Effects of Natural and Anthropogenic Stressors on Biomarkers of Fish Health in Spottail Shiners (*Notropis hudsonius*)

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ABSTRACT

Effects of natural and anthropogenic stressors on biomarkers of fish health in spottail shiners (*Notropis hudsonius*)

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Parasites and pollution are natural and anthropogenic stressors respectively in fishes. A range of biomarkers have been used to assess the sublethal effects of pollutants in fish, but few studies have examined sublethal effects of parasites and pollution in combination. I examined seven biomarkers (pigmented macrophage centres and pigmented macrophage counts in the spleen, pigmented macrophage counts in the liver, white blood cell counts, haematocrit, leucocrit, and condition factor) and parasite abundances in 204 (age 1+ and 2+) spottail shiners (*Notropis hudsonius*) collected at three polluted and two reference localities in the St. Lawrence River, Quebec, Canada. The number of pigmented macrophage centres and pigmented macrophages in the spleen was significantly higher at polluted localities than at reference localities. Seven of the nine species of parasites found in 1+ fish showed significant correlations with biomarkers. More parasites (18 species) but few significant correlations were observed in 2+ fish, indicating that parasite effects are more pronounced in young spottail shiners. A significant negative relationship was observed between condition factor and *Neoechinorhynchus rutili* (Acanthocephala) in 1+ fish, indicating its potential pathological significance in fish. High abundance of *Plagioporus sinitsini* (Digenea) was associated with increased spleen macrophage

counts and decreased condition factor at polluted localities. Results suggest that counts of spleen macrophages and macrophage centres are good indicators of exposure to pollution in spottail shiners. Furthermore, infection by *P. sinitsini* in under polluted conditions appears to a greater negative impact on fish health than either stressor alone.

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Introduction

Pollution is a worldwide problem. Terrestrial ecosystems have their own unique problems, but aquatic ecosystems seem to be particularly vulnerable to the effects of pollutants. Nutrient loading, agricultural runoff, contamination with heavy metals and organic compounds, acidification, and thermal loading all contribute to the degradation of aquatic ecosystems. Pollutants are stressors on exposed organisms whose reactions to them are both multiple and varied. The effects of pollutants on fish and other aquatic organisms are well known and are reflected as lesions, tumors and carcinomas in various organs and as changes in biochemical, histological, haematological, reproductive and physiological parameters (reviewed in Snieszko, 1974; Sindermann, 1983; Anderson, 1990; Hinton et al., 1992; Shugart et al., 1992; Wolke, 1992; Morgan and Iwama, 1996; Adams, 2001; Bols et al., 2001; Myers and Fournie, 2002; Agius and Roberts, 2003). Some examples of the toxic effects related to pollution stress in fish include the high prevalence of hepatic tumors and preneoplastic hepatic lesions in lake whitefish (*Coregonus clupeaformis*) from the St. Lawrence River (Mikaelian et al., 2002); high prevalence of cholangiocellular carcinomas in English sole (*Parophrys vetulus*) living in chemically contaminated localities in Puget Sound, Washington (Stehr and Myers, 1990); impaired gonad development in carp (*Cyprinus carpio*) from contaminated sites in Lake Mead, Nevada (Patino et al., 2003); high prevalence of caudal fin necrosis and changes in various physiological indices in winter flounder (*Pseudopleuronectes americanus*) captured adjacent to a pulp and

paper mill in St. George's Bay, Newfoundland (Barker et al., 1994); significantly higher values of detoxification enzymes (e.g., 7-ethoxyresorufin-O-deethylase and cytochrome *c* reductase) in adult redbreast sunfish (*Lepomis auritus*) collected from contaminated sites in Oak Ridge, Tennessee (Adams and Ryon, 1994); and changes in the liver enzyme cytochrome P450, blood urea nitrogen, haematocrit, leucocrit, the number of spleen pigmented macrophage aggregates, liver somatic index, gonadal-somatic index and condition factor in southern flounder (*Paralichthys lethostigma*) and spot (*Leiostomus xanthurus*) from polluted sites in Pamlico Sound, North Carolina (Adams et al., 2003).

Various stressors, including pollution, may lead to changes in resistance to disease and it is generally accepted that infectious agents are more likely to cause disease in animals living under stressed conditions than those that are not (Wedemeyer, 1970; Esch et al., 1975; Sindermann, 1983). Moreover, existing sub-clinical infections may develop into pathogenic forms following exposure to stressors (Sindermann, 1983; Gopal, 1992). Some examples of infectious disease linked to pollution stress include the high prevalence of red-sore disease (*Aeromonas hydrophila*) (bacteria) in largemouth bass (*Micropterus salmoides*) from thermally-altered sites in Savananah River, South Carolina (Esch and Hazen, 1978); high mortality of Atlantic salmon (*Salmo salar*) and white sucker (*Catostomus commersonii*) due to *Aeromonas liquefaciens* (bacteria) in polluted sites in the Miramichi River, New Brunswick (Pippy and Hare, 1969); and the high

prevalence of black spot disease (Digenea) in stream fishes living in polluted localities near Toronto, Ontario (Steedman , 1991).

Fish are typically infected with a wide variety of parasites. Parasites are natural stressors that by definition are detrimental to their host and the effects of several species on fish health are well documented. Parasites can affect vision, behavior, body condition, physiology and even survival of fish (Post, 1987). Some examples of parasite induced pathology include cataract formation associated with metacercariae of *Diplostomum* spp. (Digenea) in the eye, gastrointestinal diseases associated with *Echinorhynchus gadi* and *Pomphorhynchus laevis* (Acanthocephala), high mortality and damage to internal organs by *Raphidascaris acus* (Nematoda), a thickening of the mucus layer of the skin by *Ichthyobodo necatrix* and *I. costia* (Mastigophora), malnutrition and anemia caused by *Heximita* sp. (Mastigophora), and whirling disease due to *Myxobolus cerebralis* (Myxozoa) (Post, 1987; Schaperclaus, 1991; Paperna, 1995; Hoffman, 1999).

Many field and experimental studies have examined the effects of pollution on fish parasites. Some suggest pollution increases parasite infections, while others indicate decreased or no change in infections levels (reviewed in Moller, 1987; Khan and Thulin, 1991; Poulin, 1992; MacKenzie et al., 1995; Lafferty, 1997; Sures, 2004; Marcogliese, 2004, 2005). Higher infection levels of *Rhipidocotyle fennica* (Digenea) in roach (*Rutilus rutilus*) in Hungary (Jeney et al., 2002), *Echinorhynchus gadi* (Acanthocephala) in cunner (*Tautogolabrus adspersus*) in Newfoundland (Billiard and Khan, 2003), *R. acus* larvae in roach (*Rutilus*

rutilus) in central Finland (Valtonen et al., 1994), myxozoan parasites in spottail shiners in Quebec (Marcogliese and Cone, 2001), *Glugea stephani* (Microspora) in winter flounder in Newfoundland (Khan, 2004) and *Trichodina* sp. (Ciliophora) in three-spined stickleback (*Gasterosteus aculeatus*) in England and Wales (Yeomans et al., 1997) have been reported in fish from polluted sites compared to fish from clean sites. Khan and Kiceniuk (1988) found a higher prevalence and intensity of *Gyrodactylus* sp. (Monogenea) in Atlantic cod (*Gadus morhua*) experimentally exposed to high levels of petroleum aromatic hydrocarbons compared to those exposed to lower concentrations. Marcogliese et al. (1998) described a similar increase in the abundance of *Gyrodactylus* sp. (Monogenea) in American plaice (*Hippoglossoides platessoides*) experimentally exposed to contaminated sediments rich in hydrocarbons and biphenyls. In contrast, reports of decreasing infections in relation to pollution include a decline in species richness and abundance of metazoan parasites in American eels (*Anguilla rostrata*) in Nova Scotia due to acidification (Cone et al., 1993; Marcogliese and Cone, 1996) and a decline in species richness of metazoan and protozoan parasites of mosquitofish (*Gambusia holbrooki*) in Mississippi and Texas exposed to organic toxicants and heavy metals (Overstreet, 1997). In another study, Thulin et al. (1988) found no change in parasitic infections of perch (*Perca fluviatilis*) and roach exposed to bleached kraft mill effluents in Sweden.

Several experimental studies have shown that the effects of parasitic infection are generally more serious in fish exposed to contaminants than in

those that are not. Boyce and Yamada (1977) demonstrated increased mortality in sockeye salmon (*Oncorhynchus nerka*) smolts infected with *Eubothrium salvelini* (Cestoda) and exposed to zinc compared to uninfected fish. Pascoe and Cram (1977) demonstrated that cadmium was more toxic to three-spined sticklebacks infected with plerocercoids of the cestode *Schistocephalus solidus* than to uninfected ones and that mortality was higher in fish subjected to both stressors than to either stressor alone. In another study, Pascoe and Woodworth (1980) exposed three-spined sticklebacks to three stressors (*S. solidus*, dietary restriction and cadmium). They tested all possible combinations of these stressors jointly and separately and found significantly higher mortality in fish exposed to combined stressors than to single ones. Moles (1980) showed that coho salmon (*Oncorhynchus kisutch*) fry infected with glochidia of *Anodonta oregonensis* (Mollusca) were more susceptible to effects of crude oil, toluene and naphthalene than uninfected fish. Jacobson et al. (2003) showed that juvenile chinook salmon (*Oncorhynchus tshawytscha*) experimentally infected with metacercariae of *Nanophyetus salmonicola* (Digenea) and injected with a pollutant (a mixture of PCBs) were more likely to be killed by the pathogenic bacterium *Listonella anguillarum* than those that were not infected. More recently Gheorgiu et al. (2005) observed higher mortality of guppies (*Poecilia reticulata*) experimentally exposed to high concentrations of waterbone zinc and the monogenean *Gyrodactylus turnbulli* than those exposed to zinc alone.

Although the lethal effects of parasites and pollutants have been investigated in a number of natural and experimental systems, less is known of the sublethal effects of the combined stressors on fish. Sakanari et al. (1984) demonstrated that striped bass (*Morone saxatilis*) infected with larval nematodes (*Anisakis* sp.) and exposed to zinc and benzene had lower haematocrit values than fish exposed to single stressors alone. Jacobson et al. (2003) showed that the plaque forming cell response of anterior kidney leukocytes *in vitro* was significantly lower in fish infected with *N. salmonicola* and injected with Aroclor 1254 than with either stressor alone. Marcogliese et al. (2005) found that levels of lipid peroxidation in the livers of yellow perch (*Perca flavescens*) were higher at a polluted locality than at a clean locality and that this effect was more pronounced in fish infected with *R. acus* and *Apophallus brevis* (Digenea) at the polluted site.

Biomarkers and Bioindicators

Biological monitoring using biomarkers and bioindicators can be an informative, cost effective and powerful tool to detect potential toxicants in the environment and to assess the impact of environmental stress on an ecosystem (Shugart et al., 1992). Bioindicators can be described as biological responses occurring at higher levels of biological organization such as the individual, the population or the community. They are less sensitive indicators of environmental stress than biomarkers but are good indicators of long term exposure to pollutants (Feely, 1995; Adams, 2001; Adams and Greeley, 1999). A measurement

of survival rate of fish in environments with different levels of pollution is an example of a bioindicator (Shugart et al., 1992).

Biomarkers (as opposed to bioindicators) are defined as xenobiotically induced variations in cellular or biochemical components or processes. These may be structural (e.g., histological changes in tissues) or functional (e.g., impairment of enzyme activity) changes that are measurable in a biological system or sample (Shugart et al., 1992; Rice et al., 1996; Bols et al., 2001). The use of biomarkers in evaluating the sublethal stress in fish is a common practice. A variety of biological and biochemical variables have been used as biomarkers to evaluate the health of fish and several examples illustrating the use of various biomarkers are shown in Table 1. As would be expected, the use of multiple biomarkers is the recommended method of evaluating sublethal stress in a particular ecosystem (Broeg et al., 1999).

Many biomarkers are specific to particular stressors. A number of others including a variety of histological, haematological and physiological biomarkers, are non-specific and can be used to demonstrate the general effects of a stressor on an organism. Histological (e.g., Wolke, 1992; Hinton et al., 1992; Couillard and Hodson, 1996; Blazer et al., 1997; Couillard et al., 1999; Fournie et al., 2001; Agius and Roberts, 2003), haematological (e.g., Barker et al., 1994; Morgan and Iwama, 1996; Hoole, 1997) and general condition indices (e.g., Adams et al., 2003; Billiard and Khan, 2003) have been used in fish to demonstrate the existence of natural and anthropogenic stressors in the environment. Histological biomarkers such as

pigmented macrophage aggregates are more suitable in evaluating chronic exposure to environmental stress whereas elevated levels of hematological parameters indicate exposure to acute stress (Hinton et al., 1992; Morgan and Iwama, 1996; Agius and Roberts, 2003). Condition factor is a biomarker that can be used to assess the nutritional and physiological condition of fish (Busacker et al., 1990; Khan and Payne, 1997; Adams et al., 2003; Billiard and Khan, 2003). However, ecological factors such as competition and predation risk may also affect the condition factor and care is necessary in the interpretation of results.

Histological biomarkers

Toxicant levels in the aquatic environments are often sublethal to the organism and cause little mortality; however, damage may be detected at the tissue or cellular level. This may be manifested as morphological changes in cells and tissues and may include cell mutations (neoplasia) and cell death (Hinton et al., 1992). Many histological biomarkers in vertebrates are well validated experimentally and are useful tools to evaluate past and current exposure to environmental stressors (Hinton, 1992; Myers and Fournie, 2002). The advantage of histology is that it allows the visual localization of an injury to unique cells and tissues. Toxicants that interact with cellular metabolism can lead to biochemical and physiological dysfunction of cells. Changes detected in the cells themselves or lesions in tissues or organs that result from the cumulative effects of physiological and biochemical stressors can therefore be linked to exposure

and subsequent metabolism of chemical contaminants (Couillard et al., 1988; Myers and Fournie, 2002).

Pigmented macrophages and pigmented macrophage aggregates

Pigmented macrophages are involved in a variety of functions including the detoxification and recycling of exogenous and endogenous material, responses to foreign material or infectious agents, and antigen recognition (Aguis, 1979; Payne and Fancy, 1990; Blazer et al., 1997; Agius and Roberts, 2003). Pigmented macrophage aggregates (= melanomacrophage centers of some authors) are focal accumulations of pigmented macrophages found in the spleen, kidney, liver and other organs of fish. They are considered a nonspecific, persistent and generalized tissue response to environmental stress and indicate possible ill health in fish (Blazer et al., 1994; Couillard et al., 1999; Bols et al., 2001). Pigmented macrophage aggregates may respond to toxicants or exposure to infectious agents such as viruses or bacteria either by increasing in number and size or by changing the shape of the aggregation (Kranz, 1989; Wolke, 1992; Bols et al., 2001; Agius and Roberts, 2003). Changes such as increased numbers of pigmented macrophages or pigmented macrophage aggregates (centers) can be used as histological biomarkers to assess fish health including exposure to pollution or disease (Wolke, 1992; Hinton et al., 1992; Blazer et al., 1997).

Hematological biomarkers

The number of circulating blood leukocytes is a useful parameter in accessing fish health, including disease and sublethal effects of pollutants. The leucocrit is an approximate and rapid measurement of white blood cell abundance and can provide an evaluation of fish health. Elevated levels of leucocrit and total white blood cell (WBC) counts indicate exposure to disease (including parasitic infections) or pollution (Morgan and Iwama, 1996; Adams et al., 2003) while reduced levels have been suggested to be effects of chronic stress such as prolonged exposure to certain pollutants (Barker et al., 1994; Hoole, 1997). Elevated levels of blood hemoglobin and red cell number (hematocrit) may reflect either acute or chronic stress. Decreased levels of haemoglobin are suggested to result from exposure to chronic environmental stress, as well as viral and parasitic infections (Sakanari et al., 1984; Barker et al., 1994; Morgan & Iwama, 1996; Adams et al., 2003).

General condition biomarkers

Overall health and condition of fish can be assessed through general condition parameters such as condition factor, liver somatic index, visceral somatic index and health assessment index. Changes in these parameters may indicate exposure to pollutant stress, parasitic infections and other diseases (Adams et al., 2003; Billiard and Khan, 2003).

