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Disease Ecology of a Microsporidian Parasite and its Effects on Mottled Sculpin

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Disease ecology of a microsporidian parasite and its effects on mottled sculpin

Jared Joseph Homola

A Thesis Submitted to the Graduate Faculty of
GRAND VALLEY STATE UNIVERSITY

In
Partial Fulfillment of the Requirements
For the Degree of
Master of Science in Biology

Biology Department

August 2013
DEDICATION

This work is dedicated to my wife, Shannon, and my parents, Ken and Mary, whose boundless love, support, and encouragement has given me the courage to always reach further.
ACKNOWLEDGEMENTS

I thank my graduate committee for helping me to form, refine, and execute the ideas that are included in this finished work. My major advisor, Dr. Carl Ruetz invested a significant amount of effort to ensure the success of this project and in my professional development over the past few years. As members of my graduate committee, Drs. Ryan Thum, Steven Kohler, and Mark Luttenton generously provided their expertise and guidance throughout the research process. The support of numerous colleagues also helped to make this project successful. Brandon Harris, Stacy Provo, Alex Wieten, Jesse Wesolek, Dr. David Janetski, Julie Ryan, and Kurt Thompson provided technical and collegial support. Jeremy Newton and Dustin Wcisel lent their invaluable expertise to genetic aspects of this research. I am grateful to Doug and Judy Ledbetter, John Brown, and the Wilder Creek Conservation Club for welcoming me onto their properties to conduct research. Financial support was provided by a research assistantship from Annis Water Resources Institute and research funding from the Grand Valley State University Presidential Research Grant.
ABSTRACT

DISEASE ECOLOGY OF A MICROSPORIDIAN PARASITE AND ITS EFFECTS ON MOTTLED SCULPIN

By Jared Joseph Homola

Infectious disease can influence organisms at all levels of ecological organization, from individuals to ecosystems. Likewise, the ecosystems where pathogens exist directly influence their success. Recent theoretical studies have tied disease prevalence to biotic factors such as genetic diversity, biodiversity, and host behavior, and abiotic factors that include temperature and increased nutrient concentrations. Parasites included in the phylum Microspora are increasingly recognized for being ubiquitous in nature, although their ecological roles are generally unknown. This study examined several environmental, community, and host-related metrics to compare the biotic and abiotic aspects of 16 small streams; 6 with mottled sculpin (Cottus bairdii) populations infected by the microsporidian Glugea sp., and 10 without the parasite. Comparisons were made between the condition of infected and uninfected mottled sculpin. Relatively high water temperatures were implicated in the presence of the parasite, although the fish assemblages did not differ significantly between streams with and without Glugea. Evidence of the consequences of infection was limited to reductions in liver somatic indices and increases in the somatic mass at age for infected individuals, as well as reductions in gene diversity and Wright’s inbreeding coefficient. No significant
differences were detected in host densities, host sex ratios, relative abundances, or mortality rates, and there was an absence of genetic bottlenecks in infected mottled sculpin populations. Together, these findings suggested that host population dynamics were generally unaffected by the disease. Contrary to previous ecological research on microsporidian species, mottled sculpin populations appear to be robust to infection, which is likely due to the strong density-dependent population dynamics of mottled sculpin that allow for losses due to disease to be compensatory and quickly offset. This study provides basic ecological insight into the role of microsporidian parasites in natural ecosystems.
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CHAPTER 1

DISEASE IN NATURAL ECOSYSTEMS WITH AN EMPHASIS ON PHYLUM MICROSPORA

INTRODUCTION

The term “disease” can refer to impaired functionality caused by various reasons, such as developmental shortcomings, nutritional deficiency, genetic error, or environmental stress. Infectious disease (hereafter “disease”) is the altered state of functionality caused by parasitic infection. Diseases are capable of influencing and being influenced by ecosystems at all levels of ecological structure. For instance, environmental pressures such as stress caused by changing temperatures or famine may instigate a disease epidemic by weakening the immune system of a host species, making individuals more susceptible to infection. That epidemic may in turn cause steep declines in the abundance of a keystone species, which could trigger a trophic cascade, resulting in indirect, but strong, changes that reverberate throughout the ecosystem. Because of the complexity of these types of scenarios, disease ecology (i.e. the study of how environmental and community interactions influence pathogen-host dynamics) has developed out of the need to understand how disease interacts with the ecosystems it affects.

Much of the current disease ecology literature is composed of theoretical modeling that has provided a general understanding of the dynamics of pathogen-host interactions, but applicability of those often simplistic models to the complexity of actual ecosystems has been challenging (Hedrick 1998; Christensen et al. 2010; Roche et al.
2012). Early theoretical efforts published by Anderson and May (1978, 1979, 1981), and May and Anderson (1979) typically applied to infections with simple life histories; however, they provided a solid quantitative foundation for studying disease that has grown to include increasingly intricate parasite and host biologies. Over the past decade, empirical approaches to studying disease have become increasingly common; however, a focus on simple systems (e.g. livestock) dominates the literature, resulting in a need for information on disease in natural ecosystems (Holomuzki et al. 2010).

The immense number of transmission routes used by parasites makes universal statements regarding disease ecology principles difficult to form (Lafferty and Kuris 2002). However, by examining the disease ecology literature and seeking similarities among research results, this review aims to coalesce the most important research themes into directions for continued progress. The specific goals of this review are to (a) summarize existing literature pertaining to environmental and community influences on disease and (b) host individual and population effects on disease, (c) discuss ways that disease can influence host individuals and communities, (d) highlight the unique biology and ecological roles of the parasites that comprise the phylum Microspora, and (e) suggest future research directives.
ENVIRONMENTAL AND COMMUNITY INFLUENCES ON DISEASE

The role of biotic and abiotic factors in promoting or depressing parasite prevalence is fundamental to disease ecology (Roche et al. 2012). Although disease dynamics can most directly be traced to host behaviors and contact networks of the pathogen, vector, and host, the influence of the ecosystem on those entities cannot be overstated. For instance, theoretical modeling of environmental stressors that are capable of reducing nutrient resources, host fecundity, and host survivorship resulted in a notable reduction in carrying capacity for the host population, which would likely reduce contact among infected and susceptible hosts, lowering disease prevalence (Lafferty and Holt 2003). Moreover, simulations have suggested that host-specific pathogens generally decline in prevalence as host stress is increased, while non-specific pathogens often undergo expansions with increasing stress (Lafferty and Holt 2003). Temperature is another abiotic factor that is frequently implicated in determining success of infectious diseases. For instance, development in many microsporidian species is slowed by low temperatures (Glugea plecoglossi, Takahashi and Egusa 1977; G. stephani, Olson 1981; Loma salmonae, Becker et al. 2006; Microsporidium takedai, Dyková 2006).

Anthropogenic habitat alterations and their epidemiological consequences account for some of the most critical environmental issues facing society (Hassan 2005). One of the most dramatic changes in aquatic ecology has been the anthropogenic eutrophication of waterways (Bennett et al. 2001). Eutrophication typically results from excessive nutrient input into lakes and streams that can artificially inflate the ecosystem’s carrying capacities, resulting in fish kills, nuisance algal blooms, impaired water clarity, and other
degradative effects (Hassan 2005). Parasites are believed to benefit from eutrophication by increases in host density, which often increases contact rates (Lafferty and Holt 2003) through improved health of well-fed hosts (Smith et al. 2005). Alternatively, increased stress on hosts that are impaired by eutrophication may result in immunosuppression, making them more vulnerable to disease (Lafferty and Holt 2003). Land-use changes and habitat fragmentation caused by the construction of barriers such as cities, roadways, and dams can also shape patterns of infection (Patz et al. 2000). These obstructions can increase contact among infected and susceptible hosts trapped on one side of the barrier. However, universal rules regarding the effects of fragmentation are elusive, as exemplified by Lyme disease in its white-footed mouse (Peromyscus leucopus) vector, where prevalence was instead found to decrease linearly with decreasing patch size (Allan et al. 2003). Additionally, anthropogenic habitat fragmentation can lead to unnatural co-habitation by reservoir, vector, and host species, creating sharp increases in disease prevalence. Such a scenario has been implicated in the decline of African wild dog packs infected with rabies from domestic and feral dogs (Kat et al. 1995). Climate change also has influenced disease in detectable ways. For instance, the spruce bark beetle (Dendroctonus rufipennis), which induces mortality in Alaskan white spruce (Picea glauca), has caused increased damage as warming temperatures have allowed the parasite to complete its life cycle in one year instead of two (Chaplin et al. 2008).

Diseases can be affected by interspecific interactions in the communities where they occur. For instance, a reduction in the number of non-susceptible predators can result in a spike in the proportion of the disease susceptible prey population as their
abundances increase (Packer et al. 2003; Holt and Roy 2007). Moreover, because predators are more likely to target diseased prey (Moore 2002), the predator may act as a control agent for the parasite. In these cases, the removal of a predatory species may actually be counterproductive to management goals aimed at increasing the abundance of a prey population.

