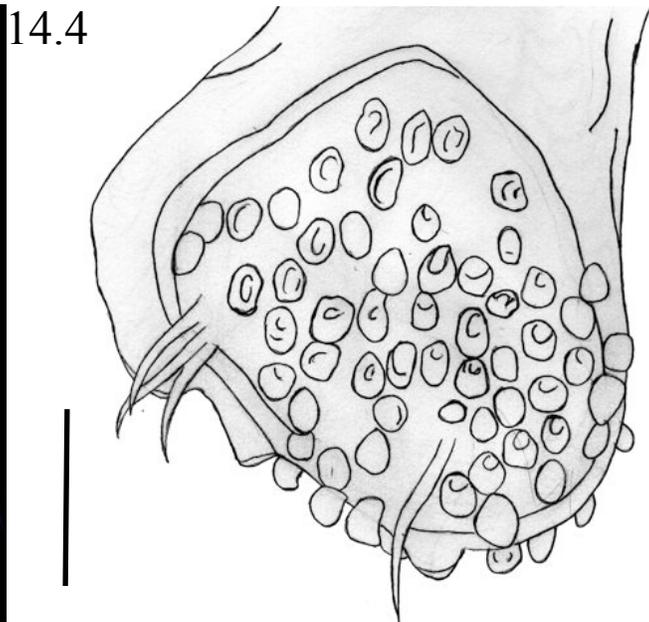
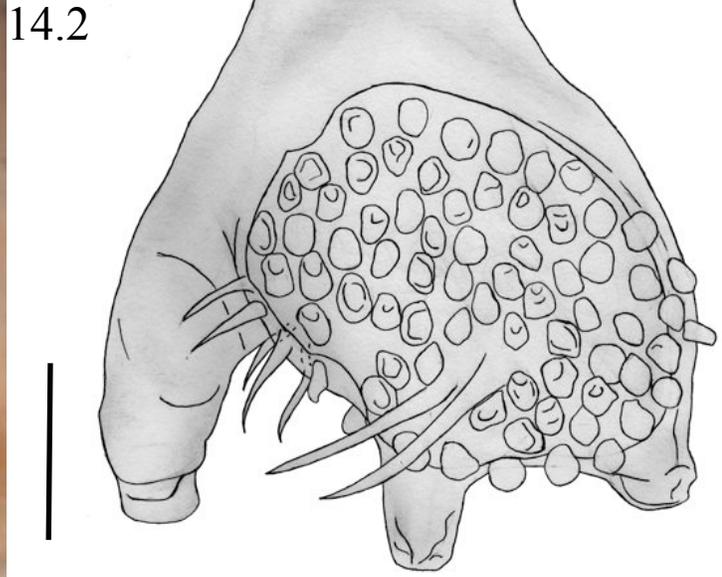
SITE(S) OF INFECTION: Buccal cavity (Fig. 14.1).

DIAGNOSTIC CHARACTER(S): Base of second maxilla with large pad of cycloid-like scales, three elongated basal teeth, with 5 ventral and 2 dorsal setae (Fig. 14.2).

**PLATE 13. Figures 13.1–13.4.** Copepoda (Arthropoda: Ergasilidae) infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. **Fig. 13.1.** Low magnification view of *Ergasilus* sp. in gill (7.5X). **Fig. 13.2.** Second antenna of *Ergasilus* cf. *arthrosis* Roberts, 1969 (400X). **Fig. 13.3.** High magnification view of *Ergasilis* sp. attached to gill filament (75X). **Fig. 13.4.** Second antenna of *Ergasilus funduli* Krøyer, 1863 (200X).



**PLATE 14. Figures 14.1–14.4.** Branchiura (Arthropoda, Argulidae) infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. Scale bars equal to 30  $\mu\text{m}$ . **Fig. 14.1.** Dorsal view of *Argulus* sp. infecting buccal cavity (20X). **Fig. 14.2.** Base of second maxilla of *Argulus* n. sp. **Fig. 14.3.** Ventral view of *Argulus* cf. *funduli* Krøyer, 1863 (40X). **Fig. 14.4.** Base of second maxilla of *A.* cf. *funduli*.



REFERENCE(S): Wilson, 1905; Cressy, 1978; Kabata, 1988.

***Argulus* n. sp. 2** (Plate 14)

SITE(S) OF INFECTION: Buccal cavity (Fig. 14.1).

DIAGNOSTIC CHARACTER(S): Base of second maxilla with 3 short basal teeth, four ventral setae (Fig. 14.4).

REFERENCE(S): Wilson, 1905; Cressy, 1978; Kabata, 1988.

**Isopoda**

**Cymthoidae**

***Lironeca* Leach, 1818**

***Lironeca ovalis* Say, 1818** (Plate 15)

SITE(S) OF INFECTION: Buccal cavity (Figs. 15.1–15.2).

DIAGNOSTIC CHARACTER(S): 5<sup>th</sup> thoracic segment wider than 3<sup>rd</sup> thoracic segment (Fig. 15.2).

REFERENCE(S): Price and Schlueter, 1980; Williams and Bowman, 1994; Hoffman, 1999.

**1.6.7. Protozoa**

*Calyptospora* cf. *funduli* (Duszynski, Solangi, and Overstreet, 1979) Overstreet, Hawkins, and Fournie, 1984, (Apicomlexa: Calyptosporidae) primarily infected the liver of *F. grandis* collected from Barataria Bay, LA. Other infection sites included fat, mesentery, and peritoneum of the body cavity. *C. cf. funduli* also infected the cysts of digenean metacercariae, metacestodes, and encapsulations of nematodes. Solangi and Overstreet (1980), Upton and Duszynski (1982), Hawkins et al. (1983), Fournie and Overstreet (1993), and Fournie et al. (2000) have published information regarding the taxonomy and biology of this parasite.

Other morphologically distinct coccidia were occasionally viewed infecting the apical

**PLATE 15. Figure 15.1–15.2.** *Lironeca ovalis* Say 1818 (Arthropoda, Isopoda, Cymthoidae) infecting buccal cavity of *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) collected from Barataria Bay, Louisiana. **Fig. 15.1.** Lateral view in buccal cavity (7.5X). **Fig. 15.2.** Dorsal view in exposed buccal cavity (10X).

15.1



15.2



surface of the urinary bladder. As this work mainly deals with major metazoan parasite groups, no plates were constructed for protozoan parasites of *F. grandis* in this thesis.

## **1.7. Discussion**

The parasite component community of *F. grandis* collected from Barataria Bay consisted of 44 species (31 endoparasites and 13 ectoparasites) that infected 23 tissue types. Of these, 10 are putatively new to science (Table 1), 24 constitute new host records, and 42 are putatively new locality records in Louisiana. Including 29 previous records, there are 60 total metazoan parasite species reported to infect *F. grandis*. Only 7 nominal accepted species have been originally described from *F. grandis* (Table 1), which underscores the probability of discovering species that are new to science.

Herein, I present information regarding the taxonomy and systematics of each major metazoan parasite group wherever applicable. Additional remarks are provided for each putative species including new species and taxa that could not be identified to the level of species.

### **1.7.1. Myxozoa (Plates 2–3)**

The Myxozoa are histozoic and coelozoic endoparasites of freshwater and marine fishes and mature in invertebrates but infect fishes (Kent et al., 1994), including chondrichthyans, as second intermediate hosts (Benz and Bullard, 2004; Blaylock et al., 2004). Initially considered to be Protozoa, Myxozoans have since been classified as Metazoa but the systematics of this group is still debated today. In this thesis, I followed current classification and regarded the Myxozoa as a major metazoan parasite phylum (Dyková and Lom, 2007).

#### ***Kudoa* Meglitsch, 1947**

As of 2004, *Kudoa* consisted of 49 named species with distributions in the northern Gulf of Mexico, Western Atlantic Ocean, and Western Pacific Ocean (Blaylock et al., 2004). Three

**Table 1. Systematic listing of the species members of the parasite (metazoans only) component community of Gulf killifish,**

***Fundulus grandis* Baird and Girard, 1853 in Barataria Bay and from previously-published records.** Taxa with (\*) comprise the

parasite component community of *F. grandis* in Barataria Bay. *Abbr.* Locality: Mississippi (MS), Louisiana (LA), Texas (TX), Florida

(FL), Alabama (AL), not reported (NR). Life history: adult (a), asexual myxospore (am), copepodid (co), cystacanth (cy), third stage

larva (L3), larval form (lf), metacercaria (me), metacystode (ms), pleurocercoid larva (p); Life cycle type: direct,

ectoparasite (D), indirect, endoparasite (I).

Taxon	Site of Infection	Locality	Life History	Life Cycle	New Host Record	New Locality Record	Reference(s)
<b>MYXOZOA</b>							
<b>Kudoidea</b>							
<i>Kudoa</i> sp.	sm	MS	am	I	X	X	Dyková et al., 1994
<i>Kudoa</i> cf. <i>funduli</i> *	sm	LA	am	I	X	X	present study
<b>Myxobolidae</b>							
<i>Myxobolus</i> n. sp. 1*	g	LA	am	I	X	X	present study
<i>Myxobolus</i> n. sp. 2*	sk	LA	am	I	X	X	present study
<i>Myxobolus</i> n. sp. 3*	bs	LA	am	I	X	X	present study
<i>Myxobolus</i> n. sp. 4*	e	LA	am	I	X	X	present study
<i>Myxobolus</i> n. sp. 5*	bf	LA	am	I	X	X	present study
<i>Myxobolus</i> n. sp. 6*	sb	LA	am	I	X	X	present study
<b>DIGENEA</b>							
<i>Digenea</i> sp. 1*	st	LA	a	I	X	X	present study
<i>Digenea</i> sp. 2*	sb	LA	a	I	X	X	present study

<b>Bucephalidae</b>									
Bucephalidae sp. 1*	vm	LA	me	I	X				present study
Bucephalidae sp. 2*	k	LA	me	I	X				present study
Bucephalidae sp. 3*	sm	LA	me	I	X				present study
Bucephalidae sp. 4*	sb	LA	me	I	X				present study
<i>Prosorhynchoides strongyluræ</i>	nr	TX	me	I					Sparks, 1960
<i>Rhipidocotyle lintoni</i>	nr	TX	me	I					Sparks, 1960
<b>Diplostomidae</b>									
<i>Posthodiplostomum minimum</i>	nr	FL	me	I					Bangham, 1940
<i>Posthodiplostomum</i> sp.*	f, ms, sb	LA	me	I	X				present study
<b>Heterophyidae</b>									
<i>Ascocotyle angrense</i>	g	LA	me	I					Sogandares-Bernal & Lumsden, 1963
<i>Ascocotyle diminuta</i> *	g	LA	me	I					Sogandares-Bernal & Bridgman, 1960; present study
<i>Ascocotyle lageniformis</i>	g	TX	me	I					Martin, 1953
<i>Ascocotyle tenuicolis</i> *	ba	LA	me	I	X				present study
<i>Ascocotyle</i> sp.*	p	LA	me	I	X				present study
<i>Euhaplorchis</i> sp.*	n	NR; LA	me	I	X				Bullard & Overstreet, 2008; present study
Heterophyidae sp. 1*	fr	LA	me	I	X				present study
Heterophyidae sp. 2*	fr	LA	me	I	X				present study
Heterophyidae sp. 3*	fr	LA	me	I	X				present study
<i>Stictodora cursitans</i>	sm	FL	me	I	X				Kinsella & Heard, 1964

<b>Strigeidae</b>													
Strigeidae sp.*	f	LA	me	I	X	X							present study
<b>MONOGENOIDEA</b>													
<b>Ancyrocephalidae</b>													
<i>Salsuginus</i> n. sp. 1*	g	LA	a	D	X	X							present study
<i>Salsuginus</i> n. sp. 2*	g	LA	a	D	X	X							present study
<i>Salsuginus spirae</i>	g	AL	a	D									Williams, 1980
<b>Gyrodactylidae</b>													
<i>Gyrodactylus</i> cf. <i>stephanus</i> *	g, sk	FL; LA	a	D	X	X							Hargis, 1955; present study
<i>Fundulotrema prolongis</i> *	sk, g	FL; AL	a		X	X							Hargis, 1955; Rogers, 1969
<i>Swingleus polyclithroides</i> *	sk	AL	a	D	X	X							Rogers, 1969
<b>CESTODA</b>													
<b>Bothriocephalidae</b>													
<i>Bothriocephalus</i> sp.	nr	FL	p	I									Bangham, 1940
<b>Gryporhynchidae</b>													
Gryporhynchidae sp.*	bc, ms, sd, l, go	LA	ms	I	X	X							present study
<b>Proteocephalidae</b>													
Proteocephalidae sp.*		LA	p	I	X	X							present study
<i>Proteocephalus</i> sp.		FL	p	I									Bangham, 1940
<b>NEMATODA</b>													
Nematoda sp. 1*	i	LA	a	I	X	X							present study
Nematoda sp. 2*	i	LA	a	I	X	X							present study
<b>Camallanidae</b>													
<i>Spirocamallanus cricotus</i>	i	MS	a	I									Fusco & Overstreet, 1978

<b>Diectophymatoidea</b>									
<i>Eustrongylides ignotus</i>	bc	NR	L3	I					Overstreet, 2003
<i>Eustrongylides</i> sp.*	bc, h, k, l, ms, sb	LA	L3	I			X		present study
<b>Spiuridae</b>									
Spiuridae sp.*		LA	L3	I			X		present study
<b>Trichinellidae</b>									
<i>Capillaria cyprinodonticola</i>		FL	a	I					Huffman & Bullock, 1973
<b>ACANTHOCEPHALA</b>									
<i>Acanthocephala</i> sp.*		LA	cy	I			X		present study
<b>Illiosentidae</b>									
<i>Dollfusentis</i> cf. <i>chandleri</i> *		TX; LA	a	I			X		Bullock, 1957; present study
<b>Neoechinorhynchidae</b>									
<i>Octospiniferoides chandleri</i> *		TX; FL; LA	a	I			X		Bullock, 1957; Bullock, 1966; present study
<b>Polymorphidae</b>									
<i>Southwellina hispida</i>	ms	TX	cy	I					Bullock, 1957
<b>Rhadinorhynchidae</b>									
<i>Leptorhynchoides thecatus</i>	i, ms	FL; AL	cy	I					Bangham, 1940; Williams & Gains, 1974
<b>HIRUDINIDA</b>									
<b>Piscicolidae</b>									
<i>Malmiana philotherma</i> *	sk	AL; LA	a	D			X		Williams, 1979; present study

<i>Myzobdella lugubris</i> *	sk	MS; LA	a		D		X	Sawyer et al., 1975; present study
<b>PENTASTOMIDA</b>								
<i>Sebekia mississippiensis</i>	ct	MS	If	I				Overstreet et al., 1985
<b>COPEPODA</b>								
<b>Caligidae</b>								
Caligidae sp.*	f	LA	co	D	X		X	present study
<b>Ergasilidae</b>								
<i>Ergasilus</i> cf. <i>arthrosis</i> *	g	LA	a	D	X		X	present study
<i>Ergasilus funduli</i> *	g	AL, FL, MS; LA	a	D			X	Johnson & Rogers, 1973; present study
<i>Ergasilus lizae</i>	g	FL; AL	a	D				Bere, 1936; Johnson & Rogers, 1973
<b>BRANCHIURA</b>								
<b>Argulidae</b>								
<i>Argulus</i> n. sp. 1*	bc	LA	a	D	X		X	present study
<i>Argulus</i> n. sp. 2*	bc	LA	a	D	X		X	present study
<b>ISOPODA</b>								
<b>Cymthoidae</b>								
<i>Lironeca ovalis</i> *	bc	FL, LA	a	D			X	Price & Schlueter, 1980; present study

unnamed congeners have been reported from fundulids that were not identified to species, the western mosquitofish, *Gambusia affinis* (Baird and Girard, 1853), (Cyprinodontiformes: Poeciliidae), and the inland silverside, *Menidia beryllina* (Cope, 1867), (Atheriniformes: Atherinopsidae) in the northern Gulf of Mexico (Dykova et al., 1994). It is supported that molecular tools should be used when describing species of *Kudoa* because workers have shown that spore morphometrics cannot differentiate all species in the genus (Blaylock et al., 2004).

***Kudoa cf. funduli* (Hahn, 1915) Meglitsch, 1947 (Plate 2)**

Specimens examined in this study most closely resemble *Kudoa funduli* (Hahn, 1915) Meglitsch, 1947, (Multivalvuvidea: Kudoidae). Spores of *K. funduli* are described as rounded posteriorly and “sharply attenuated” anteriorly in sutural view (Meglitsch, 1948). However, Akaishi et al. (2004) used scanning electron microscopy to also show that the posterior end of spores of *K. funduli* is slightly pointed in sutural view but also showed that this feature was not readily apparent in formalin fixed wet-mounted spores. In contrast, wet-mounted spores of *K. cf. funduli* studied herein contained conspicuously but not sharply pointed posterior and anterior ends in sutural view (Figs. 2.3–2.4). Also in agreement with supplemental descriptions of *K. funduli*, (Meglitsch et al., 1948; Moran et al., 1999; Akaishi et al., 2004) spores studied herein were contained in elongated cysts (Figs. 2.1–2.2) in somatic muscle and possessed apical projections on spore valves (Figs. 2.3–2.4).

***Myxobolus* Bütschli, 1882**

A total of 6 species of *Myxobolus* were identified during this study (Table 2, Plate 3). These are putatively new species and would be the first records of *Myxobolus* infecting *F. grandis* in the Gulf of Mexico. An attempt was made to scrutinize the morphological and morphometric characteristics of spores based on sites of infection. I have included a table showing

morphometric and morphological data from species of *Myxobolus* observed herein along with published data from species of *Myxobolus* infecting other species of *Fundulus* (Table 2). This table is provided to demonstrate morphometric and morphological differences between described species of *Myxobolus* and the congeners studied herein.

The presence or absence of a vacuole within the sporoplasm of myxospores was once used to diagnose species of *Myxobolus* and *Myxosoma* (see Landsberg and Lom, 1991). Because of the reported unreliability of this feature (Lom and Noble, 1984), all species of *Myxosoma* (those species without an iodophilous vacuole [Kudo, 1918]), were assigned to *Myxobolus* (Landsberg and Lom, 1991). The unreliability of this feature was evident in specimens studied herein, but the beginners mind requires consideration of this feature, and thus, the presence or absence of a conspicuous unstained vacuole in the sporoplasm of myxospores is noted (Table 2).

