

# Joint effects of parasitism and pollution on oxidative stress biomarkers in yellow perch *Perca flavescens*

David J. Marcogliese\*, Lila Gagnon Brambilla, François Gagné, Andrée D. Gendron

Environment Canada, St. Lawrence Centre, 105 McGill, 7th Floor, Montreal, Quebec H2Y 2E7, Canada

**ABSTRACT:** Yellow perch *Perca flavescens* were collected from a contaminated site and a reference site in the St. Lawrence River, Quebec, Canada. Fish were assessed for oxidative stress (lipid peroxidation and reduced glutathione levels) and parasitism by the nematode *Raphidascaris acus* and metacercariae of the digenean *Apophallus brevis*. Lipid peroxidation is not only considered a measure of oxidative stress, but of stress in general, and thus serves as an indicator of fish health. Fish from the contaminated site exhibited higher levels of lipid peroxidation than those from the reference site, independent of parasitic infections. However, fish infected with *R. acus* at the contaminated site tended to have higher levels of lipid peroxidation than uninfected fish at the same site, whereas no difference was observed between infected and uninfected fish at the reference site. Yellow perch infected with >10 metacercariae of *A. brevis* expressed higher levels of lipid peroxidation than those infected with <10 metacercariae at both the contaminated and the reference sites. No differences were found in levels of reduced glutathione in liver or muscle in relation to site or either parasite species. Results support the use of lipid peroxidation as a biomarker of water contamination. They further suggest that lipid peroxidation may be used as a biomarker of pathological effects caused by parasitism. Most importantly, results demonstrate that contaminants and parasites occurring together exacerbate oxidative stress in fish, suggesting that parasitized fish in polluted environments are in a poorer state of health than uninfected fish.

**KEY WORDS:** Lipid peroxidation · Pollution · Parasitism · Stress · Yellow perch · *Perca flavescens* · *Raphidascaris acus* · *Apophallus brevis*

Resale or republication not permitted without written consent of the publisher

## INTRODUCTION

Aquatic organisms are exposed to multiple stressors, including contaminants, disease and temperature extremes, throughout their lives. Extensive research has been conducted on the impact of various kinds of contaminants and other stressors on the growth, reproduction, physiology and behaviour of these organisms, primarily fish. However, rarely have studies examined the impact of multiple kinds of stressors on animals.

In particular, while it is important to know the effects of anthropogenic stressors on organisms for

proper management of aquatic resources and control of the production and release of toxic chemicals, environmental scientists must realize that animals in nature are also subject to continual or repeated exposure to an important natural stressor, that is, parasitism. Given that the detrimental effects of parasites are exacerbated when their hosts are stressed, it thus becomes paramount to determine the combined effects of anthropogenic stressors such as contaminants and natural stressors such as parasites. In combination, pollution and parasitism may act additively or synergistically on the health of an organism, thus rendering it in poorer condition than would be pre-

\*Email: david.marcogliese@ec.gc.ca

dicted by toxicological tests on healthy, unparasitized animals. Given the ubiquity of parasitism, and the fact that virtually all organisms are parasitized, this question has serious implications for environmental management.

For example, in a recent study Jacobson et al. (2003) effectively demonstrated that a combination of anthropogenic stressor, the PCB mixture Aroclor 1254, and a natural stressor, the digenean parasite *Nanophyetes salmonicola*, has a greater negative effect than either of these alone, on B cell function and immunosuppression in juvenile Chinook salmon *Oncorhynchus tshawytscha*.

Our study was conducted in the St. Lawrence River, where pollution levels are considered low to moderate depending on the location. Thus, most toxicological effects in the river are typically of a sublethal and chronic nature. We measured oxidative stress as an indicator of tissue damage and stress, using lipid peroxidation (Di Giulio et al. 1989, Kelly et al. 1998) and thiols in tissue homogenates (Dickinson & Forman 2002).

Increased reactive oxygen species in tissues during inflammatory or toxic conditions leads to the oxidation damage to polyunsaturated lipids (lipid peroxidation), proteins (e.g. formation of imino-propene bridges) and the genome (DNA damage) (Storey 1996). Parasites have been shown to induce oxidative stress in host-parasite systems. An increased enzyme antioxidant response was observed in carp *Cyprinus carpio* parasitized by the cestode *Ptychobothrium* sp. compared to healthy fish, indicating that some components of the fish antioxidant system can be modulated (Dautremepuits et al. 2002, 2003). Belló et al. (2000) demonstrated an increased tert-butyl hydroperoxide-initiated chemiluminescence, a measure of lipid peroxidation, in the muscle of fish infected with metacercariae of the trematode *Clinostomum detrunctum*. Gastrointestinal nematodes of mammals rely on proteinases and oxidative damage for tissue penetration and nutrients (Koski & Scott 2003).

We focused on 2 parasites common in our study areas as representative of potential natural stressors. The nematode *Raphidascaris acus* is known to cause liver pathology, poorer condition and parasite-induced host mortality in yellow perch *Perca flavescens*, its intermediate host, and other fishes (Bauer & Zmerzlaja 1973, Dergaleva & Markevich 1976, Szalai & Dick 1991, Szalai et al. 1992). Metacercariae of the digenean *Apophallus brevis* cause a reduction in growth and reproduction in yellow perch, in addition to host mortality (Johnson & Dick 2001). Biomarkers of oxidative stress were measured in yellow perch with both low and high parasite levels from relatively contaminated and pristine sites in the river.