Species diversity in host communities can influence disease prevalence, although our understanding of the dynamics between community diversity and disease are still largely theoretical (Keesing et al. 2006). For example, researchers have formed hypotheses through quantitative models stating that high species diversity may limit infection by limiting host density (Rudolf and Antonovics 2005); however, in other cases, high diversity could result in increased prevalence if the parasite is not host-specific or more vectors become available (Holt and Pickering 1985; Schmidt and Ostfeld 2001; Dobson 2004). Because hosts typically vary in their competency for parasite success, communities with high species diversity will likely reduce the relative number of competent hosts and increase the likelihood of the parasite attempting to infect inferior host species (i.e. dilution effect; Norman et al. 1999; Schmidt and Ostfeld 2001).

Additional studies have added theoretical (Dobson 2004; Rudolf and Antonovics 2005) and experimental (Mitchell et al. 2002) support for the dilution effect, although most empirical evidence is biased toward vector-borne infections, such as Lyme disease (Borrelia burgdorferi infection; Ostfeld and Keesing 2000; Schmidt and Ostfeld 2001).
HOST INDIVIDUAL AND POPULATION EFFECTS ON DISEASE

Certain aspects of host populations can influence disease dynamics. The diversity that exists at the genetic, individual, and population levels can impact the success of a parasite (reviewed by Ostfeld and Keesing 2012). Genetic diversity has been theorized to have an important role in disease dynamics (Springbett et al. 2003; Lively 2010), which is an idea that has been gaining experimental support (Pearman and Garner 2005; Whiteman et al. 2006; Altermatt and Ebert 2008). Keesing et al. (2006) suggest an encounter reduction mechanism where susceptible hosts are less likely to encounter effective parasites if the genetic structure of the host population is more diversified. Advancing that idea, Lively (2010) suggests a matching allele model where increased host genetic diversity reduces the likelihood of susceptible hosts and compatible pathogens encounters, assuming a system where only specific host genotypes are able to be infected by specific pathogen genotypes. Using the matching alleles hypothesis, modeling has indicated that disease likely spreads rapidly throughout a naïve host population until susceptible host genotypes fall below a critical level, which would result in a decline in disease prevalence (Lively 2010). The interaction of host genetic diversity and disease has been frequently observed in agricultural settings, where crop and livestock populations with intentionally depressed genetic diversity become exceptionally susceptible to disease outbreak (i.e. monoculture effect; Springbett et al. 2003; Altermatt and Ebert 2008). Some ecologically-relevant mechanisms for reducing genetic diversity, thereby potentially increasing disease susceptibility, include increased inbreeding (Whiteman et al. 2006), range expansion (Pearman and Garner 2005), and habitat
isolation (Campbell et al. 2010). Additionally, Whiteman et al. (2006) identified a reduction in naturally produced antibody levels for inbred populations. Other mechanisms can increase genetic diversity of host populations. For instance, Haldane (1949) hypothesized that coevolution among diseases and host populations could be responsible for increasing genetic diversity in hosts. Theoretical and experimental evidence has provided compelling support for this situation (Bérénos et al. 2011). Overdominance (i.e. heterozygote advantage) commonly has been implicated in host-disease relationships, which could lead to an excessive number of heterozygotes, thereby increasing genetic diversity (MacDougall-Shackleton et al. 2005).

Variation in host behavior has long been recognized as an important determinant in shaping the contact networks responsible for disease transmission. Modeling contact networks of diseases transmitted directly from host to host has been refined from the basic susceptible-infected-recovered (SIR) model that assumes consistent levels of contact to more realistic models that allow for more ecologically plausible variation (Christensen et al. 2010). Similarly, varying behaviors among infected and uninfected conspecifics that are capable of altering inter-individual contact frequencies have been observed in nature. For instance, Behringer et al. (2006) observed clustering of parasite infected lobsters in conjunction with the active avoidance of infected individuals by those without the parasite. Similar avoidance behavior along with an increased level of shoaling of infected individuals has been observed in threespine stickleback (Gasterosteus aculeatus) co-existing with the microsporidian parasite Glugea anomala
(Ward et al. 2008). Whether these traits are a cause or consequence of infection remains a point of contention (Blanchet et al. 2009).

The abundance of the potential hosts can impact parasite success. Parasites with low transmission efficiency typically can only be sustained if the host population abundance is relatively high, while those with higher transmission efficiency can be sustained despite reduced abundance (Anderson and May 1981). More recently, researchers have begun to recognize the importance of frequency-dependent transmission, rather than density dependent. Frequency-dependent transmission occurs when susceptible host individuals make a fixed number of contacts with infected hosts, regardless of the population density (e.g. sexually transmitted diseases; Begon et al. 1999). Further study has found theoretical and empirical support for the role of frequency-dependent selection in some ecological scenarios (Norman et al. 1999; Rudolf and Antonovics 2005). The large degree of variability in parasite transmission dynamics has led to the recognition of the importance of the biology of the host species in dictating the frequency of contact rates (Fenton et al. 2002).
DISEASE EFFECTS ON HOST COMMUNITIES AND ECOSYSTEMS

The impact that parasites have on ecological systems is increasingly being recognized at the community and ecosystem level. Infectious disease has the potential to reshape communities and ecosystems through altering species ranges, predation, competition, and biodiversity (reviewed by Smith et al. 2009). One of the ways that parasites can impact an ecosystem is through direct impacts on keystone species (i.e. those species that have disproportionately large ecosystem impacts compared to their relative abundances; Kotliar 2000). Disease induced changes in the population dynamics of these species could result in major shifts in community composition and ecosystem function. One of the more well studied instances of keystone species being impacted by disease is in the black-tailed prairie dogs (Cynomys ludovicianus) of western North American (reviewed in Collinge 2002). These keystone species influence the abundance and distribution of several small mammals, many of which act as reservoirs for an often fatal prairie dog plague (Culley et al. 1997, 2000; Kotliar et al. 1999). Plague epidemics typically trigger a die-off of prairie dogs, resulting in a spike in their prey (reservoir) species, which consequently sustains the pathogen in the ecosystem despite the temporary extirpation of the primary host. Moreover, researchers have hypothesized that because of high prairie dog mortality caused by the plague, scavengers may become concentrated on groups of deceased prairie dogs, potentially increasing the likelihood of disease transmission among vector and reservoir species (Collinge 2002).

Pathogens themselves have the ability to alter ecosystem composition and function by selecting for rare species and altering entire food webs. The Red Queen
community hypothesis (reviewed by Clay et al. 2008) expands an existing genetic ideology to explain how disease can influence diversity at the community level. The hypothesis states that frequency-dependent, host-specific pathogens target and reduce the abundance of a common species below a threshold capacity, consequently increasing the relative abundances of rare species. To do this, the hypothesis assumes that pathogens have an adaptable genetic control over host-specificity and can switch to a new host species once the originally targeted species falls below a critical threshold (Clay et al. 2008). Through these cyclic shifts in relative abundance among species, ecosystems can undergo vast changes in function and composition. Parasites have been shown to spur such cycles in red grouse (Lagopus lagopus scoticus, Hudson et al. 1998) and southern pine beetles (Dendroctonus frontalis, Turchin et al. 1999), both of which experienced stability in abundance once their parasites were experimentally removed. Similar cycles consistent with the Red Queen hypothesis have been experimentally induced in Daphnia magna populations using the bacterium Pasteuria ramosa. Infectious disease also has been found capable of reshaping entire food webs through trophic cascades (i.e. when predatory pressure changes result in altered predation in other levels of the food web). One of the more infamous examples of a disease-induced bottom-up trophic cascade is the Irish potato famine of the mid-1800s. During that time, a fungal parasite devastated the potato crop that Ireland was dependent upon for a food source, results in death or emigration for much of the country’s human population (Donnelly 2001). Other well-known examples come from once dominate tree species such as the American chestnut (Castanea dentate), which was decimated by chestnut blight (Cryphonectria parasitica)
leading to the near-extirpation of the species from the eastern United States (Paillet 2002). The loss of this once prolific native tree is thought to have resulted in a dramatic increase in oak trees (*Quercus* spp.), leading to wide-ranging trophic effects for various small mammals and invertebrates dependent on the oak’s inconsistent acorn crops (Collinge et al. 2008). Another trophic cascade trigged by a tree infection is the Dutch elm disease, which resulted in a loss of habitat for many tree-nesting bird species, but created an increase in resources for organisms that require dead trees (Osborne 1985).
PHYLUM MICROSPORA

The phylum Microspora constitutes a unique group of eukaryotic, intracellular parasites with potentially wide-ranging ecological effects. Widely distributed throughout aquatic and terrestrial ecosystems, microsporidian species have been found to parasitize all vertebrate and most invertebrate orders (Keeling and Fast 2002), including humans (Mathis et al. 2005). Despite being ubiquitous, these parasites are rarely studied outside of laboratory settings, leaving their role in natural ecosystems largely unknown.

Single-celled microsporidian organisms are highly organized and contain relatively few internal structures. A dense chitin-rich cell wall allows the microsporidian spore to remain viable outside of a host cell for several years (Wittner and Weiss 1999). There are three specialized structures that are used during the infection process, which is reviewed in detail by Williams (2009). First, the polaroplast (a series of membranes) swell through increases in osmotic pressure once an environmental trigger for infection has been detected. Next, the building pressure causes the rupturing of the anchoring disk, which is located at the cell’s anterior extreme. Once the anchoring disk is compromised, the compressed polar tube, which is coiled within the microsporidian cell, projects outward through the cell wall, piercing into a targeted host cell. The polar tube is a defining characteristic of the microsporidian phylum and occasionally used for morphological species identification (Ghosh and Weiss 2009). Infectious sporoplasm containing the nucleus and cytoplasm is subsequently released through the polar tube into the host cell, triggering a series of division and reproductive events as the microsporidia transitions into a new stage, the meront (Keeling and Fast 2002, reviewed by Williams
2009; Dunn and Smith 2001). The entire infection event occurs very rapidly, taking only about 2 s to complete (Frixione et al. 1992).