Pollution in the St. Lawrence River

The Saint Lawrence River extends 1600 km from the Great Lakes to the Atlantic Ocean. The freshwater portion of the river stretches for more than 400 km and nearly five million people live on its shores. Several sources of contaminants exist along the river. Direct discharge from industrial and municipal outfalls, and agricultural and urban runoff are among the major sources of pollutants. The City of Montreal wastewater treatment plant, which discharges partially treated sewage with a flow rate of 20-30 $m^3 s^{-1}$ via a single outfall, represents a major pollutant source to the St. Lawrence River. In addition, pollutants from other rivers such as the Ottawa and the Saguenay as well as from the Great Lakes also flow into the St. Lawrence River (Loiselle et al., 1997; Pham et al. 1999).

A number of toxic organic and inorganic substances occur in the water and sediments of the St. Lawrence River system. Organic substances include polychlorinated biphenyls (PCBs), dioxins and furans, polycyclic aromatic hydrocarbons (PAHs), and pesticides such as chlordane, dieldrin, chlorophenols, DDT and mirex. Some of the toxic inorganic substances present are cadmium, copper, arsenic, lead, chromium, nickel, zinc, and mercury (Loiselle et al., 1997; Quemerais et al., 1996; Pham and Proulx, 1997; Gagnon and Saulnier, 2003; Kwan et al., 2003). Most of these compounds are highly toxic and affect immune, endocrine and reproductive systems of aquatic wildlife as well as humans that consume them (Chambers et al., 1997; Loiselle et al., 1997).

Choice of fish

The spottail shiner, *Notropis hudsonius,* is a small cyprinid fish found in the St. Lawrence River, throughout the Great Lakes and in larger lakes and rivers in Manitoba and Saskatchewan. They feed mainly on plankton, aquatic insect larvae, crustaceans and algae. Spottail shiners living in Canadian waters spawn over sandy shoals in June or July. It is an important forage fish (Scott and Crossman, 1973), is easily identifiable and easy to catch and has been used previously for pollution monitoring in Great Lakes (Suns et al., 1983, 1985, 1993). There are 73 species of parasites recorded from spottail shiners, 33 of which are larval forms (Margolis and Arthur, 1979; McDonald and Margolis, 1995; Hoffman, 1999; Cone et al., 2004; Marcogliese et al., submitted). Appendix 1 provides a list of parasites reported from spottail shiners in North America along with those known to occur in the St. Lawrence River.

General Hypothesis

The St. Lawrence River contains pollutants and toxic substances that can act as stressors in fish. Histological, physiological and haematological alterations in fish tissues such as changes in number of pigmented macrophage aggregates in the spleen and liver, changes in haematocrit, leucocrit (an indirect measure of WBC) and white blood cell counts, and changes in the condition factor are non-specific biomarkers that occur in response to exposure to environmental stressors (Busacker et al., 1990; Hinton et al., 1992; Morgan and Iwama, 1996; Couillard et

al., 1999; Adams et al., 2003; Agius and Roberts, 2003). In addition to anthropogenic contaminants in polluted systems, parasites are ubiquitous natural stressors that occur in virtually all fish species (Sinderman, 1983; Khan and Thulin, 1991). Although there is evidence demonstrating the combined lethal effects of parasites and pollution on fish (Boyce and Yamada 1977; Pascoe and Cram, 1977; Pascoe and Woodworth, 1980; Moles, 1980), little is known about the joint sublethal effects of anthropogenic and natural stressors in fish (Sakanari et al., 1984; Jacobson et al., 2003; Marcogliese et al., 2005). In this study I examine the hypothesis that altered levels of seven non-specific biomarkers reflect the health of fish exposed to two general stressors (pollution and parasitic infections) in the St. Lawrence River.

I compared condition factor, haematocrit, leucocrit, WBC counts, number of spleen pigmented macrophage centers (SPMC), spleen pigmented macrophage (SPM) counts and liver pigmented macrophage (LPM) counts in spottail shiners from polluted and relatively clean localities in the St. Lawrence River. Specifically, I predict that differences should be detectable in these seven biomarkers in fish from polluted localities compared to those from cleaner ones and these changes should be more pronounced in heavily infected fish at each locality.

Materials and methods

Sampling sites

Spottail shiners were collected from five sampling localities in the St. Lawrence River near Montreal (Fig. 1). Selection of the localities was based on previous pollution studies in the St. Lawrence River (Loiselle et al., 1997; Marcogliese et al., submitted). Two localities, Île Beauregard (45°44.965'N; 73°24.910'W) and Îlet Vert (45°42.230'N; 73°27.143'W) lie downstream and two, Île Dorval (45°26.016'N; 73°44.234'W) and Îles de la Paix (45°20.022'N; 73°51.362'W), lie upstream from the City of Montreal wastewater treatment plant outfall. The concentrations of metals and other contaminants are higher downstream of the City of Montreal sewage treatment plant outfall than upstream (Pham et al., 1999; Gagnon and Saulnier, 2003). Île Dorval and Îles de la Paix are comparatively clean and were used as reference localities (Aravindakshan et al., 2004). The fifth locality at Beauharnois (45°19.051'N; 73°53.020'W) is close to mouth of the St. Louis River. This locality is heavily polluted with industrial contaminants such as mercury, copper, arsenic, lead, chromium, nickel and zinc. Mercury levels are particularly high at this locality and exceed the Toxic Effects Threshold, which is the level at which 90% of benthic organisms are believed to be harmed by a specific pollutant (Loiselle et al., 1997).

Fish collection

Spottail shiners were collected from all localities within a 7 day period (June 3-10, 2004) to minimize possible temporal effects on the physiological state of the fish that can influence biomarker measurements (Wolke, 1992; Adams and Ryon, 1994). Fish were captured using a large beach seine (22.6 m X 1.15 m, 3 mm mesh) towed by hand or partially deployed from a boat (Marcogliese and Compagna, 1999). Spottail shiner density varied from moderate to high among the localities. Diversity of other fishes ranged from low to high among the localities. Water temperature and pH measurements were also taken at each collection locality with a VSI model 63 pH meter. Age determination was based on length frequency histograms. Originally, I intended to collect 50 fish with no obvious deformities or lesions from each locality; 25 belonging to age groups 1+ and 2+ respectively. Unfortunately, despite intensive sampling, only two 1+ fish and two 2+ fish were caught at Îles de la Paix and at Île Beauregard, respectively. For this reason data were limited to 4 localities for each age group. Fish were transported live to the laboratory for processing.

Biomarker measurements

Blood

Fish were anesthetized in clove oil (50 mg/L H_2O) as described by de Lafontaine et al. (2001). The fish were measured (fork length, mm) and weighed (g). Blood samples for haematocrit and leucocrit measurements were taken from

each individual in a heparinized microhematocrit tube. Haematocrit and leucocrit measurements require at least 40 µl of blood and unfortunately it was impossible to collect a sufficient volume of blood from 1+ fish to perform these. Accordingly these measurements could only be made in 2+ fish. The blood was centrifuged for 5 minutes at 13,500 g in a standard microhematocrit centrifuge. The volume of packed red cells (haematocrit) was measured using a microhematocrit capillary tube reader. The thickness of the white blood cell layer that separates packed red blood cells from the plasma layer was measured using a dissecting microscope. The leucocrit value was calculated as the percentage of white blood cell volume (buffy coat) relative to the total blood volume (Morgan and Iwama, 1996). To compensate for the lack of haematocrit and leucocrit data from 1+ fish, blood smears were made from each fish in order to estimate the total white blood cell counts (WBC). Blood smears were stained in Wright's solution. The number of WBCs in each of 10 fields was counted on each slide. Each field contained an average of 500 red cells. This value and mean WBC counts were used to estimate the number of WBC per 10,000 red blood cells.

Condition factor

The condition factor (K) (Busacker et al., 1990) was calculated using the following formula:

$$K = \text{Weight (g)} / \text{Length (mm)}^3 \times 10^5$$

The condition factor reflects the physiological and nutritional state of fish but it can also be affected by the population density, competition, food availability, food quality, and predation risk, in addition to exposure to pollutants and / or parasite infection.

Tissue

The liver and spleen of each fish were removed and fixed in 10% phosphate buffered formalin for histological study. The rest of the carcass was placed in an individual bag and frozen at -20^0 C for subsequent parasitological examination. The spleen and liver were embedded in paraffin, sectioned at 6 μm and the sections mounted on slides. Two sections from each block were selected for staining. One was stained with Mayer's hematoxylin and eosin, the other in Periodic Acid Schiff following protocols described in Hinton (1990).

Initially, spleen and liver sections were scanned at 40X on a compound microscope fitted with an ocular grid (Fisher Scientific, Catalog Number 12-561-RG 3). The grid had a 1 mm^2 area and was divided into 100 smaller squares. A sketch of each section was drawn on paper fitted with a series of grid lines corresponding to those on the ocular grid. The number of squares occupied by the section was counted and its area was calculated (each square = 0.0625 mm^2 at 40X). All pigmented macrophage centers in the spleen sections were recorded and the mean number of macrophage centers per mm^2 of spleen tissue was calculated. There were no pigmented macrophage centers present in the liver

sections. To determine the mean number of pigmented macrophages present in spleen and liver sections, five areas of each section were selected at random and examined at 1000X. The total number of pigmented macrophages within the whole grid in each of the five areas was counted and the average was used to estimate mean number of pigmented macrophage per mm^2 of each tissue section (whole grid area = 0.01 mm^2 at 1000X).

Parasites

Individual fish were examined for external and internal parasites using standard parasitological techniques (Marcogliese 2002, http://www.eman-rese.ca/eman/ecotools/protocols/freshwater/parasites). Digeneans, acanthocephalans, cestodes, crustaceans and leeches were preserved in 70% ethanol. Nematodes were preserved in 5% glycerol and 70% ethanol. Except for nematodes, all of the parasites were stained in acetocarmine and mounted in Permount for identification. Nematodes were cleared and mounted in glycerol. Specimens were identified using the following keys: Molnar and Fernando (1974) (coccidians), Arai (1989) (acanthocephalans), Moravec (1994) (nematodes), Gibson (1996) (digeneans) and Hoffman (1999) (cestodes and other parasites). Data were summarized using standard epidemiological measures. Prevalence is defined as the percentage of fish infected with a particular parasite species. The mean abundance is defined as the mean number of parasites of a given species in the host sample which includes both infected and uninfected fish. Mean intensity

is defined as average number of particular parasite species in all infected hosts (Bush et al., 1997).

Statistical analysis

Statistical analysis were performed with NCSS 6.0 (Number Cruncher Statistical Systems, Dr. Jerry L. Hintze, Kaysville, Utah 1995), Minitab (http://www.minitab.com) and KaleidaGraph (Synergy Software, 2457, Perkiomen Ave., Reading, PA). The critical level of significance was set at $p \leq 0.05$, unless otherwise stated.

All dependent variables, i.e., the biomarkers: (WBC counts, hematocrit, leucocrit, condition factor, spleen and liver macrophage counts, number of spleen macrophage centers) and independent variables (parasite counts) were tested for normality. Variables that were not normally distributed were transformed using standard techniques, namely log (X+1), square root and rank transformation (Zar, 1984) and tested again for normality (Appendix 2, 3).

Chi-square tests used to compare prevalence of infection between 1+ and 2+ fish within each locality. Student's t-tests were used to compare physiological parameters and parasite counts between 1+ and 2+ fish within localities. Where variables could not be normalized Mann-Whitney U tests were used.

Chi-square tests were used to compare prevalence in of infection in 1+ and in 2+ fish among localities. Comparison of physiological parameters and parasite counts in 1+ or 2+ fish among localities was performed using Analysis of

Variance. A Tukey-Kramer *a posteriori* test was used where necessary to distinguish among groups. Where variables could not be normalized, the Kruskal-Wallis test, followed by Kruskal-Wallis Z test, was used. A Bonferroni correction was applied where multiple comparisons were made.

Separate MANOVAs were performed to assess the effects of polluted verses non polluted localities (pollution status) on biomarkers and on parasite species (> 2% prevalence) in 1+ and 2+ fish.

Canonical correlation analysis was used to identify relationships between biomarkers and parasite numbers in species whose overall prevalence was 2% or greater. Biomarkers and parasite numbers were plotted separately by locality. Correlations between associated biomarkers and parasite species (determined from CCA) were tested using Pearson correlations for normally distributed variables and Spearman rank correlations for non-normal variables.

To determine if there was an interaction between effects of locality and parasitism on biomarkers, MANOVA was performed on parasite species common enough to obtain comparable sized groups for statistical analysis. Fish were fish into two categories: high and low parasite intensity. The cutoff was determined separately for each parasite by examining its frequency distribution to obtain two similarly sized groups for low and high infection levels. The cutoff in age 1+ fish is as follows: *Plagioporus sinitsini,* 0 and ≥ 1 and *Diplostomum* spp., ≤ 3 and > 3. For 2+ fish the cutoffs were: *Plagioporus sinitsini, 0 and ≥ 1; Diplostomum* spp., ≤ 20 and > 20; and *Ornithodiplostomum ptychocheilus,* 0 and ≥ 1.

Results

Fish collection and sampling localities

Between 20-50 spottail shiners were collected at each locality (Table 2). Samples of 1+ and 2+ fish were collected at three localities; only two 2+ fish were collected at Îles de la Paix and only two 1+ fish were collected at Île Beauregard, respectively. The water temperature and the pH measurements at each locality are also shown in Table 2. The water temperature was lower at Île Beauregard than at the other locations and it was mildly acidic whereas it was alkaline at the other localities.

Biomarkers

Comparisons of white blood cell (WBC) counts, condition factor, spleen pigmented macrophage center (SPMC) counts, spleen pigmented macrophage (SPM) counts and liver pigmented macrophage (LPM) counts for age 1+ and 2+ fish within localities are presented in Table 3. SPM and SPMC counts were significantly higher in 2+ fish than in 1+ fish within the same locality (two sample t-test and Mann-Whitney U test, $p < 0.001$ respectively). Condition factor was significantly higher in 2+ fish at Île Dorval and in 1+ fish at Îlet Vert than in the other cohort (two sample t-test, $p < 0.05$ and $p < 0.001$ respectively). No age-related differences were seen in the WBC and LPM counts (Mann-Whitney U test, $p > 0.05$).

Separate MANOVAs were used to test whether biomarker levels were affected by the pollution status of the locality (polluted versus clean) in 1+ and 2+ fish. The overall MANOVAs were significant (Wilks $\lambda = 0.20$, $p = <0.001$; Wilks $\lambda = 0.64$, $p = < 0.001$, respectively for 1+ and 2+ fish). SPM counts, SPMC counts and condition factor differed significantly between polluted and clean localities in 1+ fish; SPM counts and SPMC counts in 2+ fish differed between polluted and clean localities.

One way ANOVAs were used to test whether biomarkers in 1+ fish differed among localities (Table 4). SPM and SPMC counts were significantly higher at polluted localities than at the reference locality (One-way ANOVA, $p < 0.001$). Condition factor and WBC counts also differed significantly among localities (one-way ANOVA, $p < 0.001$) but these were not related to the pollution status of the locality. There was no significant difference in LPM counts among localities (one-way ANOVA, $p > 0.05$).

Comparison of WBC counts, leucocrit, haematocrit, condition factor, SPM, SPMC and LPM counts for 2+ fish among localities (Table 5) revealed that SPM and SPMC counts in 2+ fish were significantly higher at polluted localities compared to reference localities (one-way ANOVA, $p < 0.001$). WBC counts (one-way ANOVA), leucocrit and LPM counts (Kruskal-Wallis test) also differed significantly among the localities ($p < 0.001$) but were unrelated to pollution status. There was no significant difference in haematocrit or condition factor of 2+ fish among localities (one-way ANOVA, $p = 0.20$ and $p = 0.39$ respectively)

Parasites

Eighteen species of parasites were found in 2+ fish; nine of these were present in the 1+ fish. Overall, nine digenean, two cestode, two nematode, one acanthocephalan, one crustacean, one leech, one myxozoan and one coccidian species were found (Appendix 4). Ten species were present as larval stages; the remainder occurred as adults. Five species were found at all five localities, three species at four localities, four at three localities, three at two localities and three at one locality.

Only five species, *Diplostomum* spp., *Plagioporus sinitsini*, *Posthodiplostomum minimum*, *Ornithodiplostomum ptychocheilus* and *Neoechinorhynchus rutili* occurred with sufficient frequency and numbers within each age group to permit comparison between 1+ and 2+ fish. Except for *N. rutili* infections at Dorval, 2+ fish had heavier infections where significant differences in prevalence and / or abundance occurred (Table 6).

