Immunomodulation in Blue Mussels (Mytilus edulis and Mytilus trossulus) Exposed to Municipal Effluent in Eastern Canada

Camille Coray,1,4 Sylvie D. St.-Jean,3,4 and Shannon Mala Bard1,2*

1 Department of Biology and 2 Environmental Science, Dalhousie University, Room 820, Life Science Centre, 1355 Oxford Street, Halifax, NS B3H 4J1
3 Environment Canada, Burlington, ON L7R 4A6
4 Present address: Jacques Whitford Limited, Moncton, NB E1H 3T3

The effects on the immune response of the mussels Mytilus edulis and Mytilus trossulus from of life-time exposure to marine pollution found in an industrial shipping port with a military history (Halifax Harbour, Nova Scotia, Canada) were investigated. Parameters measured included phagocytic activity, cellular production of hydrogen peroxide, number of circulating haemocytes, and cellular viability. Mussels sampled within Halifax Harbour had significantly reduced phagocytic activity and significantly reduced production of hydrogen peroxide in comparison with mussels sampled from a reference site outside of Halifax Harbour, indicating that pollution induced immunomodulation. No significant differences were found in number of circulating haemocytes or in cellular viability between mussels sampled within Halifax Harbour and mussels sampled outside Halifax Harbour. Results are discussed in terms of using a multi-assay approach for monitoring environmental pollution.

Key words: immunomodulation, phagocytic activity, hydrogen peroxide production, Mytilus edulis, Mytilus trossulus, Halifax Harbour

Introduction

Mussels, both freshwater and marine, are well suited to measure aquatic environmental impacts from anthropogenic contaminants. Mussels are widely distributed, sessile filter-feeders, and accumulate certain contaminants at levels exceeding those found in ambient water due to metabolic limitations (Widdows and Donkin 1992). Mussels have often been used as indicator species because they are pollution tolerant and are able to survive in highly contaminated sites (The Gulf of Maine Council on the Marine Environment 1998; Martel et al. 2003; Moles and Hale 2003).

Previous studies have examined immunological responses in mussels as a way of pinpointing negative effects of specific contaminants on bivalve health (Coles et al. 1995; Grundy et al. 1996; López et al. 1997; Pipe et al. 1999; St-Jean et al. 2002a; St-Jean et al. 2002b). In marine environments, Mytilus mussels exposed to a variety of chemicals found in municipal effluent have been shown to exhibit signs of immunological stress including, but not limited to, changes in phagocytic activity (Grundy et al. 1996; St-Jean et al. 2002a; St-Jean et al. 2002b; St-Jean et al. 2003), changes in haemocyte counts (St-Jean et al. 2002a; St-Jean et al. 2002b; St-Jean et al. 2003; Luengen et al. 2004), reduced ability to clear bacteria from their systems (Mayrand et al. 2005; St-Jean et al. 2002a; St-Jean et al. 2002b), and changes in the release of reactive oxygen metabolites (Pipe and Coles 1995). Phagocytosis and cytotoxicity through the production of reactive oxygen intermediates (O₂⁻, H₂O₂, NO) have been cited as the main immune functions executed by haemocytes in bivalves (Pipe 1992; López et al. 1997).

Previous pollution monitoring studies in Halifax Harbour, Nova Scotia, Canada have focused primarily on measuring contaminant levels in abiotic components (water and sediments) (Buckley and Winters 1992; Buckley et al. 1995; King et al. 2002), or assessing water quality through bacteriological surveys aimed at assessing human health risks (Halifax Harbour Cleanup Project 1993; Halifax Regional Municipality 1999). Indiscriminate discharge of untreated sewage and industrial waste for over 250 years has resulted in Halifax Harbour being ranked amongst the most highly contaminated of marine harbours (Gearing et al. 1991; Buckley and Winters 1992).

