PARASITE COMMUNITIES AND EFFECTS ON MUMMICHOG

(*FUNDULUS HETEROCLITUS*) PHYSIOLOGY, ANATOMY AND BEHAVIOR

by

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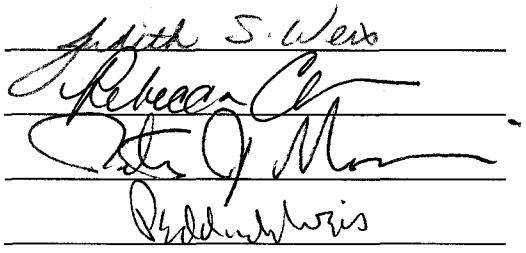

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ABSTRACT OF THE DISSERTATION

PARASITE COMMUNITIES AND EFFECTS ON

MUMMICHOG (FUNDULUS HETEROCLITUS)

PHYSIOLOGY, ANATOMY & BEHAVIOR

by

Celine Santiago Bass

Dissertation Director: Judith S. Weis, Ph.D.

Fundulus heteroclitus, the killifish or the mummichog, is commonly found in estuarine waters. *Fundulus* spp. have played important roles in advancing our understanding of different aspects in biology such as physiology, behavior, and genetics due to their hardiness and distribution and abundance.

Because parasites are so ubiquitous and can affect host physiology, behavior, and ultimately ecology, it is important to know how parasites are distributed among host populations and which populations are more susceptible to infection. A baseline survey was conducted over two-years, examining the parasite communities of 280 *F. heteroclitus* (138 males, 142 females) from seven sites throughout New Jersey and New York in early and late season collections. The gills, digestive tract, liver, body cavity and swim bladder were examined and all macroparasites were recorded. Parasite communities varied spatially over site and habitat, and temporally by year and season. Host sex did not play a significant role. Salinity appeared to play a large part in structuring communities as did site disturbance (e.g., restoration).

Heavy gill infections (>2,000 parasites) were found in fish from a restored site during the baseline survey so a more focused investigation of *F. heteroclitus* gills

ensued. Using fish from three restored and three unrestored sites from the Hackensack Meadowlands, behavior, physiology, anatomy and gill parasite abundance were examined. Fish from restored sites had the greatest number of digenean trematode metacercariae gill infections (*Ascocotyle phagicola diminuta* and *Echinochasmus schwartzi*) compared to fish from unrestored sites. Heavily parasitized individuals spent more time at the water's surface and exhibited more conspicuous behaviors, which could enhance trophic transmission. Heavily parasitized fish also had greater stamina, lower respiration rates, larger red blood cells and greater blood volume. They also induced gill tissue growth, forming additional branches as a response to the metacercariae, probably as a way to compensate for reduced oxygen extraction.

This study has shown that parasite-host relationships are highly dynamic interactions and that heavy gill infections with digenean trematode metacercariae can significantly shape host's physiology, behavior and anatomy.

DEDICATION

First and foremost, praise to my heavenly Father for providing me with the strength and endurance to complete this goal, as well as the many blessings He has bestowed upon me and my family over the years. Many thanks to my husband; Sefton, who has supported me through this process, both financially and emotionally – I love you. Additional thanks and love to my children; Sefton III (Jimmie), Allazandra (Wawa) and my milk chocolate baby; Trinity, for helping me in the field despite their reluctance, and for their patience because 'Mommy' (C) was always working. To my parents Ignacio and Amparo Santiago, and sisters Diane Rodriguez and Lisa Santiago – thank you for cheering me on, always believing in me, and making me feel so special. I love you so much. Thank you to my mother-in-law Norene Bass, and 'pseudo' mother-in-law Odessa Robinson for helping me out with the children when school schedules clashed, and for allowing me the ability to take much needed mental health days from time to time. Last but not least, to my other 'children' – the kitties Digit, Mitsie and Coco, who give me unconditional love and always know how to help me relax. As my family, you have all been my rock throughout the years. Thank you all for your love and support!

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CHAPTER I. INTRODUCTION

I.1 Parasites

Parasites are commonly defined as organisms that grow, feed, and are sheltered on or in a different organism whereby the parasite benefits and the net effect on the host is negative (+/- relationship). However, despite the detrimental effects on hosts, accumulating evidence suggests that healthy ecosystems are rich in parasite species (Overstreet, 1997; Hudson *et al.*, 2006). They may be found throughout the world, from the cold polar regions to the warmth of the tropics. In fact, half of all biodiversity might comprise parasitic species (Toft, 1986). Parasites include a large realm of organisms from tiny bacteria (*e.g., Mycobacterium bovis*) to larger multi-cellular organisms like the brown-headed cowbird (*Molothrus ater*). Parasitic life-styles have evolved over evolutionary time, resulting from the organism's inability to perform certain functions (e.g., digestion, nest building). The parasite subsequently uses its host to fill this void.

For clarity's sake, organisms that live within a host are referred to as endoparasites and those living attached to the outer surface of a host are described as ectoparasites. Macroparasites (May and Anderson, 1979) are an artificial group of metazoan parasites, comprised mainly of members of the platyhelminthes (flatworms, cestodes, monogeans and trematodes), nemathelminthes (nematodes and acanthocephalans), annelids (such as leeches) and arthropods (true lice and parasitic copepods) (Barber *et al.*, 2000). Parasites sexually mature in a final, or definitive host and may utilize one of two distinct life-cycles – either direct or indirect (also known as a complex life cycle). Those that are transmitted from one definitive host to another of the same species without the use of an intermediate host are said to have direct life-cycles. In an indirect life cycle, there is at least one intermediate host involved whereby parasites undergo some sort of development, and/or replication, but can not reach

sexual maturity until transferred to the definitive host (Moore, 2002). Oftentimes, in indirect life cycles, where parasite transmission is between species, the two hosts are usually on different trophic levels, which is critical to the parasite's overall development (Dobson, 1988).

Phylum Platyhelminthes – Class Monogenea

Monogeneans are flatworms ranging in size from small (microscopic) to medium (5 mm) with direct life-cycles. Immature worms are morphologically similar to adults. Although most primarily use a posterior haptor with chitinoid hooks or clamps for attachment to hosts, some species also utilize suckers. Some species live on the gills only, some on the body and fins only, while others can live on both. This group feeds on mucus, epithelium and sometimes blood. The monogeneans can become serious threats to fish in high numbers due to their potential to cause significant damage to gill tissues (Hoffman, 1999).

Phylum Platyhelminthes – Class Trematoda (Digenea)

Like others in this phylum, adult digeneans are dorsoventrally flattened and are typically found in the digestive tract of fishes. Although not extensively studied, adults appear to do little or no harm. Other digeneans may by found in blood, body cavity and various organs. Larval trematodes, called metacercariae, may be found in all tissues of fish. Both adults and metacercariae have distinct oral and acetabulum suckers. Initial damage to the host occurs as the trematodes penetrate (as cercariae) and migrate into the tissues of the fish causing mechanical damage and hemorrhaging. Once encysted, the metacercariae do little damage to the host unless enough accumulate to interfere with the fish's metabolism. The life cycle is completed once the host fish is eaten by the appropriate final host (Hoffman, 1999).

Phylum Platyhelminthes – Class Cestoidea

Cestodes are common in fishes and have two life cycle stages; adult and plerocercoid. Some adults are segmented, and are typically found in the intestine and pyloric caeca, while the plerocercoids may be found in the viscera and musculature. Cestodes have a scolex, typically bearing suckers, hooks or suctorial grooves. Once in the intestine, adults do no detectable damage unless in large numbers at which point growth and survival of the host are jeopardized. Plerocercoids migrating in the visceral cavity produce adhesions which can damage organs, impair metabolism and cause death (Hoffman, 1999).

Phylum Nemathelminthes – Class Nematoda

Nematodes have cylindrical bodies with a rigid cuticle. Most adult nematodes are found in the intestinal tract. Larval nematodes however, may be found in almost every organ but most commonly in the mesenteries, liver and musculature. Nematodes have an indirect life cycle, utilizing two or more hosts for their development. Although considered weakly pathogenic, some species can cause substantial damage in high numbers (Hoffman, 1999).

Phylum Acanthocephala – Classes Eoacanthocephala & Palaeacanthocephala

Acanthocephalans get their common name "Thorny-Headed Worm" due to their prominent hook-bearing eversible proboscis. They also use an indirect life cycle. Larval stages are called cystacanths and may or may not be encased in a cyst from either the larval tissues, or from the host's tissues. If numerous, acanthocephalans can cause serous damage to the intestines of its host due to the morphology of its proboscis (Hoffman, 1999).

Phylum Arthropoda – Class Crustacea

The Crustacea comprise three parasitic groups: the Branchiura (fish lice), Entomostraca (parasitic copepods) and parasitic isopods. Characteristics found are similar to those describe for others in the Class Crustacea. Adult fish lice and isopods may be found either on the outer body of the fish host (scales, fins) as well in the gill chamber. However, adult copepods are found solely attached to the gill filaments of their host. Although each group follows a parasitic life style, they may also be found free-swimming in the environment (Hoffman, 1999).

I.2 Parasite Communities

Communities are groups of organisms found in a particular area. They are structured by numerous biotic and abiotic factors including climate, diet, competition, etc. In the formative years of community ecology, ecologists were primarily concerned with listing species found in different areas (Elton, 1966). Today, community ecology has grown beyond that initial simplistic grouping of organisms, and has now moved forward to identifying and examining those biotic and abiotic processes that actually created the patterns observed in the community. Community ecologists classify communities in several ways including: 1) physically, by using distinct habitat boundaries; 2) taxonomically, by identifying the dominant species present; 3) interactively, by selecting species demonstrating strong inter-relationships; and 4) statistically, using association patterns among species (Morin, 1999). Typically, it has been more difficult to divide marine communities into distinct classifications than it is for terrestrial systems. Where terrestrial communities are classified based on their vegetation components, marine communities are normally classified according to physical components such as proximity to shore, light penetration, bottom type and wave energy (Villa and Ceroni, 2004).

Since the pioneering work of Anderson and May (1978; May and Anderson, 1978, 1979), ecologists have slowly, but steadily shown increased interest in the undeniable impact that disease and parasites have, not only on individual hosts but also on their population dynamics (Lafferty, 1993; McCallum and Dobson, 1995; Ives and Murray, 1997 and others), and community structure of their host populations (Scott, 1988; Scott and Dobson, 1989; Minchella and Scott, 1991 and others). It has been suggested repeatedly that parasites could act as key species in ecosystems as they control host population dynamics (Grenfell and Gulland, 1995; Timi and Poulin, 2003), reproductive effort (Ferrari *et al.*, 2004; Pélabon *et al.*, 2005), behavior (Santiago Bass and Weis, 1999; Baldauf *et al.*, 2007), food webs (Marcogliese and Cone, 1997; Sukhdeo and Hernandez, 2005), growth and metabolism (Anderson, 1975; Arnott *et al.*, 2000), etc.

From the parasite's perspective a host may be looked at as a habitat (Bush *et al.*, 1997; Poulin and Morand, 2000) for parasite communities. Hosts represent well-defined and almost perfectly replicated habitats with intrinsically hierarchical populations and communities of parasites (Esch and Fernandez, 1993). Parasites of many invertebrates, birds, and marine fishes exhibit characteristics of interactive communities (Esch *et al.*, 1990) whereby a series of interactions are required in order for the parasites to complete their life cycles. For example, Torchin *et al.* (2005) found that when the Japanese mud snail (*Batillaria cumingi* [=*B.attramentaria*]) invaded coastal marshes and replaced the native snails (*Cerithidea californica*), the life cycles of many digenean trematodes (e.g., *Acanthoparyphium spinulosum*) were disrupted because key hosts required for the completion of their life cycles were missing. Parasite communities are generally formed in much the same way as other communities with the host (not only the definitive host, but intermediate hosts as well) acting as the resource base. This resource base is formed in response to the host's geographical range, density, and body size (Price,

1990). An infracommunity includes all parasites of different species contained within the same host species (be it intermediate or final) at a particular time (Bush *et al.*, 1997). Recently, several studies have been conducted which analyzed parasite infracommunity structure either across sites or over time (Timi and Poulin, 2003; Vidal-Martinez and Poulin, 2003; Gonzalez and Poulin, 2005). Results varied, with some parasite communities exhibiting high variability over time and/or space and others showing predictable structuring. For example, Gonzalez and Poulin (2005) established that parasite communities in the benthic marine fish *Sebastes capensis* were predictable both spatially and temporally and the authors suggested that host biology (e.g., habitat, behavior) could be a significant contributing factor.

Although studies have shown that most parasite infracommunities are species poor (Rohde, 1998), individuals reduce potential overlap by functioning within niches, many of which will remain empty (Rohde, 1998; Mouillot *et al.*, 2003). In a study of cestode communities of two elasmobranch hosts: the round stingray (*Urobatis halleri*) and the skate (*Leucoraja naevus*), it was shown that parasites select and occupy distinct niches or microhabitats (e.g., liver, gills) just like free-living species (Friggens and Brown, 2005). These niche choices are thought to result from environmental tolerance, reproductive segregation and/or competitive displacement (Friggens and Brown, 2005). Parasites, like their free-living counterparts, are also subject to competition for resources such as limited space and/or nutrients. Competition is an important regulatory process and may result in less food intake, reduced growth and fitness of the parasite and ultimately in its host. Competition between parasites has been demonstrated using adult helminthes in vertebrate hosts (Shostak and Scott, 1993) and larval trematodes in their first intermediate host (snail) (Kuris, 1990). Using the amphipod *Paracalliope novizealandiae* and one of its trematode parasites, *Maritrema novaezealandensis,* it was determined that larval helminth infracommunities within intermediate hosts are not static

but rather dynamic. The number, size and egg-production of the trematode parasites were dependent on interactions not only between conspecifics and heterospecifics but also on the host they shared (Fredensborg and Poulin, 2005).

Measuring diversity within the community is a crucial aspect in illustrating how the system is functioning. Determining species richness (the total number of species present) is often the only measurable indication of diversity. However, diversity is not only how many species are within the community but also how evenly they are distributed. Evenness measures differences among the number of individuals representing each species. How evenly the total number of individuals are distributed across the total number of species present, is also a determinant of biodiversity within a community. Species diversity indices (e.g., Shannon-Weiner Index) are fundamental measurements used whereby two separate characteristics: the total number of species in a community (species richness) and the relative abundances of those individual species (evenness), are compared. When you include parasite species into the biodiversity count, species richness can potentially double. Communities with a high species richness, evenness or both are considered to be more diverse when compared to communities with lower species richness, evenness or both (Bush *et al.*, 1997).

I.3 Parasite and Host Behavior

Parasites are known to affect practically every aspect of a host's being. Typically, when an individual thinks of parasites and their effects, the first thing that may come to mind is actually parasite-related virulence and disease, as there are numerous well documented instances where parasites are directly responsible for significantly increasing host mortality. Two classic examples include the myxomatosis virus in wild European rabbits (*Orycotalagus cuniculus*) in the mid-1950s, and rinderpest (also known as cattle plague) in the game herds of the Serengeti in the early 1980s (Plowright, 1982).

Both of these highly pathogenic microparasitic diseases had considerable impacts on their subsequent wild animal populations that were felt for many years after the initial pandemic ran its course. However, parasite-induced changes to host behavior are also a matter of importance as the parasites are changing many aspects of the host's life history including growth, fecundity and survival (O'Brien and Van Wyk, 1985; Oppliger and Clobert, 1997), among others.

Parasite-induced changes in host behavior may be divided into two broad classes: 1) changes that increase or initiate its transmission to another member of the same species; and 2) changes that increase its transmission to a different host species (Dobson, 1988). The behavior of parasitized individuals has been described as a 'mixed phenotype' (Dawkins and Krebs, 1979) in that both the host's and parasite's genotypes are being expressed in the behavior. Parasites with life cycles requiring at least one intermediate host (an indirect life cycle) where the sexually immature form resides before being transmitted to the definitive host have been shown to commonly use behavioral modification in order to increase trophic transmission (Kuris, 1974; Dobson, 1988). It is well documented that some parasites can alter host (both intermediate and definitive) behaviors to increase the host's susceptibility to predation, and some cases, it may even be a requirement for parasites to evolve strategies that will modify their host's behavior in order to enhance trophic transmission. These behaviors are often induced by the Nemathelminthes (acanthocephalans) and the Platyhelminthes (trematodes).

Parasite-induced behavioral modifications have been reported since the early-1900s (Wheeler, 1907). Early studies focused primarily on parasites of insects. Some of these studies included the southern grass worm (*Laphygma frugiperda*) infected with a polyhedral virus causing them to crawl to the tips of grass blades (Allen, 1921); red locust (*Cyrtacanthacris septemfasciata*) infected with the fungus *Empusa grylli* that

causes them to seek higher elevations (Skaife, 1925); and the grasshopper (*Melanoplus* spp.) infected with the nematode *Tetrameres americana*, which decreased the host's overall activity (Cram, 1931). However, the most popular example of parasite-induced behavioral modifications comes from the classic cases described by both Anokhin (1966) and Carney (1969). They examined ants infected with digenean trematode metacercariae (*Dicrocoelium dendriticum*) which take up residence in the ant's subesophageal ganglion (between the nerves of the mouthparts). *D. dendriticum* normally reaches sexual maturity in large herbivorous mammals which aren't normally considered to be insectivores. However, once infected, the ant which behaves normally during the day, when the temperature drops, climbs to the top of a grass blade and anchors itself at the tip with its mandibles, remaining in a state of torpor. Herbivores that are grazing during the cooler evening and morning hours will then inadvertently ingest both the ant and its hitchhiker. Another convincing example of host behavioral modification comes from infection with the trematode *Meiogymnophallus fossarum* in its second intermediate host, the European aurora venus clam *Venerupis aurea.* When infected with *M. fossarum*, the clam host reverses its position in the mud and migrates toward the surface, becoming more conspicuous than unparasitized conspecifics. In modifying its behavior, it is preferentially predated upon by the oystercatcher *Haemalopus ostralegus*, which is the definitive host of *M. fossarum* (Bartoli, 1974).

Mesa *et al.* (1994) reviewed whether "all prey were created equal," and determined that in 27 of the 37 (73%) experiments, "substandard" prey were captured in higher than expected proportions. There are several possible mechanisms that could potentially increase vulnerability of an organism to predation, including an increase in conspicuousness. Today, it is widely accepted that any noticeable change in prey appearance, condition, or behavior may attract attention, stimulate an increase in

predatory attacks, and ultimately lead to differential predation (Bams, 1967; Mesa *et al.*, 1994). Two studies in particular have shown very strong direct linkages between parasite infection and increased trophic transmission. The first was a study by van Dobben (1952), where he reported that 30% of roach (*Rutilus rutilus*) captured by cormorants were infected with the parasite *Ligula intestinalis* from a population of roach that only supported an infection of approximately 6.5%. The second study by Lafferty and Morris (1996), examined the California killifish (*Fundulus parvipinnis*) as an intermediate host of a brain-encysting trematode (*Euhaplorchis californiensis*). Their work revealed that birds were 30 times more likely to eat infected fish than uninfected ones. Predation rates were significantly intensified due to an increase in conspicuousness of the infected host (e.g., jerking, scratching, shimmying).

Other means of increasing conspicuousness include altering the hosts' physical appearance. One example of parasites changing host appearance was reported by Gibson *et al.* (2002), in which the digenean parasite; *Leucochloridium macrostomum*, turns the eye stalks of the freshwater snail *Succinea putris* into colorful "blinker lamps," making the snails easy prey for the aquatic songbird, the water ouzel (a.k.a. the American Dipper; *Cinclus mexicanus*). In another example, the aquatic sowbug (*Asellus aquaticus*), parasitized with *Acanthocephalus* spp., undergoes a conspicuous color change and increases surface seeking behavior, thus increasing predation risk (Pilecka-Rapacz, 1986). Not only do these behavioral modifications increase the risk of predation, they also affect the host's relationships with conspecifics. Examples have been documented in birds and fish in which unparasitized or lightly parasitized individuals generally avoid heavily parasitized conspecifics. This has been shown with three-spine sticklebacks (*Gasterosteus aculeatus*) parasitized by *Argulus canadensis* (Dugatkin *et al.*, 1994) and in fledgling cliff swallows (*Hirundo pyrrhonota*) with

hematophagous swallow bugs (*Oeciacus vicarius*) and fleas (*Ceratophyllus celsus*) (Brown and Brown, 1992).

When behavioral modifications are evident, parasites have often been associated with a specific host organ (e.g., eyes, brain) and the magnitude of said modification is usually correlated with parasite intensity. For example, the eyefluke *Diplostomum spathaceum* causes blindness in its host the Dace (*Leuciscus leuciscus*), thus altering the fish's anti-predator behavior (Crowden and Broom, 1980; Seppälä *et al.*, 2004). Significant changes in minnow (*Pimephales promelas*) schooling behavior were observed as a result of the fluke *Ornithodiplostomum ptychocheilus,* infecting the brain. The differences in schooling behavior led to an increase in predation risk (Radabaugh, 1980). As discussed above, the host-parasite system (*F. parvipinnis* and *E. californiensis*) that Lafferty and Morris studied, dealt with a site-specific parasite found only in the brain of its host (Lafferty and Morris, 1996). With the specificity of digenean trematode metacercariae in the gills, coupled with what is known about parasites' potential to induce behavioral modifications in their intermediate hosts, this is an area of research that still remains unexamined. Further research to determine the potential of gill parasites to induce behavioral modifications (either directly or indirectly) in their host is needed.

I.4 Parasites and Host Physiology

Stress has been defined as the nonspecific response of the body to any demand made upon it (Selye, 1973), and parasites clearly pose a significant stress to the host. Parasite-host systems are very complex relationships and there are numerous accounts detailing the high energetic costs to a host normally associated with infection. Parasite-induced disruptions to host physiology are well documented across taxa. For example,

Hurst and Walker (1935) found higher levels of heat production in parasitized snails compared to unparasitized conspecifics. Similarly, Vernberg and Vernberg (1971) reported significant differences between the metabolic rates of worms infected with *Zoogonus lasius* metacercariae and the rates of uninfected worms. However, Becker (1964) found that metabolic rates of the gastropod *Stagnicola palustris* decreased as a result of parasitism by digenean trematodes. Dahlman and Herald (1971) found that the parasite *Apanteles congregatus* had a dampening effect on the normal oxygen consumption cycle of the tobacco hornworm (*Manduca sexta*) larvae. Contrary to what was expected, parasitized larvae in active feeding phases consumed less oxygen than inactive phases (during apolysis and ecdysis). Despite the obvious costs of being parasitized, there are also instances where infection has worked to the host's advantage by enhancing its growth. For instance, gigantism in pulmonate snails is not uncommon when parasitized with life stages of various digenean trematodes which are known to customarily castrate their host. Once partially or fully castrated, hosts can allocate more resources toward growth, which results in the larger size of infected snails when compared to unparasitized conspecifics (Ballabeni, 1995; Probst and Kube, 1999; Krist, 2000). Whether this increased growth in parasitized snails is an adaptive host response to outlive the infections, or an adaptive manipulation by the parasite to increase transmission success is still under debate. Another study examining three-spined sticklebacks (*Gasterosteus* sp.), reported accelerated growth when infected with the cestode *Schistocephalus solidus.* Overall, the infected fish were able to maintain a similar (or better) body condition than uninfected individuals, with the exception of enlarged spleen development (Arnott *et al.*, 2000). In the case of the snails, castration is detrimental to the host population overall due to the loss of gametes, however, in both the snail and fish examples, individual hosts may benefit as they reach a 'size refugia' which may also ultimately help reduce predation.

There are two mechanisms thought to induce behavioral modifications in hosts. The first mechanism deals with indirect effects on the host's system and the second mechanism deals with direct effects. Altering the host's physiology either directly or indirectly can ultimately increase parasite transmission. There are a number of cases whereby parasites have altered some physiological condition within a host that lead to an increase in trophic transmission. For example, in a study using BALB/c mice and the cestode parasite *Taenia crassiceps*, Gourbal *et al.* (2001) found that parasitized mice did not generate any physiological stress responses (e.g., release of opioid and nonopioid compounds) which are normally found in the presence of a predator. Several other studies have reported parasite-induced disruptions in olfactory cues in the presence of a predator's odor whereby activity levels are altered by either being hyperactive (Jakobsen and Wedekind, 1998) or hypoactive (Dezfuli *et al.*, 2003). Increases in host attraction to predators have also been found (Berdoy *et al.*, 2000; Baldauf *et al.*, 2007). The red grouse (*Lagopus lagopus*) infected with the cecal nematode *Trichostrongylus tenuis*, found that *T. tenuis* develops cecal pathology that hinders hens from reducing cecal fecal odor which in turn facilitates predation. Birds killed by their natural predators had higher nematode intensities than those that were shot by hunters (Hudson *et al.*, 1992). The tapeworm *Echinococcus granulosus* also relies on the predator-prey relationship between its intermediate host, the moose (*Alces alces*) and its definitive host, the wolf (*Canis lupis*) to complete its life cycle. The parasite resides in the lungs of the moose, debilitating it so much so that transmission to its final host is facilitated (Joly and Messier, 2004).

Little to no research has been conducted on the potential of gill parasites (specifically digenean trematode metacercariae) to induce either direct or indirect physiological modifications in their host.

I.5 Parasites and Sex

Parasites have strong influences over every aspect of their host's life, and that includes reproductive effort (or lack thereof). Many species of parasites are known to castrate and feminize their hosts, with molluscs and crustaceans commonly affected. Studies using brown mussels (*Perna perna*) (Calvo-Ugarteburu and McQuaid, 1998) and scallops (*Pecten fumatus* and *Chlamys (Mimachlamys) asperrima*) (Heasman *et al.*, 1996) and their digenean trematode parasite *Bucephalus* sp. have consistently shown that castration occurs, which ultimately redirects energy normally expended in reproduction, to the parasite. One classic example of host feminization and castration comes from the Rhizocephalan which is essentially a highly modified barnacle. *Sacculina* spp. have been very popular research subjects since the late 1800s (Giard, 1887). For instance, the rhizocephalan *Sacculina granifera* preferentially attacks young crabs (e.g., *Portunus pelagicus*) and considerably modifies the males. Modifications include behavioral changes, whereby males behave like egg-bearing females (e.g., grooming their egg masses, moving seaward rather than staying inshore), and morphological changes such as shortening of chelar propodus to resemble those of females, and broadening of abdomen (Reinhard, 1956; Phillips and Cannon, 1978).

Many studies have also been conducted demonstrating how sex hormones affect the rate of parasitism of hosts. General differences in parasite infection between the sexes have been explained as differences in immunocompetence (Klein, 2000), body size (Arneberg, 2002), lifespan (Wiklund *et al.*, 2003), foraging patterns (Anderson *et al.*, 2004), social behavior (Altizer *et al.*, 2003), mobility, and home range (Greenwood, 1980). To further complicate matters, elevated testosterone levels have been linked to immunosuppressive effects which could increase males' susceptibility to parasite infection and disease (Folstad *et al.*, 1989; Zuk, 1990; Wedekind and Jakobsen, 1998).

Male-biased infection rates are most notable for protozoan and nematode parasites. Bateman's principle (Bateman, 1948) suggests that females maximize their fitness by investing in longevity, whereas males invest more in mating success. Therefore, to increase longevity, females invest more energy into immune response, thus warding off more potential parasite infections (Rolff, 2002).

Differential parasitism between the sexes has been reported for a wide range of taxa including amphibians (Tinsley, 1989), mammals (Beitel *et al.*, 1974; Folstad *et al.*, 1989), crustaceans (Wedekind and Jakobsen, 1998), fish (Reimchen, 2001) and birds (Poulin, 1996). Poulin (1996) explored the question of whether there was a cost to being male by examining 30 years of parasite literature and found that prevalence of infection tended to be higher in males in many types of host-parasite relationships. Another review paper by Klein (2004) reported that of the 58 parasite species for which sex differences in hosts have been reported (protozoa, nematodes, trematodes, cestodes and arthropods), 84.5% of the studies found male-biased infections. Alexander and Stimson (1988) found that microfilariae in mice infected with *Loa loa* were consistently higher in males than females. Tinsley (1989) reported four times as many monogeans (*Pseudodiplorchis americanus*) in male desert toads (*Scaphiopus couchii*) than females. Similarly, males of three species of cichlids were found to have higher helminth infections than females (Batra, 1984).

Although males are more susceptible than females to many parasites, there are parasites for which males are more resistant than females. For example, male mice (*Mus musculus*) are less susceptible than females to several parasites, including *Babesia microti* (Aguilar-Delfin *et al.*, 2001), and *Schistosoma mansoni* (Eloi-Santos *et al.*, 1992). *Toxoplasma gondii* is said to be one of the best studied parasites demonstrating increased female susceptibility (Klein, 2004). Female mice develop more severe brain inflammation and are more likely to die following an infection than their

male counterparts (Walker *et al.*, 1997). Another study examining the intestinal cestode *Taenia crassiceps* and its rodent intermediate host, found that females developed more cysticerci than their male counterparts (Larralde *et al.*, 1995). It was determined that *T. crassiceps* development and growth is inhibited by androgens, whereas oestrogens promoted it. Another case of female-biased infection has been documented for free-ranging golden lion tamarins (*Leontopithecus rosalia*). Prevalences were greater in females for all helminth species observed, especially for *Onicola* sp. Researchers hypothesize that the differences found in parasite prevalence may be associated with changes in sexual steroid levels that accompany age and reproductive status (Monteiro *et al.*, 2007).

I.6 Parasites and the Environment

Parasites often alter the tolerance of hosts to environmental factors. When coupled with the frequent stressors inherent to estuarine systems where abiotic parameters such as temperature, salinity and oxygen vary rapidly and widely, problems ensue. For instance, when mud snails (*Nassarius obsoletus* and *N. reticulatus*) are infected with larval trematodes, they have been found to exhibit reduced resistance to high temperature (Tallmark and Norrgren, 1976). Similarly, the gastropod *Hydrobia ulvae* has been found to be less resistant to desiccation and osmotic stress when infected with larval trematodes (Lauckner, 1987). Calvo-Ugarteburu and McQuaid (1998) found that brown mussels (*Perna perna*) infected with bucephalid sporocysts (*Proctoeces* sp.) were easier to open and lost significantly more water than non-infected individuals.

In our post-industrial revolution, post-baby boom era, it is difficult to find habitats that aren't stressed and/or haven't been impacted in some way by human disturbance. There are scores of detectable toxicants in our environment of both organic (i.e., PAHs,

PCBs), and inorganic (e.g., metals, nutrients) origin. Like all organisms, parasites are also affected by changes in environmental conditions. As such, they have been used as indicators of degradation (Valtonen *et al.*, 1997) and disturbance. The first reports on the effects of parasites on the resistance of their hosts to environmental contaminants were published as early as 1977 using zinc resistance of sockeye salmon (*Oncorhynchus nerka*) infected with *Eubothrium salvelini* (Boyce and Yamada, 1977), and effects of cadmium on infected three-spined sticklebacks (*Gasterosteus aculeatus*) (Pascoe and Cram, 1977). In recent years, an increasing number of papers by parasitologists and ecologists alike have focused on the interrelationship among host-parasite associations and environmental pollution, and the health of the parasitized host (see reviews by Lafferty, 1997; Overstreet, 1997; MacKenzie, 1999; Sures, 2003). As a general rule, infections with endoparasitic helminthes (usually organisms with indirect, more complex life cycles) decrease, while infections with ectoparasites (most typically having direct life cycles) tend to increase with increasing levels of pollution. This is thought to occur because ectoparasites are in constant contact with the external environment, and have had to develop a resistance to certain natural changes (e.g., salinity, pH, pollutants) in the course of their evolution (MacKenzie, 1999). There are several reasons why there is a growing interest in the use of parasites as bioindicators: 1) there are more parasitic than free-living species; 2) many parasites have complex life-cycles containing different stages requiring a wide range of conditions. Therefore, each stage must be assessed separately, increasing the number of potential indicators; and 3) many parasite stages are extremely sensitive to even minor changes in the environment – a quality that is very useful in sentinel species (MacKenzie, 1999).

Pollutants can stimulate as well as depress parasitic activity, depending on several factors such as the type and level of pollutant, and the host-pathogen system examined. The effects of combined stressors (e.g., pollutants and parasites) have been

reported throughout the literature. One study by Klar and Sures (2004) on the effects of cadmium exposure on parasitized laboratory rats, found a strong additive effect on the rat's stress hormone levels. In another study, a significant increase in the infection intensity of the trematode *Posthodiplostomum minimum* metacercariae was found in bluegill sunfish (*Lepomis macrochirus*) exposed to heptachlor contamination, despite heptachlor being toxic to the parasite at high concentrations (Andrews *et al.*, 1966). Similar findings were reported for the European eel (*Anguilla anguilla*) where nematode (*Anguilliocola carssus*) infections were significantly higher in the more polluted upstream region of the estuary than in the less polluted downstream area (Loukili and Belghyti, 2007).

Some stressors (e.g., metals) may decrease parasitism by either killing or reducing the parasite population, or the hosts directly (Soucek and Noblet, 1998). In other cases, parasitized individuals fared better than their unparasitized counterparts as higher contaminant levels are found in some parasites than in their hosts (Sures *et al.*, 1994; Taraschewski and Sures, 1996). The parasite caused a lower body burden of the contaminant in its host. This phenomenon has been widely studied using aquatic species and much less so in terrestrial ones. Using both field and laboratory studies, Sures (2003) found that acanthocephalans stored metal concentrations at levels several thousand times higher than in host tissues, and reached a steady-state concentration orders of magnitude higher than the ambient water level. The parasites demonstrated an ability to respond quickly to changes in the surrounding environment. Another aquatic study using two host-parasite systems (minnow – cestode): *Vimba vimba – Caryophillaeus brachycollis*, and *Alburnus alburnus – Ligula intestinalis* and metals (Cu, Cr and Zn), revealed higher concentrations of these metals in cestodes than in their hosts' tissues (Gabrashanska and Nedeva, 1996). Galli *et al.* (1998) found that Cr levels in the acanthocephalan parasite were 60 times higher than those found in the chub

host's (*Leuciscus cephalus*) liver. Additionally, Pb concentrations were about 200 times higher in the parasite than in the *L. cephalus*' liver (Galli *et al.*, 1998). A study experimentally infecting male Wistar rats (CD-M strain) with the tapeworm *Hymenolepis diminuta* found lead concentrations within the cestodes to be 17 times higher than levels found in the host's kidney (Sures *et al.*, 2002). Similarly, another study using the wood mouse *Apodemus sylvaticus* and its intestinal cestode *Skrjabinotaenia lobata* found lead levels greater than 81 times higher in *S. lobata* than in the tissue of *A. sylvaticus* (Torres *et al.*, 2006).

