Combined effects of parasitism and pollution on the antipredator behaviour of

Etheostoma nigrum (Percidae: Etheostomatinae)

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Abstract

Combined effects of parasitism and pollution on the antipredator behaviour of *Etheostoma nigrum* (Percidae: Etheostomatinae)

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Pollution can have significant effects on the parasite communities and behaviour of freshwater fish. This study compares the helminth communities and predator avoidance behaviour of johnny darters (*Etheostoma nigrum*) collected from two reference localities and three polluted localities in the St. Lawrence River in southwestern Quebec, Canada. Both reference localities and one locality impacted with industrial and agricultural contaminants were located upstream of Montreal; two sites impacted with municipal sewage and urban effluent were located downstream of the city. Overall, darters were infected with 24 species of helminths, 16 of which were larval stages. Fish from the upstream polluted locality (industrial and agricultural) had a higher mean species richness than the two reference localities, which had higher richness than the two downstream localities (sewage). Fish from the upstream and reference localities had higher total parasite numbers than the two downstream localities. A non-parametric, permutational multivariate ANOVA (PERMANOVA) using Bray-Curtis dissimilarities between communities of individual fish revealed that the parasite communities differed by locality, pollution status of locality and type of pollution (upstream versus downstream). A capture time experiment and a flight distance experiment were performed, to test the effects of parasitism and pollution on susceptibility to predation. A PERMANOVA demonstrated that abundance of a brain-encysting parasite, *Ornithodiplostomum* sp. and locality explained capture time, but failed to detect a relationship between pollution status

and antipredator behaviour. Fish with high intensity infections of the brain encysting parasite, *Ornithodiplostomum* sp., were more difficult to capture, reflecting increased activity of infected individuals. Abundances of *Ornithodiplsotomum* sp. metacercariae were greater at reference localities than at impacted ones, possibly reflecting sensitivity to pollution of transmission stages or snail hosts of this parasite. Pollution may have an indirect effect on johnny darter antipredator behaviour, by decreasing abundance of a behaviour-modifying parasite at polluted localities.

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Contributions of Authors

Both chapters of this thesis have been prepared as manuscripts for submission for publication. Prof. J. Daniel McLaughlin and Dr. David J. Marcogliese contributed to the study design and editing of both chapters. Prof. James Grant provided equipment and contributed to design of the behaviour experiments in Chapter 2.

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<center>**Literature Review**</center>

Parasitism and Parasite Transmission

Parasitism is a unique category of symbiosis in which the parasite uses the host as both habitat and food source but parasites differ from other symbionts by their potential to harm their hosts (Bush *et al.* 2001). Parasitism is a highly successful life history strategy and parasitic species can be found in almost all animal phyla (Roberts and Janovy 2005). It is believed that parasites, in the broadest sense, make up the majority of living organisms on Earth (Hudson 2005).

Fish are infected by a variety of parasites, and for the purposes of this review only the macroparasites (parasitic worms and arthropods) will be considered. Some parasites, called ectoparasites, attach to the external surfaces of the fish, including the skin, fins and gills. These include monogeneans, copepods and branchiurans. Others, called endoparasites, inhabit internal tissues of the host. In this category are digenetic trematodes, cestodes, nematodes and acanthocephalans (Roberts and Janovy 2005).

Macroparasites display two basic life cycle strategies. Directly transmitted parasites are spread by production of eggs or larvae by the adult parasite that are shed into the environment and consumed by the next host where they develop directly into another adult individual. Some ectoparasites may also be spread through physical contact with an infected host. In the context of the parasites encountered in the present study, directly transmitted parasites would include ectoparasites such as monogeneans and crustaceans, and some endoparasitic nematodes. In other endoparasites, the life cycles are indirect and the transmission process is more complex. Here, passage through two or more different species of hosts is required to reach maturity. Infective stages produced by

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adults are discharged into the environment where they infect a second species of host and develop into larval stages that in turn become infective to the next host in the life cycle. Digenetic trematodes, cestodes, acanthocephalans and many species of nematodes are transmitted indirectly and require two or more host species to complete their development. In indirect life cycles, the host species in which the parasite attains sexual maturity is referred to as the definitive host; hosts infected with larval stages are referred to as intermediate hosts. Depending on the parasite, fish acquire infections through contact with or consumption of infective stages directly from the environment or through consumption of intermediate hosts. Fish serve as definitive hosts for a number of parasites, however, they also serve as intermediate hosts for many parasites that use predatory fish, birds and mammals as definitive hosts. In natural conditions, individual fish commonly act as a definitive host for some species and as an intermediate host for others simultaneously. Most adult parasites occur on the body surface or digestive tract of the host. The larvae of many species infect the musculature while other species infect the brain and eyes (Roberts and Janovy 2005).

Parasites may have important effects on host populations. For example, parasites have the potential to regulate the populations of their hosts (Anderson and May 1978; May and Anderson 1978). This has been demonstrated primarily in laboratory studies with model systems, such as in *Gyrodactylus turnbulli* infections of guppies (*Poecilia reticulata*) (Scott and Anderson 1984). However, regulation has also been reported in a few wild and semi-domesticated species, such as red grouse (*Lagopus lagopus scoticus*) infected with *Trichostrongylus tenuis* (Dobson and Hudson 1992; Hudson *et al.* 1992) and mountain hares (*Lepus timidus*) infected with *T. retortaeformis* (Newey and Thirgood

2004; Newey *et al.* 2004; 2005) To date, regulation has only been shown in systems involving parasites with direct life cycles, but it is believed that regulation may be possible, although more difficult to detect, in systems with parasites with indirect life cycles (Scott and Dobson 1989).

Parasites are cryptic members of biotic communities and have long been viewed as a negligible part of the biomass of ecosystems when compared to that of free-living organisms. However a study of parasite biomass in the Carpenteria salt marsh in California demonstrated that parasites make up more biomass than top bird predators in that system (Kuris *et al.* 2008). The authors suggest that parasite biomass is likely considerable in other ecosystems as well.

Parasites and fish behaviour

Parasitism may alter host fish behaviour in a variety of ways. Examples include changes in locomotion, foraging, predator avoidance and resistance, habitat use and reproduction (Barber *et al.* 2000). These changes may arise through adaptations of the host to infection, manipulation by the parasite to increase its fitness, or pathology of parasitism that benefits neither host nor parasite (Poulin and Thomas 1999).

Adaptive changes in the host include behaviour to avoid parasites or eliminate infections after acquiring them (Poulin 1995; Moore 2002; Barber and Rushbrook 2008). Fish may alter their behaviour as a first defense in response to a perceived risk of infection. They can limit directly transmitted parasites by avoiding conspecifics with detectable infestations or in visibly poor health. They can limit indirectly transmitted parasites with penetrating larval stages by avoiding areas where free-living larval

parasites are found (Moore 2002; Barber and Rushbrook 2008; Perrot-Minnot and Cézilly 2009). For example, individual rainbow trout (*Oncorhynchus mykiss*) experimentally exposed to infective cercariae of the trematode *Diplostomum spathaceum* acquired fewer infections when they were able to move freely than when they were confined to areas with high concentrations of the parasite (Karvonen *et al.* 2004). Following infection, fish may exhibit behaviours that limit or eliminate the parasites. Such behaviour include scraping against hard substrates and visiting cleaner fish or shrimp to remove ectoparasites (Barber and Rushbrook 2008).

Changes in host behaviour that increase transmission success are adaptive for the parasite; this is essentially an extension of the parasite's genotype to the host (Dawkins 1976; Combes 2001). Such changes are generally considered to be in this category if they appear after the parasite becomes infective to its next host (Poulin 1995). Behavioural manipulations are often found in intermediate hosts of parasites with indirect life cycles which require trophic transmission, and make the host more susceptible to predation by the parasite's definitive host (Lafferty 1999; Barber *et al.* 2000; Moore 2002). Such changes have been reported from a number of systems (reviewed by Barber *et al.* 2000; Moore 2002; Barber and Rushbrook 2008). For example, rainbow trout with infective metacercariae of *D. spathaceum* displayed fewer attempts to escape when presented with a simulated bird shadow, and were more commonly caught in simulated predation by a bird than non-infected fish (Seppälä *et al.* 2004; 2005). Similarly, California killifish (*Fundulus parvipinis*) infected with metacercariae of the brain-encysting trematode, *Enhaplorchis californiensis*, exhibited more erratic swimming behaviour, including increased flashing and surfacing, and abnormal movements such as jerky swimming and

contortion of the body in dorsal-ventral bends. These changes corresponded with

increased susceptibility to bird predation (Lafferty and Morris 1996). Roach (*Rutilus*

rutilus) infected with plerocercoids of the cestode *Ligula intestinalis* showed a greater

preference for the top layers in the water column than non-infected fish. When exposed to

a heron predator model, infected fish joined their non-infected conspecifics in deeper

water, but exhibited significantly less movement, which the authors suggest might make

them more susceptible to predation (Loot *et al.* 2002).

Parasite-induced behavioural changes may also include manipulations to decrease

predation by non-host predators. Although this type of behaviour has not been reported

from fish systems, freshwater amphipods (*Gammarus roeseli*) infected with the

acanthocephalan *Polymorphus minutus* are known to change their habitat use in the

presence of a non-host predator, the threespine stickleback (*Gasterosteus aculeatus*),

resulting in a lower predation rate of infected versus non-infected amphipods (Medoc *et

al.* 2009). A similar experiment was conducted with rainbow trout infected with *D.*

spathaceum, however in this study, infected hosts were as susceptible to the non-host

predatory pike (*Esox lucius*) as non-infected hosts, suggesting that the parasite was not

manipulating behaviour to avoid predation by dead-end hosts (Seppälä *et al.* 2006).

Seppälä *et al.* (2006) suggest that specific changes to limit predation by non-host

predators will likely only be selected for if predation pressure on the intermediate host is

already high, and not when ambient pressure is low.

There may be competition between manipulative parasites when multiple species

are present within a single host (Barber *et al.* 2000). There is limited experimental

evidence of from freshwater amphipods that multiple infections can cause unpredictable

changes in host behaviour. *G. roeseli* infected with only larvae of the acanthocephalan *P.*

minutus showed a greater preference for positions higher in the water column than non-

infected individuals, while hosts co-infected with the acanthocephalan and the

microsporidia, *Dictyocoela* sp., displayed a weakened preference (Haine *et al.* 2005). The

authors suggest that multiple infections may account for the variation in the behaviour of

infected hosts.

Finally, some changes arise out of inevitable pathology of infection, and do not

benefit host or parasite (Poulin 1995; Barber and Rushbrook 2008). Infected fish often

exhibit lethargy, increased or decreased foraging, and reduced social interactions. Barber

et al. (2000) argue that any behavioural changes, whether considered pathological or

adaptive, will likely have consequences for the ecology and ultimately the fitness of the

individual host, and so cannot be considered evolutionarily neutral.

Pollution and fish behaviour

Pollution enters the aquatic environment from a variety of sources, including

sewage treatment facilities, industry, mining, and agriculture. Contaminants from these

activities include metals, polychlorinated biphenyls (PCBs), polycyclic aromatic

hydrocarbons (PAHs), pesticides, organochlorines such as dioxins, and nutrient

enrichment in the form of chemical fertilizers, organic material and bacteria. At high

concentrations, many of these substances can have lethal effects on organisms, but

chronic exposure to sublethal levels can also have significant effects on aquatic life

(Jobling 1995).

Pollution can have ecological effects for fish when they respond behaviourally to avoid it. Avoidance response tests have shown that fish are often capable of detecting metal contamination, and will avoid it when non-contaminated areas are available to them (Atchison *et al.* 1987, Jobling 1995). This behaviour has been verified in field studies, where fish avoid habitats or stretches of streams close to point sources of contamination. This can have ecological effects, such as limiting spawning habitat available to sensitive fish species (reviewed by Atchison *et al.* 1987). Other contaminants may elicit an attractive response in some fish species, which is maladaptive and leads to increased levels of exposure (Atchison *et al.* 1987).

Toxicants often disrupt the normal functioning of sensory, endocrine, neurological and metabolic systems, all of which can have consequences for fish behaviour (Scott and Sloman 2004; Sloman 2007). For example, toxins may limit the ability of fish to detect visual or olfactory cues, which are necessary for interpreting and assessing the behaviour or chemical cues of others, identifying kin and non-kin, and detecting and assessing predation risk. These cues may be lost through the malfunctioning of sensory organs, or through the inability of the nervous system to process sensory information (Atchison *et al.* 1987; Scott and Sloman 2004; Sloman 2007). Pollutants can also interfere with the endocrine system, changing hormone production which can increase or decrease aggressiveness of individuals (Sloman 2007). Effects on metabolism may result in decreased swimming performance, or increased or decreased time spent on activities such as foraging.

In recent years, the use of behavioural endpoints has become more common in ecotoxicology, because they are often sensitive to relatively low concentrations of

toxicants and are an ecologically relevant measure of environmental stress (Atchison *et al*. 1987; Gerhardt 2007). The advantage of behavioural endpoints over other measures, such as changes in morphology, life history attributes or community diversity, is that they appear on much shorter timescales (Gerhardt 2007). Simple, individual behaviours directly linked to physiology, including swimming performance and respiratory movements, have been commonly used in ecotoxicology because they are particularly sensitive to pollution and these studies are relatively simple to perform and interpret (Atchison *et al*. 1987). More complex individual and interactive behaviours are also affected by toxicants, and some have been used as endpoints in ecotoxicology tests. These include foraging, predator detection and avoidance, learning, reproductive and other social interactions such as the formation and maintenance of social hierarchies (Atchison *et al*. 1987; Scott and Sloman 2004; Gerhardt 2007; Sloman 2007). They are more difficult to interpret, not only because of the subjective nature of scoring these particular endpoints, but also because their causes and effects may not be independent. For example, pollution exposure can lead to increased foraging, however greater foraging rates may also lead to an increase in exposure if food sources are contaminated.