Studies of Mytilus edulis and Mytilus trossulus populations in Halifax Harbour have assessed bioaccumulation of metals (Ward 1990; Hellou et al. 2003), organotins (Carter et al. 2004), polycyclic aromatic compounds (PACs) (Hellou et al. 2002; Hellou et al. 2005), and chlorobiphenyls (King et al. 2002). Stress responses of M. edulis and M. trossulus sampled within the harbour showed that shorter survival times in air were linked with higher bioaccumulation of PACs (Hellou and Law 2003). Several bioindicators of health, including lipid content and vitellins (egg yolk proteins) were compared with bioaccumulation of PACs, coprostanol, and metals in M. edulis collected at three sites within the harbour (Hellou et al. 2003). Male mussels displaying the highest levels of PACs, coprostanol, silver (Ag), and tin (Sn) had high lipid content (possibly indicating low use of energy reserves due to hindered gametogenesis), and female mussels displaying the highest levels of PACs, coprostanol, Ag, and Sn had a delayed production of vitellins. Coprostanol...
is a sewage marker derived from the bacterial reduction of cholesterol in the human intestine, so its presence in mussel tissues confirms exposure to sewage effluents and illuminates the possibility that exposure to sewage effluent may be impacting the reproductive health of mussels within Halifax Harbour (Hellou et al. 2003).

Immune responses to contaminants have been shown to differ between *Mytilus* species, with *M. trossulus* tending to be more sensitive than other *Mytilus* species (Hellou and Law 2003; Luengen et al. 2004). Both *M. edulis* and *M. trossulus* are native to the Halifax Regional Municipality area of Nova Scotia, Canada. Despite numerous laboratory studies conducted to assess adverse immunological affects of specific chemicals on freshwater and marine bivalves, limited information is available concerning the potential effects caused by long-term exposure to municipal pollution in *M. edulis* and *M. trossulus*. The objective of the present study was to augment this limited information through the investigation of immune parameters in both *M. edulis* and *M. trossulus* exposed to untreated municipal effluent at sites within Halifax Harbour, and compare measurements of immune function with those obtained from *M. edulis* and *M. trossulus* collected from a relatively pristine control site outside of Halifax Harbour. Parameters included haemocyte count and cellular viability, and production of hydrogen peroxide (H$_2$O$_2$) and phagocytic activity (PA). Sewage is such a complex mixture of substances that it can be very difficult to choose appropriate endpoints with which to assess effects of sewage exposure on the ecological health of the receiving environment. The immune parameters investigated in this study have been shown to be modulated by a number of chemicals found in municipal sewage, and are thus a useful way to detect general health effects of sewage exposure on biota.

**Materials and Methods**

**Study Sites and Physical/Chemical Environment**

Halifax Harbour is fed by over 100 untreated sewage outfalls, 39 of which are municipal and alone discharge 135 million litres of raw effluent daily (Halifax Harbour Solutions Project 1993). Throughout Halifax Harbour, Nova Scotia, Canada, contaminants associated with fluvial drainage systems and sewer outfalls have been identified in surface sediments (Buckley and Winters 1992). Sediment contamination has been divided into four types for Halifax Harbour: primary contamination from untreated sewage outfalls, surface drainage contamination from direct land runoff and freshwater flumes, secondary contamination from leaching from the city dump and from the shipyards, and diagenetic contamination (by-products generated during the chemical and physical change associated with the conversion of deposited sediment into rock) (Buckley and Winters 1992). Primary contamination is characterized by high levels of organic carbon (C), iron (Fe), zinc (Zn), copper (Cu), lead (Pb), chromium (Cr), nickel (Ni), cadmium (Cd) and mercury (Hg); surface drainage is characterized by high levels of aluminum (Al), magnesium (Mg), potassium (K), lithium (Li), Fe, and manganese (Mn); secondary contamination is characterized by high levels of Cu; diagenetic contamination is characterized by high levels of silicon (Si), titanium (Ti), and Mn (Buckley and Winters 1992). Within Halifax Harbour, three sites (Birch Cove, Dartmouth Cove, Point Pleasant) with varying levels and types of contamination were sampled for the current investigation, and one reference site (Long Cove Pond) was chosen well outside the plume of effluent emanating from the harbour, as determined by levels of faecal coliform in water samples (Environment Canada 2006) (Fig. 1).