*Myxobolus* spp. infected gill lamellae (Fig. 3.1), skin beneath scales (Fig. 3.3), branchiostegal skin (Fig. 3.5), skin of eye (Fig. 3.7), the base of pectoral fins (Fig. 3.9; putatively dermis or somatic muscle), and the swim bladder (Fig. 3.11) of *F. grandis*. Myxozoans also infected the serosa of the intestine, kidney, spleen, branchial vessels of gill arches, and the musculature of the head. Myxozoans infecting these sites were not studied and are not included in this thesis.

The high number of infection sites observed in *F. grandis* poses interesting taxonomic questions. While some myxozoan species are regarded as highly host specific and infect specific infection sites across a limited number of ecologically and phylogenetically related hosts (Ferguson et al., 2008), others infect a high number of distantly related hosts (Bond, 1937; Eiras et al., 2005). Myxozoans that infect *F. heteroclitus* have been experimentally shown to only infect other species of *Fundulus* collected from the same habitat (Bond, 1937). Noteworthy is

that the type species, *Myxobolus muelleri* Bütshchli, 1882, (Bivalvuvida: Myxobolidae) has been reported from more than 80 hosts, most of which are Eurasian cyprinid hosts (Lom and Dyková, 2006).

*Myxobolus* spp. are generally histozoic parasites that infect freshwater, estuarine, and marine fishes including species of *Fundulus* in Canada (Wiles, 1975), the eastern United States (Hahn, 1915, 1917a, 1917b), and the northern Gulf of Mexico; i.e., *Fundulus similis* (Baird and Girard, 1853), (Cyprinodontiformes: Fundulidae) (see Rigdon and Hendricks, 1955). Approximately 792 named species of *Myxobolus* exist in the literature including 7 that infect amphibian hosts (Lom and Dyková, 2006). However, many species of *Myxobolus* that infect fishes may be synonymous, which has partly resulted from difficulty in differentiating species based on morphology and morphometrics (Lom and Dyková, 2006), confusion in the taxonomy of the *Myxobolus/ Myxosoma* group (Landsberg and Lom, 1991), and the lack of studies utilizing molecular tools (SSU rRNA gene sequences for only 170 species of *Myxobolus* exist in GenBank, see Ferguson et al., 2008) to diagnose and identify species (Cone and Easy, 2005). Furthermore, it is suggested by some workers that marine species, (30 of approximately 792 [3.8%]) may belong in another genus due to the predominance of freshwater species (Lom and Dyková, 2006), however, studies utilizing molecular tools are lacking to support this idea.

Species of *Myxobolus* have pyriform, ellipsoid, ovoid, or sub-circular spores in valvular view, and appear biconvex in sutural view. In addition, spores consist of two anteriorly-positioned pyriform or rounded polar capsules bearing a coiled filament. Posterior ridges are present in some species (Bond, 1938) along with posterior sutural markings (Kudo, 1918); herein referred to as posterior sutural bumps. In addition, the sporoplasm of spores is with or without an iodophilous vacuole (Landsberg and Lom, 1991).

### ***Myxobolus* n. sp. 1** (Fig. 3.1–3.2)

The three spore types of *Myxobolus* sp. 1 studied herein have overlapping spore lengths with *Myxobolus bilineatum* Bond, 1938, (Bivalvuvida: Myxobolidae) but have significantly different widths (Table 2). *Myxobolus* n. sp. 1 is differentiated from *Myxobolus diaphanus* (Fantham, Porter, and Richardson, 1940) Landsberg and Lom, 1991, (Bivalvuvida: Myxobolidae), *Myxobolus hudsonis* Bond, 1938, (Bivalvuvida: Myxobolidae), *Myxobolus subtecalis* (Bond, 1938) Landsberg and Lom, 1991, (Bivalvuvida: Myxobolidae), and *Myxobolus funduli* (Hahn, 1915) Kudo, 1920, (Bivalvuvida: Myxobolidae), by having spores less than 12  $\mu\text{m}$  in length and polar filaments with no more than 8 coils. *Myxobolus* n. sp. 1 is differentiated from *Myxobolus* n. sp. 2 by rarely possessing dense bodies in the sporoplasm (relatively common in *Myxobolus* n. sp. 2) and by sometimes bearing posterior crescentic ridges (Fig. 3.2) (absent in *Myxobolus* sp. 2). *Myxobolus* n. sp. 1 is differentiated from *Myxobolus* n. sp. 3 by having a significantly shorter spore length, rarely containing a vacuole within the sporoplasm (relatively common in *Myxobolus* n. sp. 3), and by sometimes bearing posterior crescentic ledges (absent in *Myxobolus* sp. 3). *Myxobolus* n. sp. 4 spores were significantly longer and wider than *Myxobolus* n. sp. 1 spores and lacked a vacuole and dense bodies within the sporoplasm and lacked posterior sutural bumps.

This thesis includes 3 spore types that were drawn from spores occupying the same plasmodium. The presence of a caudal process was rarely viewed in spores occupying the same plasmodium. Posterior sutural bumps were viewed on all spores except those bearing a caudal process, perhaps indicating these spores (with sutural bumps) are more developed forms.

### ***Myxobolus* n. sp. 2** (Fig. 3.3–3.4)

*Myxobolus* n. sp. 2 is a putatively distinct from *M. diaphanus*, *M. subtecalis*, and *M.*

*funduli* by having a significantly shorter spore length. The number of filament coils in these species are far more than that observed for *Myxobolus* n. sp. 2 while *M. bilineatum* and *M. hudsonis* have overlapping filament coils. *Myxobolus* n. sp. 2 differs from *M. bilineatum* by having pyriform spores compared to ovate or nearly rounded spores. *M. hudsonis* differs from *Myxobolus* n. sp. 2 mainly by bearing polar capsules with anterior ends that are slightly offset relative to the bilateral plane in valvular view (parallel to the bilateral plane in *Myxobolus* n. sp. 2). The pyriform spores of *Myxobolus* n. sp. 2 differ from the ovate to rounded spores of *Myxobolus* n. sp. 5 and 6. *Myxobolus* n. sp. 3 and 4 are significantly longer than those of *Myxobolus* n. sp. 1 (Table 2).

### ***Myxobolus* n. sp. 3** (Figs. 3.5–3.6)

This species is most morphometrically (overlapping spore length and width and polar capsule length and width) and morphologically (both pyriform spores with prominent vacuole in sporoplasm) similar to *M. funduli*. The species demonstrated herein, however, has fewer coils contained within polar capsules, i.e. 7–9 in *Myxobolus* n. sp. 3, and 11–14 in *M. funduli*.

*M. subtecalis* and *M. diaphanus* also possess a greater number of filament coils per polar capsule compared to *Myxobolus* n. sp. 3. The spore shape of *M. bilineatum* is ovate to rounded and not pyriform as in *Myxobolus* n. sp. 3. *M. hudsonis* differs from *Myxobolus* n. sp. 3 by bearing polar capsules with anterior ends that are slightly offset relative to the bilateral plane. The spore length, width, and number of filament coils overlaps with *Myxobolus* n. sp. 4, however, these two putative species are differentiated by the complete lack of a vacuole and dense bodies in the sporoplasm and absence of posterior sutural bumps in *Myxobolus* n. sp. 4.

I identified two spore types that will tentatively be regarded as conspecific. These spores occupied the same plasmodium (cyst) with one spore type bearing 2 dense bodies within the

sporoplasm.

***Myxobolus* n. sp. 4** (Figs. 3.7–3.8)

This species is most morphometrically similar to *M. funduli*. They are morphologically distinct in that *M. funduli* is reported to have 11–14 coils per polar capsule, whereas this species from the eye (putatively the sclera of the eye) of *F. grandis* only has 6–7 visible coils. In addition, and in contrast to *M. funduli* and *Myxobolus* n. sp. 3, this species lacks a vacuole within the sporoplasm.

Another spore type was observed to originate from the same plasmodium as *Myxobolus* n. sp. 4 perhaps indicating different stages of development. The primary feature that differed between the two types was the overall shape where the probable immature spore was ovoid compared to the mature, pyriform-shaped spore. I assigned these as a single species based on multiple observations of the 2 spore types from different plasmodia collected from the same infection site (sclera of eye). Furthermore, the presence of posterior crescentic ridges in both morphotypes, along with polar capsules loosely containing a filament with 6–7 coils, led me to consider these types as conspecifics.

***Myxobolus* n. sp. 5** (Figs. 3.9–3.10)

This species is morphologically and morphometrically similar to *Myxobolus* n. sp. 6 and *M. bilineatum*. *Myxobolus* n. sp. 5 differs from *M. bilineatum* by lacking a vacuole within the sporoplasm, and by having no more than five filament coils within polar capsules. The primary difference between *Myxobolus* n. sp. 5 and 6 besides sites of infection is the presence of a vacuole in *Myxobolus* sp. 6.

***Myxobolus* sp. 6** (Figs. 3.11–3.12)

*Myxobolus* n. sp. 6 most closely resembles *M. bilineatum*, which was originally described as

having an ovate to rounded spore with an iodophilus vacuole. The following prompted me to question these as conspecifics: *M. bilineatum* possesses wider spores and the congener identified herein possesses no more than 5 coils within polar capsules whereas *M. bilineatum* possesses 7–9 coils. All other spore types reported from *Fundulus*, excluding *Myxobolus* n. sp. 5, are distinctly pyriform in shape.

### **1.7.2. Platyhelminthes**

#### **Digenea (Plates 4–6)**

##### **Bucephalidae**

At present, the four putative species listed below have not been confidently identified to genus or species. However, the relative abundance of these metacercariae in their respective infection sites leads me to question them as conspecifics. Species descriptions of genera reported from *Fundulus*, i.e., *Rhipidocotyle* Diesing, 1858 and *Proisorhynchoides* Dollfus, 1929, include descriptions of stained internal structures. Stained taxonomic vouchers have not been prepared, thus, these metacercariae are identified to the Bucephalidae based on Hopkins (1954), Kinsella and Heard (1974), Stunkard (1976), Hoffman, 1999, and Curran and Overstreet (2009).

##### **Bucephalidae sp. 1 (Figs. 4.1–4.2)**

This putative species differs from all other bucephalids presented herein by having a hemispherical anterior sucker with a distinct, flattened anterior edge.

##### **Bucephalidae sp. 2 (Figs. 4.3–4.4)**

This putative species differs from all other bucephalids presented herein by having an inflated hemi-spherical and well-demarcated anterior sucker with a rounded anterior edge.

##### **Bucephalidae sp. 3 (Figs. 4.5–4.6)**

This putative species differs from all other bucephalids presented herein by having an non

inflated hemi-spherical anterior sucker with a rounded anterior edge that is not well demarcated from the rest of the body.

#### **Bucephalidae sp. 4** (Figs. 4.7–4.8)

This putative species differs from all others presented herein by having a nearly spherical anterior sucker with 4 prominent papillae-like structures.

#### **Diplostomidae**

As with the bucephalids, diplostomid metacercariae have not been stained and permanently mounted. The diplostomid reported herein may belong to *Posthodiplostomum* Dubois, 1936. Members of this genus are endoparasites that infect freshwater and estuarine fishes including *F. grandis* (Hoffman, 1958). *Posthodiplostomum minimum* (MacCallum, 1921) Hoffman, 1958 metacercariae have been reported from 97 species of primarily freshwater fish across 18 families, (Hoffman, 1958). *P. minimum* metacercariae have been reported from kidney, spleen, liver, and pericardium (Hoffman, 1958). Tentative identification of specimens of *Posthodiplostomum* is based on the aforementioned references and Bullard and Overstreet (2008).

#### ***Posthodiplostomum* sp.** (Figs. 4.9–4.10)

This species is morphologically distinct from all other digenea reported herein by having a bulbous anterior end.

#### ***Ascocotyle* Looss, 1899**

Species of *Ascocotyle* are constituents of the “*Ascocotyle* complex”, which is a series of proposed genera and subgenera that have undergone much debate (Sogandares-Bernal and Bridgman, 1960). *Ascocotyle* is limited with regard to life cycle research that links metacercarial stages with adult forms. Even so, some species in this genus have been described on the basis of life cycle information (experimental) and morphology of cercaria and metacercaria (Ostrowski

de Núñez, 2001). Since species in this genus are primarily described on the basis of adult features (Scholz et al., 1997) and since descriptions of metacercariae are poor and depauperate in the literature, the armature of the oral sucker was used as a primary diagnostic feature (i.e. number and length of 1 or 2 rows of oral spines), which has been shown to coincide with that of adults (Sogandares-Bernal and Bridgman, 1960; Sogandares-Bernal and Lumsden, 1963). Caution was taken when describing the oral spines of species because these structures are vulnerable to fixation artifact after storage in formalin (Font et al., 1984).

A total of 6 putative species of *Ascocotyle* (Heterophyidae) are presented herein. Members of this genus possess round, ellipsoid, or ovoid cysts that are with or without melanin-like pigment on the apical surface of cyst walls. The cuticle of species are characteristically spinose and the oral sucker is with or without oral (crown) spines.

***Ascocotyle diminuta* Stunkard and Haviland, 1924** (Figs. 5.1, 6.1)

Supplemental diagnosis of the metacercarial stage of this species can be found in Martin (1953). In this work, metacercaria of *Ascocotyle lageniformis* (Chandler, 1941) Martin, 1953 were described before Sogandares-Bernal and Lumsden (1963) synonymized this species with *A. diminuta*. The status of *A. diminuta* as a valid species is confusing since specimens of this species were originally reported to have a single row of 16 oral spines (Stunkard and Haviland, 1924) and apparently were without two dorsal accessory spines. I lack a complete understanding of synonymies that have been proposed in the literature for *A. diminuta*, however, it should be noted that variation in oral spine counts has been reported for metacercariae and adult specimens of this species (Ostrowski de Núñez, 1993). Furthermore, I have learned that congeneric metacercariae and adult heterophyids cannot be solely identified with oral spine counts since there are species with overlapping or exact counts (Ostrowski de Núñez, 2001). In this thesis, I

maintained identification of specimens of *A. diminuta* from Barataria Bay based on the aforementioned publications and a key provided by Scholz et al. (1997), which affirms that this species has an oral sucker with 16 circumoral spines plus 2 accessory (dorsal) oral spines.

***Ascocotyle tenuicolis* Price, 1935** (Figs 5.5, 6.2)

This is a putatively new host record for *A. tenuicolis* in Louisiana. As per Scholz et al. (1995) and Brock and Font (2009), specimens of this species possess 2 complete rows of 16 circumoral spines (=32 total; no figure illustrating oral spines is provided in this thesis).

***Ascocotyle* sp.** (Figs. 5.2, 6.3)

This species has 2 complete rows of 18 circumoral spines (=36 total; no figure illustrating oral spines is provided in this thesis). This oral spine count does not coincide with any references listed above and may constitute a new species pending acquisition of more properly fixed and mounted material and additional references pertaining to *Ascocotyle* in *Fundulus* or any other cyprinodontiform host.

***Euhaplorchis* Martin, 1950** (Fig. 5.6)

Species of *Euhaplorchis* encyst in the neurocranium, i.e., beneath the meningeal layer and surface of the brain, of fundulids (Martin, 1950; Abbott, 1968; Bullard and Overstreet, 2008; Shaw et al., 2010). The life cycle involves three hosts, i.e., a snail, a fish host, and piscivorous birds (Martin, 1950). Cercariae are shed from the snail host and directly penetrate the skin of the fish host before migrating to the braincase and developing into metacercariae (references in Shaw et al., 2010).

**Heterophyidae sp. 1** (Figs. 5.4, 6.4)

Specimens of this species and the two other heterophyids that follow (below) may belong to a genus other than *Ascocotyle*. Currently, no records of morphologically similar heterophyids

infecting species of *Fundulus* have been obtained and these species certainly are not reported from *F. grandis*. Heterophyidae sp. 1 possesses a broad (wide) oral sucker with 2 rows of 24 oral spines (=48 total oral spines) with the anterior row of spines much longer than the posterior row .

**Heterophyidae sp. 2** (Figs. 5.4, 6.5)

Heterophyidae sp. 2 possesses a broad oral sucker with 1 row of 28 oral spines that are in length.

**Heterophyidae sp. 3** (Figs. 5.4, 6.6)

Heterophyid sp. 3 possesses a broad oral sucker with 1 row of 24 oral spines.

**Strigeidae sp.** (Figs. 4.11–4.12)

This species is morphologically distinct from all other digenean metacercariae reported herein by having slightly tapered anterior and posterior ends.

**Monogenoidea** (Plates 7–8)

The Monogenoidea, also referred to as Monogenea in the primary literature, are ectoparasitic flatworms that infect the skin, gill epithelia, and olfactory organs of fishes. A few species are reported from the skin of amphibians and reptiles (Benz and Bullard, 2004). This group is characterized as having simple, direct-life cycles. Many species lay eggs on the host, wherein an oncomiracidia larva emerges and either develops and matures on the same host or within the water column where infection (colonization) on another host may occur. The group is generally regarded as host specific; however, the lifecycles of the majority of species are unknown (Benz and Bullard, 2004).

***Salsuginus* Beverley-Burton, 1984**

Before the diagnosis of this genus, several species were assigned to *Ancyrocephalus* (Beverley-Burton, 1984; Murith and Beverley-Burton, 1985) including *Salsuginus spirae*

(Williams, 1980) Murith and Beverley-Burton, 1985, (Monogenoidea: Ancyrocephalidae). This species was originally described from the gill lamellae of *F. similis* collected from Dauphin Island, AL. In the description, *F. grandis* is documented as an additional host, however, Murith and Beverley Burton (1985) suggested that specimens collected from *F. grandis* should be re-examined. Specimens of *Salsuginus* studied herein bear distinct morphological characteristics from other species that infect *Fundulus* in the United States and Canada (*see* Murith and Beverley-Burton, 1985). As with the gyroductylids, members of this genus are primarily described on the basis of haptoral sclerite morphology and morphometrics. Morphology of copulatory sclerites is also an important diagnostic feature. Herein, I have identified two putatively new species of *Salsuginus* collected from *F. grandis*.