Parasitism and pollution

As discussed above, free-living aquatic organisms such as fish may be affected by chronic exposure to sublethal levels of pollution. Exposure may also have effects on parasitic organisms in these environments. Pollution tends to lead to a decrease in diversity of parasite communities (Cone *et al*. 1993; Mackenzie *et al*. 1995; Kennedy

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1997; Lafferty 1997; Overstreet 1997; Mackenzie 1999; Sures 2004; Marcogliese 2005; Blanar et al. 2009). This may be due to direct or indirect effects of pollution on parasites.

Ectoparasites such as monogeneans and crustaceans are continuously exposed to the external environment; others like the free-living larval stages of trematodes and cestodes only briefly (Poulin 1992). Laboratory studies of effects of metals, acidification, pesticides and chemical fertilizers on larval stages of some digenean and cestode species have shown toxic effects (reviewed by Morley et al. 2003; Pietrock and Marcogliese 2003). These toxins may work in a variety of ways to alter infection levels in the next host. These include decreasing longevity, survival and infectivity of larval stages or by impairing their ability to encyst (Morley et al. 2003; Pietrock and Marcogliese 2003). The relationship between pollutants and loss of free-living larval stages is not a simple one, however, and some studies have shown an increase in longevity or infectivity after exposure to low concentrations of some toxins (Pietrock and Marcogliese 2003). Adult ectoparasites also show sensitivity to environmental contaminants. For example, the monogenean ectoparasite G. turnbulli of guppies exposed to zinc had decreased survival, reproduction and growth (Gheorghiu et al. 2007).

Parasites may be indirectly affected when they use intermediate hosts that are sensitive to pollutants. This is commonly seen with molluscan first intermediate hosts of digenetic trematodes, which are particularly sensitive to environmental stressors such as metals (Coeurdassier et al. 2003; 2005; Grosell et al. 2006; De Schamphelaere et al. 2008; Pietrock et al. 2008). Infection with trematode sporocysts or rediae may also increase the rate of toxin-induced mortality of snail hosts, which limits both the number

of cercariae produced and the duration of the shedding period in those snails that survive

long enough to produce them (Morley *et al.* 2003; Morley *et al.* 2006).

Pollution can also indirectly affect parasites through immunosuppression of their

hosts. In stressed environments, a proliferation of some directly transmitted parasites with

low sensitivity to toxins, such as ciliates, copepods and some monogeneans, is observed

on fish (Khan and Thulin 1991; Poulin 1992; Morley *et al.* 2006). Populations of hosts

exposed to pollution may also show lower average parasite diversities due to higher

toxin-induced mortality rates of highly infected fish than fish with low or no infections

(Lafferty 1997; Barber *et al.* 2000; Sures 2008).

This pattern of decreased overall parasite diversity has been demonstrated in a

number of field studies of parasitized fish in polluted environments (reviewed by

Marcogliese 2005). For example, winter flounder (*Pleuronectes americanus*) exposed to

pulp and paper mill effluent (Khan 2006; Khan and Billiard 2007) and crude oil (Khan

and Payne 2004) had lower abundances of intestinal helminths than flounder that were

not exposed to toxins. Grey mullet (*Liza aurata* and *L. ramada*) in a polluted estuary had

lower abundances of helminth parasites compared with fish in a nearby reference estuary

(Dzikowski *et al.* 2003). Flounder (*Platichthys flesus*) exposed to urban effluent in the

German Bight in the North Sea had lower parasite species richness than flounder in

reference areas (Schmidt *et al.* 2003). Perch (*Perca fluviatilis*) in two acidified reservoirs

had impoverished parasite infracommunities, compared to a reference lake, due to a loss

of digenetic trematodes and ectoparasites in the acidic environments (Halmetoja *et al.*

2000). Similarly, American eels (*Anguilla rostrata*) in acidic streams in Nova Scotia were

missing trematodes and monogeneans present in nearby reference streams (Marcogliese and Cone 1996).

Pollution stress and parasitism may have combined effects on hosts. Fish exposed to both pollution and parasite stress may have higher mortality rates than fish exposed to only one stressor. For example, Sockeye salmon (*Oncorhynchus nerka*) smolts infected with the intestinal cestode *Eubothrium salvelini* had a higher mortality rate when exposed to zinc than non-infected smolts (Boyce and Yamada 1977). Similarly, threespine sticklebacks infected with pleroceroids of *Schistocephalus solidus* (Cestoda) were more likely to succumb to the toxic effects of cadmium exposure than sticklebacks without plerocercoids (Pascoe and Cram 1977). Guppies infected with *G. turnbulli* suffered higher rates of mortality as zinc concentration increased, whereas uninfected fish showed little mortality at any concentration (Gheorgiu *et al.* 2006). Pollution and parasitism may also have combined sublethal effects on fish. Juvenile Chinook (*Oncorhynchus tschawytscha*) salmon experimentally infected with metacercariae of the trematode *Nanophyetus salmincola* and exposed to PCBs had lower immune function and were more susceptible to infection with the bacterium *Listonella anguillarum* than salmon exposed to only PCBs or *N. salmincola* (Jacobson *et al.* 2003). Yellow perch (*Perca flavescens*) collected from areas exposed to industrial and agricultural pollution in the St. Lawrence River in southwestern Quebec and naturally infected with larvae of the nematode *Raphidascaris acus* had higher oxidative stress levels than perch exposed to either stressor alone (Marcogliese *et al.* 2005). Spottail shiners (*Notropis hudsonius*) collected from polluted localities in the St. Lawrence River and naturally infected with the trematode *Plagioporus sinitsini* had elevated numbers of pigmented macrophages in

the spleen (a general measure of fish health) and lower condition indices than spottail shiners from reference localities (Thilakaratne *et al.* 2007).

Concluding remarks

Behaviour is only one aspect of fish biology affected by stressors such as parasitism and pollution. However, behavioural changes are of particular ecological interest because disruptions of adaptive behaviour can have immediate consequences for individuals' fitness that may scale up to the population level or higher. (Poulin 1992; Mackenzie *et al.* 1995; Kennedy 1997; Lafferty 1997; Marcogliese 2005). Current studies of pollution effects on fish behaviour are largely limited to tests of single chemicals at a time. While this design simplifies interpretation of results, it may have limited application to field conditions where water pollution is typically a "cocktail" with many chemical components. Effects become even more complex if chemicals have interactive or synergistic effects on parasites and fish hosts, as suggested by several researchers (Poulin 1992; Mackenzie *et al.* 1995; Kennedy 1997; Lafferty 1997; Marcogliese 2005). Further confounding this is the effect abiotic conditions such as temperature, pH, water hardness, alkalinity and humic content have on toxicity and bioavailability of toxins (Atchison *et al.* 1987; Jobling 1995). Paralleling single-pollutant ecotoxicology studies are behavioural ecology studies of fish infected with a single parasite species. Again, this design simplifies interpretation of results, however single infections show little resemblance to parasite infections of wild fish, where the majority are infected by multiple species (Barber *et al.* 2000). Limited studies of multiple parasite infections and animal behaviour suggest that there may be significant interactions between parasites

infecting a single individual, leading to unpredictable effects, such as reduced effects of parasites that modify behaviours in single-species infections. Further, current work on parasite infracommunity composition suggests that infection diversity may be an ecologically relevant measure of parasite stress and play an important role in the behavioural ecology of infected hosts (Bordes and Morand 2009). Tests of individual contaminants and parasites are important steps in determining effects of these stressors; however studies of environmentally relevant combinations of mixtures of both contaminants and parasites are required to put them into their proper ecological context.

Finally, no studies published thus far have considered combined effects of pollution and parasitism on fish behaviour. Studies of individual stressors suggest this may be a fruitful topic of study, however study design is challenging and will require choosing behavioural endpoints that are appropriate for both types of stressors. The common co-occurrence of these two stressors in natural environments suggests that any interactions between them could be of great ecological significance.

General Introduction

Parasites and pollution are both commonly encountered by fish in the St.

Lawrence River in southwestern Quebec. Past studies of pollution from this area show

some areas of the river are contaminated with metals, PCBs and organochlorines

surpassing the Canadian Environmental Quality Guidelines (CWQG) Probable Effects

Level (PEL) for aquatic life (http://ceqg-rcqe.ccme.ca/), while other areas have

sufficiently low levels of all contaminants to be considered reference localities (Loiselle

et al. 1997; Marcogliese *et al.* 2005; 2006; Dautremepuits *et al.* 2009; Magella Pelletier,

Environment Canada, pers. comm.). Previous studies suggest that pollution at localities in

this area of the St. Lawrence River may impact parasite communities in spottail shiners

(*Notropis hudsonius*) (Marcogliese *et al.* 2006).

One fish species commonly found in the St. Lawrence River is the johnny darter

(*Etheostoma nigrum*). Johnny darters are small, cryptically coloured benthic fish

commonly found in nearshore areas (Page 2000). As expected from their small size and

colouration, they typically exhibit cryptic behaviour in response to perceived predation

risk (Smith 1979). Unlike some darter species, johnny darters do not exhibit spawning

migrations (Ingersoll *et al.* 1984). Surveys of johnny darter parasites from other systems

show that they are host to a variety of macroparasites (Bangham 1955; Dechtiar 1972).

They are recorded hosts for over 54 species of macroparasites, including cestodes,

trematodes, monogeneans, nematodes, acanthocephalans and crustaceans (Hoffman

1999).

Following is a two-part study that examines the effects of pollution on the parasite

communities in johnny darters, and the combined effects of parasitism and pollution on

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antipredator behaviour of johnny darters. This is the first comprehensive study of parasites of johnny darters in the St. Lawrence River. It is also the first study (to our knowledge) that explores the combined effects of pollution and parasitism on fish behaviour. Johnny darters are an appropriate test subject for a study of this nature for a number of reasons. First, they are known hosts of a variety of parasites, making possible significant variation between parasite communities at different localities and at the same time allowing the potential for parasite-induced stress. Second, they are relatively common locally, making study design and collection easier. Third, they are known to be relatively sedentary and do not exhibit annual migrations, leading us to be relatively certain that they had lived their entire lives in the area from which they were collected, which is important for a study of long term chronic exposure to toxicants. Finally, they are benthic fish, presenting an interesting opportunity to compare this system with previous studies on parasites of the pelagic fish, the spottail shiner, in the same ecosystem (Marcogliese *et al.* 2006).

In Chapter 1 I present the results from a survey of parasites of johnny darters from five localities in the St. Lawrence River. I compare parasite communities between the localities, and in particular examine differences between polluted and reference localities, and between polluted localities affected by different sources of contamination. I predict that parasite communities of fish from polluted localities will have lower diversity and abundances than those from reference localities, following general theory in environmental parasitology (Kennedy 1997; Lafferty 1997; Mackenzie 1999; Marcogliese 2005).

In Chapter 2, I use parasite data collected for Chapter 1 and information on pollution status of the five localities to examine the combined effects of parasite and pollution stress on the antipredator behaviour of johnny darters. Previous studies have shown that pollution stress (reviewed by Atchison *et al.* 1987; Clotfelter *et al.* 2004; Scott and Sloman 2004) and parasitism (reviewed by Barber *et al.* 2000; Moore 2002; Barber and Rushbrook 2008) may lead to greater susceptibility of fish to predation. Previous studies of combined effects of parasitism and pollution suggest that fish exposed to both stressors experience poorer health than those exposed to a single stressor (Jacobson *et al.* 2003; Marcogliese *et al.* 2005; Thilakaratne *et al.* 2007). I predict that fish exposed to both parasitism and pollution will perform more poorly in antipredator experiments than non-infected fish from polluted localities or fish from the reference localities.

Chapter 1.   **Parasite fauna of *Etheostoma nigrum* (Percidae: Etheostomatinae) in**

localities of varying pollution stress in the St. Lawrence River,

Quebec, Canada.

Introduction

Aquatic systems are affected by a variety of anthropogenic activities that decrease

water quality through the introduction of organic and inorganic pollutants. One general

trend reported from polluted aquatic environments is a decrease in diversity of

macroparasite communities in fishes (Cone *et al.* 1993; Mackenzie *et al.* 1995; Kennedy

1997; Lafferty 1997; Overstreet 1997; Mackenzie 1999; Sures 2004; Marcogliese 2005;

Blanar *et al.* 2009). This typically occurs through the loss of parasites with short-lived

free-living stages that are assumed to be sensitive to pollutants (Pietrock and Marcogliese

2003), or through the loss of parasites with indirect life cycles that use intermediate hosts

which are sensitive to pollution (Mackenzie *et al.* 1995; Marcogliese 2005; Pietrock *et al.*

2008). Digenetic trematodes in particular may be lost from communities under

contaminant stress because of reductions in survival, longevity or infectivity of their

sensitive free-living stages and/or reductions in survival of their molluscan intermediate

hosts (Marcogliese and Cone 1997; Marcogliese 2005). Diversity also may be lower in

polluted environments due to higher rates of toxin-induced mortality of infected hosts

(Lafferty 1997; Barber *et al.* 2000; Sures 2004; Sures 2008). In contrast, fish hosts in

stressed environments often have a proliferation of directly transmitted ectoparasites,

such as ciliates and monogeneans, due to immunosupressive effects of toxicants on hosts

(Khan and Thulin 1991; Poulin 1992; Morley *et al.* 2006).

