

Histopathologic and Histochemical Biomarker Responses of Baltic Clam, *Macoma balthica*, to Contaminated Sydney Harbour Sediment, Nova Scotia, Canada

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Sediments in Sydney Harbour, Nova Scotia, are highly contaminated by polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals. Histopathologic and histochemical evaluations were made on the Baltic clam, *Macoma balthica*, exposed to 11 Sydney Harbour sediment samples. Histologic lesions in digestive gland (tubular dilation or atrophy, macrophage aggregates, tubular cell necrosis, and tissue inflammation) and gonads (macrophage aggregates, supporting cell, germ cell, and ovarian cell necroses) were frequently detected in clams exposed to the most contaminated sediments from the harbor. Clams exposed to these contaminated sediments also had the highest acid phosphatase activity. The average scores of tubular dilation or atrophy, ovarian cell necrosis, and the sums of mean digestive gland lesions correlated significantly with sediment PCBs, and the activities of acid phosphatase correlated significantly with sediment heavy metals, PAHs, and PCBs. Among the lesions, digestive gland tubular dilation or atrophy, tubular cell, germ cell, and ovarian cell necroses, and the activity of acid phosphatase are the best sublethal effect indicators in *Macoma* exposed to Sydney Harbour sediments. **Key words:** biomarkers, chronic biologic effects, clams, histology, histochemistry, *Macoma balthica*, marine sediment, polynuclear aromatic hydrocarbons, polychlorinated biphenyls. *Environ Health Perspect* 111:273–280 (2003). doi:10.1289/ehp.5437 available at <http://dx.doi.org/> [Online 25 October 2002]

Sydney Harbour is located on the northeast coast of Cape Breton Island, Nova Scotia, Canada. Since the early 1900s it has received contamination from various industrial and domestic sources including marine transport, ship repair, fish processing, coal mining and processing facilities, steel mills, and untreated sewage.

Concerns about the environmental quality of Sydney Harbour were raised in the 1980s when high levels of a carcinogen, benzo[*a*]pyrene, were detected in lobsters, leading to closure of the lobster fishery in the south arm of the harbor (1,2). At the same time, other studies revealed that harbor sediments were highly contaminated with polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals (3–7). The major sources of these contaminants, especially PAHs and PCBs, are believed to originate from the adjacent Muggah Creek, which received effluents from the Sysco steel operation and runoff from the tar ponds associated with a coking facility. Sediment PAH concentrations at the mouth of Muggah Creek are among the highest reported for contaminated marine sites in the world (5,8,9).

Recently, with the support of the municipal, provincial, and federal governments, a cleanup program was initiated for Muggah Creek and its vicinities. At the same time, Canada's Toxic Substance Research Initiative (TSRI) has funded a 3-year study to investigate the ecologic and health risk of contaminated

sediments in Sydney Harbour. The aim of this study is to assess the cumulative ecosystem and human health effects resulting from exposure to mixtures of toxic substances in the harbor. In addition, the study will assist in monitoring the effectiveness of remediation efforts under way at Muggah Creek.

The present study was part of the TSRI ecologic and health risk assessment conducted in 1999. It was designed to assess the chronic biologic effects of Sydney Harbour sediments on marine organisms. The study used a standard bioaccumulation test method developed by the U.S. Environmental Protection Agency (EPA) in 1989 (10) to expose the Baltic clam (*Macoma balthica*) to sediments collected from various parts of Sydney Harbour. The exposed clams were then subject to extensive histopathologic and histochemical observations to look for early indication of cytologic and physiologic alterations in the tissue and organs. The goal was to evaluate use of these biomarkers, which may link to death of cells and organs, reproductive failure, and potential tumor formation in the clams, to assess the harbor sediments. The other goal was to develop a suite of histopathologic and histochemical biomarkers to complement the U.S. EPA bioaccumulation test method and other standard sediment bioassays such as the amphipod lethality, sea urchin fertilization, Microtox solid-phase and bacterial exoenzyme tests, which are currently recommended by Environment Canada for sediment assessment.

Most of the existing standard sediment bioassays use short-term lethal and sublethal end points, whereas end points for histopathologic and histochemical biomarkers are chronic measurements based on changes or alterations of cells, tissues, and organs. These biomarkers maintain *in situ* cellular, tissue, and organ-system relationships, allowing the investigator to observe biologic effects associated with toxicity in localized portions of an organ and the subsequent alterations in fluids, tissues, or cells at other locations. In addition, they can be used to reveal target organs for the toxic effects of a contaminant; reveal the routes of exposure and uptake of contaminants in test organisms; reflect bioavailability of toxic substances; and serve as early warning of population and community stress (11,12).