Parasites of 1+ fish included six digeneans (*Diplostomum* spp., *P. sinitsini*, *P. minimum*, *O. ptychocheilus*, *Rhipidocotyle papillosa* and *Centrovarium lobotes*), one acanthocephalan (*N. rutili*), and one myxozoan (*Myxobolus* sp.) and one coccidian (*Eimeria degiustii*) (Table 7). The prevalences of *P. minimum*, *O. ptychocheilus*, *N. rutili* and *Myxobolus* sp. were significantly different among the localities (chi-square test, $p < 0.05$). For species found at two or more localities, the lowest prevalences of *P. minimum* and *Myxobolus* sp. and the highest prevalence of *N.*

rutili were found at the reference locality; differences in the prevalence of the other species were independent of pollution status.

The overall MANOVA comparing the abundances of the species found in 1+ fish was significant (Wilks $\lambda = 0.86$, $p = <0.05$) and indicated that the abundances of *P. minimum* and *O. ptychocheilus* differed significantly between the reference locality and the polluted localities. Subsequent analyses revealed that the differences observed in these species and those of *Diplostomum* spp. and *N. rutilii* varied among localities (one-way ANOVA or Kruskal-Wallis tests $p < 0.001$) were not related to the pollution status of the locality (Table 7).

Parasites of 2+ fish included nine digeneans (*Diplostomum* spp., *P. sinitsini, P. minimum, O. ptychocheilus, R. papillosa, C. lobotes, Azigia* sp., *Ichthyocotylurus platycephalus, Apatemon gracilis*), two cestodes (*Triaenophorus nodulosus, Proteocephalus* sp.), two nematodes (*Rhapidascaris acus, Hysterothylacium* sp.), one acanthocephalan (*N. rutili*), one crustacean (*Argulus* sp.), one leech (*Myzobdella lugubris*), one myxozoan (*Myxobolus* sp.) and one coccidian (*E. degiustii*) (Table 8). The prevalence of *P. minimum, Azigia* sp., *A. gracilis, R. papillosa, C. lobotes, N. rutili, T. nodulosus, R. acus, Hysterothylacium* sp., and *E. degiustii* differed significantly among localities (chi-square test, $p < 0.05$). Only the prevalences of *P. minimum, A. gracilis, Hysterothylacium* sp. and *E. degiustii* were higher at contaminated localities than at the reference localities; differences in the prevalence of the other species were independent of pollution status of locality.

The overall MANOVA comparing the abundances of the species found in 2+ fish was significant (Wilks $\lambda = 0.55$, $p = <0.001$) but only two species of parasites, *P. minimum* and *Hysterothylacium* sp., differed significantly between polluted and clean localities. Subsequent one-way ANOVAs failed to confirm the differences in the abundance of *Hysterothylacium* sp. but did detect differences in the abundance of *Diplostomum* spp., *Azygia* sp., *N. rutili, T. nodulosus* and in the density of *E. degiustii* (Kruskal-Wallis test, $p < 0.001$), but the differences were not related to the pollution status of the locality. *Centrovarium lobotes* was found only at the reference localities.

Canonical correlation (CCA) of biomarkers and parasite abundance

Canonical correlation was used to explore the relationship between biomarkers and abundance of those parasite species with a total prevalence ≥ 2%. In 1+ fish, five biomarkers and seven species of parasites were used in the canonical correlations. The first two pairs of canonical variates were significant (Table 9, $p < 0.0001$ and $= 0.001$ respectively). Correlations and standardized canonical coefficients of the first two pairs of canonical variates are shown in Table 10. For purposes on interpretation, only variables with correlations of 0.3 or greater were considered. In 1+ fish, four of five biomarkers and six of seven parasite species were correlated with the first canonical variate. The results indicate that fish with elevated SPM, SPMC and LPM counts tended to be associated with higher infection levels of *P. sinitsini, P. minimum, O. ptychocheilus,*

R. papillosa, N. rutili and *E. degiustii,* whereas these parasites were associated with reduced condition factor scores. WBC counts and condition factor scores had the highest correlation with the second canonical variate; correlations between SPM and SPMC with this variable were lower. Two parasite species *O. ptychocheilus* and *E. degiustii* were positively correlated with the second canonical variate; two others *P. sinitsini* and *N. rutili* had negative correlations. Taken together, these results suggest that high pigmented macrophage counts, particularly in the spleen (cells and centers) and in liver tissue are associated with increased levels of parasite infection whereas condition factor scores are negatively affected by infection levels of *P. sinitsini* and *N. rutili,* parasites that are found in the gall bladder and intestine respectively.

Seven biomarkers and 14 species of parasites (≥ 2% total prevalence) were used in the canonical correlations in 2+ fish (Tables 11 and 12). Only the first pair of canonical variables was significant (Table 11, $p = 0.01$). Correlations and standardized canonical coefficients for the first pair of canonical variables of 2+ fish are shown in Table 12. Few correlations were observed in 2+ fish. With a cutoff correlation of 0.3, only three biomarkers (SPM, SPMC and LPM counts) and two species of parasites (*Hysterothylacium* sp. and *E. degiustii*) were correlated with the first pair of canonical variables.

Correlations between biomarkers and parasites abundances

The associations detected in the canonical correlation analysis were subjected to further correlation analyses (Pearson for 1+ fish; Spearman for 2+ fish) to determine which effects were related to pollution status. Correlations from 1+ fish are shown in Table 13. Only five species identified in the canonical analysis were examined; *R. papillosa,* found only found at a single locality, was excluded. Few correlations were found at the reference locality and there was no discernable pattern among localities. Over half of the correlations observed involved SPM and / or SPMC counts. The greatest number of correlations involved *E. degiustii* which was positively correlated with SPM and SPMC counts at the reference locality and at two of the three contaminated localities. Positive correlations between *P. sinitsini* and SPM counts and negative correlations between this species and condition factor scores were found at two contaminated localities. Positive correlations were also found between *P. minimum,* SPMC counts and LPM counts at contaminated localities. There was a positive correlation between *O. ptychochelius* and WBC counts at one contaminated site. Negative correlations were found between *N. rutili* and condition factor scores at the reference locality and one contaminated locality.

Comparisons for 2+ fish are shown in Table 14. *E. degiustii* was positively correlated with SPM and SPMC counts in one reference and one contaminated locality. Although a correlation was found between *Hysterothylacium* sp. and

various biomarkers in the canonical correlation analysis, no significant correlations were found between this parasite and any biomarker at any locality.

Despite all of the correlations observed, only the effects of *P. sinitsini,* in 1+ fish seem to be related to pollution. This parasite was positively correlated with SPM counts (Fig 2) and negatively correlated with condition factor (Fig. 3) at two of the three contaminated localities studied.

Interactive effects of pollution and parasitism on biomarkers

Separate MANOVAs were performed to assess biomarker levels in relation to the infection status of *P. sinitsini* and *Diplostomum* spp. in 1+ fish (Table 15) and *P. sinitsini, Diplostomum* spp. and *O. ptychocheilus* in 2+ fish (Table 16). In age 1+ fish, MANOVA on WBC counts was significant for locality ($p < 0.05$) in *P. sinitsini* and *Diplostomum* spp. infections but infection status and the interaction between locality and infection status were not significant for either parasite species. Infection status ($p < 0.001$), but not locality, was significant for condition factor in *P. sinitsini,* as was the interaction between infection status and locality ($p = 0.01$). Only locality was significant for condition factor in *Diplostomum* spp. ($p = 0.01$). MANOVA on SPM counts was significant for locality ($p < 0.001$) in *Diplostomum* spp. and *P. sinitsini,* but not for infection status or for the interaction. Locality only was significant for SPMC counts in *P. sinitsini.* There was no significant variation in LPM counts for any of the parameters (Table 15).

For the 2+ fish, MANOVA on SPM and SPMC counts were significant for locality ($p < 0.01$) in *Diplostomum* spp. and *P. sinitsini.* But neither the infection status nor the interaction between the infection status and locality was significant for either species. WBC counts were significant for both locality ($p = 0.01$) and infection status ($p < 0.001$) in *Diplostomum* spp., but not for the interaction between the two. SPM and SPMC counts were not significant for locality or infection status for *O. ptychocheilus,* but the interaction between two was significant ($p < 0.001$ and $p = 0.03$ respectively). There was no significant variation in LPM counts for any of the parameters (Table 16).

Discussion

Biomarkers and pollution

Of the seven biomarkers measured, only SPM and SPMC counts in 1+ and 2+ spottail shiners appeared to be affected by pollution. Counts were significantly higher at polluted localities than at reference localities. Previous studies also show that numbers of pigmented macrophage centres are good biomarkers of pollution. Higher numbers of pigmented macrophage centers occurred in the spleen and kidney of fish collected from contaminated localities or those experimentally exposed to pollutants compared to fish from reference localities or control fish (Hinton et al., 1992; Wolke, 1992; Blazer et al., 1994; Agius and Roberts, 2003). Examples are summarized in Table 17. Although SPM and SPMC counts are suitable biomarkers in 2+ fish, only SPM counts are suitable in 1+ fish, given their low SPMC counts. While SPMCs have been quantified previously in fish (Blazer et al., 1987; Khan, 1991; Couillard and Hodson, 1996; Fournie et al., 2001), this is the first study to quantify SPM counts. My results suggest that SPM counts are useful biomarkers; SPMC counts are useful biomarkers in 2+ fish but are of limited use in 1+ fish due to their low numbers.

Previous work has shown that the number of macrophage centres increases with age in the liver and spleen of rainbow trout (Agius, 1981), largemouth bass (Blazer et al., 1987) and white sucker (Couillard and Hodson, 1996). The results from spottail shiners are consistent with these studies and age

effects must be considered when using biomarkers in fish (Agius, 1981; Blazer et al., 1987).

Numbers of pigmented macrophage centres also vary depending on tissue and species of host (Blazer et al., 1994; Wolke, 1992; Agius and Roberts, 2003). Unlike the spleen, no pigmented macrophage centres were observed in the liver of spottail shiners. Moreover, LPM counts were lower than SPM counts in both age groups, and did not vary with the pollution status of the localities. Other studies also demonstrate that fewer macrophage centers occur in the liver than in the spleen of mummichog (*Fundulus heteroclitus*), northern pike (*Esox lucius*) and white sucker (Blazer et al., 1987; Meinelt et al., 1997; Couillard et al., 1999) and are not affected by pollution (Couillard et al., 1999). Results obtained from spottail shiners in this study are constant with those of other studies and it does not appear that LPM counts are a useful biomarkers of pollution in this species.

Condition factor is directly associated with the nutritional state of fish (Busacker et al., 1990) and is relatively insensitive to small changes in body metabolism (Adams and McLean, 1985). The results with condition factor were inconsistent, varying among localities in 1+ fish, but not 2+ fish. Among 1+ fish, those from Beauharnois (polluted locality) had a higher mean condition factor compared to those from Île Dorval (reference locality). The Beauharnois site is located at the mouth of the St. Louis River which is polluted with agricultural and industrial waste (Loiselle et al., 1997) that likely augments nutrient availability, leading to increased feeding rate and a higher condition factor

(Adams et al., 1992; Gagnon et al., 1995). Studies by Adams and McLean (1985), Khan and Payne (1997) and Barton et al. (2002) of other fish species suggest that condition factor may not be a good biomarker to assess pollution stress in fish. Barker et al. (1994) found significantly lower condition factor values in winter flounder captured near a pulp and paper mill outfall compared to those from a reference locality, whereas Khan and Payne (1997) found just opposite from the same species exposed to similar kinds of pollutants. Adams et al. (2003) reported no differences in condition factor of southern flounder and spot living in polluted and reference localities.

Blood parameters also did not vary with pollution status, although significant differences were detected among localities for WBC counts in both age classes and for leucocrit in 2+ fish. Exposure to acute stress and disease may lead to elevated blood parameters (Morgan & Iwama, 1996; Adams et al., 2003), while chronic stress results in lower values (Sakanari et al., 1984; Barker et al., 1994; Hoole, 1997; Taylor and Hoole, 1989). In contrast, other studies found no changes in blood hemoglobin levels, red cell numbers and haematocrit values of fish exposed to acute or chronic stress (reviewed by Barton et al., 2002). These results indicate that blood parameters examined in spottail shiners do not appear to be good indicators of pollution.

Whereas condition factor and blood parameters varied inconsistently among localities, SPM and SPMC counts which reflected the pollution status of the localities in this study are good biomarkers to evaluate pollution stress in

these fish. In addition, these fish are considered to consist of local populations (Marcogliese et al., submitted) that reflect local environmental conditions. Therefore, SPM counts in spottail shiners are useful biomarkers with which to evaluate water quality in field studies in the Great Lakes and St. Lawrence River basin.

Biomarkers and Parasites

General biomarkers of fish health will be affected by parasites and disease as well as by pollution. Indeed, certain biomarkers are believed to respond to pathogens (Snieszko, 1974; Sindermann, 1983; Moller, 1987; Khan and Thulin, 1991; Overstreet, 1997; Marcogliese, 2005). Nevertheless few studies have examined the effects of parasites on general biomarkers used in water quality evaluations (Sakanari et al., 1984; Billiard and Khan, 2003; Adams et al., 2003; Marcogliese at al., 2005).

The first canonical correlation coefficients for 1+ fish confirm the positive relationship between the abundance of certain parasites and stress biomarkers. Previous studies also demonstrate that parasites have effects on certain biomarkers including pigmented macrophage aggregates, liver index and blood parameters (Buchmann, 1986; Vogelbein et al., 1987; Jeney et al., 2002; Dezfuli et al., 2005).

Significant correlations were observed between SPM and/or SPMC counts and the number of *E. degiustii* (all localities), *P. sinitsini* (three localities), *P.*

minimum (two localities) and *Diplostomum* spp. (one locality) in 1+ and 2+ fish. Pigmented macrophage aggregates (centres) are believed to occur in response to disease and parasitism (Agius and Roberts, 2003). Mangrove rivulus (*Rivulus marmoratus*) experimentally exposed to the parasite *Calyptospora funduli* had more hepatic pigmented macrophage centres than uninfected fish (Vogelbein et al., 1987). Taylor and Hoole (1989) observed more frequent distribution of pigmented macrophage centers and pigmented macrophages in the spleen of roach and gudgeon infected with plerocercoids of *Ligula intestinalis* compared to uninfected fish. Overstreet and Thulin (1989) found a higher occurrence of pigmented macrophage centers and pigmented macrophages in the heart of leopard coralgrouper (*Plectropomus leopardus*) infected with the digenean *Pearsonellum corventum* than in uninfected fish. Recently, Dezfuli et al. (2005) demonstrated that aggregations of pigmented macrophages surround cysts of the trematode *Ichthyocotylurus erraticus* in the heart of powan (*Coregonus lavaretus*). These examples indicate that parasitic infections may lead to an increase in the number of pigmented macrophages and to the formation of pigmented macrophages centres in various tissues of fish. Significant positive relationships between numbers of *P. sinitsini, P. minimum Diplostomum* spp. and *E. degiustii,* and SPM and/or SPMC counts in individual spottail shiners in certain localities further demonstrate that metazoan parasites can affect the expression of these biomarkers.

Eimeria degiustii contributed to the most significant relationships with SPM and SPMC counts in both age groups among various locations. Correlations of *E. degiustii* with biomarkers were independent of the pollution status of localities. Species of *Eimeria* are host and site specific coccidian parasites that occur in various tissues of fish (Molnar, 1995; Hoffman, 1999). Coccidian infections cause pathological effects in the spleen tissue of fish and are surrounded by pigmented macrophage cells (Molnar, 1995). Given the high SPM and SPMC counts in the spleen, *E. degiustii* is potentially pathological in spottail shiners.

Condition factor was negatively correlated with the numbers of *N. rutili* and *P. sinitsini* in 1+fish. Previous studies demonstrate negative correlations between acanthocephalan infections and body lipid in trout (*Salmo trutta*) (Bristol et al., 1984) and liver index in Baltic cod (*Gadus morhua*) (Buchmann, 1986), but attempts to relate acanthocephalan infections to the condition factor of fishes have been unsuccessful (Miller, 1995). However, the relatively large size of *N. rutili* in spottail shiners may lead to detrimental effects on fish condition at high intensities at both polluted and clean localities. This parasite, which absorbs nutrients from intestinal contents of its host, may compete for nutrients with the fish. In addition, the armed proboscis may cause intestinal pathology (Post, 1987; Schaperclaus, 1991). Thus, the negative relationship between the condition factor and the number of *N. rutili* in the fish may be attributed to the high nutrient demand of the acanthocephalan and its pathological effects on the host. *Plagioporus sinitsini* occurs in the gall bladder. Effects on condition factor in 1+

fish at certain localities suggest that this parasite is pathogenic at least in some circumstances. However there are no other studies that demonstrate pathological effects of *P. sinitsini* in fish. In other host parasite system, extraintestinal trematodes are potentially pathogenic in fish (Paperna, 1995).