Despite these commonly occurring patterns of decreased diversity and/or increased infections of particular parasites, the role of pollutants in creating these patterns is complex and not always predictable. Particular effects of pollutants on parasite communities may vary by type of pollution (Mackenzie *et al.* 1995; Lafferty 1997; Marcogliese 2005; Blanar *et al.* 2009). In natural environments, pollutants typically occur as combinations of chemicals. These complex mixtures can produce unpredictable or nonlinear affects on aquatic life (Pietrock and Marcogliese 2003; Dautremepuits *et al.* 2009). Their toxicity may be further affected by other abiotic factors such as pH and temperature (Poulin 1992; Lafferty 1997; Pietrock and Marcogliese 2003; Marcogliese 2005).

The St. Lawrence River in southwestern Quebec receives anthropogenic inputs from a number of sources, including municipal sewage, industrial activity and agriculture. Various locations are exposed to differing amounts of these contaminants. Within this system, effects of pollution have been examined on immune response, stress response and parasitism in spottail shiners (*Notropis hudsonius*) and yellow perch (*Perca flavescens*) (Marcogliese and Cone 2001; Marcogliese *et al.* 2005; 2006; Thilakaratne *et al.* 2007; Dautremepuits *et al.* 2009), however no effects of pollution have been measured for a benthic fish in this river. Effects of pollutants on benthic fish may be of interest because of the close proximity of these fish to the sediments, Similar studies have been done on flatfish in marine environments (e.g. Schmidt *et al.* 2003; Khan and Billiard 2007), but to our knowledge this is the first in freshwater.

Johnny darters (Percidae: *Etheostoma nigrum*) are small, benthic fish commonly found in both clean and polluted areas of the St. Lawrence River. They are recorded to be

infected with 54 species of macroparasites in North America (Margolis and Arthur 1979; McDonald and Margolis 1995; Hoffman 1999). Unlike some darter species that undergo spawning migrations, johnny darters tend to remain in the same area over the entire year (Ingersoll *et al.* 1984). This characteristic makes them appropriate subjects on which to study the chronic effects of local long term exposure to sublethal concentrations of pollutants on benthic fish (Marcogliese 2005). We examined parasite communities of johnny darters from reference and contaminated localities in the St. Lawrence River to determine if pollution mixtures from different sources had a similar or different impact on diversity and species composition.

Materials and Methods

Study localities

Fish were collected from three polluted and two reference localities in the St. Lawrence River in southwestern Quebec in June, 2008. The pollution status of all five localities has been previously characterized based on sediment measurements of metals and polychlorinated biphenyls (PCBs) (Loiselle *et al.* 1997; Marcogliese *et al.* 2005; 2006; Dautremepuits *et al.* 2009). Sediment measurements are appropriate because they are relatively stable over time (Dautremepuits *et al.* 2009). They are also of particular biological importance for our study system because johnny darters are benthic fish and therefore are in close contact with them.

Two of the polluted localities, Îlet Vert (IVT; 45°42.230' N; 73°27.143' W) and Île Beauregard (IBE; 45°44.965' N; 73°24.910' W), are 4km and 10km downstream of the City of Montreal sewage treatment plant outfall, respectively (Figure 1.1). They both

have high concentrations of fecal coliforms, which is typical of areas receiving sewage input (Marcogliese and Cone 2001; Marcogliese *et al.* 2006). Concentrations of contaminants such as metals, PCBs, organochlorines and xenoestrogens are elevated downstream of the outfall (Pham *et al.* 1999; Sabik *et al.* 2003; Marcogliese *et al.* 2006; Dautremepuits *et al.* 2009; Magella Pelletier, Environment Canada, pers. comm.). Levels of chromium at IVT and levels of PCBs at IBE surpass the Canadian Environmental Quality Guidelines (CWQG) Probable Effects Level (PEL) for aquatic life (http://ceqg-rcqe.ccme.ca/) (Marcogliese *et al.* 2006; Magella Pelletier, Environment Canada, pers. comm.).

The third polluted locality, Beauharnois (BEA; 45°19.051' N; 73°52.020' W) is upstream of the sewage treatment plant, at the mouth of the St. Louis River. The water quality at this locality is affected by industrial and agricultural activity upstream, and constitutes a different type of contaminant mixture than that downstream of the Montreal sewage treatment outfall. BEA has high concentrations of PCBs, organochlorines and heavy metals including copper, arsenic, lead, chromium, nickel and zinc. Mercury levels are particularly high, and surpass the PEL (Marcogliese *et al.* 2005; Dautremepuits *et al.* 2009).

The two reference localities, Îles de la Paix (IPA; 45°20.022' N; 73°51.362' W) and Île Dorval (IDO; 45°26.016' N; 73°44.234' W), are located in Lake St. Louis upstream of the sewage outfall. No contaminants surpassing the PEL have been measured at either locality (Marcogliese *et al.* 2006).

Study Organisms

Fish were collected using a beach seine (22.6 × 1.15m; 3mm mesh). They were

transported to the laboratory, where they were kept in 60L gravel-lined tanks of

dechlorinated tap water. Fish from each locality were kept in separate tanks at room

temperature, and fed with Nutrafin™ fish flakes *ad lib*. They were maintained in the lab

for 6 to 14 days for an accompanying behaviour study. Following this study, fish were

killed by an overdose of clove oil (50 mg/L) solution and individually packaged and

frozen at -20°C for later necropsy. All fish used were presumed to be from the same age

class, 1+, as determined by the length frequency distributions seen at each locality

(Bagenal and Tesch 1978). All animal collection and handling procedures were

conducted in accordance with guidelines of the Canadian Council on Animal Care in

effect at the time of the study.

Examination for parasites

Frozen mass (mg) and standard length (mm) were measured for each fish prior to

necropsy. Tissues and organs, including skin, fins, gills, eyes, brain, body cavity, liver,

gastrointestinal tract, heart, spleen, gonads and muscle of each fish were examined for

macroparasites, following protocols from Marcogliese (2002). During necropsy, all

parasites were identified to genus (except non-gyrodactylid monogeneans,

acanthocephalans and a few rare trematodes, which could only be identified to a higher

taxonomic level) and counted. A subset of parasites representing all taxa found at each

locality was preserved in 70% ethanol for species identification. Trematodes, cestodes,

acanthocephalans and some monogeneans were stained with acetocarmine and cleared

with xylene before being mounted in either Permount or Canada balsam. Nematodes, copepods and some monogeneans were cleared with glycerine alcohol and examined in temporary mounts. Parasite identifications were based on keys and descriptions in Beverly-Burton (1984), Caira (1989), Kabata (1988), Moravec (1994), Gibson (1996), Scholz (1997) and Hoffman (1999).

Data analysis

Standard length was compared among localities and between polluted and reference localities. One-way ANOVAs were used for comparisons among localities, and between polluted and reference localities. Because there were significant differences in standard length between some localities and types of localities, it was initially included as a covariate in analyses using permutational multivariate ANOVAs (PERMANOVA), as described below.

Differences in total parasite numbers and species richness among localities were examined with an ANOVA using ranked data (Scheirer *et al.* 1976) followed by a Tukey-Kramer HSD test. Differences in standard length, total parasite numbers and species richness were tested using JMP® 7.0.1 (© 2007 SAS Institute Inc.).

Nonparametric statistics based on the Bray-Curtis dissimilarity index were used to further characterize parasite communities with PRIMER and PERMANOVA+ add-on (© 2009 Plymouth Routines In Multivariate Ecological Research, Plymouth, UK), following procedures outlined in Clarke and Gorley (2001), Clarke and Warwick (2001) and Anderson *et al.* (2008). The Bray-Curtis dissimilarity index is commonly used in describing community data because it takes into account both species identity and

abundances in a single measure of dissimilarity between two samples, independent of all other samples. The Bray-Curtis index emphasizes abundant species, so parasite abundance data were square root transformed to decrease the weight of common species relative to rare species in the index. A non-metric multidimensional scaling plot (MDS) was used to analyze ranked within-group and between-group Bray-Curtis dissimilarities. Relative similarities between localities based on average community composition, as calculated by centroids, were qualitatively assessed using a dendrogram. The magnitude of relative differences between communities at different localities was tested using a one-way Analysis of Similarity (ANOSIM) on ranked square root-transformed Bray-Curtis dissimilarities. ANOSIM is a nonparametric test analogous to a one-way Analysis of Variance (ANOVA). The resulting test statistic "R," a measure of the between-group to within-group dissimilarity, varies from 0 to 1, where R = 0 is the null hypothesis, and R = 1 when within-group dissimilarities of all samples are smaller than between-group dissimilarities. A Similarity Percentages (SIMPER) analysis was performed to identify the parasite species driving the differences between communities at different localities.

The dendrogram and pairwise ANOSIM suggested that the grouping of parasite communities of different localities was correlated with the pollution status of localities, so a PERMANOVA was used to test for community differences among localities and between types of localities. PERMANOVA is a nonparametric multivariate test analogous to a multivariate ANOVA. The "Pseudo-F," the test statistic calculated in PERMANOVA, is analogous to the "F" statistic of parametric tests, and is a measure of between-group to within-group variability. Because there are low numbers of replicate localities, differences between localities of different pollution status were tested for using

contrasts. Two contrasts were tested: polluted vs. reference localities (BEA, IBE and IVT vs. IDO and IPA), and upstream versus downstream polluted localities (BEA vs. IBE and IVT), reflecting different sources of pollution.

All parasite community ecology terminology follows definitions from Bush *et al.* (1997). Prevalence is the percentage of hosts from a sample infected with a particular parasite. Mean abundance is the average number of parasites over a sample. Mean intensity is the average number of parasites in infected individuals in a sample. Infracommunity refers to all parasites within a single fish.

Results

All of the darters were infected with at least one species of helminth. Twenty-four species were found, including 15 digeneans (4 adults, 11 metacercariae), 2 monogeneans, 2 cestodes (1 adult, 1 plerocercoid), 3 nematodes (1 adult, 2 juveniles), 1 larval acanthocephalan and 1 juvenile copepod. The prevalence and mean intensity of each species at each locality is presented in Table 1.1. Mean abundance of each species at each locality can be found in Appendix 1. Gyrodactylids were present at all localities and were included in the preliminary analysis. However, as they have very short generation times and proliferate rapidly under laboratory conditions (Scott and Anderson 1984), prevalence and intensities after the 6 to 14 day acclimation period were likely inflated. They were therefore excluded from the final analysis. Analysis with and without the gyrodactylids showed no major differences.

Standard length of fish from BEA (51.03 mm ± 6.92 SD) was significantly greater than those from IDO (46.88 ± 5.28 mm) ($F_{4,173}$ = 3.62, p = 0.007) but there was no

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significant difference in the standard lengths of fish from other localities (Figure 1.2).

When fish were pooled by treatment, those from the polluted localities (49.67 mm ± 5.46

SD) were significantly longer than those from the reference localities (47.48 mm ± 5.16

SD) ($F_{1,176} = 7.18$, p = 0.0081).

Total parasite numbers differed between localities; fish from BEA and IDO had

significantly greater numbers of parasites than fish from IVT and IBE ($F_{4,173} = 31.73$, p <

0.0001) (Figure 1.3), suggesting length is not correlated with pollution status. Species

richness also differed significantly between localities ($F_{4,173} = 38.48$, p < 0.0001). It was

greater in fish at BEA than at IPA and IDO which in turn was greater than at IVT and

IBE (Figure 1.4). Differences in total parasite numbers and species richness were not

tested between treatments because the greatest differences in both these measures were

between the upstream and downstream polluted localities. Analysis of Bray-Curtis

dissimilarities of infections in fish from all five localities revealed that those from the

upstream polluted locality (BEA), those from the 2 reference localities (IDO, IPA) and

those from the downstream polluted localities (IVT, IBE) formed 3 distinct clusters

(Figure 1.5, 1.6). MDS poorly represents fish without infections, so one fish from IPA

infected only with gyrodactylids was excluded from the plot.

The difference between communities at the polluted and reference localities was

primarily due to higher abundances of *Ornithodiplostomum* sp. at the reference localities

(Table 1.2). Differences between the upstream and downstream polluted localities was

mainly due to higher abundances of *Neochasmus* sp., *Ichthyocotylurus* sp. and

Cryptogonimus sp. at the upstream polluted locality.

The global ANOSIM (Global R = 0.51, P = 0.001) and the pairwise ANOSIMs

(Table 1.3) were significant. Based on the pairwise comparisons, BEA appeared to be the

most distinct of the five localities. Distinctions between reference and downstream

polluted localities were greater than within these localities. A PERMANOVA performed

on the Bray-Curtis dissimilarities confirmed ANOSIM results of differences between

localities, pollution status and the upstream / downstream polluted localities (Table 1.4).

Discussion

This is the first study to examine parasite communities of johnny darters in the St.