The Dartmouth Cove site has a high relative influence (68 to 100% loading) from primary contamination and a low relative influence (0 to 33% loading) from surface drainage, secondary contamination, and diagenetic contamination; the Birch Cove site has a low relative influence from primary contamination, moderate relative influence (34 to 67% loading) from surface drainage, and moderate relative influence from secondary and diagenetic contamination; the Point Pleasant site has a low relative influence from primary contamination, high relative influence from surface drainage, moderate relative influence from secondary contamination, and high relative influence from diagenetic contamination (Buckley and Winters 1992) (Fig. 2). While all sites within the harbour are considered to be highly contaminated, Dartmouth Cove has the highest relative influence from primary contamination, Point Pleasant has the highest relative influence from surface drainage and diagenetic contamination, and Birch Cove has low relative influence from primary contamination, but moderate influence from all other sources. Other pollutants not characterized by Buckley and Winters (1992), but documented throughout the harbour, include PACs, chlorobiphenyls, butyltins, and pharmaceuticals and personal care products (Halifax Harbour Cleanup Project 1993; Hellou et al. 2002; King et al. 2002; Carter et al. 2004). The Long Cove Pond reference site is not exposed to any known source of contaminants (Fig. 1).

At all sites, the beach substrate consists of a mix of small boulders and emergent bedrock, with some sand at the Long Cove Pond site. Salinity was 32‰ at all sites, as measured by a Fisher handheld refractometer (±1.0‰ accuracy). Seawater temperature was 15°C at all three harbour sites and 14°C at the Long Cove Pond site.

**Mussel Collection**

Sixty *Mytilus* mussels measuring between 6 and 9 cm were collected from each site at the low tide mark on September 10th (Birch Cove), September 11th (Long Cove Pond), September 12th (Point Pleasant), and September 13th (Dartmouth Cove), 2006 (Fig. 1). Each individual was measured (shell length, height, and width) (Fig. 3)
Coray et al.

with digital vernier calipers to the nearest 0.01 mm, and digital electronic balances were used to measure weight to the nearest 0.01 g. Mytilus includes a mix of M. edulis and M. trossulus, which cannot be identified externally with precision. Other measurements were made as described below in the section termed “morphological measurements.”

Reagents Used for Immunological Assays

All dyes used in the immunological assays described below were certified grade while solvents were pesticide grade, and all chemical reagents were purchased from Sigma Chemical (Oakville, Ont., Canada). A stock solution of tris-buffered saline (TBS), pH 8.4, was used as the dilution medium for the dyes and consists of 17.3 ml of 0.1 N HCl made up to 100 ml with distilled water added to 25 ml of 0.2 M aqueous Trizma base. NaCl 2.5% (wt/vol) was added to maintain osmolarity between 1,000 and 1,200 mOsm (Grundy et al. 1996), and the TBS solution was filtered (0.2 μm) before each sampling day.

Haemolymph Extraction and Haemocyte Count

Mussel valves were pried open around the byssus, mantle fluid was drained, a 20-gauge needle was inserted into the posterior adductor muscle sinus, and 0.5 ml of haemolymph was extracted into a cold 3-ml syringe preloaded with 0.5 mL of TBS. This haemocyte/TBS suspension was divided into four subsamples: 0.1 ml for the haemocyte counting, 0.2 ml for the phagocytosis assay, 0.1 ml for production of hydrogen peroxide, and 0.1 ml for the Trypan Blue exclusion dye assay used to assess cellular mortality. An improved Neubauer haemocytometer (American Optical, Buffalo, N.Y., U.S.A.) was used to determine haemocyte numbers in 0.1 ml of haemolymph, and haemocyte concentration was expressed as cells per millilitre of undiluted haemolymph.

Fungal Infection

During haemocyte counts, presence of filamentous fungi in haemolymph was noted as fungal infection.

Cellular Viability

Cellular viability was established by adding 20 μl of trypan blue (vital dye) (3%) to a slide containing a drop (20 μl) of haemolymph. After a 10 min incubation, the number of live cells (able to eliminate the dye) and dead cells were counted. Data were reported as percentage of dead cells.