Materials and Methods

Sediment sampling. Sediment sampling was conducted in Sydney Harbour 20–22 October 1999. We used a 0.1-m² Van Veen grab to collect samples at 10 preselected sampling stations and one reference station from the mouth of the harbor (Figure 1). At each station, whole sediments from two to three grabs were pooled in a 20-L bucket lined with a clean polyethylene bag and were homogenized by stirring with a stainless-steel potato masher or spoon. A subsample of 4 L was collected for this study and another 4 L for other toxicity tests including the marine amphipod 10-day whole sediment lethality test and two sediment sublethal toxicity tests, the Microtox solid-phase and

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echinoderm fertilization tests. We collected additional subsamples for chemical and grain size analyses.

The 4-L samples were placed in clean polyethylene buckets maintained at 6–11°C and shipped on the same day to the Environment Canada Environmental Quality Laboratory in Moncton, New Brunswick. Upon arrival to the laboratory, samples were checked, labeled, and stored at 4°C before use in biologic tests.

Macoma balthica exposure test. We collected the lamellibranch *M. balthica* (L.) (Baltic clam), mean (\pm SD) shell length of 1.5 cm \pm 0.2 (0.9–1.5 cm shell length; 0.17–0.7 g wet weight), at Walton Beach, Nova Scotia, Canada, on 15 November 1999 and shipped them to the Moncton Environment Canada Laboratory on the same day. Animals were collected for similar size ranges to minimize differences in age and maturity. Clams were acclimated to 10 \pm 2°C and 28 \pm 2 ppt salinity and maintained in this environment until used for testing on 22 November. The sediment samples collected from Sydney Harbour plus a control sample from Walton Beach were used in the test following the U.S. EPA sediment bioaccumulation test method (10), which is currently used by the Environment Canada Disposal at Sea Program to assess uptake of contaminants in marine organisms (13).

Three days before starting the test, we homogenized test sediments and added 2 L portions to 4-L test chambers (29 cm internal diameter and 23 cm high). The depth of the test sediments in the chambers was approximately 10 cm. The test chambers were then filled with approximately 2 L of clean sea water (30 \pm 2 ppt) and aerated with oil-free compressed air at a rate of approximately 150 mL/min.

Three days later, *M. balthica* were removed from their holding sediment by sieving the contents through a 0.5-cm sieve. Animals were double-counted, and animals were added to each of the test chambers. We replaced any animals not burrowed within the first 24 hr to avoid using unhealthy animals and empty shells at the start of the test.

We used 12 test chambers including the control and reference with no replicates for the test. Testing was performed at 15 \pm 1°C with a 16-hr light and 8-hr dark photoperiod with lighting provided by overhead fluorescent fixtures at an intensity of 400–600 lux. Tests were checked daily for observations, aeration, and temperature. Twice a week, each chamber was monitored for temperature, pH, salinity, and dissolved oxygen. Approximately 75% of the overlying water was renewed two times a week with clean seawater. A sample of overlying water was taken at the start and end of the test for ammonia analysis.

At the end of 28 days, the contents of each test chamber were sieved through a 0.5-cm

sieve. We recorded the number of dead clams. The live clams were shucked and tissues were removed and processed for histopathologic and histochemical evaluations.

Histopathologic and histochemical studies. Fifteen of the 37 exposed clams (except station 23, which had 33 clams) from each treatment including the control and reference were shucked. The tissue samples were fixed in 10% neutral buffered formalin for histopathologic and immunohistochemical analysis. Another 15 unexposed clams from the original group collected from the field were processed in the same manner to serve as additional control. The fixed samples were

dehydrated, cleared, and embedded in paraffin. Sections (5–7 μ m) were cut in series from each sample and mounted onto two glass slides (two sections per slide). We stained the first slide in Harris hematoxylin and eosin for histopathologic analysis and processed the second slide for immunolocalization of CYP1A (P450) antibodies (14). We used 191 clams for the histopathologic survey and 52 clams for P450 observation.