Lawrence River. The majority of parasite species encountered were larval stages ($\geq$16),

most of which could at best only be identified to the generic level. The majority of these

were trematodes, consistent with results of other studies on fish parasite communities in

the St. Lawrence River (Marcogliese *et al.* 2006; Thilakaratne *et al.* 2007). Assuming

each larval type identified represents a single species, two of the larval trematodes

recovered, *Ichthyocotylurus* sp. and *Tylodelphys* sp. are new reports for johnny darters, as

is the intestinal nematode, *Cammallanus lacustris*. A further two trematodes,

Phyllodistomum etheostomae and the metacercariae of *Cryptogonimus* sp. are new

reports for this host in Canada (Margolis and Arthur 1979; McDonald and Margolis

1995; Gibson 1996; Hoffman 1999). Recent studies have shown that the larval trematode

communities of fish in the St. Lawrence River are much more diverse than previously

recognized (Moszczynska *et al.* 2009; Locke *et al.* in press), and two of the "species"

identified in this study (*Diplostomum* spp. and *Apatemon* spp.) are known to occur as

species complexes in fish. Thus, the estimate of number of larval trematode species is likely conservative.

Parasite communities at polluted and reference localities showed some of the trends consistent with predicted parasite diversity in stressed environments. The two downstream polluted localities, IVT and IBE, had significantly different parasite communities from the reference localities, in both abundance and composition. Fish from the former two localities had the lowest mean total parasite numbers and mean species richness. They also had significantly lower abundances of metacercariae of the trematode *Ornithodiplostomum* sp. (Chapter 2). A large percentage of the differences between these localities and the reference localities was due to higher abundances of *Ornithodiplstomum* sp. at the reference localities. This is consistent with studies showing decreased longevity and infectivity of trematode cercariae, (Morley *et al.* 2003; Pietrock and Marcogliese 2003), and in particular decreased infectivity of cercariae of *Ornithodiplostomum* following exposure to metal pollution (Pietrock and Goater 2005). Parasite communities of a pelagic fish, spottail shiners (*Notropis hudsonius*), from these localities show a similar general pattern. Marcogliese *et al.* (2006) found that fish downstream of the sewage outfall had lower total parasite abundances than fish from reference localities in Lake St. Louis. Thilakaratne *et al.* (2007) reported a lower diversity of helminths at IVT than at the reference localities in Lake St. Louis. These patterns of lower parasite abundance and diversity in polluted environments have also been reported in field studies from other systems (reviewed by Marcogliese 2005). For example, parasite communities of grey mullet (*Liza aurata* and *L. ramada*) in a polluted estuary were missing helminth parasites with indirect life cycles that were present in a nearby

reference estuary (Dzikowski *et al.* 2003). Exposure to urban effluent was reflected in lower species richness of macroparasite communities of flounder (*Platichthys flesus*) in the German Bight in the North Sea (Schmidt *et al.* 2003). Winter flounder (*Pleuronectes americanus*) in the vicinity of a pulp and paper mill had significantly lower abundances of intestinal helminths than those at a nearby reference locality (Khan and Billiard 2007).

However, the relationship between pollution and parasite community diversity is seldom simple to interpret, and may be confounded by the type of pollution, or other abiotic or ecological factors (Poulin 1992; Mackenzie *et al.* 1995; Kennedy 1997; Lafferty 1997; Marcogliese 2005). The upstream polluted locality, BEA, differs from the pattern of parasite community impoverishment seen in the two downstream polluted localities. Composition of parasite communities at BEA differed from both of the two downstream polluted localities and the two reference localities. BEA had the highest species richness and among the highest overall parasite abundances of both polluted and reference localities. In particular, differences between BEA and the other localities were due to higher abundances of three trematodes, *Neochasmus* sp., *Ichthyocotylurus* sp. and *Cryptogonimus* sp. at BEA. Similar to the other polluted localities, BEA had lower abundances of *Ornithodiplostomum* sp. than the reference localities. The different parasite community composition between the upstream and downstream polluted localities may reflect differences in the types and sources of pollution. IVT and IBE are impacted primarily by effluent from a major sewage treatment facility that empties into the St. Lawrence River directly upstream of them. In contrast, BEA is downstream of industrial and agricultural activity along the St. Louis River. Differences in pollution include chemicals and metals, but also nutrient inputs. Both upstream and downstream

polluted localities experience eutrophication, however the specific type of nutrient inputs differ, due to their different sources. Agricultural runoff typically includes chemical fertilizers, while sewage is composed of organic material and bacteria (Khan and Ansari 2005). There are also differences in the free-living biotic communities between the upstream and downstream polluted localities, which may reflect the type of eutrophication experienced. Diets of organisms downstream of the municipal effluents are more detritus-based than those upstream, possibly reflecting simpler food webs resulting in relatively low parasite infracommunity diversity (de Bruyn *et al.* 2003; Marcogliese *et al.* 2006). Conversely, BEA has high productivity and fish diversity (David Marcogliese, Environment Canada, pers. comm.), both of which may lead to an increase in parasite abundance and diversity. Thus, our data suggest that the type of contaminant mixture may impact parasite communities of fish differently, resulting in measures of diversity and abundance that do not always follow previously widely-accepted patterns (see Mackenzie *et al.* 1995; Lafferty 1997; Marcogliese 2005; Blanar *et al.* 2009).

Figure 1.1. Map of the St. Lawrence River in southwestern Quebec, Canada, showing the

five localities sampled in June 2008: Beauharnois (BEA), Îles de la Paix (IPA), Île

Dorval (IDO), Îlet Vert (IVT) and Île Beauregard (IBE).
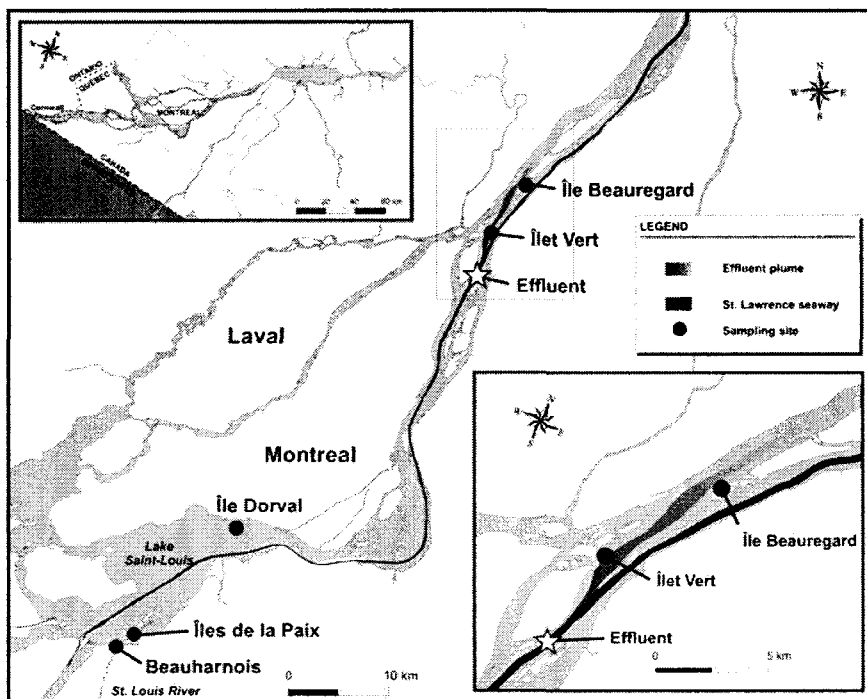
Table 1.1. Prevalence (Prev) and mean intensity (MI) of macroparasites found in johnny darters at five localities in June 2008 in the St. Lawrence River, Quebec, Canada: Beauharnois (BEA), Îlet Vert (IVT), Île Beauregard (IBE), Îles de la Paix (IPA) and Île Dorval (IDO). Larval stages are indicated by (*). Previous records are based on Gibson (1996), Hoffman (1999), Margolis and Arthur (1979), McDonald and Margolis (1995) and Scholz (1997). New host records are indicated by (°) and new Canadian records by (°).

Digenea	BEA (n = 36)		IVT (n = 36)		IBE (n = 35)		IPA (n = 36)		IDO (n = 35)	
	Prev (%)	MI ± SD	Prev (%)	MI ± SD	Prev (%)	MI ± SD	Prev (%)	MI ± SD	Prev (%)	MI ± SD
Allocreadium boleosoma	0	0	0	0	0	0	0	0	6	1 ± 0
Apatemon sp.*	47	3.9 ± 3.5	58	7.4 ± 10.0	80	8.5 ± 9.6	53	10.8 ± 19	77	4.1 ± 5.3
*Apophallus brevis**	31	2.6 ± 1.6	25	1.1 ± 0.3	23	1.4 ± 1.1	42	2.1 ± 1.4	57	2.2 ± 1.6
Azygia angusticauda	25	2.1 ± 1.3	8	1 ± 0	6	1 ± 0	3	1 ± 0	11	1.3 ± 0.5
*Clinostomum complanatum**	11	1.5 ± 0.6	0	0	0	0	8	1 ± 0	0	0
Crepidostomum isostomum	6	2 ± 0	0	0	6	1 ± 0	6	1 ± 0	0	0
Cryptogonimus sp.*°	75	11.5 ± 22.2	33	1.1 ± 0.3	23	1.8 ± 1.2	39	9.8 ± 9.0	26	2.7 ± 4.0
Diplostomum spp.*	33	1.5 0.8	0	0	0	0	28	1 ± 0	20	1.3 ± 0.6
Ichthyocotylurus sp.**	92	10.2 ± 9.9	19	3.4 ± 2.9	80	3.5 ± 4.3	42	5.2 ± 4.8	71	3.1 ± 2.9
Neochasmus sp.*	97	14.0 ± 14.5	14	14.6 ± 17.9	40	2 ± 1.4	6	1 ± 0	26	1.1 ± 0.3
Ornithodiplostomum sp.*	83	4.6 ± 4.8	72	3.5 ± 2.2	54	2.5 ± 2.4	92	13.6 ± 14.3	97	45.3 ± 35.5

Table 1.1. Continued

	BEA (n = 36)		TVT (n = 36)		IBE (n = 35)		IPA (n = 36)		IDO (n = 35)	
	Prev (%)	MI ± SD	Prev (%)	MI ± SD	Prev (%)	MI ± SD	Prev (%)	MI ± SD	Prev (%)	MI ± SD
Phyllodistomum etherostomae[b]	0	0	0	0	0	0	3	1 ± 0	0	0
Tylodelphys sp.**	0	0	0	0	0	0	17	1.7 ± 0.8	0	0
metacercaria sp. 1*	11	2 ± 2	0	0	0	0	0	0	0	0
tetracotyle sp. 1*	0	0	3	1 ± 0	6	1 ± 0	25	3.2 ± 2.0	0	0
Monogena										
Gyrodactylus etherostomae	94	7.7 ± 5.0	97	5.3 ± 4.8	94	6.5 ± 9.5	97	7.4 ± 5.6	94	7.5 ± 4.9
Aethycleson nigrei	61	2.3 ± 1.6	75	5.5 ± 3.7	23	1.1 ± 0.4	22	1.5 ± 0.8	60	1.7 ± 1.1
Cestoda										
Botheriocephalus caspidatus	25	1.3 ± 0.5	0	0	0	0	0	0	0	0
*Trigenophorus nodulus***	6	1 ± 0	0	0	0	0	0	0	0	0
Nematoda										
Dichelyne sp.*	72	3.5 ± 2.5	8	1 ± 0	29	1.5 ± 0.8	50	4.3 ± 5.5	54	2.6 ± 3.0
Raphidascaris acus	61	1.7 ± 0.9	6	1.5 ± 0.7	3	1 ± 0	31	1.5 ± 1.2	6	1 ± 0
Cammallanus lacustris	6	1 ± 0	0	0	0	0	3	1 ± 0	0	0
Acanthocephala*	25	1.9 ± 1.3	8	1.7 ± 1.2	17	1.2 ± 0.4	19	1.6 ± 0.5	6	1 ± 0
Copepoda										
Lernaea sp.*	0	0	0	0	0	0	69	2.8 ± 2.1	0	0

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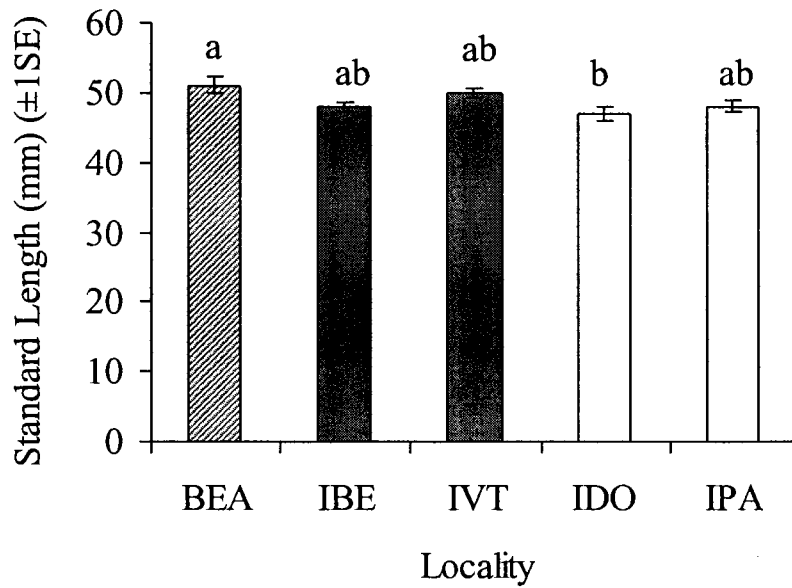
Figure 1.2. Mean standard length (mm) ± standard error of johnny darters (*Etheostoma*

nigrum) from five localities in June 2008 in the St. Lawrence River, Quebec, Canada: one

upstream polluted locality (light grey), Beauharnois (BEA); two downstream polluted

localities (dark grey), Île Beauregard (IBE), Îlet Vert (IVT); and two reference localities