***Salsuginus* n. sp. 1** (Figs. 7.1–7.3)

The primary distinguishing characteristics of *Salsuginus* n. sp. 1 studied herein include highly arched transverse dorsal and ventral bars and sharply recurved hamuli that differs from both *Salsuginus angularis* (Mueller, 1934) Beverley-Burton, 1984, (Monogenoidea: Ancyrocephalidae) and *Salsuginus heteroclitii* Murith and Beverley-Burton, 1985, (Monogenoidea: Ancyrocephalidae). *Salsuginus* n. sp. 1 also has ventral hamuli that are consistently longer than dorsal hamuli, which differs from both *Salsuginus umbraensis* (Mizelle, 1938) Murith and Beverley-Burton, 1985, (Monogenoidea: Ancyrocephalidae) and *Salsuginus fundulus* (Mueller, 1934) Beverley-Burton, 1984, (Monogenoidea: Ancyrocephalidae), which bear dorsal and ventral hamuli that are consistently of the same length. *S. spirae* possesses hamuli with relatively long and slender roots that are very distinct from the broad roots of *Salsuginus* n. sp. 1 studied herein.

***Salsuginus* n. sp. 2** (Figs. 7.1, 7.3–7.4)

The primary distinguishing characteristic of *Salsuginus* n. sp. 2 studied herein that differs from *Salsuginus* n. sp. 1 is the morphology of the ventral bar. This structure bears three prominent transverse ridges that are absent in all previously discussed species of *Salsuginus*. The hamuli of this species are most identical to *S. heteroclitii* (cf. Fig. 18 in Murith and Beverley-Burton) but in addition to the presence of ridges on the ventral bar, shorter (non-overlapping) ventral bar lengths were recorded from specimens studied herein (29–31  $\mu\text{m}$  in *Salsuginus* n. sp. 2 and 32–36  $\mu\text{m}$  in *S. heteroclitii*).

### **Genus *Gyrodactylus* Nordmann, 1832**

Over 400 species of *Gyrodactylus* infect the external surfaces of fishes worldwide (Boeger et al., 2003; Harris et al., 2004). All gyrodactylids are ectoparasites with direct life cycles and many species are regarded as highly host specific (Boeger et al., 2003). Although there are inconsistencies in the published literature regarding morphometrics, diagnosis of this genus and subsequent genera within Gyrodactylidae are based on morphology of sclerotized structures associated with the haptor (Vignon, 2011). Members of this genus have a haptor with 16 marginal hooks and a single pair of hamuli that articulate with dorsal and ventral bars.

### ***Gyrodactylus stephanus* Mueller, 1937 (Figs. 8.1–8.2)**

The specimens observed herein are most likely *G. stephanus*, however, additional specimens should be mounted, measured, and compared to available type material. The supplemental description provided by King and Cone (2009) described the dorsal bar of this species as possessing a median notch that was not mentioned in the original description (Mueller, 1937), which also lacks morphometric data concerning the dorsal bar. The redescription provided by Hargis (1955) coincided with my observations of the lack of a median notch in the dorsal bar of this species.

### **Genus *Fundulotrema* Kritsky and Thatcher, 1977**

Members of this genus bear a haptor with 16 marginal hooks arranged in two groups (three pairs of anterolateral hooks and five pairs of posterior hooks) and a single pair of relatively long anchors (hamuli) supported by a dorsal and ventral bar.

***Fundulotrema cf. prolongis* (Hargis, 1955) Kritsky and Thatcher, 1977** (Figs. 8.2, 8.4)

The putative specimens of *F. cf. prolongis* observed herein possess a dorsal bar that appears morphologically distinct from the original description along with the re-description of the species. According to the original description (Hargis, 1955), the dorsal bar of *F. prolongis* is “stout, butterfly-shaped” while no such comparison was made for the re-described species, which appears to have a thin, fragile, medially constricted, and steeply arched (anteriorly-directed) dorsal bar. Our specimens differ from specimens described in both of the aforementioned accounts by having an arched (also anteriorly directed) dorsal bar that is uniform in width, not as steep as that reported in the re-description, and certainly not butterfly-shaped as in the original description. Additional morphometric data and the acquisition of type material are required to solidify these observations. Furthermore, specimens from Barataria Bay should be compared to other published descriptions of species within *Fundulotrema*; i.e., *Fundulotrema foxi* (Rawson, 1973) Kritsky and Thatcher, 1977, (Monogenoidea: Gyrodactylidae), *Fundulotrema megacanthus* (Wellborn and Rogers, 1967) Kritsky and Thatcher, 1977, (Monogenoidea: Gyrodactylidae), *Fundulotrema stableri* (Hathaway and Herlevich, 1973) Kritsky and Thatcher, 1977, (Monogenoidea: Gyrodactylidae), and *Fundulotrema trematoclithrus* (Rogers, 1967) Kritsky and Thatcher, 1977, (Monogenoidea: Gyrodactylidae).

### **Genus *Swingleus* Rogers, 1969**

Species within this genus bear a haptor with 16 marginal hooks arranged in two groups (three pairs of anterolateral hooks and five pairs of posterior hooks), a single pair of relatively long hamuli bearing dense caps of tissues anteriorly and supported by a ventral bar, and have lateral wing-like bars instead of a dorsal bar.

***Swingleus polyclithroides* Rogers, 1969** (Figs. 8.2, 8.6)

Specimens of *S. polyclithroides* identified herein coincide with the original description (Rogers, 1969). This species was compared with the species descriptions for *Swingleus ancistrus* Billeter, Klink, and Mangel, 2000, (Monogenoidea: Gyrodactylidae) a congener that infects *F. heteroclitus* on the east coast of the United States (Billeter et al., 2000), and *Swingleus* sp. of Billeter (1974) infecting *F. heteroclitus* and *Fundulus majalis* (Walbaum, 1972), (Cyprinodontiformes: Fundulidae) on the east coast of the United States. *S. ancistrus* and *Swingleus* sp. both bear a conspicuous mid-sagittal notch on the anterior margin of the haptor and have digitiform extensions on the dense cap of tissue on the anterior ends of hamuli. These features were absent in specimens of *S. polyclithroides* identified herein.

**Cestoda** (Plate 9)

At least two species of tapeworms were collected from *F. grandis*. However, since these species are larval (metacestode and plerocercoid) forms, identification to species is not currently possible. However, I have provided information below regarding possible genera that may be infecting *F. grandis* from Barataria Bay based on host records from other species of *Fundulus*; e.g. *Fundulus grandissimus* Hubbs, 1936, (Cyprinodontiformes: Fundulidae), *F. heteroclitus*, *F. majalis*, and *Fundulus persimilis* Miller, 1955, (Cyprinodontiformes: Fundulidae).

**Gryporhynchidae**

**Gryporhynchidae sp. 1** (Figs. 9.1–9.2)

Members of Gryporhynchidae were once placed within the Dilepididae (Scholz et al., 2004). Phylogenetic studies based on comparative morphology (Hoberg et al., 1999) and molecular data (Mariaux, 1998) have shown that Dilepididae is a distinct lineage from that of species within the Gryporhynchidae. Species of Gryporhynchidae mature in piscivorous birds and metacestodes only infect fish second intermediate hosts that include 110 fish species across 27 families and 12 orders (Scholz et al., 2004). Identification of this group requires examination of the number, arrangement, size, and shape rostellar hooks (Scholz and Salgado-Maldonado, 2001). The metacestodes observed herein are possibly species of *Glossocercus* Chandler, 1935 or *Cyclusteria* Fuhrmann, 1901 (see Scholz et al., 2004). These genera have been reported from fundulids in the United States and Mexico (Chandler, 1935; Scholz and Salgado-Maldonado, 2001; Scholz et al. 2002; Scholz and Harris, 2006).

#### **Proteocephalidae sp.** (Figs. 9.3–9.4)

Proteocephalid pleurocercoids infect the stomach mucosa of fishes and are nearly impossible to identify as immature forms (Hoffman, 1999) especially when specimens are cold-fixed in formalin. Preliminary identification to family is based on the lack of a rostellum and the presence of four unarmed suckers and an apical organ (Freze, 1969; Hoffman, 1999). Specimens studied herein may be species of *Proteocephalus*, which has been reported from *F. diaphanus*, *F. grandis*, *F. heteroclitus* and *F. similis* (see Harris and Vogelbein, 2006 for host records).

#### **1.7.3. Nematoda** (Plate 10)

##### ***Eustrongylides* sp. Jägerskiöld, 1909** (Figs. 10.1–10.2)

Identification of *Eustrongylides* spp. is based on descriptions of adult specimens (Lichtenfels and Pilitt, 1986). Specimens of this putative genus infecting *F. grandis* (mainly determined from published host records) are third stage larvae that cannot be identified to species at this time.

## **Spiuridae** (Fig. 10.3)

Larval nematodes infected the gut mucosa of *F. grandis* but were not identified to species. This species was relatively abundant (compared to putative *Eustrongylides* sp.) and was mostly viewed in sediment after washing and scraping the gut mucosa of individuals of *F. grandis*. Preliminary observations of the sharply tapered posterior end of specimens suggest that this may be a species of Spiuridae (Hoffman, 1999).

### **1.7.4. Acanthocephala** (Plate 11)

#### ***Dollfusentis* cf. *chandleri*, 1969** (Figs. 11.1–11.2)

*D. chandleri* is reported from a diversity of fishes (Salgado-Maldonado, 1976; Kohn and Macedo, 1984; Noronha et al., 1986; Vicente et al., 1989; Luque et al. 1996a, 1996b; Alves and Luque 2001; Santos et al., 2008), which has resulted in confusion and subsequent synonymy of *D. chandleri* with other congeners in the northern Gulf of Mexico (Buckner et al., 1978). While specimens of *D. cf. chandleri* from Barataria Bay identified herein most likely are species of *Dollfusentis*, visualization of only 6 enlarged hooks (8 in *D. chandleri*; see Golvan, 1969) at the base of the proboscis and the inability to visualize the position of sensory papillae and the number of hooks per longitudinal row limited my identification.

#### ***Octospiniferoides chandleri* Bullock, 1957** (Figs. 11.3–11.4)

Specimens of *O. chandleri* identified herein matched the original description of this species (Bullock, 1957). This species is differentiated from *Octospiniferoides incognita* Schmidt and Huggins, 1973, (Neoechinorhynchida: Neoechinorhynchidae) by having markedly shorter hooks (*O. chandleri* hook length= 12–24 apical, 10–19 medial, 8–15 basal; *O. incognita*= 80–90 apical, 60 medial, 46–50 basal). Furthermore, *O. incognita* has 10 hooks in each circular row on the proboscis, whereas *O. chandleri* only has 8 per row. *Octospiniferoides australis* Schmidt and

Hughhins, 1973, (Neoechinorhynchida: Neoechinorhynchidae) (hook length 28–32 apical, 22–26 medial, 12–14 basal) also possesses longer hook armature than *O. chandleri* (Schmidt and Hughhins, 1973).

#### **1.7.5. Annelida** (Plate 12)

##### ***Malmiana philotherma* Sawyer, Lawler, and Overstreet, 1975** (Figs. 12.1–12.2)

Specimens of *Malmiana philotherma* studied herein were identified using keys provided by Sawyer et al. (1975) and Sawyer and Kinard (1980). Key characters include a flattened body and a large disc-like caudal sucker that is well demarcated and slightly larger or equal to the maximum body width. Because this species has a single pair of eyes on the oral sucker, it has been suggested that this species may belong to another genus; i.e., other species of *Malmiana* have at least 2 pairs of eyes on the oral sucker (Sawyer et al., 1975; Sawyer, 1986; Burreson and Kalman, 2006). The species is reported in this thesis as *M. philotherma* because no record of its reassignment to another genus has been published. This is a new locality record for this parasite in the Gulf of Mexico; a previous locality (Dauphin Island, AL) and host record for this parasite infecting *F. grandis* is published by Williams, 1979.

##### ***Myzobdella lugubris* Leidy, 1851** (Figs. 12.3–12.4)

Specimens of this species were identified using keys provided by Sawyer et al. (1975) and Appy and Dadswell (1980). Key characters include a single pair of eye spots (this feature was variably present in conspecifics studied herein but may be a result of fixation [Appy and Dadswell, 1980]) and a terminal caudal sucker that is not distinct from the posterior end (urosome) of the body.

#### **1.7.6. Arthropoda**

##### **Copepoda** (Plate 13)

### ***Ergasilus* Nordman, 1832**

As of 1999, *Ergasilus* Nordman, 1832 was comprised of about 33 freshwater or estuarine species in North America (Hoffman, 1999). This group of ectoparasites has a direct life-cycle where only females are parasitic. Although life cycle studies are generally lacking, it is accepted that eggs hatch while the female is infecting the host (primarily gill) and juveniles undergo up to seven morphologically distinct free-living stages before reaching reproductive maturity (Paperna and Zwerner, 1975). To my knowledge, no checklist or keys to species specifically regarding species of *Ergasilus* in the Gulf of Mexico exists today.

### ***Ergasilus* cf. *arthrosis* Roberts, 1969** (Figs. 13.1–13.2)

*Ergasilus* cf. *arthrosis* Roberts, 1969, (Copepoda: Ergasilidae) is morphologically similar to *Ergasilus lizae* Krøyer, 1863, (Copepoda: Ergasilidae). In the key provided by Kabata (1988), *E. arthrosis* is not included and by using the morphological feature in couplet 2 that states “no inflation between first and second segments of second antenna,” I initially identified one specimen as *E. lizae*. However, this species, like *E. arthrosis*, has a slender second antenna with a small spine-like protuberance on the inner margin of the second segment (Roberts, 1970). Furthermore, and also similar to *E. lizae*, the specimen studied herein possesses a proximal and distal setule on the inner margin of the 3<sup>rd</sup> segment of the second antenna. The final identification is based on the length of the 4<sup>th</sup> segment of the second antenna relative to the third segment. The 4<sup>th</sup> antennal segment in *E. lizae* is reported to be 50-60% of the length of the 3<sup>rd</sup> segment, however, there is variability that has been reported for this character (Roberts, 1970). The 4<sup>th</sup> antennal segment in *E. arthrosis* is 80-90% of the length of the third segment, which coincides with specimens identified herein.

### ***Ergasilus funduli* Krøyer, 1863** (Figs. 13.3–13.4)

According to Kabata (1986) the type material for *Ergasilus funduli* Krøyer, 1863 has been destroyed. In this work, the bio-illustrations in Figs.1–4 now serve as the lectotype for the species. This re-description was also based on weathered and partially compressed specimens. Nonetheless, preliminary identification of our specimens of this species was supplemented with the key and diagnosis provided by Roberts (1970).

## **Branchiura (Plate 14)**

### ***Argulus* Müller, 1785**

Of the four genera comprising the Branchiura, *Argulus* is the largest and most diverse group that infect the external skin surfaces of fishes (Møller, 2009). Approximately 129 nominal accepted species of *Argulus* exist in the literature today, 44 of which are estuarine and marine species, with the remaining 85 considered freshwater species (Poly, 2008). There are approximately ten species of *Argulus* that range in the Gulf of Mexico (Poly, 2009). The specimens of *Argulus* observed herein were compared to descriptions of 5 of those species; i.e., *Argulus alosae* Gould, 1841, (Branchiura: Argulidae), *Argulus floridensis* Meehan, 1940, (Branchiura: Argulidae), *Argulus funduli* Krøyer, 1863, (Branchiura: Argulidae), *Argulus megalops* Smith, 1873, (Branchiura: Argulidae), and *Argulus laticauda* Smith, 1873 (Branchiura: Argulidae). To better scrutinize specimens collected in this study, descriptions must be acquired for the remaining species that occur in the Gulf of Mexico and eastern Atlantic Ocean; i.e., *Argulus bicolor* Bere, 1936, (Branchiura: Argulidae), *Argulus fuscus* Bere, 1936, (Branchiura: Argulidae), *Argulus rotundus* Wilson, 1944, (Branchiura: Argulidae), *Argulus varians* Bere, 1936, (Branchiura: Argulidae), and *Argulus yucatanus* Poly, 2005 (Branchiura: Argulidae).

*Argulus* is a group of dioecious fish ectoparasites that utilize the habitat of the host to lay eggs after mating (Benz and Bullard 2005; Poly, 2008). *Argulus* exhibit a number of sexual

dimorphisms (Poly, 2008) that must be considered in the taxonomy of this group. Knowledge of life history characteristics associated with molting stages is sparse in the literature (Shimura, 1981; Møller, 2009), however, such information could prove valuable to taxonomy of *Argulus* in Barataria Bay.

During this study, larval forms of *Argulus* sp. were observed infecting fins and skin of the body but are not demonstrated or bioillustrated in this thesis. Species of *Argulus* undergo nine molting stages throughout development (Shimura, 1981; Benz and Bullard, 2005) and each stage can possess or lack certain morphological characteristics that can make identification of species rather difficult. Our putative larval specimens from Barataria Bay contained the following larval characteristics deduced from Wilson (1907) and Shimura (1981): carapace margins with minute cilia-like structures, numerous posteriorly projecting spine-like structures on ventral surface of carapace, and under-developed first maxillary suckers (sucker replaced by claw-like structure and lacking maxillary support rods).

***Argulus* n. sp. 1** (Figs. 14.1–14.2)

*Argulus* n. sp. 1 is most morphologically similar to *A. funduli*. I do not regard the specimens of *Argulus* n. sp. 1 observed herein to be conspecific with *A. funduli* due to the following distinctions: *Argulus* n. sp. 1 possesses a relatively large pad of cycloid-like vs. ctenoid-like scales (deduced from bio-illustrations in Kabata, 1988) that covers a larger area on the ventral base of the second maxilla compared to *A. funduli*. Also, the three basal teeth of the second maxilla in specimens studied herein are longer than those of *A. funduli* illustrated in Kabata (1988). Furthermore, the presence of 5 ventral and 2 dorsal setae at the base of the second maxilla in our specimens is not mentioned in any description or key concerning *A. funduli*. I have not encountered any statements about morphological variation or sexual dimorphism of the

structures associated with the base of second maxilla in adults, but I acknowledge that such an account may be in existence for other species of *Argulus*.

***Argulus n. sp. 2*** (Figs. 14.3–14.4)

Specimens of *Argulus n. sp. 2* also possess three basal teeth on the second maxilla that are distinctly shorter than those of *Argulus n. sp. 1* studied herein. The presence of four ventral setae at the base of the second maxilla further differentiates *Argulus n. sp. 2* from *Argulus n. sp. 1*.