Microsporidians have interesting evolutionary histories that create taxonomic challenges for researchers. Although over 1,200 species belonging to over 150 genera have been described, only a small percentage of the number of extant species has been identified (Keeling and Fast 2002). Although initially identified by polar tube morphology, sequencing of the small subunit of the microsporidian ribosomal DNA has provided the best means of identifying spores to the species level (Vossbrinck and Debrunner-Vossbrinck 2005). Despite these advances, confusion regarding phylogenies remains pervasive due to only minute genomic difference among species (Lom and Nilsen 2003; Vossbrinck and Debrunner-Vossbrinck 2005). Phylogenetic analyses place the microsporidians in a position most closely related to fungi, as early branching eukaryotes, but the lack of clarity regarding their evolutionary history is reinforced by continual controversy of their taxonomic placement (Vossbrinck and Debrunner-Vossbrinck 2005; Lee et al. 2008). Genome sizes of sequenced individuals range from only 2.9 megabases (Mb; Katinka et al. 2001) to about 23 Mb (Belkorchia et al. 2008). Interestingly, the microsporidian genome sequenced by Katinka et al. (2001) exhibited no evidence of possessing introns and had numerous overlapping transcripts. These multigene transcripts are found in microsporidians of larger genomic size and may be unique to the phylum (Belkorchia et al. 2008).

Certain microsporidians that initiate extreme hypertrophy in their host cells, creating cyst-like structures (xenomas; Sprague and Vernick 1968; Weissenberg 1968),
are especially evident in aquatic ecosystems. Xenomas can range up to 2 cm in diameter and contain very large numbers of developed spores (see Figure 2.1). The disfiguring nature of xenomas is commonly thought to result in energetic costs and behavioral changes to host individuals (Ward et al. 2005), although evidence of these effects is scarce because most studies concerned with xenomas have occurred at the cellular level rather than assessing effects on host individuals and populations (Lom and Dyková 2005). One study that did look at individual-level effects of a xenoma-inducing microsporidian (*Loma branchialis*) found emaciation, significantly reduced condition factors, marked decreases in food consumption of Atlantic cod (*Gadus morhua*) experimentally infected with the parasite (Khan 2005). Another study of experimentally infected threespine stickleback indicated a metabolic cost to infection as infected hosts lost more mass than uninfected individuals during a period of food deprivation (Ward et al. 2005).

While the ecological role of microsporidians is largely unknown, some research on these species have been conducted in natural ecosystems. Commonly documented results of these studies included high parasite prevalence and reductions in the host population abundance. The microsporidian parasite *Cougourdella* spp. and host caddisfly (*Glossosoma nigror*; Kohler and Wiley 1992, 1997) constitute one well-studied example. Using a long-term dataset that included years prior to the introduction of the microsporidian, the researchers were able to make strong inferences between the presence of the parasite and the collapse of host populations (Kohler and Wiley 1992). Further analysis revealed an increase in the abundance of competing, non-susceptible species.
following the collapse of the host caddisfly (Kohler and Wiley 1997). Again using a long-term dataset involving a microsporidian (*Microsporidium* sp.) and a caddisfly species (*Brachycentrus americanus*), Kohler and Hoiland (2001) implicated the parasite’s presence in driving the density-dependent growth of the host species. The parasite (*Nosema ceranae*) presents another case of a microsporidian that is capable of collapsing its host population. In this case, populations of various bee species from around the world have experienced sudden die-offs once the parasite enters their colony despite the existence of otherwise plentiful resources for the host (Higes et al. 2008).

An increasing number of human infections, especially in immunocompromised individuals, has hastened the need for information regarding disease ecology of microsporidians (Mathis et al. 2005; Didier 2005). However, it should be noted that while some microsporidians have been cultured in human cells *in vitro* (Trammer et al. 1999; Lowman et al. 2005), the vast majority of microsporidians are host specific and do not naturally parasitize humans. Of those found to infect humans, at least one utilizes a mosquito vector (Coyle et al. 2004). The lack of major differences among the phylogenies of human infecting and non-infecting microsporidian species is suggestive that only slight mutations are required of the phylum’s relatively simple genome in order for host-specific adaptations to shift (Mathis et al. 2005).
FUTURE RESEARCH DIRECTIONS

As disease ecology matures as a field of study, there are several research foci that should be targeted. Most of these objectives involve how infectious disease is incorporated in ecosystem level processes, both as a native cohabitor and as a disturbance agent. To understand these processes, multi-disciplinary integrated research will be essential.

The ways that changing ecosystems and anthropogenic influences can impact disease are some of the most important issues confronting disease ecology. Invasions of non-native species, which tend to reduce overall species diversity and increase native and exotic parasite prevalence, are already having profound effects on ecosystems (Vandegrift et al. 2010; Poulin et al. 2011). Invasive species can thrive in their non-native ecosystems due to a release from their native predators and parasites (Torchin et al. 2003). Additionally, invading species can introduce new parasites into the native, naïve ecosystem (i.e. enemy-release hypothesis), although recent studies suggest this phenomenon may be less important than previously thought (Tompkins et al. 2011). As climates continue to change across the globe, parasites will likely adapt to the changing environments (Slenning 2010). Increases in the prevalence of some parasites coinciding with increasing temperatures are likely, as supported through findings such as those regarding fish microsporidians (Li et al. 2003; Lowman et al. 2005) and range expansion of Lyme-disease transmitting ticks (Lindgren et al. 2000). Additionally, reassessment of anthropogenic land use strategies must consider how these ecosystem disruptions influence infectious diseases (Patz et al. 2004). Specific research objectives must focus
on metapopulation dynamics and specifically on how changes to ecosystem patch sizes will alter contact networks among and within species. Finally, a basic research goal must be to continue to gather baseline data regarding the status of disease in contemporary ecosystems in order to detect changes as they occur.

Disease caused by microsporidian parasites must continue to see increases in research attention as more species are discovered and the need to better understand their roles in natural ecosystems and human-mediated population increases. As an example, the microsporidian *Nosema ceranae*, implicated in colony collapse disorder in honeybee populations (Higes et al. 2008), could have devastating ecological and economical consequences if these critical pollinators become extirpated. Additionally, increases in aquaculture efforts have coincided with increases in *Loma salmonae*, which can account for up to 30% mortality in farmed salmon (Kent and Speare 2005). Evolutionary biological research also must continue to trace the evolutionary pathways of microsporidians to better understand how they have co-evolved with their hosts and the frequency of host-shifting that occurs (Smith 2009). As genomic sequencing technologies become more accessible, scientists will be able to learn how this unique group of organisms has managed to evolve its physical components down to only the most essential organelles and genomic units.

Future research for disease’s role in natural ecosystems will include improving our understanding of population-level impacts and transmission dynamics using cross-disciplinary research approaches. For example, bringing together experts in micro- and macro-ecological processes will be necessary to gain an understanding of how processes
that occur throughout biologically realistic systems influence pathogen transmission (Roche et al. 2012). A growing body of theoretical research also will demand empirical investigation. Increases in the resolution of theoretical efforts through the addition of variables such as abundances, birth and death rates, contact rates, and susceptibilities also will need to be tested in both experimental and ecological settings (Roche et al. 2012). Finally, disease ecology’s role in human health must be investigated through the melding human medicine and ecological sciences to discover the ties that exist among disease, biodiversity, ecosystem health, and human health.
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CHAPTER 2
EFFECTS OF A NOVEL MICROSPORIDIAN PARASITE ON A BENTHIC STREAM FISH IN MICHIGAN STREAMS

ABSTRACT

Despite microsporidian parasites occurring in all vertebrate and many invertebrate orders, research regarding their effects on natural populations remain uncommon. I used several individual and population-level metrics to evaluate the costs of a microsporidian infection (genus: *Glugea*) on 6 mottled sculpin populations using comparisons to individuals from 10 nearby uninfected populations. Additionally, I sought to determine biotic and abiotic factors that influence the presence or absence of the parasite in each stream. Infected individuals ($n = 129$) had significantly lower condition as measured by their liver somatic index and slope of weight-length relationship, and were significantly heavier at each sampled age group when compared to uninfected fish ($n = 331$). Population-level metrics indicated few negative effects of infection with no significant differences in mortality rates, mean age, sex ratios, and likelihood of experiencing a genetic bottleneck between infected and uninfected populations. Warmer water temperature was significantly associated with the presence of the parasite. Given the disfiguring nature of infection and the severity of infection consequences for microsporidians presented in the literature, the lack of extensive costs of infection to mottled sculpin was unexpected.
INTRODUCTION

Infectious disease can have profound effects on host species in natural ecosystems, such as regulating abundance (Hudson et al. 1998) shaping genetic variation (Burdon and Shattock 1980), and limiting coexistence with other species or even conspecifics (Holt and Pickering 1985; Behringer et al. 2006). While the influence of disease on populations has long been recognized (Belding 1927; Mitchell 2001), the difficulty in accessing study organisms and environments in aquatic ecosystems has resulted in deficiencies in our knowledge of how disease affects natural fish populations (Hedrick 1998). Consequently, most studies of fish disease focus on captive individuals and populations, whereas knowledge of how diseases affect wild populations is comparatively smaller (Mitchell 2001). Discovering how disease influences natural populations is important to better manage aquatic ecosystems (Riley et al. 2008; Fisher et al. 2012) and improve basic understanding of trophic interactions (Holomuzki et al. 2010).