Significant correlations were observed between haematological biomarkers and *O. ptychocheilus* and *E. degiustii* in 1+ fish. Results from other studies are inconsistent. Some found that parasites lowered blood parameters in fish (Murad and Mustafa, 1998; Jeney et al., 2002), while others showed no effects (Palikova and Navratil, 2001). The limited and inconsistent results of this study suggest that the blood parameters measured herein are poor indicators of sublethal effects of parasitic infections in spottail shiners.

The relationship between parasites and biomarkers is more prominent in 1+than in 2+fish. The stressing effects of parasitic infections as revealed by biomarkers may be neutralized once the stress has been terminated or after the organism has been exposed continuously for long periods of time (Barton, 1996). Thus, pathological effects of parasites may be attenuated in 2+ fish once they have adjusted or adapted to the presence of parasites. Young fish may also be more vulnerable to pathological effects compared to older fish. Alternatively, highly stressed 1+ fish may have been eliminated from the population before they reach their second year. These results further support the use of 1+ spottail shiners as bioindicators of fish health, as they appear to be more sensitive to effects of pollution and parasitism than older fish.

Parasite and pollution effects

Combined effects of parasites and pollution status were detected with certain biomarkers. The first canonical correlation coefficients for 1+ fish indicate the overall negative effect of parasite infection and stress on fish condition. Although *E. deguistii* and *P.sinitsini* accounted for most significant correlations with biomarkers in polluted localities in 1+ fish, only those of *P. sinitsini* were restricted to polluted localities. Unfortunately, there is no literature available on the impact of *P. sinitsini* on fish health. These findings demonstrate there are pathological impacts on spottail shiners, but only in polluted localities suggesting that the effects of this parasite may be manifested in fish already subjected to stress from contaminants. This parasite is abundant at all localities in the St. Lawrence River and may be detrimental to the fish population at impacted localities in the river.

To date, few other studies have examined the interaction between parasites and pollution in fish. Previous studies show that the combined stress of parasites and pollution are more lethal than either stressor alone (Boyce and Yamada, 1977; Pascoe and Cram, 1977; Moles, 1980; Pascoe and Woodworth, 1980; Jacobson et al., 2003; Gheorgiu et al., 2005). Still fewer studies have been examined the sublethal effects of pollution and parasites in fish. Combined sublethal effects of larval nematodes (*Anisakis* sp.) and pollutants (zinc and benzene) in striped bass caused lower haematocrit values than in fish exposed to single stressors alone (Sakanari et al., 1984). Khan (1987) demonstrated that

Atlantic cod (*Gadus morhua*) exposed to combined chronic stress (continuous exposure to petroleum hydrocarbons) and infected with the blood protozoan, *Trypanosoma murmanensis,* had lower condition factor scores than fish exposed to individual stressors alone. Immune activity of juvenile Chinook salmon was more depressed when jointly exposed to parasitic infection (*N. salmonicola*) and Aroclor 1254 than with either stressor alone (Jacobson et al., 2003). Higher levels of lipid peroxidation were observed in yellow perch caught in a polluted locality than those from a clean locality and this effect was more pronounced in fish infected with *R. acus* and *A. brevis* (Digenea) at the polluted site (Marcogliese et al., 2005). This study contributes to a limited but growing body of literature which demonstrates that parasites and pollutants together affect fish health more than either stressor alone. Interestingly, *P. sinitsini* does not appear to significantly affect fish health in the absence of pollution. Similarly Marcogliese et al. (2005) found that *R. acus* only affected oxidative stress in perch at a polluted locality, while *A. brevis* affected fish at both polluted and reference localities, though the effects were enhanced at the polluted site.

Ecological considerations

All species of parasites found (18) were present in 2+ fish while nine species were present in 1+ fish. Two species, *Azigia* sp. and *E. degiustii*, have not been reported previously in spottail shiners (Margolis and Arthur, 1979; McDonald and Margolis, 1995; Hoffman, 1999; Marcogliese et al., submitted).

Prevalence and/or abundance of five parasite species in 1+ fish and 11 species in 2+ fish were significantly different among localities but none were related to the pollution status of the localities, except for the abundance of *P. minimum* in 2+ fish. This may simply be due to chance, as there no reason to expect *P. minimum* to be more abundant at polluted localities than at reference localities. Indeed, this pattern was not observed in 1+ fish.

Many studies have examined the effects of pollution on fish parasites. Some suggest pollution increases abundance of certain parasites (Valtonen et al., 1994; Yeomans et al., 1997; Jeney et al., 2002; Khan, 2004), while others report decreases (Cone et al., 1993; Marcogliese and Cone, 1996) or no change (Thulin et al., 1988). However, abundance of parasites may not always be a suitable indicator of pollution. Parasite abundance may not be greatly affected when environmental impacts are limited or pollution levels are low (reviewed in Moller, 1987; Khan and Thulin, 1991; Poulin, 1992; Marcogliese, 2004, 2005). In a three year study of the effects of municipal effluents on parasites on spottail shiners, Marcogliese et al. (submitted) found no definitive effects on parasite populations or communities. The localities downstream of the Montreal sewage outflow are exposed to only moderate levels of PCBs, PAHs and heavy metals (Pham et al., 1999; Gagnon and Saulnier, 2003; Kwan et al., 2003). The degree of contamination may be insufficient to impact significantly on parasite abundance and richness (Marcogliese et al. submitted).

Diplostomum spp. is the most common parasite species in both age groups at all localities. Prevalence and /or abundance of this parasite was higher at Île Dorval, Îles de la Paix and Beauharnois, which are located in Lake St. Louis, than at other sites. This observation is in agreement with previous studies where the distribution of this parasite is related to the abundance and distribution of definitive (gull) and first intermediate (snail) hosts (Marcogliese et al., 2001a, 2001b). *Centrovarium lobotes* is found only at Île Dorval and Îles de la Paix (reference localities) in both age groups. Piscivorous fish that are required to complete the life cycle are abundant in the Lake St. Louis (Marcogliese et al., submitted), perhaps explaining this restricted distribution.

High abundance of *N. rutili* in Lake St. Louis is noteworthy. Many studies found a reduced abundance of acanthocephalans in polluted localities (Kussat, 1969; Barker et al., 1994; Khan and Payne, 1997; Billiard and Khan, 2003) and Lafferty (1997) suggested that acanthocephalans may be good indicators of pollution. However, *N. rutili* was abundant at both a polluted and a reference locality, suggesting that pollution does not affect the occurrence of this particular acanthocephalan. Therefore the generalization that acanthocephalan populations are good pollution indicators does not appear to be correct in this case.

From this study, it can be concluded that some general biomarkers of fish health are a useful means to examine sublethal effects of pollution and certain parasites in fish. Increased SPM and SPMC counts in spottail shiners are good biomarkers of exposure to pollutants. Results also suggest that 1+ spottail shiners

are suitable bioindicators to evaluate the impact of pollution in fish. Moreover, one parasite, *P. sinitsini*, had an impact on biomarkers, but only at some polluted localities. These results further substantiate other studies that demonstrate combined sublethal effects of parasites and pollutants on fish. More importantly, it suggests that parasites must be considered in studies that evaluate the sublethal effects of pollution on biomarkers of aquatic animal health. In addition, given that all fish species are infected with parasites, fish health is further compromised under polluted conditions as result of the combined effect of parasitism and pollution.

Table 1: Examples of various kinds of biomarkers used to study the effects of pollution and other stressors in fishes.

Name of biomarker	Examples	Reference
1. Serum enzyme levels	Increased serum enzyme levels e.g. cytochrome P450 and stress proteins	Schlenk and Giulio, 2002
2. Hematological parameters -blood cell volumes	Increased levels of leucocrit and hematocrit	Murad and Mustafa, 1988; Morgan and Iwama, 1996
3. Hepatic biomarkers	Hepatocelluler necrosis, bile duct hyperplasia, hepatomegaly	Hinton et al., 1992; Myers and Fournie, 2002
4. Pigmented macrophage aggregates	Increased levels of macrophage aggregates in internal organs	Wolke, 1992; Hinton et al., 1992; Blazer et al., 1997; Agius and Roberts, 2003
5. Physiological and reproductive indices	Lower condition factor, delayed spawing, fecundity, follicular atrasia of oocytes or ovary, abnormal hepatosomatic and gonadosomatic index	Barker et al, 1994; Adams et al., 2003; Patino et al., 2003
6. Morphological deformities	Scale disorientation, mouth/lip protrusion, deformities of eyes, gills, fins and skeleton	Sun et al., 1998

Table 2: Summary of general pollution status, water parameters and spottail shiner (*Notropis hudsonius*) collection data from the five sampling localities in the St. Lawrence River in June, 2004.

Locality	Pollution status	Temperature (C^0)	pH	Number of Age 1+	Number of Age 2+	Total fish
Île Dorval	Clean	17.0	8.3	30	18	50
Îles de la Paix	Clean	17.2	8.8	02	38	40
Îlet Vert	Polluted	18.5	8.4	35	15	50
Île Beauregard	Polluted	14.5	6.6	18	02	20
Beauharnois	Polluted	16.0	8.4	27	21	48

Table 3: Comparison of mean (± SD) of white blood cell (WBC) counts, condition factor, spleen pigmented macrophage (SPM) counts, liver pigmented macrophage (LPM) counts and spleen pigmented macrophage center (SPMC) counts in 1+ and 2+ spottail shiners (*Notropis hudsonius*) collected at each of three localities in St. Lawrence River in June, 2004.

Locality	Île Dorval (Clean)		Beauharnois (polluted)		Îlet Vert (Polluted)	
Biomarkers	Age 1+	Age 2+	Age 1+	Age 2+	Age 1+	Age 2+
	N = 30	N = 18	N = 27	N = 21	N = 35	N = 15
WBC counts	64.5 ± 45.8	54.0 ± 28.4	50.6 ± 26.1	53.0 ± 32.4	85.3 ± 40.4	95.6 ± 47.3
Condition factor	0.90 ± 0.11 [a]	0.97 ± 0.10 [b]	1.03 ± 0.18	1.01 ± 0.13	1.06 ± <0.01 [a]	0.96 ± <0.01 [b]
SPM counts	51.4 ± 15.2 [a]	102.4 ± 19.6 [b]	133.2 ± 35.7 [a]	198.5 ± 28.4 [b]	112.5 ± 23.6 [a]	193.4 ± 15.6 [b]
LPM counts	8.9 ± 4.6	8.3 ± 3.5	8.9 ± 2.6	8.5 ± 2.7	7.7 ± 4.0	7.0 ± 1.7
SPMC counts	<0.01 ± 0.1 [a]	2.1 ± 0.5 [b]	0.15 ± 0.4 [a]	6.9 ± 3.1 [b]	0.14 ± 0.4 [a]	4.0 ± 1.7 [b]

Differences between age groups were tested using two sample t-test or Mann-Whitney U test and superscripts indicates a significant differences between the age group within the locality.

Table 4: Comparison of mean (± SD) white blood cell (WBC) counts, condition factor, spleen pigmented macrophage (SPM) counts, SPM center (SPMC) counts and liver pigmented macrophage (LPM) counts of age 1+ spottail shiners (*Notropis hudsonius*) collected from four localities along the St. Lawrence River in June, 2004.

Localities	Île Dorval	Île Beauregard	Îlet Vert	Beauharnois
Biomarkers	(Clean)	(Polluted)	(Polluted)	(Polluted)
	N = 30	N = 18	N = 35	N = 27
WBC counts	64.5 ± 45.1 [a]	78.2 ± 60.5 [b]	85.3 ± 40.5 [c]	50.6 ± 26.1 [a]
Condition factor	0.90 ± 0.11 [a]	0.85 ± 0.13 [a]	1.06 ± <0.01 [b]	1.03 ± 0.18 [b]
SPM counts	51.4 ± 15.2 [a]	160.8 ± 27.1 [b]	112.5 ± 23.6 [b]	133.2 ± 35.7 [b]
SPMC counts	<0.01 ± 0.2 [a]	0.54 ± 0.7 [b]	0.14 ± 0.5 [b]	0.15 ± 2.9 [b]
LPM counts	8.9 ± 4.7	7.3 ± 2.9	7.7 ± 4.0	8.9 ± 2.6

Superscripts indicate significant differences; One-way ANOVA or Kruskal-Wallis test.

Table 5: Comparison of mean (± SD) white blood cell (WBC) counts, hematocrit, leucocrit, condition factor, spleen pigmented macrophages (SPM) count, SPM center (SPMC) counts and liver pigmented macrophages (LPM) counts of 2+ spottail shiners (*Notropis hudsonius*) collected from four localities of St. Lawrence River in June, 2004.

Localities Biomarkers	Île Dorval (Clean) N = 18	Île de la Paix (Clean) N = 38	Îlet Vert (Polluted) N = 15	Beauharnois (Polluted) N = 21
WBC counts	54.0 ± 28.8 [a]	70.9 ± 30.2 [b]	95.6 ± 43.8 [c]	54.2 ± 32.8 [a]
Haematocrit	49.3 ± 5.7	52.0 ± 7.4	46.2 ± 1.5	49.3 ± 7.3
Leucocrit	0.6 ± 0.6 [a]	0.8 ± 0.7 [b]	0.6 ± 0.1 [a]	1.0 ± 0.9 [c]
Condition factor	0.97 ± 0.11	1.01 ± <0.01	0.96 ± <0.01	1.00 ± 0.13
SPM counts	102.4 ± 19.6 [a]	95.9 ± 20.3 [a]	193.3 ± 15.6 [b]	203.6 ± 17.5 [b]
SPMC counts	2.1 ± 0.5 [a]	2.4 ± 0.8 [a]	3.9 ± 1.7 [b]	6.9 ± 3.1 [b]
LPM counts	8.3 ± 3.1 [a]	5.3 ± 1.6 [b]	7.1 ± 1.7 [a]	8.5 ± 2.7 [a]

Superscripts indicate significant differences; One-way ANOVA or Kruskal-Wallis test.

Table 6: Comparison between age groups of prevalence and abundance (mean ± SD) of the most common parasite species in spottail shiners (*Notropis hudsonius*) collected from three localities of St. Lawrence River in June, 2004.

Locality	Île Dorval (Clean)		Beauharnois (Polluted)		Îlet Vert (Polluted)	
Parasite species	Age 1+ N = 30	Age 2+ N = 18	Age 1+ N = 27	Age 2+ N = 21	Age 1+ N = 35	Age 2+ N = 15
Diplostomum spp.						
Prevalence	93	100	85	100	69 [a]	93 [b]
Abundance	4.7 ± 3.0 [a]	27.3 ± 16.4 [b]	7.5 ± 6.5 [a]	27.9 ± 17.2 [b]	3.6 ± 3.6 [a]	8.4 ± 6.1 [b]
Ornithodiplostomum ptychocheilus						
Prevalence	0 [a]	39 [b]	19 [a]	48 [b]	23 [a]	47 [b]
Abundance	0 [a]	0.5 ± 0.8 [b]	0.3 ± 0.7 [a]	0.7 ± 0.7 [b]	0.6 ± 1.6	1.4 ± 2.3
Plagioporus sinitsini						
Prevalence	47	44	37	38	37	47
Abundance	2.3 ± 3.2	5.9 ± 11.6	3.1 ± 4.7	4.1 ± 6.8	2.7 ± 4.8	9.7 ± 12.8
Posthodiplostomum minimum						
Prevalence	3 [a]	17 [b]	11 [a]	57 [b]	26 [a]	60 [b]
Abundance	0.2 ± 0.9	0.2 ± 0.4	0.5 ± 1.3 [a]	2.0 ± 4.9 [b]	0.7 ± 1.4	0.9 ± 0.9
Neoechinorhynchus rutili						
Prevalence	17 [a]	6 [b]	52	52	0	0
Abundance	0.3 ± 0.7	<0.01 ± 0.2	2.3 ± 3.5	2.2 ± 2.4	0	0

Superscripts indicate significant differences between the two age groups; Chi-square test or two sample t-test or Mann-Whitney U test.

Table 7: Comparisons of prevalence and mean abundance (± SD) of parasite species in 1+ spottail shiners (*Notropis hudsonius*) collected at four localities in the St. Lawrence River in June, 2004.