(white), Île Dorval (IDO) and Îles de la Paix (IPA). Different letters indicate significant

differences between localities.
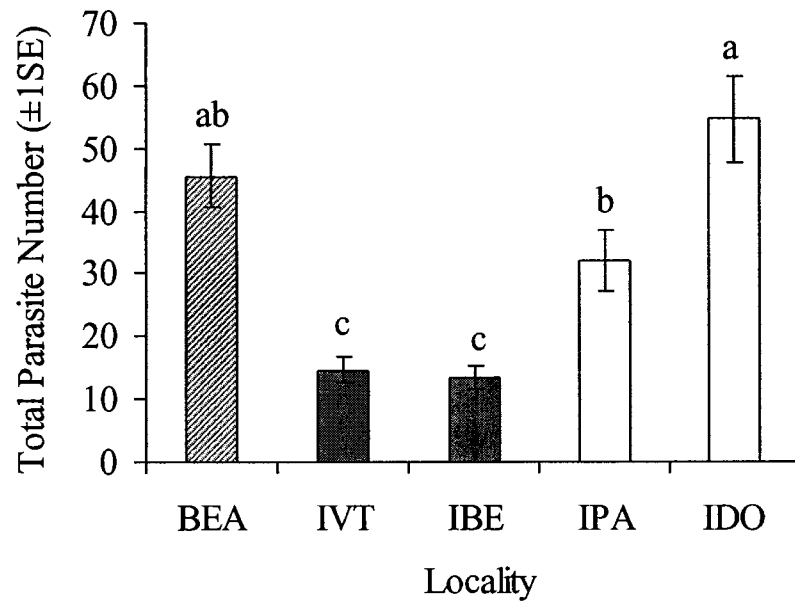
Figure 1.3. Mean total parasite numbers ± standard error in johnny darters (*Etheostoma nigrum*) from five localities in June 2008 in the St. Lawrence River, Quebec, Canada: one upstream polluted locality (light grey), Beauharnois (BEA); two downstream polluted localities (dark grey), Île Beauregard (IBE), Îlet Vert (IVT); and two reference localities (white), Île Dorval (IDO) and Îles de la Paix (IPA). Different letters indicate significant differences between localities.
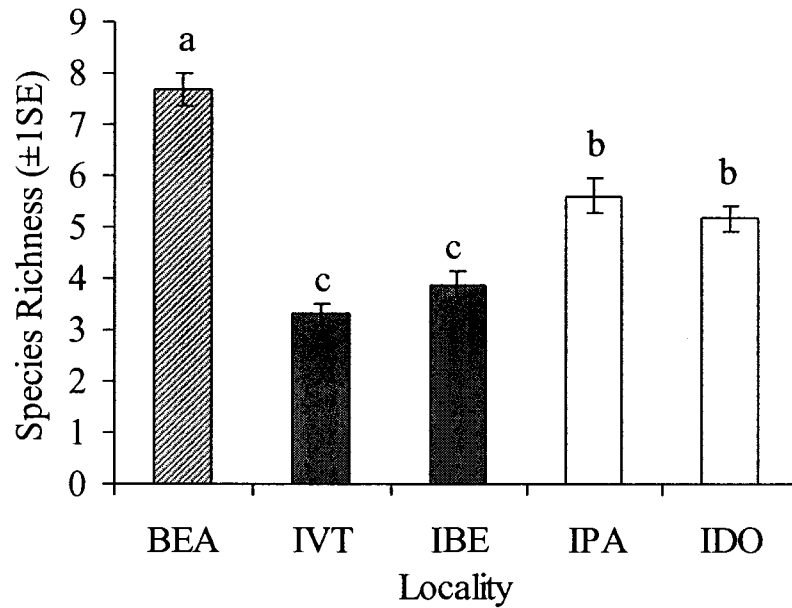
Figure 1.4. Mean species richness ± standard error in johnny darters (*Etheostoma nigrum*) from five localities in June 2008 in the St. Lawrence River, Quebec, Canada: one upstream polluted locality (light grey), Beauharnois (BEA); two downstream polluted localities (dark grey), Île Beauregard (IBE), Îlet Vert (IVT); and two reference localities (white), Île Dorval (IDO) and Îles de la Paix (IPA). Different letters indicate significant differences between localities.
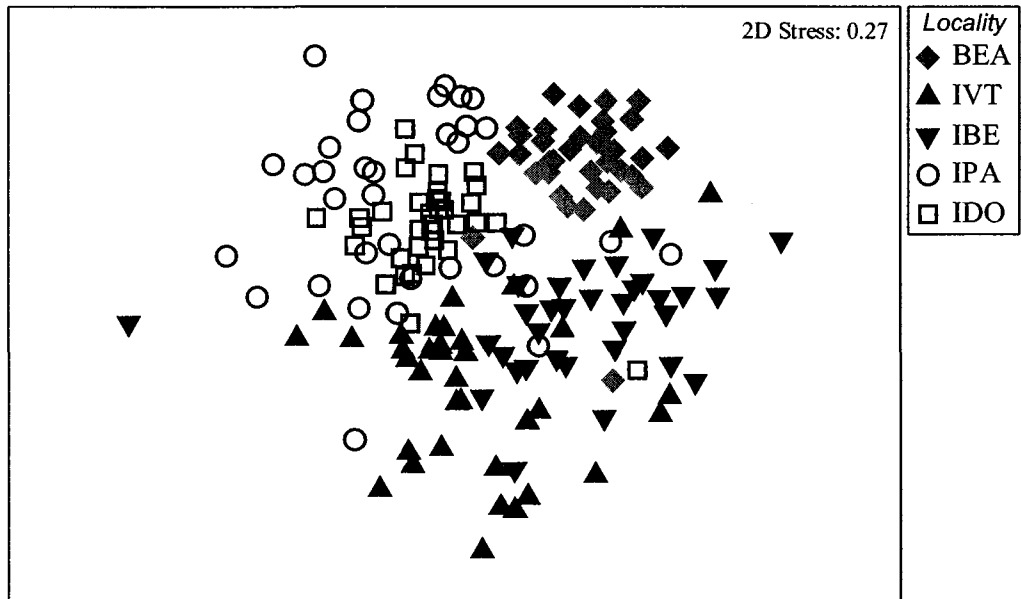
Figure 1.5. Two-dimensional nonmetric multidimensional scaling plot of ranked square root transformed Bray-Curtis dissimiliarities between parasite communities in johnny darters (*Etheostoma nigrum*) from five localities in June 2008 in the St. Lawrence River, Quebec, Canada one upstream polluted locality (light grey), Beauharnois (BEA); two downstream polluted localities (dark grey), Île Beauregard (IBE), Îlet Vert (IVT); and two reference localities (white), Île Dorval (IDO) and Îles de la Paix (IPA). The stress level of the plot is 0.27
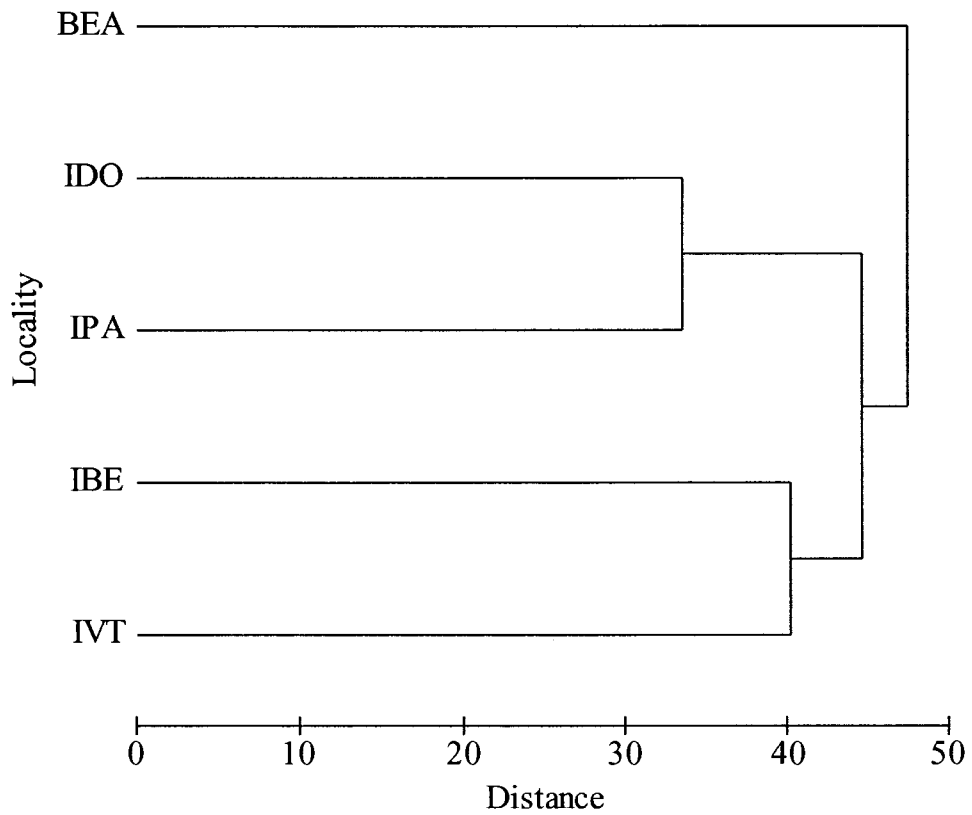
Figure 1.6. Dendrogram of average parasite communities in johnny darters from five

localities in June 2008 in the St. Lawrence River, Quebec, Canada: Beauharnois (BEA),

Île Beauregard (IBE), Îlet Vert (IVT), Île Dorval (IDO) and Îles de la Paix (IPA).

Distances on the figure represent square root transformed Bray-Curtis dissimilarities

between localities.

Table 1.2. Pairwise similarity percentages analysis for macroparasites in johnny darters (*Etheostoma nigrum*) from five localities in June 2008 in the St. Lawrence River, Quebec, Canada: Beauharnois (BEA), Île Beauregard (IBE), Îlet Vert (IVT), Île Dorval (IDO) and Îles de la Paix (IPA). "Contribution %" gives the percent of the overall dissimilarity explained by the parasite, and "Cumulative %" is the cumulative percent, starting with the parasite contributing the most to the dissimilarity. Only parasite species contributing a minimum of 10% to the dissimilarity are included.

Localities	Parasite	Relative Abundance	Contribution %	Cumulative %
BEA, IBE	*Neochasmus* sp.	BEA > IBE	19.25	19.25
	Ichthyocotylurus sp.	BEA > IBE	11.95	31.20
	Cryptogonimus sp.	BEA > IBE	11.87	43.07
	Apatemon spp.	BEA < IBE	11.76	54.85
BEA, IVT	*Neochasmus* sp.	BEA > IVT	20.33	20.33
	Ichthyocotylurus sp.	BEA > IVT	15.16	35.49
	Cryptogonimus sp.	BEA > IVT	10.80	46.29
BEA, IDO	*Ornithodiplostomum* sp.	BEA < IDO	25.33	25.33
	Neochasmus sp.	BEA > IDO	16.92	42.25
	Ichthyocotylurus sp.	BEA > IDO	10.27	52.25
BEA, IPA	*Neochasmus* sp.	BEA > IPA	18.03	18.03
	Ichthyocotylurus sp.	BEA > IPA	11.85	29.88
	Ornithodiplostomum sp.	BEA < IPA	10.84	51.23
	Cryptogonimus sp.	BEA > IPA	10.52	51.23
IBE, IDO	*Ornithodiplostomum* sp.	IBE < IDO	43.55	43.55
	Apatemon spp.	IBE > IDO	12.77	56.31
IBE, IPA	*Ornithodiplostomum* sp.	IBE < IPA	18.40	18.40
	Apatemon spp.	IBE > IPA	16.07	34.46
	Ichthyocotylurus sp.	IBE > IPA	10.94	45.41

Table 1.2. *Continued.*

Localities	Parasite	Relative Abundance	Contribution %	Cumulative %
IDO, IVT	*Ornithodiplostomum* sp.	IDO > IVT	40.11	40.11
	Apatemon spp.	IDO ≈ IVT	11.70	51.81
	Aethycteron nigrei	IDO < IVT	11.25	63.06
IPA, IVT	*Ornithodiplostomum* sp.	IPA > IVT	16.52	16.52
	Aethycteron nigrei	IPA < IVT	13.59	30.11
	Apatemon spp.	IPA ≈ IVT	13.21	43.32
IDO, IPA	*Ornithodiplostomum* sp.	IDO > IPA	28.15	28.15
	Apatemon spp.	IDO ≈ IPA	10.69	38.84
IBE, IVT	*Apatemon* spp.	IBE > IVT	21.39	21.39
	Aethycteron nigrei	IBE < IVT	19.13	40.52
	Ichthyocotylurus sp.	IBE > IVT	15.32	55.84
	Ornithodiplostomum sp.	IBE < IVT	13.38	69.22

Table 1.3. Pairwise analysis of similiarities (ANOSIM) of parasite communities in johnny darters (*Etheostoma nigrum*) from five localities in June 2008 in the St. Lawrence River, Quebec, Canada: Beauharnois (BEA), Île Beauregard (IBE), Îlet Vert (IVT), Île Dorval (IDO) and Îles de la Paix (IPA). The ANOSIMs compared actual Bray-Curtis dissimilarities with data generated through 999 random permutations of the data set. All randomly generated permutations of the data were original, and each comparison had a significance level of 0.01%.