Anthropogenic environmental stress is not limited to pollution however. The act of marsh restoration, although well meaning, is in fact quite a significant form of disturbance as it oftentimes requires the removal of invasive species (e.g., via mechanical or chemical means), and the regrading of banks and channels to improve tidal flow (e.g., large machinery). As previously discussed, the use of digenean trematode parasites as indicators of disturbance, hence, wetland restoration success is plausible given their need for two or more hosts to complete their complex life cycles and their ubiquity in these ecosystems. Parasites do well in wetland habitats because predation rates are high, as is host density (Thomas *et al.*, 1997). Additionally, habitat quality is being improved, thus attracting additional intermediate hosts and definitive hosts. All of these factors tend to increase the probability of parasite transmission. For example, estuarine snails are the first intermediate host for numerous trematode species. The brackish water snail, *Cerithidea californica;* is the first intermediate host of at least 19 species of trematodes (Lafferty *et al.*, 2006). Several studies have demonstrated positive associations between abundance (Hechinger and Lafferty, 2005; Fredensborg *et al.*, 2006) and species richness (Hechinger and Lafferty, 2005) of birds and trematodes in snails on local levels. Although bird abundance and diversity are more important in structuring trematode communities in first intermediate host snails

(Matthews *et al.*, 1985; Smith, 2001), the presence of second intermediate hosts such as *Fundulus heteroclitus* also plays a critical role. Therefore, the use of parasite abundance or intermediate host abundance should be further examined for their utility in determining wetland restoration progress and ultimate success.

I.7 The Host – Fundulus heteroclitus

Fundulus heteroclitus (Linneaus 1766) (Figure 1); commonly known as the common killifish or the mummichog, is in the Family Cyprinodontidae and occurs in sheltered coastal waters along the Atlantic coast of North America from Newfoundland to northern Florida (Kneib, 1986). Attempts have been made to quantify their densities (Nixon and Oviatt, 1973; Kneib, 1984; Kneib and Wagner, 1994), and it is the general consensus that populations of *Fundulus* spp. are very dense relative to most other fish species within this habitat (Valiela *et al.*, 1977; Abraham, 1985). In fact, the name "mummichog" is an Indian word meaning "going in crowds" (Nichols and Breder Jr., 1927). Schools may number from a few fish to several hundred or more (Hildebrand and Schroeder, 1972).

F. heteroclitus has a short, rounded snout and caudal fin, 31-35 scales along the lateral line (Rosen, 1973), 11-12 dorsal fin rays, and a terminal mouth with protruding lower jaw (Hildebrand and Schroeder, 1972). Mummichogs are sexually dimorphic, with adult males obtaining a dark green or olive coloration dorsally, a yellowish hue ventrally (during the spawning season), numerous white or yellowish spots, and approximately 15 narrow silvery vertical stripes along their sides. Males may also have a dark spot on the posterior 4-5 rays of their dorsal fin which the females lack (Rosen, 1973). These sex-specific color patterns appear when fish are between 38-44 mm long (Hildebrand and Schroeder, 1972). Females are less colorful than their male counterparts, having a simple brownish color dorsally, and a paler white hue on their ventral surface.

Experiencing their fastest growth during their first two growing seasons (Kneib and Stiven, 1978), adults can reach lengths upwards of 146 mm in total length (pers. obsv.). Females are typically larger than males after the first year, however, males are generally found to be heavier. *F. heteroclitus* are oviparous with females reaching sexual maturity at 38 mm, and males at 32 mm (Hildebrand and Schroeder, 1972). Mummichog age can be determined using otoliths after a fish has overwintered once. Fritz and Garside (1975) calculated the following age-length correlations for *F. heteroclitus* in Nova Scotia: 1 yr old=35-55 mm; 2 yr old=51-74mm; 3 yr old=68-83; and 4 yr old=78-95 mm, with size and age distributions varying considerably in contaminated estuaries. Many population studies have been performed using *F. heteroclitus* and investigators agree that the species does not survive beyond a fourth growing season (Valiela *et al.*, 1977; Kneib and Stiven, 1978; Samaritan and Schmidt, 1982). They are non-migratory with respect to breeding, and while some may move as much as 375 m, their typical home range is approximately 38 m along tidal creek banks (Lotrich, 1975), and up to 15 ha within [restored] salt marshes (Teo and Able, 2003). Although they may occasionally be found in freshwater (Denoncourt *et al.*, 1978; Samaritan and Schmidt, 1982; Weisberg, 1986), the species is most often found in tidal salt marshes, which are known to be relatively extreme physical environments to which mummichogs are well adapted (Bigelow and Schroeder, 1953). In the winter, they migrate to the mouth of the tidal channel where they have been living and return to the same channel the following spring (Butner and Brattstrom, 1960). *Fundulus* spp. are also one of the few species that can be found in heavily contaminated environments where less hardy species often find survival difficult (Huver, 1973; Weis and Weis, 1989; Vogelbein *et al.*, 1990).

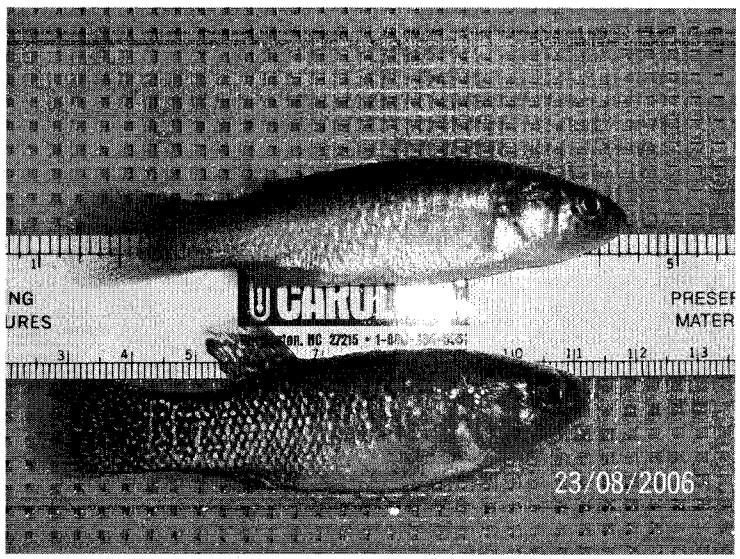


Figure 1-1. Adult *Fundulus heteroclitus* (common Mummichog) – female (top) and male (bottom).

F. heteroclitus occupies an intermediate level trophic position within the structure of salt marsh communities on the eastern coast of the United States. Although they are typically found near the bottom, *Fundulus* spp. are capable of feeding at the surface, mid-pelagic and benthic regions (Huver, 1973). They function as both predator and prey in the trophic structure of east coast tidal marshes (Nixon and Oviatt, 1973; Kneib, 1986). As predators, the diet of adult *F. heteroclitus* is comprised primarily of small crustaceans (e.g., *Palaemonetes pugio*) and annelids (Kneib and Stiven, 1978; Samaritan and Schmidt, 1982) which account for 40% of their diets from May to June (Wiltse *et al.*, 1984). As prey, *Fundulus* spp. are important prey items for organisms such as the blue crab (*Callinectes sapidus*) (Kneib, 1986), and various birds such as herons and egrets (Valiela *et al.*, 1977). Additionally, their movements on and off the marsh surface are thought to be an important aspect in the export of organic materials from the marsh surface (Valiela *et al.*, 1977). *Fundulus* spp. are an important link in energy transfers between the marsh surface and the subtidal systems. Additionally,

Fundulus spp. are economically significant due to their extensive use by the sport fishing industry as bait for summer flounder (*Paralichthys dentatus*) and young bluefish (*Pomatomus saltatrix*) in New York and New Jersey (Perlmutter, 1961) as a result of their seemingly inexhaustible numbers. Over time, *Fundulus* spp. have played important roles in advancing our understanding of many different aspects in the biological sciences such as physiology and anatomy (Weis and Weis, 1976), behavior (Weis *et al.*, 1999; Zhou *et al.*, 2001), and genetics (Cashon *et al.*, 1981) due to their hardiness, distribution and abundance (Huver, 1973).

I.8 Rationale

Past studies that have examined parasites of *F. heteroclitus* have focused on individual parasite species (Bergey *et al.*, 2002; Schmalz Jr. *et al.*, 2002), or more recently, given a species checklist (Harris and Vogelbein, 2006). Studies of gill parasites of *F. heteroclitus* in particular have limited their focus to a specific species (Bond, 1937, 1938; Stunkard and Uzman, 1955; Lawler, 1967 and others), with only a handful of studies considering parasite suites (Dickinson and Threlfall, 1975; Marcogliese, 1995). Only one study has looked at gill parasites of *F. heteroclitus* on a broader ecological scale that included effects of season, locality, host sex and size (Barse, 1998). Little to no research has been conducted on the potential of gill parasites (specifically digenean trematode metacercariae) to induce either direct, or indirect physiological modifications in their host, nor their potential to modify either the anatomy or behavior of their host.

Parasites are common in marine communities and it is probable that all species in marine habitats are infected with parasites and/or are parasitic themselves at some point in their life cycle (Price, 1980). Because parasites are so ubiquitous and can affect host physiology, behavior, and ultimately ecology, it is essential to know how parasites are distributed among host populations and which populations may potentially become

more susceptible to infection. Although higher order predators (e.g., birds) tend to serve disproportionately as hosts, consumers at mid-trophic levels such as the mummichog (*Fundulus heteroclitus*), are extremely vulnerable as they are subjected not only to various predators, but to a wide array of parasite species as well (Lafferty *et al.*, 2006). Parasites of fish may also be good indicators of the life habits and environment of the fish, including their interactions with the benthic, planktonic and other fish communities. In nature, it is rare for individual fish to be infected with just one single parasite species, rather a suite of infections is typical (Barber and Poulin, 2002). A recent study using *F. heteroclitus* found 22 parasite taxa infecting this particular species (Harris and Vogelbein, 2006). To understand the structure of parasite infracommunities, we must begin with the identification of the parasite species present, their relative abundances, and their distribution within their fish host. Therefore, the aim of this study is to examine the following hypotheses:

1. Hosts in cleaner habitats will have greater parasite species richness and evenness than those occupying more contaminated sites, as cleaner sites should support a greater diversity of intermediate hosts, thus parasites.

2. Parasite abundance will increase with increased contamination, as more stress tolerant parasite species have less competition and are allowed to proliferate.

3. Parasite abundance will increase with increased disturbance (specifically wetland restoration processes), due to the creation of more suitable habitats for both intermediate and definitive hosts, thus facilitating parasite transmission.

4. Parasite loads will increase with time – early season collections will have fewer parasites than late season, as numbers of available intermediate hosts also increase.

5. Males will have greater parasite loads than females resulting from potential differences in behavior and/or physiology (e.g., testosterone).

6a. Fish with heavily parasitized gills will have LOWER activity levels than those with low to moderate infections resulting from blocked respiratory structures, thus compromising dissolved oxygen (DO) uptake; or

6b. Fish with heavily parasitized gills will have HIGHER activity levels than those with lower infections as a response to parasite manipulation of host behavior to increase trophic transmission rates.

7. Fish with heavily parasitized gills will exhibit the greatest number of conspicuous behaviors (e.g., jerking, flashing) compared to less parasitized conspecifics, resulting from either direct or indirect parasite behavioral modifications.

8. Fish with heavily parasitized gills will spend significantly more time at the water's surface than less parasitized conspecifics as a result of either direct or indirect parasite behavioral modification.

9. Fish with heavily parasitized gills will have lower respiration rates than those with lower infections, as respiratory structures are compromised.

10. Fish with less parasitized gills will have higher stamina versus those with significantly higher infections as a consequence of healthier gills.

11. Excessive parasite loads will induce physiological (e.g., erythrocyte volume) and/or morphological changes (e.g., additional branching) in the gills to compensate for presumed oxygen loss.

The following study will explore the parasite communities of *F. heteroclitus*, as well as host-parasite interactions as they relate to a number of factors, including

temporal and spatial effects (Chapter 2), behavior (Chapter 3), anatomy and physiology (Chapter 4), as well as environmental conditions (Chapters 2 & 5).

CHAPTER II. BASELINE SURVEY

INTRODUCTION

Historically, most of the literature on community ecology has been dedicated to free-living species. In the last two decades, interest in parasite community ecology has grown. A healthy ecosystem is one that persists, maintains high productivity, organization (biodiversity and predictability) and resilience (Costanza and Mageau, 2000). Accumulating research suggests that as parasite species diversity increases, overall ecosystem functioning improves. The biodiversity of free-living organisms is shadowed by parasites as each individual host can be considered a habitat to be colonized and exploited (Hudson *et al.*, 2006). In fact, it has been argued that half of all biodiversity likely comprises parasitic species (Toft, 1986). Vertebrates are usually exposed to more than one parasite species at any given time, and as such, are often infected with suites of parasites (Esch *et al.*, 1990; Barber and Poulin, 2002), so when examining the dynamics of parasite infection, it is best understood when looking at the entire host-parasite community level (Bottomley *et al.*, 2005). Parasites are known to cause various effects (e.g., physiological, behavioral) in their hosts, which in turn may potentially have larger ecosystem-wide effects. Consequently, gaining a better understanding of the host-parasite relationship and how parasite communities function is crucial.

Due to their complexity, estuarine systems are ideal for parasites as they offer numerous potential hosts, as resident and migratory species from both aquatic (fresh and marine) and terrestrial habitats rely heavily on these ecosystems for many aspects of their life histories. Wetland ecosystems are not immune to the strong structuring influence that parasite species have, which is due in part to this inherent complexity. Salt marshes in particular, are highly variable environments where

conditions such as salinity, temperature, turbidity and oxygen concentration of the water can fluctuate rapidly, both temporally and spatially (Nixon and Oviatt, 1973). They are also among the most productive estuarine environments in mid- to high-latitudes worldwide and, like other estuarine habitats, they function as nurseries for a variety of nektonic species (Boesch and Turner, 1984).

In fish, a number of factors have been found that influence the prevalence and intensity of their parasites, including host age, sex, diet, and season (Hogue and Peng, 2001, 2003). One of the several ways in which parasites may affect a fish host is by mechanical effects that include damage to tissues, rupturing protective layers, complete or partial atrophy of internal organs, obstruction of the alimentary canal or vascular system, etc. (Bauer, 1970). Because parasites are such an integral part of the ecosystem as a whole, they may be good indicators of the life habits of the fish, including their interactions with the benthic, planktonic and other fish communities. Many researchers rely of the use of gut contents to determine diet but they are only a snapshot in time of what a host ate just prior to capture. Parasites on the other hand, can reveal long-term trends in host ecology, as parasites can live in hosts from months to years (Marcogliese and Cone, 1997). Furthermore, each parasite reflects the presence of different organisms that must participate in its life cycle (Marcogliese, 2005), thus providing a picture of overall ecosystem health. Gibson (1972) has proposed the use of parasites as biological tags to trace the origin of migratory fish such as the flounder *Platichthys flesus*, to follow its movements. Having a basic understanding of the spatial differences in parasite composition and abundance can also help researchers determine habitat similarity or dissimilarity. Examining spatial differences in parasitism using the Patagonian toothfish (*Dissostichus eleginoides*, a.k.a. Chilean sea bass), Brickle *et al.* (2006) noted that parasite communities on the Falklands-Patagonian shelf and deep water were different, whereas those caught at

intermediate depths on the shelf slope had parasite communities that were intermediate, containing a mixture of shelf and deeper-water parasites.

II.1 Effects of Pollution on Host-Parasite Systems

Behavioral effects of toxic contaminants may have profound effects on a host organism's ability to obtain live food (Weis *et al.*, 2001b; Zhou *et al.*, 2001), avoid predation (Weis *et al.*, 1999), and reproduce successfully (Toppin *et al.*, 1987) in its natural environment. There are several possible outcomes when parasites are added to an already stressed system. Studies have shown both positive and negative correlations between fish parasitism and contaminant levels (Lafferty, 1997; MacKenzie, 1999). Some stressors may make hosts more susceptible to parasitism, which may also increase mortality rates of infected hosts. For example, juvenile rainbow trout (*Salmo gairdneri*) exposed to pollutants caused an increase in dermal mucus secretion that helped facilitate parasite infestation (Glodes *et al.*, 1988). Khan (1990) found highly significant increases in gill parasite (*Trichodina* spp.) intensity on longhorm sculpins (*Oligocottus maculosus*) and Atlantic cod (*Gadus morhua*) on petroleum-exposed hosts. However, some stressors may decrease parasitism by either killing or reducing the parasite population (Pietrock *et al.*, 2002), or other the directly (Soucek and Noblet, 1998). Although stressors may reduce the parasite's intermediate host's abundance, thereby reducing transmission rates, in other cases these stressors may serve to increase the number of intermediate hosts thus facilitating transmission (see review by Lafferty and Kuris, 1999). Subsequently, parasites with life cycles that utilize multiple hosts may prove to be useful in providing specific information about the condition of a particular ecosystem (Cone *et al.*, 1993; Lafferty, 1997; Overstreet, 1997).

Although it seems intuitive that pollution and parasites are harmful to hosts, there are cases whereby hosts may benefit. For example, environmental stress often result in lowered predation pressure because predators may be either rare or very inefficient (Kerfoot and Sih, 1987) in these areas. Next, it has been demonstrated that some parasites accumulate high levels of contaminants resulting in parasitized hosts having lower contaminant levels than unparasitized conspecifics. For example, Bergey *et al.* (2002) reported that *F. heteroclitus* parasitized with the nematode *Eustrongylides* sp., and grass shrimp (*Palemonetes pugio*) infected with the isopod *Probopyrus pandalicola*, accumulated lower concentrations of mercury than unparasitized conspecifics. Similarly, periwinkles (*Littorina littorea*) infected by either *Cryptocotyle lingua* rediae or *Renicola roscovita* sporocysts had lower copper and iron concentrations compared to uninfected snails of the same sex (Cross *et al.*, 2003).

II.2 Seasonality and Parasite Abundance

Season is an important factor in structuring parasite communities and is particularly well known in hosts found in higher latitudes where seasonal temperature changes are greatest. There are several factors believed to influence the seasonal fluctuations in parasite abundance and prevalence found among hosts including feeding habits, immunological alterations, hormonal changes, temperature, and availability of infected intermediate hosts (Mukbel *et al.*, 2001). For example, in the Baltic Sea, most endoparasitic species of the flounder (*Platichthys flesus*) and all of the intestinal parasites of the cod (*Gadus morhua*) have seasonal oscillations (Möller, 1974), due to the seasonality of the intermediate hosts containing the parasite's infective stages. Fluctuations may also occur due to changes in abiotic cues (e.g., temperature, rain) such that parasites time their peak reproductive effort in

coordination with these environmental conditions. This system has been described by using cichlids (*Cichlasoma urophthalmus*) and their digenean trematodes; *Oligogonotylus manteri* (Jimenéz-García and Vidal-Martínez, 2005).

Many host-parasite systems have been studied to determine when parasite abundance peaks within its intermediate and/or definitive host. Results vary with parasite, host, and season. For example, Strømnes and Andersen (2000) examined the nematode parasite *Anisakis simplex* and three of its host species, saithe (*Pollachius virens*), cod (*Gadus morhua*) and redfish (*Sebastes marinus*) to observe potential seasonal oscillations. They found that there was an increase in parasite abundance in all three species in the spring (Strømnes and Andersen, 2000). Examining *Curtuteria arguinae* trematode metacercariae and their host, the cockle (*Cerastoderma edule*), Desclaux *et al.* (2006) found that the maximum parasite load was in autumn. Distinct temporal differences were observed using two species of gill monogeneans (*Polylabris mamaevi* and *Tetrancistrum nebulosi*) on caged and wild-caught rabbitfish (*Siganus fuscescens*), with infection intensities increasing from February to May and disappearing from June to August (Yang et al., 2006). So, although distinct seasonal differences occur, the variability of the host-parasite association being examined, coupled with geographic region, play significant roles.

II.3 The Influence of Host Sex

There are both ecological and biological reasons behind the consistent reports of males being more likely to be parasitized than females. Males and females exhibit differences in the quantity of food consumed, their body size, behaviors (e.g., territoriality, aggression) and of course, hormones. Hierarchal social relationships among vertebrates are normally mediated by sex hormones and those individuals with the highest levels of steroids are usually at the greatest risk of parasite infection

(Davis and Read, 1958). For example, Batra (1984) found that three species of male cichlids had higher helminth infections than female conspecifics. Similarly, infestations by the monogenean *Gyrodactylus salaris* were greatest on male Arctic charr (*Salvelinus alphinus*) than on females (Robertsen *et al.*, In press 2007). Experimental manipulations of parasite loads have shown that infection rates can not only alter and hinder courtship (Pélabon *et al.*, 2005) and sex ratios (Ehman and Scott, 2002) but also the expression of secondary sex characteristics, which are normally regulated by testosterone. Therefore, females choosing well-developed secondary sex characteristics are likely to choose relatively parasite-free males (Zuk, 1990; Hillgarth and Wingfield, 1997; Thompson *et al.*, 1997). For example, Plath (2004) demonstrated that female cave mollies (*Poecilla mexicana*) preferred males that were not infected with the pathogenic bacterium *Mycobacterium* sp. However, choosiness is not limited to females. Male pipefish (*Syngnathus typhle*) have also been shown to avoid females parasitized with the trematode *Cryptocotyle* sp. by using visual cues (Rosenqvist and Johansson, 1995).

II.4 Rationale

Due to the ubiquity of parasites in the natural environment, and the fact that they often affect host physiology, behavior, and ultimately ecology, it is of interest to understand how parasites are distributed among host populations of *F. heteroclitus*, and if some populations could potentially be more susceptible to infection than others. In general, it is believed that parasites of fish may also be good indicators of the life habits and environment of their host, including their interactions with the benthic, planktonic and other fish communities. Therefore, it is important to identify the parasite species present, their relative abundances, and their distribution within their fish host. For the purposes of this baseline study, only endoparasitic macroparasites

were examined. The aim of this study was to investigate the following four hypotheses:

1. Hosts in cleaner habitats will have greater parasite species richness and evenness than those occupying more contaminated sites, as cleaner sites should support a greater diversity of intermediate hosts, thus parasites.

2. Parasite abundance will increase with increased contamination, as more stress tolerant parasite species have less competition and are allowed to proliferate.

3. Parasite loads will increase with time – early season collections will have fewer parasites than late season, as numbers of available intermediate hosts also increase.

4. Males will have greater parasite loads than females resulting from potential differences in behavior and/or physiology (e.g., testosterone).

MATERIALS AND METHODS

II.5 Study Sites

Seven sites were examined, six of which were located within New Jersey and one located in Long Island, New York (Appendices B and C). Please refer to Table 2-1 for mean sediment metal contaminant levels for each study site.

Tuckerton (Graveling Point)

Graveling Point (TK) is part of the Edwin B. Forsythe National Wildlife Refuge (Brigantine Division) which is located in a non-industrialized area adjacent to Tuckerton, NJ (39.588149 N, 74.364586 W). It contains more than 17,400 ha of southern New Jersey coastal habitats and was established in 1939 to protect tidal

wetland and shallow bay habitat for migratory water birds. In 1986, it was designated a Wetland of International Importance under the Ramsar Convention of 1971. Consequently, in 1997 the Jacques Cousteau National Estuarine Research Reserve (JC NERR) was created. JC NERR is one of 26 research reserves across the country. TK is the southern most site examined, with salinities of approximately 25 – 30 ppt.

Sandy Hook

Sandy Hook (SH) is a peninsula projecting northward into Raritan Bay at the northern most point of the New Jersey coast, in Monmouth County (40.402377 N, 73.990912 W). The Sandy Hook Unit Gateway National Recreation Area is controlled by the National Park Service. It is a 827 ha, mostly undeveloped with a variety of habitats including beach and dunes, mudflats, holly forest, deciduous woods, freshwater ponds, salt marshes, coastal scrub/shrub, and successional fields. Like TK, SH falls along a major bird migration flyway in both the spring and fall, attracting approximately 340 bird species to its various habitats. Fish were collected in SH Bay, located at the eastern end of Raritan Bay. Salinity in this area is approximately 25 – 30 ppt.

Union Beach

East Creek is located in Union Beach (UB) in the BayShore region of Monmouth County, NJ (40.439862 N, 74.199563 W). It is a tidally-influenced creek that flows into Raritan Bay. UB is surrounded by a nearby residential area and is used for both commercial and recreational fishing, and shellfish harvesting. The two creeks in this area; the Chingarora and Flat Creeks, are both tidally-influenced and have been identified by the New Jersey Audubon Society as important natural areas. Salinity in this area is approximately 20 – 25 ppt.

Piles Creek

Piles Creek (PC) is a tributary of the Arthur Kill located in Linden, New Jersey (40.62505 N, 74.23844 W). PC is surrounded by industrial sites, a sewage treatment plant, a power plant, and a major highway (the New Jersey Turnpike). There is limited freshwater flow into the area and as a result, both the sediments and the organisms living within the creek have high concentrations of many pollutants. Numerous studies have been conducted within this system to investigate various aspects of organismal health and ecological interactions (Santiago Bass *et al.*, 2001; Weis *et al.*, 2001b; Weis *et al.*, 2002). Salinity at this site is approximately 10 – 20 ppt.

Mill Creek

Mill Creek Marsh (MC), in the Hackensack Meadowlands District, is bordered by the New Jersey Turnpike and a shopping center (40.79101 N, 74.063416 W). MC is a 55 ha tidal marsh that was restored in 1999 to reestablish tidal flow across the site, create additional open water habitats and develop low marsh and upland habitats. The MC restoration has increased the diversity of this estuarine system. More than 260 species now use the area. Migratory shore birds can be found, along with a variety of other waterfowl that utilize it for various activities (Meadowlands, 2004). MC does not have direct estuarine influence, and as such has lower salinities than the other sites examined (approximately 5 – 10 ppt).

Richard W. DeKorte Park

Richard W. DeKorte Park (RD) is a 45 ha site in Lyndhurst NJ (40.809433 N, 74.12453 W), centrally located within the Hackensack Meadowlands District (Meadowlands, 2004). It is located within the Saw Mill Creek Basin, and was initially part of a large marsh system influenced by Kingsland Creek and Sawmill Creek but

has since been cut off from full tidal inundation due to construction activities. Salinity is approximately 5 – 10 ppt. Much of this area is still dominated by *Phragmites australis* that fringes large open water areas. RD hosts 265 bird species (resident and migratory) as well as terrapins, red fox, muskrats, among others (Meadowlands, 2004).

Bullhead Bay, Long Island

Bullhead Bay (BB) is a relatively pristine habitat located on the northern shore of the south fork of eastern Long Island (Southhampton, NY), and is part of the Peconic estuary (40.904341 N, 72.410271 W). BB is surrounded by light residential use and a golf course. While the estuary as a whole continues to show signs of stress, such as brown tides, and eelgrass die-offs due to these types of anthropogenic loading practices, overall the indicators point to a relatively healthy system relative to other estuaries nationwide. The significant open space that is still available in this particular region of Long Island protects its natural habitats, groundwater recharge areas, and surface water quality (Miller, Winter 2004-2005). Salinity at this site is approximately 25 – 30 ppt.

Table 2-1. Mean sediment metal contamination for study sites (Weis *et al.*, 2001a*; Windham *et al.*, 2004**).

Site	MEAN SEDIMENT METAL LEVELS (ppm) Hg	Cu	Pb	Zn
SH*	0.02 ±0.01	113.0 ±84.0	285.0 ±147.0	181.0 ±79.0
TK*	0.19 ±0.02	43.8 ±7.6	73.2 ±8.0	141.0 ±13.4
PC*	6.30 ±1.00	485.0 ±46.7	107.0 ±20.8	525.0 ±67.1
UB*	0.03 ±0.01	112.0 ±8.5	150.0 ±12.7	284.0 ±2.8
BB*	0.01 ±0.01	47.5 ±6.3	134.0 ±1.4	189.0 ±114.0
MC**	4.27 ±0.28	143.0 ±16.0	139.0 ±4.0	342.0 ±6.0
RD** (Saw Mill Creek)	2.04 ±0.53	92.0 ±14.0	143.0 ±34.0	178 ±22.0

II.6 Collection

F. heteroclitus were collected using seines and killie-traps from each site during the summers of 2004 and 2005. To reduce variability, 2-3 yr old fish (~51-83 mm) were selected. Sampling was divided according to season: early- (May/June) and late-season (August/September) collections were made. Fish were euthanized with an overdose of buffered MS-222 (3-aminobenzoic acid ethyl ester), and individuals were placed in separate 50 mL Falcon™ tubes filled with 10% buffered formalin in the field during each sampling event.

II.7 Parasite Infracommunities

Baseline data on fish hosts including total length (cm), weight (gm) and sex were recorded. During dissection, organs were placed in separate Petri dishes with

deionized (DI) water so as not to mix parasite habitats. Macroparasites were viewed under a dissection microscope (20X – 40X), removed and recorded from the following organs: gills, body cavity, digestive system, swim bladder and liver. Parasites collected were identified to genus and when possible to species (Hoffman, 1999), and placed in separate vials of 10% buffered formalin. Parasite species richness and abundance, and Shannon Wiener diversity and evenness were determined and the results compared. Prevalence was determined by taking the number of hosts infected by a particular parasite species, divided by the total number of hosts (Bush *et al.*, 1997).

II.8 Statistics

ANOVA analysis was run using Statistix 8® statistical package and MANOVA analysis was performed using SAS 9.1®. A Canonical Correspondence Analysis (CCA) was performed using PC-ORD 4.25®. *P* values <0.05 were considered statistically significant.

RESULTS

II.9 Fish Data

A one-way ANOVA was used to analyze fish length and weight. Significant differences (p=0.005; N=140; $F_{1,278}$=7.84) were found in mean fish length between years 2004 (mean=6.66 ±0.07 cm) and 2005 (mean=6.91 ±0.05 cm) (Table 2-2). Furthermore, significant differences were found for mean fish weight (p=0.003; N=140; $F_{1,\ 278}$=8.83) between years 2004 (mean=4.55 ±0.16 gm) and 2005 (mean=5.15 ±0.12 gm) (Table 2-3).

II.10 Parasite Overview

Of the 280 fish examined over the two-year period, approximately 165,000 parasites were collected from 16 different taxa (Appendix A). Highly significant differences ($p<0.001$) in mean parasite abundance among organs was found using a one-way ANOVA. Gill parasites (mean=38,788 ±13,188SE) accounted for the majority of parasites observed (94.2%), most of which were digenean trematode metacercaria (*Echinochasmus scharwtzi* and *Ascocotyle* (*Phagicola*) *diminuta*). 90% of all fish examined had metacercariae encysted in their gills, ranging from zero to 9,450. The digestive tract had 3.5% of the total number of parasites observed, and had the second highest mean number (1,457 ±570 SE). Most of these parasites were either digenean trematodes or nematodes. The adult digenean trematode, *Homalometron pallidum* and an unidentified trematode thought to be newly excysted *H. pallidum* adults contributed to a large proportion of this percentage. This was followed by the body cavity (480 ±67 SE), swim bladder (209 ±44 SE), and liver (34 ±6 SE) which accounted for 1.2%, 0.5% and <0.1% of the total respectively (Figure 2-2).

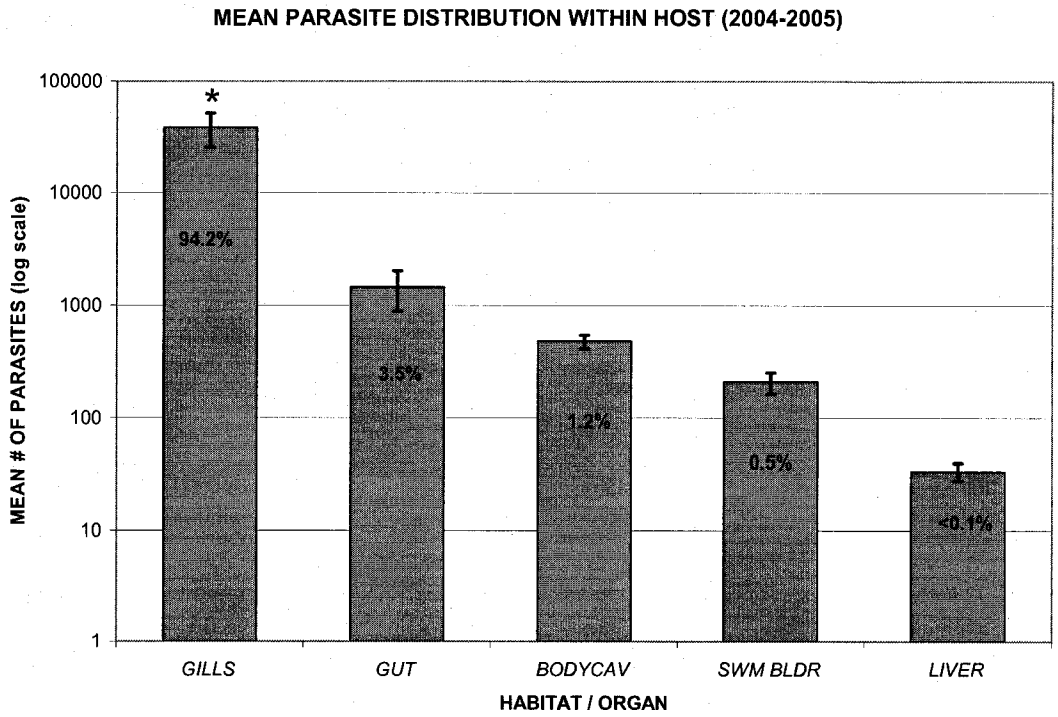


Figure 2-2. Mean (±SE) parasite distribution within hosts for 2004 and 2005.

Using a MANOVA, highly significant differences (Wilks' Lambda $p<0.001$, $F_{1,278}=5.46$) were found between years 2004 and 2005 for parasite abundance (Figure 2-3). There were significant differences ($p<0.001$, $F_{1,278}=15.37$) found in overall total parasite abundance, with a general increase in parasite number from 2004 (mean=304.79 ±31.91) to 2005 (mean=897.21 ±155.51). SH, MC, RD and PC had increases in overall parasite abundance from 2004 to 2005, while TK, UB and BB experienced slight decreases. This significance is attributed to differences found in the gills ($p<0.001$, $F_{1,278}=15.37$) likely due to *A. p. diminuta* and *E. schwartzi*, and the body cavity ($p<0.039$, $F_{1,278}=4.30$), which was likely due to increases in the unidentified acanthocephalan. No significance ($p>0.05$) was found for the digestive tract, liver or swim bladder. Consequently, each year will be analyzed separately.