Fig. 1. 2006 sample sites for investigation of immune parameters in Mytilus edulis and Mytilus trossulus in the Halifax Regional Municipality, Nova Scotia, Canada. Effluent plume boundary assessed by the Canadian Shellfish Sanitation Program based on levels of faecal coliform in water samples (Environment Canada 2006).

Fig. 2. Relative influence of primary contamination, surface drainage, secondary contamination, and diagenetic contamination in sediment samples taken at sites sampled for immune parameters in Mytilus edulis and Mytilus trossulus. A high influence indicates sediment loading between 68 to 100%, a moderate influence indicates sediment loading between 34 to 67%, and a low influence indicates sediment loading of 0 to 33% of organic and labile metals, acids, and organics associated with each type of contamination (Buckley & Winters 1992). Primary contamination is characterized by high levels of organic carbon (C), iron (Fe), zinc (Zn), copper (Cu), lead (Pb), chromium (Cr), nickel (Ni), cadmium (Cd), and mercury (Hg); surface drainage is characterized by high levels of aluminum (Al), magnesium (Mg), potassium (K), lithium (Li), iron (Fe), and manganese (Mn); secondary contamination is characterized by high levels of copper (Cu); and diagenetic contamination is characterized by high levels of silicon (Si), titanium (Ti), and manganese (Mn) (Buckley & Winters 1992).
Production of Hydrogen Peroxide

Aliquots of 100 μl of haemolymph per mussel were added to 96-well microplates and incubated in the dark for 1 hour. One hundred microlitres of phenol red solution (PRS) were added to haemolymph and incubated for 30 min. The PRS contained phosphate buffered saline at pH 7.4, 900 mOsm, 5.5 mM dextrose, 0.56 mM phenol red, and 8.5 U·ml⁻¹ horseradish peroxidase (Type II). The reaction was stopped by addition of 20 μl of NaOH 1 N and optical density readings were determined in a microplate reader ELx808IU (Bio-Tek Instrument Inc.) at 620 nm. Data reported are mean optical density per million of cells.

Phagocytic Activity

Duplicate aliquots of 100 μl of haemolymph/TBS were added to 96-well microplates. Ten microlitres of neutral-red stained zymosan type 1 yeast (6.6 mg·ml⁻¹) were added to each well for a 30 min incubation. After this period, 100 μl of Baker’s fixative (Lowe and Pipe 1994) was added to each well. The neutral red and/or zymosan not absorbed by phagocytes was removed by two washes with TBS (centrifuged for 3 min at 2,000 g). The cells are washed with 100 μl of extraction solution to extract the zymosan and dye from the interior of cells. The remaining steps and more details of this technique followed St-Jean et al. (2003). Optical density readings were determined in a microplate reader ELx808IU (Bio-Tek Instrument Inc.) at 540 nm. Data reported are mean optical density per million of cells.

Morphometric Measurements for Mytilus Species Identification

Shell characters classified by McDonald et al. (1991) as having a high absolute standardized canonical coefficient were used to distinguish M. edulis from M. trossulus. The characters used were: (i) the length of the anterior adductor muscle scar (AAM); (ii) the length of the hinge plate (HP); (iii) the distance between the anterior edge of the posterior adductor muscle scar and the shell margin (PADP); (iv) the distance between the ventral edge of the posterior adductor muscle scar and ventral shell margin (PADV); (v) the distance between the pallial line and the ventral shell margin midway along the shell (PAL); (vi) shell height (HEI); and (vii) shell width (WID) (Fig. 3). All shell measurements were made with digital vernier callipers to 0.01 mm.

Data Analyses

Statistical analysis on shell morphology was conducted using Systat 11.1 software (SPSS Inc., Chicago, Ill., U.S.A.). A canonical discriminant analysis was used to derive a canonical function that separated the two mussel species (M. edulis and M. trossulus). Each shell character was first standardized using log₁₀ and divided by log₁₀ of the shell length. The accuracy of the discriminant analysis in comparison to electrophoretic analysis for separating the two species of mussels was tested by Mallet and Carver (1995). Only 7 of 143 mussels were misclassified on the basis of morphology, giving the discriminant analysis a 95% accuracy rate.