Comparisons	R Statistic
BEA, IDO	0.798
BEA, IVT	0.669
BEA, IBE	0.591
IBE, IDO	0.584
BEA, IPA	0.564
IDO, IVT	0.536
IPA, IVT	0.432
IBE, IPA	0.429
IBE, IVT	0.336
IDO, IPA	0.327

Table 1.4. Permutation multivariate ANOVA of square root transformed Bray-Curtis

dissimilarities of parasite communities among five localities localities in June 2008 in the

St. Lawrence River, Quebec, Canada: Beauharnois (BEA), Île Beauregard (IBE), Îlet

Vert (IVT), Île Dorval (IDO) and Îles de la Paix (IPA). Localities were further divided

into two contrasts: "Pollution status" (BEA, IBE and IVT vs. IDO and IPA) and

"Upstream/downstream polluted" (BEA vs. IBE and IVT). (*) designates significance (P

< 0.05).

Source		df	SS	MS	Pseudo-F	P
Locality		4	1.41×10^5	35145	21.676	0.001*
Contrasts:	Pollution status	1	48276	48276	22.791	0.001*
	Upstream/downstream	1	43857	43857	22.438	0.001*
Residuals		173	2.18×10^5	1621.4		
Total		177	4.21×10^5			

Connecting Statement

As suggested in Chapter 1, pollution can have significant effects on the parasite communities of freshwater fish species such as the johnny darter. Both pollution and parasitism can have effects on other aspects of fish biology, such as immune system function or behavioural ecology (Atchison *et al.* 1987; Poulin 1995; Barber *et al.* 2000; Jacobson *et al.* 2003; Scott and Sloman 2004; Marcogliese *et al.* 2005; Sloman 2007; Barber and Rushbrook 2009). One aspect of fish behavioural ecology that may be affected by both pollution and parasitism is the response to predation threat. Both stressors may disrupt the normal antipredator response of fish by interrupting normal functioning of systems such as sensory, neurological and metabolic systems (Sloman 2007). The disruption of these will have effects on fish's ability to detect and respond appropriately to predators (Atchison *et al.* 1987; Scott and Sloman 2004). Parasites can also affect antipredator behaviours of fish through parasite-induced manipulations to aid in its trophic transmission.

Antipredator behaviours are of particular interest for two reasons: they have immediate consequences for the future fitness of fish, and they are the most commonly modified behaviours of hosts with behaviour-manipulating parasites. They are also ideal for use in behaviour studies using wild-caught fish, because fish likely retain antipredator behaviours in the lab, whereas reproductive and foraging behaviours may be disrupted (James Grant, Concordia University, pers. comm.). Chapter 2 uses simulated predation events to examine the effects of both parasitism and pollution on the antipredator behaviour of johnny darters.

Chapter 2.   Do infections with parasites and exposure to pollution affect susceptibility to predation in *Etheostoma nigrum* (Percidae: Etheostomatinae)?

Introduction

Parasites and pollution are common stressors in aquatic systems, and both may affect fish behaviour. Fish are host to a variety of helminth parasites, many of which occur as larval stages that develop to maturity in hosts further up the food chain. The effects of parasites on fish behaviour may be adaptive or simply reflect pathology caused by the parasite (Poulin 1995; Barber *et al.* 2000; Moore 2002; Barber and Rushbrook 2008). Larval stages of trophically transmitted parasites commonly manipulate behaviour in fish intermediate hosts. These parasites may increase their transmission success by modifying their host's behaviour to increase its susceptibility to predation by the downstream host in the life cycle. Such changes include increased flashing and surfacing, reduced schooling, and altered habitat use. Pathological changes include lethargy, increased or decreased foraging activity and altered social interactions (Barber *et al.* 2000; Moore 2002; Barber and Rushbrook 2008).

Chronic exposure to sublethal levels of pollutants can also cause changes in fish anti-predator behaviours. Studies of direct effects of exposure to metals, organic chemicals and pesticides show that exposed fish may be more susceptible to predation (reviewed by Atchison *et al.* 1987; Clotfelter *et al.* 2004; Scott and Sloman 2004). This may be due to effects of pollution on physiological performance, sensory perception or information processing (Sloman 2007).

Parasitism and pollution stress may have combined effects on fish health. Juvenile Chinook salmon (*Oncorhynchus tschawytscha*) experimentally infected with

metacercariae of the digenetic trematode *Nanophyetus salmincola* and exposed to PCBs

had lower immune function and were more susceptible to infection by the bacterium

Listonella anguillarum than fish exposed to only individual stressors (Jacobson *et al.*

2003). Yellow perch (*Perca flavescens*) exposed to environmental levels of industrial and

agricultural pollution and naturally infected with larvae of the nematode *Raphidascaris*

acus had higher oxidative stress levels than fish exposed to only one of these stressors

(Marcogliese *et al.* 2005). Spottail shiners (*Notropis hudsonius*) exposed to

environmental levels of urban and industrial effluents and naturally infected with the

trematode *Plagioporus sinitsini* had more pigmented macrophages in their spleens, a

general indicator of stress, and lower condition indices than fish exposed to either stressor

alone (Thilakaratne *et al.* 2007). Pollution can also indirectly affect fish behaviour by

eliminating, decreasing or increasing the abundance of behaviour-modifying parasites

(Mackenzie *et al.* 1995; Kennedy 1997; Lafferty 1997; Overstreet 1997; Mackenzie

1999; Sures 2004; Marcogliese 2005).

Behaviour is an important indicator of stress in fish, linking the physiological

effects of parasites and pollution with ecological processes (Scott and Sloman 2004;

Barber and Rushbrook 2008). Changes in antipredator behaviour are of particular

ecological relevance because they have direct consequences for future host fitness.

Although both parasitism and pollution have the potential to affect fish behaviour, no

published studies to date have considered the combined effects of these two stressors. In

this study, we test the combined effects of parasitism and pollution in field conditions on

johnny darters (*Etheostoma nigrum*), a small, cryptically coloured benthic fish commonly

found in the St. Lawrence River in southwestern Quebec, Canada. Johnny darters inhabit

both relatively pristine and polluted areas of the river and are host to a diverse

community of parasites. The parasite communities of johnny darters from the St.

Lawrence River show differences that are correlated with pollution status of sampling

localities, as well as the type of pollution (Chapter 1). Herein, we examine the combined

effects of pollution and parasitism on antipredator behaviours in this host, using fish from

replicated contaminated and reference localities in the river. Specifically, we examine

whether effects of pollution and parasitism together are greater than effects of either

stressor alone. What further differentiates this study is that, by using fish collected in

field situations, we are examining the effects of both natural parasite communities

(Bordes and Morand 2009), including individual species, in addition to mixtures of

contaminants that are typical of real environmental situations in contrast to simplified

laboratory experiments that focus on single species and chemicals.

Materials and Methods

Study localities

Fish were collected in June 2008 from five localities in the St. Lawrence River in

southwestern Quebec, Canada. These included two reference localities, Îles de la Paix

(IPA; 45°20.022' N; 73°51.362' W) and Île Dorval (IDO; 45°26.016' N; 73°44.234' W),

and three polluted localities, Beauharnois (BEA; 45°19.051' N; 73°52.020' W), Îlet Vert

(IVT; 45°42.230' N; 73°27.143' W) and Île Beauregard (IBE; 45°44.965' N; 73°24.910'

W) (Figure 1.1). Localities were characterized in previous studies, based on

concentrations of metals, polychlorinated biphenyls (PCBs), and other contaminants in

the sediments (Loiselle *et al.* 1997; Marcogliese *et al.* 2005; 2006; Dautremepuits *et al.*

2009). These measures are considered an accurate representation of pollution status because sediment contamination is relatively stable over time in this system (Dautremepuits et al. 2009). They are also biologically significant, because johnny darters are benthic organisms that spend their lives in close contact with the sediment and feed on benthic invertebrates (Strange 1991). IPA and IDO are located upstream of the Island of Montreal in Lake St. Louis. No contaminants surpassing the Canadian Environmental Quality Guidelines Probable Effects Level (PEL) (http://ceqg-rcqe.ccme.ca/for aquatic life) were detected at either locality (Marcogliese et al. 2006). BEA is also located in Lake St. Louis, at the mouth of the St. Louis River. It is primarily affected by industrial and agricultural activity upstream in the St. Louis River. BEA has high levels of PCBs, orgnanochlorines, and several metals, particularly mercury, which surpasses the PEL (Loiselle et al. 1997; Marcogliese et al. 2005; Dautremepuits et al. 2009). IVT and IBE are located downstream of Montreal in the plume of the Montreal sewage treatment plant outfall. They both have high levels of organochlorines, PCBs and some metals. PCB levels at IBE and chromium levels at IVT surpass the PEL (Marcogliese et al. 2006; Magella Pelletier, Environment Canada, pers comm.).

Study organisms

One hundred and seventy-eight johnny darters were collected using a beach seine (22.6 × 1.15m; 3mm mesh) and transported live to the laboratory. Fish from each locality (n = 35-36 per locality) were kept in separate tanks (90cm × 45cm × 35cm). Tanks were lined with aquarium gravel, filled with 60L of dechlorinated tap water and continuously

aerated. Fish were kept at 20°C, in a 14:10 light:dark regime. Fish were fed *ad lib* with Nutrafin™ fish flakes.

Behaviour experiments

Fish were kept in the laboratory for an acclimation period of at least six days prior to testing (Smith 1979). Behaviour experiments for fish from each locality were conducted over two to three consecutive days, within 14 days of collection. Behavioural metrics were chosen based on results from preliminary experiments on johnny darters from two localities in the St. Lawrence River, IPA and IVT, in September 2007. Two experiments were conducted. The first experiment measured capture time of each fish (n = 178), defined as the time taken to catch individual fish, and was considered a proxy for susceptibility to predation. Capture time was tested in the same tanks used for acclimation, to minimize unnecessary handling of the fish. The experiment consisted of catching fish one by one from the large tank using a hand-held dip net (43cm long, 13cm × 16cm opening). During the experiment, the dip net was placed in the middle of the water column in the centre of the tank and shaken vigorously to alert the fish to the "predator." The net was then moved in a regular manner counterclockwise along the walls of the tank, at an approximately constant speed of 20cm/s around the tank until a fish was caught at random. This was repeated until all fish were caught. The order in which fish were captured from each tank was recorded as "capture order" and examined as an additional behaviour measure.

After fish were removed in the capture time experiment, they were transferred into test tanks for the second behaviour experiment, a measurement of flight distance.

Fish were paired in narrow "flight distance" test tanks (90cm × 30cm × 35cm, 50L) and then left to acclimate in the tanks for two hours before beginning the experiment. During the acclimation period and between experiments, two carbon water filters were run in the flight distance tanks to remove any chemical cues that may have been left by fish previously tested in the tanks. After the acclimation period the water filters were turned off. Flight distance was measured by moving a predator model from the end of the tank opposite the two fish towards them at an approximate speed of 10 cm/s. Flight distance measurements were made on one "focal" fish (n = 89), the second "dither" fish being placed in the tank to reduce the stress level of the focal fish (Brown et al. 2006). The fish closest to the approaching predator was designated as the focal fish. The experiment was filmed and flight distance, defined as the distance from the predator model at which the fish initiated movement, was measured from the video recording.

Following the behaviour experiments, fish were killed with an overdose of clove oil (50 mg/L) solution and frozen for later necropsy. All animal collection and experimental procedures were in accordance with guidelines of the Canadian Council on Animal Care in effect at the time of the study.

Examination for parasites

Fish frozen mass (mg) and standard length (mm) were measured for each fish, followed by a complete necropsy. Parasites from tissues and organs, including fins, skin, gills, eyes, brain, body cavity, gastrointestinal tract, liver, heart, spleen, gonads and muscle were collected and enumerated, following standard parasite examination protocol (Marcogliese 2002). During the necropsy, all parasites were enumerated and identified to

genus, with the exception of acanthocephalans, non-gyrodactylid monogeneans, and a few rare trematodes, which could only be identified to a higher taxonomic level. Representative samples of parasites recovered from each locality were preserved in 70% ethanol for later identification. Trematodes, cestodes, acanthocephalans and some monogeneans were stained with acetocarmine and cleared with xylene before mounting in Permount or Canada balsam. Other monogeneans were mounted unstained in Hoyer's medium. The remaining monogeneans and all nematodes and copepods were cleared in glycerine alcohol and examined in temporary mounts. Identifications were made using keys in Beverly-Burton (1984), Caira (1989), Kabata (1988), Moravec (1994), Gibson (1996), Scholz (1997) and Hoffman (1999).