Locality	Île Dorval (Clean)	Île Beauregard (Polluted)	Îlet Vert (Polluted)	Beauharnois (Polluted)
Parasite species	**N = 30**	**N = 18**	**N = 35**	**N = 27**
Diplostomum spp.				
Prevalence	93	94	69	85
Abundance	4.7 ± 3.1[a]	4.7 ± 2.3 [a]	3.6 ± 3.6 [b]	7.4 ± 6.5 [c]
Plagioporus sinitsini				
Prevalence	47	56	37	37
Abundance	2.3 ± 3.0	5.9 ± 7.5	2.7 ± 4.8	3.1 ± 4.7
Posthodiplostomum minimum				
Prevalence	3 [a]	50 [d]	26 [c]	11 [b]
Abundance	0.2 ± 0.9 [a]	1.4 ± 2.6 [b]	0.7 ± 1.4 [a]	0.5 ± 1.4 [a]
Ornithodiplostomum ptychocheilus				
Prevalence	0	44 [a]	23 [c]	19 [b]
Abundance	0	1.2 ± 1.7 [a]	0.6 ± 1.6 [b]	0.3 ± 0.7 [b]

Table 7 continued

Rhipidocotyle papillosa				
Prevalence	0	39	0	0
Abundance	0	0.9 ± 1.4	0	0
Centrovarium lobotes				
Prevalence	7	0	0	0
Abundance	0.1 ± 0.4	0	0	0
Neoechinorhynchus rutili				
Prevalence	17 [b]	6 [a]	0	52 [c]
Abundance	0.3 ± 0.7 [a]	0.0 ± 0.2 [b]	0	2.3 ± 3.5 [c]
Myxobolus sp.				
Prevalence	3 [a]	17 [b]	0	0
Eimeria degiustii				
Prevalence	10	6	9	7
Density	11.1 ± 35.0	2.8 ± 11.8	7.9 ± 25.9	11.3 ± 41.6

Values with no or the same superscript do not differ significantly; Chi-square test or One-way ANOVA or Kruskal-Wallis test.

Table 8: Comparison of prevalence and mean abundance (± SD) of parasite species in 2+ spottail shiners (*Notropis hudsonius*) collected at four localities in the St. Lawrence River in June, 2004.

Locality Parasite species	Île Dorval (Clean) N = 18	Îles de la Paix (Clean) N = 38	Îlet Vert (Polluted) N = 15	Beauharnois (Polluted) N = 21
Diplostomum spp.				
Prevalence	100	100	93	100
Abundance	27.3 ± 16.4[b]	30.7 ± 16.4 [b]	8.3 ± 6.1 [a]	27.9 ± 17.2 [b]
Ornithodiplostomum ptychocheilus				
Prevalence	39	37	47	38
Abundance	0.5 ± 0.8	0.5 ± 0.7	1.3 ± 2.26	0.6 ± 0.7
Plagioporus sinitsini				
Prevalence	44	26	47	38
Abundance	5.9 ± 11.5	3.0 ± 5.7	9.7 ± 12.8	4.1 ± 6.8
Posthodiplostomum minimum				
Prevalence	17 [a]	18 [a]	60 [b]	57 [b]
Abundance	0.2 ± 0.4 [a]	0.3 ± 0.9 [a]	0.9 ± 0.2 [b]	2.0 ± 4.9 [b]
Apatemon gracilis				
Prevalence	11 [b]	5 [a]	20 [c]	0
Abundance	1.9 ± 7.8	<0.1 ± 0.2	0.9 ± 2.8	0

Table 8 continued

Azigia sp.				
Prevalence	22 [b]	5 [a]	0	19 [b]
Abundance	0.2 ± 0.4	0.1 ± 0.6	0	0.2 ± 0.5
Ichthyocotylurus platycephalus				
Prevalence	17	18	0	14
Abundance	0.6 ± 1.7	0.3 ± 0.6	0	0.6 ± 1.7
Rhipidocotyle papillosa				
Prevalence	0	5 [a]	20 [b]	5 [a]
Abundance	0	<0.1 ± 0.2	0.3 ± 0.6	<0.1 ± 0.2
Centrovarium lobotes				
Prevalence	17 [a]	5 [b]	0	0
Abundance	0.2 ± 0.5	0.1 ± 0.5	0	0
Triaenophorus nodulosus				
Prevalence	28 [a]	8 [b]	0	5 [b]
Abundance	0.3 ± 0.5 [a]	<0.1 ± 0.3 [b]	0	<0.1 ± 0.2 [b]
Proteocephalus sp.				
Prevalence	0	3	0	0
Abundance	0	<0.1 ± 0.5	0	0
Hysterothylacium sp.				
Prevalence	6 [a]	3 [a]	13 [b]	19 [b]
Abundance	<0.1 ± 0.2	<0.1 ± 0.2	0.1 ± 0.3	0.3 ± 0.7

Table 8 continued

Raphidascaris acus				
Prevalence	0	8 [a]	7 [a]	0
Abundance	0	0.1 ± 0.4	<0.1 ± 0.3	0
Neoechinorhynchus rutili				
Prevalence	6 [a]	50 [b]	0	52 [b]
Abundance	<0.1 ± 0.2 [a]	1.3 ± 1.8 [b]	0	2.2 ± 2.4 [b]
Myzobdella lugubris				
Prevalence	0	5	0	0
Abundance	0	< 0.1 ± 0.5	0	0
Argulus sp.				
Prevalence	0	0	0	5
Abundance	0	0	0	<0.1 ± 0.2
Myxobolus sp.				
Prevalence	11	0	0	0
Eimeria degiustii				
Prevalence	28 [a]	5 [b]	0	10 [b]
Density	30.0 ± 49.0 [b]	4.7 ± 20.6 [a]	0	9.9 ± 30.1 [a]

Values with no or the same superscript do not differ significantly; Chi-square test or One-way ANOVA or Kruskal-Wallis test.

Table 9: Canonical correlations between biomarkers and parasite abundance variables for 1+ spottail shiners (*Notropis hudsonius*) collected from four localities in the St. Lawrence River in June, 2004.

Canonical variate	Canonical correlation	R-squared	F-value	Degrees of freedom	P value
1	0.640	0.41	2.85	40	<0.0001
2	0.540	0.29	2.08	28	0.0013

Table 10: Correlations and standardized canonical coefficients between biomarkers, parasites and their corresponding variables for 1+ spottail shiners (*Notropis hudsonius*) collected in June, 2004.

	First canonical variate				Second canonical variate			
	Correlation		Coefficient		Correlation		Coefficient	
Y Variables	*X1*	Y1	*X1*	Y1	*X2*	Y2	*X2*	Y2
WBC count	*-0.05*	-0.07		-0.10	*0.41*	**0.76**		0.63
Condition factor	*-0.31*	**-0.48**		-0.42	*0.27*	**0.50**		0.56
SPM counts	*0.51*	**0.80**		0.73	*0.17*	**0.32**		-0.01
SPMC counts	*0.43*	**0.67**		0.22	*0.23*	**0.42**		0.52
LPM counts	*0.20*	**0.31**		0.21	*-0.10*	-0.19		-0.09
X Variables								
Diplostomum spp.	*0.12*	0.08	*0.02*		*0.03*	0.02	*0.15*	
Plagioporus sinitsini	***0.44***	0.28	*0.31*		***-0.36***	-0.20	*0.36*	
Posthodiplostomum minimum	***0.56***	0.36	*0.48*		*0.28*	0.15	*0.10*	
Ornithodiplostomum ptychocheilus	***0.47***	0.30	*0.19*		***0.43***	0.23	*0.19*	
Rhipidocotyle papillosa	***0.46***	0.29	*0.21*		*0.07*	0.04	*0.10*	
Neoechinorhynchus rutili	***0.38***	0.25	*0.47*		***-0.55***	-0.30	*-0.55*	
Eimeria degiustii	***0.32***	0.21	*0.45*		***0.51***	0.28	*0.39*	

Significant values are shown in bold.

Table 11: Canonical correlations between biomarkers and parasite variables for 2+ spottail shiners (*Notropis hudsonius*) collected from four localities in the St. Lawrence River in June, 2004.

Canonical variate	Canonical correlation	R-squared	F-value	Degrees of freedom	P value
1	0.75	0.57	1.43	105	0.01
2	0.67	0.46	1.14	84	0.21

Table 12: Correlations and standardized canonical coefficients between biomarkers, parasites and their corresponding variables of age 2+ spottail shiners (*Notropis hudsonius*) collected in June, 2004.

	First canonical variate			
	Correlation		Coefficient	
	X1	Y1	X1	Y1
Y Variables				
WBC count	-0.08	-0.10		-0.37
Haematocrit	0.07	0.09		-0.24
Leucocrit	0.21	0.29		0.51
Condition factor	0.02	0.03		-0.21
SPM counts	-0.55	**-0.73**		-1.44
SPMC counts	-0.28	**-0.38**		0.65
LPM counts	-0.38	**-0.51**		-0.07
X Variables				
Diplostomum spp.	-0.01	-0.00	0.03	
Ornithodiplostomum ptychocheilus	-0.09	-0.06	-0.05	
Plagioporus sinitsini	-0.23	-0.17	-0.33	
Posthodiplostomum minimum	0.03	0.02	-0.14	
Azigia sp.	-0.11	-0.09	0.01	
Ichthyocotylurus platycephalus	0.08	0.06	0.17	
Apatemon gracilis	-0.24	-0.18	-0.30	
Centrovarium lobotes	0.24	0.18	0.24	
Rhipidocotyle papillosa	-0.15	-0.11	0.12	
Neoechinorhynchus rutili	-0.09	-0.07	-0.30	
Hysterothylacium sp.	**-0.68**	-0.51	-0.71	
Raphidascaris acus	0.18	0.14	0.18	
Triaenophorus nodulosus	-0.12	-0.09	-0.21	
Eimeria degiustii	**-0.35**	-0.27	-0.52	

Significant values are shown in bold.

Table 13: Pearson correlation coefficients and p values (in parentheses) of white blood cell (WBC) counts, condition factor (CF), spleen pigmented macrophage (SPM) counts, spleen pigmented macrophage centers (SPMC) counts, liver pigmented macrophage (LPM) counts and abundance of parasite species of age 1+ spottail shiners (*Notropis hudsonius*) collected from Île Dorval (DL), Île Beauregard (IB), Îlet Vert (IV) and Beauharnois (BH) in the St. Lawrence River in June, 2004. Significant values are shown in bold.

Parasites	*Plagioporus sinitsini*	*Posthodiplostomum minimum*	*Ornithodiplostomum ptychocheilus*	*Rhipidocotyle papillosa*	*Neoechinohynchus rutili*	*Eimeria degiustii*
WBC-DL	0.075 (0.69)	0.094 (0.61)	Not found	Not found	0.009 (0.96)	0.216 (0.25)
CF-DL	-0.024 (0.89)	-0.003 (0.98)	Not found	Not found	**-0.424 (0.02)**	-0.150 (0.43)
SPM-DL	-0.042 (0.82)	0.202 (0.29)	Not found	Not found	0.119 (0.53)	**0.438 (0.01)**
SPMC-DL	-0.165 (0.39)	-0.035 (0.85)	Not found	Not found	-0.075 (0.69)	**0.700 (0.00)**
LPM-DL	-0.306 (0.09)	-<0.001 (0.99)	Not found	Not found	0.123 (0.52)	0.143 (0.45)
WBC-IB	-0.069 (0.79)	0.378 (0.13)	**0.500 (0.04)**	0.018 (0.94)	0.284 (0.27)	-0.218 (0.40)
CF-IB	-0.193 (0.44)	-0.035 (0.88)	-0.105 (0.67)	0.076 (0.76)	0.247 (0.32)	0.080 (0.73)

Table 13 continued

SPM-IB	**0.566 (0.01)**	-0.051 (0.84)	0.284 (0.25)	0.224 (0.37)	-0.167 (0.50)	0.449 (0.06)
SPMC-IB	0.271 (0.27)	**0.502 (0.03)**	0.464 (0.05)	0.086 (0.73)	-0.206 (0.41)	-0.206 (0.41)
LPM-IB	0.076 (0.77)	-0.174 (0.50)	-0.199 (0.44)	-0.049 (0.85)	0.108 (0.67)	-0.080 (0.75)
WBC-IV	-0.108 (0.54)	-0.087 (0.62)	0.097 (0.58)	Not found	Not found	**0.463 (0.00)**
CF-IV	**-0.350 (0.03)**	-0.078 (0.65)	0.079 (0.64)	Not found	Not found	-0.041 (0.81)
SPM-IV	0.025 (0.88)	-0.030 (0.86)	0.214 (0.22)	Not found	Not found	**0.838 (0.00)**
SPMC-IV	-0.233 (0.18)	-0.040 (0.81)	-0.028 (0.87)	Not found	Not found	**0.588 (0.00)**
LPM-IV	-0.242 (0.16)	**0.391 (0.02)**	0.086 (0.62)	Not found	Not found	0.265 (0.13)
WBC-BH	-0.169 (0.40)	-0.379 (0.05)	-0.062 (0.76)	Not found	-0.302 (0.13)	-0.091 (0.67)
CF-BH	**-0.642 (0.00)**	-0.209 (0.29)	0.030 (0.88)	Not found	**-0.581 (0.00)**	0.100 (0.63)
SPM-BH	**0.514 (0.00)**	0.214 (0.30)	0.089 (0.66)	Not found	0.282 (0.17)	0.337 (0.09)
SPMC-BH	0.145 (0.48)	**0.449 (0.02)**	-0.208 (0.31)	Not found	0.259 (0.21)	**0.493 (0.01)**
LPM-BH	0.110 (0.59)	0.232 (0.25)	-0.222 (0.27)	Not found	0.270 (0.18)	-0.230 (0.27)

Table 14: Spearman correlation coefficients and p values (in parentheses) of spleen pigmented macrophage counts (SPM), spleen pigmented macrophage centers (SPMC) and liver pigmented macrophage counts (LPM) and abundance of selected parasite species of 2+ spottail shiners collected from Île Dorval (DL), Îles de la Paix (IP), Îlet Vert (IV) and Beauharnois (BH) in the St. Lawrence River in June, 2004. Significant values are shown in bold.

Parasites Biomarkers	*Eimeria degiustii*	*Hysterothylacium* sp.
SPM-DL	**0.762 (0.00)**	-0.023 (0.93)
SPMC-DL	**0.685 (0.00)**	-0.094 (0.71)
LPM-DL	0.152 (0.56)	-0.169 (0.50)
SPM-IP	**0.386 (0.02)**	0.127 (0.44)
SPMC-IP	**0.329 (0.04)**	-0.165 (0.32)
LPM-IP	0.137 (0.42)	0.262 (0.11)
SPM-IV	Not found	-0.274 (0.32)
SPMC-IV	Not found	-0.068 (0.80)
LPM-IV	Not found	0.464 (0.08)
SPM-BH	0.120 (0.62)	0.051 (0.82)
SPMC-BH	0.013 (0.95)	-0.258 (0.26)
LPM-BH	-0.109 (0.67)	-0.053 (0.82)

Table 15: F-Ratio and P-values of MANOVA on white blood cell (WBC) counts, condition factor, spleen pigmented macrophage (SPM) counts, SPM center (SPMC) counts and liver pigmented macrophage (LPM) counts by localities for infection levels of *Plagioporus sinitsini* (infected vs uninfected) and *Diplostomum* spp. (≤3 metacercariae vs >3 metacercariae) in 1+ spottail shiners (*Notropis hudsonius*) collected in June, 2004. Significant values are shown in bold.

Source of variation	WBC counts		Condition factor		SPM counts		SPMC counts		LPM counts	
Plagioporus sinitsini	F	P	F	P	F	P	F	P	F	P
Locality	10.75	**0.04**	2.95	0.20	67.40	**0.00**	16.82	**0.02**	1.20	0.44
Infection	0.86	0.35	8.76	**0.00**	1.89	0.17	0.64	0.43	0.82	0.37
Locality X Infection	0.43	0.73	3.88	**0.01**	1.68	0.18	0.43	0.73	1.56	0.20
Diplostomum spp.										
Locality	26.07	**0.01**	22.68	**0.01**	188.12	**0.00**	4.13	0.14	4.40	0.13
Infection	0.12	0.72	0.03	0.85	1.51	0.22	0.04	0.84	0.00	0.99
Locality X Infection	0.17	0.91	0.58	0.63	0.57	0.63	1.40	0.25	0.32	0.81

Table 16: F-Ratio and P- values of MANOVA on white blood cell (WBC) counts, condition factor, spleen pigmented macrophage (SPM) counts, SPM center (SPMC) counts and liver pigmented macrophage (LPM) counts by localities for infections of *Plagioporus sinitsini* (uninfected vs infected), *Diplostomum* spp. (≤20 metacercariae vs >20 metacercariae) and *Ornithodiplostomum ptychocheilus* (uninfected vs infected) in 2+ spottail shiners collected in June, 2004.