All other statistical analyses were conducted with SPSS 14.0. Analysis of variance (ANOVA) assumptions were verified graphically by both box and probability plots, and further verified by Levene’s and one-sample Kolmogorov-Smirnov tests. Cell viability percentages were analyzed after angular transformation (=ASIN(SQRT(n%/100))*181PI()) in Microsoft Excel (http://helios.bto.ed.ac.uk/bto/statistics/tress4.html). Results for H₂O₂ and PA were log transformed and standardized to one million cells, and haemocyte count data were square-root transformed.

A multivariate test using Pillai’s Trace, Wilk’s Lambda, Hotelling’s Trace, and Roy’s Largest Root was used to examine interaction between site location and species. Differences among sites were compared by
one-way ANOVA followed by a Tukey a posteriori test. Significant differences were reported when \( P < 0.05 \).

**Results**

**Species Numbers**

Point Pleasant (Fig. 1) was the only site surveyed where equal numbers of *M. trossulus* and *M. edulis* were documented (Fig. 4). Mussels sampled from all other sites (Fig. 1) were dominated by *M. edulis* and included 20% or fewer *M. trossulus* (Fig. 4).

**Cellular Viability**

Cell viability did not vary significantly between sites or species.

**Haemocyte Counts**

Haemocyte concentration varied significantly between sites (\( F_{3,236} = 19.23, P \leq 0.001 \)). Birch Cove had significantly higher haemocyte count than any other site (\( P \leq 0.001 \)) (Fig. 5). Haemocyte concentration also varied significantly between species (\( F_{3,136} = 5.83, P \leq 0.01 \)), with *M. trossulus* having significantly lower haemocyte counts than *M. edulis* (Fig. 6). No interaction occurred between site location and species.

**Fungal Infection**

Filamentous fungi were evident in the haemolymph of 45% of the mussels collected at the Dartmouth Cove site and 3% of the mussels collected at the Point Pleasant site. Mussels collected from the other two sites (Birch Cove, Long Cove Pond) displayed no evidence of fungal infection. Numbers of overall infected mussels collected at the Point Pleasant site, and numbers of *M. trossulus* collected at Dartmouth Cove were insufficient to enable a comparison of fungal infection rate between the two species.

**Hydrogen Peroxide Production**

\( \text{H}_2\text{O}_2 \) production varied significantly between sites (\( F_{3,236} = 357.57, P \leq 0.001 \)). Long Cove Pond had the highest \( \text{H}_2\text{O}_2 \) production of all sites, followed by Duncan’s Cove, Birch Cove, and Point Pleasant with the lowest (Fig. 5). \( \text{H}_2\text{O}_2 \) was lower in *M. trossulus* than *M. edulis*, but not significantly so (\( P = 0.09 \)) (Fig. 6). No interaction occurred between site location and species.
Phagocytic Activity

PA varied significantly between sites \( (F_{2,326} = 92.78, P \leq 0.001) \). Long Cove Pond had the highest PA of all sites, followed by Point Pleasant, Duncan’s Cove and Birch Cove with the lowest (Fig. 5). PA was lower in *M. trossulus* than *M. edulis*, but not significantly so \( (P = 0.33) \) (Fig. 6). No interaction occurred between site location and species.