## MATERIALS AND METHODS

Yellow perch were collected from 2 sites, 1 contaminated and 1 reference, in the southern part of Lake St. Louis, a fluvial lake southwest of Montreal in the St. Lawrence River in June 2002 (Fig. 1). The contaminated site was located east of Beauharnois where the Saint-Louis River enters Lake St. Louis (45° 19.051' N, 73° 53.020' W). At this site, levels of cadmium, copper, lead, zinc and PCBs exceeded the Threshold of Significant Contamination (TSC), and mercury exceeds the Toxic Effect Threshold (TET), as defined for the St. Lawrence River (Loiselle et al. 1997). The reference site was Dorval Island (45° 25.915' N, 73° 44.288' W), where no substances have been measured above the TSC (Loiselle et al. 1997). Fish from the 2 sites are considered distinct populations, as they are separated by a maritime navigation channel that acts as a barrier to perch movement (Dumont 1996). They are abundant at both sites. Moreover, there were no signs of stunting (reduced mean size in each age class) at either site, which would be indicative of competitive stress. Yellow perch were subsampled from fish collected with a beach seine (22.6 m × 1.15 m; 3 mm mesh) from Beauharnois on 28 May and 6 June 2002, and from Dorval Island on 3 June 2002, thus controlling for the confounding effects of season on expression of the biomarkers. Collections were performed until a sufficient number (≥30) of small fish were obtained from each site for analysis. Fish were transported alive in aerated polystyrene containers to the laboratory. Temperature, conductivity and pH were measured with a TSI conductivity meter. Sediment samples were collected from each site and sent to the national laboratory at the Canadian Centre for Inland Waters in Burlington, Ontario, for analysis.

In the laboratory, fish were maintained in aerated aquaria at 15°C for up to 72 h. Each individual was killed by cervical dislocation, weighed and measured for fork length. Liver and muscle tissue samples (3 g) were removed from each individual. In the case of infected individuals, parasites were removed and counted, and tissue was removed from the vicinity of an encysted parasite, without including any parasite or cyst tissue. Opercular bones and scales were removed from each fish for age determination (Jearld 1983). The right and left opercula were detached, boiled in distilled water, and the flesh removed. Approximately 10 to 15 scales were removed from the left flank under the pectoral fin. Scales were mounted on slides and covered with coverslips for readings. Age is determined by the number of annuli or year marks on the scales/opercula. In the case of yellow perch, age is primarily determined using the opercula; scales are used for verification.

Liver samples were weighed and kept on crushed ice in plastic microtubules (1.5 ml). Each liver sample was homogenized using a 'Potter-Eveljhem' tissue grinder (Teflon Homogenizer, Fisher Scientific) immediately after the tissue extraction. The tissue was mixed with a sufficient quantity of ice-cold homogenation buffer (120 mM NaCl, 25 mM Tris-acetate, 1 mM EDTA, pH 7.5) to obtain a 20% (w/v) homogenate. The homogenate was distributed in aliquots in pre-identified plastic microtubes and frozen at  $-80^{\circ}\text{C}$  until required for analysis. Muscle tissue was weighed and frozen immediately at  $-80^{\circ}\text{C}$  until required for analyses. In preparation for analysis, the muscle tissue was thawed and mixed with a sufficient quantity of homogenation buffer (120 mM NaCl, 25 mM Tris-acetate, 1 mM EDTA, pH 7.5) so that a 20% (w/v) homogenate was obtained using a Polytron™ (Fisher Scientific). The homogenate was distributed in preidentified plastic microtubes (aliquots) and conserved on crushed ice for analysis.

The thiobarbituric acid (TBA) test for malonaldehyde (MDA) was used to measure and quantify lipid

peroxidation. This assay is the most widely employed method to measure lipid peroxidation (Di Giulio et al. 1989). MDA is the major aldehyde formed during the lipid peroxidation reaction (Lefèvre et al. 1998). In a black opaque 96 well microplate, 100  $\mu\text{l}$  of a trichloroacetic acid solution (TCA 20%,  $\text{FeSO}_4$  1 mM) and 50  $\mu\text{l}$  of a thiobarbituric acid solution (TBA 0.67%) were added to 25  $\mu\text{l}$  of each tissue homogenate (diluted  $\frac{1}{10}$  in distilled water for liver and non-diluted for muscle) and to 25  $\mu\text{l}$  of the homogenization buffer, diluted or not (blank). The microplate was heated for 10 minutes at  $80^{\circ}\text{C}$  in a temperature-controlled water bath. After 10 min, the microplate was read at 516 nm (excitation) and 600 nm (emission) using a fluorimeter (Fluorolite 1000). For calibration, standard solutions of tetramethoxypropane (TMP 0.001% diluted in HCl 0.1 M) were used in the presence of the homogenization buffer. The results obtained are expressed in  $\mu\text{g}$  of thiobarbituric acid reactants (TBARS)  $\text{g}^{-1}$  of protein. All measurements were performed in duplicate for each sample and in triplicate for the standards.