We shucked and processed another four clams from each treatment including the control and reference for freeze-drying and glycomethacrylate embedment as described by Teh and Hinton (14). Four unexposed clams were

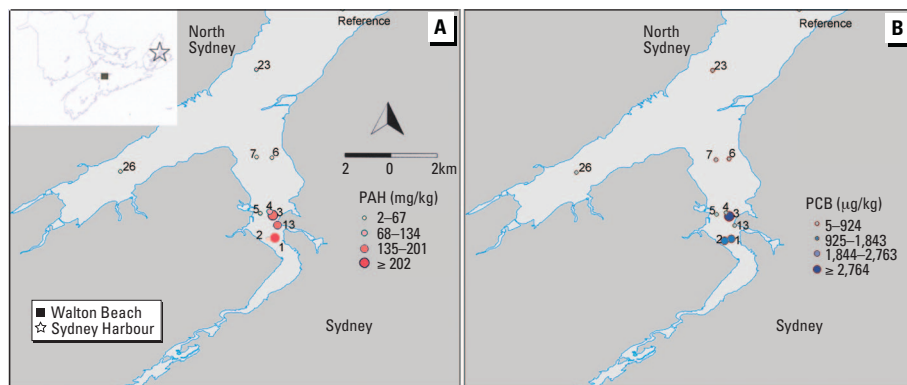


Figure 1. PAHs (A) and PCBs (B) in sediment samples from Sydney Harbour.

Table 1. Particle size, total organic carbon, PAHs and PCBs in the control, reference, and treatment sediment samples.

Station	TOC (%)	GRA (%)	Sand (%)	Silt (%)	Clay (%)	PAHs ^a (mg/kg)	PCBs (mg/kg)
Control	0.63	0.29	31.65	49.61	18.41	0.11	0.016
Reference	1.69	0.00	84.00	12.00	3.00	1.50	0.005
1	7.22	0.00	3.00	76.00	22.00	246.40	1.060
2	11.71	0.61	12.00	66.00	20.00	132.18	1.630
3	10.18	0.21	10.00	68.00	19.00	199.97	3.377
4	5.45	0.00	6.00	67.00	25.00	87.28	0.374
5	4.63	0.05	8.00	65.00	23.00	35.35	0.203
6	4.56	0.00	2.00	69.00	29.00	16.94	0.525
7	3.73	0.21	4.00	69.00	24.00	33.67	0.330
13	12.13	1.11	8.00	72.00	18.00	195.12	0.708
23	3.83	0.00	22.00	61.00	9.00	4.77	0.005
26	2.91	0.00	6.00	71.32	22.05	20.98	0.045

Abbreviations: GRA, gravel; TOC, total organic carbon.

^aTotal of 16 U.S. EPA priority PAHs.

Table 2. Heavy metals (mg/kg) in the control, reference, and treatment sediment samples.

Station	As	Hg	Cd	Cr	Cu	Pb	Mo	Ni	Ag	V	Zn
Control	21.60	0.02	0.06	—	16.78	23.79	—	—	—	—	49.00
Reference	16.00	0.01	0.10	29.00	10.00	19.50	0.08	14.00	0.20	47.00	50.00
1	29.00	0.42	1.46	76.00	110.00	408.00	8.20	27.80	1.60	113.00	360.00
2	41.00	0.48	1.54	86.00	99.00	295.00	9.40	35.80	1.95	146.00	474.00
3	29.00	0.30	1.64	72.00	70.00	206.00	7.40	27.40	1.70	113.00	380.00
4	23.00	0.14	0.94	71.00	55.00	132.50	8.40	31.00	1.00	120.00	246.00
5	21.00	0.09	0.70	74.00	51.00	105.50	7.00	35.80	0.90	121.00	182.00
6	22.00	0.10	0.72	68.00	41.00	99.50	6.80	28.60	0.80	122.00	188.00
7	23.00	0.10	0.68	78.00	45.00	111.50	7.60	34.60	0.80	142.00	214.00
13	21.00	0.35	1.56	67.00	90.00	218.00	11.20	23.40	2.00	98.00	304.00
23	16.00	0.04	0.16	47.00	19.00	25.50	2.40	21.60	0.35	75.00	78.00
26	19.00	0.09	0.54	75.00	39.00	101.50	6.40	33.60	0.70	129.00	174.00

also processed to serve as additional control. Two sections (5 μm) were serially sectioned and reacted at room temperature for localization of glycogen with periodic acid-Schiff reagent (PAS), acid phosphatase (ACP), adenosine triphosphatase (ATP), and γ -glutamyl transpeptidase (GGT). Details of each enzyme protocol were described by Teh and Hinton (14). We used 52 clams for this observation.