Microsporidia is a diverse phylum (>1200 described species in 150 genera) of eukaryotic, intracellular parasites that occur within an array of hosts but are most common among aquatic organisms (Keeling and Fast 2002). Characterized by free-living infective spores, microsporidian infections of fish often result in disfiguring hypertrophic host cells (xenomas; Weissenberg 1968; Figure 2.1) that presumably alter a host’s ability to function normally. Parasitized cell function and morphology undergo vast changes as the microsporidian becomes physiologically integrated with its host (Lom and Dyková 2005), likely resulting in physiological costs to infected individuals. While
microsporidian species are ubiquitous, only a small percentage of the number of extant species have been described (Keeling and Fast 2002) and far fewer have been studied in nature, making ecological inferences across genera difficult. Additionally, confusion regarding their phylogenetic placement remains pervasive, even in light of genetic sequencing (Lom and Nilsen 2003; Vossbrinck and Debrunner-Vossbrinck 2005). Those species that have been identified and studied occur in a diversity of hosts and have exhibited a multitude of differing biologies (reviewed in Smith 2009; Texier et al. 2010).

The role of biotic and abiotic factors in promoting or depressing parasite prevalence is foundational to disease ecology (Roche et al. 2012). Stress caused by environmental factors can have varying effects on disease (Lafferty and Holt 2003). For instance, temperature affected the prevalence and growth rate of several microsporidian species during controlled manipulation when the parasites exhibit reduced developmental rates at cooler water temperatures (e.g. below 15-16°C; Olson 1981; Becker et al. 2006). Similar patterns have been documented in natural ecosystems that experience increased prevalence coinciding with warmer summer water temperatures (Takvorian and Cali 1984; Speare et al. 1998).

Fish diseases also can be affected by the fish assemblage in the waterway where they occur. For instance, a reduction in the number of predators can result in a spike in disease prevalence in the prey population when increased prey densities result in increased contact frequencies among infected and susceptible individuals or susceptible individuals and parasites (Packer et al. 2003; Holt and Roy 2007). Species diversity in host communities also can influence disease prevalence, although the understanding of
the dynamics between diversity and disease are still largely theoretical (Keesing et al. 2006). High species diversity was hypothesized to limit infection by limiting host abundance (Schmidt and Ostfeld 2001), yet in other cases high diversity could result in increased prevalence if the parasite is not host-specific or more vectors become available (Holt and Pickering 1985; Dobson 2004).

The microsporidian genus *Glugea* constitutes one group of species that typically causes the formation of xenomas and predominantly infects fish hosts (Lom 2002). A currently undescribed *Glugea* species recently was found to infect mottled sculpin (*Cottus bairdii*) in several streams in southwest Michigan, USA (Figure 2.2). While previous research on this novel parasite is limited, the parasite exhibits large and numerous xenomas within individual hosts, host specificity, and a dichotomous nature of either high prevalence or absence in host populations (J.A. Ryan and S.L. Kohler, Western Michigan University, personal communication).
Figure 2.1. Example mottled sculpin, including (A) healthy ventral surface, (B) ventral surface of individual infected with *Glugea* spp. and (C) necropsy of infected individual showing opaque, white circular hypertrophied parasitized host cells (i.e. xenomas).

While the species of *Glugea* investigated herein has not been previously studied, costs to hosts can be inferred from other parasitic, xenoma-inducing species in the phylum Microsporidia. For instance, during a period of food deprivation, threespine sticklebacks (*Gasterosteus aculeatus*) infected by *Glugea aculeatus* were observed to lose significantly more body mass than uninfected conspecifics (Ward et al. 2005). Additionally, a lack of vigor and stability while swimming were noted in fathead minnows (*Pimephales promelas*) infected with *Glugea pimephales* (Forest et al. 2009). Reduced condition factor and difficulty retaining body mass were observed in other fish hosts of microsporidians, including American winter flounder (*Pseudopleuronectes*...
*americanus* infected by *Glugea stephani* (Cali et al. 1985). Mortality rates can be greater than 50% in experimental populations infected by *Glugea* (Cali et al. 1986). Sex-ratio distortion can be another consequence of infection by microsporidians (Terry et al. 2004; Ryan and Kohler 2010). For instance, *Glugea anomala* preferentially infected male threespine sticklebacks, resulting in a female-biased sex ratio (Arnold et al. 2003). However, other microsporidians are more likely to infect female hosts (Terry et al. 2004). When considering microsporidians in general, effects of infections can reduce condition of fish (Khan 2005) and even result in population collapse of aquatic invertebrates (Kohler and Wiley 1992).

I quantified the effect of *Glugea* infections on mottled sculpin by examining individual- and population-level responses to infection. Mottled sculpin frequently dominate North American fish assemblages (Scott and Crossman 1973; Grossman et al. 1998; Adams and Schmetterling 2007) and provide a trophic link between aquatic invertebrates and piscivorous predators (e.g. fish, birds, and snakes; Becker 1983). Mottled sculpin also has been highlighted as an indicator species for overall stream health, reaffirming its relevance to ecosystem management (Carline et al. 1994; Brinkman and Woodling 2005; Besser and Mebane 2007). Drawing on previous research, I hypothesized that *Glugea* infected individuals would be older, and exhibit reductions in liver somatic index, mass for a given age, and overall condition (measured by length-weight relationship). Additionally, I expected infected populations would have biased sex ratios (though direction was unclear), reduced levels of genetic diversity as either a cause or consequence of infection (Whiteman et al. 2006; Altermatt and Ebert 2008; Lievely 2010).
and exhibit evidence of genetic bottlenecks associated with recent disease-related declines in abundance. Finally, I expected fish assemblages and environmental characteristics to differ among streams based on the presence of *Glugea*.

Figure 2.2. Locations of 16 small streams located across five major western Michigan river basins that were surveyed for mottled sculpin populations and *Glugea* spp. with sampled stream reaches indicated.
MATERIALS AND METHODS

Field Methods

Sampling occurred during May – July 2011 and June – July 2012 in 16 tributaries of the Grand, Kalamazoo, Muskegon, St. Joseph, and White rivers in the western portion of Michigan’s Lower Peninsula (Figure 2.2). Fish sampling was conducted using a backpack electrofishing unit (ETS Electrofishing, Verona, Wisconsin), and all fish captured in a 90-m reach were identified to species and enumerated. Specific streams and sampling locations were selected based on accessibility (publically accessible land or private landowner permission) and previous observations that indicated streams containing Glugea spp. (S. Kohler, personal observation).

The first 20 mottled sculpin encountered while backpack electrofishing were collected from Silver and Buckhorn creeks and the first 30 mottled sculpin were collected from all other locations (due to higher abundance). Small individuals that were presumed to be age 0 were intentionally excluded from collections. All individuals were immediately euthanized using tricaine methanesulfonate (MS-222). Either a small fin clip (approximately 1 cm²) was taken and stored dry in individually marked vials or a white tissue sample (approximately 5 mg) was removed from below the epidermis to the right side of the anterior dorsal fin for use in genetic analyses. Fish and tissue samples were placed on ice in a cooler during transportation to the laboratory. Euthanized fish were stored at -20°C until necropsies were performed (< 2 months after collection), and white tissue samples and fin clips were stored in a freezer at -80°C immediately upon arrival to the laboratory (< 2 hours after collection) until DNA extraction occurred.
Measurement techniques for environmental variables were based on methods of Fitzpatrick et al. (1998). The length of a surveyed stream reach was determined by multiplying the channel’s mean wetted width by 20 (or at least 150 m) to ensure that a representative combination of hydrologic features were sampled, which always included the 90-m electrofishing reach. Environmental measurements were made at 11 equidistant transects in each reach. At each transect, water depth (cm) and velocity (m/s) were measured using a top-setting wading rod and Marsh-McBirney portable flow meter (Hach Company, Loveland, Colorado) in the stream’s thalweg and at half the distance between the thalweg and each bank for each transect. Percent composition of substrate types were visually estimated based on maximum diameter of particles (fine: <2 mm; gravel: 2-32 mm; cobble: >32 mm). Percent macrophyte (submerged and emergent) coverage was visually estimated at each transect. Specific conductivity was measured at half water-column depth in the thalweg of each transect using a YSI30 conductivity meter (YSI Inc., Yellow Springs, Ohio). Water temperatures were recorded using in-stream data loggers (HOBO Water Temperature Pro v2 Data Loggers, Onset Computer Corporation, Pocasset, Massachusetts) or were made available by the Michigan Department of Environmental Quality or Michigan Department of Natural Resources. Water temperature data incorporates averages from July 19th to August 1st for each stream; however, the years of record vary, including 1984 (Rice Creek), 2005 (Sevenmile Creek) and 2010-2012 (all others).
Laboratory Methods