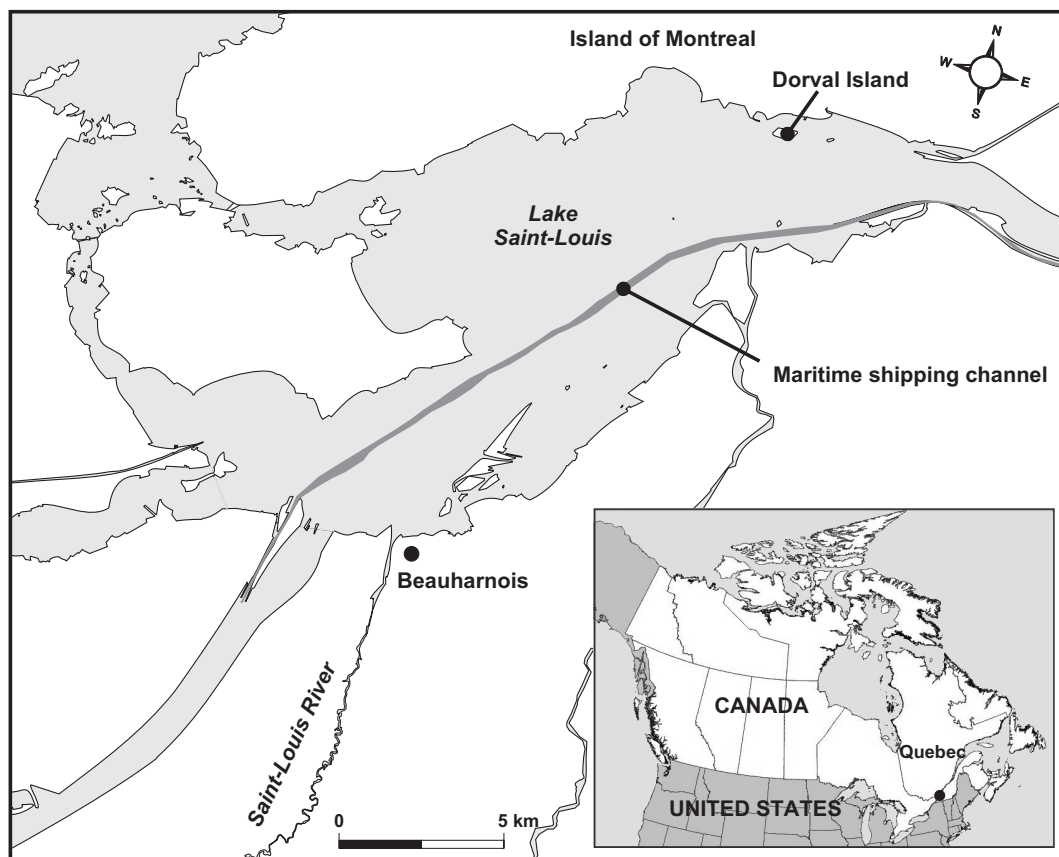


Fig. 1. Sample sites in Lake St. Louis, a fluvial lake in the St. Lawrence River. The contaminated site is located at Beauharnois, at the mouth of the Saint-Louis River. The reference site is located at Dorval Island. The sites are separated by the Maritime shipping canal

Reduced thiol (-SH) was measured using the dithionitrobenzoate (DTNB) reagent method (Sedlak & Lindsay 1968). This simple method permits the quantification of molecules having a sulfhydryl group (-SH) using a spectrophotometric method. To determine the total sulfhydryl groups (T-SH) of each sample, 100  $\mu\text{l}$  of 0.5 mM DTNB reagent (5,5'-dithiobis-2-nitrobenzoate in 100 mM glycine buffer, pH 8.5) was added to 100  $\mu\text{l}$  of each tissue homogenate (previously diluted  $\frac{1}{10}$  to  $\frac{1}{40}$  in distilled water) in a clear 96 well microplate. Absorbance (optical density) was read at 412 nm after mixing for 10 min (PowerWaveX, Bio-Tek Instruments). Calibration was achieved with reduced glutathione (100  $\mu\text{g ml}^{-1}$  stabilized in HCl 0.1 M) and results obtained are expressed in  $\mu\text{g}$  GSH equivalents per g of protein. All measurements were performed in duplicate for each sample and in triplicate for the standards. Tissue levels of reduced thiols and thiobarbituric acid reactant substances (TBARS) were normalized by total protein levels as determined by the protein-dye principle (Bradford, 1976). The optical density was read at 595 nm and serum bovine albumin was used for calibration.

Results were analyzed using the JMPin<sup>®</sup> software (SAS Institute). First, a correlation matrix was carried out to draw up the possible links between the different continuous variables. Data were normalized using rank transformation. One-way analysis of variance (ANOVA) was used to establish the relationship between the different stress biomarkers (continuous variables) and class values (i.e. discreet variables) 'site', 'sex' and 'infected/uninfected' for each parasite. Two-way ANOVAs were used to determine if there was an effect of the site, of parasitism, or any interaction between the two on the oxidative stress measurements. Significance was defined as  $p \leq 0.05$ .

Yellow perch normally spawn at age 3 and 4 for males and females, respectively (Scott & Crossman 1973), after which they disperse in Lake St. Louis (Dumont 1996). Analyses were restricted to 1+ and 2+ fish to avoid potential confounding effects of age and maturity on biomarkers. Older fish that were collected were not considered. In addition, by restricting analyses to young fish, we increased the likelihood that we were dealing with local populations that were exposed to local environmental conditions.