Before ranking the histopathologic and histochemical alterations, we established a set of measurements for the degree of cytologic alterations and staining intensity of each enzyme and antibody to ensure consistency of scoring. The scoring for histopathologic alterations considered the number and severity of each type of lesion detected in the clams. For example, specimens with no observable lesions in the tissues were rated as 0. Those with small number and slight tissue lesions were considered to be in mild condition and were given a score of 1. Those with moderate number and moderate lesions were scored as 2, and those specimens with a high number of extensive lesions were considered as severe and were scored as 3. We used the same process to score the histochemical alterations. Each enzyme and antibody was semiquantitatively ranked on a scale of -1 to 2 (-1 = decreased reaction; 0 = normal reaction; 1 = increased; and 2 = enhanced) based on the staining intensity seen in control clams.

Statistical methods. We used SYSTAT computer software to compare the number of cellular alterations in individual lesions and groups of lesions among the unexposed, control, reference, and treated clams. We used analysis of variance (ANOVA) to estimate the level of significance. A nonparametric test (Kruskal-Wallis one-way ANOVA of Mann-Whitney *U*-test) was run on the same comparisons and generally was in close agreement with the ANOVA. We calculated Pearson correlations between lesions and environmental variables (sediment content of organic contaminants and heavy metals) and determined

significance. Occurrence of apicomplexan diseases (parasite) in kidney, heart, and intestine and unidentified ovarian parasite in ovary in the unexposed, control, and exposed clams were counted. We tested the potential causes of tissue lesions and histochemical alterations by the observed parasites to ensure that their occurrence was not a factor when making assumption in the cause-and-effect measurement of the cellular alternations and histochemical studies.

Results

Concentrations of contaminants in Sydney Harbour sediments. Results of the sediment chemical analyses indicated that Sydney Harbour sediments are contaminated with PAHs, PCBs, and heavy metals (arsenic, mercury, cadmium, chromium, copper, lead,

molybdenum, nickel, silver, vanadium, and zinc) (Tables 1 and 2). The concentrations of these contaminants decreased with increasing distance from the mouth of Muggah Creek. The concentrations of organic contaminants, such as the PAHs and PCBs compounds, in the sediments collected from the stations near the mouth of Muggah Creek are many times higher than those reported for other North American major harbors such as Halifax, Vancouver, and Boston harbors (15). In contrast to the organic contaminants, heavy metals in Sydney Harbour are much lower than in the other industrial harbors.

Macoma balthica exposure test. All test clams (12 treatments of 37 clams) survived the 28-day exposure, except the station 23 clams, which had a 89.2% survival. Survival of clams was considered acceptable in all treatments.

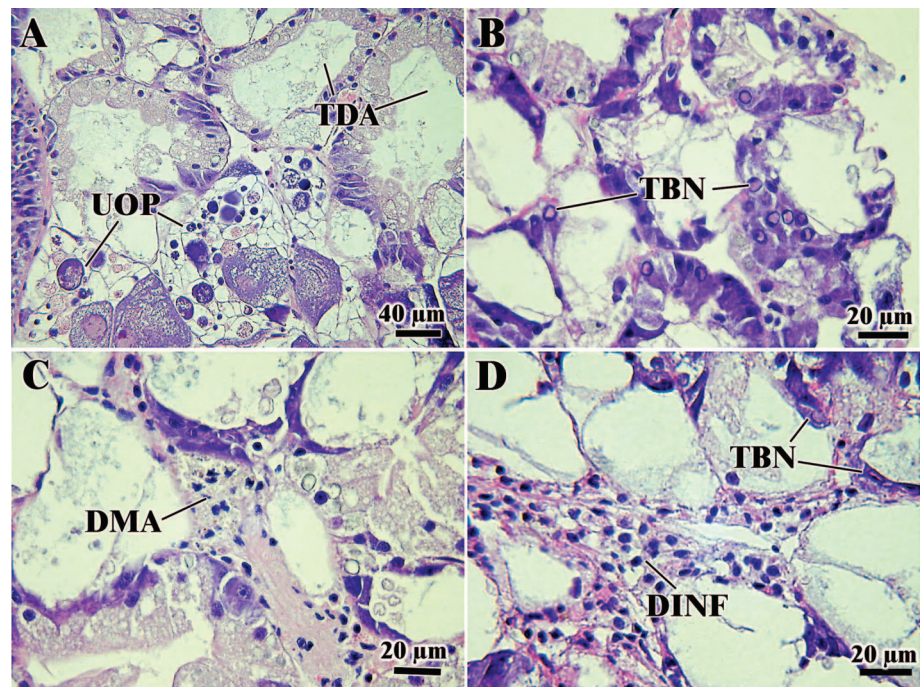


Figure 3. Digestive gland lesions in *M. balthica* exposed to Sydney Harbour sediments. (A) TDA, station 7. (B) TBN, station 1. (C) DMA, station 3. (D) DINF, station 3. UOP, unidentified ovarian parasite.