I verified the identity of the parasite using genetic barcoding techniques. To do this, I used the universal primer set V1(18f) and 1492r (Weiss and Vossbrinck 1998; Vossbrinck and Debrunner-Vossbrinck 2005) to sequence the small subunit (SSU) of the ribosomal DNA (rDNA) of spores obtained from homogenized xenomas. DNA extractions were performed using QIAGEN DNeasy kits (Qiagen, Inc. Valencia, California) on spores obtained from five different fish from each population that had xenomas. Polymerase chain reactions (PCRs) were conducted in 25-µL volumes that contained 2-µL of template DNA, 5-µL of 1x polymerase buffer (Promega), 1-µL each of 5mM V1(18f) and 1492r primers, 0.2-µL 1.0 U Taq polymerase, 2-µL of 2.5 mM MgCl₂, 2.5-µL of 0.2 mM deoxynucleotide triphosphates, and sterile water. PCR amplification occurred using a touchdown protocol that included 94°C for 3 minutes, then 10 cycles of 94°C for 45 seconds, a temperature that begins at 62°C and is reduced by 1°C each cycle for 45 seconds, and 72°C for 2 minutes, followed by 25 cycles of 94°C for 45 seconds, 52°C for 45 seconds, and 72°C for 2 minutes, and concluding with a final extension period of 72°C for 5 minutes. The PCR products were cleaned using 0.05-µL exonuclease I, 0.2-µL shrimp alkaline phosphatase, and 1.75-µL of sterile water for each reaction that was incubated at 37°C for 45 minutes and then 80°C for 20 minutes (Exo-Sap protocol). Next, sequencing reactions for each strand of DNA were performed in 10-µL volumes containing 2-µL of template, 1.5-µL 5x sequence buffer, 1-µL 3.3mM primer (V1(18f) or 1492r), 1-µL BigDye sequencing kit, and sterile water. Conditions for the sequencing reaction were as follows: 95°C for 1 minute, followed by 25 cycles of 95°C for 15
seconds, 50°C for 10 seconds, and 60°C for 4 minutes. Sequencing was subsequently conducted using an ABI 3130x1 automated genetic analyzer (Applied Biosystems, Inc. Foster City, California). Resultant sequences were trimmed and aligned by eye in the computer program MEGA4 (Tamura et al. 2007) and then queried against the GenBank nucleotide database using the BLAST algorithm to quantify similarity to known sequences (http://blast.ncbi.nlm.nih.gov/; Altschul et al. 1990; 1997).

I used a set of six microsatellite loci to evaluate the role of infection on host genetic diversity using 20 mottled sculpins from each stream. DNA was extracted from fin clips or white tissue samples using Qiagen DNeasy blood and tissue kits using manufacturer’s specifications. Following less than optimal DNA quality obtained from fin clip extractions, white tissue was used exclusively for subsequent analyses. I conducted PCRs at six polymorphic microsatellite loci (Table 2.1). PCRs were performed in 25-µL volumes containing 2-µL of template DNA, 5-µL of 1x polymerase buffer (Promega), 0.5-µL of fluorescently labeled primer, 0.5-µL of locus-specific fluorescent primer tag (Table 2.1), 1-µL of unlabeled primer, 0.2-µL 1.0 U Taq polymerase, and locus-specific amounts of 2.5 mM MgCl2, 0.2-mM deoxynucleotide triphosphates (dNTPs; Table 2.1), and sterile water. PCR conditions used the following touchdown procedure (Don et al. 1991; Korbie and Mattick 2008): initial denaturing at 94°C for 2 minutes, followed by 10 cycles of 94°C for 30 seconds, a locus specific annealing temperature (Table 2.1) that was reduced by 1°C for each cycle for 30 seconds, and 72°C for 30 seconds, followed by 30 cycles of 94°C for 30 seconds, the final temperature from the previous set of annealing cycles (i.e. initial annealing temperature minus 10°C) for 30
seconds, and 72°C for 30 seconds, and concluding with a final extension period of 72°C for 10 minutes. Resulting PCR products were multiplexed into two sets (set 1: Cba14, Cco09, Cgo18ZIM, and Cgo310MEHU; set 2: Cott112 and Cott290) before undergoing electrophoresis on an ABI Prism 3130xl automated genetic analyzer. Alleles were scored using either GeneMapper version 3.7 or Peak Scanner version 1.0 (Applied Biosystems, Inc.).

Table 2.1. Polymerase chain reaction conditions for six mottled sculpin microsatellite loci.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Initial TA (°C)</th>
<th>MgCl₂ (µL)</th>
<th>dNTPs (µL)</th>
<th>Fluorescent tag</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cba14</td>
<td>60</td>
<td>2.5</td>
<td>2</td>
<td>Pet</td>
<td>Fiumera et al. 2002</td>
</tr>
<tr>
<td>Cco09</td>
<td>57</td>
<td>3.5</td>
<td>3</td>
<td>Fam</td>
<td>Fujishin et al. 2009</td>
</tr>
<tr>
<td>Cgo18ZIM</td>
<td>55</td>
<td>2.5</td>
<td>2</td>
<td>Ned</td>
<td>Englbrecht et al. 1999</td>
</tr>
<tr>
<td>Cgo310MEHU</td>
<td>60</td>
<td>2.5</td>
<td>2</td>
<td>Ned</td>
<td>Englbrecht et al. 1999</td>
</tr>
<tr>
<td>Cgo1114PBBE</td>
<td>60</td>
<td>2.5</td>
<td>2</td>
<td>Pet</td>
<td>Englbrecht et al. 1999</td>
</tr>
<tr>
<td>Cott290</td>
<td>65</td>
<td>1.5</td>
<td>2</td>
<td>Pet</td>
<td>Nolte et al. 2005</td>
</tr>
</tbody>
</table>

Initial TA, initial annealing temperature; dNTPs, deoxynucleotide triphosphates

Necropsy was used to diagnose infection and measure effects of infection on mottled sculpin. Total length (mm) and mass of fish were measured prior to making initial incisions and all mass measurements were recorded to the nearest 0.001 g using an electronic balance. Accuracy of external disease detection was evaluated by comparing the presence or absence of xenomas assessed visually through the abdominal epidermis.
prior to making the initial incision and the internal visual examination of the body cavity for xenomas. All xenomas from a fish were weighed aggregately and any fish where xenomas were undetected was classified as uninfected. Wet liver mass was measured to calculate the liver somatic index (LSI; Heidinger and Crawford 1977), where lower values indicate impaired condition. Sex of each fish was determined by visual inspection of the gonads for circular (ovarian) or elongated (testicular) structures (Guerrero and Shelton 1974). Both sagittal otoliths were removed from each individual for aging. Once removed, otoliths were submerged in clove oil to improve annuli clarity before being photographed whole via a microscope (Grossman et al. 2002).

Statistical Analyses

Differences in fish assemblages among streams with infected and uninfected populations of mottled sculpin were evaluated using nonmetric multidimensional scaling (NMDS) to identify potential biotic factors that could influence *Glugea* presence. The Sørensen (Bray-Curtis) coefficient was used as the distance measure on the relative abundances of all species. A multi-response permutation procedure (MRPP) was used to test for significant differences in the fish assemblages between infected and uninfected streams. I retained rare species (<5% occurrence) in our multivariate analyses to improve our ability to detect subtle ecological gradients (Cao et al. 1998; Poos and Jackson 2012). However, as a precaution, analyses were conducted both with and without rare species, which indicated that inclusion of rare species did not change the interpretation of results.

A multivariate analysis of variance (MANOVA) was used to assess whether environmental factors were associated with *Glugea* infections in mottled sculpin.
Environmental variables that I included in the MANOVA were percent of fine substrate, mean wetted stream width, specific conductivity, mean daily water temperature, mean water depth, and forested land cover within the stream watershed, and none of these environmental variables were strongly correlated with each other. Because no water temperature data was available for Sand Creek (Grand River basin), this stream was excluded from all environmental analysis.

For analyses focusing on the effects of the disease on mottled sculpin at the individual level, I pooled samples across all streams. I concluded this design was better than blocking by stream because the high disease prevalence in infected streams would have created a strongly unbalanced design (i.e., comparing a small number of uninfected fish with a large number of infected fish in a given stream) and made no use of our observations of fish in uninfected streams. First, I tested whether the mean age of mottled sculpin differed depending on infection status using a t-test. I then tested whether infection intensity (i.e. xenoma mass / somatic body mass) increases with age using an analysis of variance (ANOVA). Next, I evaluated whether somatic mass (i.e. mottled sculpin mass – aggregate xenoma mass) at age differed depending on infection status using a randomized block ANOVA, where age was the blocking variable. The effect of infection intensity on somatic mass at each age was evaluated using multiple regression analysis. I assessed whether two measures of whole-body condition were affected by infection status. First, I tested whether LSI differed with infection status using a t-test. The LSI was calculated as liver mass/(somatic body mass); all measurements were wet mass (g). The influence of infection intensity on LSI was then evaluated using linear
regression. Second, I tested whether the slope of the length-somatic mass relationship (Le Cren 1951) differed with infection status (including interactions) using an analysis of covariance (ANCOVA); $\log_{10}(\text{somatic mass})$ was the response variable and $\log_{10}(\text{total length})$ was the covariate. The role of infection intensity on the length-somatic mass relationship was subsequently tested by regressing the residuals from the length-somatic mass relationship (a measure of an individual fish’s condition) against infection intensity.