**Isopoda** (Plate 15)

**Cymthoidae**

***Lironeca ovalis* Say, 1818** (Fig. 15.1–15.2)

A single specimen of this species has been collected in this study perhaps due to the relative ease of escaping capture and swimming ability. This species has been identified from *F. grandis* (Price and Schlueter, 1980) in Florida, therefore, this may be a new locality record for the species in the Gulf of Mexico. However, the primary literature must be further investigated to confirm this suspicion. This specimen was identified using a key provided in Hoffman (1999) and is characterized by having a fifth thoracic segment that is distinctly wider than the third thoracic segment.

**1.8. Conclusions**

The species-level identification of the parasites infecting *F. grandis* requires complete taxonomic exploration. This entails comparing voucher specimens from this study to available museum type material. Certain parasite groups including the Myxozoa and Digenea may also require molecular differentiation due to the lack of type material (for putatively new species) and the lack of species descriptions that describe immature and intermediate forms (e.g. metacercariae).

Although 44 species were putatively identified herein, I suspect that more species could be discovered to infect *F. grandis* upon examination of additional inventoried parasite material not included in this thesis. I did not construct species accumulation (rarefaction) curves in this study, however, with this large host sample size I expect that a rarefaction curve would likely show a distinct plateau. The parasite component community of *F. grandis* in Barataria Bay could potentially be compared to other parasite surveys conducted in other localities. For instance, the parasite component community of *F. grandis* in Perdido Bay, AL, could be characterized and the results from such a survey could be compared to my study in terms of both biodiversity and the potential use of parasites as bioindicators of environmental alterations (e.g. the effects of land-use and urbanization).

Another area of exploration would be to characterize the protozoan parasites that infect *F. grandis*, which could be facilitated with histopathology of infected tissues.

## Chapter 2

### PARASITES AS BIOINDICATORS OF ECOSYSTEM FUNCTIONING IN BARATARIA BAY, LA, AFTER THE 2010 BP *DEEPWATER HORIZON* OIL SPILL

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## 2.1. Abstract

The use of parasites as ecosystem indicators is common in the literature, but no such study has been conducted in the Gulf of Mexico nor subsequent to the 2010 BP Deepwater Horizon Oil Spill. I used the foundational knowledge of the parasite component community (Chapter 1) to test the hypothesis that decreased parasite species richness or changes in mean intensity or prevalence indicate an ecosystem disturbance. The analysis considers all metazoan parasites of Gulf killifish, *Fundulus grandis*, including ectoparasites and endoparasites. Within Barataria Bay, 4 reference sites (non-oiled) and 4 oiled sites were sampled continuously for 12 months, accounting for seasonal variation in parasite life cycle and subsequent abundance and intensity. No significant differences were detected between prevalence (aggregated) of ectoparasites (Monogenoidea, Hirudinida, Copepoda, Branchiura, Isopoda) and endoparasites (Myxozoa, Digenea, Cestoda, Nematoda, Acanthocephala) in oiled and non-oiled sites nor in mean intensity across parasite groups in oiled and non-oiled sites. However, significant differences were detected in species prevalence of acanthocephalans (21.4% in non-oiled sites vs. 3.2% in oiled sites), copepods (20.5% vs. 33.6%), and branchiurans (13.6% vs. 27.3). Seasonal effects were statistically detected in myxozoans (highest intensity of infection during May 2011) and digeneans (highest intensity in August 2011), and those seasonal patterns were not significantly different between oiled and non-oiled sites. Digenean metacercariae infecting gill and heart, monogenoideans infecting skin, and nematodes infecting body cavity each have a significantly higher log mean intensity in oiled sites. No significant differences were detected for species richness of ectoparasites and endoparasites in oiled and non-oiled sites and across seasons. Condition factor of *F. grandis* was not significantly different between oiled and non-oiled sites and across seasons.

## 2.2. Introduction to parasites as bioindicators of ecosystem functioning

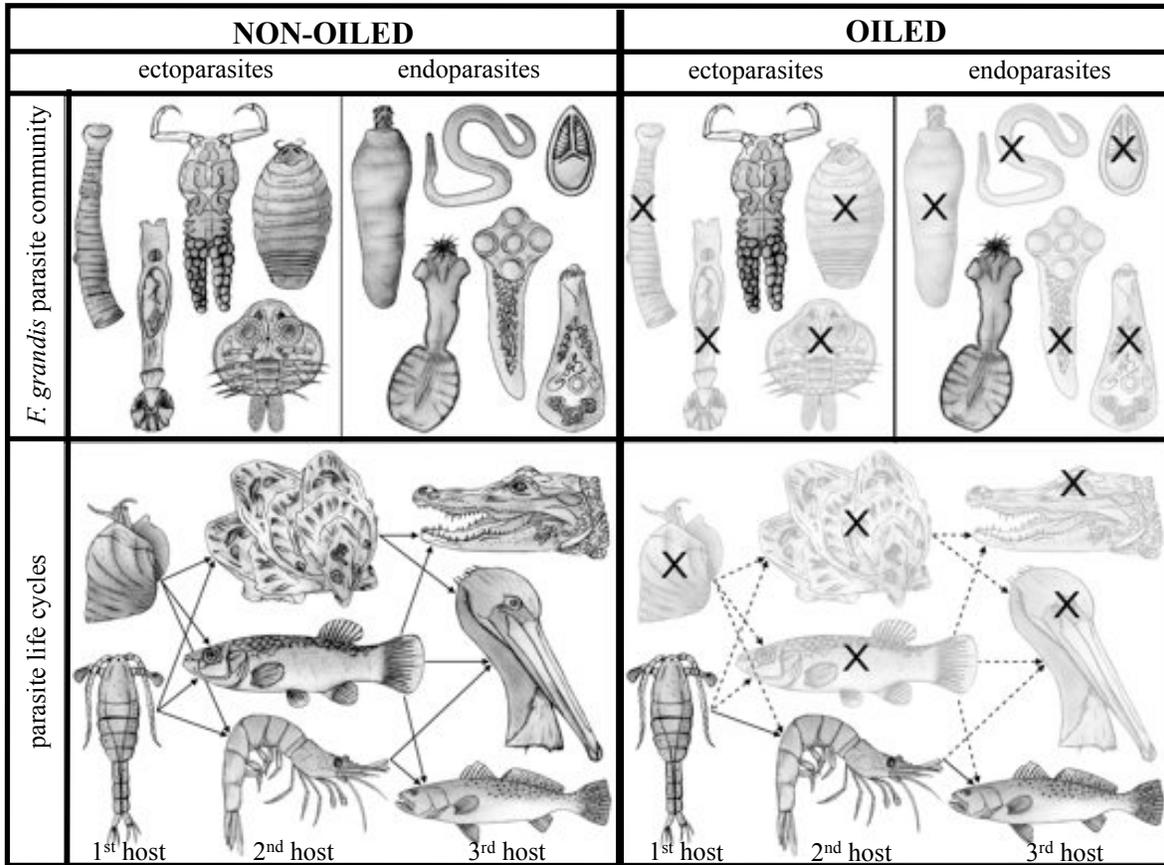
Endoparasites and ectoparasites greatly outnumber free-living organisms in freshwater and marine ecosystems (Benz, 1995), and have thus far been under-utilized as bioindicators in community-level eco-toxicological studies (Sures, 2004). Aquatic contaminants can change parasite component communities, e.g., decrease parasite species richness (Diamant, 1999; Pech, 2009), result in heavy infections (Paperna, 1975; Paperna and Overstreet, 1981; Broeg, 1999; Khan, 1990; Williams and MacKenzie, 2003; Pérez-del Olmo et al., 2007), lower prevalence (Khan and Payne, 1997; Halmetoja et al., 2000), or skew parasite diversity indices (Dušek et al., 1998; Dzikowski et al., 2003; Schmidt et al., 2003; Rueckert et al., 2009). Some published works regarding parasites as bioindicators utilize metadata to draw generalized conclusions about the possible effects of environmental toxins (including crude oil, eutrophication, heavy metals, pesticides, PCBs, and pulp mill effluents) on major parasite lineages (Sures et al., 1999; Vidal-Martinez et al., 2009). Experimental exposure studies have also provided evidence of aromatic hydrocarbon toxicity effects on parasite prevalence and intensity and host immunity (Moles, 1980; Khan and Kiceniuk, 1983; Khan, 1987; Khan and Kiceniuk, 1988; Khan, 1991; Khan et al., 1994; Marcogliese et al., 1998; Moles and Norcross, 1998; Moles and Wade, 2001; Khan and Payne, 2004). For instance, prevalence and intensity of infection has been shown to increase for *Gyrodactylus* spp. infecting the gill of Atlantic cod, *Gadus morhua* Linnaeus, 1758, (Gadiformes: Gadidae) throughout chronic exposure to petroleum hydrocarbons (Khan and Kiceniuk, 1988). Marcogliese et al. (1998) reported similar results with regard to higher intensity of *Gyrodactylus* sp. infecting the skin of the body and fins of American plaice, *Hippoglossoides platessoides* (Fabricius, 1780), (Pleuronectiformes: Pleuronectidae) after exposure to oil contaminated sediments.

Parasites can inform ecosystem functioning at various levels. This is because a given parasite species has a specific host or hosts that it requires to complete its life cycle (Plate 16).

Endoparasites have a complex indirect life cycle involving trophic interactions. Hence, the presence of one parasite species in one host is an indicator that all other required hosts such as snails, shrimp, annelids, crabs, amphipods, birds, mammals, and fishes are present in that ecosystem (Hechinger et al., 2007). Endoparasites are less vulnerable because they are insulated by their host's tissues yet over a longer temporal scale are imperiled if required host populations decrease or are extirpated (Möller, 1987; MacKenzie et al., 1995; Lafferty, 1997; MacKenzie, 1999; Khan, 2003; Hudson et al., 2006; Hechinger et al., 2007). This makes endoparasites potential bioindicators of chronic environmental perturbations; generally, high endoparasite richness correlates with pristine habitat (Hudson et al. 2006) and proxies for ecosystem functioning. Toxic events, like an oil spill, that decrease the abundance of or eliminate free-living invertebrates and/or vertebrates therefore have great potential to severely impact parasite community structure.

Not all parasites require a host other than that of its parent(s). These parasites have simple, direct life cycles because they infect a single host species. Direct life cycle parasites are primarily ectoparasites that are vulnerable to water-borne toxins because they are bathed in ambient water (MacKenzie, 1999; Sasal et al., 2007). Their life cycles are generally regarded as simple and involve a single host with no trophic interaction or intermediate hosts. These ecological qualities theoretically make ectoparasites susceptible to aquatic pollution and bioindicators of acute environmental perturbation, i.e., ectoparasites can die soon after a toxic event. High biodiversity or increasing intensity or prevalence of ectoparasites can also indicate that a host population is immunocompromised (Moles et al., 1993; Valtonen et al., 1997;

**PLATE 16.** Parasite communities as indicators of ecosystem functioning and food webs in oiled (right) and non-oiled (left) sites. Parasites are extirpated (X) and component communities change as their hosts' populations are reduced/eliminated.



Steyermark et al., 1999) or stimulated by anthropogenic eutrophication (Overstreet and Howse, 1977; Lafferty, 2008) or that parasites are evolutionarily adapted to toxic events (Lafferty and Kuris, 1999).

Herein, characterization of the parasite component community of *F. grandis* (Chapter 1) was used as a foundation for analyzing mean intensity, prevalence, and species richness of parasites in oiled and non-oiled reference sites across four collection events in order to (1) evaluate ecosystem functioning and (2) to identify and document seasonality in the parasite component community of *F. grandis*.

### **2.3. Justification**

The use of parasites as ecosystem indicators is common in the literature, but no such study has been conducted in the Gulf of Mexico nor subsequent to the 2010 BP *Deepwater Horizon* Oil Spill. Furthermore, shifts in parasite diversity, prevalence, and intensity resulting from the 2010 DHOS could indicate spill related changes to water quality, abundances and immunological health of free-living organisms, or the Gulf of Mexico food web.

### **2.4. Objectives and Hypotheses**

*Objective II:* Test for differences in prevalence, mean intensity and species richness of ectoparasites and endoparasites between oiled and non-oiled study sites, thereby testing their use as biological indicators of acute and chronic perturbations resulting from the 2010 BP *Deepwater Horizon* Oil Spill.

*Hypothesis II:* Prevalence, mean intensity, and species richness of ectoparasites and endoparasites infecting Gulf killifish collected from four oiled and four non-oiled sites in Barataria Bay, LA are not significantly different.

*Objective III:* Document seasonality of infections by members of the parasite component

community.

*Hypothesis III:* The parasite component community is not seasonal.

## **2.5. Materials and methods**

The sampling design employed in Chapter 1 of my thesis corresponds to the ecological analysis presented herein. Briefly, 100 Gulf killifish were collected from four oiled and four non-oiled reference sites in Barataria Bay, LA (see Chapter 1.5.1.). Each fish was abdominally injected in the field with 10% formalin and immersed in whirl-paks filled with 10% formalin. 480 fish were necropsied at Auburn University and their parasites were collected and tentatively identified to one of ten major metazoan parasite groups (Myxozoa, Digenea, Monogenea, Cestoda, Nematoda, Acanthocephala, Hirudinida, Branchiura, Copepoda, Isopoda). Their respective sites of infection and intensity of infection were recorded on standardized data sheets that were entered in a host-parasite matrix using Microsoft Excel. At least three specimens of each major metazoan parasite group infecting twenty-three tissue sites were identified to the lowest taxonomic level by comparing them to published species descriptions. These species comprised the parasite component community of *F. grandis* in Barataria Bay, LA and provided the foundation for hypothesis testing of ecosystem functioning after the DHOS.

### **2.5.1. Calculating prevalence and mean intensity**

Mean intensity is the average number of a parasite species in a sample of infected hosts divided by the total number of infected hosts while prevalence is the total number of infected hosts divided by the total number of examined hosts in a sample (Bush et al., 1997) (see Chapter 1.2.3.). Prevalence (aggregated) and mean intensity (aggregated) of ectoparasites (Monogenea, Hirudinida, Copepoda, Branchiura) and endoparasites (Digenea, Cestoda, Nematoda, Acanthocephala) and each major metazoan parasite group were calculated (see

Tables 5–6 in Appendices) and graphically represented for oiled and non-oiled reference sites using Microsoft Excel. (Note: Since intensity values from host to host vary with sample size, there is an associated estimate of the standard error of the mean [Norman and Streiner, 1986]. Prevalence is not an average [usually expressed as a percentage, see Bush et al., 1997] and therefore has no mean and no estimate of the standard error of the mean).

### **2.5.2. Statistical testing**

Operational taxonomic units (OTUs) for statistical analysis were established based on identification of major metazoan parasite groups and their respective sites of infection (see Chapter 1.5.). Histograms of mean intensity data for all OTUs were used to assess normal distributions. Intensity distributions were generally skewed non-normal distributions that were variably fixed with log-transformation. Thus, OTUs infecting tissue sites with >40% prevalence were statistically tested using non-parametric tests. The Wilcoxon rank-sum test was used for differences between oiled and non-oiled reference sites (OTUs= digenean metacercariae infecting gill, bulbous arteriosus, and fin rays; monogenoids infecting skin and gill; nematodes infecting body cavity). The Kruskal-Wallis test was used for differences in seasonality of gill parasites (OTU's= Myxozoa, Digenea, Monogenoidea). Tests were run using SAS 9.0 software. All significance values were set at  $\alpha = 0.05$ .

### **2.5.3. Estimating species richness between sites and seasons**

Using the putative parasite species identified in Chapter 1, species richness was estimated across four collection events for aggregated ectoparasites (Monogenoidea, Hirudinida, Copepoda, Branchiura, Isopoda) and endoparasites (Myxozoa, Digenea, Cestoda, Nematoda, Acanthocephala) in four oiled and four non-oiled reference sites. Total species richness estimates across four collection events were graphically represented using Microsoft Excel.

#### **2.5.4. Condition factor analysis**

Host condition factor ( $K=100 \times \text{weight}/\text{length}^3$ ) was calculated for 480 individuals of *F. grandis* randomly selected for necropsy and parasite component community characterization (Chapter 1.5.4.; see Table 5, 6). A t-test for significant differences in mean condition factor of necropsied fish in oiled (n=240) and non-oiled (240) reference sites was run using Microsoft Excel with significance set at  $\alpha = 0.05$ . Seasonal mean condition factor of *F. grandis* was also calculated and tested for significant differences (ttest). Seasonal mean condition factor was graphically represented using Microsoft Excel.

### **2.6. Results**

#### **2.6.1. Prevalence of parasite infections**

No significant differences were detected between aggregated prevalence of ectoparasite (Monogenoidea, Hirudinida, Copepoda, Branchiura, Isopoda) and aggregated prevalence of endoparasite (Myxozoa, Digenea, Cestoda, Nematoda, Acanthocephala) groups in oiled and non-oiled sites (Plate 19). Significant differences were detected in aggregated species prevalence of Acanthocephala, Copepoda, and Branchiura (Plate 20). Acanthocephalans infected *F. grandis* with 19.6% prevalence in non-oiled sites vs. 2.9% in oiled sites. Copepods infected *F. grandis* with 21.7% prevalence in non-oiled sites vs. 34.2% in oiled sites. Branchiurans infected *F. grandis* with 13.8% prevalence in non-oiled sites vs. 28.3% in oiled sites.

#### **2.6.2. Mean intensity of infections**

No significant differences were detected between aggregated mean intensity of ectoparasite (Monogenoidea, Hirudinida, Copepoda, Branchiura, Isopoda) and aggregated mean intensity of endoparasite (Myxozoa, Digenea, Cestoda, Nematoda, Acanthocephala) groups in oiled and non-oiled sites (Plate 21). Furthermore, no significant differences in mean intensity were detected for

any major metazoan parasite group (Plate 22). However, statistical testing of putative species-level OTU's showed some significant differences (Table 3–4). Digenean metacercariae of the gill and heart had a significantly higher log mean intensity in oiled sites (p-value= 0.0007 for gill metacercariae [see Plate 17]; p-value= 0.0025 for heart metacercariae). Monogenoidea infecting skin showed a higher log mean intensity of infection in oiled sites (p-value= 0.0001 [see Plate 18]). Nematoda (L3) infecting the body cavity of *F. grandis* had a greater mean intensity in oiled sites (p-value= 0.01).

### **2.6.3. Species richness of ectoparasites and endoparasites**

No significant differences were detected for seasonal species richness of ectoparasites and endoparasites and no significant differences were detected between oiled and non-oiled sites (Plate 23).