Source of variation	WBC counts		Condition factor		SPM counts		SPMC counts		LPM counts	
Plagioporus sinitsini	F	P	F	P	F	P	F	P	F	P
Locality	1.39	0.13	0.60	0.66	19.57	**0.01**	0.12	**0.00**	5.90	0.09
Infection	4.27	0.24	0.18	0.67	0.03	0.87	205.48	0.73	0.18	0.67
Locality X Infection	1.37	0.25	2.18	0.10	0.14	0.93	0.06	0.98	0.89	0.45
Diplostomum spp.										
Locality	18.27	**0.01**	1.04	0.49	24.01	**0.01**	180.12	**0.00**	1.53	0.37
Infection	15.99	**0.00**	0.71	0.40	0.81	0.37	0.02	0.90	0.06	0.80
Locality X Infection	0.61	0.60	1.07	0.37	0.12	0.95	0.06	0.98	1.61	0.19
Ornithodiplostomum ptychocheilus										
Locality	4.68	0.12	4.97	0.11	0.82	0.56	3.66	0.16	2.44	0.24
Infection	0.56	0.46	0.51	0.48	2.93	0.09	1.69	0.19	0.00	0.97
Locality X Infection	1.18	0.32	0.27	0.84	4.31	**0.00**	3.01	**0.03**	1.78	0.16

Table 17: Examples of the use of pigmented macrophage aggregates (centres) as biomarker in previous field and experimental studies to evaluate pollution stress in fish.

Fish	Organs	Contaminant(s)	Type	Reference
White sucker (*Catostomus commersonii*)	spleen and kidney	bleach-kraft mill	Field	Couillard and Hodson, 1996
Mummichog (*Fundulus heteroclitus*)	spleen	PAHs	Field	Blazer et al., 1997
Northern Pike (*Esox lucius*)	liver, spleen and kidney	mercury	Field	Meinelt et al., 1997
Tilapia (*Oreochromis* spp.)	spleen	industrial and municipal effluents	Field	Sun et al., 1998
Estuarine fish	spleen and liver	heavy metals, PAHs and PCBs	Field	Fournie et al., 2001
Plaice (*Pleuronectes platessa*)	spleen	potassium dichromate	Lab	Kranz and Gercken, 1987
Striped bass (*Morone saxatilis*)	spleen	arsenic	Lab	Blazer et al., 1997

Figure 1: Map showing sampling localities, City of Montreal sewage effluent plume and the St. Lawrence Seaway.

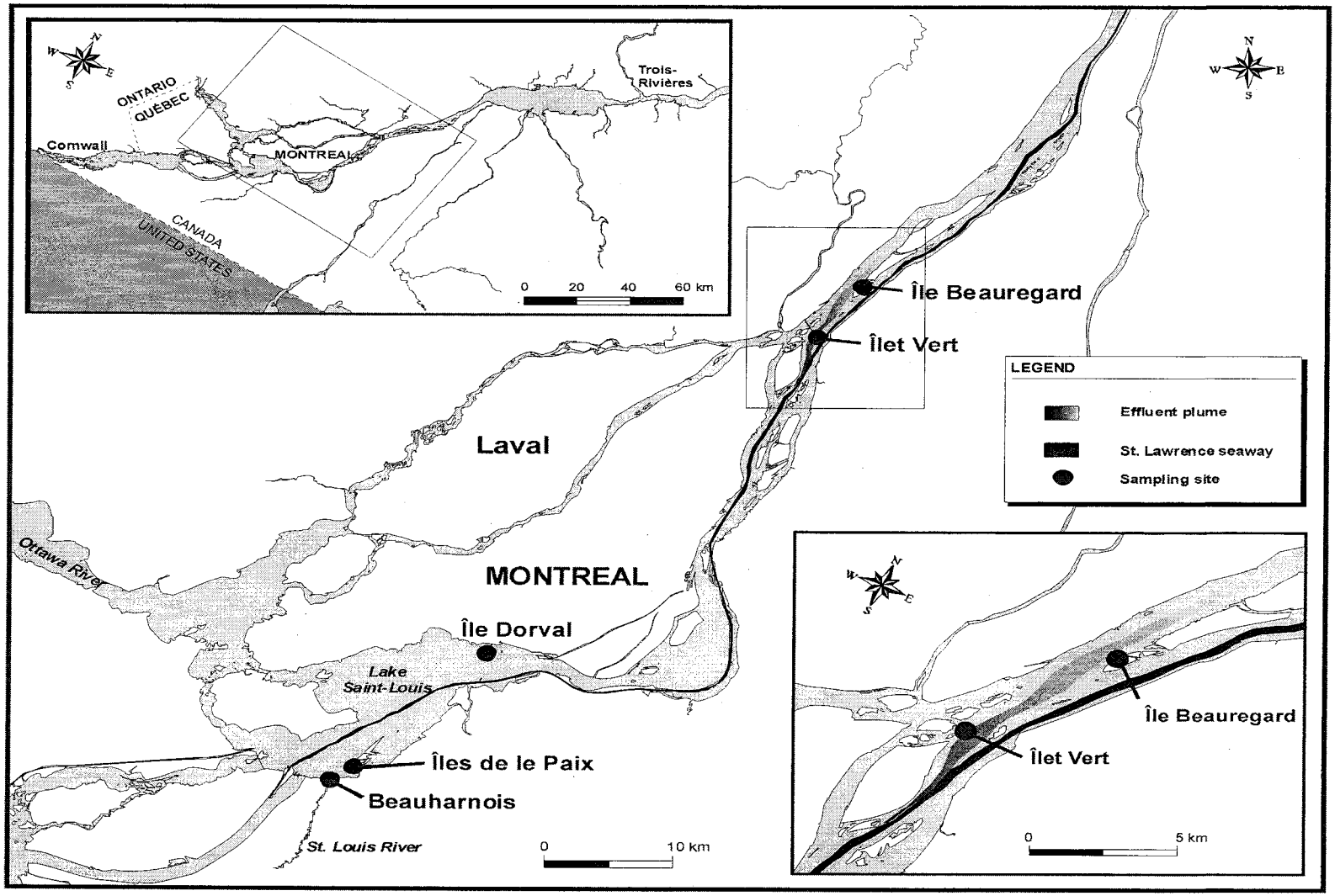


Figure 2: Relationship between spleen pigmented macrophage (SPM) counts and the number of *Plagioporus sinitsini* in 1+ spottail shiners at the reference site (Île Dorval) and at three polluted sites (Île Beauregard, Îlet Vert and Beauharnois) collected in June, 2004.

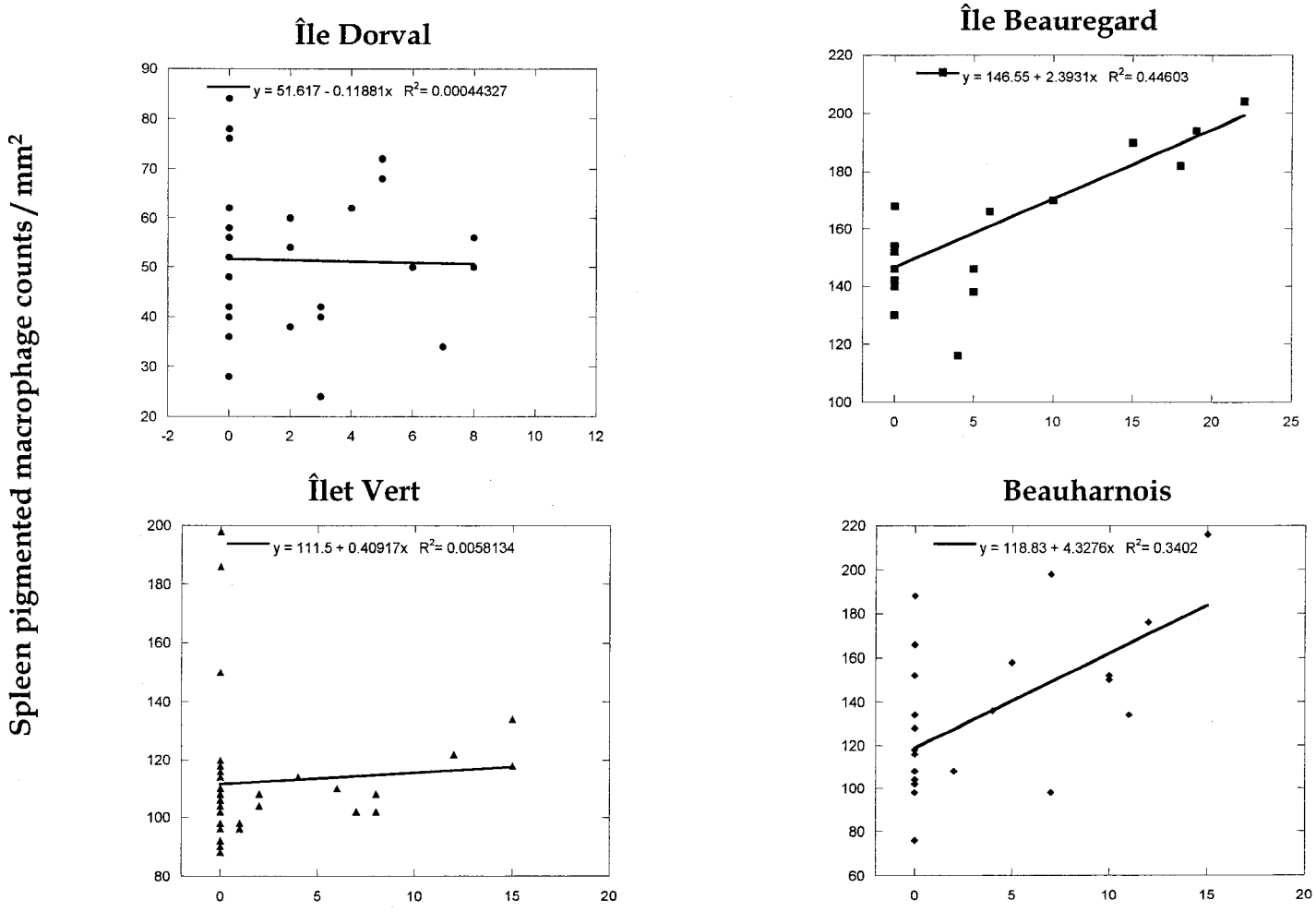
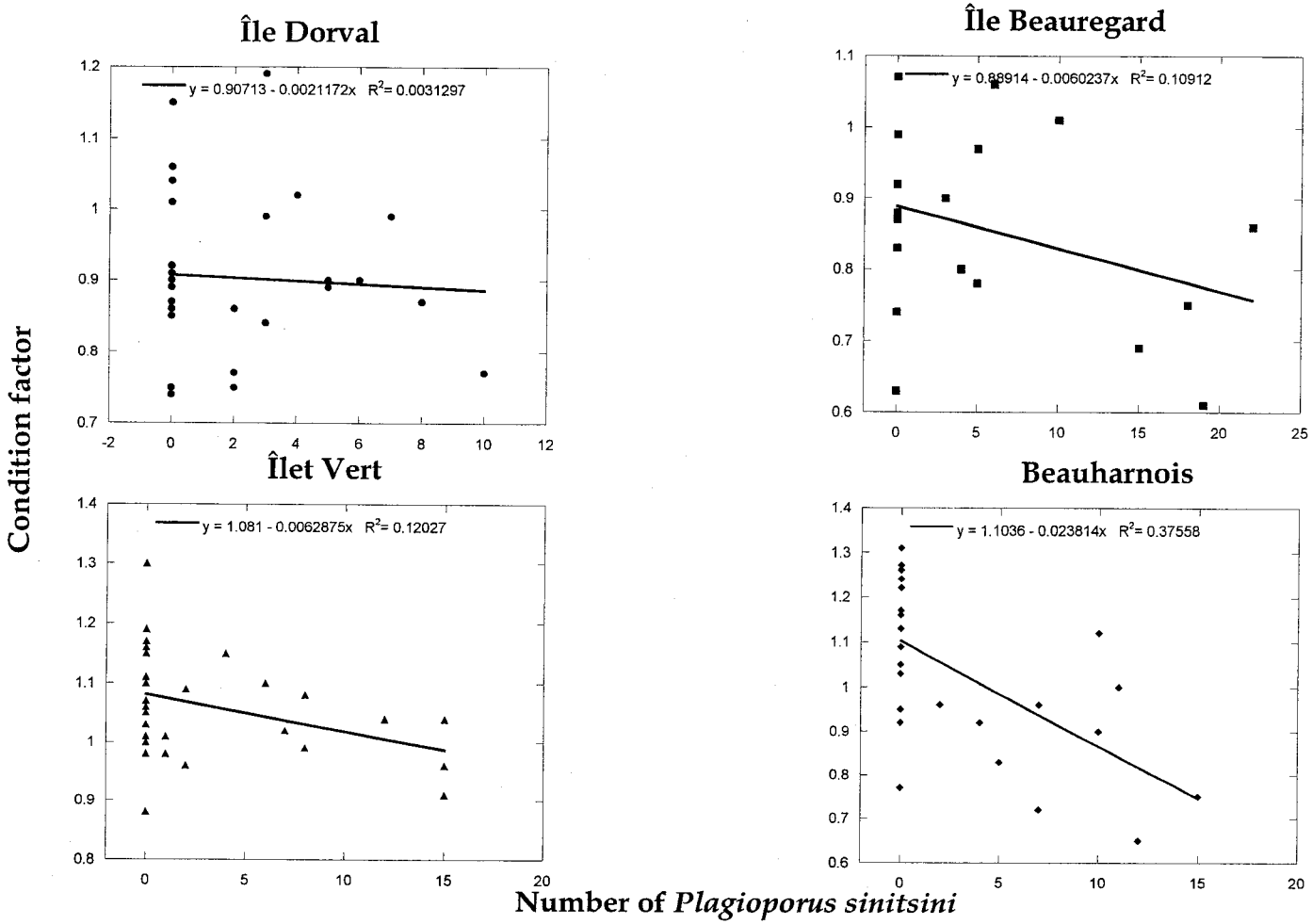


Figure 3: Relationship between condition factor and the number of *Plagioporus sinitsini* in 1+ spottail shiners at the reference site (Dorval) and at three polluted sites (Île Beauregard, Îlet Vert and Beauharnois) collected in June, 2004.



Appendix 1: Parasites of spottail shiners (*Notropis hudsonius*) reported from North America.

•Adult, ▲ larval forms, + presence, - absence

Canada (Margolis and Arthur, 1979; McDonald and Margolis, 1995)	North America (mainly USA) (Hoffman, 1999)	St. Lawrence River (Cone et al., 2004; Marcogliese et al., (submitted)
Digeneans		
Alllocreadium lobatum •	+	-
Apophallus brevis ▲		+
Bucephalus sp. ▲	+	-
Tetracotyle sp. ▲	+	-
Centrovarium lobotes ▲•	+	+
Clinostomum complanatum ▲	+	+
Crassiphiala bulboglossa ▲	-	-
Cryptogonimus chili ▲	+	-
Diplostomum spathaceum ▲	+	-
Plagioporus cooperi •	+	-
Plagioporus sinitsini •	+	+
Posthodiplostomum minimum ▲	+	+

Appendix 1 continued

Sanguinicola sp. ▲	-	-
Uvulifer ambloplitis ▲	-	+
-	*Echinochasmus donaldsoni* ▲	-
-	*Lebouria cooperi* ▲	-
-	*Lissorchis* sp. ▲	-
-	*Neascus* sp. ▲	-
-	*Petasiger nitidus* ▲	-
-	*Sanguinicola lophophora* ●	--
-	*Trigandistomum* sp. ▲	-
-	-	*Apatemon gracilis* ▲
-	-	*Caecincola* sp. ▲
-	-	*Diplostomum* spp. ▲
-	-	*Ichthyocotylurus platycephalus* ▲
-	-	*Neochasmus* spp. ●
-	-	*Ornithodiplostomum ptychocheilus* ▲
-	-	*Rhipidocotyle papillosa* ▲
-	-	*Tylodelphys scheuringi* ▲

Appendix 1 continued

Acanthocephala:		
Leptorhynchoides thecatus●	+	-
Neoechinorhynchus rutili●	+	+
Neoechinorhynchus sp.●	-	-
Pomphorhynchus bulbocolli●	+	-
Pomphorhynchus sp.●	-	-
-	*Acanthocephalus dirus* ●	-
-	*Echinorhynchus salmonis*●	-
Cestodea		
Glaridacris sp. ●	-	-
Ligula intestinalis▲	+	-
Proteocephalus ambloplitis●	+	-
Proteocephalus pinguis●	+	-
Proteocephalus sp.●	+	+
Schistocephalus sp.▲	+	-
Spartoides wardi●	+	-
Triaenophorus nodulosus▲	-	+
-	*Pliovitellaria wisconensis*●	+
-	-	Pseudophyllidea sp. ▲

Appendix 1 continued

Monogenea:		
Dactylogyrus sp.●	+	-
Gyrodactylus sp.●	+	+
Otomacrum semotili●	-	-
Nematoda:		
Camallanus oxycephalus●	+	-
Contracaecum sp. ▲	+	-
Rhabdochona cascadilla●	+	+
Spinitectus gracilis●	+	-
Paracuaria adunca ▲	-	-
-	-	*Hysterothylacium* sp. ▲
-	-	*Raphidascaris acus*▲
-	-	*Tetrameres* sp. ▲
-	-	*Phylometra* sp. ●
-	-	*Cosmocephalus obvelatus*▲
Annelida:		
Myzobdella lugubris●	-	-
-	-	Unidentified species

Appendix 1 continued

Arthropoda:		
Argulus stizostethii•	+	-
Ergasilus sp.•	+	+
-	*Lernea cyprinacea*•	-
-	*Argulus canadensis*•	-
Myxozoa:		
Myxosoma grandis	+	-
Thelohanellus notatus	+	+
Myxobolus fanthami	-	+
-	-	*Myxobolus* sp.
-	-	*Myxobolus bartai*
-	-	*Myxobolus hendricksoni*
Ciliophora:		
Trichodina sp.	-	-
-	*Goussia deguisti*	-

Appendix 2: Normality and transformation details of all dependent (biomarkers) and independent (parasites) variables pertaining to 1+ spottail shiners (*Notropis hudsonius*) collected in the St. Lawrence River in June, 2004.