## RESULTS

At Beauharnois, the temperature, conductivity and pH were 15.0°C, 321  $\mu\text{S}$ , and 8.3 respectively, and at Dorval Island, 15.7°C, 209.2  $\mu\text{S}$ , and 8.5, respectively. Measurements of metals in the sediments at both sites are shown in Table 1. The principal difference

between sites was found for mercury, this being much higher at Beauharnois.

There were no differences between levels of lipid peroxidation or reduced thiols in 1+ and 2+ fish in both the liver and the muscle (data not shown) (1-way ANOVA,  $p > 0.05$ ). Nor was there any difference in lipid peroxidation or reduced thiol levels in liver or muscle between males and females (data not shown) (1-way ANOVA,  $p > 0.05$ ). Thus, fish of the 2 age groups and sexes could be combined for further analyses of lipid peroxidation and reduced thiols.

Yellow perch from the contaminated site displayed significantly higher lipid peroxidation induction ( $531 \pm 31 \mu\text{g TBARS g}^{-1}$  protein) in the liver than those from the reference site ( $307 \pm 38 \mu\text{g TBARS g}^{-1}$  protein) (1-way ANOVA,  $p < 0.001$ ). For muscle tissue, there was no significant difference in lipid peroxidation levels in fish from the contaminated site ( $6.50 \pm 1.06 \mu\text{g TBARS g}^{-1}$  protein) and those from the reference site ( $4.83 \pm 1.06 \mu\text{g TBARS g}^{-1}$  protein) (1-way ANOVA,  $p > 0.05$ ).

Mean numbers of *Raphidascaris acus* per fish did not differ between the reference site ( $0.53 \pm 1.02$ ) and the

Table 1. Measurements of metals (ppm) in sediments from the contaminated site (Beauharnois) and the reference site (Dorval Island) in Lake St. Louis, a fluvial lake in the St. Lawrence River, Quebec, Canada

Metal	Beauharnois	Dorval Island
Aluminum	59 700	56 600
Antimony	1.0	0.3
Arsenic	2	<1
Barium	1410	604
Beryllium	1.62	1.23
Bismuth	0.2	0.1
Calcium	41 200	85 300
Cadmium	0.8	1.0
Chromium	93	49
Cobalt	19.7	9.3
Copper	56	11
Iron	36 500	27 900
Gallium	19.7	13.0
Lanthanum	34.4	32.1
Lead	58.9	23.8
Lithium	35.7	17.0
Magnesium	16 900	15 300
Manganese	918	565
Mercury	6.76	0.100
Molybdenum	1.5	0.7
Nickel	54.4	20.4
Phosphorus	845	1300
Potassium	19 600	20 100
Rubidium	88.4	61.1
Sodium	11 000	15 700
Strontium	269	450
Thallium	0.518	0.353
Uranium	1.79	1.32
Vanadium	85	67
Zinc	201	159

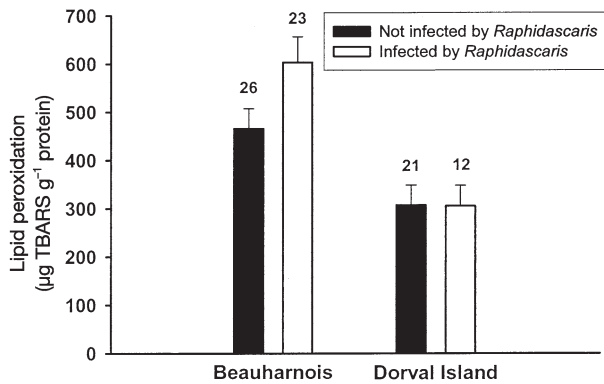


Fig. 2. Measurements of lipid peroxidation ( $\mu\text{g TBARS g}^{-1}$  protein) in the livers of yellow perch *Perca flavescens* from the contaminated site (Beauharnois) and the reference site (Dorval Island). The black histograms refer to those fish not infected with *Raphidascaris acus*, and the open histograms refer to infected fish. Error bars: SE. Numbers above histograms refer to the sample size

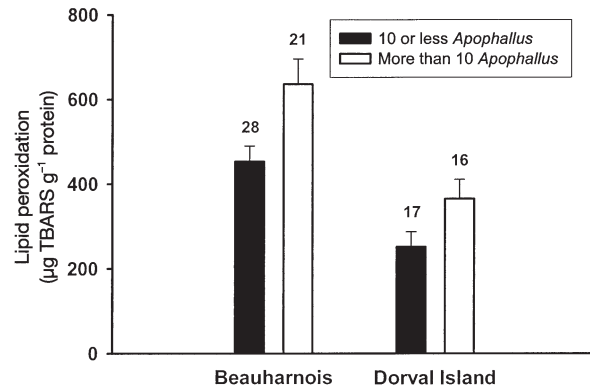


Fig. 3. Measurements of lipid peroxidation ( $\mu\text{g TBARS g}^{-1}$  protein) in the livers of yellow perch *Perca flavescens* from the contaminated site (Beauharnois) and the reference site (Dorval Island). The black histograms refer to those fish each infected with  $\leq 10$  *Apophallus brevis*, and the open histograms refer to fish infected with  $>10$  metacercariae. Error bars: SE. Numbers above histograms refer to the sample size