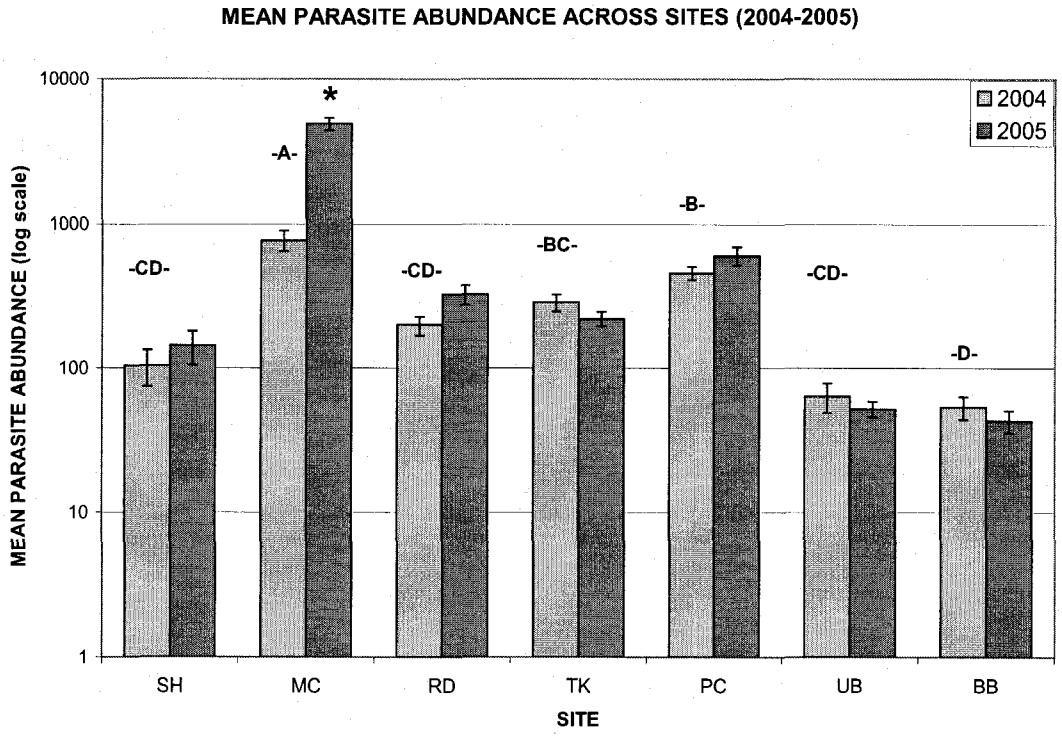


Figure 2-3. Comparison of mean (±SE) parasite abundance for 2004 and 2005 with Tukey HSD groupings.

Mean (±SE) parasite species abundance, prevalence and range of infection for each habitat type/organ for 2004 and 2005 were calculated, and are listed in Table 2-4.

Table 2-4. Summary of all parasites present in 2004 and 2005 from all sites.

PARASITE (w/Phylum)	2004 (n=140)			2005 (n=140)		
	Mean±SE	*Prev.(%)*	*Range*	*Mean±SE*	*Prev. (%)*	*Range*
Gills						
Ascocotyle (Phagicola) diminuta (P-T)	167±22.21	86	0-2153	661±124.82	98	0-7549
Dactylogryrus sp. (P-M)	10.56±1.11	94	0-88	6.85±0.55	86	0-30
Ergasilus funduli (Ar)	1.67±0.28	40	0-18	0.81±0.18	31	0-16
Echinochasmus schwartzi (P-T)	62.68±7.23	86	0-538	195.7±31.28	98	0-1887
Fundulotrema sp.(P-M)	0.46±0.09	29	0-8	1.39±0.30	38	0-26
Other	0.40±0.11	17	0-9	5.79±5.11	22	0-715
Gut						
Homalometron pallidum (P-T)	2.07±0.56	26	0-51	1.13±0.41	14	0-37
Trematode, Unkn (P-T) (*H. pallidum*?)	15.34±4.69	21	0-399	12.62±3.34	20	0-248
Dichelyne bullocki (N)	4.29±0.51	75	0-33	6.04±0.63	84	0-49
Proteocephalus sp. (P-C)	0.11±0.04	6	0-4	0.03±0.02	2	0-2
Body Cavity						
Acanthocephalan, Unkn (Ac)	1.29±0.54	19	0-63	1.45±0.42	19	0-47
Nematode, Red (N)	2.45±0.89	23	0-109	4.95±0.66	69	0-50
Eustrongylides ignotus (N)	0.21±0.06	10	0-5	0.25±0.06	14	0-4
Posthodiplostomum minimum (P-T)	2.04±0.84	13	0-92	1.06±0.34	17	0-36
Swim Bladder						
Cystidicola sp. (N)	3.49±1.03	47	0-137	2.49±0.39	41	0-18
Liver						
Neoechinorhynchus sp., cystacanth (Ac)	0.36±0.13	15	0-15	0.61±0.29	10	0-28

Ar=Arthropoda, Ac=Acanthocephala, N=Nematoda, P=Platyhelminthes
P-C=Class Cestoda, P-T=Class Trematoda, P-M=Class Monogenea

II.11 Parasite Abundance

Twenty fish from each site were examined for each year. Highly significant differences (Wilks' Lambda $p<0.001$, $F_{5,274}=5.46$) among sites with respect to parasite abundance were found for both 2004 and 2005 using a MANOVA.

Year 1 (2004)

Twenty fish from each of the seven sites were examined for a total of 140 fish. Of those 140 fish, 67 were males and 73 were females. A total of 38,417 parasites from 16 taxa were collected (Appendix A). No significant differences in fish length (N=140; $F_{6,133}$=1.07; p=0.384; Table 2-2), or weight (N=140; $F_{6,133}$=0.57; p=0.756; Table 2-3) were found among sites using a one-way ANOVA.

Table 2-2. Mean (±SE) fish total length (cm) across sites for 2004 and 2005.

SITE	2004 Mean (±SE)	RANGE (cm)	2005 Mean (±SE)	RANGE (cm)
SH	6.7 ±0.2	5.5-7.9	6.8 ±0.1	6.1-7.5
MC	6.3 ±0.3	5.0-8.3	7.2 ±0.1	6.2-8.1
RD	6.8 ±0.2	5.8-8.4	7.0 ±0.3	3.7-8.4
TK	6.8 ±0.2	5.4-8.4	6.9 ±0.2	5.6-7.9
PC	6.8 ±0.2	5.1-7.5	7.0 ±0.2	6.0-8.3
UB	6.6 ±0.2	5.7-8.0	6.7 ±0.1	5.9-8.0
BB	6.7 ±0.3	5.2-8.9	6.8 ±0.1	5.9-7.7

Overall total parasite abundance was found to be significantly different (Wilks' Lambda p<0.001, $F_{6,133}$=22.57) among sites using a MANOVA, which was followed by a Tukey HSD pairwise comparison. Three groups in which the means were not significantly different from one another were found. The first group contained MC which had the greatest number of parasite individuals per host (mean=774.5 ±124.7SE), accounting for 40% of the total number of parasites collected across the seven sites. PC was placed in the second group with a mean of 459.4 (±47.2SE) parasite individuals, or 24% of the total. TK was an intermediate with a mean of 286.2 (±38.8SE) parasites, or 14% of the total. The last group contained the remaining sites; RD (mean=198.6 ±29.4SE), SH (mean=103.8 ±29.4SE), UB

(mean=63.4 ±14.7SE) and BB (mean=53.6 ±9.7SE). Together, these sites contained approximately 22% of the total parasites examined (Table 2-5).

Table 2-5. Mean (±SE) parasite abundance by site (2004).

SITE	MEAN ±SE	TUKEY HSD
MC	774.5 ±124.7	A
PC	459.4 ±47.2	B
TK	286.2 ±38.8	BC
RD	198.6 ±29.4	C
SH	103.8 ±29.4	C
UB	63.4 ±14.7	C
BB	53.55 ±9.7	C

Using a MANOVA, it was determined that the gills (Wilks' Lambda $p<0.001$, $F_{6,133}=26.96$), digestive tract (Wilks' Lambda $p<0.001$, $F_{6,133}=5.70$), and liver (Wilks' Lambda $p=0.009$, $F_{6,133}=3.02$) were responsible for the significant site differences calculated. No significance (Wilks' Lambda $p>0.05$) was found for the body cavity or the swim bladder. Increases in gill parasites were due to *A. p. diminuta*, *E. schwartzi, Fundulotrema* sp., *E. funduli* and *Dactylogyrus* sp. Using a Tukey HSD test for the gills, four groups were found. MC was first with the largest mean number (mean=760.0 ±122.9SE) of gill parasites, followed by PC (mean=449.0 ±47.6SE) in the second group. TK was an intermediate with a mean of 266.05 (±40.92SE). A second group of intermediates; RD (mean=174.4 ±28.9SE) and UB (mean=40.8 ±8.3SE) were found. SH and BB made up the last group with means of 15.2 (±5.8SE) and 11.0 (±2.3SE) gill parasites respectively (Table 2-6).

Table 2-6. 2004 MANOVA results for site parasite abundance by organ.

SITE	GILLS **		DIGEST **		BODY CAVITY		SWM BLDR		LIVER *	
	MEAN	TUKEY	MEAN	TUKEY	MEAN	TUKEY	MEAN	TUKEY	MEAN	TUKEY
SH	15.2	*D*	85.1	*A*	3.25	*n.s.*	0.1	*n.s.*	0.15	*B*
MC	759.95	*A*	4	*B*	4.05	*n.s.*	5.75	*n.s.*	0.75	*B*
RD	174.35	*CD*	9.75	*B*	6.3	*n.s.*	7.15	*n.s.*	1.05	*B*
TK	266.05	*BC*	18	*B*	1.35	*n.s.*	0.25	*n.s.*	0.55	*B*
PC	448.95	*BC*	6	*B*	4.3	*n.s.*	0.05	*n.s.*	0.1	*B*
UB	40.75	*CD*	5.7	*B*	9.4	*n.s.*	7.35	*n.s.*	0.2	*B*
BB	10.95	*D*	23.4	*B*	6.3	*n.s.*	2.45	*n.s.*	10.45	*A*

n.s.=no significance
* $P \leq 0.01$, ** $P < 0.001$

Significant differences in the number of parasites digestive tract parasites were due to increases in *H. pallidum, D. bullocki,* and the unidentified trematode. Only two groups of homogenous means were found using the Tukey HSD test. SH made up the first group with a mean of 85.1 (±29.4SE) parasites. The other six sites were placed together in the second group as follows: BB (mean=23.4 ±7.1SE), TK (mean=18.0 ±10.0SE), RD (mean=9.8 ±2.2SE), PC (mean=6.0 ±1.0SE), UB (mean=5.7 ±1.6SE), and MC (mean=4.0 ±1.5SE, Table 2-6).

The last organ that was significantly different among sites with respect to parasite abundance (*Neoechinorhynchus* sp.) was the liver, with two groups found using Tukey HSD. BB was placed in the first group with a mean of 10.5 (±5.7SE) parasites per individual. The second group contained the remaining six sites as follows (mean ±SE): RD (1.1 ±0.8), MC (0.8 ±0.2), TK (0.6 ±0.2), UB (mean=0.2 ±0.1), SH (0.2 ±0.1), and PC (0.1 ±0.1, Table 2-6).

Year 2 (2005)

Twenty fish from seven sites were examined for a total of 140 fish. Of those 140 fish, 71 were males and the other 69 were females. A total of 125,547 parasites from 16 taxa were collected (Appendix A). Using a one-way ANOVA, no significant

differences were found in fish length (N=140; $F_{6,133}$=1.44; p=0.203; Table 2-2) among sites. However, differences in weight were determined to be significant (N=140; $F_{6,133}$=2.93; p=0.010; Table 2-3) and a Tukey HSD test was run, where two homogenous groups were found. On average, MC fish weighed the most (mean=6.2 ±0.3SE) and were placed in the first group. RD (mean=5.5 ±0.4SE), TK (mean=5.3 ±0.3SE), and PC (mean=4.9 ±0.3SE) were intermediates. The remaining sites; UB (mean=4.8 ±0.3SE), BB (mean=4.8 ±0.2SE), and SH (mean=4.7 ±0.2SE), were placed together in the second group with similar means.

Table 2-3. Mean (±SE) fish weights (gm) across sites for 2004 and 2005.

SITE	2004 MEAN ±SE (gm)	RANGE (gm)	2005 MEAN ±SE (gm) w/Tukey HSD		RANGE (gm)
MC	4.1 ±0.5	1.72-9.29	6.1 ±0.3	(A)	4.17-9.14
RD	4.7 ±0.4	2.61-9.27	5.5 ±0.4	(AB)	2.83-8.39
TK	5.1 ±0.6	1.90-10.26	5.3 ±0.3	(AB)	2.41-7.26
PC	4.5 ±0.3	1.37-5.93	4.9 ±0.3	(AB)	3.12-8.57
UB	4.3 ±0.3	2.56-7.20	4.8 ±0.3	(B)	2.93-8.51
BB	4.5 ±0.5	1.86-11.27	4.8 ±0.2	(B)	2.70-6.75
SH	4.7 ±0.4	2.12-8.82	4.7 ±0.2	(B)	3.42-6.72

For 2005, overall total parasite abundance was found to be significantly different (p<0.001, N=20, $F_{6,133}$=88.35) among sites using a MANOVA, followed by a Tukey HSD pairwise comparison with two groups of homogenous means found. MC made up the first group with the greatest mean of 4,888.4 (±485.8SE) parasites per individual. The other six sites were placed together in the second group (mean ±SE): PC (608.6 ±87.3), RD (326.4 ±50.5), TK (219.6 ±26.8), SH (142.4 ±38.0), UB (52.2 ±6.5), and BB (43.1 ±7.6, Table 2-6).

Table 2-6. Mean (±SE) parasite abundance by site (2005).

SITE	MEAN ±SE	TUKEY HSD
MC	4888.4 ±485.8	A
PC	608.6 ±87.3	B
RD	326.4 ±50.5	B
TK	219.6 ±26.8	B
SH	142.4 ±38.0	B
UB	52.2 ±6.5	B
BB	43.1 ±7.6	B

Using a MANOVA, gills (p<0.001, $F_{6,133}$=89.38), body cavity (p<0.019, $F_{6,133}$=2.64), digestive tract (p<0.001, $F_{6,133}$=7.14), and swim bladder (p<0.001, $F_{6,133}$=8.65) were found to be responsible for the significant site differences calculated. No significance (p>0.05) was found for the liver. Using a Tukey HSD test for the gills, two groups were found. MC had the largest mean number (mean=4864.0 ±484.5SE) of gill parasites (*A. p. diminuta* and *E. schwartzi*) and was placed in the first group. The second group included the other six sites (mean ±SE): PC (582.4 ±85.5), RD (298.1 ±50.6), TK (158.5 ±21.3), SH (93.2 ±35.8), UB (41.1 ±7.4), and BB (24.3 ±5.5) gill parasites per fish (Table 2-7).

Table 2-7. 2005 MANOVA results for site parasite abundance by organ.

SITE	GILLS **		DIGEST **		BODY CAV *		SWM BLDR **		LIVER	
	MEAN	TUKEY	MEAN	TUKEY	MEAN	TUKEY	MEAN	TUKEY	MEAN	TUKEY
SH	93.2	*B*	42.8	*AB*	3.75	*AB*	2.05	*B*	1.05	*n.s.*
MC	4864.9	*A*	4.2	*C*	12.3	*A*	6.25	*A*	0.75	*n.s.*
RD	298.1	*B*	11.05	*BC*	11.2	*AB*	6.05	*A*	0	*n.s.*
TK	158.5	*B*	57.95	*AB*	3.1	*B*	0	*B*	0	*n.s.*
PC	582.4	*B*	16.35	*BC*	9.45	*AB*	0.35	*B*	0.05	*n.s.*
UB	41.1	*B*	3.65	*C*	6.25	*AB*	1.05	*B*	0.15	*n.s.*
BB	24.3	*B*	4.35	*C*	9.15	*AB*	2.05	*B*	3.3	*n.s.*

n.s.=no significance
* $P \leq 0.01$, **P<0.001

Significant differences in the number of parasites found among sites within the fish's body cavity were computed using a MANOVA. Two homogenous groups of

means were calculated using Tukey HSD. MC had the greatest mean number (mean=12.3 ±3.4SE) of parasites in the body cavity (red nematodes and unidentified acanthocephalan) and was in the first group. TK had the fewest average number of body cavity parasites (mean=3.1 ±0.9SE) and was placed in the second group. The five other sites (mean ±SE); RD (11.2 ±1.9), PC (9.5 ±2.0), BB (9.2 ±3.1), UB (6.3 ±1.8), and SH (3.8 ±1.2) were considered intermediates between these two groups (Table 2-7).

Highly significant differences in the number of parasites in the digestive tract (unidentified trematode) were found using a MANOVA. Three groups of homogenous means were found using a Tukey HSD test. TK made up the first group with a mean of 58.0 (±15.9SE) parasites. This was followed by SH (mean=42.8 ±13.7SE) as an intermediate group. A second intermediate grouping was found comprised of PC (mean=16.4 ±3.0SE) and RD (mean=11.1 ±1.8SE). The final group contained BB (mean=4.4 ±1.1SE), MC (mean=4.2 ±0.8SE), and UB (mean=3.7 ±1.1SE, Table 2-7).

Significant differences were also found in the swim bladder (*Cystidicola* sp.) using a MANOVA. Two groups were found using a Tukey HSD pairwise comparison test. The first group contained MC and RD with means of 6.3 (±1.4SE) and 6.1 (±1.2SE) respectively. The second group of means included (mean ±SE): BB (2.1 ±0.9), SH (1.7 ±0.9), UB (1.1 ±0.7), PC (0.4 ±0.3), and TK (0.0, Table 2-7).

II.12 Parasite Species Richness, Diversity and Evenness

In both 2004 and 2005, 16 parasite taxa were documented and included species from Phyla Acanthocephala, Nematoda, Arthropoda, and Platyhelminthes (Appendix A). Several calculations were made to determine site biodiversity including: species richness (S), Shannon-Weiner Diversity Index (H'=-sum(Piln[Pi])) and evenness (E_H=H'/lnS). Although both years had a total species richness of 16

taxa, differences were found for both diversity and evenness between 2004 (H'=1.260, E_H=0.453) and 2005 (H'=0.786, E_H=0.284).

Year 1 (2004)

Highly significant differences (p<0.001, $F_{6,133}$=10.1) in mean number of parasite species were found among sites using a one-way ANOVA, and three groups were found with a Tukey HSD. RD (7.4 ±0.3SE) was placed in the first group with the greatest mean species richness. MC (mean=7.2 ±0.4SE), BB (mean=6.6 ±0.4 SE), and UB (mean=6.4 ±0.4 SE) were grouped together as intermediates. TK was a second intermediate with a mean species richness of 5.7 ±0.3SE. PC and SH comprised the third group with means of 4.9 (±0.2 SE) and 4.40 (±0.5SE), respectively (Figure 2-4).

BB (H'=2.049) had the highest overall diversity followed by (H'): UB (1.976), RD (1.428), SH (1.059), TK (0.959) and PC (0.928). MC had the lowest diversity (0.715) of all sites examined (Figure 2-5). UB had the greatest species evenness (E_H=0.749), followed by (E_H): BB (0.739), RD (0.557), PC (0.403), SH (0.391) and TK (0.374). MC (E_H=0.271) had the lowest evenness of all sites examined.

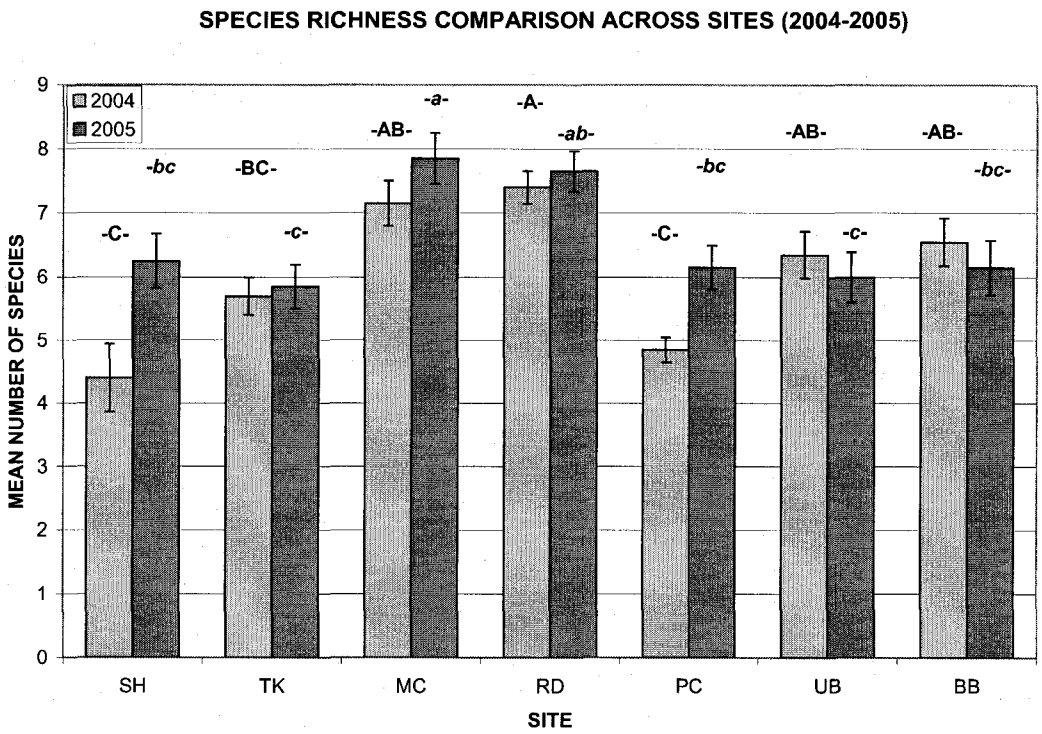


Figure 2-4. Mean species richness comparison across sites for 2004 and 2005 with Tukey HSD.

Year 2 (2005)

Highly significant differences (p<0.001, $F_{6,133}$=4.68) in mean number of parasite species were found among sites using a one-way ANOVA with three groups found using a Tukey HSD. MC made up the first group with the greatest mean species richness of 7.9 (±0.4SE) of all sites examined, followed by RD (7.7 ±0.3SE) which was placed as an intermediate. SH (6.3 ±0.4SE), PC (6.2 ±0.3SE), and BB (6.2 ±0.4SE) were placed together as a second group of intermediates. UB and TK made up the third group with 6.0 (±0.4SE) and 5.9 (±0.3SE) species respectively (Figure 2-4).

BB had the highest overall diversity (Figure 2-5) and evenness (H', E_H): (2.056, 0.779), followed by UB (1.637, 0.638), and SH (1.435, 0.560). Although RD

(1.217, 0.474) had greater diversity than TK (1.189, 0.496), RD had lower evenness. PC (0.943, 0.380) and MC (0.547, 0.213) had the lowest overall diversity and evenness of all sites examined.

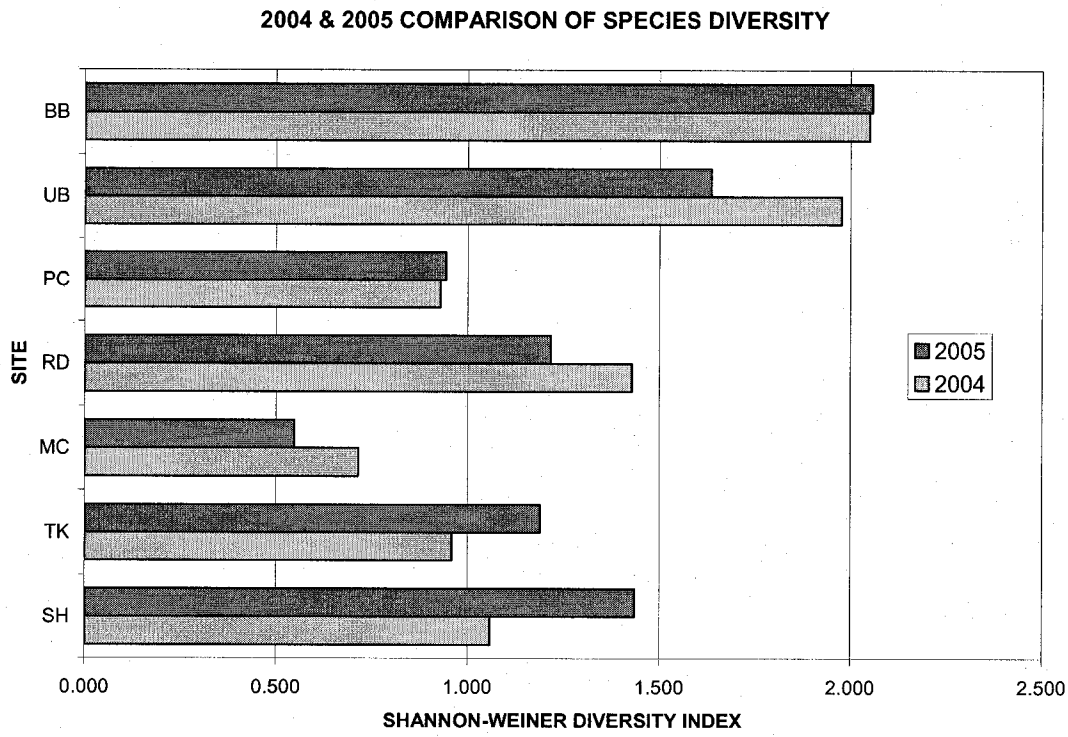


Figure 2-5. Shannon-Weiner Index comparison across sites for 2004 & 2005.

II.13 Seasonal Parasite Abundance and Richness

A total of 280 parasite communities were examined; 140 from 2004 and another 140 in 2005. Significant seasonal differences in parasite abundance were found for years 2004 (Wilks' Lambda $p<0.001$, $F_{5,134}=5.51$) and 2005 (Wilks' Lambda $p<0.013$, $F_{5,134}=3.00$) using a MANOVA (Figure 2-6).

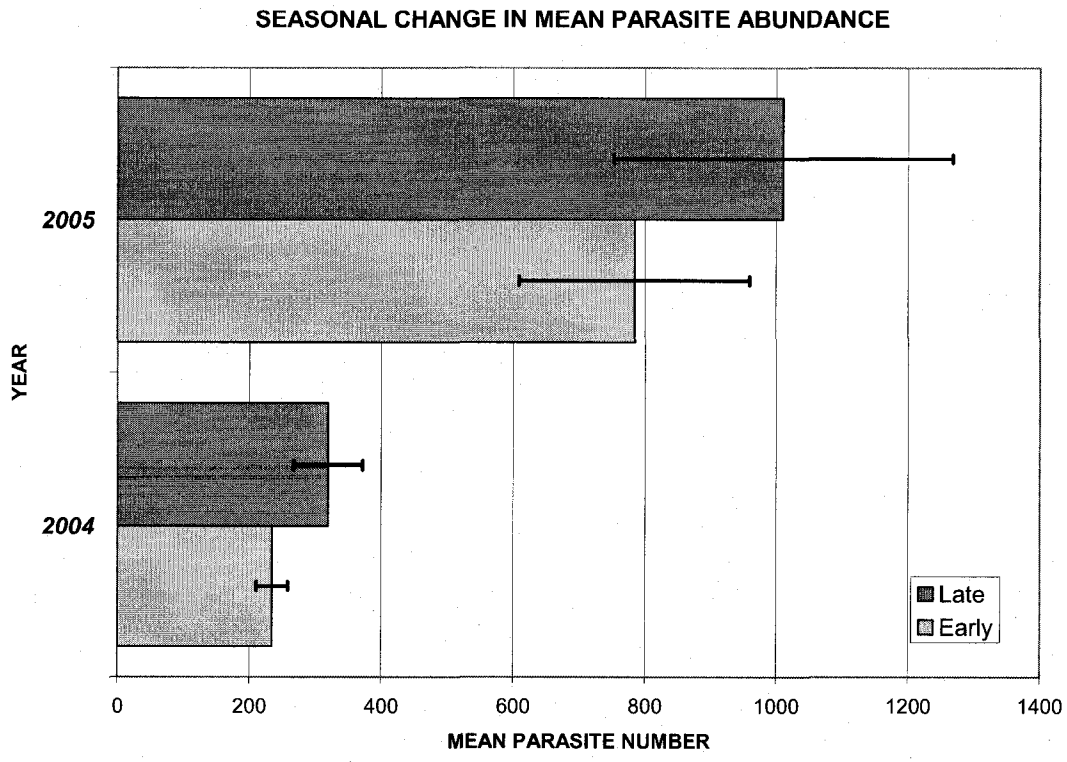


Figure 2-6. Mean (±SE) parasite abundance for 2004 and 2005 by season.

Year 1 (2004)

Significant seasonal differences were found in parasite abundance for 2004 in the gills (p=0.045, $F_{1,138}$=4.09 and digestive tract (p<0.001, $F_{1,138}$=11.78) using a MANOVA, but no difference (p>0.05) for the body cavity, liver or swim bladder. Seventy fish were collected early in the season (May/June), producing a mean of 186.4 (±25.4SE) gill parasites per fish. Later season collections (August/September) averaged 304.0 (±52.3SE) gill parasites per fish. Increases were seen in *A. p. diminuta, Dactylogyrus* sp., and *E. schwartzi* in the late season collections. In the digestive tract, an average of 38.3 (±9.3SE) parasite individuals were collected in the early sampling period, and only 5.1 (±2.6SE) in the late season. Differences were found due to *H. pallidum, D. bullocki, Proteocephalus* sp., and the unidentified trematode thought to be *H. pallidum* in the early season collections (Figure 2-7).

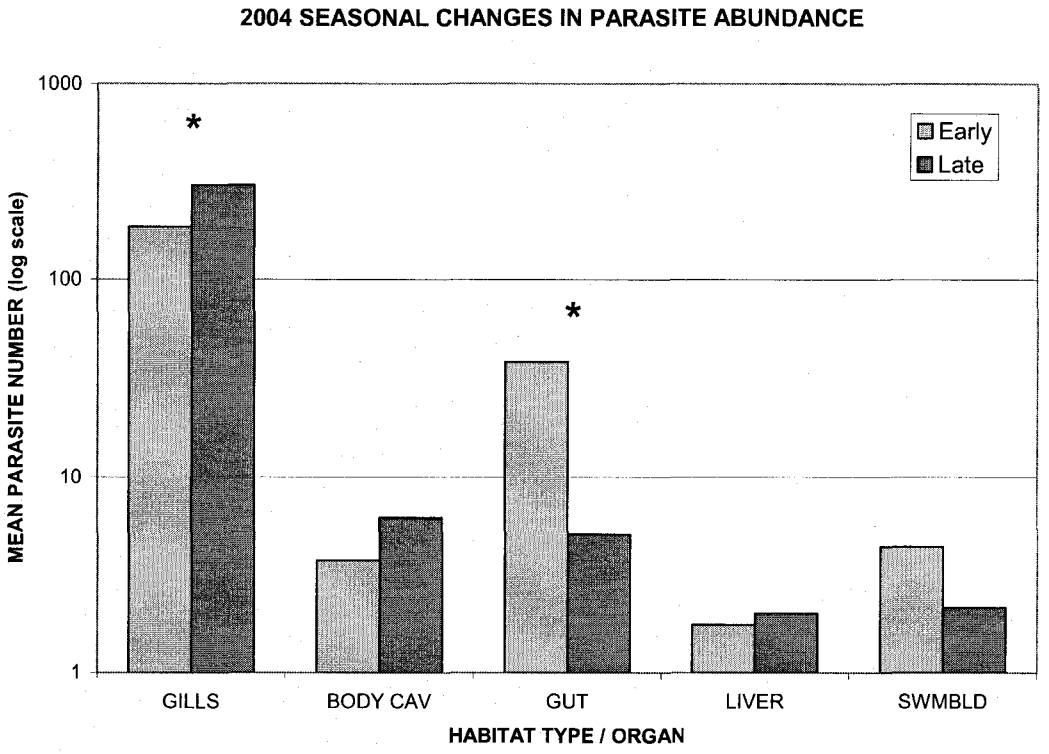


Figure 2-7. Seasonal differences in mean parasite abundance by habitat.

Using a one-way ANOVA, species richness was also found to fluctuate seasonally. MC (7.4 ±0.5SE) fish had the highest mean species richness in early season collections followed by UB (6.6 ±.06SE), SH (5.7 ±0.6SE) and TK (6.1 ±0.5SE). These sites had greater mean parasite species richness in early season collections when compared to late season (mean ±SE: 6.9 ±0.5; 6.1 ±0.5 3.1; ±0.7; and 5.3 ±0.4; respectively). RD (7.7 ±0.4SE) had the highest overall mean species richness of the seven sites examined. Peaking in the late season with PC (5.3 ±0.3SE) and BB (6.8 ±0.4SE) rather than in the early season (mean ±SE: 7.1 ±0.4, 4.4 ±0.2 and 6.3 ±0.6 respectively) (Figure 2-9).

Year 2 (2005)

Significant seasonal differences were found for parasite abundance in 2005 for the digestive tract ($p<0.001$, $F_{1,138}=11.62$) using a MANOVA, but not for the gills, body cavity, liver or swim bladder ($p>0.05$). Seventy fish were collected early in the season (May/June), producing a mean of 31.4 (±5.6SE) gut parasites per fish. Later season collections (August/September) averaged 8.9 (±3.6SE) gut parasites per fish. Differences were found due to increases in abundance of *H. pallidum, D. bullocki, Proteocephalus* sp., and the unidentified trematode thought to be *H. pallidum* in the early season collections (Figure 2-8).

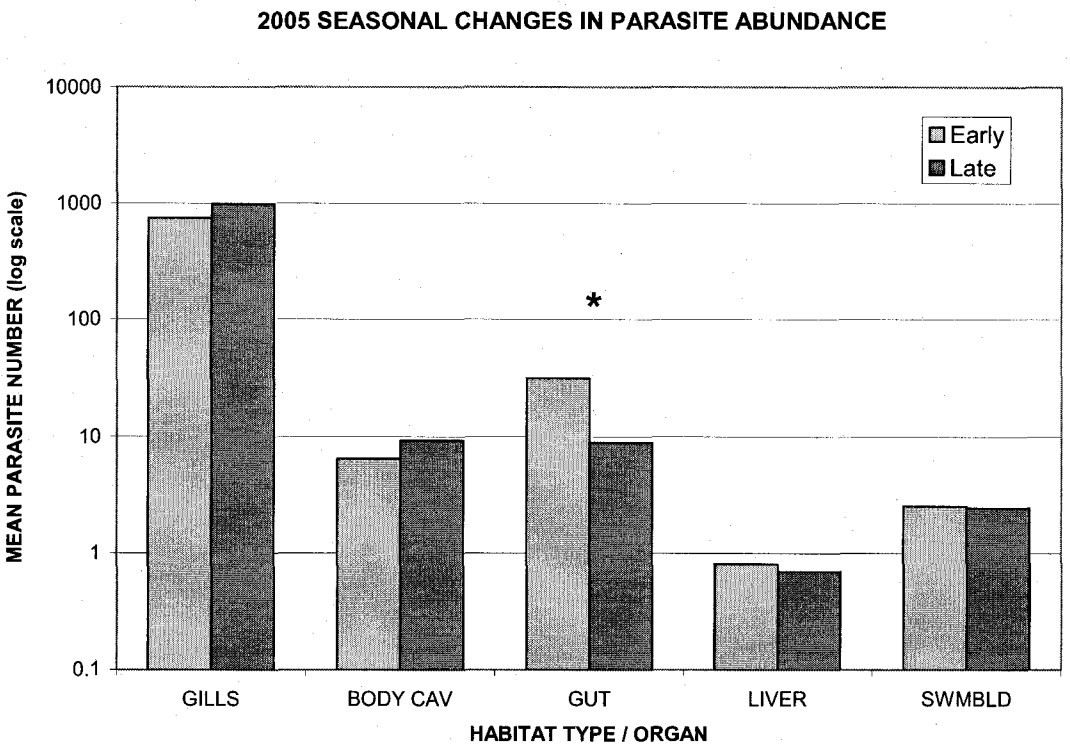


Figure 2-8. Seasonal differences in mean parasite abundance by habitat.