(Martinez-Iglewicz test, Kolmogorov-Smirnov test, D'Agostino Skewness test, D'Agostino Kurtosis test and D'Agostino Omnibus test were used to test normality and, if at least 2 of these tests did not reject the null hypothesis, the variable was considered to be normally distributed).

Variables	Normality of raw data	Normality of Log (X+1) transformed data	Normality of Square root transformed data	Normality of rank transformed data
WBC counts	No	Yes		
Condition factor	Yes	Yes		
SPM counts	No	Yes		
SPMC counts	No	No	No	No
LPM counts	No	Yes		
Diplostomum spp.	No	Yes	No	Yes
Plagioporus sinitsini	No	No	No	Yes
Posthodiplostomum minimum	No	No	No	Yes
Ornithodiplostomum ptychocheilus	No	No	No	Yes
Rhipidocotyle papillosa	No	No	No	No
Centrovarium lobotes	No	No	No	No
Neoechinorhynchus rutili	No	No	No	Yes
Myxobolus sp.	No	No	No	No
Eimeria degiustii	No	No	No	No

Appendix 3: Normality and transformation details of all dependent (biomarkers) and independent (parasites) variables pertaining to 2+ spottail shiners (*Notropis hudsonius*) collected in the St. Lawrence River in June, 2004.

(Martinez-Iglewicz test, Kolmogorov-Smirnov test, D'Agostino Skewness test, D'Agostino Kurtosis test and D'Agostino Omnibus test were used to test normality and, if at least 2 of these tests did not reject the null hypothesis, the variable was considered to be normally distributed).

Variables	Normality of raw data	Normality of Log (X+1) transformed data	Normality of Square root transformed data	Normality of rank transformed data
WBC counts	No	Yes		
Condition factor	No	Yes		
SPM counts	No	Yes		
SPMC counts	No	Yes		
LPM counts	No	Yes		
Haematocrit	Yes	Yes		
Leucocrit	No	No	Yes	
Diplostomum spp.	No	Yes	Yes	Yes
Ornithodiplostomum ptychocheilus	No	No	No	No
Plagioporus sinitsini	No	No	No	No
Posthodiplostomum minimum	No	No	No	No

Appendix 3 continued

Apatemon gracilis	No	No	No	No
Azigia sp.	No	No	No	No
Rhipidocotyle papillosa	No	No	No	No
Centrovarium lobotes	No	No	No	No
Triaenophorus nodulosus	No	No	No	No
Proteocephalus sp.	No	No	No	No
Hysterothylacium sp.	No	No	No	No
Raphidascaris acus	No	No	No	No
Neoechinorhynchus rutili	No	No	No	No
Myzobdella lugubris	No	No	No	No
Argulus sp.	No	No	No	No
Myxobolus sp.	No	No	No	No
Eimeria degiustii	No	No	No	No

Appendix 4: List of parasites species and their overall prevalence in total sample of 1+ and 2+ spottail shiners (*Notropis hudsonius*) collected from the St. Lawrence River in June, 2004.

Parasite species	Prevalence
Age 1+ fish	
Diplostomum spp.	83.6
Plagioporus sinitsini	42.7
Posthodiplostomum minimum	20.0
Ornithodiplostomum ptychocheilus	19.1
Neoechinorhynchus rutili	18.2
Eimeria degiustii	8.2
Rhipidocotyle papillosa	6.4
Myxobolus sp.	3.6
Centrovarium lobotes	1.8
Age 2+fish	
Diplostomum spp.	100.0
Ornithodiplostomum ptychocheilus	41.8
Plagioporus sinitsini	36.3
Posthodiplostomum minimum	34.1
Neoechinorhynchus rutili	34.1

Appendix 4 continued

Ichthyocotylurus platycephalus	14.3
Azigia sp.	11.0
Triaenophorus nodulosus	9.9
Eimeria degiustii	9.9
Hysterothylacium sp.	8.8
Apatemon gracilis	7.7
Rhipidocotyle papillosa	6.6
Centrovarium lobotes	5.5
Raphidascaris acus	4.2
Myxobolus sp.	2.2
Myzobdella lugubris	2.0
Argulus sp.	1.1
Proteocephalus sp.	1.1

References

Adams, S.M. (2001) Biomarker/bioindicator response profile of organisms can help differentiate between sources of anthropogenic stressors in aquatic ecosystems. *Biomarkers* **6**, 33-44.

Adams, S.M., Crumby, W.D., Greeley, M.S., Shugart, L.R. and Saylor, C.F. (1992) Responses of fish populations and communities to pulp mill effluents: a holistic assessment. *Ecotoxicology and Environmental Safety* **24**, 347-360.

Adams, S.M. and Greeley, M.S. (1999) Establishing possible link between aquatic ecosystem health and human health: an intergraded approach. In: DiGuiulo, R.T. and Monosson, E., (Eds.) *Inter-Connections between Human and Ecosystem Health*, 4th ed. Chapman and Hall, London, 91-102.

Adams, S.M., Greeley, M.S., Law, J.M., Noga, E.J. and Zelikoff, J.T. (2003) Application of multiple sublethal stress indicators to assess the health of fish in Pamlico Sound following extensive flooding. *Estuaries* **26**, 1365-1382.

Adams, S.M. and McLean, R.B. (1985) Estimation of largemouth bass (*Micropterus salmonides*) growth using condition indices and physiological variables. *Journal of Fish Biology* **26**, 111-126.

Adams, S.M. and Ryon, M.G. (1994) A comparison of health assessment

approaches for evaluating the effects of contaminant-related stress on fish populations. *Journal of Aquatic Ecosystem Health* **3**, 15-25.

Agius, C. (1979) The role of melano-macrophage centres in iron storage in normal and diseased fish. *Journal of Fish Diseases* **2**, 337-343.

Agius, C. (1981) Preliminary studies of ontogeny of the melano-macrophages of teleost haemopoitic tissues and age-related changes. *Developmental and Comparative Immunology* **5**, 597-606.

Agius, C. and Roberts, R.J. (2003) Melano-macrophage centres and their role in fish pathology. *Journal of Fish Diseases* **26**, 499-509.

Anderson, D.P. (1990) Immunological indicators: effects of environmental stress on immune protection and disease outbreaks. *American Fisheries Society Symposium* **8**, 38-50.

Arai, H.P. (1989) Acanthocephala. In: Margolis, L. and Kabata, Z., (Eds.) *Guide to the Parasites of Fishes of Canada*, part III Canadian Special Publication of Fisheries and Aquatic Sciences, No. 107, Ottawa.

Aravindakshan, J., Paquet, V., Gregory, M., Dufresne, J., Fournier, M., Marcogliese, D.J. and Cyr, D.G. (2004) Consequences of xenoestrogen exposure on male reproductive function in spottail shiners (*Notropis hudsonius*). *Toxicological Science* **78**, 156-165.

Barker, D.E., Khan, R.A. and Hooper, R. (1994) Bioindicators of stress in winter flounder, *Pleuronectes americanus*, captured adjacent to a pulp and paper mill in St. George's Bay, Newfoundland. *Canadian Journal of Fisheries and Aquatic Sciences* **51**, 2203-2209.

Barton, B.A. (1996) Stress in finfish: past, present and future - a historical perspective. In: Iwama, G.K., Pickering, A.D., Sumpter, J.R. and Schreck, C.B., (Eds.) *Fish Stress and Health in Aquaculture,* Cambridge University Press, London, 1-9.

Barton, B.A., Morgan, J.D. and Vijayan, M.M. (2002) Physiological and condition related indicators of environmental stress in fish. In: Adams, S.M., (Ed.) *Biological Indicators of Aquatic Ecosystem Stress,* American Fisheries Society, Bethesda, Maryland, 111-142.

Billiard, S.M. and Khan, R.A. (2003) Chronic stress in cunner, *Tautogolabrus adspersus*, exposed to municipal and industrial effluents. *Ecotoxicology and Environmental Safety* **55**, 9-18.

Blazer, V.S., Facey, D.E., Fourney, J.W., Courtney, L.A. and Summer, J.K. (1994) Macrophage aggregates as indicators of environmental stress. In: Stolen, J.S. and Fletcher, T., (Eds.) *Modulators of Fish Immune Response: Models for Environmental Toxicology / Biomarkers, Immunostimulators,* SOS publications Fair Haven, New Jersey, 165-189.

Blazer, V.S., Fournie, W.K. and Weeds, B.A. (1997) Macrophage aggregates: biomarker for the immune functions of fishes. In: Dwyer, F. G., Doane, T. T., and Hinmman, M. L. (Eds.) *Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment,* American Society for Testing and Materials, Conshohocken, Pennsylvania, 360-375.

Blazer, V.S., Wolke, R.E., Brown, J. and Powell, C.A. (1987) Piscine macrophage aggregates parameters as health monitors: effect of age, sex, relative weight, season and site quality of in largemouth bass. *Aquatic Toxicology* **10**, 199-215.

Bols, N.C., Brubacher, J.L., Ganassin, R.C. and Lee, L.E.J. (2001) Ecotoxicology and innate immunity in fish. *Developmental and Comparative Immunology* **25**, 853-873.

Boyce, N.P. and Yamada, Y. (1977) Effects of a parasite, *Eubothrium salvelini* (Cestoda: Pseudophyllidea) on the resistance of juvenile sockeye salmon, *Oncorhynchus nerka,* to zinc. *Journal of the Fisheries Research Board of Canada* **34**, 706-709.

Bristol, J.R., Mayberry, L.F., Huber, D. and Ehrlich, I. (1984) Endoparasite fauna of trout in the Plitvice Lake National Park. *Veterinarski Arhive* **54**, 5-11.

Broeg, K., Zander, S., Diamant, A., Korting, W., Kruner, G., Paperna, I. and Westernhagen, H.V. (1999) The use of fish metabolic, pathological and

parasitological indices in pollution monitoring: North Sea. *Helgoland Marine Research* **53**, 171-194.

Buchmann, K. (1986) On the infection of Baltic cod (*Gadus morhua*) by the acanthocephalan *Echinorhynchus gadi* (Zoega) Muller. *Nordisk Veterinaermedicin* **38**, 308-314.

Busacker, G.P., Adelman, I.R. and Goolish, E.M. (1990) Growth. In: Schreck, C.B. and Moyle, P.B., (Eds.) *Methods for Fish Biology*, American Fisheries Society, Bethesda, Maryland, 363-382.

Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W. (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* **83**, 575-583.

Chambers, P.A., Allard, M., Walker, J., Marsalek, J., Lawrence, M., Kent, R.A. and Wong, M.P. (1997) Impact of municipal waste water effluent on Canadian waters. *Water Quality Research Journal of Canada* **32**, 659-713.

Cone, D., Marcogliese, D.J. and Russell, R. (2004) The myxozoan fauna of spottail shiners in the Great Lakes basin: membership, richness, and geographical distribution. *Journal of Parasitology* **90**, 921-932.

Cone, D.K., Marcogliese, D.J. and Watt, W.D. (1993) Metazoan parasite communities of yellow eels (*Anguilla rostrata*) in acidic and limed rivers of

Nova Scotia. *Canadian Journal of Zoology* **71**, 177-184.

Couillard, C.M., Berman, R.A. and Panisset, J.C. (1988) Histopathology of rainbow trout exposed to bleached craft pulp mill effluent. *Archives of Environmental Contamination and Toxicology* **17**, 319-323.

Couillard, C.M., Courtenay, S.C. and Rawn, G.P. (1999) Histopathological evaluation of Atlantic tomcod collected at estuarine sites receiving pulp and paper mill effluent. *Aquatic Toxicology* **44**, 263-278.

Couillard, C.M. and Hodson, P.V. (1996) Pigmented macrophage aggregates: A toxic response in fish exposed to bleached kraft mill effluent. *Environmental Toxicology and Chemistry* **15**, 1844-1854.

de Lafontaine, Y.D., Turgeon, K., Kemp, A., Ménard, C., Labonté, D., Dubé, P. and Parent, M. (2001) *Protocole de mesure, d'étiquettage et de conservation des poissons à des fins de suivi environnemental et d'analyse chimique.* Rapport Scientifique et technique ST- 221. Conservation de l'environment, Centre Saint-Laurent, Environnement Canada - region du Quebec.

Dezfuli, B.S., Giari, L., Simoni, E., Shinn, A.P., Manera, M. and Bosi, G. (2005) Histopathology, ultrastructure and immunohistochemistry of *Coregonus lavaretus* hearts naturally infected with *Ichthyocotylurus erraticus* (Trematoda). *Diseases of Aquatic Organisms* **66**, 245-254.

Esch, G.W., Gibbons, J.W. and Bourque, J.E. (1975) An analysis of the relationship between stress and parasitism. *American Midland Naturalist* **93**, 339-353.

Esch, G.W. and Hazen, T.C. (1978) Thermal ecology and stress: A case history for red-sore disease in largemouth bass (*Micropterus salmoides*). *DOE Symposium Series* 331-363.

Feely, M.M. (1995) Biomarkers of Great Lakes priority contaminants: halogenated aromatic hydrocarbons. *Environmental Health Perspectives* **103**, 7-16.

Fournie, J.W., Summers, J.K., Courtney, L.A., Engle, V.D. and Blazer, V.S. (2001) Utility of splenic macrophage aggregates as an indicator of fish exposed to degraded environments. *Journal of Aquatic Animal Health* **13**, 105-116.

Gagnon, M.M., Bussieres, D., Dodson, J.J. and Hodson, P.V. (1995) White sucker (*Catostomus commersoni*) growth and sexual maturation in pulp mill - contaminated and reference rivers. *Environmental Toxicology and Chemistry* **14**, 317-327.

Gagnon, C. and Saulnier, I. (2003) Distribution and fate of metals in the dispersion plume of a major municipal effluent. *Environmental Pollution* **124**, 47-55.

Gheorgiu, C., Marcogliese, D.J. and Scott, M. (2005) Concentration-dependent effects of waterborne zinc on population dynamics of *Gyrodactylus turnbulli* (Monogenea) on isolated guppies (*Poecilia reticulata*). *Parasitology* **132**, 1-8.

Gibson, D.I. (1996) Trematoda. In: Margolis, L. and Kabata, Z., (Eds.) *Guide to Parasites of the Fishes of Canada, Part IV*, Canadian Special Publication of Fisheries and Aquatic Sciences, No. 124, Ottawa.

Gopal, K., Pathak, S.P. and Sharma, B. (1992) Stress mediated immunosupression and disease syndrome in fresh water fishes. *International Journal of Toxicology, Occupational, and Environmental Health* **3**, 39-47.

Hinton, D.E. (1990) Histological Techniques. In: Schreck, C.B. and Moyle, P.B., (Eds.) *Methods for Fish Biology,* American Fisheries Society, Bethesda, Maryland, 191-211.