contaminated site ( $1.06 \pm 1.57$ ) (*t*-test for unequal variances,  $p > 0.05$ ). Livers of 1+ and 2+ individuals infected with *R. acus* displayed significantly higher levels of lipid peroxidation than those of uninfected individuals (1-way ANOVA,  $p < 0.05$ ) at the contaminated site, but not at the reference site (Fig. 2). Results of the 2-way ANOVA indicate that site ( $p < 0.0001$ ) and parasite infection with *R. acus* ( $p = 0.05$ ) are significant, but the interaction between the two is not ( $p > 0.05$ ) (Table 2). Mean numbers of *Apophallus brevis* per fish did not differ in individuals from the reference site ( $12.38 \pm 11.52$ ) and the contaminated site ( $10.25 \pm 7.02$ ) (*t*-test for unequal variances,  $p > 0.05$ ). Livers of 1+ and 2+ yellow perch infected with  $>10$  metacercariae of *A. brevis* exhibited significantly greater levels of lipid peroxidation than those with  $\leq 10$  metacercariae independent of site (2-way ANOVA,  $p < 0.05$ ) (Fig. 3). Furthermore, there was a significant difference between sites, independent of the level of infection (2-way ANOVA,  $p < 0.05$ ; Table 2). The interaction between *A. brevis* and site was not significant (2-way ANOVA,  $p > 0.05$ ; Table 2).

For reduced thiols, no significant difference in levels in liver or muscle was observed in relation to site or to either parasite species (data not shown) (1-way ANOVA,  $p > 0.05$ ).

### DISCUSSION

Our initial results strongly suggest that the effects of stress from residing

in polluted waters can be enhanced in fish by parasitic infection, as measured by lipid peroxidation production. While we cannot conclusively state that the greater induction of lipid peroxidation is due to contaminants, certainly the high levels of mercury there strongly suggest that this is indeed the case. Levels above 1 ppm are considered elevated (Eisler 2000). However, other unknown ecological site differences that were not measured herein may have contributed to the difference. Uncontrolled variables that may affect biomarker response include age, sex, diet and gonadal status, as well as season, temperature, and toxicant concentration (Di Giulio et al. 1989). Most of these variables were controlled for in this study, with the exception of diet, which, given the differences in mercury contamination, we do not consider an overriding factor.

Table 2. Two-way ANOVA in levels of lipid peroxidation ( $\mu\text{g TBARS g}^{-1}$  protein) in yellow perch *Perca flavescens* by site (contaminated vs reference) and by parasitic infection (infected by *Raphidascaris acus* vs. uninfected; infected with  $>10$  metacercariae of *Apophallus brevis* vs. those with  $\leq 10$  metacercariae). Data were rank-transformed prior to analysis. SS = sum of squares; MS = mean of squares

Source of variation	df	SS	MS	F	p
<i>Raphidascaris acus</i>					
Site	1	11 756.00	11 756.00	28.39	<0.0001
Infection	1	1 589.43	1 589.43	3.84	0.0537
Site $\times$ Infection	1	295.53	295.53	0.71	0.4008
<i>Apophallus brevis</i>					
Site	1	11 104.57	11 104.57	29.98	<0.0001
Infection	1	3 931.51	3 931.51	10.37	0.0019
Site $\times$ Infection	1	39.93	39.93	0.11	0.7465

This is not the first study to document interactions between parasites and contaminants in aquatic organisms, but it is among the first to show enhanced sublethal stress effects in the presence of both contaminants and parasites. Parasitism effectively reduces survival of aquatic organisms in polluted conditions. Sockeye salmon *Oncorhynchus nerka* smolts infected with the cestode *Eubothrium salvelini* were less tolerant to zinc than uninfected fish (Boyce & Yamada 1977). Coho salmon fry *Oncorhynchus kisutch* infected with glochidia of *Anodonta oregonensis* were more susceptible to crude oil, toluene, and naphthalene than uninfected fish (Moles 1980). Three-spined sticklebacks *Gasterosteus aculeatus* infected with plerocercoids of the cestode *Schistocephalus solidus* experienced decreased survival upon exposure to cadmium compared with uninfected fish (Pascoe & Cram 1977, Pascoe & Woodworth 1980). The amphipod *Gammarus pulex* infected with the acanthocephalan *Pomphorhynchus laevis* suffered increased mortality compared to uninfected amphipods when exposed to cadmium (Brown & Pascoe 1989) or aluminum at low pH (McCahon et al. 1988, McCahon & Poulton 1991). The snail *Lymnaea stagnalis* infected with the trematodes *Schistosoma douthitti* or *Trichobilharzia* sp. experienced increased mortality when exposed to high concentrations of zinc when compared with uninfected conspecifics (Guth et al. 1977). However, the effects of joint stress on parasitized animals are not limited to anthropogenic stressors. For example, infection with the ectoparasitic copepod *Salmincola edwardsii* reduced resistance of brook trout *Salvelinus fontinalis* to high temperature (Vaughn & Coble 1975). Three-spined sticklebacks infected with *S. solidus* died under conditions of dietary stress before uninfected fish (Pascoe & Matthey 1977). Infection with the isopod *Bopyroides hippolytes* reduced tolerance to salinity change in the kelp shrimp *Eualus suckleyi* (Moles & Pella 1984). Gastropods infected with larval digeneans were less resistant to desiccation and hypoxia (Jensen et al. 1996, Wegeberg & Jensen 1999). Lastly, juvenile Chinook salmon *Oncorhynchus tshawytscha* infected with metacercariae of the trematode *Nanophyetus salmonicola* exposed to PCBs suffered higher mortalities when challenged with the pathogenic bacterium *Listonella anguillarum* (Jacobson et al. 2003).