Species richness also exhibited seasonal fluctuations in 2005 using a one-way ANOVA, with higher means in early season collections for SH (7.3 ±0.4SE), TK (6.7 ±0.5SE), UB (6.6 ±0.7SE) and BB (6.2 ±0.7SE) rather than late-season collections

(mean ±SE: 5.2 ±0.6; 5.0 ±0.3; 5.4 ±0.4; and 6.1 ±0.6 respectively). RD (8.5 ±0.4SE) and MC (8.3 ±0.5SE) averaged the most species of all sites examined and experienced their peaks in species richness later in the season with PC (6.9 ±0.5SE) rather than in the early (mean ±SE: 6.8 ±0.3; 7.4 ±0.6; and 5.4 ±0.4 respectively) season collections (Figure 2-9).

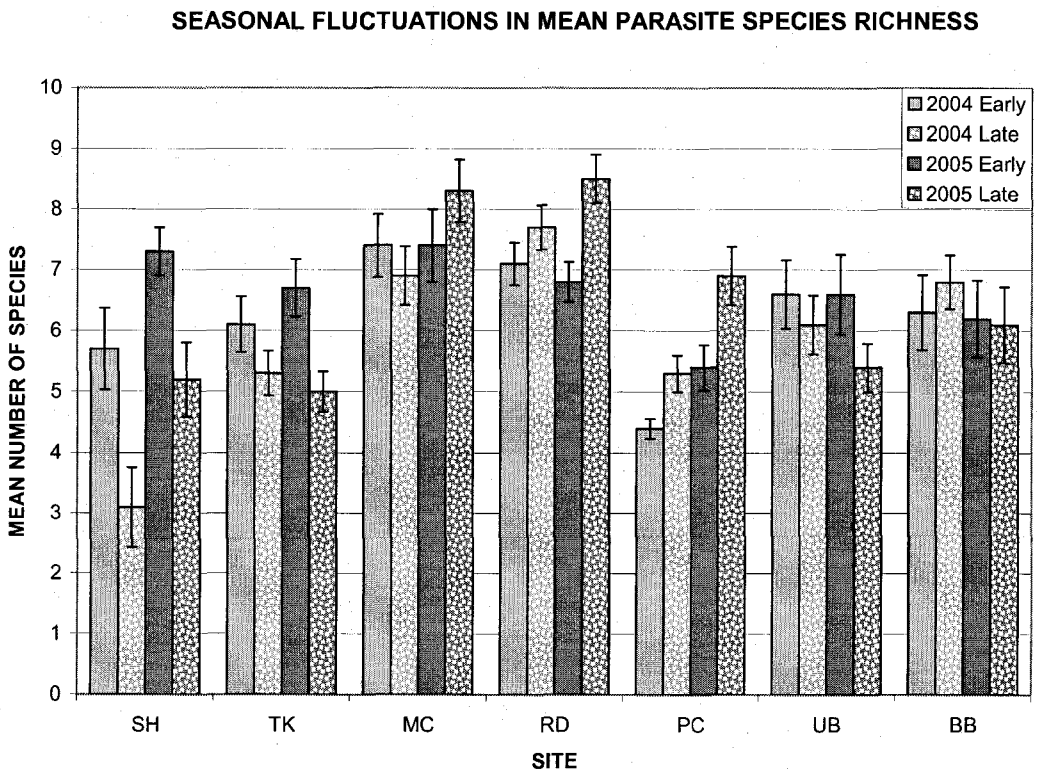


Figure 2-9. Seasonal changes in mean parasite species richness between early and late collections for 2004 and 2005.

II.14 Sex Differences in Parasite Loads

Overall, a total of 280 fish were collected, 138 of which were male, and the other 142 were female. Using a MANOVA, no significant difference (WIlks' Lambda $p>0.05$) between sexes was found for either 2004 or 2005.

Year 1 (2004)

Of the 140 fish collected, 67 were males and 73 were females. Although no significant difference was calculated for overall parasite abundance using a MANOVA (mean±SE: ♀=251.5 ±40.2; ♂=298.4 ±42.0), significance between males and females was found only for the digestive tract (p=0.044, $F_{1,138}$=4.12). On average, females were more heavily infected by gut parasites (mean=31.3 ±6.9SE) compared to their male counterparts (mean=11.2 ±7.2SE, Figure 2-10).

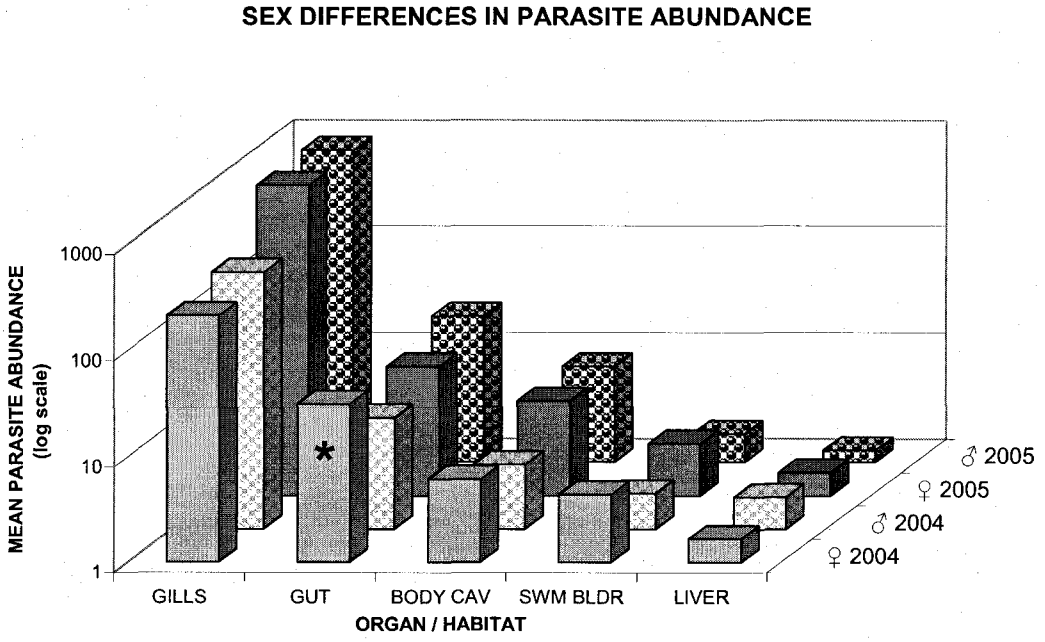


Figure 2-10. Mean parasite abundance of organ / habitat by sex and year.

Year 2 (2005)

In the second year, of the 140 fish collected, 71 were males and the other 69 were females. Females averaged 887.4 (±222.3SE) total parasites and males averaged 907.0 (±219.2SE). No significant differences (Wilks' Lambda p>0.05) in

parasite abundance were calculated between sexes for any habitat/organ using a MANOVA (Figure 2-10).

II.15 Parasites and Environment

CCA was used to illustrate potential site and parasite species relationships with environmental factors including salinity and metal levels (Hg, Cu, Pb and Zn). For clarity's sake, two plots were created – the first comparing the environmental factors with the sites, and a second comparing the environmental factors with parasite species.

Year 1 (2004)

Using CCA, significant correlations were found between sites and environmental factors (e.g., contaminants, salinity). A Monte Carlo technique using 99 permutations found significant differences for both Axes 2 and 3 (p=0.01), but not for Axis 1 (p>0.05). Approximately 85.5% of the total variation in parasite abundance was explained using the first two axes (1 and 2) (Table 2-8).

Table 2-8. 2004 CCA Summary for site and species, with abiotic variables.

Axis	Sp-Env Corr	Eigenvalues	% Variance
1	0.981	0.596	53.1
2	0.994	0.364	32.4
3	0.998	0.037	3.3

	Correlations		
Variable	Axis 1	Axis 2	Axis 3
Hg	-0.232	0.797	-0.218
Cu	-0.156	0.403	-0.551
Pb	0.677	-0.391	-0.061
Zn	-0.325	0.315	-0.302
Salinity	0.319	-0.713	0.053

	By Site				By Species			
Axis	Mean	Min	Max	*P*	Mean	Min	Max	*P*
1	0.944	0.702	1.000	0.300	0.972	0.877	1.000	0.610
2	0.814	0.461	0.994	***0.010***	0.954	0.802	0.999	0.160
3	0.732	0.369	0.979	***0.010***	0.915	0.589	0.999	***0.020***

The first plot of sites suggests strong correlations between PC, RD, MC and TK with Hg levels (0.797). UB was found along the salinity (-0.713) vector. BB did not appear to be linked with any specific environmental vector although Pb appears to play a minor role (0.677). SH was far removed from the six other sites and did not appear to be driven by any of the environmental factors examined (Figure 2-11).

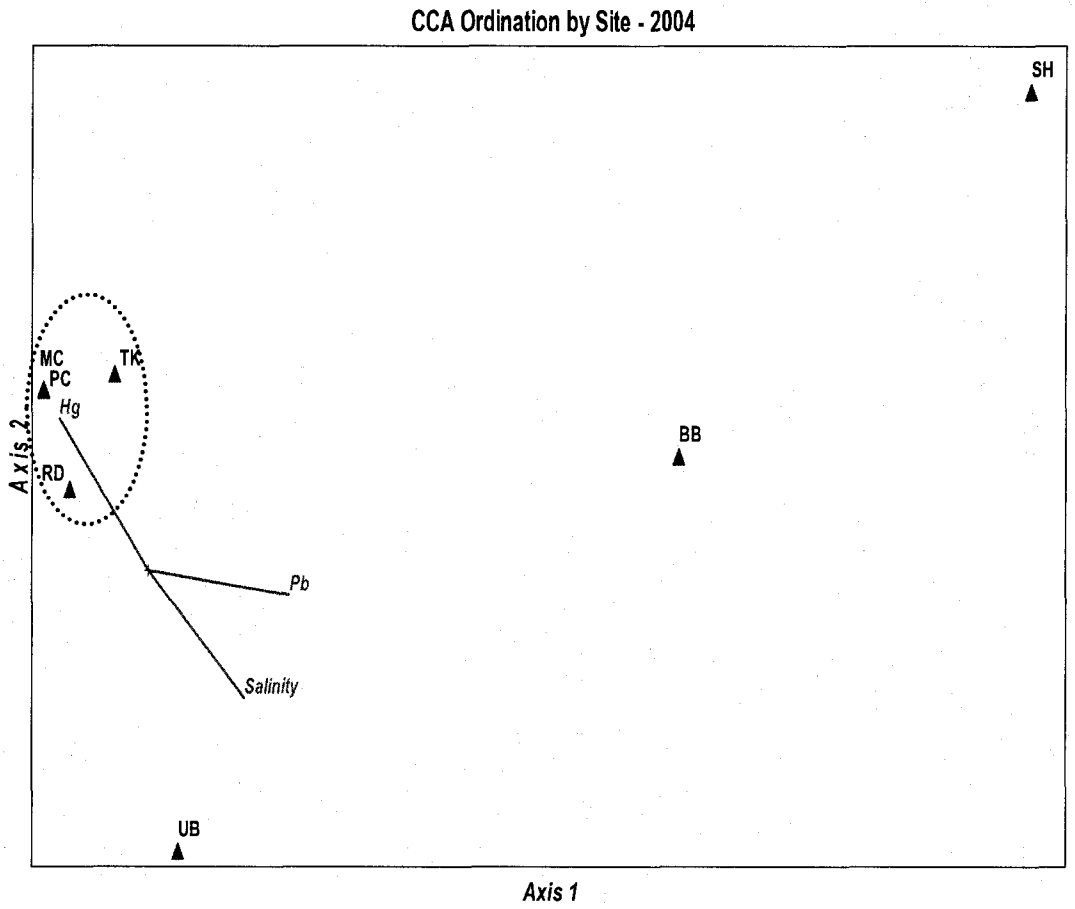


Figure 2-11. Site comparison to abiotic environmental factors (2004).

When broken down by species, only Axis 3 was determined to be significant (p=0.02) and four major groups appeared. The first group was loosely clustered around the Hg vector (0.797) and was comprised of the trematodes *A. p. diminuta, E. schwartzi*, the unknown acanthocephalan, and the cestode *Proteocephalus* sp. A second group was loosely associated with the Pb vector and included nematodes *E. ignotus* and the red nematode, in addition to the trematode *H. pallidum*. The third group: trematodes *Dactylogyrus* sp. and *P. minimum*, the nematodes D. *bullocki* and *Cysditicola* sp., the acanthocephalan species *Neoechinorhynchus*, monogenean *Fundulotrema* sp., and the parasitic copepod *E. funduli* were grouped together along the salinity vector (-0.713). Within this larger cluster, three subgroups could be made.

The unknown gut trematode (*H. pallidum*?) was found distanced from all other parasite species and not strongly related to any of the environmental factors examined (Figure 2-12).

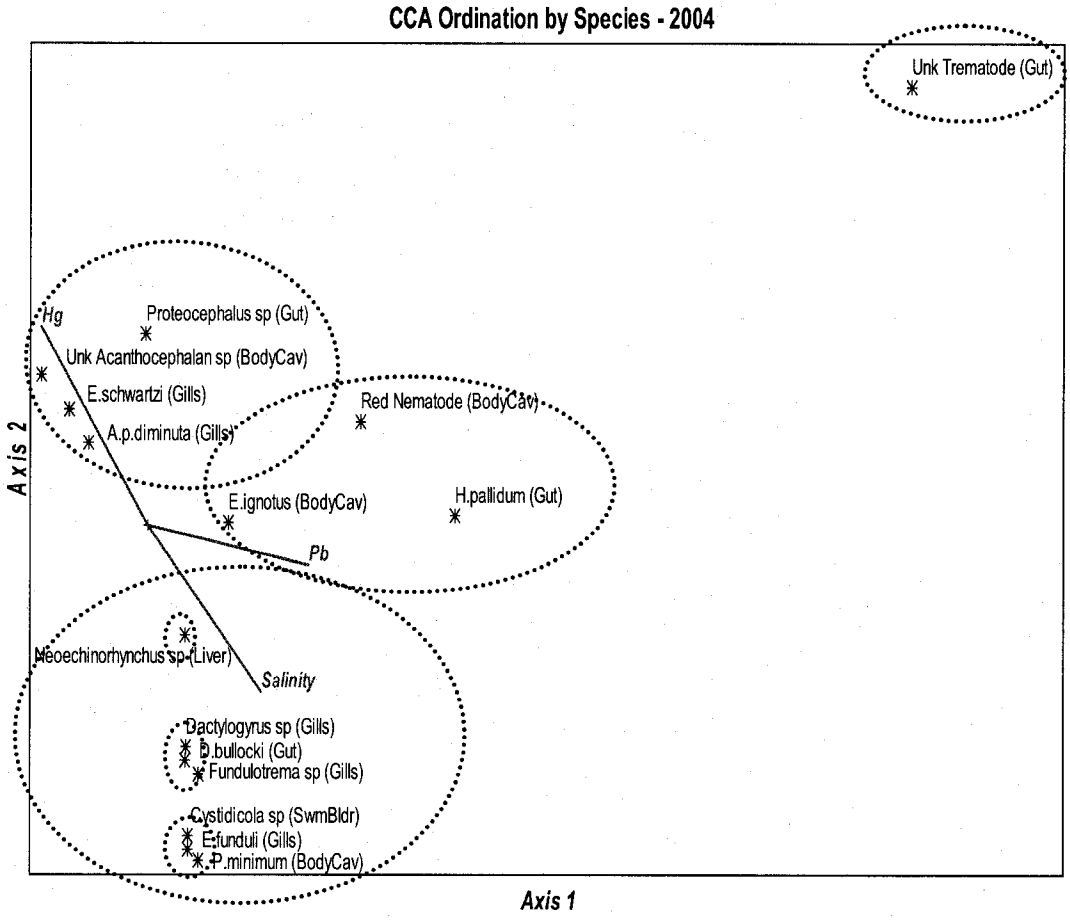


Figure 2-12. Parasite comparison to abiotic environmental factors (2004).

Year 2 (2005)

Using CCA, significant correlations were found between sites and environmental factors (e.g., contaminants, salinity). A Monte Carlo technique using 99 permutations found significant differences for only Axis 3 (p=0.02). Axes 1 and 2 were not considered significant (p>0.05). Approximately 71% of the total variation in parasite abundance was explained using the first two axes (1 and 2).

Table 2-9. 2005 CCA Summary for site and species, with abiotic variables.

Axis	Sp-Env Corr	Eigenvalues	% Variance
1	0.989	0.276	47.6
2	0.823	0.135	23.3
3	1.000	0.038	6.6

	Correlations		
Variable	Axis 1	Axis 2	Axis 3
Hg	0.584	0.211	0.778
Cu	0.627	0.147	0.751
Pb	-0.501	-0.180	-0.315
Zn	0.668	0.253	0.682
Salinity	-0.381	-0.478	-0.784

	By Site				By Species			
Axis	Mean	Min	Max	*P*	Mean	Min	Max	*P*
1	0.934	0.742	0.997	0.120	0.944	0.762	1.000	0.250
2	0.743	0.553	0.976	0.220	0.870	0.355	1.000	0.750
3	0.896	0.580	1.000	***0.020***	0.774	0.354	0.999	***0.010***

The first figure (Figure 2-13) illustrates the relationship between the abiotic variables with respect to the seven sites. The environmental vectors were found to be very short suggesting that neither contaminants nor salinity have strong influences on structuring the placement of the sites as shown below (Figure 2-13). Relationships between MC, PC and RD were found with Zn (0.668) along Axis 1. UB was distanced from these sites, yet was aligned along an 'imaginary vector' not represented in this data, and BB to a lesser extent. Salinity was negatively correlated (-0.381) with SH and TK.

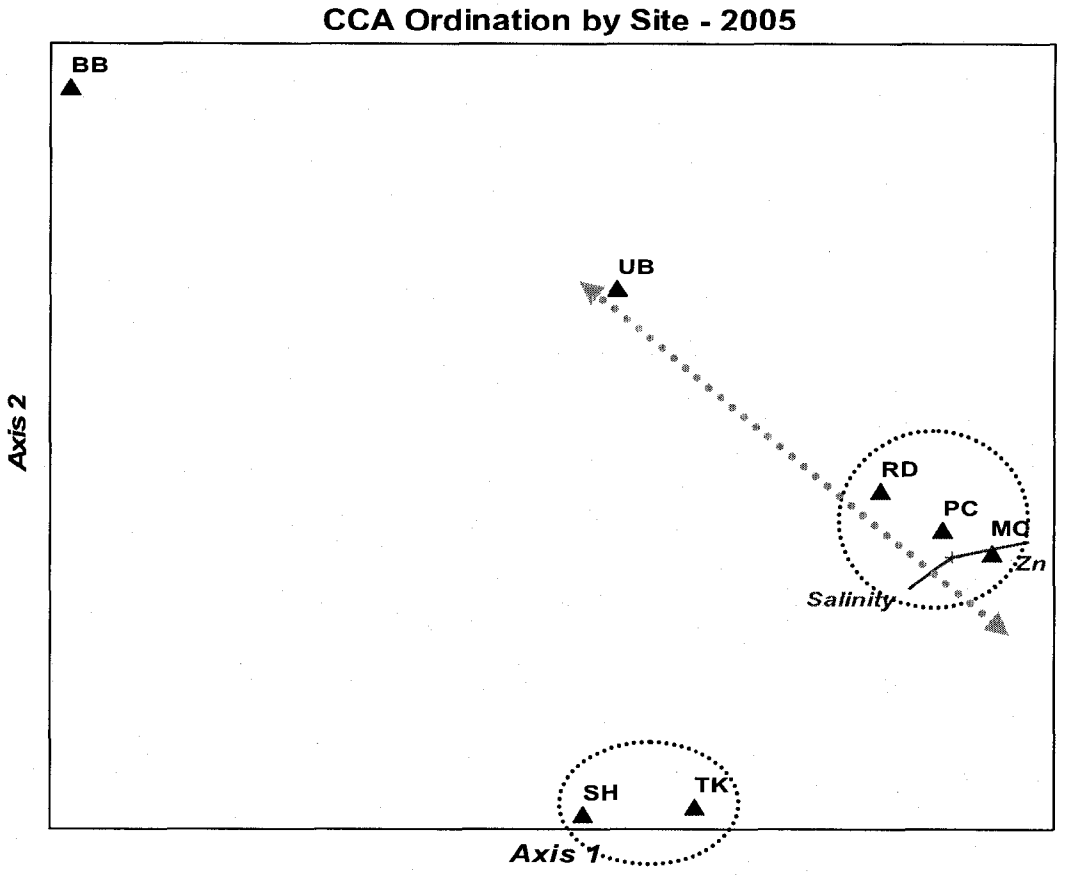


Figure 2-13. Site comparison to abiotic environmental factors (2005).

The second plot compared the abiotic variables with individual parasite species. A significant difference was found for Axis 3 (p=0.01), but not for Axes 1 or 2 (p>0.05). Four major groups of species were found clustered together. The first was loosely comprised of the cestode species *Proteocephalus*, the trematode *P. minimum*, and the acanthocephalan species *Neoechinorhynchus*. A second group was comprised of the monogenean species *Fundulotrema* and *Dactylogyrus,* nematodes *Cystidicola* sp. unknown red nematodes and *D. bullocki,* and the parasitic copepod *E. funduli.* A third group contained both *H. pallidum* and the unidentified trematode thought to be newly excysted *H. pallidum* adults. The final group contained: the trematodes *A. p. diminuta* and *E. schwartzi,* the nematode *E. ignotus,*

and the unidentified acanthocephalan (Figure 2-14). *A. p. diminuta* and *E. schwartzi* were strongly associated with Zn (0.668).

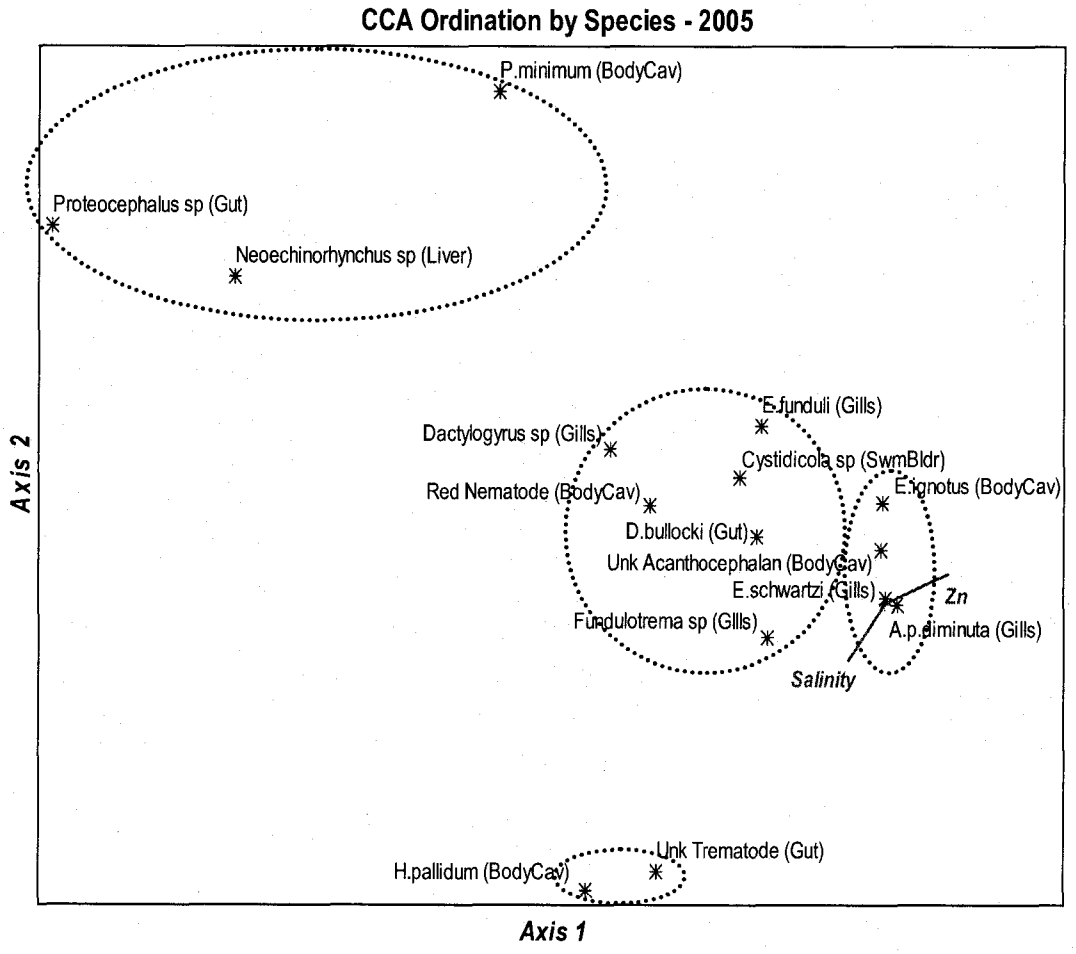


Figure 2-14. Parasite comparison to abiotic environmental factors (2005).

DISCUSSION

Parasites are important components of ecosystems due to their ability to control population dynamics and shape community structure. Research has shown that parasites are also useful in determining food web structure (Marcogliese and Cone, 1997; Sukhdeo and Hernandez, 2005; Lafferty *et al.*, 2006), biodiversity (Gelnar *et al.*, 1997; Hechinger *et al.*, 2006) and providing information on environmental stress (Lafferty, 1997; Marcogliese, 2005). One study looking at the

biodiversity of parasites in relation to parasitism, found that changes in the community structure of the metazoan parasites observed fully corresponded to the differences in the species composition of the benthic macroinvertebrate fauna (Gelnar *et al.*, 1997). This makes intuitive sense as more degraded, polluted sites would not be able to support a very diverse community of potential macroinvertebrate hosts for the parasites.

In the present study, significant differences in parasite abundance were found between years which necessitated separate analyses. In 2004, significant annual differences were found for the digestive cavity, liver and gills. Gut parasite communities from SH fish were found to be significantly different from those of the other six sites due to a surge in number of the unidentified digenean trematode thought to be immature adults of *H. pallidum*. Digenean trematodes require several hosts (e.g., mollusk, fish) to complete their life-cycle before maturing into reproductive adults in a definitive host (e.g., bird, mammal). When there are high numbers of trematodes, either adults or metacercariae, it may be inferred that all required hosts are present in the ecosystem. These results are not surprising for two reasons. First, like several of the other sites, SH is located along a major bird migration flyway, attracting hundreds of bird species. However, unlike those sites, the area where fish were collected has thousands of individuals of various mollusk species. These species could potentially act as hosts for any number of trematode species and their life-stages. Significant annual differences were also found in host livers that were attributed to large numbers of acanthocephalan infections in BB fish. While acanthocephalans have been known to cause high morbidity in mammals, fish are more tolerant of high worm intensities without displaying overt symptoms of disease (Taraschewski, 2000). Typically, acanthocephalans use crustaceans (e.g., copepod, amphipod) as their intermediate host, before maturing in their definitive fish host

(Hoffman, 1999). While examining gut contents, Weis *et al.* (2001a) found that grass shrimp (*Palemonetes pugio*) comprised 64% of *F. heteroclitus*' diet in BB. Thus, it is not surprising that acanthocephalan infections were high.

Significant annual differences were also found in the gills due to high *A. p. diminuta* and *E. schwartzi* abundances, with MC having the highest mean number of all. These two metacercariae made up approximately 90% of all parasites tallied. Trematode metacercariae are typically regarded simply as immature, nonfeeding adults, surrounded by a cyst wall. It has been accepted that in this stage of development, the trematode is dormant (Poulin and Latham, 2003). However, Goater *et al.* (2005) have found morphological evidence that metacercariae of many trematode species (e.g., *Ornithodiplostomum ptychocheilus*) continue to undergo developmental changes in their intermediate host (fathead minnows, *Pimephales promelas*) promoted by feeding activities that occur before encysting.

In 2005, significant annual differences were found for the digestive cavity, gills, body cavity and swim bladder. Once again, the significant differences in the digestive cavity were due to another surge in unidentified digenean trematode parasite abundances at SH and TK, while RD and PC experienced an increase in *D. bullocki*, a gut nematode. The gills experienced a three-fold increase in the number of *A. p. diminuta* and *E. schwartzi* between 2004 and 2005, with MC experiencing the highest number of gill parasites of all sites examined for the second year. Differences in the number of parasites found in the body cavity were due to numerous small red nematodes found encysted on the outer layer of SH fish's digestive tracts. Furthermore, highly significant differences in the number of swim bladder parasites were found, due to an increase in larval nematodes (*Cystidicola* sp.) for both MC and RD.

Two hypotheses were put forth regarding parasite abundance and parasite species richness as they relate to environmental conditions. First, it was hypothesized that hosts in cleaner habitats would have greater parasite species richness and evenness than those occupying more contaminated sites, as cleaner sites should support a greater diversity of intermediate hosts, thus parasites (Hyp 1). High species richness and diversity of parasite species may indicate a healthy environment, presumably meaning that the necessary intermediate and definitive hosts are present (Overstreet, 1997). Secondly, that parasite abundance would increase with increased contamination, as more stress tolerant parasite species would have less competition and thus be allowed to proliferate (Hyp 2).

MC fish had about 10 times as many parasites as fish from the second highest infected site (PC) and about 100 times as many parasites as fish from the least parasitized site (BB). Peaks and valleys in annual parasite abundances have been noted elsewhere throughout the literature for several taxa (Poulin and Valtonen, 2002; Vincent and Font, 2003; Brickle *et al.*, 2006; Desclaux *et al.*, 2006). However, despite normal annual fluctuations, positive associations have also been linked to host size, explaining a large proportion of variability seen among sites (Aho and Bush, 1993). There were significant differences between years for host length and weight. Fish collected in 2005 were longer and heavier, with MC fish being the largest of all. When parasites are extremely abundant, their biomass can be substantial. For example, in NJ Pine Barrens' streams, there are larval acanthocephlan populations with a total biomass projected to be comparable to that of a top predator population, and possibly a higher turnover rate (Sukhdeo and Hernandez, 2005). Perhaps having several thousand gill metacercariae, contributed to the overall biomass. Differences in host size may partially explain why many more

parasites were found in 2005, but it is doubtful that it can explain a three-fold increase between years.

MC was the second most polluted site examined (Hg, Cu and Zn), following PC. Despite this fact, mean species richness was the highest recorded for all sites in 2005 and the second highest in 2004. Although MC had one of the highest species richness, it had the lowest diversity and evenness of all sites examined due to extremely high numbers of gill parasites. However, pollution may not be the only factor involved. First, as a consequence of their indirect life cycle, digenean trematodes need several hosts to persist. Species richness is generally thought to be a useful measure of the severity of pollution (Sheehan, 1984). The fact that there are so many species, specifically digenean trematodes (metacercariae), implies that there are many first intermediate hosts and definitive hosts utilizing this system. Additionally, free-living stages such as cercariae, tend to be sensitive to anthropogenic environmental pollution and would likely die once released from their first intermediate host (Pietrock *et al.*, 2002), while in search of their second intermediate host (*F. heteroclitus*) if pollution was a serious problem.

Another possible reason could be environmental changes. 2005 was the hottest year recorded globally in the last century (Gutro, 2006). Increases in infection intensities with temperature have been reported for other parasite-host associations such as the tree swallow (*Tachycineta bicolor*) and the parasitic blow fly *Protocalliphora* (Dawson *et al.*, 2005). Water temperatures for 2004 were 21.5 °C in MC. In 2005, MC experienced the third highest recorded water temperature (26.6°C) in the last 10 years, following 1997 (32.3°C) and 1999 (29.8°C). Records indicate that 2006 was higher yet (28.7°C) (MERI, 2006). Perhaps the increase in water temperature stimulated cercariae release, thus intensifying gill parasite infection of *F. heteroclitus*. Fingerut *et al.* (2003) determined that the emergence of various

estuarine trematode cercariae from their first intermediate host (*Cerithidea californica*), was significantly correlated with water temperature, with the highest number of cercariae being released with the warmest water temperatures.

Unlike any of the other sites examined, MC was restored (in 1999). As habitat quality is improved following the restoration process, suitable substrate for hosts (e.g., gastropods, wading birds) also increases. Previous studies have found that prevalence of larval trematode parasites of the California horn snail (*Cerithidea californica*) nearly quadrupled at restored sites while control sites remained unchanged (Huspeni and Lafferty, 2004). More first intermediate and definitive hosts may be increasing the number of trematodes in this system as well. The improved habitat quality may have played a role in the surprisingly high species richness mean which would support Hypothesis 3. Finally, another plausible explanation for some of the increase in parasite abundance could be from acquiring experience in counting parasites. Several samples were recounted from 2004 to test this and numbers were somewhat comparable, although an increase of approximately 10% was noted.