**Discussion**

Cellular viability did not vary significantly between sites or species, supporting the hypothesis that new cells are resilient to the action of toxicants (Mehendale 1995). Haemocyte count, however, varied significantly between both sites and species. Mussels from the Birch Cove site had a significantly higher haemocyte count than those from all of the other sites (Fig. 5). Mussels from the Dartmouth Cove and Point Pleasant sites had slightly higher haemocyte counts than the Long Cove Pond site, but not significantly so (Fig. 5). These results correspond with field studies showing that oysters (Fisher et al. 2000) and *Mytilus* mussels (Pipe et al. 1995; Mayrand et al. 2005) exposed for years to industrialized conditions have higher haemocyte counts than those from an uncontaminated reference site. Laboratory experiments have also shown an increase in haemocyte count in reaction to various chemical stressors (Coles et al. 1995; St-Jean et al. 2002a, 2002b). An increase in circulating haemocyte numbers in response to a chemical stressor may be due to migration of blood cells from tissues rather than haemocyte proliferation. However, results of the present study support the hypothesis that haemocyte proliferation is responsible for higher haemocyte counts in mussels chronically exposed to municipal pollution compared with unexposed mussels from a relatively clean reference site. A similar trend was observed by Mayrand et al. (2005) who found that mussels chronically exposed to pollution in Pictou Harbour, Nova Scotia, Canada had approximately twice the haemocyte count and twice the mitotic activity for a given number of haemocytes than mussels native to a relatively clean site (Richibucto, New Brunswick, Canada). However, we cannot rule out that handling stress may partially explain the higher haemocyte count at the Birch Cove site (St-Jean, Personal communication). Compared with other sites where mussels were sampled within one hour of collection, haemolymph from the Birch Cove mussels was sampled almost 12 hours after collection due to field logistics (tide schedule). Although the Birch Cove mussels were refrigerated immediately after sampling, it is possible that physical stress may have compounded any haemocyte augmentation that had occurred due to chemical stress from their environment through the migration of blood cells from tissues. Additional physical stress may thus explain why Birch Cove mussels exhibited such an elevated number of haemocytes in comparison with mussels collected from other polluted sites in this study.

In addition to site differences in haemocyte counts, *M. edulis* exhibited significantly higher haemocyte counts than *M. trossulus* (Fig. 6). Although an increased count of defensive cells is a basic response when animals are under stress, low numbers of haemocytes can be indicative of a depressed immune system and decreased ability to respond efficiently against pathogens and/or chemical substances. *M. trossulus* is expected to be more sensitive to stress than *M. edulis*, which may be confirmed in this study by the significant difference observed in haemocyte counts.

Previous studies have shown that phagocytic activity can be either inhibited (Coles et al. 1995; Grundy et al. 1996; Pipe et al. 1999; Gagné et al. 2002) or increased (Cheng and Sullivan 1984; St-Jean et al. 2002b) in bivalves exposed to variety of substances that may be found in sewage. Longer-term exposures to heavy metals, polycyclic aromatic hydrocarbons, DDT (dichlorodiphenyltrichloroethane) and PCBs (polychlorinated biphenyls) have all been shown to inhibit haemocyte phagocytosis (Coles et al. 1995; Grundy et al. 1996; Pipe et al. 1999). Freshwater mussels (*Elliptio complanata*) exposed to primary treated municipal effluent for 62 days exhibited phagocytic activity inhibition along with bioaccumulation of heavy metals in their tissues (Gagné et al. 2002). Conversely, Luengen et al. (2004) reported elevated phagocytosis in *Mytilus* mussels from contaminated estuarine sites in California, U.S.A. relative to control site mussels, and St-Jean et al. (2002b) observed an increase in phagocytic activity in mussels exposed experimentally to the biocide tributyltin as well as the product of its partial degradation, dibutyltin. Cheng and Sullivan (1984) also reported an increase in phagocytosis in oysters (*Crassostrea gigas*) exposed to Cu and Fe when compared with controls. Concentration, chemical structure, and time of exposure to contaminants likely all play a part in modulating phagocytosis. The present study suggests that long-term exposure to high levels of untreated sewage effluent results in an inhibition of phagocytosis in both *M. edulis* and *M. trossulus*. Mussels from all three of the sites exposed to a variety of contaminants (Birch Cove, Dartmouth Cove, Point Pleasant) exhibited significantly lower phagocytic activity than mussels from a relatively pristine site (Long Cove Pond) (Fig. 5). The Birch Cove site exhibited significantly lower phagocytosis than the other two harbour sites (Fig. 5). As mentioned previously, the Birch Cove mussels may have suffered from more physical stress during the sampling process than mussels from the other sites, which may explain the differences observed between them and other mussels exposed to municipal effluent. It is also possible that the Birch Cove mussels are being exposed to different chemicals or concentrations of chemicals than the Dartmouth Cove and Point Pleasant site mussels. Although the general residual surface water circulation in Halifax Harbour runs from Bedford Basin (Birch Cove) to the inner Harbour (Dartmouth Cove),
tidal currents will move water and contaminants from the inner harbour back into Bedford Basin (King et al., 2002) (Fig. 1), so it is possible that the Birch Cove site is a hot-spot for certain contaminants found in lower concentrations at the other harbour sites. Although not significant ($P = 0.33$), *M. trossulus* did exhibit lower phagocytic activity than *M. edulis*, further indicating *M. trossulus* to be the more sensitive species (Fig. 6). Lack of significance may be due to the fact that only very hearty *M. trossulus* specimens survive under conditions of such extreme stress.