Next, I assessed the effect of the disease on mottled sculpin at the population level. For these analyses, I considered the stream reach our experimental unit and performed statistical analyses based on means (which included individuals that I classified as uninfected in streams containing the parasite) except when assessing sex ratios. First, I tested whether sex ratios differed among streams based on infection status using Fisher’s exact test. Second, I tested whether mortality rates differed among streams based on infection status using a t-test. Morality rate ($Z$) of each population was estimated using Chapman and Robson’s maximum-likelihood method, which estimates an instantaneous mortality rate assuming constant mortality and recruitment among years as well as an equal probability of capture for each year class (Chapman and Robson 1960). Age-frequency plots indicated no major assumption violations for my data (Murphy 1997). Third, differences in the density (fish capture/area sampled) and relative abundance of mottled sculpin were compared among streams with and without the parasite using a t-test. Fourth, I tested whether measures of genetic diversity differed with infection status using a MANOVA. Analyzed diversity measures included mean number of alleles ($N_A$) and allelic richness ($A$), which were estimated using the program FSTAT.
(Version 2.9.3.2; Goudet 2001), as well as observed heterozygosity (H₀), expected heterozygosity (Hₑ), and Wright’s inbreeding coefficient (Fᵢₛ) that were estimated using GenePop (Raymond and Rousset 1995). Finally, I tested for evidence of genetic bottlenecks in each population. Genetic bottlenecks caused by severe recent reductions in effective population size can be detected when expected heterozygosities (Hₑ) are higher than expected given mutation-drift equilibrium heterozygosities. Such evidence was evaluated using the computer program BOTTLENECK (v. 1.2.02; Piry et al. 1999). I tested for bottleneck effects under an infinite allele model (IAM), step-wise mutation model (SMM), and two-phase mutation model (70% of SMM mutations and 30% IAM mutations). Significance of heterozygosity excess over all loci was determined using a one-tailed Wilcoxon sign rank test, which is particularly powerful when fewer than 20 loci are considered (Piry et al. 1999). Considering the low number of loci I tested, I applied a Bonferroni correction to a more liberal α = 0.10 (rather than using α = 0.05), yielding a corrected critical value of 0.0063. Significant environmental variables were evaluated for their role in individual- and population-level effects by repeating analyses using only populations that co-occur with Glugea or by using linear regression to determine whether observed effects were caused by the infection or environmental variation as appropriate. Based on residual plots, I used a log₁₀ transformation (or x+1 when zero values were present) on individual length, individual mass, and liver somatic index to correct for heteroscedasticity. All calculations were performed using R version 2.13.2 (R Development Core Team, 2011).
RESULTS

Parasite Identification

Using genetic barcoding techniques, I identified the parasite as *Glugea*. The diagnostic region of the rDNA SSU was successfully sequenced for spores from a total of 26 mottled sculpin that included five from Bigelow, Rice, and Sevenmile creeks, four from Augusta and Wilder creeks, and three from Silver Creek. Variation among sequenced spores was limited (< 1%). BLAST searches confirmed the spores as *Glugea*, as they exhibited 98% similarity to five known *Glugea* species: *Glugea atherinae* (GenBank accession number: U15987.1), *G. hertwigi* (GQ203287.1), *G. plecoglossi* (AB623035.1), *G. anomala* (AF056016.1), *G. stephani* (AF056015.1), and *G. spp.* (JX852026.1). External visual identification of infection was typically not effective. Infection was evident through external observation of the ventral mid-section in 32.1% of fish that were found to be infected following dissection. No fish that appeared infected during external examination was found to be uninfected during necropsy.

Fish Assemblages and Environment

A total of 2,519 fish were captured, representing 30 different species (Appendix 1). Mottled sculpin was the most commonly caught species (69.8% of catch) followed by brown trout (*Salmo trutta*; 11.1%). I detected *Glugea* in mottled sculpin from six streams, whereas I did not detect the parasite in the remaining 10 streams (Table 2.2). A total of 460 mottled sculpin were collected for laboratory examination. The percent of infected individuals in populations where *Glugea* occurred ranged from 53.3% (Wilder Creek) to 86.7% (Bigelow Creek; Table 2.3). The NMDS of fish assemblages provided a two-
dimension solution (stress = 0.11) that indicated modest separation of fish assemblages among streams by infection status (Figure 2.3). However, the differences in the fish assemblages among streams by infection status were not significantly different based on the MRPP ($A = 0.01, p = 0.24$).

Environmental variables were found to differ significantly among streams with and without *Glugea* as indicated by the MANOVA (Wilk’s $\lambda = 0.26$, $F_{6,9} = 4.19$, $p = 0.03$). Further examination indicated significantly higher mean water temperature for streams with *Glugea* was driving the MANOVA results (Table 2.3).

### Table 2.2. Mean values of six environmental variables with standard error in parentheses and results of MANOVA ($p = 0.027$) for streams with ($n = 6$) and without ($n = 10$) *Glugea*.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Present</th>
<th>Absent</th>
<th>$F$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine substrate (%)</td>
<td>39.8 (7.24)</td>
<td>62.9 (11.44)</td>
<td>1.94</td>
<td>0.19</td>
</tr>
<tr>
<td>Mean wetted width (m)</td>
<td>6.4 (0.73)</td>
<td>5.2 (0.77)</td>
<td>0.86</td>
<td>0.37</td>
</tr>
<tr>
<td>Conductivity (µS)</td>
<td>475 (32.55)</td>
<td>428 (46.11)</td>
<td>0.49</td>
<td>0.50</td>
</tr>
<tr>
<td>Mean water temp (°C)</td>
<td>20.3 (0.47)</td>
<td>16.6 (0.71)</td>
<td>12.74</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean water depth (m)</td>
<td>0.35 (0.04)</td>
<td>0.26 (0.03)</td>
<td>1.86</td>
<td>0.35</td>
</tr>
<tr>
<td>Forested land cover (%)</td>
<td>33.6 (7.41)</td>
<td>38.2 (6.55)</td>
<td>0.16</td>
<td>0.69</td>
</tr>
</tbody>
</table>
Figure 2.3. NMDS plot of fish assemblages for six western Michigan streams with, and 10 streams without Glugea (MRPP, $p = 0.24$).

Table 2.3. Number of mottled sculpin examined by necropsy, disease prevalence, and mean ($\pm 1$ standard error) total length (TL), water depth, stream wetted width, and water temperature for 16 western Michigan mottled sculpin populations and resident streams from 5 large river basins.
<table>
<thead>
<tr>
<th>Basin</th>
<th>Stream</th>
<th>n</th>
<th>Percent infected</th>
<th>Total length (mm)</th>
<th>Depth (m)</th>
<th>Wetted width (m)</th>
<th>Water temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalamazoo River</td>
<td>Sand Creek</td>
<td>30</td>
<td>0</td>
<td>72.06 (1.94)</td>
<td>0.32 (0.02)</td>
<td>5.00 (0.39)</td>
<td>18.9 (0.10)</td>
</tr>
<tr>
<td>Kalamazoo River</td>
<td>Portage Creek</td>
<td>30</td>
<td>0</td>
<td>66.20 (1.57)</td>
<td>0.21 (0.02)</td>
<td>3.76 (0.22)</td>
<td>18.8 (0.08)</td>
</tr>
<tr>
<td>Kalamazoo River</td>
<td>Lee Creek</td>
<td>30</td>
<td>0</td>
<td>68.87 (2.17)</td>
<td>0.14 (0.01)</td>
<td>1.89 (0.12)</td>
<td>18.3 (0.10)</td>
</tr>
<tr>
<td>Kalamazoo River</td>
<td>Spring Brook</td>
<td>30</td>
<td>0</td>
<td>64.70 (2.29)</td>
<td>0.35 (0.02)</td>
<td>4.39 (0.23)</td>
<td>17.5 (0.08)</td>
</tr>
<tr>
<td>Kalamazoo River</td>
<td>Wilder Creek</td>
<td>30</td>
<td>53.3</td>
<td>66.03 (2.03)</td>
<td>0.41 (0.04)</td>
<td>4.37 (0.17)</td>
<td>19.3 (0.09)</td>
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<tr>
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<td>Sevenmile Creek</td>
<td>30</td>
<td>73.3</td>
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<td>83.3</td>
<td>69.67 (1.53)</td>
<td>0.29 (0.02)</td>
<td>8.26 (0.59)</td>
<td>23.1 (0.09)</td>
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<td>Kalamazoo River</td>
<td>Silver Creek</td>
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<td>75</td>
<td>78.8 (2.71)</td>
<td>0.16 (0.02)</td>
<td>4.88 (0.17)</td>
<td>19.9 (0.07)</td>
</tr>
<tr>
<td>Kalamazoo River</td>
<td>Rice Creek</td>
<td>30</td>
<td>83.3</td>
<td>71.97 (2.72)</td>
<td>0.46 (0.03)</td>
<td>10.14 (0.41)</td>
<td>19.0 (0.12)</td>
</tr>
<tr>
<td>Muskegon River</td>
<td>Bigelow Creek</td>
<td>30</td>
<td>86.7</td>
<td>65.10 (2.79)</td>
<td>0.38 (0.03)</td>
<td>4.79 (0.41)</td>
<td>19.7 (0.07)</td>
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<tr>
<td>Muskegon River</td>
<td>Buckhorn Creek</td>
<td>20</td>
<td>0</td>
<td>66.6 (2.62)</td>
<td>0.21 (0.03)</td>
<td>3.27 (0.34)</td>
<td>12.5 (0.03)</td>
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<tr>
<td>Muskegon River</td>
<td>Cedar Creek</td>
<td>30</td>
<td>0</td>
<td>59.17 (1.93)</td>
<td>0.38 (0.02)</td>
<td>8.25 (0.37)</td>
<td>16.8 (0.07)</td>
</tr>
<tr>
<td>White River</td>
<td>Knutson Creek</td>
<td>30</td>
<td>0</td>
<td>69.3 (2.32)</td>
<td>0.13 (0.01)</td>
<td>3.80 (0.28)</td>
<td>16.5 (0.03)</td>
</tr>
<tr>
<td>White River</td>
<td>Sand Creek</td>
<td>30</td>
<td>0</td>
<td>64.5 (2.00)</td>
<td>0.24 (0.02)</td>
<td>7.31 (0.58)</td>
<td>13.8 (0.03)</td>
</tr>
<tr>
<td>Grand River</td>
<td>Sand Creek</td>
<td>30</td>
<td>0</td>
<td>67.50 (2.05)</td>
<td>0.30 (0.02)</td>
<td>9.70 (0.41)</td>
<td>18.0 1</td>
</tr>
<tr>
<td>St. Joseph River</td>
<td>Curtis Creek</td>
<td>30</td>
<td>0</td>
<td>75.63 (1.42)</td>
<td>0.29 (0.02)</td>
<td>5.05 (0.37)</td>
<td>15.2 (0.08)</td>
</tr>
</tbody>
</table>