Statistical analysis

Mean total parasite number and infracommunity species richness, standard length, capture time and flight distance of fish were tested among localities and between polluted and reference localities. Comparisons among localities and between treatments were made using ranked data by one-way ANOVAs (Scheirer *et al.* 1976) followed by Tukey-Kramer HSD tests, except for standard length, which was tested using untransformed data. Because capture order of individual fish was dependent on capture order of other fish from within the same tank (i.e. locality), it could not be compared among localities or between fish pooled by pollution status. Separate regression comparisons for each locality were made between capture order of individual fish and their total parasite number, infracommunity species richness and abundance of individual parasite species. All univariate tests were conducted using JMP® 7.0.1 (© 2007 SAS Institute Inc.).

Multivariate analyses were conducted using the PERMANOVA+ add-on for PRIMER (© 2006 Plymouth Routines In Multivariate Ecological Research, Plymouth, UK). A stepwise regression of capture time with abundances of all parasite species was performed with a distance-based linear model (DISTLM). This test allows for a stepwise test of continuous variables that are not normally distributed. Species that significantly correlated with capture time were included as covariates in a permutational multivariate ANOVA (PERMANOVA) of capture time. PERMANOVA is a nonparametric test analogous to a multivariate ANOVA. It gives the test statistic "Pseudo-F," which is analogous to the "F" statistic in measuring the among-group to within-group variation. The initial model also included mean total parasite number, mean infracommunity species richness, mean standard length, locality, and interactions between variables. The final model included only terms that significantly explained capture time.

Mean abundances of parasite species included in the PERMANOVA were individually compared among localities using ANOVAs on ranked data, followed by a Tukey HSD tests. They were tested between polluted and reference localities using nonparametric Wilcoxon tests.

Terminology

Parasite ecology terms adhere to definitions of Bush *et al.* (1997). Prevalence is the percentage of hosts infected with a given parasite species in a sample. Abundance is the number of parasites of a given species infecting a given host, whether the host is infected or not. Mean abundance is the number of parasites of a given species averaged over the whole host sample, and includes hosts with and without infections. Intensity is

the number of parasites of a given species infecting a host, and mean intensity is the number of parasites of that species averaged across infected hosts in a sample. An infracommunity refers to all the individuals of all the parasite species within an individual host. Locality refers to the geographic area from which the host was collected, and site refers to the specific host tissue or organ from which the parasite was collected.

Results

Twenty-four species of parasites were identified in the 178 darters examined. The prevalence and mean intensity of each parasite species at each locality are presented in Chapter 1. Mean total parasite number was highest at BEA and IDO and lowest at IVT and IBE ($F_{4,173} = 31.73$, $p < 0.0001$). Mean infracommunity species richness was greatest at BEA, second highest at IPA and IDO, and lowest at IVT and IBE ($F_{4,173} = 38.48$, $p < 0.0001$). Standard length was significantly larger for fish from BEA than those from IDO ($F_{4,173} = 3.62$, $p = 0.007$), but did not differ among fish from other localities.

Capture time differed significantly among localities ($F_{4,173} = 6.20$, $p = 0.0001$), with the longest capture times at IDO and BEA and the shortest capture time at IVT (Figure 2.1). Capture time did not differ significantly between fish from polluted and reference localities ($F_{1,176} = 1.57$, $p = 0.12$).

Mean total parasite number and mean infracommunity species richness did not significantly correlate with capture time. The only parasite species that significantly explained capture time was *Ornithodiplostomum* sp. Capture time was best explained by a model including *Ornithodiplostomum* sp. abundance and locality (Table 2.1). When all samples were pooled, the relationship between *Ornithodiplostomum* sp. abundance and

capture time was significantly positive (Figure 2.2), suggesting that fish with higher

intensity infections might be less susceptible to capture than fish with low or no

infections. *Ornithodiplostomum* sp. mean abundance was highest at IDO, followed by

IPA, and was lowest at BEA, IVT and IBE ($F_{4,173} = 46.6$, $p < 0.0001$; Figure 2.3). It was

significantly higher at reference than polluted localities ($F_{1,176} = 119.40$, $p < 0.0001$).

Neither capture order nor flight distance was significantly correlated with any of

the variables measured in this study, and were not considered further. Results from the

regressions for capture order and the PERMANOVA for flight distance can be found in

Appendices 2 and 3, respectively.

Discussion

Johnny darters with high intensity infections of the brain-encysting parasite,

Ornithodiplostomum sp., had longer capture times than fish with low or no infections,

perhaps reflecting an increase in activity of infected fish. Johnny darters normally exhibit

a cessation of movement when they detect a predator (Smith 1979). Stressors that induce

hyperactivity may disrupt adaptive anti-predator behaviours. In this study, darters

exhibiting typical antipredator behaviour appeared to be more susceptible to capture,

while fish behaving abnormally, by moving quickly and erratically, and swimming to the

surface, were more difficult to catch. In natural systems, however, predators such as

piscivorous diving birds such as mergansers, the definitive hosts of *Ornithodiplostomum*

spp., typically depend on visual cues such as movement to capture their prey, and an

increase in activity may make cryptic fish such as johnny darters more susceptible to

predation (Ydenberg and Dill 1986). Therefore deviations from typical, cryptic anti-

predator behaviour of johnny darters caused by high intensities of *Ornithodiplostomum* sp. may reflect an adaptation of the parasite to increase its transmission success, although it could simply be a pathogenic by-product of infection (Poulin 1995, Poulin and Thomas 1999).

Neither parasitism nor pollution could statistically explain observed differences in either of the other two behavioural measures, capture order or flight distance. Capture time was a measurement that has not been used in previous studies, but was measured because it showed significant results in the pilot study. However, flight distance is a measure commonly used to measure fish reactions to predation risk (Ydenberg and Dill 1986). Lack of response in this study may reflect difficulties in accurately measuring flight distance in this species. Alternatively, it may be inappropriate in a species such as the johnny darter that typically exhibits a cessation of movement in response to perceived predators.

Studies of behaviour changes of fathead minnows (*Pimephales promelas*) with infections of *Ornithodiplostomum ptychocheilus* suggest that behavioural changes may be caused by adaptive manipulation by the parasite or pathology of parasite development in the host. Fathead minnows with mature infections of *O. ptychocheilus* exhibited less compact shoaling behaviour and swam higher in the water column, which may make them more susceptible to predation (Radabaugh 1980). Alternatively, minnows with new infections of *O. ptychocheilus* showed reduced standard optomotor response (OMR), likely due to damage caused at the site of infection, the optic tectum (Shirakashi and Goater 2001; Shirakashi and Goater 2002). The greatest decrease in OMR occurred during parasite development and subsided after they reached infectivity, reflecting

damage to the optic lobes during parasite growth (Shirakashi and Goater 2005). Behavioural changes induced before a parasite becomes infective are considered pathological, while those that ensue following development to the infective forms may be evidence of adaptation (Poulin 1995). The present study does not explore the specific physiological mechanisms of the observed behavioural change, nor does it measure actual predation rates of infected and non-infected fish. However, evidence from other *Ornithodiplostomum*-fish systems, as seen above, suggests that both scenarios are possible. In our study, parasites were encysted and presumably infective, lending support to the idea that the behavioural changes may be adaptive. Further experiments to test the fitness consequences for both the parasite and host are necessary to determine whether the behaviour change seen here is an adaptive modification by the parasite or merely a pathological side effect (Poulin 1995).

Behavioural changes of hosts infected with similar parasites have been demonstrated in other studies using simulated predation. For example, rainbow trout (*Oncorhynchus mykiss*) with mature infections of metacercariae of the eye fluke *Diplostomum spathaceum* were more susceptible to simulated predation, as measured by percentage of infected versus non-infected fish captured (Seppälä *et al*. 2004; 2005).

Locality was also significantly correlated with differences in fish behaviour. This may reflect a tank effect in the experimental design, because fish from each locality were kept and tested in a single tank. However, it may also be due to a parasite effect. Mean capture time of fish from different localities showed patterns similar to patterns of parasite community parameters, suggesting it was not a tank effect. Fish from BEA and IDO had higher capture times than fish from IVT, and also higher mean species richness

and mean total parasite number. Neither species richness nor total parasite number, nor any parasite species apart from *Ornithodiplostomum* sp. was significant in the model of capture time, however the pattern seen in capture time may nonetheless be partially due to these factors or interactions of specific parasites that were not statistically detectable. *Ornithodiplostomum* sp. occurred in the highest abundance of all the parasites found, and lack of effects of other species may simply be due to low infection intensities, rather than a real lack of effect. There was no interaction between locality and mean abundance of *Ornithodiplostomum* sp., suggesting that the effects of the parasite on behaviour were independent of pollution status.

There was no direct effect of pollution on fish behaviour. However, the analysis of parasite communities and pollution revealed a significant, negative effect of pollution on the abundance of *Ornithodiplostomum* sp. (Chapter 1). Free-living cercariae of digenetic trematodes are sensitive to a variety of types of pollution, including metals, acidification, chemical fertilizers and pesticides, which can reduce their survival, longevity, encystment and infectivity (Morley *et al.* 2003; Pietrock and Marcogliese 2003). Cercariae of *O. ptychocheilus* exposed to cadmium showed decreased infectivity to fish (Pietrock and Goater 2005). Therefore, metal pollution may indirectly affect johnny darter behaviour at contaminated localities, through the reduction of survival and/or infectivity of cercariae of *Ornithodiplostomum* sp.

Previous studies of effects of pollution and parasite stress on fish behaviour have focused primarily on single pollutants and single parasite species, and have not tested both stressors together. This study considers these stressors in combination, and tests naturally infected fish obtained directly from polluted localities. This approach can limit

the interpretive power of the study and does not allow hypotheses regarding effects of specific pollutants to be tested. However it represents conditions seen in nature, where pollution stress often is due to combinations of many chemicals (Jobling 1995; Mackenzie *et al.* 1995; Lafferty 1997; Marcogliese 2005) and fish are commonly infected with communities of parasites (Barber *et al.* 2000; Barber and Rushbrook 2008), conditions difficult to replicate in laboratory experiments.

In this study we found that behaviour of johnny darters was primarily affected by a brain-encysting parasite, *Ornithodiplostomum* sp. There was no direct effect of pollution on behaviour, possibly because pollution levels in the study area are considered moderate (Marcogliese *et al.* 2006) and may not be high enough to cause detectable changes in fish behaviour. Yet pollution may have had an indirect effect on behaviour by lowering abundances of *Ornithodiplostomum* sp. through effects on cercarial transmission. We suspect that effects of parasitism and pollution on fish behaviour will be context dependent and vary with parasite species composition and abundance, as well as the degree and types of contamination.
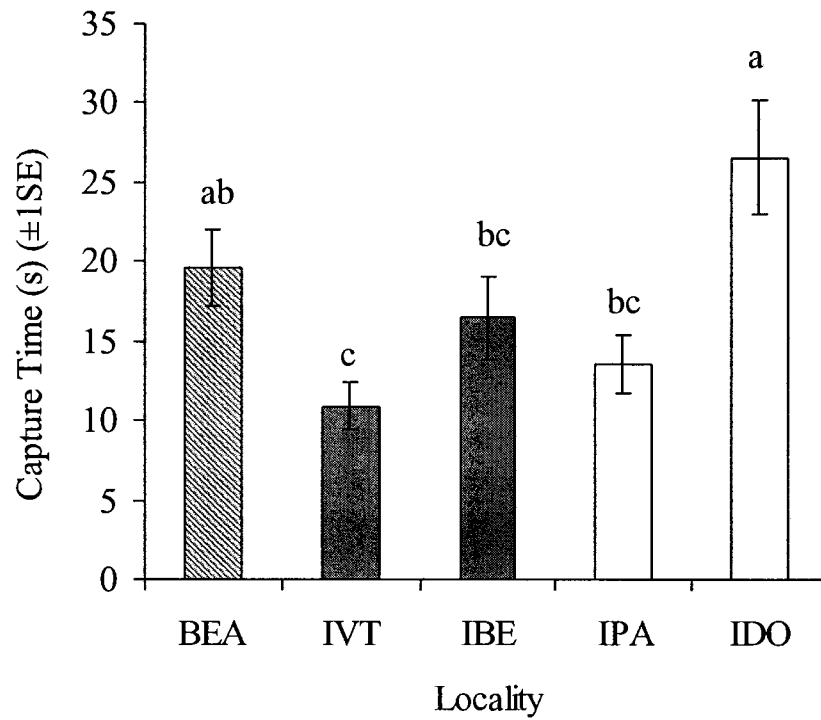
Figure 2.1. Mean capture time (s) ± standard error of johnny darters from five localities in June 2008 in the St. Lawrence River in southwestern Quebec, Canada: one upstream polluted locality (light grey), Beauharnois (BEA); two downstream polluted localities (dark grey), Île Beauregard (IBE), Îlet Vert (IVT); and two reference localities (white), Île Dorval (IDO) and Îles de la Paix (IPA). Different letters indicate significant differences between localities.

Table 2.1. Permutational MANOVA of capture time (s) of johnny darters by

Ornithodiplostomum sp. abundance and locality. Johnny darters were from five localities

in June 2008 in the St. Lawrence River in Quebec, Canada: Beauharnois (BEA), Île

Beauregard (IBE), Îlet Vert (IVT), Île Dorval (IDO) and Îles de la Paix (IPA). The

PERMANOVA is based on 999 random permutations of the data, 998 of which were

original. (*) designates significance (P < 0.05).