Hinton, D.E., Baumann, P.C., Garder, G.R., Hawkin, W.E., Hendricks, J.D. and Okihiro, M.S. (1992) Histopathologic biomarkers. In: Huggeh, R.J., Kimerle, R.A., Mehrle, P.M., Jr. and Bergman, H.L., (Eds.) *Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress,* Lewis Publishers, Chelsea, Michigan, 155-200.

Hoffman, G.L. (1999) *Parasites of North American Freshwater Fishes,* Cornell

University Press, Ithaca, New York.

Hoole, D. (1997) The effects of pollutants on the immune response of fish: implications for helminth parasites. *Rivista Di Parassitologia* **39**, 219-225.

Jacobson, K.C., Arkoosh, M.R., Kagley, A.N., Clemons, E.R., Collier, T.K. and Casillas, E. (2003) Cumulative effects of natural and anthropogenic stress on immune function and disease resistance in juvenile chinook salmon. *Journal of Aquatic Animal Health* **15**, 1-12.

Jeney, Z., Valtonen, E.T. and Jokinen, E.H. (2002) Effect of pulp and paper mill effluent (BKME) on physiological parameters of roach (*Rutilus rutilus*) infected by the digenean *Rhipidocotyle fennica*. *Folia Parasitologica* **49**, 103-108.

Khan, R.A. (1987) Effects of chronic exposure to petroleum hydrocarbon on two species of marine fish infected with a haemoprotozoan, *Trypanosoma murmanensis*. *Canadian Journal of Zoology* **65**, 2703-2709.

Khan, R.A. (1991) Effect of oil-contaminated sediment on the longhorn sculpin (*Myoxocephalus octodecemspinosus*) following chronic exposure. *Bulletin of Environmental Contamination and Toxicology* **47**, 63-69.

Khan, R.A. (2004) Effect, distribution, and prevalence of *Glugea stephani* (Microspora) in winter flounder (*Pleuronectes americanus*) living near two

pulp and paper mills in Newfoundland. *Journal of Parasitology* **90**, 229-33.

Khan, R.A. and Kiceniuk, J. (1988) Effects of petroleum aromatic hydrocarbons on monogeneids parasitizing Atlantic cod. *Bulletin of Environmental Contamination and Toxicology* **41**, 94-100.

Khan, R.A. and Payne, J.F. (1997) A multidisciplinary approach using several biomarkers including parasites as indicator of pollution: a case history from a paper mill in Newfoundland. *Rivista Di Parassitologia* **39**, 183-188.

Khan, R.A. and Thulin, J. (1991) Influence of pollution on parasites of aquatic animals. *Advances in Parasitology* **30**, 231-238.

Kranz, H. (1989) Changes in splenic melano-macrophage centres of dab (*Limanda limanda*) during and after infection with ulcer disease. *Diseases of Aquatic Organisms* **6**, 167-173.

Kranz, H. and Gerken, W.H. (1987) Effects of sublethal concentrations of potassium dichromate on the occurrence of splenic macrophage centres in juvenile plaice, *Pleuronectes platessa* L. *Journal of Fish Biology* **75**, 70-80.

Kussat, R.H. (1969) A comparison of aquatic communities in the Bow River above and below sources of domestic and industrial waste from the City of Calgary. *Canadian Fish Culture* **40**, 3-31.

Kwan, M.K.H., Chan, H.M. and Lafontaine, Y.D. (2003) Metal contamination in zebra mussels along the St. Lawrence River. *Environment Monitoring and Assessment* **88**, 193-219.

Lafferty, K.D. (1997) Environmental parasitology: What can parasites tell us about human impact on the environment. *Parasitology Today* **13**, 251-255.

Loiselle, C., Fortin G. R., Lorraine, S. and Pelletier, M. (1997) *Dynamics and Contamination of St. Lawrence River Sediment*, St. Lawrence Centre, Environmental Canada, Montreal.

MacKenzie, K., Williams, H.H., Williams, B., McVicar, A.H. and Siddall, R. (1995) Parasite as indicators of water quality and the potential use of helminth transmission in marine pollution studies. *Advances in Parasitology* **35** , 86-144.

Marcogliese, D.J. (2004) Parasites: Small players with crucial roles in the ecological theater. *Ecohealth* **1**, 151-164.

Marcogliese, D.J. (2005) Parasites of the superorganisms: Are they indicators of ecosystem health? *International Journal for Parasitology* **35**, 705-716.

Marcogliese, D.J., Brambilla, L.G., Gagne, F. and Gendron, A.D. (2005) Joint effects of parasitism and pollution on oxidative stress biomarkers in yellow perch *Perca flavescens*. *Diseases of Aquatic Organisms* **63**, 77-84.

Marcogliese, D.J. and Compagna, S. (1999) Diplostomatid eye flukes in young of the year and forage fishes in the St. Lawrence River, Quebec. *Journal of Aquatic Animal Health* **11**, 275-282.

Marcogliese, D.J., Compagna, S., Bergeron, E. and McLaughlin, J.D. (2001a) Population biology of eye flukes in fish from a large fluvial ecosystem: the importance of gulls and habitat characteristics. *Canadian Journal of Zoology* **79**, 1102-1113.

Marcogliese, D.J. and Cone, D.K. (1996) On the distribution and abundance of eel parasites in Nova Scotia: Influence of pH. *Journal of Parasitology* **83**, 389-399.

Marcogliese, D.J. and Cone, D.K. (2001) Myxozoan communities parasitizing *Notropis hudsonius* (Cyprinidae) at selected locations on the St. Lawrence River, Quebec: Possible effects of urban effluents. *Journal of Parasitology* **87**, 951-956.

Marcogliese, D.J., Dumont, P., Gendron, A. D., Mailhot, Y., Bergeron, E. and McLaughlin, J.D. (2001b) Spatial and temporal variations in abundance of *Diplostomum* spp. in walleye (*Stizostedion vitreum*) and white sucker (*Catostomus commersoni*) from the St. Lawrence River. *Canadian Journal of Zoology* **79**, 355-369.

Marcogliese, D.J., Nagler, J.J. and Cyr, D.G. (1998) Effects of exposure to contaminated sediments on the parasite fauna of American plaice (*Hippoglossoides platessoides*). *Bulletin of Environmental Contamination and Toxicology* **61**, 88-95.

Margolis, L. and Arthur, J.R. (1979) *Synopsis of the Parasites of Fishes of Canada,* Bulletin of the Fisheries Research Board of the Canada, Ottawa, No. 199.

McDonald, T.E. and Margolis, L. (1995) *Synopsis of the Parasites of Fishes of Canada: Supplement (1978-1993),* Canadian Special Publication of Fisheries and Aquatic Sciences, Ottawa, No 122.

Meinelt, T., Kruger, R., Pietrock, M., Osten, R. and Steinberg, C. (1997) Mercury pollution and macrophage centres in pike (*Esox lucius*) tissues. *Environmental Science and Pollution Research* **4**, 32-36.

Mikaelian, I., de Lafontaine, Y., Harshbarger, J.C., Lee, L.J. and Martineau, D. (2002) Health of lake whitefish (*Coregonus clupeaformis*) with elevated tissue levels of environmental contaminants. *Environmental Toxicology and Chemistry* **21**, 532-542.

Miller, D.M. (1995) Phylum Acanthocephala. In: Woo, P.T.K., (Ed.) *Fish Diseases and Disorders,* CAB International, Wallingford, 447-473.

Moles, A. (1980) Sensitivity of parasitized coho salmon fry to crude oil, toluene,

and naphthalene. *Transaction of the American Fisheries Society* **109**, 293-297.

Moller, H. (1987) Pollution and parasitism in the aquatic environment. *International Journal for Parasitology* **17**, 353-361.

Molnar, K. (1995) Phylum Apicomplexa. In: Woo, P.T.K., (Ed.) *Fish Diseases and Disorders*, CAB International, Wallingford, 263-287.

Molnar, K. and Fernando, C. H. (1974) Some new *Eimeria* (Protozoa, Coccidia) from freshwater fishes in Ontario, Canada. *Canadian Journal of Zoology* **52**, 413- 419.

Moravac, F. (1994) *Parasitic Nematodes of Freshwater Fishes of Europe,* Kluwer Academic Publishers, Dordrecht, Germany.

Morgan, J.D. and Iwama, G.K. (1996) Measurements of stressed states in the field. In: Iwama, G.K., Pickering, A.D., Sumpter, J.R. and Schreck, C.B., (Eds.) *Fish Stress and Health in Aquaculture,* Cambridge University Press, London, 249-269.

Murad, A. and Mustafa, S. (1998) Blood parameters of catfish, *Heteropneustes fossilis* (Bloch), parasitized by metacercariae of *Diplostomum* sp. *Journal of Fish Diseases* **11**, 365-368.

Myers, M.S. and Fournie, J.W. (2002) Histopathological biomarkers as indicators

of anthropogenic and environmental stressors. In: Adams, S.M., (Ed.) *Biological Indicators of Environmental Stress*, American Fisheries Society, Bethesda, Maryland, 221-287.

Overstreet, R.M. (1997) Parasitological data as monitors of environmental health. *Rivista Di Parassitologia* **39**, 169-175.

Overstreet, R.M. and Thulin, J. (1989) Response by *Plectropomus leopardus* and other serranid fishes to *Pearsonellum corventum* (Digenia: Sanguinicolidae), including melanomacrophage centres in the heart. *Australian Journal of Zoology* **37**, 129-142.

Palikova, M. and Navratil, S. (2001) Occurrence of *Anguillicola crassus* in the water reservoir Korycany (Czech Republic) and its influence on the health condition and haematological indices of eels. *Acta Veterinaria BRNO* **70**, 443-449.

Paperna, I. (1995) Digenea (Phylum Platyhelminthes). In: Woo, P.T.K., (Ed.) *Fish Diseases and Disorders*, CAB International, Wallingford, 329-389.

Pascoe, D. and Cram, P. (1977) The effect of parasitism on the toxicity of cadmium to the three-spined stickleback, *Gasterosteus aculeatus*, L. *Journal of Fish Biology* **10**, 467-472.

Pascoe, D. and Woodworth, J. (1980) The effects of joint stress on sticklebacks.

Zeitschrift Fur Parasitenkunde **62**, 159-163.

Patino, R., Goodbred, S.L., Draugelis-Dale, R., Barry, C.E., Foot, J.S. and Wainscott, M.R. (2003) Morphometric and histopathological parameters of gonadal development in adult common carp from contaminated and reference sites in Lake Mead, Nevada. *Journal of Aquatic Animal Health* **15**, 55-68.

Payne, J.F. and Fancy, L.F. (1990) Effects of polycyclic aromatic hydrocarbons on immune response of fish: changes in melano-macrophage centres in flounder (*Pseudopleuronectes americanus*) exposed to hydrocarbon-contaminated sediments. *Marine Environmental Research* **28** , 431-435.

Pham, T. and Proulx, S. (1997) PCBs and PAHs in the Montreal Urban Community (Quebec, Canada) wastewater treatment plant and in the effluent plume in the St. Lawrence River. *Water Research* **31**, 1887-1896.

Pham, T.T., Proulx, S., Brochu, C. and Moore, S. (1999) Composition of PCB's and PAH's in the Montreal Urban Community wastewater treatment plant and in the surface water of the St. Lawrence River (Canada). *Water, Air, and Soil Pollution* **111**, 251-270.

Pippy, J.H.C. and Hare, G.M. (1969) Relationship of river pollution to bacterial infections in salmon (*Salmon salar*) and suckers (*Catostomus commersoni*). *Transactions of the American Fisheries Society* **98**, 685-690.

Post, G. (1987) *Textbook of Fish Health,* TFH Publication Inc., Neptune City, New Jersey.

Poulin, R. (1992) Toxic pollution and parasitism in freshwater fish. *Parasitology Today* **8**, 58-66.

Quemerais, B., Lum, K.R. and Lemieux, C. (1996) Concentrations and transport of trace metals in the St. Lawrence River. *Aquatic Sciences* **58**, 53-66.

Rice, C.D., Kergosien, D.H. and Adams, M.S. (1996) Innate immune functions as a bioindicator of pollution stress in fish. *Ecotoxicology and Environmental Safety* **33**, 186-192.

Sakanari, J.A., Moser, M., Reilly, C.A. and Yoshino, T.P. (1984) Effects of sublethal concentrations of zinc and benzene on striped bass, *Morone saxatilis* (Walbaum), infected with larval *Anisakis* nematodes. *Journal of Fish Biology* **24**, 553-563.

Schaperclaus, W. (1991) *Fish Diseases,* Akademie-Verlag, Berlin.

Schlenk, D. and Giulio, R.T.D. (2002) Biochemical response as indicators of aquatic ecosystem health. In: Adams, S.M., (ed.) *Biological Indicators of Aquatic Ecosystem Stress,* American Fisheries Society, Bethesda, Maryland, 13-110.

Scott, W.B. and Crossman, E.J. (1973) *Freshwater Fishes of Canada,* Fisheries

Research Board of Canada, Ottawa.

Shugart, L.R., MaCarthy, J.F. and Halbrook, R.S. (1992) Biological markers of environmental and ecological contamination. *Risk Analysis* **12**, 353-360.

Sindermann, C.J. (1983) An examination of some relationships between pollution and disease. *R'eunion du Conseil international pour I'exploration de la Mer* **182**, 37-43.

Snieszko, S.F. (1974) The effects of environmental stress on outbreaks of infectious diseases of fishes. *Journal of Fish Biology* **6**, 197-208.

Steedman, R.J. (1991) Occurrence and environmental correlates of black spot disease in stream fishes near Toronto, Ontario. *Transactions of the American Fisheries Society* **120**, 494-499.

Stehr, C.M. and Myers, M.S. (1990) The ultrastructure and histology of cholangiocelleular carcinomas in English sole (*Parophrys vetulus*) from Puget sound, Washington. *Toxicologic Pathology* **18**, 362-372.

Sun, P.L., Brown-Peterson, N.J., Hawkins, W.E., Overstreet, R.M., Krol, M.R., Tsaio, S.H. and Zhu, Y. (1998) Morphological and histological abnormalities in tilapia (*Oreochromis* spp.) from two contaminated rivers in southern Taiwan. *Environmental Science* **63**, 129-152.

Suns, K., Craig, G.R., Crawford, G., Rees, G.A., Tosine, H. and Osborne, J. (1983)

Organochlorine contaminant residues in spottail shiners (*Notropis hudsonius*) from the Niagara River. *Journal of Great Lakes Research* **9**, 335-340.

Suns, K., Crawford, G. and Russel, D. (1985) Organochlorine and mercury residues in young-of-the-year-spottail shiners from the Detroit River, Lake St. Clair and Lake Erie. *Journal of Great Lakes Research* **11**, 347-352.

Suns, K.R., Hitchin, G.G. and Toner, D. (1993) Spatial and temporal trends of organochlorine contaminants in spottail shiners from selected sites in the Great Lakes (1975-1990). *Journal of the Great Lakes Research* **19**, 703-714.

Sures, B. (2004) Environmental parasitology: relevancy of parasites in monitering environmental pollution. *Trends in Parasitology* **20**, 170-177.

Taylor, M. and Hoole, D. (1989) *Ligula intestinalis* (L.) (Cestoda: Pseudophyllidea): plerocercoid induced changes in the spleen and kidney of roach, *Rutilus rutilus* (L.), and gudgeon, *Gobio gobio* (L.). *Journal of Fish Biology* **34**, 583-596.

Thulin, J., Hoglund, J. and Lindesjoo, E. (1988) Diseases and parasites of fish in a bleached kraft mill effluent. *Water Science Technology* **20**, 179-180.

Valtonen, E.T., Haaparanta, A. and Hoffmann, R.W. (1994) Occurrence and histopathological response of *Raphidascaris acus* (Nematoda: Ascaridoidea)

in roach from four lakes differing in water quality. *International Journal for Parasitology* **24**, 197-206.

Vogelbein, W.K., Fournie, J.W. and Overstreet, R.M. (1987) Sequential development and morphology of experimentally induced hepatic melano-macrophage centres in *Rivulus marmoratus*. *Journal of Fish Biology* **31**, 145-153.

Wedemeyer, G. (1970) The role of stress in the disease resistance of fishes. *Special Publication of the American Fisheries Society* **5**, 30-35.

Wolke, R.E. (1992) Piscine macrophage aggregates: A review. *Annual Review of Fish Diseases* **2**, 91-108.

Yeomans, W.E., Chubb, J.C. and Sweeting, R.A. (1997) Use of protozoan communities for pollution monitoring. *Parassitologia* **39**, 201-212.

Zar, J.H. (1984) *Biostatistical Analysis*, Prentice-Hall, Inc., Englewood Cliffs, New Jersey.