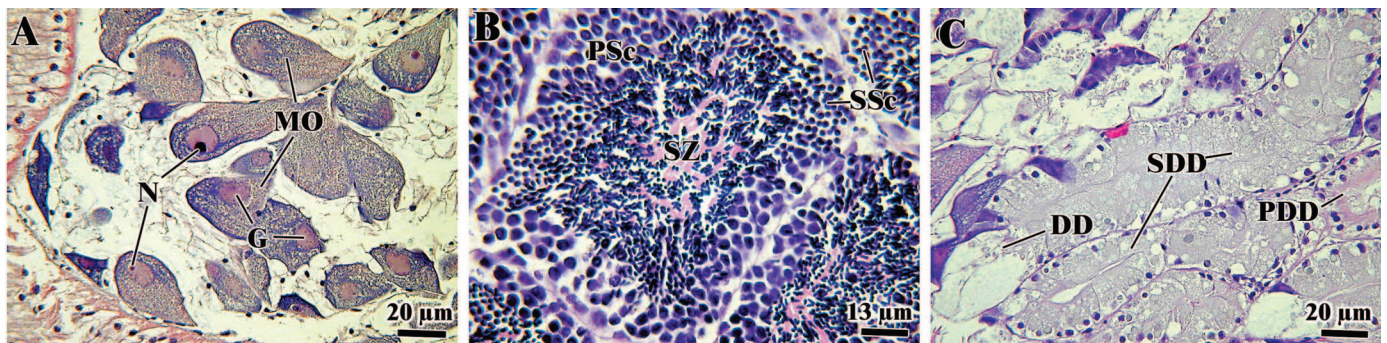


Figure 2. Ovary (A), testis (B), and digestive gland (C) of *M. balthica* exposed to control sediments. Abbreviations: DD, digestive diverticulum; G, germinal vesicle; MO, mature oocytes; N, nucleus; PDD, primary digestive duct; PSc, primary spermatocyte; SDD, secondary digestive duct; SSc, secondary spermatocyte; SZ, spermatozoon.

Digestive gland lesions. Digestive glands of control clams are shown in Figure 2. Four lesions were identified in the digestive gland of the exposed clams: tubular dilation or atrophy (TDA), macrophage aggregates (DMA), tubular cell necrosis (TBN), and inflammation (DINF) (Figure 3, Table 3). Only 1 of the 7 female clams in the control group had a mild case of TDA. Mild and moderate TDA was observed in 40% of the clams exposed to the reference sample, whereas only 1 of the 15 reference clams had a severe case of TBN. No DMA and DINF were found in the reference clams.

Forty-five percent of digestive gland lesions observed in all of the 162 reference

and treated clams were found in the 50 clams exposed to sediment samples from stations 1, 2, and 3 (Figure 4). These stations located at the mouth of Muggah Creek had the highest sediment PAHs, PCBs, and heavy metals (Tables 1 and 2). One exception is the station 13 clams. This station, which is also close to the mouth of the creek, had high concentrations of PAHs in the sediment samples. However, only TDA was detected in these clams. Also, the number of TDA observed in these clams was lower than the stations with low sediment contaminants such as station 7 at mid-harbor and station 23 at outer harbor.

Fifty-two of the 162 clams exposed to the reference and treatment sediments developed

TDA in the digestive gland. The highest TDA score occurred in clams exposed to station 3 sediments, which had the highest concentrations of PCBs and the second highest concentration of PAHs. Clams in this station also had high scores of TBN and DINF in the digestive glands. The number of TDA ($r = 0.67, p = 0.018$) and the totals of mean digestive gland lesions ($r = 0.69, p = 0.012$) were significantly correlated with the total sediment PCBs (Figure 5). Both DMA and TBN were found only in station 1, 2, 3, and 26 clams, while DINF was detected only in station 2 and 3 clams. Statistical analysis showed a significant correlation between the number of DMA and sediment Pb concentrations ($r = 0.7, p = 0.02$).

The number of digestive gland lesions observed was not significantly different between male and female clams. There was no indication that Apicomplexan diseases observed in intestine were responsible for the increase number of lesions in the digestive gland. Lesions in the reference site clams were significantly more than in control clams, indicating that the reference sediments may not be properly selected for this study. Comparing with the control clams, lesions increased significantly in clams exposed to the three most contaminated stations (stations 1, 2, and 3), station 5, which was another station closer to Muggah Creek, and station 7, which was located in a slight depression at the middle of the south arm of the harbor.