PC fish had the second highest parasite abundance. Although PC fish were some of the largest fish collected over the two year period, they had one of the lowest mean weights calculated for all sites. This poor body condition may be due to the high levels of contamination they are subjected to in their natural environment (Smith and Weis, 1997). Perhaps the poor health of PC fish are making them more susceptible to parasite infection. In this study, PC fish had as many, if not more *Dactylogyrus* sp. as fish from less contaminated sites. In the presence of certain pollutants (i.e., pulp and paper mill effluent), *Dactylogyrus* sp. have been reported to have reduced abundance and mean number of species in their fish host *Rutilus rutilus* (Siddal *et al.*, 1996). Therefore, although the types of contaminants found in PC are not impacting *Dactylogyrus* sp., it is likely that the fish hosts' compromised

body condition is increasing infection risk. Unlike MC fish, the increase in parasite abundance from 2004 to 2005 was not overwhelmingly due to an increase in gill parasites, but rather several taxa throughout the organs examined. A large increase was seen in parasites in the gills and guts (*P. minimum* and *D. bullocki*) and unidentified red nematodes. The increase in numbers of *P. minimum* may be a positive sign for PC site improvement. Soucek and Noblet (1998) have shown that *P. minimum* cercariae are very sensitive to Cu (acute toxicity at 44.6 µg/L). PC sediment was shown to have the highest levels of Cu (525.0 ppm ±67.1SE) of all sites examined. Perhaps the increase in number of this species is an indication of a slight improvement of overall system health. The fact that PC fish had very high parasite abundances resulted in low evenness values for both years. Surprisingly however, PC had high species richness. Although low in 2004 (second only to SH), in 2005 PC was comparable to SH and had higher species richness than BB, UB and TK – the 'cleaner' sites. Once again, perhaps this is indicative of slight improvements in PC ecosystem health.

RD is part of a heavily degraded brackish marsh system, and has a restricted tidal flow with salinities of approximately 10 ppt. With respect to parasite abundance, RD fish were surprisingly grouped with 'cleaner' sites (UB and SH), having even fewer parasites than TK. Although RD did not thousands of parasites, fish were expected to be grouped together with MC, and perhaps PC as well due to similarities in contaminants and salinity. Using a CCA, it was determined that RD was closely related to both MC and PC due to similarities in contaminants (e.g., Zn, Hg) for both 2004 and 2005. Similarities in parasite species were also found. In 2004, *E. schwartzi* was responsible for linking these two sites together. In 2005, similarities in *E. funduli, Fundulotrema* sp., and *D. bullocki* were very strong. RD fish had the highest number of body cavity acanthocephalans (second only to MC in 2005). As

discussed previously, acanthocephalans have the ability to take contaminants from their host and concentrate them in their own tissues at levels several thousand times that of their host (Sures, 2003). Perhaps the bioconcentration of contaminants in the acanthocephalan tissues can also benefit RD fish. Like MC, RD had one of the highest average species richness of all sites examined. However, since it did not have nearly as many parasites as either MC or PC, RD had a much higher species diversity index and greater evenness than either of these other two sites, despite coming from a heavily degraded marsh system. RD fish parasites had a diversity and evenness that rivaled those of SH and TK fish.

The fish from SH, UB, and TK were expected to be grouped together due to general similarities in location (higher salinity, more southerly), fish condition and environmental variables. Based on these criteria, it was also expected that BB might be grouped with these three sites as well, despite being located in Long Island. They are all found at higher salinities and are considered to be somewhat 'cleaner' than the more northerly urban NJ sites (MC, RD and PC). Furthermore, despite their relatively close proximity, UB and SH parasite species compositions were also not as similar as initially expected despite being relatively close to one another. For example, UB had much higher abundances of the nematode *D. bullocki* and digenean trematode *P. minimum* than SH, TK or BB, while these latter sites had higher abundances of the unidentified red nematode and the digenean trematode *H. pallidum.* UB fish and their parasites were closely linked to salinity in 2004, but not 2005. In 2005 SH and TK fish and their parasites (e.g., unidentified trematode) were linked with salinity. For both years, BB stood out from all the other sites due to the presence of *Proteocephalus* and *Neoechinoryhncus* species. BB parasite communities do not appear to be strongly influenced by the environmental factors examined in this study. Furthermore, although still relatively high, the Shannon-Weiner diversity index for SH

was much lower than that for UB and BB, and TK was even lower. This was due to a surge in number of unidentified digenean trematodes in the guts of female SH fish in 2004 and 2005. A similar increase was found in TK with what appears to be the same trematode species. This trematode was also observed in BB, but not in UB. However, if this is in fact an immature form of *H. pallidum,* this species is found in UB as an adult and just not in this newly excysted (less mature) form.

Despite the fact that overall species compositions between TK and MC were fairly similar, both species richness and abundance were very different. Furthermore, the environmental variables tested against sites (Hg, Zn, Pb, Cu and salinity) were also very different. Nonetheless, in the CCA for 2004, TK fish were surprisingly similar to MC, RD and PC, clustered closely together. These sites strongly associated with *A. p. diminuta* and the Hg vector. This was completely unexpected.

Disagreements have been ongoing for several decades regarding whether or not parasite community structure in marine fish is an unstructured assemblage with little or no resource limitation, and thus no competition (Holmes, 1961; Price, 1980). However, observations made over the course of the baseline survey support results found in previous studies (Rohde, 1998; Mouillot *et al.*, 2003; Friggens and Brown, 2005), whereby parasite individuals reduce potential overlap by functioning within niches. Depending on whether one or more species were present, parasites were consistently found grouped together with conspecifics in specific areas. For example, when the nematode, *D. bullocki*, was the only parasite present in the digestive tract, it was found throughout the length of the organ. However, when other parasite species (e.g., *H. pallidum*) were present, *D. bullocki* frequently appeared only in the lower third of the digestive tract while *H. pallidum* remained in the middle third. Another notable example includes the unidentified digenean trematode species. When it was present, it occupied the lower third of the digestive tract while restricting *H. pallidum*

to the middle third. In the gills, *Fundulotrema* sp. was typically found at the gill filament tips while *Dactylogyrus* sp. were found nestled between the filaments. Although it is uncertain why this partitioning occurs, it is suggested that reproductive interference, and/or competition for resources such as limited space or nutrients may be responsible (Holmes, 1961).

It was hypothesized (Hyp. 4) that parasite loads would increase over time with early season collections having fewer parasites than late season, as the number of available intermediate hosts increased. Overall mean parasite abundances were consistently higher for both years in the late season collections when compared to early season. However, although significant seasonal differences were found, early season samples generally had higher species richness than late season samples contrary to what was hypothesized. In 2004, significant seasonal differences were found in the gills due to an increase in *A. p. diminuta, E. schwartzi* and *Dactylogyrus* sp. abundances, with MC having the highest overall mean number of parasites of all sites. These two metacercariae made up approximately 90% of all parasites tallied with abundances higher in the late season collections. There were also significant seasonal differences found within years that can be attributed to the unidentified digenean trematode recorded in early season samples for TK in 2005, and SH for both years. Increases in the other three gut species were observed as well. So, although early season collections from every site did not overwhelmingly have higher abundances than late season collections, distinct seasonal differences were found. Rather than hypothesize that total parasite abundance would increase over time, perhaps a more suitable hypothesis would have been more explicit with respect to either changes in habitat/organ or to a specific species.

Hosts of different sexes may acquire parasites at differential rates due to differences in feeding habits, reproductive behavior, physiological requirements, etc.

It was hypothesized (Hyp. 5) that males would have greater parasite loads than females resulting from potential differences in behavior and/or physiology. Surprisingly, this was not the case. Although no significant differences were found in overall parasite abundance between sexes, a highly significant difference was found in the number of gut parasites acquired by female SH fish in the early season collections of 2004. Female SH fish had significantly more unknown digenean trematode infections than SH males. There are several possibilities that may explain this sex-biased increase in infection. First, it may be due to differences in hormonal levels accompanying reproductive status that can increase the susceptibility of females to parasites and pathogens (Zuk, 1990). Larralde *et al.* (1995) found that intestinal cestodes' (*Taenia crassiceps*) development and growth were inhibited by androgens, whereas oestrogens promoted it. Although no significant differences were found in length between sexes, sexual size dimorphism may have played a part in the differences seen. Overall, female *Fundulus* tend to be larger than their male counterparts. Esch and Fernandez (1993) determined that host size correlates with parasite burdens in many systems. Thus, it may be that the larger sex is infected because of its larger body size and not because of sexual differences per se. However, although there were still significant differences in the number of gut parasites in 2005, no sex differences were found.

Although great lengths were taken to conduct a thorough examination of the endoparasites of *F. heteroclitus*, the possibility remains that several parasite species may have gone unrecorded. According to Poulin (1998a), approximately one-third of helminth parasite species from vertebrate hosts [bird and mammal] have a prevalence of 5% or less. Due to their rarity, many species will be missed when surveys of less than 40 or 50 individual hosts are examined. Also, since many of the parasite species examined are microscopic, it is relatively easy to overlook them and

underestimate their abundance (Poulin and Morand, 2000), thus potentially compromising species richness and evenness.

Overall, it appears that environmental site conditions, especially salinity, play a significant part in shaping parasite species communities. Additionally, it appears that site disturbance (restoration) may also play a large role. One study examining the biodiversity of parasites in freshwater environments in relation to varying levels of pollution, found fewer parasite species infecting chub (*Leuciscus cephalus*) in polluted areas (28 species) compared to unpolluted sites (34 species) (Gelnar *et al.*, 1997). This was not necessarily the case as found in this study. MC, the second highest polluted site had the highest mean species richness of all sites examined. MC also had the greatest mean abundance of parasites of all sites examined – specifically digenean trematode metacercariae. The question now remains, how can a fish population living in a highly urbanized, degraded site support such high parasite species richness? Also, why are there so many of these trematode metacercariae infecting fish at this particular site? MC was the only restored site examined. Perhaps the restoration process had something to do with the high parasite species richness and the high levels of digenean trematode metacercariae associated with fish gills from this site. Also, what effects do several thousand metacercariae in the gills have on fish physiology? Do they affect host behavior? As such, the highly significant differences in gill parasite abundance found among sites warrants closer inspection. Subsequently, the following chapters discuss studies designed to look at differences in behavior (Chapter Three), anatomy and physiology (Chapter Four) and effects of restoration (Chapter Five) as they relate to high levels of gill parasitism in *F. heteroclitus*.

CHAPTER III. BEHAVIOR

INTRODUCTION

Parasites can impact fish behaviors in a number of ways. First, fish can alter their everyday behaviors to avoid being parasitized in the first place. Examples include juvenile sticklebacks (*Gasterosteus* spp.) avoiding habitats associated with infection and only swimming near the bottom when the ectoparasite *Argulus canadensis* was absent (Poulin and Fitzgerald, 1989), or banded killifish (*Fundulus diaphanus*) avoiding shoals with individuals infected with *Crassiphiala bulboglossa* trematodes (Krause and Godin, 1996). Another example is male pipefish (*Syngnathus typhle*) rejecting sexual partners parasitized with *Cryptocotyle* sp. trematodes (Rosenqvist and Johansson, 1995). Another phenomenon is hosts altering their behavior to keep existing parasite loads in check, such as coral reef fish visiting a 'cleaning station' to have ectoparasites removed (Losey, 1987; Bshary and Schaffer, 2002). Both of these behaviors are thought to be adaptations that benefit the fish host.

The third behavioral modification normally serves to benefit the parasite and can result from either direct or indirect influences, although in some cases, the behavioral outcome may be a side effect of infection that benefits neither host nor parasite (Poulin, 1998b). The majority of the literature today dealing with this phenomenon examines behavioral modifications (usually of the intermediate host) that increase trophic transmission. Lafferty (1999) has coined the phrase 'parasite increased trophic transmission' (PITT) in an effort to describe this increasingly common occurrence.

One of the best known studies investigating behavioral modifications comes from the early works of Bethel and Holmes (1973). Using an amphipod-

acanthocephalan parasite-host system, they reported overt behavioral changes of amphipods (*Gammarus lacustris*) infected with *Polymorphus paradoxus* cystacanths. Only those individuals who were either not infected or did not carry the parasite in its infective stage performed typical anti-predator behaviors such as avoiding light and if disturbed, diving and burrowing. However, those amphipods carrying stages that were infective to the definitive host (the mallard duck, *Anas platyrhynchos*), became positively phototactic spending more time at the water's surface. Not only did the amphipods increase their time at the water's surface, but they also behaved in more conspicuous ways such as skimming and clinging to floating objects. Conspicuous behaviors vary in degree and form and serve to increase predation risk. Another example comes from the bivalve (*Macoma balthica*), infected with sporocysts containing metacercariae of a gymnophallid trematode. Swennen (1969) reported that trematode-infected *Macoma* crawl just under the surface of sandy tidal flats in high intertidal zones, leaving conspicuous tracks indicating their location. In a survey, 100% of all *Macoma* recovered at the end of the tracks were infected, compared to 13% and 15% infection rates in clams from randomly sieved samples in the same area. Not only is the crawling behavior considered to be a waste of energy, but the tracks left behind increase predation by shorebirds (e.g., gulls) which are the definitive hosts of gymnophallids.

A striking example comes from Lafferty and Morris' (1996) study of California killifish (*Fundulus parvipinnis*) as an intermediate host of a brain-encysting trematode (*Euhaplorchis californiensis*). Their work revealed that birds were 30 times more likely to eat infected fish than uninfected ones. Predation rates were significantly intensified due to an increase in conspicuousness of the infected host (e.g., jerking, scratching, shimmying).

However, although rare (Moore, 2002), there have also been reports whereby parasites alter definitive host behavior in ways that decrease predation on the host. Grass shrimp (*Palaemonetes pugio*) infected with *Probopyrus pandalicola* had significantly reduced activity in the presence of a killifish predator (Santiago Bass and Weis, 1999). *P. pandalicola* uses *P. pugio* as its definitive host, so by reducing the shrimp's activity, it effectively reduces potential predation risk, thus extending its (parasite's) own life.

III.1 Digenean Trematode Gill Parasites

Data collected during the baseline survey (Chapter Two) determined that *F. heteroclitus* from MC had gills that were highly infected (several thousand parasites) with digenean trematode metacercariae relative to other sites examined (several hundred). Upon further examination, two digenean trematode metacercariae (*Ascocotyle (phagicola) diminuta* and *Echinochasmus schwartzi*) were identified and found to comprise 99% of the total number of gill parasites identified. Adult and metacercarial stages of *A. p. diminuta* have been described in detail from both definitive hosts (e.g., wading birds, rats) and their second intermediate host *F. heteroclitus* (Stunkard and Haviland, 1924; Stunkard and Uzman, 1955), while the life cycle and biology of *E. schwartzi* has been reviewed by Price (1931) and Zettergren (1972). A general review of a typical digenean trematode life-cycle follows (Figure 3-1).

Adult worms are somewhat flask shaped, with a coronet of oral spines. A 22 spine coronet has been described for *E. schwartzi* (Zettergren, 1972), which differs from the single row of 16 spines and two additional spines behind the first row dorsally in *A. p. diminuta* (Stunkard and Haviland, 1924; Stunkard and Uzman, 1955). Parasites can sexually mature in a definitive host's (e.g., piscivorous birds, mammals)

intestinal region in as little as three days (Stunkard and Uzman, 1955) and produce eggs which are passed out into the marsh or estuary with the host's feces (Stein, 1968). The worms of the *Ascocotyle* complex are hermaphroditic and one adult parasite is capable of populating a marsh with eggs (Leigh, 1956).

The first intermediate host (amnicolid and hydrobiid snails), ingest the eggs as they feed on the marsh surface (Schroeder and Leigh, 1965). In the snail, one or more asexual generations form – the sporocyst and the rediae, which are essentially sacs of asexually produced offspring. Each fertilized egg produces a sporocyst which in turn produces the rediae (Moore, 2002). The rediae, which at this stage have suckers and a small gut, then travel from the snail's digestive gland to the gonads or hepatopancreas where further development continues. The rediae grow in length and bear up to 50 cercariae in various stages of development. One redia is capable of growing 6,000 cercariae over a one year period (Leigh, 1974). The cercariae are essentially smaller, juvenile forms of the adult digenean trematode with a tail (Moore, 2002). When mature, the cercariae leave the rediae through a birth pore, mostly during daylight hours or dawn, and swim out of the snail towards illuminated areas of the water (Martin, 1950; Font *et al.*, 1984).

The cercariae swim near their second intermediate host (in this case a cyprinodontid fish), which takes the cercariae up via their respiratory current. The parasite will then attach to and penetrate the gill filaments of the fish, dropping their tails before or soon after penetration (Ostrowski de Nunez, 1992; 1993), immediately encysting in the gill by producing a multi-layered hyaline, collagen-like cyst (Lumsden, 1963; 1968). Metacercaria must be passed on from the infected fish via predation to a definitive host so that the worm can sexually mature. Metacercarial cysts are resistant to HCl which allows them to pass through the definitive host's stomach and

dissolve only within the intestine, subsequently freeing the worm to fertilize and lay eggs.

Although adults are considered non-pathogenic to their piscivorous bird and mammalian hosts (Lumsden, 1963), some metacercariae are harmful (Holmes and Bethel, 1972; Minchella and Scott, 1991), while others cause little physiological or behavioral responses in the host (Coleman and Travis, 1998). However, even those that appear to be benign can cause reduced swimming performance under stressful conditions when infections are severe (>100 cysts) (Coleman, 1993), which may cause an increase in predation rates by definitive hosts. They can also cause reduced winter survivorship of their intermediate host (Coleman and Travis, 1998).

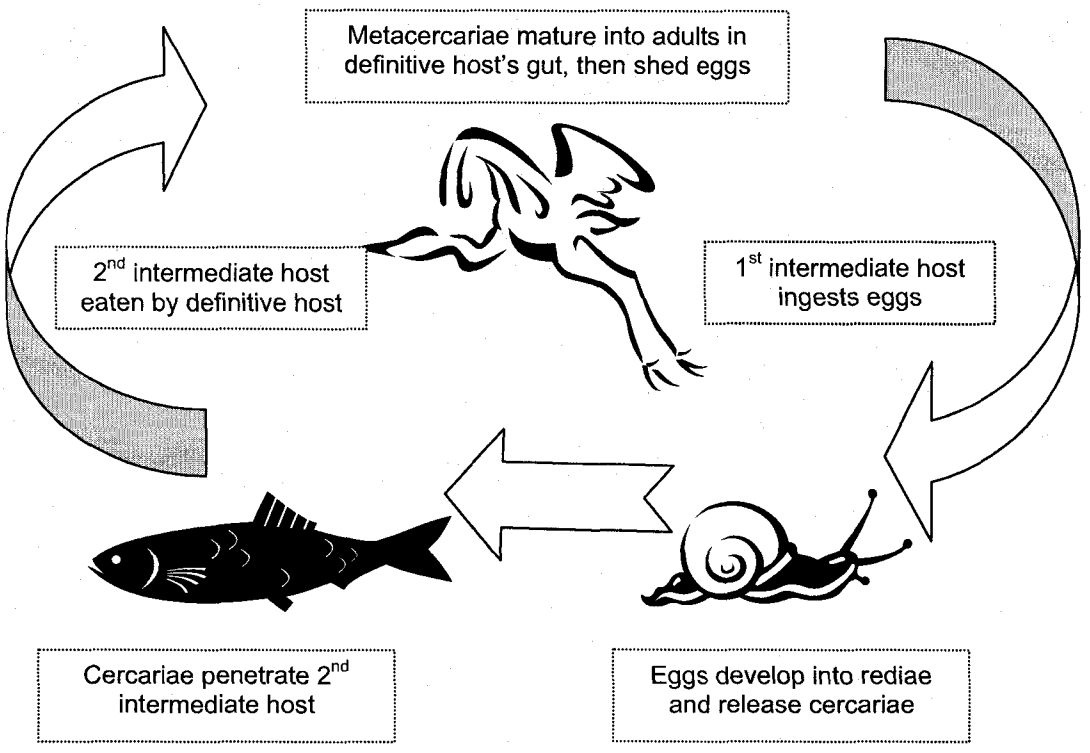


Figure 3-1. Typical digenean trematode life-cycle.

III.2 Rationale

In general, *F. heteroclitus* prefer to occupy deeper waters (40-60 cm) rather than shallow water (20 cm) habitats (Bretsch and Allen, 2006). This is a typical behavioral strategy to avoid both terrestrial and avian predators (Kneib, 1997). However, fish with substantial parasite infections may be experiencing parasite-induced behavioral modifications whereby fish occupy areas closer to the surface and/or exhibit conspicuous behaviors, which in turn could increase their risk of consumption by wading birds (the parasite's final host). This would subsequently increase the bird host's parasite load as well.

Although there are numerous accounts whereby parasites can either directly or indirectly modify intermediate host behavior, these studies have focused primarily on cestodes in the gut, acanthocephalans in the body or digenean trematodes in the brain. Few studies have examined potential behavioral effects of high parasite loads in the gills of fish, and none have examined digenean trematode metacercariae. Consequently, a more focused experimental study of fish from MC and other sites within the Hackensack Meadowlands District was undertaken to investigate gill parasites and their potential impact on *F. heteroclitus* behavior. The following hypotheses were tested:

6a. Fish with high gill parasite infections will have LOWER activity levels than those with low to moderate infections resulting from blocked respiratory structures, thus compromising DO uptake.

6b. Fish with high gill parasite infections will have HIGHER activity levels than those with lower infections as a response to parasite manipulation of host behavior to increase trophic transmission rates.

7. Fish with high gill parasite infections will exhibit a greater number of conspicuous behaviors than less parasitized conspecifics, resulting from parasite-induced behavioral modifications.

8. Fish with high gill parasite infections will spend significantly more time near the water's surface than less parasitized conspecifics as a result of parasite behavioral modifications.

MATERIALS AND METHODS

III.3 Study Sites

The Hackensack Meadowlands District (District) stretches between Bergen and Hudson Counties in northeastern New Jersey and covered 83 km^2 (7,885 ha). Although it includes a heavily degraded brackish marsh system (see Table 3-1 for sediment contamination levels), due to its size (3,399 ha), it is a significant natural component of the Hudson Raritan Estuary in a highly urbanized area (Appendix D). A majority of the wetlands located within the Meadowlands are dominated by *Phragmites australis,* an invasive wetland species that forms dense monocultures in which few, if any, other plant species can exist (Windham and Lathrop Jr., 1999). Starting in the 1800s and into the early part of the 20th century, diking and draining contributed to the increase in *P. australis* and the decrease in the tidal flow of water through the area. By the 1960s, it was believed that due to the severe pollution, *F. heteroclitus* was the sole fish species remaining in the Hackensack River due to its hardiness. Following reduction in sewage plant discharge, closing of garbage dumps, and a series of restoration projects, approximately 3,399 ha of the District now consist of open space, waterway and wetlands, and is host to more than 265 different species of birds (both resident and migratory). Shellfish and finfish have also

returned. Six research sites were examined within the District; Mill Creek, Richard W. DeKorte Park, Skeetkill Creek, Cedar Creek, Vince Lombardi, and Kingsland Creek, and are described further as follows:

Mill Creek

Mill Creek (MC) marsh is bordered by the New Jersey Turnpike and a shopping center (40.79101 N, 74.063416 W). MC is a 55 ha tidal marsh that was restored in 1999 to reestablish tidal flow across the site, create additional open water habitats and develop low marsh and upland habitats. The MC restoration has increased the diversity of this estuarine system. More than 260 species now use the area. Migratory shore birds can be found, along with a variety of other waterfowl that utilize it for various activities (Meadowlands, 2004).

Richard W. DeKorte Park

Richard W. DeKorte Park (RD) is a 44.5 ha site in Lyndhurst NJ (40.809433 N, 74.12453 W), centrally located within the Hackensack Meadowlands District (Meadowlands, 2004). It is located within the Saw Mill Creek Basin, and was initially part of a large marsh system influenced by Kingsland Creek and Sawmill Creek but has since been cut off from full tidal inundation due to construction. Much of this area is still dominated by *P. australis* which fringes large open water areas. More than 265 bird species use the area, and is home to terrapins, red fox, and muskrats, among others (Meadowlands, 2004).

Skeetkill Creek

Skeetkill Creek (SK) marsh is a 6.6 ha tidal marsh, located in an industrialized area of the Borough of Ridgefield, NJ (40.832568 N, 74.001531 W), and was acquired in 1996 by the NJ Meadowlands Commission, after which restoration

activities took place. Prior to restoration, *P. australis* was the dominant plant species. The restoration process included grading to reestablish tidal flow and the replanting of native wetland plants (Meadowlands, 2004).

Vince Lombardi

Vince Lombardi (VL) marsh is located in Ridgefield Borough, Bergen County (40.832568 N, 74.001531 W), and is approximately 4 ha in size. It is bordered to the east by the Vince Lombardi Rest Area in northern NJ, off the southbound side of the NJ Turnpike which is on the north side of the site. It is bordered on the west and southwest by the Hackensack River where three small islands create a small isolated jughandle that meanders off of the Hackensack River. These islands were restored in 1984 by the removal of *P. australis* and replacement with *S. alterniflora*. In recent years, the islands have since returned to *Phragmites* dominance.

Cedar Creek

Cedar Creek (CC) is a brackish water marsh that flows through a heavily industrialized region of the District. It is bordered by a gas pipeline and the NJ Turnpike (40.838527 N, 74.104069 W). It extends from the east side of NJ Route 120 just south of Paterson Plank Road and around the north side of the Continental Airlines Arena before emptying directly into the Hackensack River. The majority of CC's tidal wetlands were eliminated in the construction of the Meadowlands Sports Complex in the 1970s. What remains of the tidal creek is dominated by *P. australis*, with clumps of *S. aterniflora* at the edge. Various fish, birds and terrapins are still found utilizing this area.

Kingsland Creek

Kingsland Creek (KC) marsh is a channelized creek in Lyndhurst, NJ (40.809433 N, 74.12453 W). KC is a heavily degraded creek dominated by *P. australis*, flowing under the NJ Turnpike and a gas pipeline. A large portion of its channel is lined with rip-rap, which opens up to a small marsh area just before flowing into the Kingsland Tidal Impoundment. The Kingsland Tidal Impoundment is regulated by a tide gate operated by the Hackensack Meadowlands Development Commission and is known to be an important overwintering area and migratory stop-over for dozens of bird species.

Table 3-1. Mean sediment contamination for sites located within District (Meadowlands, 1997**; Windham *et al.*, 2004*; MERI, 2006***).

HACKENSACK MEADOWLANDS DISTRICT MEAN SEDIMENT CONTAMINATION (ppm)				
Site	***Hg***	***Cu***	***Pb***	***Zn***
MC*	4.27 ±0.28	143 ±16	139 ±4	342 ±6
RD* (Saw Mill Creek)	2.04 ±0.53	92 ±14	143 ±34	178 ±22
SK**	*n/a*	127.66	184.88	454.24
VL	*n/a*	*n/a*	*n/a*	*n/a*
CC	*n/a*	*n/a*	*n/a*	*n/a*
KC***	*n/a*	22.6	193	40.2

****levels reported are 2006 seasonal readings for Hackensack River (South). KC is one of 5 stations.*

III.4 Collection

F. heteroclitus were collected using seines and killie-traps from each site. Fish were returned to the lab, kept in an aerated 38 L tank and allowed to acclimate for approximately one week until tested. Fish were fed a combination of Tetramin® fish flakes and dried shrimp twice daily.

III.5 Vertical Positioning

Groups of 10 fish (5 male, 5 female) were placed in a 38 L tank that had the upper third (10 cm) delineated with a line to distinguish between the 'surface' and the lower region. After allowing the fish to acclimate secluded for one hour, they were videotaped to remove potential confounding effects (e.g., observer's presence). The first 10 min of footage was discarded to reduce any residual effects resulting from the human presence (e.g., turning camera on). The number of individuals found in the upper 10 cm were recorded each minute over 15 min.

III.6 Conspicuous Behaviors

In viewing the videotapes, each fish was observed individually for five min, and any unusual conspicuous, aberrant behaviors such as flashing and jerking were recorded.

III.7 Activity

One fish at a time was placed into a 38 L tank with a five cm^2 grid drawn on the bottom and with approximately 10 cm of water and allowed to acclimate. Following 30 min of acclimation, activity rates were determined by counting the number of lines crossed in one minute. Twenty-five fish were tested.

III.8 Gill Parasite Abundance

Following behavioral tests, fish were sacrificed in MS-222 and preserved in 10% buffered formalin. Gills were removed and placed in a Petri dish with deionized water to keep wet. Every gill filament from each arch was tallied. Following Shaw *et al.* (2005), double sampling of the gills was employed. All gill filaments on one side of the gill arch were examined under a 20X dissection microscope. The number of metacercariae found were then doubled. Direct counts of all other parasites were made using every gill. The total number of gill parasites was then recorded and their prevalence calculated. Gills were preserved in individual vials of 10% formalin for later reference. Twenty-one fish from each site were examined.

III.9 Statistics

One-way ANOVAs and Tukey HSD analyses were run using Statistix 8®, and MANOVA, Canonical Correlations and Principal Component Analyses (PCA) using SAS 9.1®. *P* values <0.05 were considered statistically significant.

RESULTS

III.10 Gill Parasite Abundance

Highly significant differences (p<0.001, $F_{5,113}$=83.3) in gill parasite abundance were found among sites using a one-way ANOVA, with four groups of homogenous means calculated using Tukey HSD (Figure 3-2). MC (mean=6052.1 ±415.7SE) had the highest total number of gill parasites of all sites examined and was placed in the first group by itself. This was followed by SK (mean=3302.9 ±246.6SE), which made up the second group. VL (mean=2815.8 ±231.3SE) was intermediate and CC (mean=2021.1 ±164.5) made up the third group. KC and RD had the fewest total number of gill parasites, making up the fourth group with means of 492.5 (±86.0SE) and 318 (±246.6SE) gill parasites respectively.

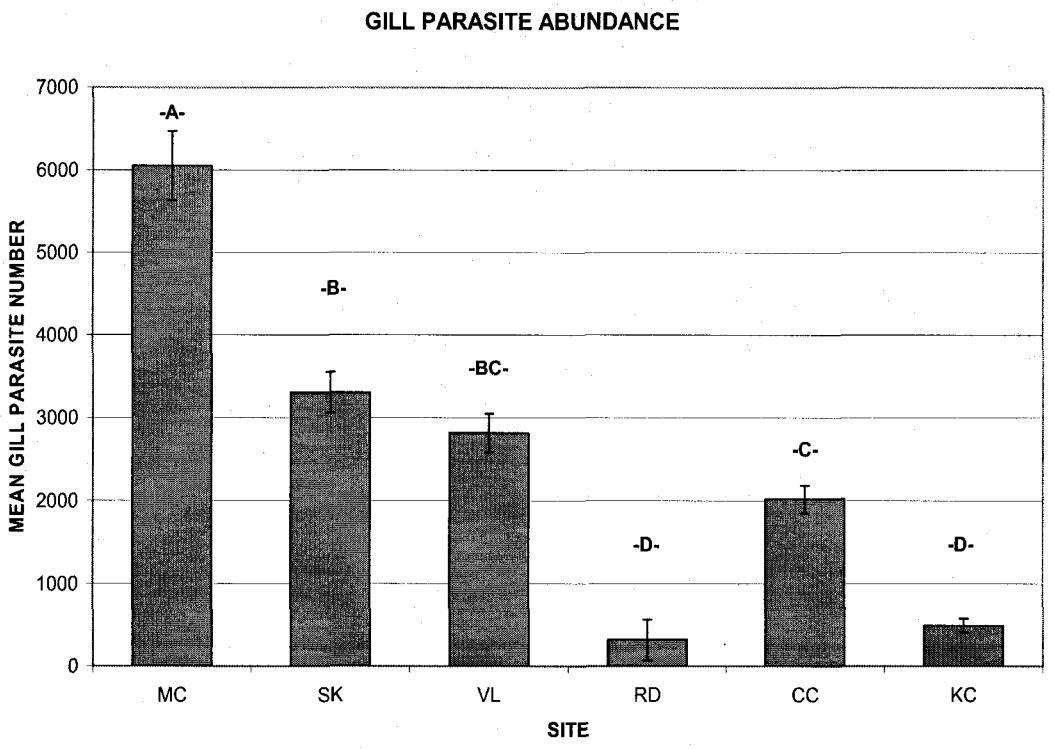


Figure 3-2. Mean (±SE) number of gill parasites, with Tukey HSD comparison across sites.

Five macroparasite species were commonly found associated with the gills: *A. p. diminuta*, *E. schwartzi*, *Dactylogyrus* sp., *Fundulotrema* sp., and *Ergasilus funduli*. A sixth species; *Argulus flavens* was rarely observed (prevalence << 1%) and therefore was not included. Both *A. p. diminuta* (range 29-7549) and *E. schwartzi* (range 54-1887) were found in 100% of all fish from the six sites examined. *Dactylogyrus* sp (range 0-56) was the second most common species with a total prevalence of 95%. 55% of all fish examined were infected by *Fundulotrema* sp. (range 0-38), and less than half (42%) were infected by *E. funduli* (range 0-28) (Table 3-2).

Table 3-2. Gill parasite mean (±SE), range, and prevalence across six sites.

SPECIES	SITE					
	MC (n=21)	SK (n=21)	VL (n=21)	RD (n=21)	CC (n=21)	KC (n=21)
A. p. diminuta						
Mean ±SE	4789 ±319	2514 ±178	2452 ±187	122 ±11	1551 ±121	111 ±22
Range	1181 - 7549	1005 - 4291	987 - 3923	36 - 233	525 - 2816	29 - 408
Prevalence (%)	100	100	100	100	100	100
E. schwartzi						
Mean ±SE	1197 ±80	629 ±45	625 ±47	182 ±16	388 ±30	445 ±88
Range	295 - 1887	251 - 1073	247 - 981	54 - 350	131 - 704	115 - 1632
Prevalence (%)	100	100	100	100	100	100
Dactylogyrus sp.						
Mean ±SE	9 ±2	6 ±1	6 ±1	11 ±2	19 ±3	6 ±2
Range	1 - 41	0 - 22	0 - 22	2 - 25	1 - 56	0 - 29
Prevalence (%)	100	86	91	100	100	95
***Fundulotrema* sp.**						
Mean ±SE	1 ±0.3	0.8 ±0.2	0.1 ±0.1	1.0 ±0.2	3.0 ±2.0	1.0 ±0.3
Range	0 - 4	0 - 3	0 - 2	0 - 3	0 - 38	0 - 4
Prevalence (%)	71	48	10	62	29	57
E. funduli						
Mean ±SE	1.0 ±0.6	0.9 ±0.3	0.8 ±0.2	0.7 ±0.3	3.0 ±0.7	4.0 ±2.0
Range	0 - 13	0 - 4	0 - 4	0 - 5	0 - 12	0 - 28
Prevalence (%)	19	52	48	33	67	33

A Principal Component Analysis (PCA) determined that of the five gill parasites observed, the digenean trematode metacercariae of *A. p. diminuta* (38%) and *E. schwartzi* (22%) were accountable for approximately 60% of the total variability seen in mean gill parasite abundance (Table 3-3).

Table 3-3. Principal Component Analysis of gill parasite species.