When phagocytosis occurs, a respiratory burst is initialized that results in the production of oxygen metabolites ($O_2^-, H_2O_2, OH^-$) (Pipe 1992). Concentration of $H_2O_2$ can thus indicate how the cells of a bivalve are responding to anthropogenic stress such as sewage exposure. Mussels exhibiting reduced phagocytic activity in this study also exhibited reduced concentrations of $H_2O_2$, with mussels from all of the harbour sites displaying significantly lower $H_2O_2$ production than mussels from the control site (Fig. 5). Mussels from the Dartmouth Cove site had the lowest production of $H_2O_2$ of all of the sites, as well as the highest input of primary contamination (Fig. 5 and 2). Although an increase of $H_2O_2$ production has been described in *M. edulis* exposed to the polycyclic aromatic hydrocarbon fluoroanthene (Coles et al. 1994), the current study shows a decrease of $H_2O_2$ production in *M. edulis* and *M. trossulus* native to Halifax Harbour and exposed for a lifetime to untreated sewage. *M. trossulus* did exhibit lower $H_2O_2$ production than *M. edulis*, although not significantly so ($P = 0.09$) (Fig. 6). As mentioned previously, lack of significance may be due to the fact that only very hearty *M. trossulus* specimens survive under conditions of such extreme stress.

Another indication of immune system depression is the inability to clear infection from bacteria or fungus. Opportunistic and toxigenic microorganisms, including many filamentous fungi, increase in number in biotopes polluted with industrial or domestic sewage, and these opportunistic filamentous fungi can cause infectious diseases in a host that is senescent or suffers from any other condition of immunodeficiency (Zvereva and Vysotskaya 2005). Fungal infection was evident in almost half (45%) of the mussels sampled from the Dartmouth Cove site—which is the receiving area for several untreated sewage outfalls—and in 3% of the mussels sampled from the Point Pleasant site, which is directly adjacent to one untreated sewage outfall. Fungal infection was not evident in any mussels sampled from the Birch Cove site, which is indirectly exposed to raw sewage, or from the Long Cove Pond site, which has no known exposure to untreated sewage effluent.

**Conclusion**

Municipal pollution, primarily in the form of untreated sewage outfalls and fluvial drainage systems, modulates immune system responses (haemocyte count, PA, production of $H_2O_2$) of mussels in Halifax Harbour in a manner that indicates impaired health in comparison to mussels from a reference site. *M. trossulus* also appears to be more sensitive than *M. edulis* to stress caused by exposure to municipal pollution. Immunological endpoints such as haemocyte count, PA, and production of $H_2O_2$ show responses to a large number of contaminants and provide a measure of mussel population health. Knowledge of mussel population health can then be extrapolated to make predictions regarding the current health of the receiving environment, which, in the case of Halifax Harbour, we judge to be seriously impaired. Advanced primary sewage treatment is anticipated to commence in Halifax in 2008. Baseline data regarding immune responses in endemic mussels will be useful in assessing potential impacts of low-level treatment of effluent on the future biological health of the Halifax Harbour receiving environment.

**References**


Immunomodulation in Blue Mussels in Eastern Canada


Received: 26 April 2007; accepted: 12 September 2007