Ovarian lesions. Ovaries of control clams are shown in Figure 2. Four lesions were observed in the gonad of exposed female clams: macrophage aggregates (GMA), supporting cell necrosis (SCN), primary and secondary germ cell necroses (GCN), and ova cell necrosis (OCN) (Figure 6, Table 4). No lesions were observed in the unexposed and reference female clams, with the exception of

Table 3. Means of digestive gland lesions in unexposed, control, reference, and treated clams.

Station	No.	TDA	DMA	TBN	DINF	Total
Unexposed	14	0.00	0.00	0.00	0.00	0.00
Control	15	0.07	0.00	0.00	0.00	0.07
Reference	15	0.47	0.00	0.20	0.00	0.67
1	15	0.33	0.20	0.20	0.00	0.73
2	15	0.40	0.07	0.00	0.07	0.54
3	15	0.93	0.07	0.20	0.13	1.33
4	15	0.07	0.00	0.00	0.00	0.07
5	15	0.47	0.00	0.07	0.00	0.54
6	14	0.14	0.00	0.00	0.00	0.14
7	15	0.60	0.00	0.27	0.00	0.87
13	13	0.31	0.00	0.00	0.00	0.31
23	15	0.40	0.00	0.00	0.00	0.40
26	15	0.13	0.13	0.07	0.00	0.33

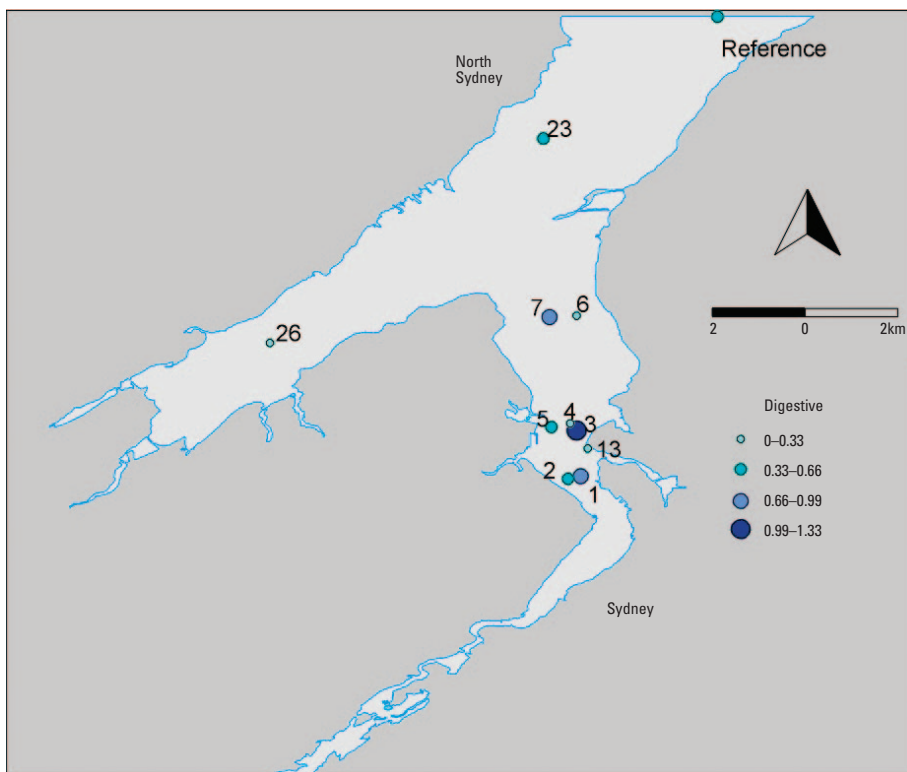


Figure 4. Totals of mean digestive gland lesions (“digestive”) in *M. balthica* exposed to Sydney Harbour sediments by location.

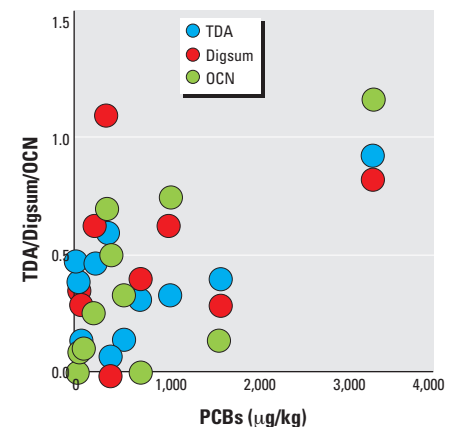


Figure 5. Relationship between PCBs and TDA ($r = 0.67, p = 0.018$); sums of mean digestive gland lesions (Digsum; $r = 0.69, p = 0.012$); and OCN ($r = 0.71, p = 0.01$).

four clams (two each in the unexposed and reference samples) that had mild to moderate GCN. No lesions were observed in control female clams.