	Eigenvalues of the Correlation Matrix			
	Eigenvalue	*Difference*	*Proportion*	*Cumulative*
1	1.9029	0.8115	0.3806	0.3806
2	1.0915	0.0822	0.2183	0.5989
3	1.0093	0.1361	0.2019	0.8007
4	0.8732		0.1746	0.9754
	Eigenvectors			
	Prin1	*Prin2*	*Prin3*	*Prin4*
A. p. diminuta	0.6982	0.0882	-0.0173	0.0453
E. schwartzi	0.6939	0.0964	-0.0402	0.1123
Dactylogyrus sp.	-0.023	0.7572	0.1123	-0.6424
Fundulotrema sp.	-0.0023	0.0862	0.9592	0.2694
E. funduli	-0.1748	0.6341	-0.2559	0.7072

III.11 Vertical Positioning

Overall, fish with the highest parasite infections (VL, SK and MC) spent significantly more time at the water's surface (p=0.004; $F_{5,84}$=3.76) than fish with lower parasite infections when using a one-way ANOVA, with two groups reported using Tukey HSD. Although MC spent much of its time at or near the surface, it was not grouped with the other two highly parasitized sites. VL (mean=4.60 min ±0.70SE) and SK (mean=3.8 min ±0.6SE) spent the most time at the surface (46% and 38% respectively) and comprised the first group. A set of intermediates included (mean ±SE): MC (3.4 min ±0.6), RD (2.7 min ±0.6) and CC (2.5 min ±0.6), spending 35%, 27% and 25% of their time at the surface respectively. The second group consisted of KC fish, which only spent approximately 14% of their time (mean=1.4 min ±0.4SE) at the surface (Figure 3-3).

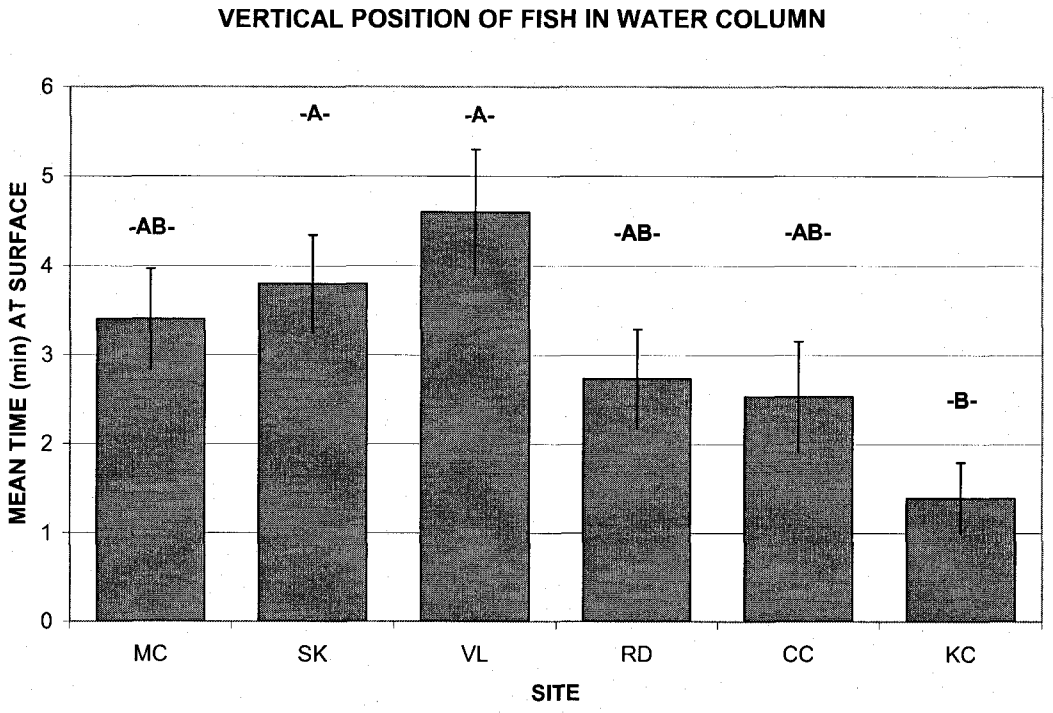


Figure 3-3. Mean amount of time (min) spent at surface of tank during 15 min of observation.

III.12 Conspicuous Behaviors

Highly significant differences (p=0.003, $F_{5,54}$=4.16) were found among sites with respect to the number of conspicuous behaviors observed using a one-way ANOVA. Tukey HSD determined that there were two groups of homogenous means (Figure 3-4). Fish from SK performed the greatest mean number (5.3 ±2.1SE) of individual conspicuous behaviors and were placed into one group. VL and MC were grouped together as intermediates with means of 4.2 (±0.7SE) and 1.8 (±0.5SE) respectively. The second group contained (mean ±SE): CC (1.2 ±0.6), KC (1.1 ±0.4) and RD (0.4 ±0.2).

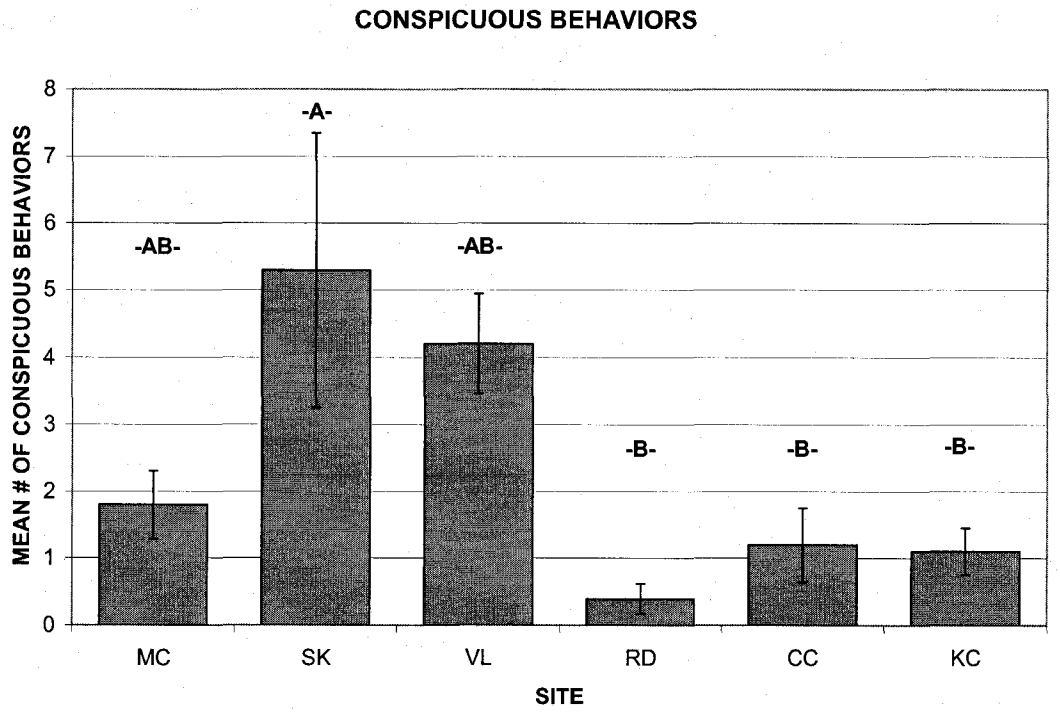


Figure 3-4. Mean number of individual conspicuous fish behaviors in 5 min per population.

III.13 Activity

No significant differences (p>0.05) were found among sites when activity levels were analyzed using a one-way ANOVA. SK fish had the highest activity with an average of 63.2 ±6.1SE lines crossed in a minute's time and CC had the lowest activity with 50.3 (±6.1SE) lines crossed in the same period (Table 3-4). However, the MC fish (and RD to a lesser extent), swam extensively up and down. This could not be recorded in this experimental set-up as activity was only looked at in two dimensions.

Table 3-4. Mean number of lines crossed per minute.

	ACTIVITY	
Site	*Mean*	*±SE*
MC	55.8	6.1
SK	63.2	6.1
VL	53.6	6.7
RD	51.0	6.1
CC	50.3	6.1
KC	55.5	6.1

DISCUSSION

Highly significant differences ($p<0.001$) were recorded among sites with respect to gill parasite abundance. MC, SK and VL had the highest average number of gill parasites of all sites examined, averaging several thousand metacercaria per individual fish host. Though five species were commonly found across all sites, there were two digenean trematode species that were dominant in both abundance and prevalence, *A. p. diminuta* and *E. schwartzi*. It was determined that the majority of all variation in gill parasite abundance observed (60%) was due to these two digenean trematode species.

Highly significant differences were found among sites with respect to conspicuousness and vertical positioning. Sites with highest gill parasite abundances (MC, SK and VL) exhibited the greatest number of aberrant behaviors (e.g., jerking and scratching) per fish. *F. heteroclitus* with higher numbers of gill parasites also spent significantly more time at or near the water's surface than less parasitized conspecifics (RD, KC and CC). Fish with one of the greatest number of gill parasites spent as much as 46% of their time at or near the water's surface versus significantly less parasitized conspecifics (14%), making them about 3.5 times as likely to be predated upon. Add to this factor unusual, conspicuous behaviors, and the risk of

predation should significantly increase. Although MC had the highest parasite abundance overall, it was not reflected in the surfacing or conspicuousness experiments. Upon reviewing the videotape, MC fish swam throughout the tank with much more vertical movement rather than horizontal. It was not that MC fish remained any closer to the bottom than VL or SK, but rather when the data was recorded at that specific moment, they were either heading back to the surface, or on their way back down. To test this, I went back and staggered the data collection time by 30 sec (e.g., from 01:00 to 01:30). In doing so, their mean increased from 3.4 ±0.6SE to 4.0 ±0.7SE. Although no significant differences were found in activity among sites, MC did have the second highest activity (after SK, another heavily parasitized site) of all sites examined. It is possible that if activity had been recorded in all three dimensions, MC may have had the highest activity.

Radabaugh (1980) studied the schooling behavior of fathead minnows (*Pimephales promelas*) parasitized with metacercariae of *Ornithodiplostomum ptychocheilus* and found that they not only performed more conspicuous behaviors (e.g., diving frequently, less compact schools), but also remained closer to the water's surface than control schools. Both atypical behaviors (surfacing, conspicuousness) encourage the vulnerability of the minnow, an intermediate host, to its definitive host, an avian predator. Crowden and Broom (1980) reported that dace (*Leuciscus leuciscus*) with higher intensities of *Diplostomum spathaceum* eye infections, spent the most time in the upper 10 cm of the water column. Helluy (1982) found that it only took one metacercaria of *Microphallus papillorobustus* in the protocerebrum to initiate behavioral modifications in *Gammarus insensibilis* and *G. aequicauda*, including positive phototaxis and hyperactivity when disturbed. Although the parasite-host system that Helluy (1982) studied is different than the one examined here, it shows how influential even the smallest number of metacercariae can be on their

host's behavior. The fish from MC, SK, VL and CC had several thousand metacercariae in their gills which in itself, is likely to be extremely stressful due to their sheer number. In the vertical positioning experiment, RD which only averaged several hundred metacercariae per fish was grouped together with MC and CC, both of which had over 1,000 per fish. This anomaly may have occurred not due to abundance of gill parasites but to other factors such as dissolved oxygen limitations. RD is a part of the former 440 landfill site (now called Richard DeKorte Park), which is currently closed. Reclamation of the 1.4 ha 'garbage island' took place in the late 1980s through the early 1990s and the possibility still exists that leachates or other environmental stressors are causing oxygen fluctuations. There's also the possibility of other parasites in other tissues doing something to affect fish behavior. RD fish were found to have a relatively high prevalence (95%) and abundance (averaging seven per fish) of larval nematodes infecting their swim bladder. Nematode life cycles always use an invertebrate first intermediate host. As the second intermediate host, fish take the nematode in while feeding and it develops into an adult in the intestinal tract of piscivorous fish, birds and mammals. This may be another case of parasite-induced behavioral modification or perhaps it is pathological where damage to the swim bladder results in fish surfacing frequently. For example, infections with the nematode *Anguillicola crassus* in European eels (*Anguilla Anguilla*) are known to cause swim bladder pathology (Nimeth *et al.*, 2000) and a change in the composition of swim bladder gases (Wurtz *et al.*, 1996).

Although it was hypothesized that significant differences in activity levels would be found, differences were not found. The freshwater snail *Semisulcospira libertina* with various trematode infections (*Metagonimus* sp., *Centrocestus armatus* and *Cercaria yoshidae*) also did not exhibit differences in activity levels compared to unparasitized conspecifics (Shinagawa *et al.*, 2001). Perhaps during their co-

evolution, those parasites inducing behavioral differences (surfacing and conspicuousness) were more successful in facilitating trophic transmission than those parasites that changed their host's activity levels. As such, the 'behavioral changing genes' would have been passed on to subsequent generations and 'activity-altering genes' would have been lost. It is also possible that the factors that might have caused activity to increase were counteracted by those that would have caused it to decrease (lowered respiration). Considering that highly significant differences were observed in other behaviors (e.g., surfacing, conspicuousness), it does not appear that an increase or decrease in overall activity would be important as predation of infected fish would already be enhanced.

The behavioral effects seen in the most heavily parasitized fish in this study may be induced through direct or indirect means (Milinski, 1990). Indirect behavioral alterations can include an increase in foraging behavior of fish infected with nutritionally demanding parasites (Giles, 1987). A parasite can directly impact its host's behavior by damaging the brain, or through biochemical secretions (e.g., neurotransmitters) that act directly on the host's system (Helluy and Holmes, 1990). However, within the context of direct impacts, it is uncertain whether host behavior is changed due to the physical damage inflicted by the parasite, a build-up of metabolic compounds from the parasite or by the secretion of behavior-modifying chemicals (Barber and Wright, 2006). From the data here, there appears to be an infection threshold of approximately 1,000 gill metacercariae to cause fish to exhibit significant behavioral modifications. Once fish have more than 1,000 metacercariae, direct and/or indirect parasite effects appear to take place, altering the fish's 'normal' behavior. Crowden and Broome (1980) noted similar findings wherein behavioral changes noted for *L. leuciscus* were greatest for those with largest *D. spathaceum* trematode parasite abundances. Smaller numbers of parasites appeared to have

very little effect. Although the mechanism behind the differences in swimming near the surface and exhibiting conspicuous behaviors is unknown, it appears that extremely high numbers of gill parasites can exert a powerful influence on their hosts' behavior. This influence facilitates the parasite's transmission to avian predators, which are the digenean trematode's definitive host. Additional research into the physiological aspect of the behavioral modifications seen in this study (and others) should be pursued to expand the overall scientific knowledge base of parasite-host interactions.

CHAPTER IV. ANATOMY AND PHYSIOLOGY

INTRODUCTION

Parasite-induced physiological alterations differ with each parasite-host system as the requirements for each association vary. For example, some nematomorph parasitoids alter their insect host's behavior by increasing their thirst which facilitates transmission of the parasite's adult stages to appropriate habitats (water) (Poinar, 1991). Three-spined sticklebacks (*Gasterosteus aculeatus*) parasitized with *Schistocephalus* plerocercoids have higher respiration rates (Lester, 1971), and higher resting, routine and maximum activity levels than unparasitized fish (Giles, 1987) which could facilitate their detection by a predator. The trematode *Ascocotyle pachycystis* which encysts in the bulbus arteriousus of sheepshead minnows (*Cyprinodon variegates*), obstructs blood flow, significantly decreasing the time infected fish can swim at their maximum sustainable velocity before becoming exhausted (Coleman, 1993). Similarly, significant reductions in swimming speed have also been reported in sockeye salmon (*Oncorhynchus nerka*) infected with the myxosporidian parasite *Myxobolus arcticus* (Moles and Heifetz, 1998).

IV.1 Gill Anatomy & Respiration

Respiration and metabolism are intricately related. Respiration in fish is the process in which water passes across the gill region and oxygen is extracted, while metabolism is the conversion of glucose and oxygen to use as energy. As such, fish require oxygen to produce sufficient energy to support their overall metabolic needs. In fish, the rate of oxygen consumption is often used as an indicator of metabolic rate (Diana, 1995).

Water contains < 0.01% oxygen by volume as compared with 21% for air. Despite the fact that the gills of most fish are extremely efficient at extracting oxygen from water, a fish may use 10% (or more) of the oxygen it obtains just to operate its respiratory muscles (Jones and Schwarzfeld, 1974). The high density and viscosity of water forces fish to work harder to move it across the gills than terrestrial organisms do with air going into lung tissue.

The gills are the major organ for respiratory gas exchange, but they also have vital roles in ionic and osmotic balance, and are a primary site for the excretion of nitrogenous wastes (Mommsen, 1984). Overall, the general morphology of fish gills ensures that maximum oxygen diffusion will occur by optimizing total gill surface area, which is typically a function of body size (Hughes, 1984). The optimization of total gill surface area is achieved via three structures: the bony gill arches, the primary lamellae, and secondary lamellae. The primary lamellae (gill filaments) are found in alternating pairs and subsequently have their surface area increased by the secondary lamellae found regularly spaced on both sides of each filament. The secondary lamellae are considered to be the most important structure in gas exchange (Hughes, 1984). A layer of thin epithelial cells covers the outside of the lamellae, and under the basement membrane are supportive pillar cells and blood vessels running in opposite directions to the water flow, ensuring very efficient gas exchange, with oxygen utilization as high as 80% (Birx, 2002). As with other fishes of intermediate activity, *F. heteroclitus*' gill arches are well developed, supporting primary and secondary lamellae of average size.

Almost all fish have a unidirectional flow of water over their gills. At faster swimming speeds, fish will switch to ram ventilation in order to conserve energy. At that point, the branchial pump is switched off and fish regulate the flow of water over the gills by opening their mouth in relation to their speed and oxygen demand (Birx,

2002). Additionally, muscles in the gill filaments can change the filament's angle on the arch, altering the flow pattern over the secondary lamellae, thus increasing the amount of oxygen they can extract. All gill functions require a metabolic rate that is higher than all other fish tissues (in a resting fish, on a per gram basis) (Johansen and Pettersson, 1981). This high metabolic requirement is due to the oxygen demands of the gill filament musculature and pillar cells (Pasztor and Kleerekoper, 1962), in addition to the needs of the numerous chloride cells (Shirai and Utida, 1970), and mucus cells (Morgan and Tovell, 1973). The gill tissue alone may require up to 7% of the fish's total oxygen consumption for its own metabolism (Mommsen, 1984).

Many studies have looked at effects of gill ectoparasites. Montero *et al.* (2004) found that heavy infestations of the monogenean ectoparasite *Zeuxapta seriolae* on amberjacks (*Seriola dumerili*) reduced hematocrit values and resulted in significant mortalities of the hosts. All dead amberjacks had high parasite abundances and egg strings entangled in the gills. *Z. seriolae* caused lamellar synechiae (adhesion of tissues), lamellar clubbing, and disrupted the epithelial and vascular structures of the gills. Goodwin (1999) studied channel catfish (*Ictalurus punctatus*) and bighead carp (*Hypophthalmichthys nobilis*) infected with the copepod *Lernaea cyprinacea*, and reported large losses of *I. punctatus* from massive infections of *L. cyprinacea*. The copepods were found grazing on the gill tissue which caused severe gill damage including epithelial hyperplasia, telangiectasis (dilated blood vessels) and hemorrhage. No studies to date have examined the physiological effects of gill endoparasites (e.g., digenean trematode metacercariae) on fish respiration.

Fish with gills highly infected by digenean trematode metacercariae were shown to experience behavioral modifications (see Chapter Three). Perhaps this

occurs through physiological changes induced by the parasite. As fish gills are increasingly saturated with parasites, their respiration may become severely compromised, simulating hypoxic conditions and thus response. Reed *et al.* (1996) looked at effects of monogean ectoparasites and found that large numbers on either the skin or gills resulted in significant damage and mortality. Heavy gill infestations resulted in respiratory disease with swollen, pale gills and an increase in respiration rate. Ultimately, heavy infestations led to reduced tolerance of low oxygen conditions.

Hosts with severe gill parasite infections may compensate for the decrease in oxygen extraction by countering the parasite invasion in various ways, including behaviorally (i.e., surface breathing, reduced activity) or physiologically (i.e., increase gill surface area or red blood cell number). For example, nine-spined sticklebacks (*Pungitius pungitius*) infected with *Schistocephalus* sp. exhibited an increased frequency of aquatic surface respiration and had higher lethal oxygen levels when exposed to hypoxic conditions (Smith and Kramer, 1987).

IV.2 Blood

Parasites may also change hematological parameters of fish. Fish blood is a dynamic metabolic system. It is the first line of defense against environmental disturbances and is a vital respiratory tissue. Oxygen enters the blood via the gills and is transported via the circulatory system to various tissues and organs and then released. Fish erythrocytes are long-lived with half-lives of approximately 51 days (but as long as 270 days) (Fischer *et al.*, 1998). Unlike the enucleated red blood cells (RBC) of mammals, fish erythrocytes have very large nuclei, occupying up to 29% of the cell's total volume (Wilhelm Filho *et al.*, 1992). Erythrocyte characteristics help determine the efficiency of oxygen transport from the respiratory surface to tissues

(Holland and Forster, 1966). Hemoglobin (Hb), the oxygen-carrying protein found within RBCs, binds to oxygen for transport around the body (Helfman *et al.*, 1997).

The normal composition of fish blood and tissue may undergo changes resulting from environmental factors, including disease, stress and water chemistry (Wedemeyer and Yasutake, 1977). The percentage of RBCs in fish blood (hematocrit) is the largest range of any vertebrate group, ranging from zero to >50%. Physiological state (e.g., stress, parasites) and environmental factors (e.g., DO, pH) are known to influence hematocrit values (Gallaugher and Farrell, 1998). Changes in erythropoietic activity may be reflected by changes in hematological parameters such as hematocrit, Hb concentration and RBC counts (Valenzuela *et al.*, 2006). These variables are then regulated by several factors such as hypoxia (Valenzuela *et al.*, 2002), exercise (Kita and Itazawa, 1989), reproductive state (Cech and Wohlschlag, 1982) and seasonal variations (Thomas *et al.*, 1999).

Parasites are one of the stressors encountered by fish in their natural habitat that can change hematological parameters. For example, Khan (1977) found a decrease in Atlantic cod (*Gadus morhua*) blood volumes in those parasitized with *Trypanosoma murmanensis* compared with unparasitized conspecifics. Parasitized fish had hematacrit values of 15% compared to 24% for unparasitized fish. Montero *et al.* (2004) also found that heavy infestations of the monogenean ectoparasite *Zeuxapta seriolae* on amberjacks (*Seriola dumerili*) reduced hematocrit values and resulted in significant mortalities of the hosts. Some parasites (e.g., Sanguinicolid and heterophyid digenean flukes) reduce the blood's carrying capacity and its ability to exchange gases due to mechanical obstruction, by altering the number and type of blood cells, and by causing hemorrhage (Smith, 1972).

IV.3 Rationale

No studies have examined potential physiological effects on hosts caused by high gill endoparasite infections, specifically digenean trematode metacercariae, which should be stressful. *F. heteroclitus* may be experiencing conditions similar to hypoxia as their respiration becomes compromised from parasite-saturated gills. They may try to compensate for decreased oxygen extraction by responding to the parasite invasion physiologically. Consequently, a more focused experimental study of fish from MC and other sites within the Hackensack Meadowlands District was undertaken to investigate the relationship of gill parasites to physiology (respiration, stamina), and possible compensatory responses such as increased number of red blood cells, and anatomical modifications of gills to increase gill surface area. The aim of this study is to investigate the following hypotheses:

9. Fish with very high numbers of gill parasites will have lower respiration rates than those with lower infections, as respiratory structures are compromised.

10. Fish with more gill parasites will have reduced stamina than those with significantly fewer parasites as a consequence of gill disease.

11. Excessive parasite loads will induce physiological (e.g., increase RBC) and/or morphological changes in the gills to compensate for presumed oxygen deficiency.

IV.4 Study Sites

Fish from the six sites within the Hackensack Meadowlands District; Mill Creek (MC), Skeetkill Creek (SK), Richard W. DeKorte Park (RD), Kingsland Creek (KC), Vince Lombardi (VL), and Cedar Creek (CC) described previously (see Chapter Three), were used for this study.

MATERIALS AND METHODS

IV.5 Collection

F. heteroclitus were collected with seines and killie-traps at each site. Fish were returned to the lab and kept in an aerated 38 L tank and allowed to acclimate for approximately one week until tested. Fish were fed a combination of Tetramin® fish flakes and dried shrimp twice daily.

IV.6 Respiration Rate

F. heteroclitus resting respiration rates were measured by placing weighed fish in individual 2.8 L Erlenmeyer flasks with a magnetic stirrer on a Themolyne Nuova II stirring plate, to keep the water mixed continuously. Initial dissolved oxygen (DO) levels were measured using a YSI DO meter (model 51B), and the flasks sealed with parafilm. Fish were kept in a quiet area where they were not disturbed, and DO was measured again after 30 min. The difference between initial and final readings was recorded. Respiration rate was calculated per gram fish (mg/g/30 min). Twenty-five fish from each site were tested.

IV.7 Stamina

Fish stamina was measured by placing individual fish in a 2.8 L Erlenmeyer flask. A 63 mm magnetic stirrer was added to the flask/fish set-up and positioned on a Themolyne Nuova II stirring plate. Fish swam against a current generated (~127 cm/sec) by the fixed stirring action over a 15 min period and the time to exhaustion was recorded. Fish were deemed exhausted when they succumbed to the current's force, and were subsequently caught-up in the vortex. Twenty-five fish from each site were tested.

IV.8 Blood Collection & Analysis

Following standard fish hematological methods (Wedemeyer and Yasutake, 1977), whole blood was taken directly from the severed caudal peduncle of 15 fish that had been lightly anesthetized with MS-222. Blood was put into a 75 mm (50 µl) heparinized microcapilliary tube (Fisherbrand™) and sealed with tube sealant compound (Chāseal™). Microcapillary tubes were placed in an IEC microcapillary centrifuge and spun for five minutes at approximately 10,000 rpm, after which the tubes were read immediately to avoid CO_2-induced swelling of RBCs. The color of the supernatant was recorded and the hematocrit or percent (%) of packed red blood cells (PRBC) was calculated as follows:

$$\text{Hematocrit (\%)} = \frac{\text{Length RBC column}}{\text{Total length of blood column.}} \times 100$$

Using rainbow trout (*Salmo gairdneri*), Houston and DeWilde (1968) concluded that hematocrit values may be substituted for RBC counts and hemoglobin (Hb) determinations in routine evaluations of hematological status.

Differential blood smears were prepared from the 15 fish and slides were air dried, stained using Quickstep Wright-Giemsa stain (Fischer Scientific), rinsed in DI water, and allowed to air dry. The surface area of 100 RBCs was calculated using the ImageJ program (National Institutes of Health, 2006). Fish were subsequently placed in individual 50 mL Falcon™ tubes and fixed in 10% formalin.

IV.9 Gill Morphology

Gills were removed from preserved fish and placed in a Petri dish with DI water to keep wet and examined under a dissection microscope. Twenty fish from

each site were examined. Individual gill filaments and additional branches were counted and recorded, as were any other potential abnormalities.

IV.10 Statistics

One-way ANOVA, Tukey HSD analyses and Principal Component Analysis (PCA) were performed using Statistix 8® statistical package. Pearson and Canonical Correlations were run using SAS 9.1®. *P* values <0.05 were considered statistically significant.

RESULTS

IV.11 Respiration

Using a one-way ANOVA, highly significant differences ($p<0.001$, $F_{5,140}=7.9$) in respiration rates (mg/g) were found among sites, and two homogenous groups were revealed by a Tukey HSD test (Figure 4-1). RD had the highest mean respiration rate (mean=0.165 mg/g ±0.011SE) of all sites examined and made up the first group. VL (mean=0.127 mg/g ±0.012SE) was placed in an intermediate group. The remaining four sites were placed together in the second group as follows (mean ±SE): SK (0.110 mg/g ±0.011), MC (0.105 mg/g ±0.011), KC (0.086 mg/g ±0.011), and CC (0.085 mg/g ±0.011). Thus, all the sites with high parasite loads had low respiration rates, but so did KC.

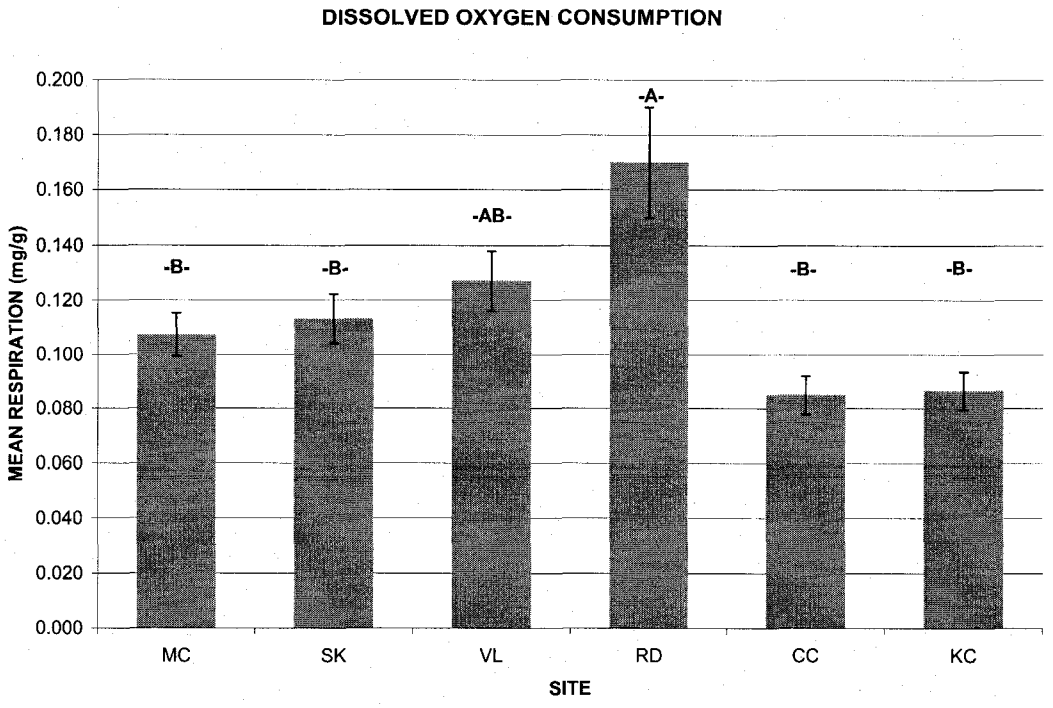


Figure 4-1. Mean dissolved oxygen consumption (mg/g) over 30 min.

A PCA determined that of the five gill parasites observed, the digenean trematode metacercariae of *A. p. diminuta* and *E. schwartzi* were accountable for approximately 60% of the total variability seen in mean gill parasite abundance (Chapter Three, Table 3-3). A Canonical Correlation was used to analyze potential correlations between these two species (*A. p. diminuta* and *E. schwartzi*) and various physiological components. Although a highly significant difference (Wilks' Lambda $p<0.001$) was found for all physiological components when compared against the two parasite species, only a small, insignificant relationship (0.1727) was found with respiration (Table 4-1).

Table 4-1. Canonical Correlation structure comparing *A. p. diminuta* and *E. schwartzi* against physiological variables.

	Canonical Variables	
Original Variables	*CV1*	*CV2*
A.diminuta	***0.9999***	0.0120
E. schwartzi	-0.1041	***-0.9946***
	W1	W2
PRBC	-0.0392	***0.7129***
RBC size	***0.9902***	-0.0292
Respiration	0.1727	-0.0100
Stamina	0.0492	***0.5164***

Wilks' Lambda $P < 0.001$, $F_{10,98}$

IV.12 Stamina

Significant differences (p=0.002, $F_{5,140}$=4.10) were found among sites when analyzed using a one-way ANOVA, and two groups of similar means found using Tukey HSD. VL had the greatest stamina averaging 11.46 min (±1.28SE). SK and RD were in the second group with the lowest mean stamina of 6.17 ±1.17SE and 4.19 ±1.17SE min respectively. The remaining sites were placed between the two groups as intermediates (mean ±SE): CC (8.77 ±1.17), KC (7.20 ±1.17), and MC (6.60 ±1.17) (Figure 4-2). Surprisingly, the populations with high gill parasites did not generally show reduced stamina as expected. Using a Canonical Correlation (Table 4-1), a significant (Wilks' Lambda p<0.001) moderately negative correlation was found between stamina (0.5164) and *E. schwartzi* (-0.9946), but not *A. p. diminuta* (0.0492).

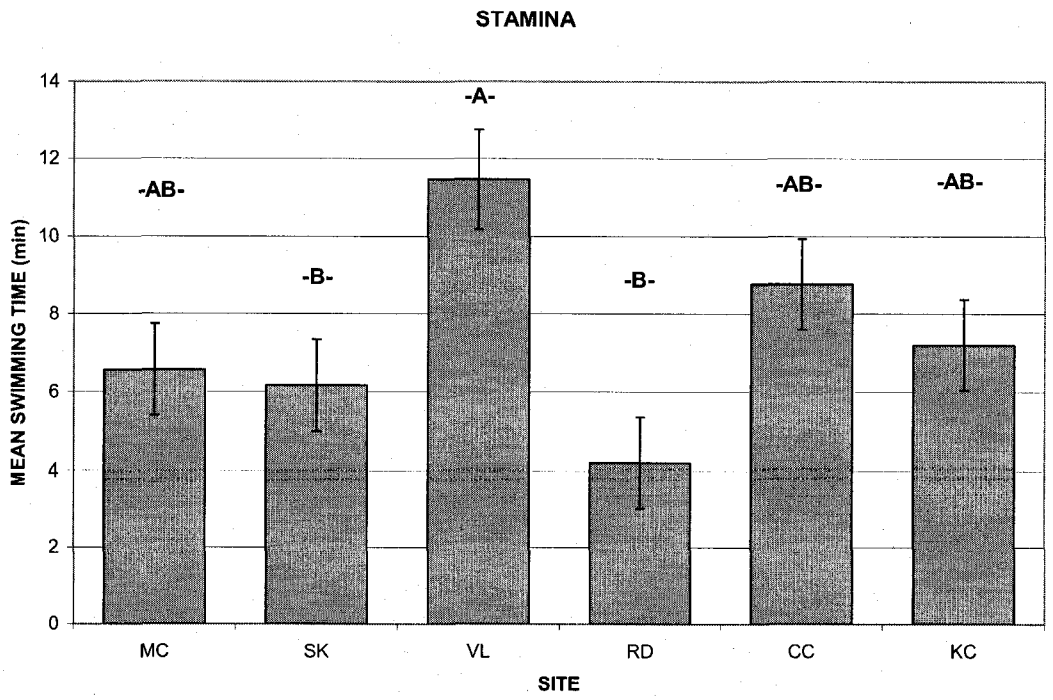


Figure 4-2. Mean (±SE) fish stamina (min) with Tukey HSD comparison for all sites.