### **2.6.4. Seasonality of gill parasites**

Myxozoa infecting gill showed a seasonal trend where the greatest intensities of infection were during May 2011 (Plates 24–25). This trend was consistent and not significantly different between oiled and non-oiled sites. Digenean metacercariae infecting gill also showed a seasonal trend where the greatest intensities of infection were during August 2011 (Plates 26–27). This trend was also consistent and not significantly different between oiled and non-oiled sites.

### **2.6.5. Condition factor analysis**

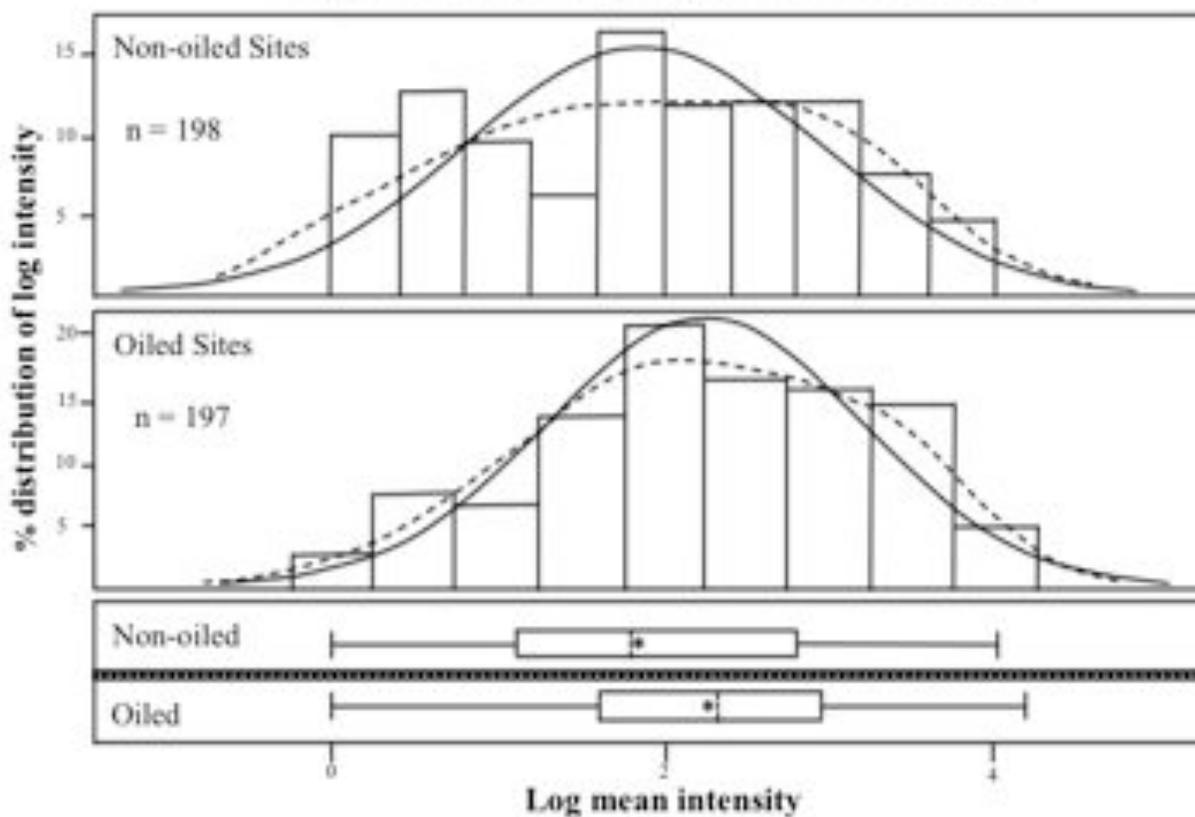
No significant differences were detected for mean condition factor (Plate 28) of *F. grandis* between oiled and non-oiled sites (p-value= 0.37) and across seasons (October 2010 p-value= 0.13; February 2011= 0.45; May 2011= 0.45; August 2011= 0.05). The greatest difference was 6.7% in August 2011 (condition=1.5 [oiled], 1.4 [non-oiled]).

**Table 3. Summary of statistical tests and conclusions for the parasite component community of *Fundulus grandis*.**

Taxon	Site of Infection	Null hypothesis	Test	P-value(s)	Conclusion
<b>Myxozoa</b>	gill	No seasonal difference in mean intensity	Kruskal-Wallis	0.0001 (O); 0.001 (NO)	Reject null; Mean intensity significantly greater in May for both (O) and (NO) sites
		No seasonal difference in mean intensity	Kruskal-Wallis	0.0001 (O); 0.001 (NO)	Reject null; Mean intensity significantly greater in May for both (O) and (NO) sites
<b>Digenea</b>	gill	No difference in mean intensity between sites	Wilcoxon	0.0007	Reject null; Mean intensity significantly greater in (O) sites
		No difference in mean intensity between sites	Wilcoxon	0.0025	Reject null; Mean intensity significantly greater in (O) sites
		No difference in mean intensity between sites	Wilcoxon	0.2623	Fail to reject null; Mean intensity not significantly different between sites
<b>Monogenoidea</b>	gill	No seasonal difference in mean intensity	Kruskal-Wallis	0.7529 (O); 0.0563 (NO)	Fail to reject null; Mean intensity not significantly different between seasons
		No difference in mean intensity between sites	Wilcoxon	0.8506	Fail to reject null; Mean intensity not significantly different between sites
		No difference in mean intensity between sites	Wilcoxon	0.0001	Reject null; Mean intensity significantly greater in (O) sites
<b>Cestoda</b>	–	–	–	–	–
<b>Nematoda</b>	body cavity	No difference in mean intensity between sites	Wilcoxon	0.01	Reject null; Mean intensity significantly greater in (O) sites
<b>Acanthocephala</b>	–	–	–	–	–
<b>Hirudinida</b>	–	–	–	–	–
<b>Copepoda</b>	–	–	–	–	–
<b>Branchiura</b>	–	–	–	–	–
<b>Isopoda</b>	–	–	–	–	–

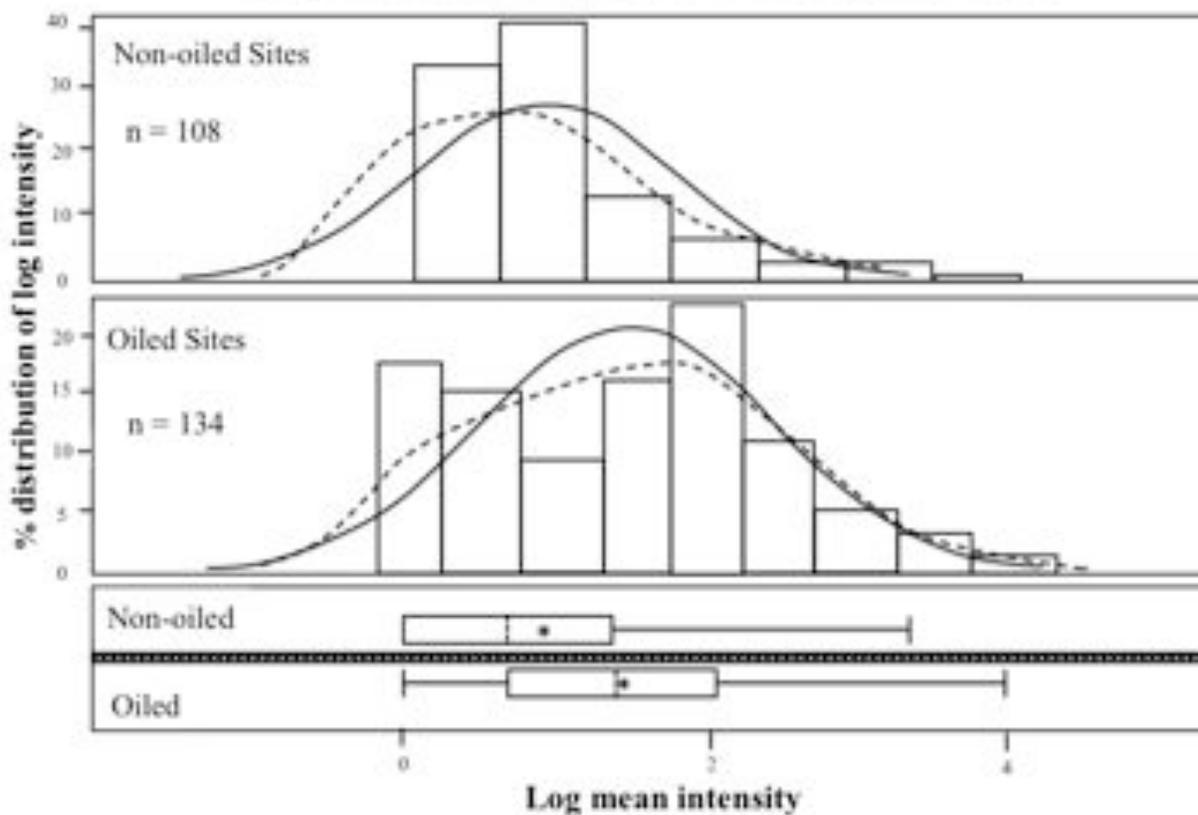
**PLATE 17.** Log mean intensity of metacercariae infecting gill lamellae of *Fundulus grandis*  
Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in oiled and non-oiled sites.

### Log mean intensity of gill metacercariae



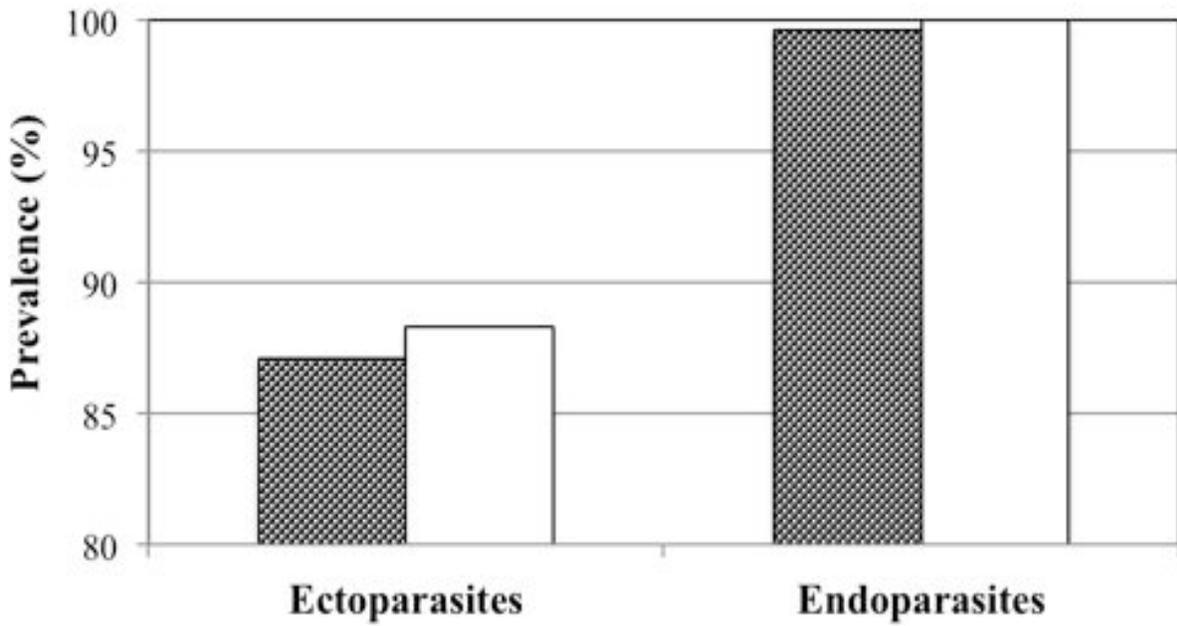
**PLATE 18.** Log mean intensity of Monogenoidea infecting skin of *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in oiled and non-oiled sites.

### Log mean intensity of skin monogenoideans



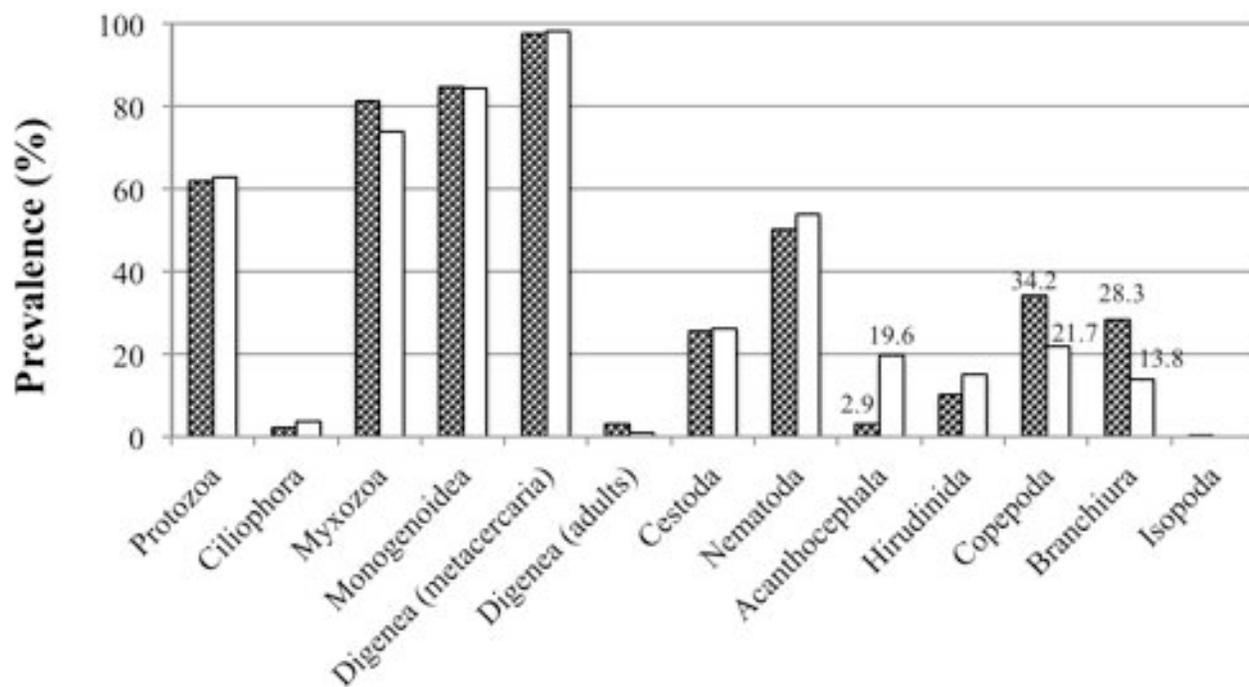
**PLATE 19.** Prevalence of ectoparasites (direct life cycles) and endoparasites (indirect life cycles) infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in oiled and non-oiled sites. Spotted bars indicate oiled sites and open bars indicate non-oiled sites.

**Prevalence of ectoparasites and endoparasites  
infecting *Fundulus grandis***



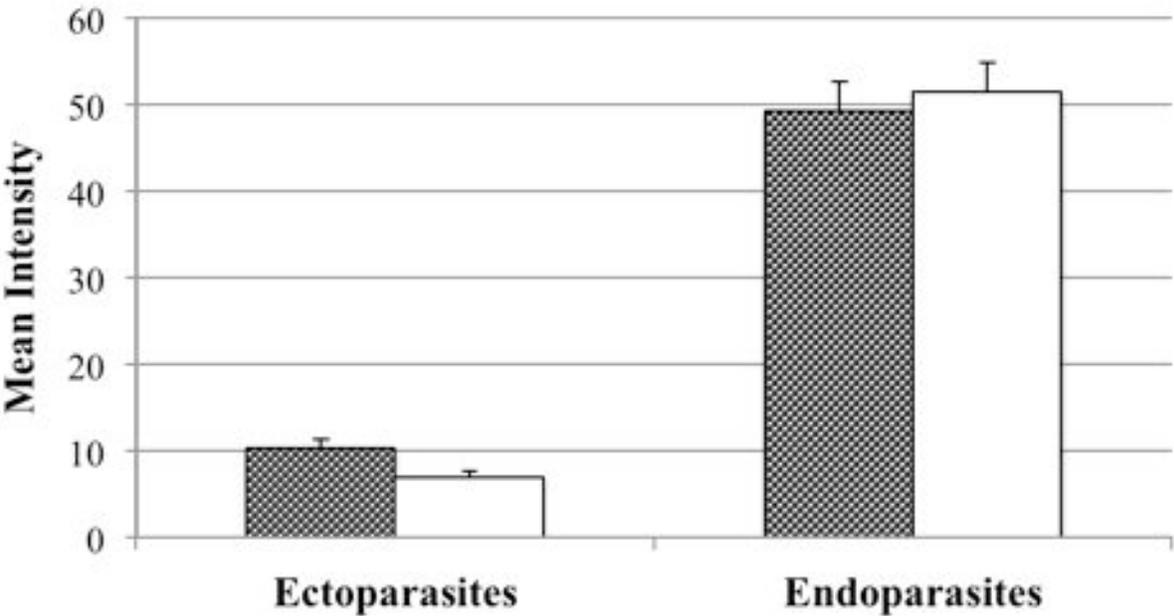
**PLATE 20.** Prevalence of major metazoan parasite groups infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in oiled and non-oiled reference sites. Prevalence of Protozoa (Apicomplexa and Ciliophora) and adult digeneans are included. Spotted bars indicate oiled sites and open bars indicate non-oiled sites. Significantly different prevalence values above bars.