Source	df	SS	MS	Pseudo-F	P
Ornithodiplostomum sp.	1	6545	6545	18.82	0.002*
Locality	4	1941	485	2.45	0.039*
Residuals	172	34063	198		
Total	177	42549			
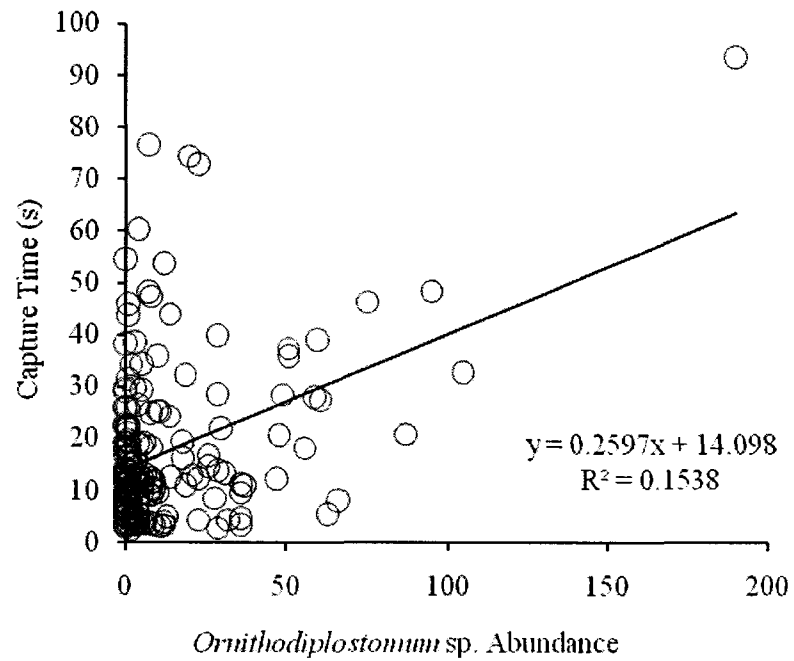
Figure 2.2. Capture time (s) by *Ornithodiplostomum* sp. abundance for johnny darters from five localities in June 2008 in the St. Lawrence River, Quebec, Canada. The linear correlation is significant ($R^2 = 0.15$, $p < 0.0001$)
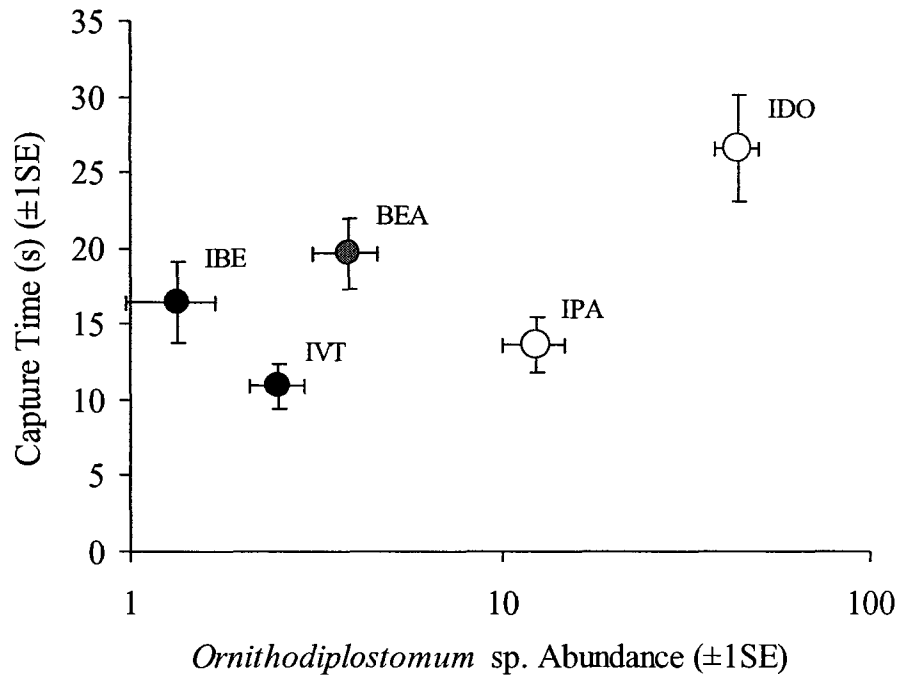
Figure 2.3. Mean capture time (s) and mean *Ornithodiplostomum* sp. abundance in johnny darters from five localities in June 2008 in the St. Lawrence River in Quebec, Canada: one upstream polluted locality (grey circle), Beauharnois (BEA); two downstream polluted localities (black circles), Île Beauregard (IBE) and Îlet Vert (IVT); and two reference localities (white circles), Île Dorval (IDO) and Îles de la Paix (IPA). Error bars represent standard errors.

General Conclusions

The results presented in this thesis show that pollution can have an effect on parasite community composition of johnny darters, reducing the total number of parasites and species richness. Among the parasite species most affected was *Ornithodiplostomum* sp., which had lower abundances at the polluted localities than the reference localities. There was a significant effect of *Ornithodiplostomum* sp. abundance on antipredator behaviour of fish. Therefore, differences in behaviour between fish from polluted and reference localities may be an indirect effect of pollution-induced changes in the parasite community.

Results from Chapter 1 demonstrated that pollution may influence parasite communities, however the type of pollution may also be an important factor as well. As predicted, the two polluted localities downstream of the Montreal sewage treatment outfall did show low parasite species richness and abundance, however the upstream polluted locality, which is affected primarily by industrial and agricultural pollution, had high parasite species richness and total parasite numbers. Differences between these localities may be due to the type of pollution affecting them. They may also be due to the ecological context of the different localities: the two downstream localities have lower fish diversity and compacted food webs, while the upstream locality has higher fish diversity and productivity. The abundance of *Ornithodiplostomum* sp. was significantly higher at the references localities than the polluted localities. This is consistent with previous work on susceptibility of transmission stages of this parasite to metal contamination.

Chapter 2 examined the behavioural response of johnny darters to simulated predation events. The infection intensity of a single parasite, *Ornithodiplostomum* sp., had a significant positive effect on capture time, reflecting an increase in swimming activity of infected fish. This is likely a maladaptive change in johnny darters, which typically display cryptic behaviour in response to perceived predation risk. Locality also had a significant effect on capture time, and showed a similar pattern to differences in species richness and total parasite numbers among localities. This may be a reflection of parasite effects that were present but not statistically detectable. Pollution did not have a significant direct effect on behaviour. However, the relationship between *Ornithodiplostomum* sp. intensities and behaviour, and the significant differences in abundance of this parasite between polluted and reference localities suggest that pollution may be indirectly affecting fish behaviour by reducing infections of this behaviour-modifying parasite.

The results presented in this study highlight two important points. First, not only will pollution affect parasite communities of fish, but the type of contaminant mixtures occurring in polluted situations may have differential effects on those communities. Second, parasites can be a confounding factor in behaviour studies using wild-caught fish. This has been found in studies of environmental contamination and bioenergetics (Kelly and Janz 2008), and is likely also true in ecotoxicological studies of wild fish that use behavioural endpoints.

Pollution of aquatic systems is pervasive, and can have wide-reaching, cascading effects on ecosystems. The results presented here demonstrate that pollution can have significant effects at multiple ecological levels. Understanding the magnitude of these

effects and interpreting the changes seen will require a better understanding not only of the effects on charismatic free-living organisms, but on more cryptic members of the biotic community, the parasites.

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Appendix 1. Abundances ± standard deviations of macroparasites in johnny darters from five localities in June 2008 in the St. Lawrence River in southwestern Quebec, Canada: Beauharnois (BEA), Ilet Vert (IVT), Île Beauregard (IBE), Îles de la Paix (IPA) and Île Dorval (IDO).

	BEA (n = 36)	IVT (n = 36)	IBE (n = 35)	IPA (n = 36)	IDO (n = 35)
Digenea					
Allocreadium boleosoma	0	0	0	0	0.05 ± 0.24
Apatemon spp.	1.83 ± 3.08	4.31 ± 9.39	6.77 ± 9.19	5.72 ± 14.98	3.17 ± 4.98
Apophallus brevis	0.81 ± 1.51	0.28 ± 0.51	0.31 ± 0.76	0.89 ± 1.39	1.26 ± 1.62
Azygia angusticauda	0.53 ± 1.11	0.08 ± 0.28	0.06 ± 0.24	0.03 ± 0.17	0.14 ± 0.43
Clinostomum complanatum	0.17 ± 0.51	0	0	0.08 ± 0.28	0
Crepidostomum isostomum	0.11 ± 0.46	0	0.06 ± 0.24	0.06 ± 0.23	0
Cryptogonimus sp.	8.61 ± 19.76	0.36 ± 0.54	0.40 ± 0.91	3.18 ± 7.33	0.71 ± 2.28
Diplostomum spp.	0.50 ± 0.85	0	0	0.28 ± 0.45	0.26 ± 0.61
Ichthyocotylurus sp.	9.39 ± 9.87	0.67 ± 1.82	2.77 ± 4.04	2.17 ± 4.01	2.20 ± 2.85
Neochasmus sp.	13.61 ± 14.43	2.03 ± 7.93	0.80 ± 1.30	0.06 ± 0.23	0.29 ± 0.52
Ornithodiplostomum sp.	3.86 ± 4.75	2.50 ± 2.47	1.34 ± 2.17	12.47 ± 14.23	43.97 ± 35.76
Phyllodistomum etheostomae	0	0	0	0.03 ± 0.17	0
Tylodelphys sp.	0	0	0	0.28 ± 0.70	0
metacercaria sp. 1	0.22 ± 0.87	0	0	0	0

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Appendix 1. *Continued.*

	BEA (n = 36)	IVT (n = 36)	IBE (n = 35)	IPA (n = 36)	IDO (n = 35)
tetracotyle sp. 1	0	0.03 ± 0.17	0.06 ± 0.24	0.81 ± 1.72	0
Monogena					
Gyrodactylus etheostomae	$7.28 + 5.22$	5.19 ± 4.79	6.17 ± 9.39	7.17 ± 5.66	7.11 ± 5.03
Aethycteron nigrei	1.42 ± 1.71	4.11 ± 3.98	0.26 ± 0.51	0.33 ± 0.72	1.00 ± 1.16
Cestoda					
Bothriocephalus cuspidatus	0.33 ± 0.63	0	0	0	0
Triaenophorus nodulus	0.06 ± 0.23	0	0	0	0
Nematoda					
Dichelyne sp.	2.56 ± 2.66	0.08 ± 0.28	0.43 ± 0.81	2.14 ± 4.38	1.40 ± 2.52
Raphidascaris acus	1.06 ± 1.09	0.08 ± 0.37	0.03 ± 0.17	0.47 ± 0.97	0.06 ± 0.24
Cammalanus lacustris	0.06 ± 0.23	0	0	0.03 ± 0.17	0
Acanthocephala	0.47 ± 1.03	0.14 ± 0.54	0.20 ± 0.47	0.31 ± 0.67	0.06 ± 0.24
Copepoda					
Lernaea sp.	0	0	0	2.00 ± 2.23	0

Appendix 2. Linear regressions of capture order by standard length, total parasite

number, species richness, and *Ornithodiplostomum* sp. abundance, for johnny darters

from five localities in June 2008 in the St. Lawrence River in southwester Quebec,

Canada: Beauharnois (BEA), Îlet Vert (IVT), Île Beauregard (IBE), Îles de la Paix (IPA)

and Île Dorval (IDO).

	BEA	IVT	IBE	IPA	IDO
Standard length	$R^2 = 0.08$	$R^2 = 0.04$	$R^2 = 0.05$	$R^2 = 0.005$	$R^2 = 0.02$
	$p = 0.11$	$p = 0.21$	$p = 0.18$	$p = 0.69$	$p = 0.40$
Total parasite number	$R^2 = 0.01$	$R^2 = 0.09$	$R^2 = 0.01$	$R^2 = 0.01$	$R^2 = 0.001$
	$p = 0.63$	$p = 0.07$	$p = 0.66$	$p = 0.55$	$p = 0.84$
Species richness	$R^2 = 0.06$	$R^2 = 0.06$	$R^2 = 0.05$	$R^2 = 0.01$	$R^2 = 0.01$
	$p = 0.14$	$p = 0.16$	$p = 0.19$	$p = 0.60$	$p = 0.64$
Ornithodiplostomum sp.	$R^2 = 0.02$	$R^2 = 0.01$	$R^2 = 0.02$	$R^2 = 0.04$	$R^2 = 0.001$
	$p = 0.45$	$p = 0.51$	$p = 0.43$	$p = 0.26$	$p = 0.83$

Appendix 3. Permutational multivariate ANOVA of flight distance versus standard

length, locality and pollution status, for johnny darters from five localities in June 2008 in

the St. Lawrence River in southwester Quebec, Canada: Beauharnois (BEA), Îlet Vert

(IVT), Île Beauregard (IBE), Îles de la Paix (IPA) and Île Dorval (IDO).

Source	df	SS	MS	Pseudo-F	P
Locality	3	70.91	23.64	0.537	0.68
Standard length	1	5.52	5.52	0.125	0.71
Total parasites	1	3.87	3.87	88.0	0.78
Ornithodiplostomum sp.	1	2.10	2.10	47.8	0.79
Species richness	1	0.18	0.18	4.06×10^{-4}	0.95
Residuals	77	3389.4	44.02		
Total	85	3527.4			