IV.13 Blood Analysis

Using a one-way ANOVA, highly significant differences (p<0.001, $F_{5,84}$=52.6) in RBC area (mm) were calculated, and three groups of similar means were found using Tukey HSD. VL had the largest RBCs (mean=1.17E-09 ±4.22E-12SE) making up the first group. KC (mean=1.15E-09 ±5.39E-12); CC (mean=1.14E-09 ±4.78E-12); SK (mean=1.14E-09 ±4.04E-12); and MC (mean=1.13E-09 ±5.79E-12) were placed together in the second group. Finally, RD was determined to have the smallest RBCs (mean=1.07E-09 ±3.20E-12SE) and made up the third group (Figure 4-3). Highly significant differences (Wilks' Lambda p<0.001) in the size of fish RBCs (0.9902) were found to negatively correlate strongly with *A. p. diminuta* (0.9999) but not *E. schwartzi* (-0.0292) using a Canonical Correlation (Table 4-1). Thus, the site with the

fewest parasites had the lowest RBC size, suggesting that fish may increase RBC size to compensate for the added stress of high parasite infection.

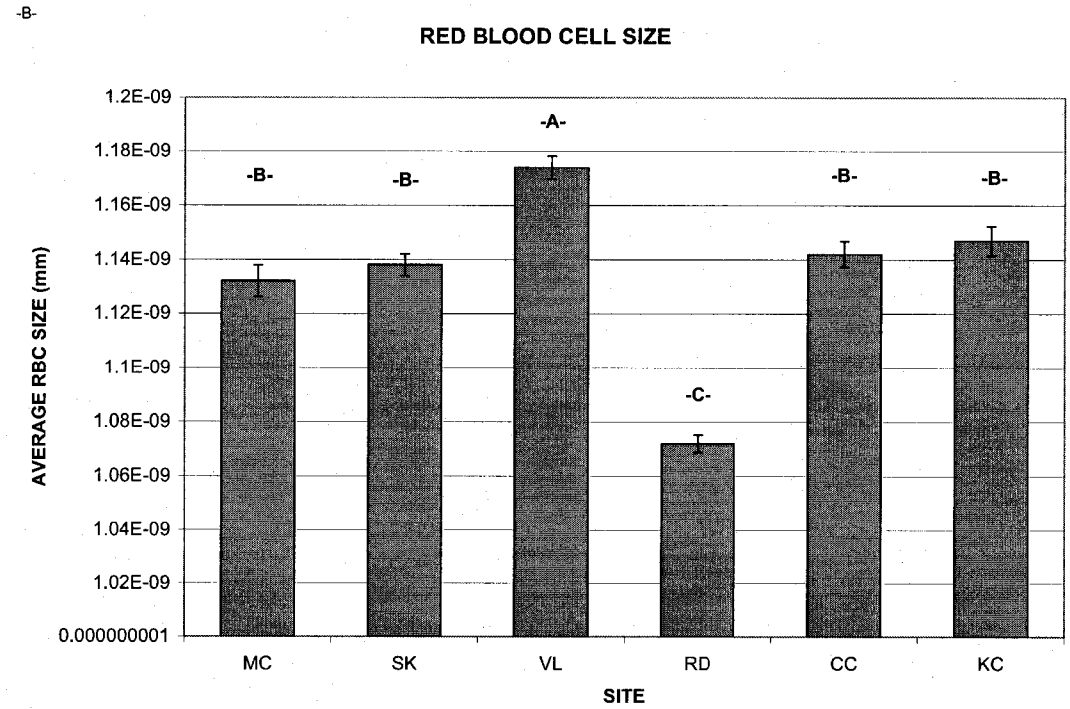


Figure 4-3. Mean (±SE) red blood cell size (mm) with Tukey HSD.

Highly significant differences (p=0.007, $F_{5,84}$=3.46) were also found among sites for PRBC volume (%) using a one-way ANOVA. Tukey HSD revealed two homogenous groupings. KC (mean=40% ±1.4SE) and SK (mean=38% ±1.9SE) were in the first group, followed by an intermediate group containing CC (mean=37% ±1.6SE), VL (mean=37% ±1.4SE) and MC (mean=36% ±1.7SE). RD had the smallest percentage and made up the last group with a mean of 32% (±1.0SE). Thus, the site with the fewest gill parasites had the lowest blood volume percentage and smallest RBCs, suggesting that high parasite infections may trigger erythropoiesis. The percentage of clear supernatant ranged from 93% (MC) to 73% (RD) (Table 4-2). Highly significant (Wilks' Lambda p<0.001) strong negative

correlations were found between PRBC (0.7129) and *E. schwartzi* (-0.9946) but not *A. p. diminuta* (-0.0392) using a Canonical Correlation (Table 4-1).

Table 4-2. Mean (±SE) PRBC % of total whole blood volume with Tukey HSD comparison and percentage of clear supernatant.

SITE	MEAN (±SE) PRBC%	TUKEY HSD	CLEAR SUPERNATANT
KC	39.9 ±1.4	A	80%
SK	38.0 ±1.9	A	93%
CC	37.3 ±1.6	AB	87%
VL	36.5 ±1.4	AB	87%
MC	35.3 ±1.6	AB	87%
RD	31.5 ±1.0	B	73%

IV.14 Gill Morphology

Highly significant ($p<0.001$, $F_{5,114}=6.98$) differences in the number of additional gill branches (Figure 4-4) were found among sites. Tukey HSD reported two groups in which the means were not significantly different from one another. The first group contained CC, VL and MC. CC had the greatest number of additional branches per fish (mean=10.7 ±2.7SE), followed by MC (mean=10.4 ±2.1SE) and VL (mean=9.5 ±1.5SE). SK (mean=6.9 ±1.2SE) was an intermediate. The second group contained KC (mean=2.5 ±0.6SE) and RD (mean=0.9 ±0.2SE). Some VL and KC fish had significant physical damage to their gills where numerous filaments were fused together, or large sections were missing altogether.

Figure 4-4. Abnormal gill filament (~6 mm) of *F. heteroclitus* with two additional branches emerging from the primary axis on right-hand side. Image taken at 300x magnification. Photo enlarged further to view detail.

A Pearson Correlation was used to compare branching and each of the five gill parasite species. Highly significant ($p<0.001$), positive correlations were found between branching and *A. p. diminuta*, (r=0.3606) and *E. schwartzi* (r=0.3131), but not *Dactylogyrus* sp., *F. prolongis* or *E. funduli* ($p>0.50$). Figure 4-5 below details the number of metacercariae (both species combined) found in relation to the number of additional gill branches observed. Two or more additional branches form with infections greater than 1000 metacercariae.

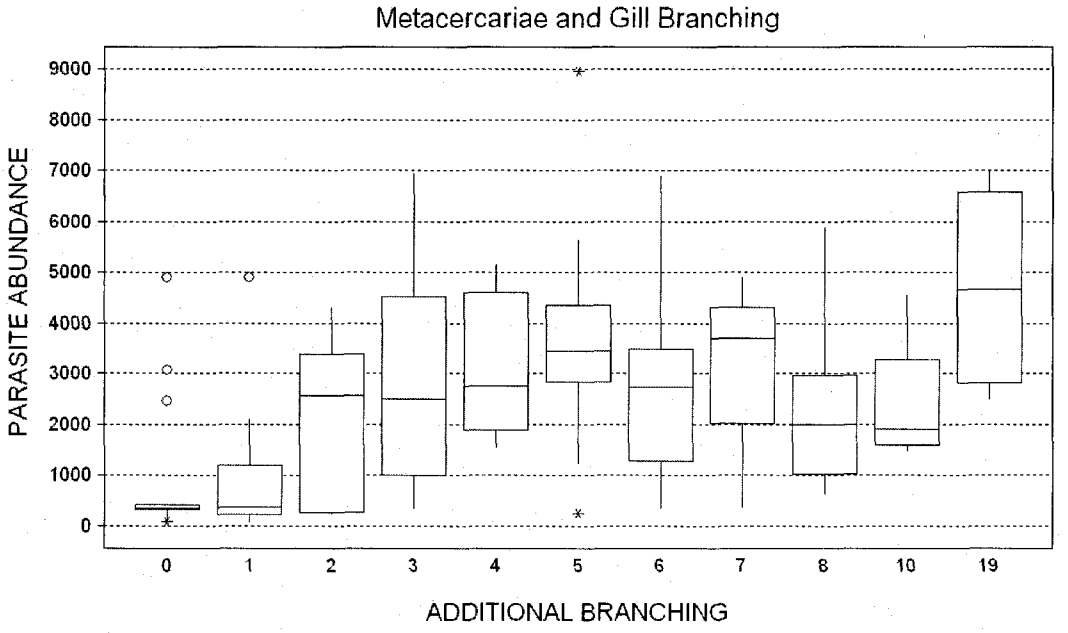


Figure 4-5. Box Whisker Plot comparing gill metacercariae parasite abundance (*A. p. diminuta* and *E. schwartzi* combined) and number of additional branches formed.

No significant differences ($p>0.05$) in the total number of gill filaments per individual were found among sites using a one-way ANOVA (Table 4-3).

Table 4-3. Mean number of filaments (±SE) with Tukey HSD comparison.

SITE	MEAN FILAMENTS	SE
KC	847.6	14.4
SK	809.9	10
CC	838.3	11.9
VL	835.4	12.5
MC	827.7	14.9
RD	824.1	10.3

A second Canonical Correlation (Table 4-4) was used to compare anatomical and physiological components against the physiological outputs of respiration and stamina. A highly significant (Wilks' Lambda $p<0.001$, $F_{8,168}=3.60$), strong negative correlation was found between respiration (-0.9885) and the number of gill filaments

(0.8012) per fish and a moderately positive correlation with RBC size (0.4488). Also, highly significant, strong positive correlations were found between stamina (1.0000) and the number of additional branches (0.7249), and a moderately positive correlation with PRBC (0.4312).

Table 4-4. Canonical Correlation structure comparing anatomical and physiological components against physiological outputs.

	Canonical Variables	
Original Variables	*CV1*	*CV2*
Additional Branches	0.1040	***0.7249***
Filament Number	***0.8012***	-0.4312
RBC Size	0.4488	0.1378
PRBC	0.3191	0.4608
	W1	W2
Respiration	***-0.9885***	-0.1512
Stamina	-0.0080	***1.0000***

Wilks' Lambda $P < 0.001$, $F_{3,60}$

DISCUSSION

As discussed previously (Chapter Three), highly significant differences in gill parasite abundance were found among sites, with two digenean trematode species (*A. p. diminuta* and *E. schwartzi*) making up the majority of parasites found. Highly significant differences in respiration were found among sites with RD having the highest overall respiration and the more parasitized fish (MC, SK, VL and CC) having lower respiration. However, KC unexpectedly had one of the lowest respiration rates. Although KC fish did not have heavily parasitized gills, some fish had significant physical damage to their gills where filaments were fused together, or large sections

were missing altogether. This physical damage may have contributed to the reduction in respiration observed.

There was a highly significant, strong negative correlation found between respiration and both gill filament number and RBC size. KC fish had the highest number of gill filaments and larger RBCs. As the number of gill filaments and RBC size increased, respiration rates decreased. While no significant differences in the number of gill filaments was found among sites, KC did have the greatest number of gill filaments overall (which was not due to body size as KC fish were neither the heaviest nor the largest). Fluctuations between hypoxic/anoxic and normoxic conditions have been reported in other studies throughout the Hackensack Meadowlands (Kraus and Bragin, 1989; Raichel *et al.*, 2003) and remains a seasonal problem (Iannuzzi and Ludwig, 2004). KC feeds into the Kingsland Tidal Impoundment which is served by a tide gate. Oxygen levels are known to fluctuate widely under these types of conditions and hypoxia (DO less than 3 mg/L) is not an uncommon occurrence. KC fish may be exposed to episodic hypoxic events during juvenile stages of development, which could have stimulated the growth of additional filaments and caused an increase in the size of erythrocytes (Weber, 1982) in adults. Both conditions have been associated with hypoxia. Another possibility is that parasites in other organs (that were not examined), may also be contributing to the results found here.

Highly significant differences in stamina were also found among sites. Contrary to numerous studies (Giles, 1987; Kunz and Pung, 2004; Östlund-Nilsson *et al.*, 2005 and others) in which parasitized organisms were found to have reduced stamina, fish with higher gill parasite abundances (MC, SK, VL and CC) surprisingly had higher stamina than less parasitized conspecifics (RD). Strong negative correlations were found between *E. schwartzi* and stamina. KC was grouped with fish

having high parasite abundances, but this may be explained by the number of additional branches and PRBC volumes calculated for these fish as a highly significant, moderately positive correlation was found between stamina and PRBC. Although KC fish had few metacercariae in their gills and did not have many additional branches, they did have the highest PRBC volume of all sites tested which may be a response to environmental conditions such as hypoxia, rather than parasites. Erythropoiesis is activated by hypoxia as a compensatory response (Härdig *et al.*, 1978) and may contribute to elevated Hb levels under prolonged hypoxic conditions which would explain the high percentage of PBRC reported for KC. The heavily parasitized fish from SK were also grouped with KC, followed by the other heavily parasitized fish suggesting that fish with heavy metacercariae loads may respond similarly to those experiencing hypoxic conditions.

Several previous studies (Dawson, 1933; Holland and Forster, 1966; Graham *et al.*, 1985; Wells and Baldwin, 1990) have found inverse relationships between RBC size and aerobic swimming ability. This seems to occur due to the larger surface area to volume ratios and shorter diffusion distances allowing more rapid oxygenation and deoxygenation of hemoglobin in smaller RBCs (Jones, 1979; Wilhelm Filho *et al.*, 1992). However, this is contrary to what was found in the present study. Although the least parasitized fish from RD had the smallest RBCs and highest respiration, they also had the lowest stamina. Conversely, VL which was one of the most parasitized sites had the largest RBCs, lower respiration and the greatest stamina. Although an increase in the size of erythrocytes has been associated with hypoxia, it is generally considered a stress response (Weber, 1982), which in this case may reflect the high number of parasites infecting the gills. The differences shown here for stamina are likely due to the added benefits of additional gill tissue produced by branching.

The only reference to gill branching (filament division) in the literature is made briefly by Hughes (1984) in his review of gill anatomy, stating that in some species (e.g., giant sturgeons [*Huso huso*] and the mudskipper [*Periopthalmus* sp.]), filaments sometimes divide at their tips, as either an anomaly during development, or perhaps as a result of physical damage. He also notes that it may be more common in some species than others. In this study, it appears clear that 'physical damage' (excessive endoparasite loads) is largely responsible for the significant differences found in gill branching as reflected in the highly significant correlation between gill branching and *A. p. diminuta* and *E. schwartzi* abundances. There appears to be a threshold of approximately 1,500 gill metacercariae that elicits an anatomical response in the fish to form additional branches. Once infections are greater than 1,500 metacercariae, the fish's gills are likely becoming compromised and no longer able to function as efficiently. Although there is a general relationship between the number of branches formed and the number of parasites, it is unclear why the most heavily infected individuals do not have the greatest number of branches. The wide scattering of points suggests that other factors may be at work (e.g., physiological, environmental). RD had the lowest number of additional branches while VL had one of the highest.

A highly significant, strong positive correlation was found between stamina and the number of additional branches. By increasing the overall respiratory surface area (and number of RBCs), it appears that increased stamina may be an unexpected side-effect, helping to explain why KC was more similar to MC, SK and CC, rather than RD, as KC averaged more than two additional branches per fish.

Wu (2002) describes three responses to hypoxia by marine organisms. First, fish will try to maintain oxygen delivery by increasing their respiration rate, number of RBCs, or the oxygen binding capacity of Hb. Next, fish will conserve energy (e.g., metabolic depression), and under prolonged exposure, will resort to anaerobic

respiration. *F. heteroclitus* with very high numbers of metacercariae are likely experiencing conditions similar to hypoxia as suggested by their larger RBC size. Although the parasitized fish in this study were unable to increase their respiration rate due to the number of parasites and/or physical condition of their gills, they did respond with an increased output of RBCs, resulting in higher PBRC volumes. More importantly, the anatomical anomalies (gill branching) reported herein appear to be a novel response by fish to counteract heavy parasite infections and should be placed with the first set of responses described above. The formation of additional branches appears to help heavily parasitized fish increase their overall stamina. Their ability to increase their respiratory surface in response to high parasite loads demonstrates the flexibility of these dynamic tissues and provides a glimpse into the co-evolutionary 'arms race' being waged between parasites and their hosts (Price, 1980; Seger and Hamilton, 1988). The next step in this 'arms race' is seen by the appearance of parasites in the new gill tissue of the branches.

CHAPTER V. PARASITES AND RESTORATION

INTRODUCTION

V.1 Parasites as Indicators of Restoration Success

Historically, wetlands were considered nuisances due to their mucky sediments, odors, and the insects they bred – a wasted land with no beneficial use. Today, salt marshes and other types of wetlands are gaining an increase in understanding and respect by the populace for their many vital functions. They are important in controlling floodwaters, recharging groundwater, contributing to improved air quality and filtering pollutants. They serve as critical habitat for various fish and wildlife including threatened and endangered species, and are known to act as nurseries for many commercially valued fish. Furthermore, these ecosystems offer societal value by providing recreation areas, education, fisheries, research, and aesthetic significance as well (Ferren *et al.*, 1995). Wetlands in general are among the most productive ecosystems in the world, due to their ability to not only capture significant amounts of solar energy, but to store it (chemical energy) and efficiently recycle what is produced (Niering, 1998).

As far back as the 19th century, governments have tried to rid the landscape of wetlands by filling or draining them in to make the land "more productive" (e.g., agriculture), with legislation such as the Federal Swamp Land Act of 1850. Over time, fragmentation, water quality degradation, the introduction of invasive plants and animals, and other forms of environmental disturbance have impacted the remaining estuarine habitats (Flack and Benton, 1998; Zampella and Bunnell, 1998). Loss of tidal marsh habitat over the last several decades, combined with the importance of these habitats as nurseries for commercially important finfish and shellfish (Talbot

and Able, 1984; Miltner *et al.*, 1995), and as breeding grounds for many bird species (Erwin, 1987) has led to an increased effort to replace what has been lost due to human recklessness.

Restoration activities vary with the extent of degradation the environment has experienced including ditching, filling, diking, and pollution. Along the eastern coast of the United States, typical restoration activities are regulated and include the removal of invasive plant species such as *Phragmites australis* through chemical means (herbicides), and/or mechanical (dredging, mowing, burning) processes. More desirable, native plant species (e.g. *Spartina* spp.) are then put in their place for sediment stabilization and wildlife use. This process also commonly includes the resurfacing of the marsh topography (e.g., reduction of uplands) that not only increases the available area of mud flat, but also improves tidal flow to both the lower and upper marsh.

In the past, evaluations of the success of a restoration project have been limited to vegetation communities (LaSalle *et al.*, 1991; Rozas and Reed, 1993) and some animal/infaunal communities (Rozas and Reed, 1993; Posey *et al.*, 1997). To date, only one group of scientists using the Carpinteria salt marsh in California as their study site, have looked into the potential use of parasites as indicators of wetland restoration success. Over a six year period following the restoration, Huspeni and Lafferty (2004) used larval trematode parasites of the California horn snail (*Cerithidea californica*) as an indicator of restoration success and found that trematode prevalence nearly quadrupled at restored sites while control sites remained unchanged. Also, species richness at the restored sites increased two-fold, while unrestored sites remained the same. The restoration grading process likely increases potential habitat for both pelagic (e.g., fish as second intermediate host) and benthic (e.g., snails as first intermediate host) organisms. These newly

established communities then attract wading birds (final/definitive hosts) (Seigel *et al.*, 2005), thus enriching the wetland's trematode community (Zampella and Bunnell, 1998; Huspeni and Lafferty, 2004).

As a consequence of their indirect life cycle, digenean trematodes typically require a mollusc as its first intermediate host. As habitat quality is improved following the restoration process, suitable substrate for these benthic invertebrates also increases. As such, more gastropods should be found in restored sites than unrestored. Common estuarine species include the coastal marsh snail *Littoridinops tenuipes,* the periwinkle *Littorina littorina*, the mud snail *Ilyanassa obsoleta,* and the salt marsh hydrobe *Spurwinkia salsa*. Due to their life histories, each species could potentially act as the first intermediate host. They are highly gregarious, and are found in the intertidal zone (most commonly on mudflats and the salt marsh), which provides an abundant food base as they are all detritivores and deposit-feeders (Thompson, 1968; Davis *et al.*, 1982; Curtis, 2005).

V.2 Rationale

Early studies (see Chapter 2) found that fish from two restored tidal marshes located in the Hackensack Meadowlands District (MC and SK) had several thousand gill metacercaria, compared to fish in an unrestored tidal marsh within the same region (RD), which had only several hundred metacercaria per individual. Little research has been done to examine relationships of parasites to restored tidal marshes. A more focused experimental effort on the fish of restored and unrestored sites within the District was undertaken to investigate if the process of restoration is influencing either parasite or snail (first intermediate host) abundance (Hyp. 3).

V.3 Study Sites

Six sites were examined within the Hackensack Meadowlands District, three of which were restored; Mill Creek (MC), Skeetkill Creek (SK), Vince Lombardi (VL), and three which were not restored; Kingsland Creek (KC), Richard W. DeKorte Park (RD), and Cedar Creek (CC).

MATERIALS AND METHODS

V.4 Snail Abundance

The coastal marsh snail *Littoridinops tenuipes* (Couper) was chosen as the potential first intermediate host because other snail species (e.g., *L. littorina*, *I. obsoleta, S. salsa*) were not found despite intensive searches. Snail populations of *L. tenuipes*, believed to be the first intermediate host for the digenean trematode species found encysted in *F. heteroclitus* gills were estimated. The coastal marsh snail *L. tenuipes*, also commonly known as Henscomb Hydrobe, is a small snail with a conical shell that's approximately 2.4 to 4.8 mm in length, with five to six whorls. The shell is light brown or olivaceous in color, with an oily luster. The snail itself has a heavily pigmented foot, pedicel and snout. They are presently known from Maine to Florida, typically inhabiting fresh- and brackish waters, and found on detrital mats of the mudflat (Thompson, 1968). Like others in the Hydrobiidae Family, *L.* tenuipes is a browser, consuming fine particulate matter and other microorganisms found on the marsh surface. Little is known about their life history, but it is thought that they live two to three years (Natural_Heritage, 2004).

The numbers of snails occupying the immediate area surrounding the study sites were estimated by sampling mud flats at low tide between June and September of the summer of 2006. Six surficial sediment samples were taken using watch

glasses (area=23.75 cm^2) at approximately 2 m intervals, parallel to the marsh surface. Samples were returned to the lab where they were rinsed through a 1 mm^2 mesh sieve. Snails were collected once a month over a three month period and their numbers recorded.

V.5 Environmental Conditions

Dissolved oxygen and salinity were checked at all six sites to determine whether there were any fundamental abiotic differences between sites. Salinity readings were taken using a hand-held refractometer. Three separate DO readings were taken at each site using a YSI DO meter (model 51B), both at the surface and at approximately 60 cm below surface (bottom). All readings were taken on one occasion in August 2006, within a two hour window of one another.

V.6 Statistics

The Kruskal-Wallis test was used to compare mean intensities of snails among sites. A Pearson correlation was used to analyze relationships between snail abundance and parasite abundance. A two-sample *t*–test was used to compare salinity and DO means between restored and unrestored sites. *P* values <0.05 were considered statistically significant. Analyses were performed using Statistix 8 statistical package.

RESULTS

V.7 Snail Abundance

Highly significant differences ($p<0.001$, $F_{5,102}=126$) were calculated among sites sampled using a Kruskal-Wallis one-way nonparametric ANOVA, with three

groups of homogenous means found with an all-pairwise comparisons test (Figure 5-1). Overall SK, VL and MC; the three restored sites, had more snails than the three unrestored sites (CC, RD and KC). SK and VL were placed together with similar means in group one (Kruskal-Wallis χ^2=93.83 and 83.56 respectively). MC and CC were placed as two separate intermediates (Kruskal-Wallis χ^2=65.61 and 42.58 respectively). The third group contained RD and KC with (Kruskal-Wallis χ^2=23.19 and 18.22 respectively).

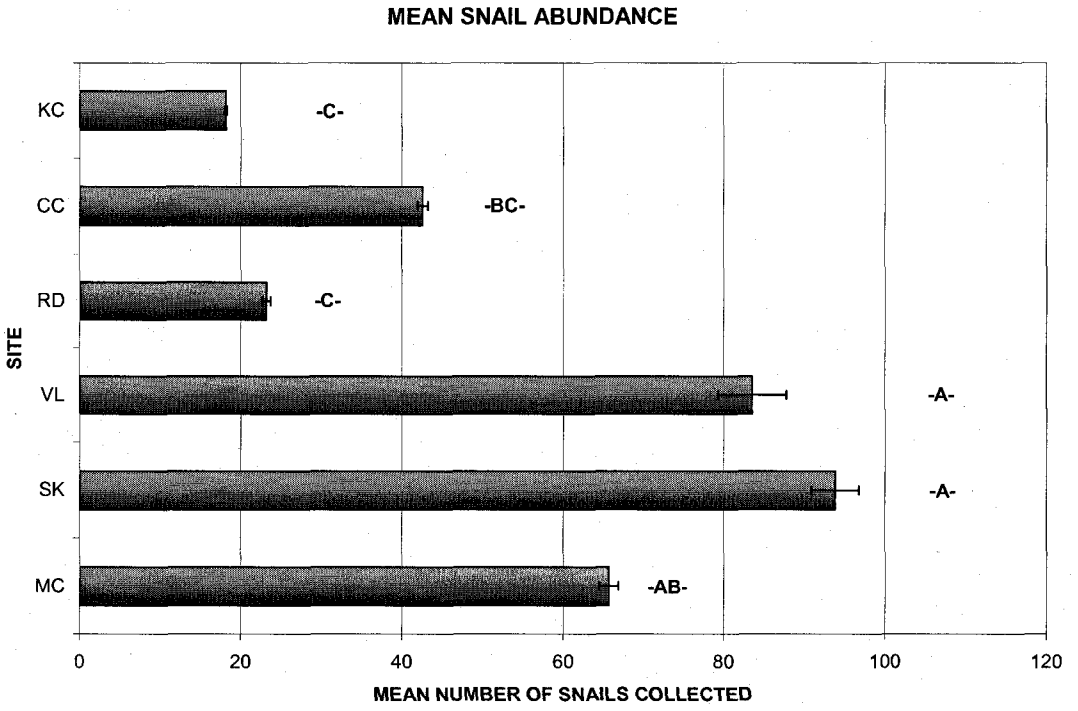


Figure 5-1. Mean number of snails collected from restored and unrestored sites.

Several snail specimens from the restored sites were dissected to determine whether snails maintained any viable digenean trematode infections. Large cercariae were found occupying the reproductive organs within the visceral mass. These cercariae are brownish with pronounced black eyes, with two bands of black pigment dorsolaterally in position. Additionally, extremely large masses of metacercariae were

found in the reproductive organs within the visceral mass (M. Mazurkiewicz pers. comm.).

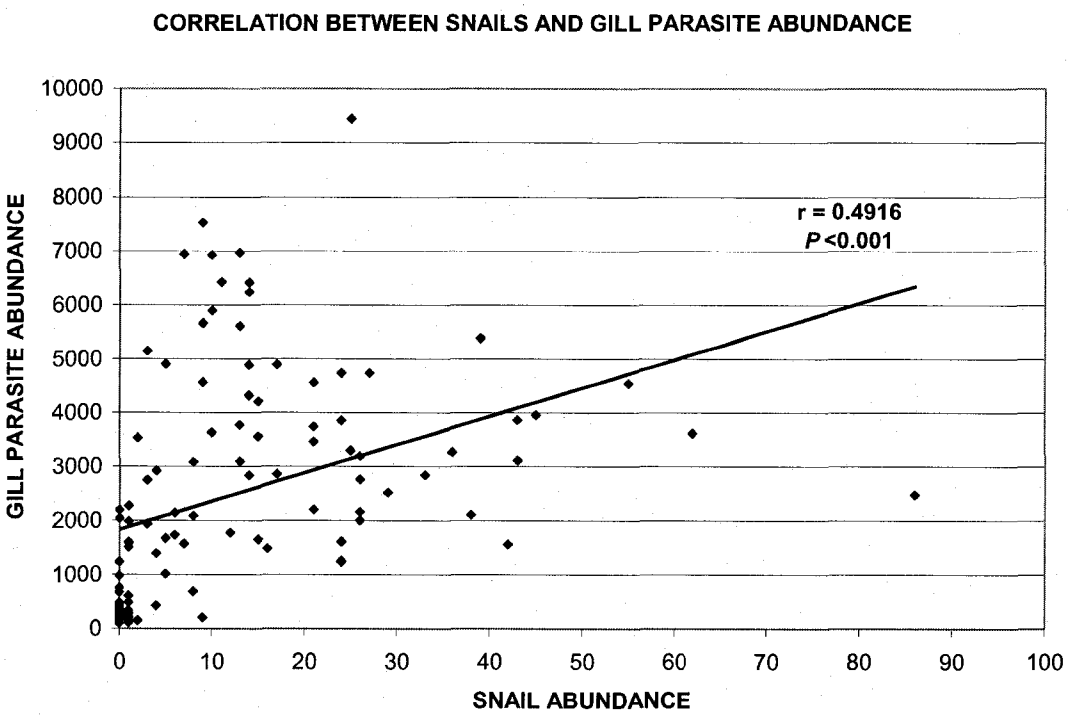


Figure 5-2. Correlation between gill parasites and snail abundance.

A Pearson correlation was used to examine the relationship between mean gill parasite abundance and mean snail abundance. Although a highly significant difference (p<0.001) was found among sites, a correlation (r=0.4916) was found between the two factors (Figure 5-2).

V.8 Environmental Conditions

Significant differences in dissolved oxygen for both surface (p<0.001) and bottom (approximately 60 cm) (p=0.010) samples were found between the restored (MC, SK, VL) and unrestored (RD, CC, KC) sites using a two-sample t-test. Surface DO levels at restored sites ranged from 5.3 to 5.6 mg/g and were higher (mean=5.4

±0.1SE, N=9, t=4.81) than unrestored sites (mean=4.9 ±0.1SE, N=9, t=4.81) that ranged from 4.5 to 5.4 mg/g. Bottom DO readings were also higher (mean=5.0 ±0.1SE, N=9, t=3.03) at restored sites than unrestored sites (mean=4.5 ±0.2SE, N=9, t=3.03) with a range of 4.7 to 5.4 mg/g versus 4.1 to 5.2 mg/g at the unrestored sites. Overall, of the six sites tested, RD had the lowest DO range (surface mean=4.6 mg/g; bottom mean=4.2 mg/g), while MC had the highest (surface mean=5.5 mg/g; bottom mean=5.3 mg/g, Table 5-1).

Highly significant differences (p<0.001) in salinity were also found between restored (mean=5.3 ±0.2SE, N=9, t=-21.21) and unrestored sites mean=10.3 ±0.2SE, N=9, t=-21.21). Salinity was lowest (5-6 ppt) at the restored sites (MC, SK, VL), and higher (10-11 ppt) at the unrestored sites (RD, CC, KC) as shown in Table 5.1.

Table 5-1. Salinity and dissolved oxygen readings across sites.

SITE	AVG SALINITY (ppt)	AVG SURFACE DO (mg/g)	AVG BOTTOM DO (mg/g)
MC	6	5.5	5.3
SK	5	5.4	5.0
VL	5	5.4	4.8
RD	10	4.6	4.2
CC	10	5.3	5.1
KC	11	4.7	4.3

DISCUSSION

As discussed earlier in this study (Chapter 3), highly significant differences were recorded among sites with respect to gill parasite abundance. Restored sites (ML, SK, and VL) had the highest mean number of gill parasites overall. Though five species were commonly found across all sites, there were two digenean trematode species that were dominant in both abundance and prevalence: *A. p. diminuta* and *E.*

schwartzi, either of which could potentially use this gastropod (*L. tenuipes*) as their first intermediate host. Highly significant ($p<0.001$) differences in the number of snails collected were also found with the greatest mean number recorded from the three restored sites (MC, SK, VL). Snails from the restored sites were found to contain extremely high numbers of parasite cercariae. A significant, yet small correlation (($r=0.4916$, $p<0.001$) between gill parasite and snail abundance was calculated.

Subsequent changes incurred to salt marsh habitats following restoration include an increase in the total area of mud flats which are important contributors to primary production and to the breakdown of organic materials. The bacteria and algae that thrive on both the mud flat and detrital mat are important components to the health and well-being of gastropods and other grazers like *L. tenuipes,* as they require fine particulate matter and other microorganisms for their survival. One study comparing the population structure, growth and fecundity of snails (*Melampus bidentatus*) from natural and restored marshes, found that snails from a restored marsh grew more rapidly and were larger than conspecifics from the natural marsh. Furthermore, not only did these larger snails deposit almost twice as many egg masses, but the masses were also significantly larger than those from the natural marsh (Spelke *et al.*, 1995). Thus, with the increase in suitable substrate (mud flats), there should not only be a subsequent increase in the number of organisms that utilize the available resources (producers), but also a potential increase in those consumer's total biomass. Subsequently, there will be an overall improvement in the trophic complexity including primary consumers (gastropods), secondary consumers (fish) and tertiary consumers (wading birds). With the increased utilization of these areas by wading birds (Seigel *et al.*, 2005), the abundance and diversity of parasites

may also potentially increase (Joklea and Lively, 1995; Galaktionov, 1996; Huspeni and Lafferty, 2004).

Although there seems to be an increase in both gill parasite and snail abundance following the restoration process, there are several plausible explanations behind this phenomenon. Whether they are acting singly or in conjunction with other factors needs to be studied further. One such explanation has been put forth by Coyner *et al.* (2003). They discuss two ways by which anthropogenic eutrophication (with sewage effluent) of wading bird foraging areas may be contributing increased prevalence of infected intermediate hosts (fish). "First, eutrophication can result in increased fish densities that will attract larger numbers of birds. Secondly, the attraction of larger numbers of birds increases the chances of an infected bird contaminating the site." The Hackensack Meadowlands is a highly urbanized wetland system. SK, MC and VL all experience varying levels of anthropogenic impacts and nutrient enrichment. SK and MC both have direct inputs of local stormwater runoff into their creeks via outfalls. VL is adjacent to a NJ Turnpike rest stop and also receives a great deal of runoff. Although KC runs underneath a major traffic thoroughfare (the NJ Turnpike), and RD has connections to a former (now closed) landfill, KC, RD, and CC do not have any known direct discharge outfalls. This possible nutrient enrichment, coupled with the restoration grading process, also likely serves to increases primary production, hence consumer abundance.