## Prevalence of parasites infecting *Fundulus grandis*



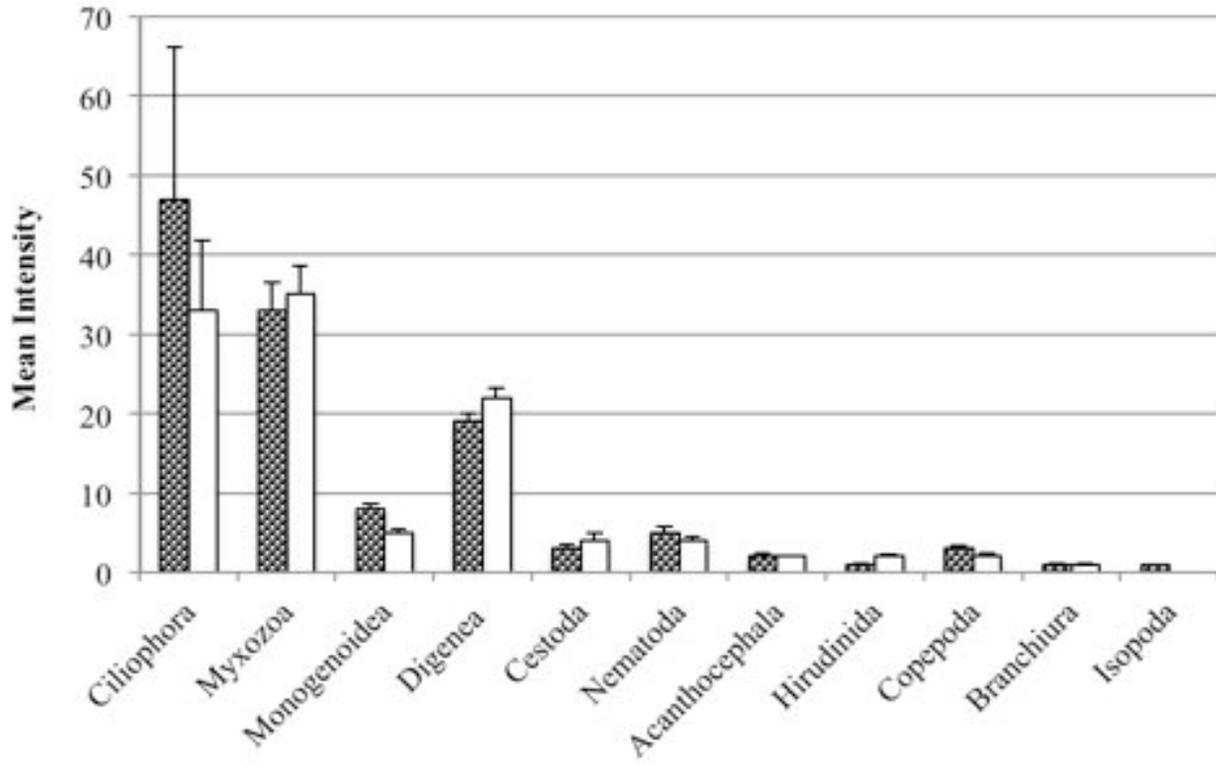
**PLATE 21.** Mean intensity (+S.E.) of ectoparasites (direct life cycles) and endoparasites (indirect life cycles) infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in oiled and non-oiled reference sites. Spotted bars indicate oiled sites and open bars indicate non-oiled sites.

**Mean intensity of ectoparasites and endoparasites infecting *Fundulus grandis***



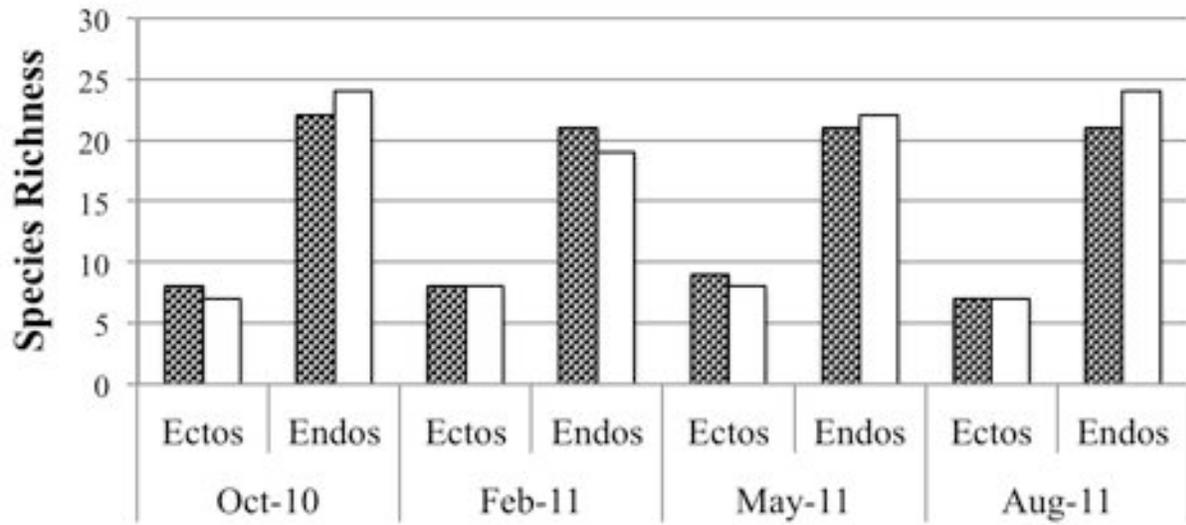
**PLATE 22.** Mean intensity (+S.E.) of major metazoan parasite groups infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in oiled and non-oiled sites. Mean intensity of Ciliophora (Protozoa) is included. Spotted bars indicate oiled sites and open bars indicate non-oiled sites.

Mean intensity of parasites infecting *Fundulus grandis*



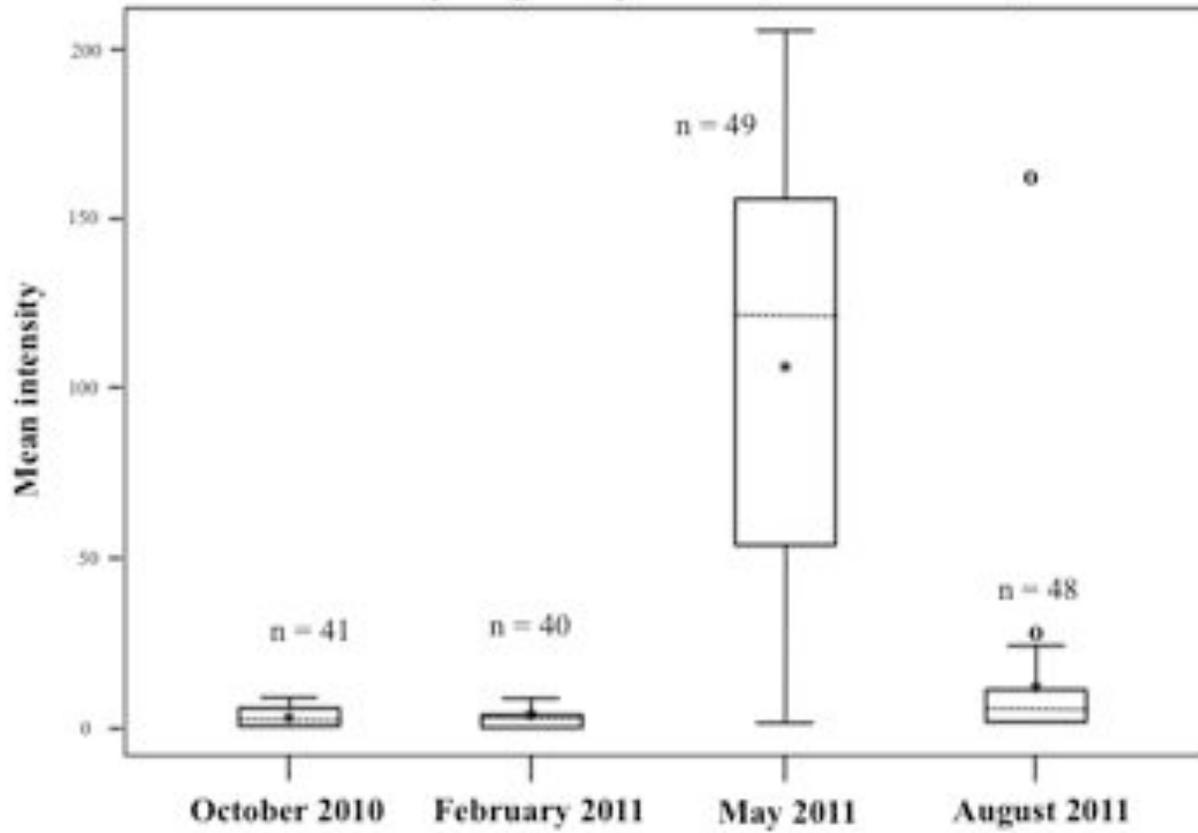
**PLATE 23.** Seasonal species richness of ectoparasites and endoparasites infecting *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in oiled and non-oiled reference sites. Spotted bars indicate oiled sites and open bars indicate non-oiled sites.

**Species richness of parasites infecting *Fundulus grandis***



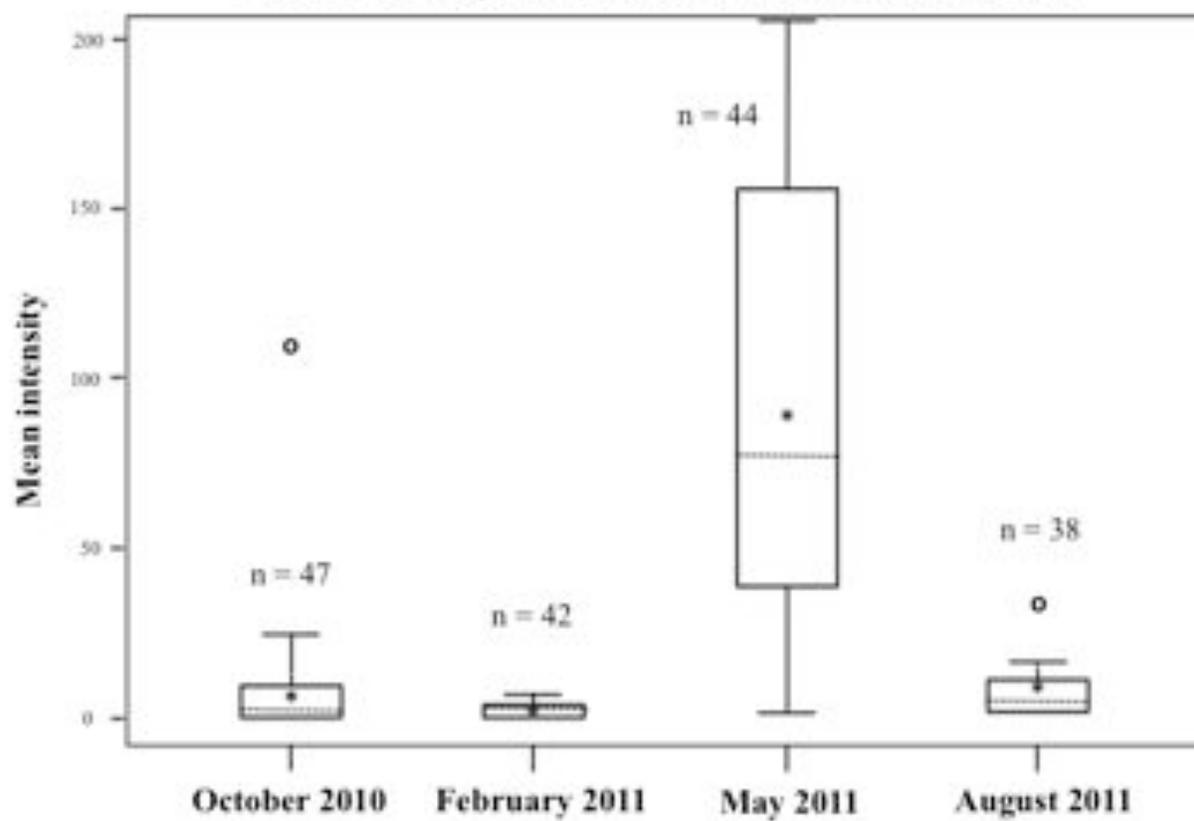
**PLATE 24.** Seasonal mean intensity of myxozoans infecting gill lamellae of *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in all oiled sites. Outliers represented by circles above error bars. Dotted lines within box-plots represent median values. Asterisks represent mean values.

### Intensity of gill myxozoa (all oiled sites)



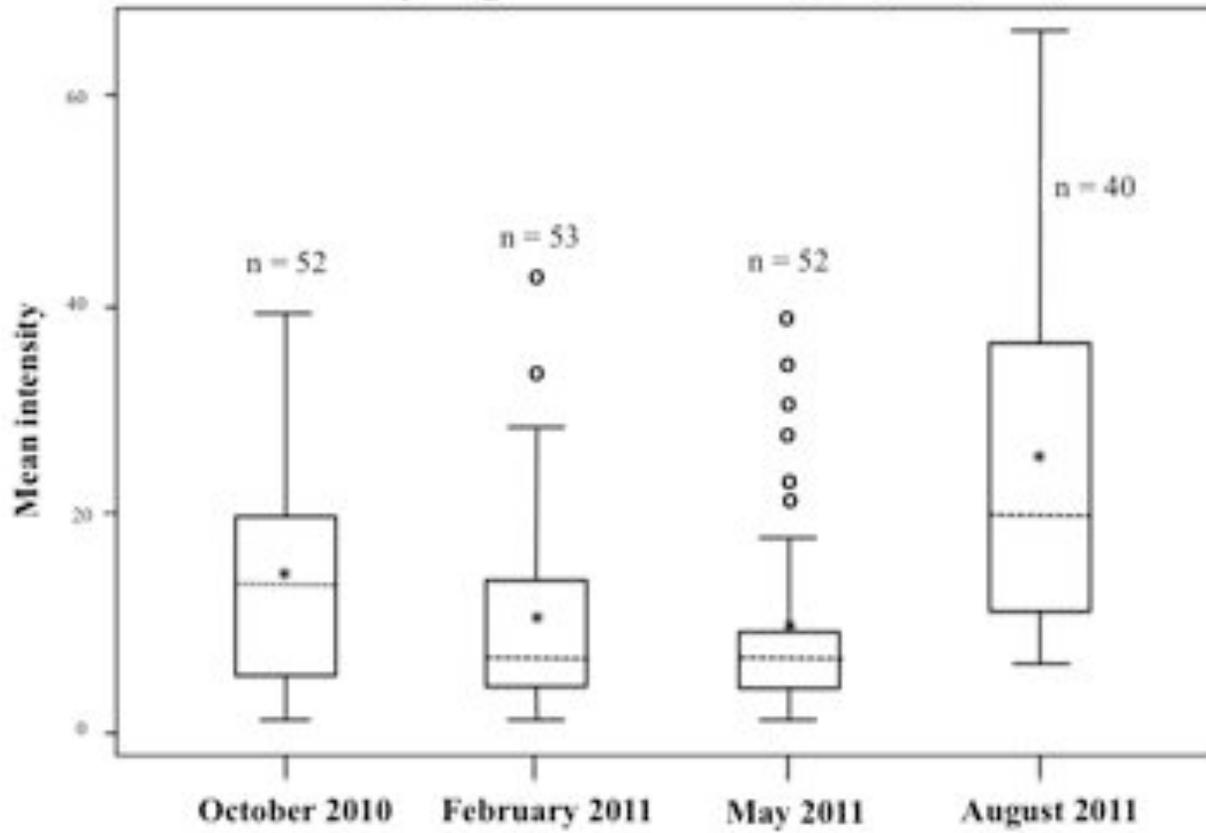
**PLATE 25.** Seasonal mean intensity of myxozoans infecting gill lamellae of *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in all non-oiled sites. Outliers represented by circles above error bars. Dotted lines within box-plots represent median values. Asterisks represent mean values.

### Intensity of gill myxozoa (all non-oiled sites)



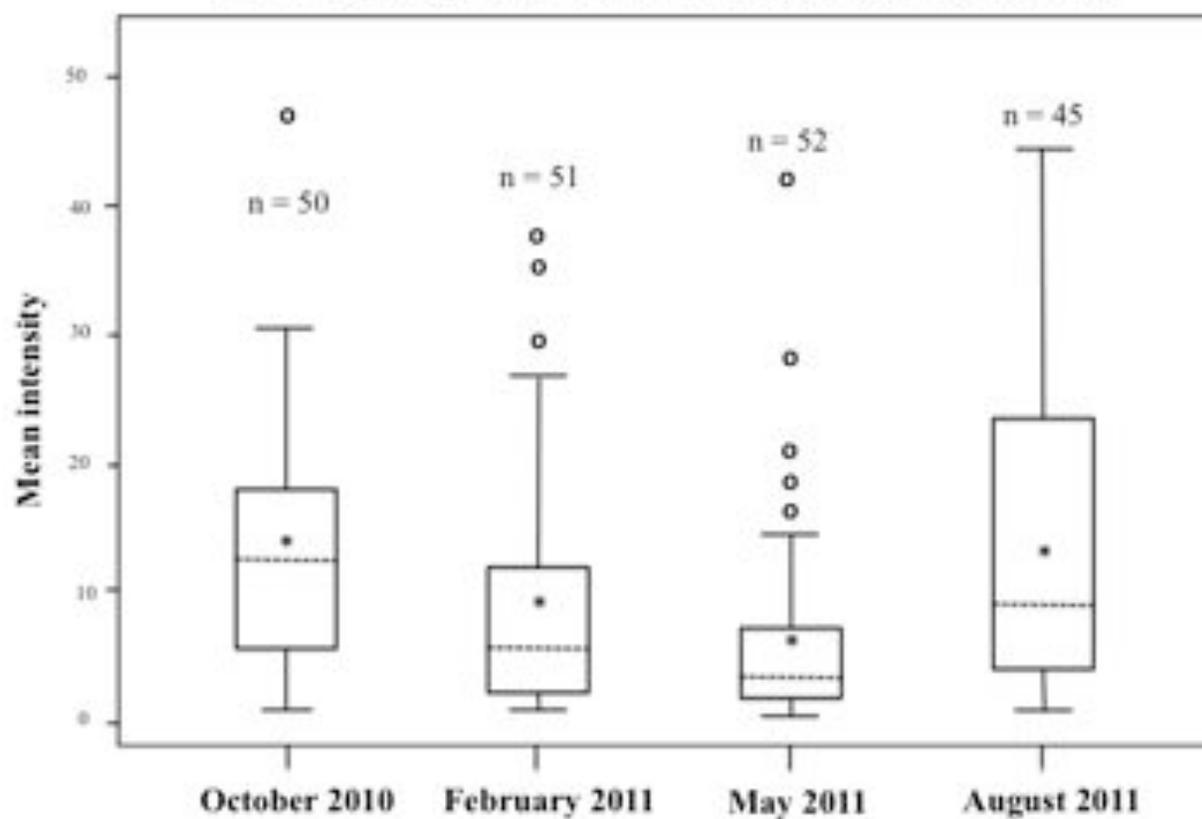
**PLATE 26.** Seasonal mean intensity of metacercariae infecting gill lamellae of *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in all oiled sites. Outliers represented by circles above error bars. Dotted lines within box-plots represent median values. Asterisks represent mean values.