1 actual data missing. Reported result is mean of existing values and was used in multivariate analyses.
Individual-Level Analyses

I evaluated four hypotheses regarding the effects of *Glugea* infection on individual mottled sculpin. First, I found mean age was not significantly different between infected and uninfected individuals (*t*$_{458}$ = 0.22, *p* = 0.82; Figure 2.4A); however, when mottled sculpin from only infected populations were considered, mean age was significantly higher for infected individuals (*t*$_{168}$ = -2.02, *p* = 0.05; Figure 2.4B). Additionally, the slope of the relationship between infection intensity and age was not significant (*t*$_{127}$ = -0.83, *p* = 0.41). Second, infected mottled sculpins were significantly larger in terms of somatic mass at a given age than uninfected individuals (*t*$_{451}$ = 7.01, *p* < 0.01; Figure 2.5) and the interaction between infection status and age was not significant (*t*$_{451}$ = 0.28, *p* = 0.97). To account for the potential effects of water temperature on growth (because warmer streams tended to have the infection), analyses of mass at age were repeated using only individuals from populations that co-occur with *Glugea*, which yielded similar results. Additionally, using a multiple regression model, I found that somatic mass of mottled sculpin increased with age as expected but there also was a significant interaction between age and infection intensity (Table 2.4, Figure 2.6). For older mottled sculpin, growth rates (i.e. somatic mass at age) were lower for individuals with more severe infections (Figure 2.6). I also found that water temperature did not significantly influence mean somatic mass (*t*$_{13}$ = 0.93, *p* = 0.37; Figure 2.7), suggesting observed differences in mass are likely a cause of infection. Third, LSI was significantly lower for infected individuals (*t*$_{458}$ = 3.63, *p* < 0.01; Figure 2.8A); however, when reanalyzing LSI using only individuals that co-occurred with *Glugea*, LSI values were not significantly different depending on infection status (*t*$_{85}$ = 0.14, *p* = 0.89; Figure 2.8B). Although warmer streams tended to have the *Glugea*
infection, the slope of the relationship between mean stream LSI and water temperature was not significant \((t_{14} = 0.25, p = 0.80)\). Testing LSI as a function of infection intensity also yielded a nonsignificant result \((t_{458} = 1.86, p = 0.07, r^2 = 0.02)\). Fourth, I tested whether infection caused a reduction in the slope of the length-weight relationship (as an index of overall condition). I found the effect of infection on the slope of the total length-somatic mass relationship was greatest for small mottled sculpin and the difference diminished as size increased \((t_{458} = 2.52; p = 0.01; \text{Figure 2.9A})\). Additionally, total length \((t_{457} = 60.72, p < 0.01)\) was found to be a significant covariate and infection was a significant factor \((t_{457} = 4.51, p < 0.01)\). Nevertheless, the effect size (i.e. difference in regression lines) was small. For instance, the somatic mass of small (44 mm) mottled sculpin with the infection was 0.193 g greater than uninfected fish, which was only a difference of about 4.2%. When limiting analyses to individuals from populations co-occurring with *Glugea*, there was not a significant difference between the slopes \((t_{166} = 0.45, p = 0.66)\) or intercepts \((t_{166} = 1.05, p = 0.30; \text{Figure 2.9B})\), although somatic mass still increased significantly with total length \((t_{166} = 36.31, p < 0.01; \text{Figure 2.9B})\). For only infected fish, the residuals from the regression between somatic mass and total length were not significantly associated with infection intensity \((t_{127} = 1.01, p = 0.32, r^2 = 0.01)\), further supporting the lack of an ecologically meaningful difference in the slopes of the length-mass relationship. To evaluate the influence of water temperature on the length-weight relationships, I regressed residuals of the total length-somatic mass relationship against water temperature. This yielded a significant result \((t_{428} = 20.69, p < 0.01; \text{Figure 2.10})\), suggesting that observed differences in the length-weight of infected and uninfected mottled sculpin (Figure 2.9A), may be driven by differences in stream water temperature.
Figure 2.4. Relationship of mean ages (with standard error bars) among infected and uninfected mottled sculpin including data for individuals from (A) all sampled populations ($n = 460$ individuals) and (B) only from populations co-occurring with *Glugea* spp. ($n = 170$ individuals).
Figure 2.5. Mean (±1 standard error) somatic mass at a given age for mottled sculpin infected \((n = 129)\) and uninfected \((n = 331)\) with \textit{Glugea} spp.
Table 2.4. Results of multiple regression analysis evaluating the relationship of somatic mass to infection and age (i.e. somatic mass = infection intensity * age).

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.46</td>
<td>0.09</td>
<td>5.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age</td>
<td>0.12</td>
<td>0.05</td>
<td>2.50</td>
<td>0.01</td>
</tr>
<tr>
<td>Intensity</td>
<td>0.08</td>
<td>0.04</td>
<td>2.38</td>
<td>0.02</td>
</tr>
<tr>
<td>Age*Intensity</td>
<td>-0.04</td>
<td>0.02</td>
<td>-2.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Figure 2.6. Relationship between *Glugea* spp. infection intensity (i.e. xenoma mass/somatic mass) and somatic mass among mottled sculpin from four age groups ($n = 129$).
Figure 2.7. Relationship between mean water temperature and mean somatic mass for 15 populations of mottled sculpin.
Figure 2.8. Mean (±1 standard error) liver somatic index of infected and uninfected mottled sculpin for (A) all sampled populations (n = 460) and (B) only populations co-occurring with Glugea spp. (n = 170).
Figure 2.9. Relationship between somatic mass and total length for infected and uninfected mottled sculpin for (A) all sampled populations (n = 460; uninfected: $y = -5.14 + 3.13x$; infected: $y = -4.60 + 2.85x$; $y = \log_{10}[\text{somatic mass}]$ and $x = \log_{10}[\text{total length}]$) and (B) only populations co-occurring with Glugea (n = 170; uninfected: $y = -4.77 + 2.94x$; infected: $y = -4.60 + 2.85x$).
Figure 2.10. Distribution of the residuals of a total length-somatic mass regression versus water temperature for mottled sculpin \((n = 430)\) from 15 populations.
Population-Level Analyses

Most population-level analyses did not find significant effects of *Glugea* infection. Mottled sculpin susceptibility to infection did not differ significantly with sex (males: 28.8% prevalence, females: 27.1% prevalence; Fisher’s exact test two-sided: $p > 0.875$). Consequently, sex ratios did not differ among streams regardless of infection status (males/females = 1.152) or uninfected (1.148). No significant difference was found between mottled sculpin densities in streams with and without *Glugea* (with *Glugea* [mean ± 1 SE]: 0.10 ± 0.04 mottled sculpin/m$^2$; without: 0.16 ± 0.04 mottled sculpin/m$^2$; $t_{14} = 1.05, p = 0.31$). Similarly, mottled sculpin relative abundances did not differ significantly with infection status (with *Glugea*: 0.55 ± 0.06; without: 0.63 ± 0.11; $t_{14} = 0.93, p = 0.37$). Mortality rates also were not significantly different among streams based on infection status (infected mean $Z$: 0.57 ± 0.03 yr$^{-1}$; uninfected $Z$: 0.52 ± 0.02 yr$^{-1}$; $t_{14} = -0.79, p = 0.45$).