Less information exists on sublethal effects of parasites, which are naturally occurring stressors, and anthropogenic contaminants. Striped bass infected with larval nematodes *Anisakis* sp. exposed to zinc and benzene experienced decreased haematocrit values compared to uninfected fish (Sakanari et al. 1984). More recently, Jacobson et al. (2003) elegantly demonstrated that juvenile Chinook salmon infected with *Nanophyetus salmonicola* and exposed to PCBs dis-

played a decreased immune response measured by a hemolytic plaque-forming cell assay. Thus, these studies along with the results herein provide ample evidence suggesting that fish exposed to sublethal levels of contaminants experience greater stress when infected by parasites, or in other words, parasites and toxicants may act together to increase stress.

Our results also provide further support for the use of lipid peroxidation as an indicator of stress resulting from exposure to contaminants. Peroxidation of cellular lipids results from oxyradical production, which is produced upon exposure to xenobiotics (Kelly et al. 1998). Lipid peroxidation is essentially a toxic response to oxidative damage to cellular and tissue components (Di Giulio et al. 1989, Storey 1996, Kelly et al. 1998). Yet, there is a need for studies that link oxidative stress and disease (Kelly et al. 1998). The use of lipid peroxidation as an indicator of general stress is also supported in that it can be induced by parasitic infection with both *Raphidascaris acus* and *Apophallus brevis*. In measuring impacts of joint stressors, general biomarkers are essential, as specific biomarkers may not respond to one of the stressors, or indeed, may even be compromised by another agent of stress. However, measurements of biomarkers may vary with age, reproductive status, sex, season, temperature and other uncontrolled variables (Di Giulio et al. 1989). In this study, we controlled for age and reproductive status by sampling immature fish of specific ages, and for season and temperature by sampling at the same time. Nor were any differences found between the 1+ and 2+ age groups or between the sexes.

In addition, our results also suggest that lipid peroxidation can be used in a comparative manner to measure the degree of pathogenicity exerted by different parasites. While infection with *Raphidascaris acus* did not induce greater lipid peroxidation in individuals compared to infected fish from the reference site, fish infected with >10 metacercariae of *Apophallus brevis* demonstrated enhanced lipid peroxidation compared to those infected with ≤10 metacercariae at the same site. These results imply that *A. brevis* may be more pathogenic than *R. acus*, at least at the infection levels considered. This conjecture must be accepted with caution because intensities of *R. acus* were lower than those of *A. brevis*. High infection levels of both *R. acus* and *A. brevis* are known to be pathogenic, but little is known of effects at low intensities. Dezfuli et al. (2000) observed an increase in rodlet cells in the liver and pancreas of minnows *Phoxinus phoxinus* infected with *R. acus* and suggested that these cells were involved in an inflammatory response to infection. Our results further support the existence of an inflammatory reaction to infection with this nematode, even at low intensities. Furthermore, results suggest that infection with *A.*

*brevis* can cause pathological effects away from the site of infection, as metacercariae are found on the skin and in muscle, but effects were measured in liver. This parasite is unique in that it induces a host reaction consisting of the formation of a mineralized ossicle or cyst (Taylor et al. 1993) which conceivably may pose an oxidative stress to the host as a result of the chronic inflammation at the infection site (Taylor et al. 1994). The effects of parasitic infection are all the more interesting because they were evident at relatively low intensities, especially for *R. acus*. Measurements of oxidative stress may be an effective means of indicating pathological effects of parasites at low intensities, where often typical pathology is not manifest.

We do not know why no differences were observed in reduced thiols between the various groups of yellow perch. Presumably oxidative stress should be detectable through an examination of reduced thiols. Perhaps results herein reflect a lower sensitivity of this parameter compared to the measurement of lipid peroxidation.

Few other studies have demonstrated sub-lethal effects of parasites through measurements of biomarkers. Metacercariae of *Clinostomum detrunctum* induce lipid peroxidation production in the muscle of the freshwater fish *Rhamdia quelen* (Belló et al. 2000), which the authors attributed to oxidative stress and membrane damage caused by the parasite. Carp infected with the intestinal cestode *Ptychobothrium* sp. displayed an increased antioxidant response compared to uninfected fish as measured by activities of the enzymes catalase and glutathione reductase in liver and glutathione s-transferase in both liver and kidney (Dautremepuits et al. 2002, 2003). The authors attributed this response to the increased metabolic activity of infected fish. Shrimp *Palaemonetes argentinus* infected with the isopod *Probopyrus ringueleti* exhibited lower activity of the antioxidant enzyme superoxidase dismutase, which presumably lowers the capacity of the organism to deal with reactive oxygen species and thus to prevent cellular damage (Neves et al. 2000). Other examples are reviewed in Sures (2004), who also recently reviewed the influence of parasites on biomarkers and discussed their possible impact on the physiological homeostasis of their hosts.