### Intensity of gill metacercaria (all oiled sites)



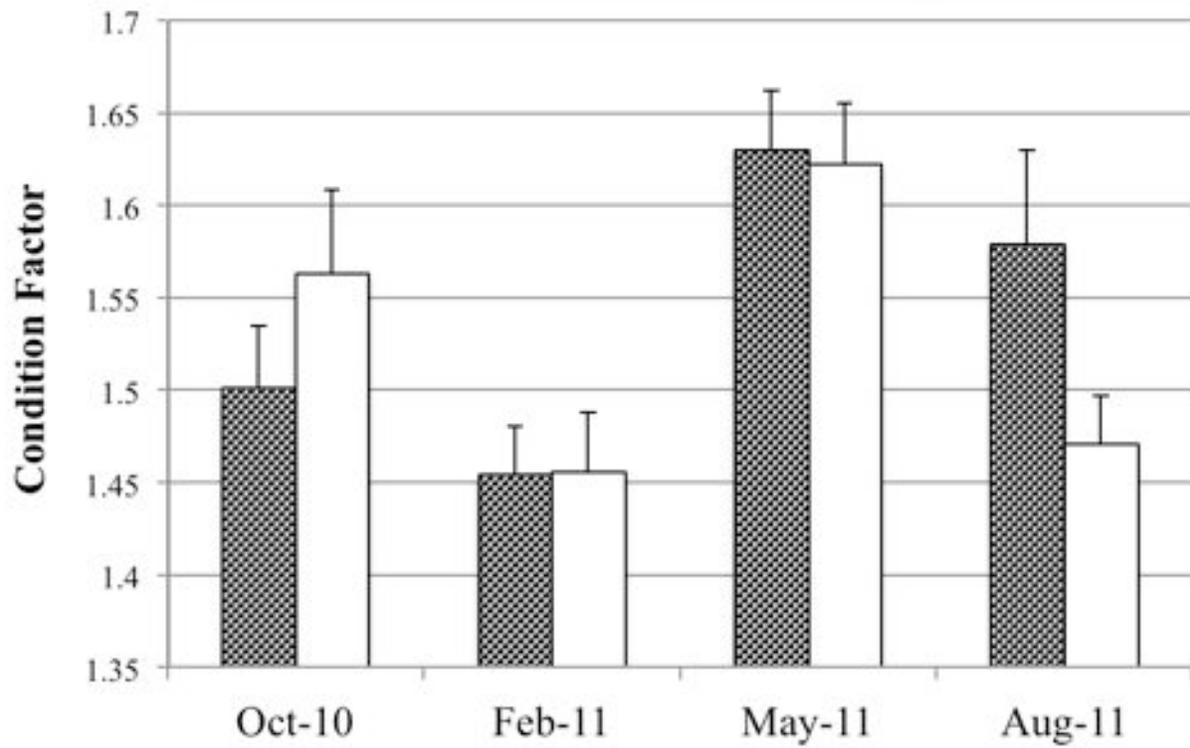
**PLATE 27.** Seasonal mean intensity of metacercariae infecting gill of *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in all non-oiled sites. Outliers represented by circles above error bars. Dotted lines within box-plots represent median values. Asterisks represent mean values.

### Intensity of gill metacercaria (all non-oiled sites)



**PLATE 28.** Seasonal condition factor (+S.E.) of *Fundulus grandis* Baird and Girard, 1853, (Cyprinodontiformes: Fundulidae) in oiled and non-oiled sites. Spotted bars indicate oiled sites and open bars indicate non-oiled sites.

### Seasonal condition of *Fundulus grandis*



## 2.7. Discussion

### 2.7.1. Prevalence of parasite infections

No significant differences were detected for aggregated prevalence of ectoparasite and endoparasite groups in oiled and non-oiled reference sites (Plate 19). My results differ from those reported by Dzikowski et al. (2003) for the parasite community infecting *Liza aurata* (Risso, 1810), (Mugiliformes: Mugilidae) and *Liza ramada* (Risso, 1810), (Mugiliformes: Mugilidae) from the Mediterranean Sea. These authors showed that prevalence of aggregated endoparasites was significantly higher at a non-polluted site while prevalence of ectoparasites was greater in an anthropogenically-polluted site. They stated that high concentrations of heavy metals and nutrients were previously reported in their polluted site and concluded that these conditions may be toxic to endoparasites including their free-swimming larval stages but beneficial to ectoparasites infecting an immunocompromised population of *Liza* spp.

Higher prevalence was detected for acanthocephalans infecting the intestine of *F. grandis* in non-oiled reference sites (Plate 20, Table 4). These differences in prevalence can indicate two ecological perturbations: (1) populations of an intermediate host, likely an ostracod (DeMont and Corkum, 1982), in Barataria Bay were being reduced or extirpated through direct toxicity in oiled sites, or (2) the adult form itself was directly killed because fishes in a saltmarsh drink seawater in order to osmoregulate. Khan and Kiceniuk (1983) experimentally showed that prevalence of the acanthocephalan, *Echinorhynchus gadi* Zoega, 1776, (Palaeacanthocephala: Echinorhynchidae) was significantly greater in *G. morhua* control groups vs. those exposed to crude oil fractions. Furthermore, Dzikowski et al. (2003) showed a complete absence of gut helminths infecting *Liza* spp. in one anthropogenically polluted site compared to a pristine reference site along the Mediterranean coast. Both the latter and former study speculated that

aquatic pollutants may have had a direct toxic effect on parasites inhabiting the digestive system of fishes.

Statistically, the Acanthocephala requires a larger host sample size before tests for significant differences (with an associated p-value) can be applied. However, continued longterm sampling of the parasite component community of *F. grandis* in Barataria Bay could reveal noteworthy results especially if coupled with population assessments and parasite surveys of ostracod intermediate hosts in Barataria Bay.

Herein, Copepoda and Branchiura had greater prevalences in oiled sites (Plate 20, Table 4). These results also coincide with Dzikowski et al. (2003) who showed increased prevalence in copepods infecting *Liza* spp. from an anthropogenically-polluted site in the Mediterranean. The authors suggested that increased copepod populations could have been facilitated by immune suppression of hosts in the polluted site. The authors also showed that fishes examined from the polluted site had a significantly lower mean condition factor than fishes from the reference site. My study showed no significant differences in condition factor that could suggest impaired host immunity and the subsequent increase in prevalence of arthropod ectoparasites.

### **2.7.2. Mean intensity of infections**

Metacercariae infecting the bulbous arteriosus of *F. grandis* had a significantly higher mean intensity in oiled sites (Table 4). In contrast, Pech et al. (2009) showed that the mean intensities of two species of digenean metacercariae, *Apharyngostrigea* sp. and *Stephanostomum* sp. infecting the brain and kidney, respectively, of checkered puffer, *Sphoeroides testudineus* (Linnaeus, 1758), (Tetraodontiformes: Tetraodontidae) were negatively correlated with increasing levels of pollution including PAH concentrations in coastal lagoons of the Yucatan. The authors suggested that certain abundant parasite species may respond differently to varying

degrees of pollution. Herein, I tentatively agree with these authors and speculate that the species of metacercariae infecting the bulbous arteriosus of *F. grandis*, putatively identified as *Ascocotyle tenuicolis* Price, 1935, (Digenea: Heterophyidae) has an indirect life cycle mediated by a specific intermediate host population that is potentially pre-adapted to oiled conditions in Barataria Bay.

Differences in mean intensity were observed in monogenoids infecting skin of the body and fins of *F. grandis* (Plate 18, Table 4). Putative skin monogenoids identified in Chapter 1 include *Gyrodactylus stephanus* Mueller, 1937, (Monogenoidea: Gyrodactylidae), *Fundulotrema prolongis* (Hargis, 1955) Kritsky and Thatcher, 1977, (Monogenoidea: Gyrodactylidae) and *Swingleus polyclithroides* Rogers, 1969, (Monogenoidea: Gyrodactylidae) (see Plate 8). My results show that mean intensity of skin monogenoid infection in oiled sites is greater than non-oiled reference sites. Similar results by Pérez-del Olmo et al. (2006) showed greater intensities of two species of Monogenoidea, *Cyclocotyla bellones* (Otto, 1821), (Monogenoidea: Dicliphoridae) and *Microcotyle erythrini* Van Beneden and Hesse, 1863 (Monogenoidea: Microcotylidae) in two oiled sites after the 2002 *Prestige* Oil Spill off the Galician coast of Spain. These differences were recorded in 2004 and 2005 and compared to historical data from the same sites in 2001 (survey data prior to the *Prestige* spill). The authors of the latter study stated that these differences might have been attributed to chronic toxicant exposure effects on final host immunology and greater adaptability of ectoparasites to polluted environments.

Nematoda (L3) putatively identified as *Eustrongylides* sp. infected the body cavity of *F. grandis* with a significantly greater mean intensity in oiled sites. This is in contrast to a laboratory exposure study conducted for *Anisakis simplex* larvae infecting the body cavity and mesentery of Pacific herring *Clupea pallasii pallasii* Valenciennes, 1847 (Clupeiformes:

Clupeidae) (see Moles et al., 1993). A field component of this study accompanied laboratory results showing that intensities of this nematode were significantly lower in two oil-polluted sites in Prince William Sound, Alaska, two weeks after the *Exxon Valdez* Oil Spill. The authors attributed decreased intensities of *Anisakis* larvae to direct oil toxicity and noted that larvae also infected somatic muscle more often in hosts sampled from oil-contaminated sites.

### **2.7.3. Species richness of ectoparasites and endoparasites**

No significant differences were detected for species richness of ectoparasite and endoparasite groups in oiled and non-oiled reference sites (Plate 23). Herein, endoparasite species richness was consistently greater than ectoparasite species richness across the study period. A survey by Rueckert et al. (2009) comparing parasite species richness of *Mugil cephalus* (n=70) and *Scatophagus argus* (n=70) between an oil-polluted mangrove site located near an oil processing plant and a reference site in Indonesia showed that total species richness of ectoparasites was greater than that of endoparasites. The authors did not speculate on these results but perhaps biogeographical differences between an Asian-Pacific mangrove and the Gulf of Mexico can account for differences in parasite biodiversity composition. Furthermore, Rueckert et al. (2009) showed that endoparasite richness was greater in their reference site. The authors concluded that the absence of endoparasites in their polluted site was likely a result of industrialization effluents that prevented the completion of multiple-host life cycles.

### **2.7.4. Seasonality of gill parasites**

My results showed that mean intensity of Myxozoa, putatively identified as *Myxobolus* n. sp. 1, infecting the gill lamellae of *F. grandis* was greatest in May 2011 (Plates 24–25, Table 4), which could indicate a seasonal effect that is mediated through some abiotic factor such as water temperature. Hiner and Moffitt (2002) showed that significant differences in seasonal intensity of

*Myxobolus cerebralis* Hofer, 1903, (Bivalvuvida: Myxobolidae) were positively correlated with water temperature and abundance of an oligochaete definitive host, *Tubifex tubifex*. The authors also showed that population abundances of *T. tubifex* were correlated with water temperature. *T. tubifex* were examined from three river drainages in Idaho and had a lower prevalence of *M. cerebralis* infection compared to the fish intermediate hosts, rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), (Salmoniformes: Salmonidae) and cut-throat trout, *Oncorhynchus clarkia clarkii* (Richardson, 1836), (Salmoniformes: Salmonidae). Furthermore, the abundance of *T. tubifex* was positively correlated with habitat containing heavy silt and organic debris. My results showed no significant differences between oiled and non-oiled reference sites. This could indicate that oil-laden sediments may not have affected populations of the appropriate definitive host (likely an annelid) up to a year after the DHOS. My results do not allow me to conclude that oil from the DHOS provided an organically enriched habitat correlating with abundance of a definitive host.

Metacercariae infecting gill of *F. grandis* also showed a seasonal difference with the highest intensities recorded in August 2011 (Plates 26–27, Table 4). There were no significant differences detected between oiled and non-oiled reference sites. In contrast to my results, a five year field study by Khan (2004) showed significantly higher intensities of metacercariae infecting the gill of *Pleuronectes americanus* (Walbaum, 1792), (Pleuronectiformes: Pleuronectidae) collected from five polluted sites on the coast of Newfoundland. Khan (2004) attributed these differences to chronic nutrient loading in polluted sites that proliferated intermediate host populations.

## **2.8. Conclusions**

The process of testing the use of parasites as biological indicators of ecosystem functioning is

not simple. It requires species-level identification, long-term sampling that allows one to obtain data with which to test natural seasonal cycles of each parasite taxon, and a large sample size to account for rare infections. Acute or chronic perturbations resulting from the DHOS were not apparent, especially when assessing parasite species richness and even condition factor of *F. grandis* across seasons. My results showed that generally comparing ecto- and endoparasites did not differentiate oiled and non-oiled study sites; however, particular taxonomic groups showed significant differences in prevalence and intensity between the oiled and non-oiled study sites. Acanthocephalans decreased in prevalence in the oiled sites, perhaps indicating the extirpation of a required invertebrate intermediate host (likely an ostracod of the benthos). Copepods and branchiurans were more frequently encountered on Gulf killifish from oiled sites, perhaps indicating that the fish were immunocompromised.

Definitively stating the biological process that explains these statistically significant differences requires at least laboratory experiments and field trials involving caged or tagged fish. In the strict sense, I cannot address why these differences in prevalence or intensity came about; however, the differences observed could indicate habitat integrity differences in the oiled and non-oiled sites.

As previously stated, seasonality of infections among some taxa was detected, indicating that my sampling design indeed could differentiate between the natural processes that lead to fluctuations in parasite abundance, prevalence, and intensity from an oil spill.

**Table 4. Significant results across major metazoan parasite groups.**

Taxon	Non-oiled references sites	Oiled sites
<b>Myxozoa</b>		Intensity highest in May (gill)
<b>Digenea</b>		Intensity higher (gill and heart)
		Intensity highest in August (gill)
<b>Monogenoidea</b>		Intensity higher (skin)
<b>Cestoda</b>	–	–
<b>Nematoda</b>		Intensity higher (body cavity)
<b>Acanthocephala</b>	Prevalence higher (intestine)	
<b>Hirudinida</b>	–	–
<b>Copepoda</b>	–	Prevalence higher (gill)
<b>Branchiura</b>	–	Prevalence higher (skin)
<b>Isopoda</b>	–	–

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## **Appendices**

**Table 2. *Myxobolus* infecting *Fundulus* in North America (ordered by decreasing spore length). *Abbr.* Length × width (L × W); filament coils (FC); spore shape (SS): pyriform (py), ovate-round (ov); vacuole (V): absent (ab), common (co), present (pr), rare (ra); posterior bumps (PB); posterior ridges (PR); polar capsule (PC); infection site: testes (t), ct (connective tissue), fin (f), fat of brain (fb), skin of eye (se), integument (in), muscle (mu), gill (g), branchiostegal skin (bs), base of fin (bs), skin (sk), brain (b), kidney (k), urinary bladder (ub); Locality: Nova Scotia (NV); Maryland (MD), Louisiana (LA), Massachusetts (MA), New York (NY).**

Taxon	Spore (L × W)	FC	SS	V	DB	PB	PL	PC (L × W)	Host species	Infection site	Locality	Reference(s)
<i>Myxobolus diaphanus</i>	16–20 × 5–8	11–15	py	ab	ab	ab	ab	7–10 × 2	<i>diaphanus</i>	t	BC	Fantham et al., 1940; Landsberg and Lom, 1991
<i>Myxobolus subtecalis</i>	15–18 × 7–8	11–12	py	ab	pr	ab	1	7–8 × 2	<i>heteroclitus</i>	ct, f, fb	MD	Bond, 1938; Landsberg and Lom, 1991
<i>Myxobolus n. sp. 4</i>	15 (14–15) × 8 (8–9)	7 (6–7)	py	ab	ab	ab	1–2	9 (8–10) × 2 (1–2)	<i>grandis</i>	se	LA	present study
<i>Myxobolus funduli</i>	14 × 8	11–14	py	pr	ab	7–10	ab	8 × 2	<i>heteroclitus majalis</i> , <i>diaphanus</i>	in, mu, g	MA	Hahn, 1915; Kudo, 1920
<i>Myxobolus n. sp. 3</i>	14 (12–15) × 8 (7–8)	9 (7–9)	py	co	ra	3–5	ab	8 (6–10) × 2 (1–2)	<i>grandis</i>	bs, fr	LA	present study
<i>Myxobolus hudsonis</i>	12–13 × 7	7–9	py	ab	pr	ab	ab	4–5 × 2–3	<i>heteroclitus</i>	bf	NY	Bond, 1938
<i>Myxobolus n. sp. 2</i>	11 (10–11) × 7 (7–8)	7 (5–7)	py	ra	co	3–5	ab	6 (5–6) × 2 (2–3)	<i>grandis</i>	sk	LA	current study
<i>Myxobolus n. sp. 1</i>	10 (10–12) × 6 (5–7)	8 (7–8)	py	ra	ra	3–10	1–2	6 (5–6) × 2 (1–2)	<i>grandis</i>	g	LA	present study
<i>Myxobolus</i>	10 (9–11)	5 (4–5)	ov	ab	ab	ab	ab	4 (3–4)	<i>grandis</i>	bf	LA	present study