Highly significant differences in abiotic conditions (DO and salinity) were found between restored and unrestored sites with restored sites having the highest DO and lowest salinity. Lower salinities were expected at the restored sites due to their more northerly, upstream location. Higher DO levels were found at the restored sites for both surface and bottom readings compared to the unrestored sites. Although brackish water organisms are highly tolerant of rapid and widespread changes in

abiotic conditions, it is unknown how, or if, these significant differences in DO and salinity affected parasite/snail/fish associations, or if they contributed to the high levels of infection found. Although these readings were taken at essentially the same time (within a 2 hr window) at all sites, they were measured on only one occasion, which creates a significant level of uncertainty.

One unrestored site (CC) had comparable mean parasite abundances to the restored sites (MC, SK and VL) yet had significantly fewer snails. One reason for this could be that the site is relatively close to a rather large (83 ha) wetland mitigation bank, Marsh Resources, Inc. (MRI). Doctor's Creek flows through this restored tidal marsh. It connects directly to the Hackensack River, which in turn connects to Cedar Creek further downstream. If MRI is similar to the other restored sites there may be a large snail population supporting parasite reproduction. Most digenean trematode cercariae live approximately 24 hours (Fingerut *et al.*, 2003) and are strong swimmers with muscular tails (Lafferty, 2002). This infective stage has been previously observed (Jimenéz-García and Vidal-Martínez, 2005) traveling distances of approximately 50 m by means of both active (e.g., swimming) and passive (e.g., water current) transport. Some cercariae emerging during flood tides have been transported 100 – 300 m/h (Fingerut *et al.*, 2003). However, it is unknown whether the tidal currents within the Hackensack River are strong enough to carry the cercariae far enough downstream to CC, or whether the cercariae will survive and/or remain infective long enough to reach CC. Another possibility is that there is a mixing of *F. heteroclitus* populations between the two sites. If MRI is similar to the other restored sites, its fish should also be highly infected with digenean metacercariae. Either or both of these scenarios could help explain the large number of digenean trematodes observed at CC.

The wetland restoration process, however beneficial in the long run, is still an act of disturbance with activities such as grading to increase tidal flow, removal of invasive vegetative species, etc. Restoration may not be beneficial to all species. If it is beneficial to parasites, it may not be beneficial to their hosts. Problems related to parasites may include competition among prey species that have now been released from their parasites or difficulties in catching parasite-free fish by piscivorous birds (Huspeni and Lafferty, 2004). Conversely, avian predators may potentially be at risk by eating a disproportionate number of highly infected prey items. Moore (1983) reported that higher than expected numbers of infected starlings (*Sturnus vulgaris*) were observed given the prevalence of the parasitized isopods they feed on. This was probably due to the higher encounter rates between the birds and their behaviorally modified prey. It has already been shown that migratory birds are more apt to suffer parasite infections than their residential cohorts (Figuerola and Green, 2000), and in response, have subsequently increased the size of their immune defense organs (the bursa of Fabricius and the spleen) (Møller and Erritzøe, 1998). Although many researchers have not found differences in host [bird] fitness when comparing infected and non-infected individuals, differences in the level of infection intensity (e.g., heavy vs. light), may occur (Hudson, 1999). It has been suggested by McNeil *et al.* (1994) that heavy helminth infections may be an important factor contributing to aberrant migratory behaviors demonstrated in shorebirds. Other potential implications include an increase in mortality rates of both first (Huxhma *et al.*, 1993) and second intermediate (Coleman and Travis, 1998) hosts, and the likelihood of significant behavioral (Lafferty and Morris, 1996; Levri, 1999) and physiological (Probst and Kube, 1999; Dezfuli *et al.*, 2003) changes in the host. Having gills saturated with parasite metacercariae would be expected to pose some stress to the fish.

Typically, high prevalence and abundance of parasite species indicate a healthy environment that likely has all of the necessary intermediate and definitive hosts (Overstreet 1997). However, when species richness is reduced due to the dominance of one or two species as found in the restored sites, it is likely that there are other factors in play. Perhaps this is a stress response resulting from restoration. If so, once the restored sites 'mature' over time, perhaps the fish will begin to resemble unrestored populations more. The significant differences observed in the levels of digenean trematode infections of fish between restored and unrestored habitats necessitates additional investigations into potential consequences to all of the hosts involved in their life cycle and their relation to the restoration process.

CHAPTER VI. DISCUSSION

Several hypotheses were tested to investigate the infracommunities of the fish *F. heteroclitus* and how they vary in abundance and diversity both spatially and temporally. More focused examinations ensued, specifically on those parasites occupying the gill region and their effects on host behavior, anatomy and physiology. These effects were then put into context with respect to environmental conditions including site restoration.

VI.1 Environment

Several hypotheses were put forth with respect to parasite infracommunities. First, it was hypothesized that parasite infracommunities of fish from polluted sites would have higher abundances, and lower species richness, diversity and evenness when compared to relatively 'cleaner' sites. Parasitized hosts have an additional stressor compromising their health in an already stressed (polluted) environment. Jokela *et al.* (2005) found that freshwater clams (*Anodonta piscinalis*) infected with digenean trematodes (*Rhipidocotyle campanula* and *R. fennica*), had increased mortality rates compared to uninfected clams when starved or exposed to anoxic conditions. Although it was found that more polluted sites typically did have greater overall parasite abundance, especially in the gills and digestive cavity, a general increase in parasite species richness was also found at these sites, which was contrary to what was hypothesized. MC was the second most polluted site, yet had the highest species richness. However, despite the surprisingly high species richness reported, due to the high abundances found at these stressed sites, overall species diversity and evenness were reduced as expected.

It was also hypothesized that temporal differences would be found; with an increase in parasite abundance in late season collections. Overall, there were

differences found for seasonal collections both between- and within-years. Higher annual parasite abundances were found in late season samples as hypothesized. However, unexpectedly, there were surges in numbers of specific parasite species within particular organs/habitats, specifically digenean trematodes of the gut in early season samples. Rather than generate a broad sweeping hypothesis about seasonal change in abundance, perhaps a more precise question should have been formulated testing differences between organ/habitat or species/guild. Differences in species richness did not generally follow the same pattern as abundance, as mean species richness was usually higher for early season samples. Additionally, results indicate that the parasite infracommunities of *F. heteroclitus* resemble structured assemblages with individuals occupying distinct niches within their host.

The last hypothesis examined whether *F. heteroclitus* males would be more heavily parasitized than female conspecifics. Although there were no significant differences found between sexes for mean parasite abundance, differences were found for a specific organ/habitat, the gut. Females had significantly more digenean trematodes in their gut than their male counterparts, with the greatest abundance in early season collections in fish from a high salinity site (SH). These trematodes are believed to be newly excysted adults of *H. pallidum*. While examining *H. pallidum* in *F. heteroclitus*, Manter (1926) also found numerous metacercariae and very young worms in the guts of *F. heteroclitus*. Despite the large number of the immature worms, infection with the adult form was never heavy and he found that typical infections ranged from two or three worms per fish host.

Environmental factors including contaminants and salinity were examined with respect to sites and their parasite infracommunities. It was determined that both contaminants and salinity play a roles in structuring these communities, such as the case described above for the unidentified trematode thought to be *H. pallidum*.

Parasite communities in fish from TK, SH and UB were associated with salinity at times, yet fish from MC, PC and RD were consistently associated with contaminants. Although gill metacercariae (*A. p. diminuta* and *E. schwartzi*), *E. ignotus,* and the unknown acanthocephalan species were associated with pollutants (e.g., Hg, Pb, Zn), most of the other 11 parasite species were influenced either by salinity or another unexamined environmental factor.

Species richness is generally thought to be a useful measure of the severity of pollution (Sheehan, 1984). The fact that MC stood out from the other six sites (e.g., species richness and parasite abundance) despite its sediment contaminant levels, indicates that pollution may not have been the only factor involved. However, if the diversity index were only considered, MC was very low (due to the extremely high numbers of gill parasites), which could reflect a stressed environment. As MacKenzie *et al.* (1999) pointed out, it is very difficult to link parasite population levels with pollution without considering other abiotic and biotic factors. As such, it was important to determine whether the fact that MC was restored had any influence on the differences observed. Site disturbance of any kind, even with good intentions (e.g., restoration), has the potential to change community structure for either or both parasites and hosts.

VI.2 Restoration

In this study, a relationship of high gill parasites to restored habitats was seen. The importance of saving and restoring degraded wetland habitats and the species affiliated with these systems is undeniable. However, the conservation of their parasitic counterparts has received little attention (Rozsa, 1992). For example, the removal of a parasite species could have negative effects on the system as a whole because they are partially responsible for the evolution of their host's genetic diversity

(Dawkins and Krebs, 1979; Rozsa, 1992). Also, high parasite species diversity could protect hosts against selective pressures exerted by high numbers of a single parasite species (e.g., competition between parasite taxa) (Holmes, 1979). To date, only one group of scientists has looked into the potential use of parasites as indicators of wetland restoration success. Huspeni and Lafferty (2004) found that trematode prevalence nearly quadrupled at restored sites while control sites remained unchanged. Also, species richness at the restored sites increased two-fold, while unrestored sites remained the same. The restoration grading process likely increases potential habitat for pelagic and benthic organisms, which then attracts wading birds (Seigel et al., 2005), thus enriching the wetland's trematode community (Zampella and Bunnell, 1998; Huspeni and Lafferty, 2004). In this study, MC was also found to have the highest species richness and the highest species abundance (due to gill metacercariae) but the lowest diversity when compared to unrestored sites.

Hechinger *et al.* (2006) proposed the use of larval trematode abundance as an indicator of free-living diversity. However, they found inconsistent associations between larval trematodes in *C. californica* and fish communities, which they attributed to inadequate sampling methods for highly mobile fish. In the present study, some evidence in support of the use of trematode metacercaria as a 'bioindicator' has been found. Higher digenean trematode metacercariae infections were found in fish from restored sites (>2,000 metacercariae) compared to unrestored sites (<2,000 metacercariae), with MC having the greatest overall mean abundance. Snail abundance was also found to be higher at these restored sites, many of which were also highly infected with trematode cercariae.

Similar to free-living organisms, parasites occupy suitable habitats (hosts) that are limited with regard to the amount of space and resources available. It had been

generally assumed that once inside the intermediate host, parasites became dormant as they waited to be transferred upward to the definitive host. However, recent studies now reveal just how dynamic these parasite populations truly are, and what implications extremely high levels of infection may have on both its host and the parasite's future. Fredensborg and Poulin (2005) examined natural populations of the crab; *Macrophthalmus hirtipes*, and its parasite community (an acuariid nematode; trematodes *Maritrema novaezealandensis*, and *Levinseniella* sp.; and an acanthocephalan, *Profilicollis* spp.). They showed that when large parasite infrapopulations of the *M. novaezealandensis* metacercariae were present within an intermediate host, the parasites were smaller on average. These smaller metacercariae then produced smaller in vitro adult worms, which in turn produced fewer, smaller eggs (Brown *et al.*, 2003; Fredensborg and Poulin, 2005).

VI.3 Behavior

A relationship between high numbers of gill metacercariae and altered behavior was seen in this study. These behaviors, swimming closer to the water's surface and making more conspicuous movements, are likely to make the infected hosts more apt to be seen and consumed by predators that would be the final hosts for the trematodes. Kramer *et al.* (1983) confirmed that the risk of predation increases for fish nearer the surface when using a heron as a predator. Two independent studies using two different fish-parasite systems; the ninespine stickleback (*Pungitius pengitius*) with the cestode *Schistocephalus* sp. (Smith and Kramer, 1987) and the fathead minnow (*Pimephales promelas*) with the fluke *Ornithodiplostomum ptychocheilus* (Radabaugh, 1980), both reported finding parasitized fish swimming closer to the water's surface than unparasitized conspecifics. Similar findings have been reported by others, including Medoc *et al.*

(2006) where field studies examining the vertical distribution of *Gammarus roeseli* reported a higher proportion of individuals infected with the acanthocephalan *Polymorphus minutus* at the water's surface compared to uninfected individuals. Furthermore, the size distribution of infected gammarids revealed predation pressure on infected individuals by piscivorous birds, the definitive host. The body cavities of MC (restored) and RD (unrestored) fish were also examined for acanthocephalans in Chapter One and were found to be comparable mean=9.1 and 8.3 respectively). Yet when tested, MC fish remained closer to the water's surface and performed more conspicuous behaviors than RD fish. This suggests that other factors are likely contributing to these behavioral changes, namely the high metacercariae infections in the gills.

One argument against parasite-induced behavioral alterations has been the possibility of interactions between site conditions and learned response. A question that had been posed was whether the sites with the highest numbers of gill parasites also might have had the lowest DO. If that were the case, the argument could be made that perhaps these fish were found to surface more frequently in response to hypoxic conditions, rather than to a parasitic manipulation (either indirect or direct). What was seen in the lab could have been fish expressing a learned behavior of surfacing to remain closer to the air/water interface where DO levels are highest. However, the investigation of the abiotic conditions found with respect to DO levels found that the restored sites had the highest levels of DO. The fact that RD had the lowest overall DO of all sites examined may have been a contributor to its surface seeking behavior of fish from that site. Although there was only one data point per site for DO conditions, it helps support the idea that the behavioral differences seen at the restored sites is probably not due to environmental stressors like hypoxia, but rather a parasite manipulation causing the fish to remain at the surface, thus

increasing trophic transmission. Bethel and Holmes (1973) tested this argument using the amphipod-acanthocephalan association and found that oxygen did little to influence the surfacing behavior of *G. lacustris* when infected with *P. polymorphus*.

The mechanism behind these behaviors is still unknown. Consequently, behavior should not be looked at without also considering host physiology. Perhaps the parasites are releasing a sort of allomone (a chemical produced to evoke physiological or behavioral reactions that favor the parasite) into the fish's bloodstream as suggested by Whittaker and Feeney (1971) that could induce both the surfacing behavior and conspicuous behaviors reported herein. When injected with serotonin, uninfected amphipods briefly exhibited similar skimming, clinging and photophilia behaviors as their infected (*P. paradoxus*) counterparts (Bethel and Holmes, 1973; Helluy and Holmes, 1990). Similarly, Maynard *et al.* (1996) found evidence that the ventral nerve cords of infected amphipods also contained more serotonin-like substances than uninfected amphipods. Thus, the behavioral responses shown in this study (surfacing, conspicuousness) by fish from the restored sites (MC, VL and SK), could result from either a direct or indirect response to the high levels of gill parasite infections observed.

There appears to be an infection threshold of approximately 1,500 gill metacercariae when fish begin to exhibit significant behavioral modifications. Once these infections are greater than 1,500 metacercariae, direct and/or indirect parasite affects appear to take place, altering the fish's normal behavior. A study examining the behavior of mosquito larvae (*Aedes aegypti*) infected with the metacercariae of *Plagiorchis noblei* also found a threshold with the level of parasite infection and behavioral modifications (Webber and Rau, 1986). Using four groups of larvae with infections ranging from zero to more than three metacercariae, Webber and Rau (1986) found that larvae infected with three or more metacercariae were less active

and spent more time suspended from the surface of the water than uninfected larvae. This behavior facilitates transmission to the parasite's definitive mammal host (e.g., the mole *Microtus pennsylvanicus*) that forages at the water's edge. Two or fewer metacercariae resulted in larvae spending more time at the bottom of the tank where *P. noblei* cercariae settle, thus increasing their susceptibility to additional infection.

Although it was hypothesized that significant differences in activity levels could either increase or decrease, no significant differences were found. However, should this activity study be repeated in the future, in could be modified and more time could be given in each trial (rather than only one min), and/or fish could be videotaped rather than have an observer present. However, considering that highly significant differences were observed in other behaviors (e.g., surfacing, conspicuousness), it does not appear that an increase or decrease in overall activity would be important as predation of infected fish will already be enhanced.

Avian predators in these systems may be at risk by eating a disproportionate number of highly infected prey items. It has already been shown that migratory birds are more apt to suffer parasite infections than their residential cohorts (Figuerola and Green, 2000), and in response, have subsequently found ways to increase their immunity (Møller and Erritzøe, 1998). Although researchers have not been able to find differences in host [bird] fitness when comparing infected and non-infected individuals, differences in infection intensity are present (Hudson, 1999). McNeil *et al.* (1994) suggested that heavy helminth infections may be an important factor in aberrant shorebird migratory behaviors due to the physiological disorders they may cause.

VI.4 Physiology

The mechanisms involved in the modification of host behaviors are not well understood because there has been a significant lack of general research looking at the physiological aspects of host-parasite associations and their influence on behavior. Barber and Wright (2006) gave two fundamental reasons why the physiology behind parasite-induced behavioral changes should be studied further. First, there are little data supporting key hypotheses that state that parasite-induced behavioral changes arose as parasitic adaptations to maximize transmission success (see reviews by Barber *et al.*, 2000; and Moore, 2002). Second, because behavioral effects of parasites ultimately arise from physiological changes in the host, gaining a clearer understanding of those mechanisms involved in parasite-induced alterations of host behavior can also provide information about healthy organisms (Helluy and Holmes, 1990).

Highly significant differences were found between fish populations when *F. heteroclitus* physiology (respiration, stamina, RBC size and volume) was examined. However, those differences did not appear to be linked strongly to gill parasitism. A study using the cardinal fish *Cheilodipterus quinquelineatus* demonstrated that individuals parasitized with the parasitic isopod *Anilocra apogonae* had higher rates of oxygen consumption than non-parasitized fish (Östlund-Nilsson *et al.*, 2005). Parasitized three-spined sticklebacks were also found to have higher respiration rates, but also higher resting, routine and maximum activity levels than healthy, unparasitized fish (Lester, 1971; Giles, 1987 and others). This is contrary to what was observed in this study where more parasitized individuals generally had lower respiration rates than less parasitized individuals, although it should be noted that fish from a less parasitized site (KC) were grouped with those more parasitized sites,

possibly due to the gill damage observed in these fish. A study by Anderson (1975) using shrimp (*Palaemonetes pugio*) infected with the parasitic isopod *Probopyrus pandalicola,* reported that in most instances, infected shrimp had lower rates of oxygen consumption than unparasitized conspecifics, which is somewhat comparable to what is reported herein. Similarly, it was reported that copepods (*Eudiaptomus gracilis*) infected with plerocercoid larvae (*Diphyllobothrium latum*) also had lower mean oxygen consumption rates than noninfected copepods (Klekowski and Guttowa, 1968). The results reported herein may be more similar to the Anderson (1975) study as both examine parasites that directly affect host respiratory structures. Adult *P. pandalicola* attach to their shrimp hosts' branchial chamber. The other studies examining the affects of the *S. solidus* plerocercoids on *G. aculeatus*, used body cavity parasites and found what is likely an indirect effect. It is reasonable that gills that are heavily infected with parasites would be less able to take in oxygen. Erythropoiesis and larger erythrocytes are usually indicative of a stress response which in this case may be caused by the heavy gill parasite loads. However, in generating additional blood cells, the fish may be trying to circumvent the reduced respiration rate and get more oxygen to its tissues.

However, despite having a general decrease in respiration rates and larger RBCs, heavily parasitized individuals surprisingly did better in the stamina experiment overall. This may be attributed to increases in RBC volume and the development of additional gill tissue. Additional branches appear to be the fish's major strategy to compensate for the stress induced by heavy parasite loads.

The physiological results, although significant, do not point at gill parasitism as the driving force. Perhaps other parasites that were not examined were behind the differences seen. Other possibilities include habitat differences (e.g., impoundment) or various environmental effects. Any of these factors singly or in combination with

one another, could be responsible for the physiological differences found among sites.

VI.5 Gill Anatomy

Highly significant differences in gill anatomy were also found among sites, with fish from heavily parasitized sites (restored) having the greatest number of additional branches compared to fish with fewer parasites from unrestored sites. The branchial epithelium of fish gills is an extremely sensitive, highly dynamic and metabolically active tissue, responsible not only for gas exchange, but also ion regulation, acid-base regulation and nitrogenous waste excretion (McDonald and Wood, 1993). As such, when faced with various environmental stressors (e.g., pH, hypoxia, parasites), the gills can undergo certain morphological changes in an attempt to acclimate and ultimately tolerate the ensuing conditions. Any form of respiration has some costs associated with it. Under most circumstances, as the oxygen content of the water changes, a fish should adopt the mode of respiration that minimizes the cost to it (Kramer, 1987). The thousands of metacercariae found in the gill filaments are likely interfering with normal gill functions such as impairing gas exchange efficiency between the environment and gill tissue, making this situation comparable to low oxygen conditions. This has been shown in other studies (Ishimatsu *et al.*, 1996; Dezfuli *et al.*, 2003) where gill ectoparasites cause damage to the filaments and epithelia of the secondary lamellae, causing an increase in host mucous secretion. However, in this study, fish with severe gill parasite infections appear to be responding with both morphological (additional gill branches) and behavioral adaptations (conspicuousness, vertical positioning) as well as physiological parameters (RBC size). There appears to be a threshold of approximately 1,500 gill parasites whereby fish ellicit an anatomical response to form additional branches.

High numbers of digenean trematode infections have been shown to induce tissue proliferation in other taxa, specifically anurans. Johnson *et al.* (1999; 2001) found that increased intensity of *Ribeiroia* sp. metacercariae in limbs of Pacific treefrogs (*Hyla regilla*) and western toads (*Bufo boreas*) hindlimbs, caused an increase in extra limbs. Linzy *et al.* (2003) also found correlations between limb abnormalities in marine toads (*Bufo marinus*) and whistling frogs (*Eleutherodactylus johnstonei*) and high metacercariae infections in limbs. In the present study, it appears that once gill infections are greater than approximately 1,500 metacercariae, the fish's gills become compromised and no longer able to efficiently extract oxygen. The growth of gill tissue, in the form of additional branches, may help the host compensate for oxygen deficits.

Pollutants are one of the most commonly used stressors researchers have tested with respect to morphological changes in the gills. There are a number of ways that the gills are affected and these changes have been put into two broad categories: accumulated damage, and repair and compensatory response (Mallatt, 1985). To further complicate matters, it is not always clear where the line is drawn between these two categories. In a comprehensive review of morphological effects of metal exposure, Mallatt (1985) found that damage constitutes changes such as separation of epithelial layers, tissue oedema, and clubbing of lamellae at moderate levels of exposure. At more severe levels, tissue necrosis, and rupture and fusion of secondary lamellae were more prominent. Compensatory and repair responses include hypertrophy and hyperplasia of mucous cells and chloride cells, and a general thickening of the filamental and lamellar epithelia. No previous references to branching or additional growth of gill tissue have been made. Most if not all responses to contaminants were shown to be reversible over time by increasing mitotic activity and rapidly changing over cellular components (McDonald and Wood,

1993). The additional gill branching observed in *F. heteroclitus* in this study is a permanent morphological change that is strongly correlated with high numbers of digenean trematode metacercariae, namely those of *A. diminuta* and *E. schwartzi.*

Interactions between parasites and their host populations over time have often been likened to an evolutionary arms race, where for each defense that the host evolves against being parasitized, the parasite is evolving a counter-adaptation to said defense (Price, 1980; Seger and Hamilton, 1988). The fact that the newly produced gill tissue then becomes infected with metacercariae is the next step in this arms race.

This study was designed to address parasite community structure in context with different variables, and to investigate affects of high gill parasite loads on host behavior, physiology and anatomy. In conclusion, the parasite infracommunities studied herein have been found to be dynamic over space and time. These communities are affected by environmental factors including contaminants and salinity. Site disturbance also plays a role and is not limited to pollution. Site disturbance in the form of restoration is also shown to change community structure for both the parasite and host. Parasites are shown to influence host behavior such that trophic transmission is potentially increased. Physiology and anatomy were also shown to differ from that of less parasitized hosts and it appears that hosts induce several physiological and anatomical mechanisms against large parasite infections. As such, parasites are important components of estuarine habitats and have the ability to structure wetland systems, affecting all trophic levels including those of benthic, pelagic and terrestrial organisms.

APPENDIX A

Parasite List

Phylum Acanthocephala

Neoechinorhynchus sp. (cystacanth) – liver

Unknown sp. – mesenteries, viscera

Phylum Arthropoda, Subphylum Crustacea

Argulus funduli, Krøyer, 1863 – skin, fins

Ergasilus funduli, Krøyer, 1863 – gills

Phylum Platyhelminithes, Class Cestoda

Proteocephalus sp. – intestine

Phylum Platyhelminithes, Class Trematoda (Digenean)

Homalometron pallidum, Stafford, 1904 – intestine

Unknown trematode – intestine

Posthodiplostomum minimum subsp. *minimum* (metacercaria), MacCallum, 1921; Dubois, 1936; Hoffman, 1958 – liver, mesenteries, viscera

Ascocotyle (Phagicola) diminuta (metacercaria), Stunkard and Haviland, 1924 – gills

Echinochasmus schwartzi (metacercaria), Price, 1931 – gills

Phylum Platyhelminithes, Class Trematoda (Monogean)

Fundulotrema prolongis, Hargis, 1955; Kritsky and Thatcher, 1977 – fins, gills

Dactylogryrus sp. – gills

Phylum Nematoda

Eustrongylides ignotus, Jägerskiöld, 1909 (larval form) – mesenteries, viscera

Dichelyne bullocki, Stromberg and Crites, 1972 – intestine

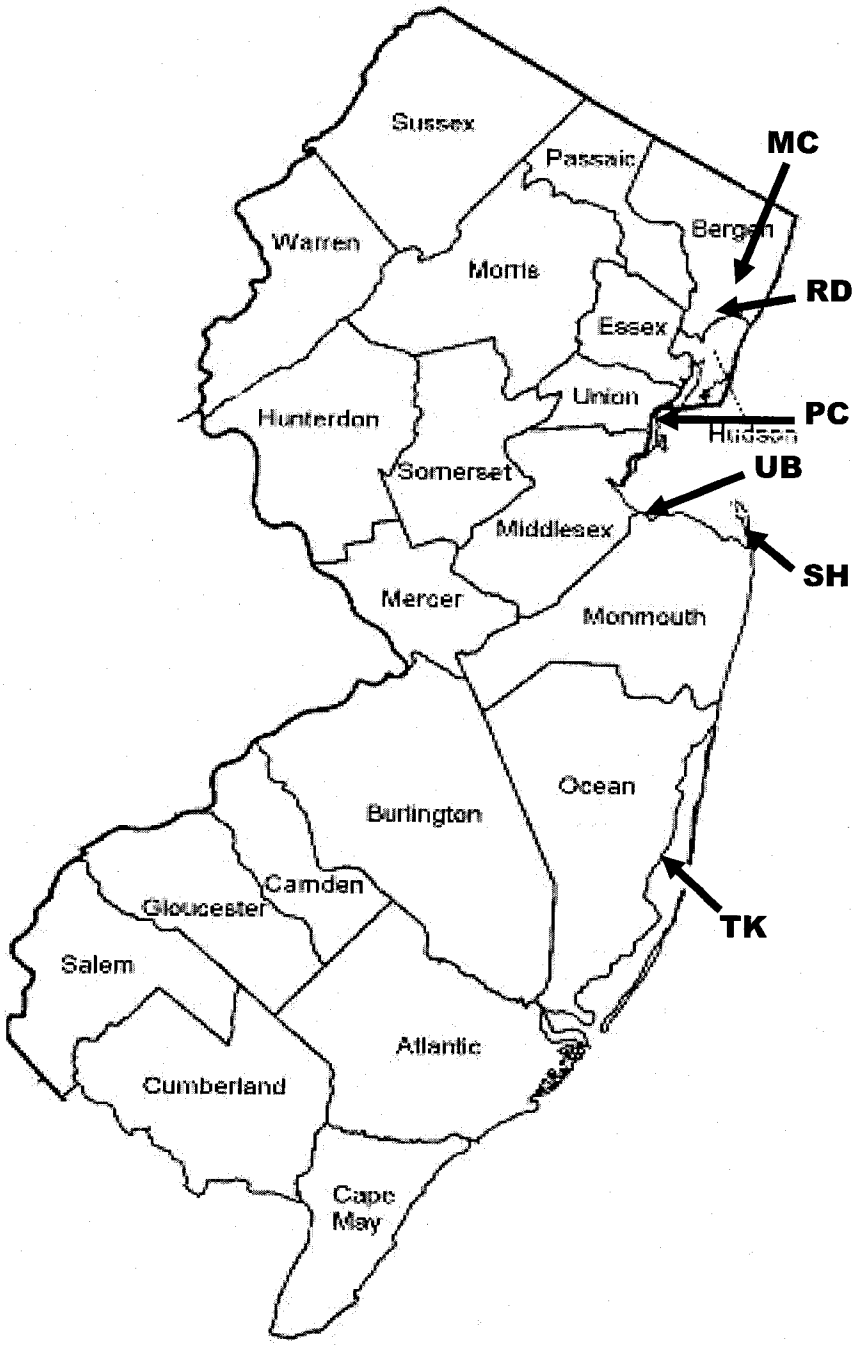
Spiroxys sp., Schneider, 1866 (larval form) – mesenteries

Cystidicola sp. Fischer, 1798 – swim bladder

Unknown sp. (red) – mesenteries, viscera

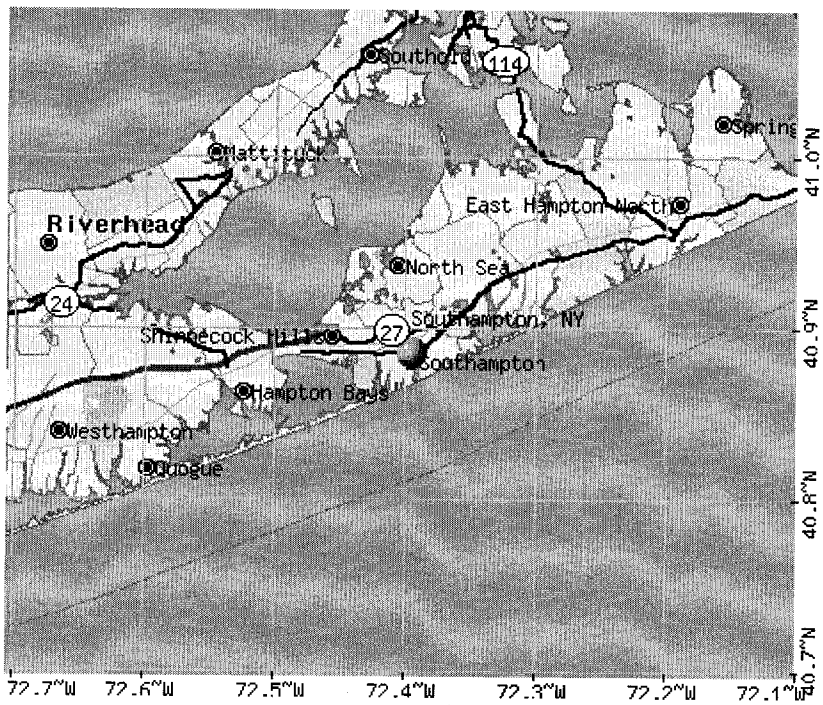
APPENDIX B

New Jersey Map



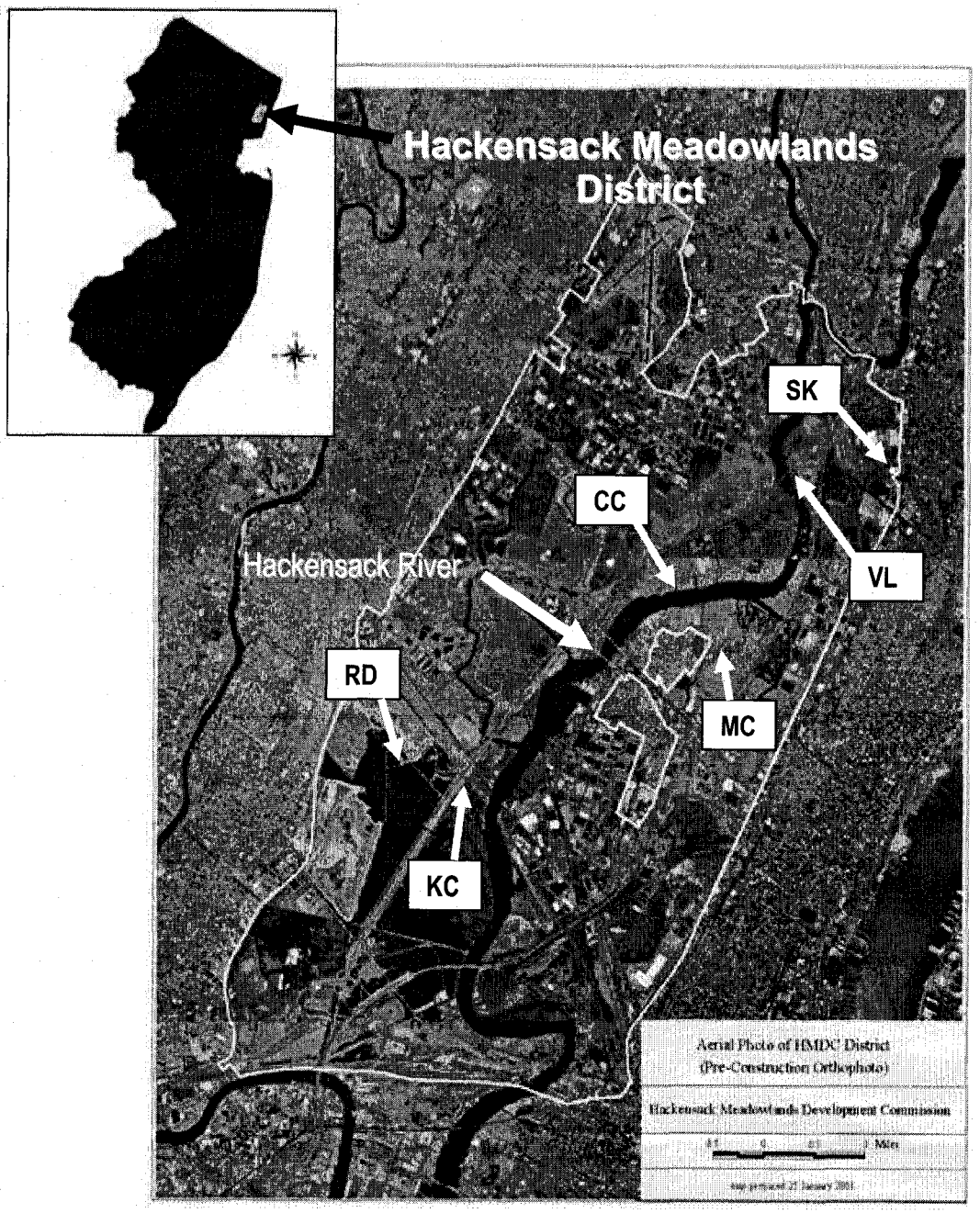
APPENDIX C

Bullhead Bay, Southampton, Long Island, New York



APPENDIX D

Hackensack Meadowlands District Map



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