Forty-four percent of female gonad lesions were observed in clams exposed to sediments collected from stations 1, 2, and 3, which had the highest sediment concentrations of PAHs, PCBs, and heavy metals. One exception is the station 13 clams. Though the sediment sample from this station had very high concentrations of PAHs, clams exposed to this sample had the lowest number of female gonad lesions than all other harbor sediments except the reference site. The highest scores of GMA, SCN, and OCN (Figure 7) were found, respectively, in clams from stations 1, 2, and 3. The exception is GCN, which had the highest scores in station 7 clams.

GCN was the most frequently observed ovarian lesion in the treatment clams. The number of GCN in all the treatment clams

including the reference site was significantly different from the control. Only clams in stations 1, 6, and 7 had significantly more lesions than the reference. Statistical analyses showed that only the number of OCN had a significant correlation with the sediment concentrations of PCBs ($r = 0.71$, $p = 0.01$; Figure 5). There was a weak, but insignificant, correlation between the total heavy metal concentrations in sediments and the sums of mean female gonad lesions ($r = 0.55$, $p = 0.08$). OCN, GMA, and SCN had zero incidences in the unexposed, control, and reference clams and showed increase in the treatment clams. No correlation was observed between ovarian lesion and the unidentified ovarian parasite that was detected in a significant number of female clams (Figure 6C).

Testicular lesions. Testes of control clams are shown in Figure 2. GMA, gonadal inflammation (GINF), GCN, SCN, and male primordial germ cells were observed in testes of male clams used in this study (Table 5). There were no significant differences in the scores of these lesions among the unexposed, control, reference, and treated clams. In 1 of the 15 clams exposed to the station 7 sediments, a hermaphroditic (intersex) gonad was detected (Figure 8).

Histochemical study. Three enzymes (ACP, ATP, and GGT), one antibody (CYP1A; P450), and a cytoplasmic glycogen (PAS) were

localized, and their staining intensities were compared between the control and treatment clams (Figure 9, Table 6). The control clams have normal activities of ACP, GGT, and P450, and variable activities of ATP and PAS. Decreased activities of ACP (stations 6 and 7) (Figure 7) and ATP (station 7) only occurred in clams exposed to sediment samples from the middle section of the south arm of the harbor. Increased and enhanced ACP activities occurred more frequently in clams exposed to sediments collected from the mouth of Muggah Creek, with the exception of station 13. ACP was the only enzyme correlated significantly with sediment PAHs ($r = 0.83$, $p = 0.003$), PCBs ($r = 0.77$, $p = 0.01$; Figure 10), and heavy metals (Ag, As, Cd, Cu, Hg, Pb and Zn; $r = 0.63$ – 0.72 ; $p = 0.01$ – 0.04). Only 11 of the 48 clams used for histochemical observation have increased activities of P450.

Discussion

In this study, we used a suite of histopathologic and histochemical biomarkers and a semiquantitative scoring system to assess the chronic effects of contaminated sediments on *M. balthica* in a controlled laboratory environment. A screening evaluation was conducted on major organs and tissues of randomly selected clams to identify lesions that can be used as indicators of biologic effects. Based on the results of this evaluation,