I found evidence of reduced genetic diversity for mottled sculpin populations infected with *Glugea*. Microchecker analyses identified one locus exhibiting evidence of null alleles and no evidence of stuttering or allelic dropout. The locus *Cgo310MEHU* showed evidence of null alleles in the Bigelow Creek and Cedar Creek populations (estimated frequency: 0.15 for each population). Due to the relatively low estimated frequency of null alleles and evidence only being recorded in two populations, *Cgo310MEHU* was retained for genetic diversity estimates. Populations infected with *Glugea* had significantly lower $H_E$ ($t_{14} = -2.27, p = 0.04$) and $F_{IS}$ ($t_{14} = -2.51, p = 0.03$; Table 2.5). No population was found to have significant evidence of having undergone a population bottleneck (Table 2.5).
Table 2.5. Comparison of mean genetic diversity measures with standard error in parentheses for *Glugea* infected (*n* = 6) and uninfected (*n* = 10) mottled sculpin populations estimated using six microsatellite loci and significance assessed using *t*-tests with 14 degrees of freedom.

<table>
<thead>
<tr>
<th></th>
<th>Infected Mean</th>
<th>Uninfected Mean</th>
<th><em>t</em></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N_a</strong></td>
<td>2.77 (0.13)</td>
<td>2.94 (0.14)</td>
<td>-0.85</td>
<td>0.407</td>
</tr>
<tr>
<td>A</td>
<td>2.64 (0.13)</td>
<td>2.84 (0.13)</td>
<td>-0.99</td>
<td>0.340</td>
</tr>
<tr>
<td><strong>H_o</strong></td>
<td>0.31 (0.05)</td>
<td>0.34 (0.02)</td>
<td>-0.60</td>
<td>0.557</td>
</tr>
<tr>
<td><strong>H_e</strong></td>
<td>0.30 (0.03)</td>
<td>0.39 (0.02)</td>
<td>-2.27</td>
<td>0.039</td>
</tr>
<tr>
<td><strong>F_is</strong></td>
<td>-0.03 (0.07)</td>
<td>0.14 (0.03)</td>
<td>-2.51</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Mean number of alleles (N_a), allelic richness (A), observed heterozygosity (H_o), expected heterozygosity (H_e) and Wright's inbreeding coefficient (F_is)
Table 2.6. Results of significance testing for genetic bottlenecks using infinite allele model (IAM), two-phase model (TPM), and step-wise mutation model (SMM); Bonferroni corrected alpha level = 0.0063.

<table>
<thead>
<tr>
<th>Population</th>
<th>Basin</th>
<th>IAM</th>
<th>TPM</th>
<th>SMM</th>
</tr>
</thead>
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<td>Sand Creek</td>
<td>Kalamazoo</td>
<td>0.594</td>
<td>0.688</td>
<td>0.922</td>
</tr>
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<td>Portage Creek</td>
<td>Kalamazoo</td>
<td>0.906</td>
<td>0.906</td>
<td>0.938</td>
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<tr>
<td>Lee Creek</td>
<td>Kalamazoo</td>
<td>0.063</td>
<td>0.094</td>
<td>0.094</td>
</tr>
<tr>
<td>Spring Brook</td>
<td>Kalamazoo</td>
<td>0.719</td>
<td>0.945</td>
<td>0.977</td>
</tr>
<tr>
<td>Wilder Creek</td>
<td>Kalamazoo</td>
<td>0.047</td>
<td>0.109</td>
<td>0.109</td>
</tr>
<tr>
<td>Sevenmile Creek</td>
<td>Kalamazoo</td>
<td>0.008</td>
<td>0.008</td>
<td>0.023</td>
</tr>
<tr>
<td>Augusta Creek</td>
<td>Kalamazoo</td>
<td>0.781</td>
<td>0.922</td>
<td>0.922</td>
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<td>Silver Creek</td>
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<td>0.156</td>
<td>0.438</td>
<td>0.938</td>
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<td>Kalamazoo</td>
<td>0.313</td>
<td>0.500</td>
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</tr>
<tr>
<td>Bigelow Creek</td>
<td>Muskegon</td>
<td>0.281</td>
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<td>0.781</td>
</tr>
<tr>
<td>Buckhorn Creek</td>
<td>Muskegon</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
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<td>Muskegon</td>
<td>0.594</td>
<td>0.891</td>
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<td>Knutson Creek</td>
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<tr>
<td>Sand Creek</td>
<td>White</td>
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</tr>
<tr>
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<tr>
<td>Curtis Creek</td>
<td>St. Joseph</td>
<td>0.906</td>
<td>1.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>
DISCUSSION

Given the ability of xenomas to disfigure their hosts (e.g. Figure 2.1), I found considerably lower costs of infection to infected individuals than expected. My findings suggest that the *Glugea* infection has only subtle effects on host mottled sculpin. This study also linked increased water temperature with the presence of the infection in streams, which could be helpful in predicting streams that contain the parasite.

Contrary to my expectations, mottled sculpin infected with *Glugea* were larger at all given ages. This result was particularly surprising given the presumed metabolic costs of infection inferred by xenoma size. One potential cause of this unexpected relationship is a re-allocation of resources within infected mottled sculpin. For instance, if fecundity is diminished for infected individuals, more energy may be allocated to somatic growth. Alternatively, larger individuals can have naturally higher foraging rates that may have increased risk of exposure to *Glugea* spores (Hutchings et al. 2002; Hall et al. 2007; Blanchet et al. 2009). Because significant differences in the total length-somatic mass relationship of infected and uninfected individuals is likely of little ecological consequence (see above), maintaining comparable length-weight relationships regardless of infection status is indicative of proportional increases to length and mass as infected individuals grow, further supporting a lack of metabolic costs due to infection.

The ability of the host species to remain largely unaffected at the population-level suggests disease-associated individual losses are likely compensatory. Highly stable population dynamics of mottled sculpin despite highly variable environmental conditions have been previously explained using a model of simple density dependence (Grossman et al. 2006). Moreover, Grossman et al. (2006) also found that when adult sculpin temporarily were removed
from stream reaches, they were quickly replaced by juveniles (whom are generally competitively inferior). The absence of genetic bottlenecks that I documented also supports the host populations’ ability to maintain demographic stability despite infection.

This study provides basic ecological insight into the role of microsporidian parasites in natural ecosystems. For instance, my findings support the importance of warm water temperatures for the success of microsporidians (Takahashi and Egusa 1977; Olson 1981; Becker et al. 2006). Streams that experience warming because of global climate change and watershed deforestation should be more likely to have microsporidian parasites. My results also convey much less disruptive consequences of infection compared with other studies of microsporidians. For example, while mottled sculpin are only modestly affected by Glugea, cyclic collapses have been observed in caddisfly populations infected with Microsporidium spp. (Kohler and Wiley 1992), and the microsporidian Nosema ceranae has been implicated in colony collapse disorder of various bee species (Higes et al. 2008). Insights into the host species’ ecology can help to form additional inferences about the parasite. For instance, drawing on the species’ defensive territoriality (Petty and Grossman 2004; 2007), the high prevalence of the parasite is likely the result of the free-living stage of the microsporidian lifecycle being present in high densities throughout the water column (Keeling and Fast 2002) rather than a result of excessive mottled sculpin intra-species contact. Finally, a lag time between infection and xenoma development is suggested by the significantly greater mean age of infected mottled sculpin compared to uninfected individuals when considering only populations that co-occur with Glugea (Figure 2.4B). The similarity of LSI values regardless of infection status for fish from infected populations (Figure 2.7B) also supports the idea of an infection latency period, presuming some
individuals classified as uninfected were actually being impacted by the parasite. Despite microsporidian parasites being ubiquitous in nature, this study represents a rare effort to investigate one in a natural setting. Given the apparent variability in microsporidian effects on hosts and with very little known regarding the parasite’s ecological roles, future investigation will be required to understand this unique group of organisms.
LITERATURE CITED


Olson, R.E. 1981. Effects of low temperature on the development of the microsporidian Glugea stephani in English sole (Parophrys vetulus). J. Wildl. Dis. 17(4): 559-562. doi:


## Appendix 1. Number and species of fish captured via backpack electrofishing of 90-m stream reaches 2011-2012.

<table>
<thead>
<tr>
<th>Species</th>
<th>Kalamazoo River</th>
<th>Muskegon River</th>
<th>White River</th>
<th>Grand River</th>
<th>St. Joseph River</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sand Creek</td>
<td>Portage Creek</td>
<td>Lee Creek</td>
<td>Spring Brook</td>
<td>Wilder Creek</td>
</tr>
<tr>
<td>Mottled sculpin</td>
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<td>60</td>
<td>104</td>
<td>245</td>
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<tr>
<td>Brown trout</td>
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<td>42</td>
<td>17</td>
<td>6</td>
<td>50</td>
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<td>4</td>
<td>1</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Green sunfish</td>
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<td></td>
<td></td>
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<tr>
<td>Pumpkinseed</td>
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<td></td>
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</tr>
<tr>
<td>Bluegill</td>
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<tr>
<td>Creek chub</td>
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<td>Northern hog sucker</td>
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<td>Grass pickerel</td>
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<td>Rainbow darter</td>
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### Appendix 1 continued

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<th>Fish Species</th>
<th>Kalamazoo River</th>
<th>Muskegon River</th>
<th>White River</th>
<th>Grand River</th>
<th>St. Joseph River</th>
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<tbody>
<tr>
<td></td>
<td>Sand Creek</td>
<td>Portage Creek</td>
<td>Lee Creek</td>
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<td>Wilder Creek</td>
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<tr>
<td>Black-sided darter</td>
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<th>Knutson Creek</th>
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