The typical approach in studying disease in an environmental context is to assume that pollution reduces the immunocompetence of the host, thus leading to increased disease and parasitism (Wedemeyer 1970, Snieszko 1974, Sindermann 1979, Möller 1985). More recently, researchers have acknowledged the potential for compounding and interactive effects of parasitism and anthropogenic factors (Khan & Thulin 1991, Overstreet 1993). It appears that parasitism in the presence of pollution may further compromise the health of the

host, even at low intensities. Given that most wild organisms are parasitized, biomarker measurements from ecotoxicological testing may underestimate the impact of pollutants in that results typically pertain to uninfected hosts in controlled laboratory situations. The combination of contaminants and parasites may prove to be more deleterious, and parasitism should not be neglected in environmental impact studies in order to obtain a more precise determination of the impacts of contaminants on natural ecosystems and the organisms found therein. In the unlikely scenario that some aspect of habitat quality other than contaminants caused a difference between sites in this study, clearly parasitism has an impact on lipid peroxidase production. It is evident that effects of these and other parasites on the physiological responses and biomarker measurements in their hosts should be explored further.

*Acknowledgements.* We thank Germain Brault, Stephanie Barbeau and Valérie Paquet for assistance in the field.

#### LITERATURE CITED

- Bauer ON, Zmerzlaja EI (1973) Influence of *Raphidascaris acus* (Nematoda, Anisakidae) on the bream, *Abramis brama*. Verh Internat Verein Limnol 18:1723–1728
- Belló ARR, Fortes E, Belló-Klein A, Belló A, Llesuy SF, Robaldo RB, Bianchini A (2000) Lipid peroxidation induced by *Clinostomum detrunctum* in muscle of the freshwater fish *Rhamdia quelen*. Dis Aquat Org 42:233–236
- Boyce NP, Yamada Y (1977) Effects of a parasite, *Eubothrium salvelini* (Cestoda: Pseudophyllidea), on the resistance of juvenile sockeye salmon, *Oncorhynchus nerka*, to zinc. J Fish Res Board Can 34:706–709
- Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
- Brown AF, Pascoe D (1989) Parasitism and host sensitivity to cadmium: an acanthocephalan infection of the freshwater amphipod *Gammarus pulex*. J Appl Ecol 26:473–487
- Dautremepuits C, Betoulle S, Vernet G (2002) Antioxidant response modulated by copper in healthy or parasitized carp (*Cyprinus carpio* L.) by *Ptychobothrium* sp. Biochim Biophys Acta 1573:4–8
- Dautremepuits C, Betoulle S, Vernet G (2003) Stimulation of antioxidant enzyme levels in carp (*Cyprinus carpio* L.) infected by *Ptychobothrium* sp. (Cestoda). Fish Shellfish Immunol 15:467–471
- Dergaleva ZT, Markevich NB (1976) The dynamics of the infestation of the silverside *Atherina mochon pontica* by the larvae of *Raphidascaris acus* (Nematoda, Ascaridata) in the Aral Sea and its influence on the physiological state of the fish. J Ichthyol 16:866–868
- Dezfuli BS, Simoni E, Rossi R, Manera M (2000) Rodlet cells and other inflammatory cells of *Phoxinus phoxinus* infected with *Raphidascaris acus* (Nematoda) Dis Aquat Org 43:61–69
- Dickinson DA, Forman HJ (2002) Cellular glutathione and thiols metabolism. Biochem Pharmacol 64:1019–1026
- Di Giulio RT, Washburn PC, Wenning RJ, Winston GW, Jewell CS (1989) Biochemical responses in aquatic animals: a