III	O4-A025	4.4	65.0	6.5	1.6	1	88	1	3	1	5	1	7	1	3	1	100	1
III	O4-A026	6.2	72.0	7.2	1.7	1	182	1	3	1	6	1	5	1	5	1	193	1
III	O4-A027	4.1	62.0	6.2	1.7	1	66	1	2	1	5	1	1	13	1	1	85	1
III	O4-A028	3.3	61.0	6.1	1.5	1	92	1	2	1	11	1	2	1	22	1	127	1
III	O4-A029	3.4	62.0	6.2	1.4	1	151	1	2	1	4	1	8	1	8	1	171	1
III	O4-A030	4.3	64.0	6.4	1.7	1	134	1	1	1	11	1	2	1	32	1	179	1
III	O1-A051	7.9	76.0	7.6	1.8	1	120	1	14	1	29	1	1	1	1	1	150	1
III	O1-A052	7.8	77.0	7.7	1.7	1	30	1	16	1	20	1	2	1	5	1	150	1
III	O1-A053	5.6	71.0	7.1	1.6	1	72	1	3	1	28	1	4	1	4	1	50	1
III	O1-A054	4.6	67.0	6.7	1.5	1	75	1	10	1	32	1	7	1	4	1	113	1
III	O1-A055	5.1	68.0	6.8	1.6	1	40	1	4	1	28	1	17	1	4	1	90	1
III	O2-A051	6.3	74.0	7.4	1.6	1	45	1	9	1	16	1	5	1	10	1	85	1
III	O2-A052	15.2	102.0	10.2	1.4	1	2	1	14	1	11	1	1	1	1	1	78	1
III	O2-A053	5.3	71.0	7.1	1.5	1	37	1	10	1	9	1	1	1	4	1	15	1
III	O2-A054	3.9	62.0	6.2	1.6	1	28	1	2	1	12	1	1	1	1	1	47	1
III	O2-A055	4.7	71.0	7.1	1.3	1	31	1	9	1	9	1	1	1	1	1	43	1
III	O3-A051	4.6	65.0	6.5	1.7	1	30	1	2	1	7	1	3	1	1	1	43	1
III	O3-A052	5.0	70.0	7.0	1.5	1	8	1	1	1	8	1	1	1	1	1	37	1
III	O3-A053	8.8	99.0	9.9	0.9	1	1	1	1	1	1	1	1	1	1	1	8	1
III	O3-A054	5.8	71.0	7.1	1.6	1	3	1	1	1	3	1	1	1	1	1	1	1
III	O3-A055	7.8	72.0	7.2	2.1	1	30	1	5	1	22	1	1	1	1	1	4	1
III	O4-A051	8.5	83.0	8.3	1.5	1	141	1	10	1	19	1	3	1	1	1	52	1
III	O4-A052	4.1	66.0	6.6	1.4	1	191	1	1	1	9	1	8	1	1	1	160	1
III	O4-A053	7.3	82.0	8.2	1.3	1	5	1	12	1	12	1	1	1	3	1	208	1
III	O4-A054	4.4	66.0	6.6	1.5	1	107	1	5	1	6	1	1	1	1	1	13	1
III	O4-A055	9.9	88.0	8.8	1.5	1	1	1	5	1	22	1	1	1	5	1	115	1
IV	O1-A031	1.2	48.0	4.8	1.1	1	1	1	4	1	41	1	1	1	4	1	23	1
IV	O1-A032	1.0	44.0	4.4	1.2	1	3	1	2	1	16	1	2	1	4	1	46	1
IV	O1-A033	1.1	47.0	4.7	1.0	1	4	1	3	1	7	1	2	1	2	1	21	1
IV	O1-A034	1.4	48.0	4.8	1.2	1	8	1	2	1	15	1	1	1	3	1	22	1
IV	O1-A035	1.3	46.0	4.6	1.3	1	2	1	1	14	1	1	5	1	2	1	23	1
IV	O1-A036	1.3	42.0	4.2	1.7	1	15	1	2	1	39	1	2	1	2	1	56	1
IV	O1-A037	1.0	41.0	4.1	1.4	1	4	1	1	19	1	2	1	1	2	1	23	1
IV	O1-A038	3.6	59.0	5.9	1.7	1	4	1	2	1	30	1	2	1	2	1	30	1
IV	O1-A039	0.9	40.0	4.0	1.4	1	58	1	3	1	58	1	1	1	1	1	58	1
IV	O1-A040	0.8	40.0	4.0	1.2	1	18	1	3	1	22	1	1	1	3	1	41	1
IV	O2-A031	17.7	102.0	10.2	1.7	1	24	1	4	1	23	1	5	1	2	1	52	1
IV	O2-A032	3.1	60.0	6.0	1.4	1	2	1	3	1	14	1	6	1	3	1	22	1
IV	O2-A033	10.6	85.0	8.5	1.7	1	51	1	7	1	12	1	1	1	2	1	64	1
IV	O2-A034	7.3	73.0	7.3	1.9	1	7	1	1	1	50	1	1	1	1	1	59	1
IV	O2-A035	7.1	76.0	7.6	1.6	1	30	1	2	1	84	1	1	1	3	1	115	1
IV	O2-A036	10.3	76.0	7.6	2.3	1	29	1	6	1	23	1	1	1	7	1	52	1





**Table 6. Raw parasite prevalence and intensity data for non-oiled reference sites.** *Abbr:* Collection event (CE); weight in grams (W); length (L); condition factor (CF); Protozoa (1); Ciliata (2); Myxozoa (3); Monogeneoidea (4); Digenea [metacercariae] (5); Digenea [adults] (6); Cestoda (7); Nematoda (8); Acanthocephala (9); Hirudinida (10); Copepoda (11); Branchiura (12); Isopoda (13); aggregated ectoparasites (AEc); aggregated endoparasites (AEn); intensity (int); a value of 1 under column (n) denotes infection by a parasite group [1–13] and the sum of this column was used to calculate prevalence.

CE	Host	W (g)	L (mm)	L (cm)	CF	1	2	3	4	5	6	7	8	9	10	11	12	13	AEc n	AEn n		
						n	int	n	int	n	int	n	int									
I	N1-A001	5.1	74	7.4	1.3	1	2	1	2	1	18	1	2	1	1	1			2	1	23	1
I	N1-A002	17.6	95	9.5	2.1	1	4	1	2	1				2	1	1			3	1	6	1
I	N1-A003	10.2	88	8.8	1.5	1	11	1	3	1	8	1		3	1				3	1	22	1
I	N1-A004	3.6	50	5	2.9	1			1	1	1	1	3	1					1	1	4	1
I	N1-A005	13.7	95	9.5	1.6	1	15	1	1	1	2	1							1	1	17	1
I	N1-A006	7.8	75	7.5	1.9	1	11	1	1	1	28	1	2	1					1	1	41	1
I	N1-A007	5.0	70	7	1.4	1			1	1	10	1	1	1					1	1	11	1
I	N1-A008	5.8	73	7.3	1.5	1			1	1	12	1				1	1		2	1	12	1
I	N1-A009	11.3	92	9.2	1.5	1	46	1	1	1	10	1				1	1		2	1	56	1
I	N1-A010	5.0	72	7.2	1.3	1			3	1	6	1							3	1	6	1
I	N2-A001	9.0	80	8	1.8	1			2	1	44	1	1	1	2	1			2	1	47	1
I	N2-A002	4.6	70	7	1.3		1	1	6	1	15	1	1	1					6	1	17	1
I	N2-A003	7.4	75	7.5	1.8	1			1	1	12	1							4	1	13	1
I	N2-A004	24.5	111	11.1	1.8	1	49	1	3	1	15	1	12	1	4	1			9	1	80	1
I	N2-A005	3.3	66	6.6	1.1	1	83	1	3	1	45	1			2	1			5	1	128	1
I	N2-A006	19.8	107	10.7	1.6	1	64	1	3	1	25	1	2	1	3	1	2	1	8	1	91	1
I	N2-A007	4.8	70	7	1.4	1	14	1			10	1		1	1						25	1
I	N2-A008	4.9	62	6.2	2.1	1			2	1	29	1									29	1
I	N2-A009	4.6	68	6.8	1.5						12	1	53	1	1	1					67	1
I	N2-A010	21.0	105	10.5	1.8	1	11	1			20	1	1	1	1	3	1		4	1	33	1
I	N3-A001	4.8	70	7	1.4	1	8	1	1	1	9	1							1	1	17	1
I	N3-A002	11.0	89	8.9	1.6	1	23	1	4	1	21	1		2	1	1	1	1	6	1	46	1
I	N3-A003	2.2	56	5.6	1.2		12	1	1	1	9	1	1	1	1				2	1	23	1
I	N3-A004	15.9	99	9.9	1.6	1	10	1			6	1	4	1							20	1
I	N3-A005	6.2	77	7.7	1.4	1	3	1	3	1	10	1							3	1	13	1
I	N3-A006	9.4	89	8.9	1.3	1	2	1	4	1	11	1	2	1					4	1	15	1
I	N3-A007	3.4	65	6.5	1.2	1	2	1	5	1	53	1	5	1		1	1		6	1	60	1
I	N3-A008	13.0	95	9.5	1.5	1	31	1	9	1	25	1							10	1	56	1



II	N2-A011	3.1	60	6	1.4	1	1	3	1	4	1	2	1	1	1	3	1	8	1	
II	N2-A012	8.0	77	7.7	1.8	4	1	24	1	10	1				1	1	25	1	14	1
II	N2-A013	4.7	67	6.7	1.6	1	1	16	1	14	1	2	1	1	1	16	1	17	1	1
II	N2-A014	4.3	62	6.2	1.8	1	2	1	5	1	1	2	1	1	1	2	1	9	1	1
II	N2-A015	5.2	69	6.9	1.6	1	1	4	1	11	1	1	1	1	2	1	6	1	14	1
II	N2-A016	5.1	70	7	1.5	5	1	9	1	8	1	1	2	1	1	11	1	15	1	1
II	N2-A017	4.8	68	6.8	1.5	1	1	4	1	8	1	1	1	1	1	5	1	9	1	1
II	N2-A018	1.8	52	5.2	1.3	2	1	4	1	4	1	2	1	1	1	5	1	8	1	1
II	N2-A019	8.7	86	8.6	1.4	17	1	4	1	2	1	1	1	1	3	1	8	1	21	1
II	N2-A020	3.6	64	6.4	1.4	1	1	1	1	5	1	1	1	1	1	2	1	8	1	1
II	N3-A011	4.4	66	6.6	1.5	3	1	17	1	2	1	1	1	30	1	1	1	8	1	1
II	N3-A012	13.0	82	8.2	2.4	1	16	1	29	1	1	1	1	1	1	19	1	36	1	1
II	N3-A013	6.6	73	7.3	1.7	1	13	1	1	1	1	10	1	1	1	16	1	29	1	1
II	N3-A014	6.1	75	7.5	1.4	1	15	1	7	1	3	1	1	1	1	16	1	11	1	1
II	N3-A015	7.0	74	7.4	1.7	1	1	29	1	10	1	6	1	19	1	23	1	28	1	1
II	N3-A016	2.3	56	5.6	1.3	4	1	4	1	13	1	2	1	4	1	31	1	17	1	1
II	N3-A017	4.5	64	6.4	1.7	1	67	1	11	1	1	1	1	5	1	5	1	19	1	1
II	N3-A018	5.9	70	7	1.7	1	1	1	4	1	1	5	1	3	1	67	1	19	1	1
II	N3-A019	2.1	54	5.4	1.3	1	1	3	1	11	1	1	1	1	1	2	1	4	1	1
II	N3-A020	6.3	73	7.3	1.6	1	3	1	14	1	13	1	20	1	3	1	4	1	13	1
II	N4-A011	3.2	61	6.1	1.4	1	2	1	8	1	49	1	8	1	1	11	1	59	1	1
II	N4-A012	3.2	58	5.8	1.6	4	1	4	1	28	1	1	1	1	1	4	1	28	1	1
II	N4-A013	4.8	65	6.5	1.7	15	1	15	1	8	1	1	1	1	2	1	17	1	9	1
II	N4-A014	3.8	63	6.3	1.5	14	1	14	1	29	1	1	1	1	3	1	17	1	29	1
II	N4-A015	2.7	59	5.9	1.3	6	1	4	1	12	1	1	1	1	1	5	1	20	1	1
II	N4-A016	2.7	58	5.8	1.4	1	7	1	7	1	13	1	1	1	4	1	10	1	18	1
II	N4-A017	2.6	60	6	1.2	1	2	1	3	1	28	1	1	1	1	4	1	48	1	1
II	N4-A018	2.7	59	5.9	1.3	6	1	6	1	45	1	1	1	1	4	1	6	1	50	1
II	N4-A019	2.2	56	5.6	1.2	4	1	4	1	3	1	1	1	1	1	5	1	4	1	1
II	N4-A020	3.1	62	6.2	1.3	5	1	5	1	21	1	1	1	1	2	1	70	1	22	1
II	N1-A046	1.8	53	5.3	1.2	7	1	1	1	5	1	2	1	2	1	2	1	2	1	1
II	N1-A047	3.2	61	6.1	1.4	28	1	1	1	7	1	1	1	1	1	1	1	14	1	1
II	N1-A048	1.9	54	5.4	1.2	1	28	1	1	1	1	1	1	1	1	1	1	35	1	1
II	N1-A049	2.1	52	5.2	1.5	1	1	2	1	8	1	1	1	1	1	2	1	10	1	1
II	N1-A050	1.3	45	4.5	1.4	1	3	1	3	1	1	3	1	1	1	6	1	6	1	1
II	N2-A046	3.8	67	6.7	1.3	1	1	1	4	1	1	2	1	2	1	1	1	8	1	1
II	N2-A047	2.7	67	6.7	0.9	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1
II	N2-A048	4.4	67	6.7	1.5	1	28	1	13	1	1	1	1	1	1	28	1	13	1	1
II	N2-A049	3.5	69	6.9	1.1	3	1	3	1	3	1	1	1	1	1	3	1	3	1	1
II	N2-A050	6.0	75	7.5	1.4	5	1	8	1	11	1	5	1	1	1	9	1	16	1	1
II	N3-A046	6.2	73	7.3	1.6	4	1	14	1	8	1	4	1	1	1	14	1	12	1	1
II	N3-A047	6.3	77	7.7	1.4	1	5	1	7	1	1	1	1	1	1	5	1	9	1	1





IV N2-A032	3.7	63	6.3	1.5	1	10	7	1	19	1	2	1	7	1	31	1		
IV N2-A033	3.4	60	6	1.6	1	3	4	1	10	1				5	1	10	1	
IV N2-A034	2.7	56	5.6	1.6		3	6	1	41	1	2	1		6	1	46	1	
IV N2-A035	15.6	101	10.1	1.5	1	22	12	1	84	1			2	1	1	1	109	1
IV N2-A036	9.3	80	8	1.8	1	42	1	1	57	1				1	1	100	1	
IV N2-A037	13.0	87	8.7	2.0		36	7	1	29	1			9	1	7	1	74	1
IV N2-A038	1.9	54	5.4	1.2		4	5	1	23	1				5	1	27	1	
IV N2-A039	13.9	89	8.9	2.0	1	31	6	1	46	1	7	1	5	6	1	89	1	
IV N2-A040	17.5	98	9.8	1.9	1	20	15	1	13	1	3	1	1	1	1	37	1	
IV N2-A056	1.4	48	4.8	1.3	1	5	4	1	4	1				1	1	9	1	
IV N2-A057	2.9	56	5.6	1.6	1	5	2	1	13	1				2	1	18	1	
IV N2-A058	2.0	51	5.1	1.5	1	4	1	1	23	1	3	1		1	1	30	1	
IV N2-A059	2.3	56	5.6	1.3	1	19	1	1	19	1		1		2	1	38	1	
IV N2-A060	2.1	51	5.1	1.6		13	5	1	26	1				5	1	39	1	
IV N3-A031	3.4	63	6.3	1.4	1	3	2	1	12	1	4	1	1	3	1	20	1	
IV N3-A032	3.1	63	6.3	1.2	1	9	9	1	9	1	2	1	1	1	1	22	1	
IV N3-A033	2.8	59	5.9	1.4	1	3	3	1	24	1			4	3	1	31	1	
IV N3-A034	3.3	57	5.7	1.8	1	12	1	1	14	1			1	1	27	1		
IV N3-A035	11.7	85	8.5	1.9	1	21	1	1	60	1		1	1	3	1	83	1	
IV N3-A036	3.7	67	6.7	1.2	1	9	7	1	3	1			1	7	1	13	1	
IV N3-A037	4.1	62	6.2	1.7		3	3	1	3	1					6	1		
IV N3-A038	5.2	69	6.9	1.6	1	30	4	1	15	1	1	1	10	4	1	56	1	
IV N3-A039	2.9	57	5.7	1.6	1	13	1	1	5	1				1	1	18	1	
IV N3-A040	2.2	54	5.4	1.4			3	1	4	1	1	1	1	3	1	6	1	
IV N3-A056	1.6	50	5	1.3					8	1					8	1		
IV N3-A057	2.8	60	6	1.3	1	34	1	1	19	1				1	1	53	1	
IV N3-A058	2.2	54	5.4	1.4	1	7	7	1	3	1	3	1	1	1	1	14	1	
IV N3-A059	1.9	53	5.3	1.3	1	3	3	1	11	1			3	2	1	17	1	
IV N3-A060	1.8	52	5.2	1.3	1	2	2	1								2	1	
IV N4-A031	2.7	58	5.8	1.4	1	1	1	1	103	1	3	1	1		108	1		
IV N4-A032	1.9	52	5.2	1.3	1	10	1		50	1				1	61	1		
IV N4-A033	1.9	53	5.3	1.2			2		49	1			5		54	1		
IV N4-A034	4.5	67	6.7	1.5	1				56	1					56	1		
IV N4-A035	2.7	57	5.7	1.4	1	11	1	1	73	1			7	1	1	91	1	
IV N4-A036	1.9	51	5.1	1.4		4	4	1	59	1					63	1		
IV N4-A037	1.8	51	5.1	1.3	1	1	2	1	27	1	1	1		2	1	29	1	
IV N4-A038	12.9	91	9.1	1.7	1	112	1	1	99	1			1	1	1	212	1	
IV N4-A039	3.6	63	6.3	1.4	1	4	4	1	43	1				4	1	43	1	
IV N4-A040	3.0	60	6	1.4	1	4	1	1	64	1			6	1	1	74	1	
IV N4-A056	3.2	61	6.1	1.4					17	1					17	1		
IV N4-A057	3.9	62	6.2	1.6					12	1					12	1		
IV N4-A058	2.4	55	5.5	1.5		7	7	1	66	1				1	1	73	1	

IV	N4-A059	12.2	92	9.2	1.6	93	1	42	1	1.6	1.6	1.6	1.6	2.3	1.1	1	1	1	1	135	1
IV	N4-A060	13.8	93	9.3	1.7	93	1	60	1	1.6	1.6	1.6	1.6	2.3	1.1	1	1	1	1	153	1
	<b>MEAN</b>	5.8	68.433	6.843	1.5	33	35	4.7	22	12	3.8	4.2	4.2	2.3	1.1	1	1	1	1	6.86	51.5
	<b>TOTAL INFECTED</b>			151		9	177	202	236	2	63	129	129	36	52	33	0	0	213	240	
	<b>PREVALENCE</b>			63		4	74	84	98	1	26	54	54	15	22	14	0	0	89	100	
	<b>STANDARD ERROR INTENSITY</b>			8.8		3.6	0.4	0.4	1.3	11	1	0.5	0.1	0.2	0.3	0.1	0	0	0.71	3.36	