- review of determinants of oxidative stress. *Environ Toxicol Chem* 8:1103–1123
- Dumont P (1996) Comparaison de la dynamique des populations des perchaudes (*Perca flavescens*) soumises à niveaux différents de stress anthropiques. Ministère de l'Environnement et de la Faune, Service de l'aménagement et de l'exploitation de la faune, Montréal
- Eisler R (2000) Handbook of chemical risk assessment. Health hazards to humans, plants and animals, Vol 1. Metals. Lewis Publishers, Boca Raton, FL
- Guth DJ, Blankespoor HD, Cairns J Jr (1977) Potentiation of zinc stress caused by parasitic infection of snails. *Hydrobiologia* 55:225–229
- Jacobson KC, Arkoosh MR, Kagley AN, Clemons ER, Collier TK, Casillas E (2003) Cumulative effects of natural and anthropogenic stress on immune function and disease resistance in juvenile Chinook salmon. *J Aquat Anim Health* 15:1–12
- Jearld A Jr (1983) Age determination. In: Nielson LA, Johnson DL (eds) Fisheries techniques. Am Fisheries Soc, Bethesda, MD, p 301–324
- Jensen KT, Latama G, Mouritsen KN (1996) The effects of larval trematodes on the survival rates of two species of mud snails (Hydrobiidae) experimentally exposed to desiccation, freezing and anoxia. *Helgol Meeresunters* 50: 327–335
- Johnson MW, Dick TA (2001) Parasite effects on the survival, growth, and reproductive potential of yellow perch (*Perca flavescens* Mitchell) in Canadian Shield lakes. *Can J Zool* 79:1980–1992
- Kelly SA, Havrilla CM, Brady TC, Abramo KH, Levin ED (1998) Oxidative stress in toxicology: established mammalian and emerging piscine models. *Environ Health Perspect* 106:375–384
- Khan RA, Thulin J (1991) Influence of pollution on parasites of aquatic animals. *Adv Parasitol* 30:201–238
- Koski KG, Scott ME (2003) Gastrointestinal nematodes, trace elements, and immunity. *J Trace Elem Exp Med* 16: 237–251
- Lefèvre G, Beljean-Leymarie M, Beyerle F, Bonnefont-Rousselot D, Cristol JP, Théron P, Torreilles J (1998) Évaluation de la peroxydation lipidique par le dosage des substances réagissant avec l'acide thiobarbiturique. *Ann Biol Clin* 56 :305–319
- Loiselle C, Fortin GR, Lorraine S, Pelletier M (1997) Dynamics and contamination of St. Lawrence River sediment. State of the Environment, St. Lawrence Centre, Environment Canada, Montreal
- McCahon CP, Poulton MJ (1991) Lethal and sub-lethal effects of acid, aluminium and lime on *Gammarus pulex* during repeated simulated episodes in a Welsh stream. *Freshw Biol* 25:169–178
- McCahon CP, Brown AF, Pascoe (1988) The effect of the acanthocephalan *Pomphorhynchus laevis* (Müller 1776) on the acute toxicity of cadmium to its intermediate host, the amphipod *Gammarus pulex* (L.). *Arch Environ Contam Toxicol* 17:239–243
- Moles A (1980) Sensitivity of parasitized coho salmon fry to crude oil, toluene, and naphthalene. *Trans Am Fish Soc* 109:293–297
- Moles A, Pella JJ (1984) Effects of parasitism and temperature on salinity tolerance of the kelp shrimp *Eualus suckleyi*. *Trans Am Fish Soc* 113:354–359
- Möller H (1985) A critical review on the role of pollution as a cause of fish disorders. In: Ellis AE (ed) Fish and shellfish pathology. Academic Press, London, p 169–182
- Neves CA, Santos EA, Baily ACD (2000) Reduced superoxide dismutase activity in *Palaemonetes argentinus* (Decapoda, Palaemonidae) infected by *Probopyrus ringueleti* (Isopoda, Bopyridae) *Dis Aquat Org* 39:155–158
- Overstreet RM (1993) Parasitic diseases of fishes and their relationship with toxicants and other environmental factors. In: Couch JA, Fournie JW (eds) Pathobiology of marine and estuarine organisms. CRC Press, Boca Raton, FL, p 111–156
- Pascoe D, Cram P (1977) The effect of parasitism on the toxicity of cadmium to the three-spined stickleback, *Gasterosteus aculeatus* L. *J Fish Biol* 10:467–472
- Pascoe D, Matthey D (1977) Dietary stress in parasitized and non-parasitized *Gasterosteus aculeatus* L. *Z Parasitenkd* 51:179–186
- Pascoe D, Woodworth J (1980) The effects of joint stress on sticklebacks. *Z Parasitenkd* 62:159–163
- Sakanari JA, Moser M, Reilly CA, Yoshino TP (1984) Effects of sublethal concentrations of zinc and benzene on striped bass, *Morone saxatilis* (Walbaum), infected with larval *Anisakis* nematodes. *J Fish Biol* 24:553–563
- Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. Bulletin 184. Fisheries Research Board of Canada, Ottawa
- Sedlak J, Lindsay RH (1968) Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 25:192–205
- Sindermann CJ (1979) Pollution-associated diseases and abnormalities of fish and shellfish: a review. *Fish Bull* 76: 717–749
- Snieszko SF (1974) The effects of environmental stress on outbreaks of infectious diseases of fishes. *J Fish Biol* 6: 197–208
- Storey KB (1996) Oxidative stress: animal adaptations in nature. *Braz J Med Biol Res* 29:1715–1733
- Sures B (2004) Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Trends Parasitol* 20:170–177
- Szalai AJ, Dick TA (1991) Role of predation and parasitism in growth and mortality of yellow perch in Dauphin Lake, Manitoba. *Trans Am Fish Soc* 120:739–751
- Szalai AJ, Lysack W, Dick TA (1992) Use of confidence ellipses to detect effects of parasites on the growth of yellow perch, *Perca flavescens*. *J Parasitol* 78:64–69
- Taylor LH, Hall BK, Cone DK (1993) Experimental infection of yellow perch (*Perca flavescens*) with *Apophallus brevis* (Digenea, Heterophyidae): parasite invasion, encystment, and ossicle development. *Can J Zool* 71:1886–1994
- Taylor LH, Hall BK, Miyake T, Cone DK (1994) Ectopic ossicles associated with metacercariae of *Apophallus brevis* (Trematoda) in yellow perch, *Perca flavescens* (Teleostei): development and identification of bone and chondroid bone. *Anat Embryol* 190:29–46
- Vaughan GE, Coble DW (1975) Sublethal effects of three ectoparasites on fish. *J Fish Biol* 7:283–294
- Wedemeyer G (1970) The role of stress in the disease resistance of fish. *Spec Publ Am Fish Soc* 5:30–35
- Wegeberg AM, Jensen KT (1999) Reduced survivorship of *Himasthla* (Trematoda, Digenea)-infected cockles (*Cerastoderma edule*) exposed to oxygen depletion. *J Sea Res* 42: 325–331