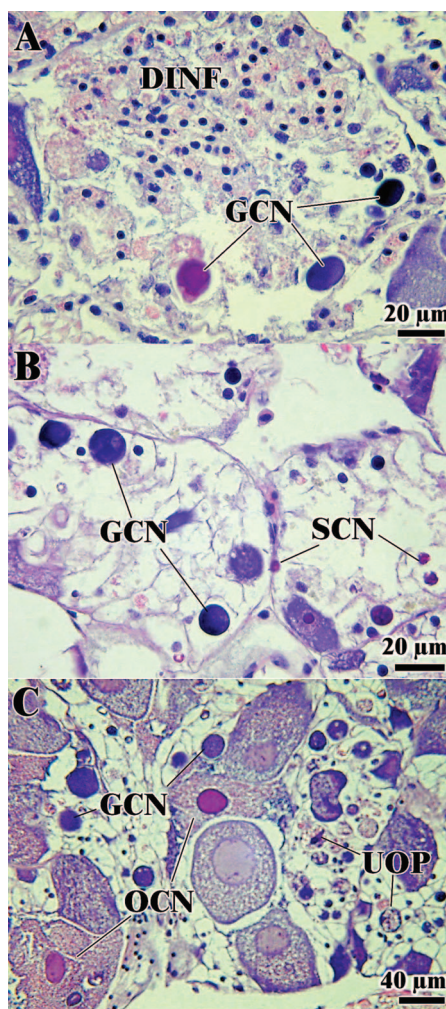


Figure 6. Female gonad lesions in *M. balthica* exposed to Sydney Harbour sediments. (A) DINF and GCN in station 6. (B) GCN and SCN in station 1. (C) GCN and OCN in station 7. UOP, unidentified ovarian parasite.

Table 4. Means of female gonad lesions in unexposed, control, reference, and treated clams.

Station	No.	GCN	OCN	GMA	SCN	Total
Unexposed	5	0.60	0.00	0.00	0.00	0.60
Control	7	0.00	0.00	0.00	0.00	0.00
Reference	4	0.50	0.00	0.00	0.00	0.50
1	8	1.50	0.75	0.75	0.38	3.38
2	7	1.14	0.14	0.00	0.86	2.14
3	6	1.50	1.17	0.33	0.00	3.00
4	6	1.17	0.50	0.17	0.00	1.84
5	8	1.63	0.25	0.00	0.00	1.88
6	6	1.83	0.33	0.00	0.80	2.96
7	10	2.30	0.70	0.00	0.20	3.20
13	5	1.00	0.00	0.00	0.00	1.00
23	11	1.27	0.09	0.09	0.00	1.45
26	10	1.00	0.10	0.50	0.30	1.90

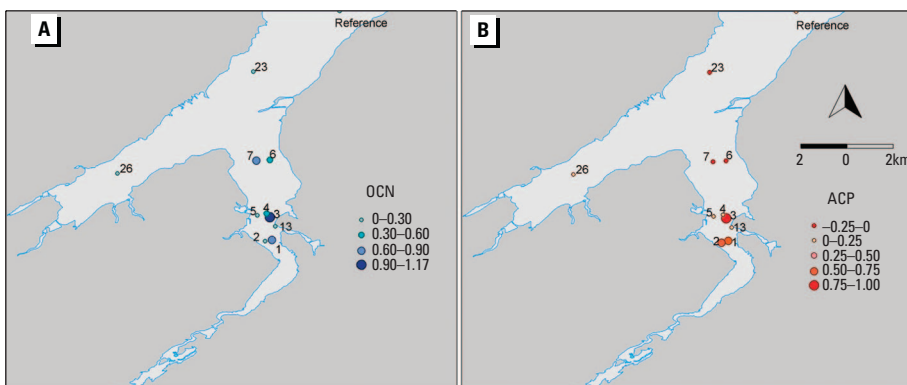


Figure 7. Means of OCN (A) and ACP (B) in *M. balthica* exposed to Sydney Harbour sediments by location.

the digestive gland and gonads were selected for more detailed observation.

Studies using both histopathologic and histochemical biomarkers to assess chronic effects of sediment contaminants on *M. balthica* are rare. The most recent study, which used only histopathologic lesions in *M. balthica* to assess pollution, was reported by Peters and Lehtonen (16). They found degenerative and inflammatory changes in the kidney, pericardial gland, heart, and digestive gland in *M. balthica* collected from the Gulf of Riga in the Baltic Sea. Neoplasms of possible hemic and germ cell origin were also found in clams from the station with potentially higher organic and heavy metal contamination.

Unlike the findings of Peters and Lehtonen (16), we found no neoplastic lesions in the present study.

The digestive gland of mollusks has been known as a target organ for contaminant effects because this organ plays a major role in contaminant uptake and metabolism of inorganic and organic chemicals in the organisms (17). Digestive gland lesions including tubular dilation, cell vacuolation, and tissue inflammation induced by exposure to contaminated sediments were reported in mussels, *Mytilus edulis* (18–23), and oysters, *Ostrea edulis*, *Crassostrea virginica*, and *C. gigas* (24,25). Teh et al. (26) reported severe inflammation and moderate atrophy of primary ducts and diverticula in the digestive gland of an euryhaline species of bivalve mollusk, the Asian clam (*Potamocorbula amurensis*), collected from Sacramento River of the San Francisco estuary in California, where high concentration of heavy metals were found. Working in a controlled laboratory environment, they also observed similar lesions in Asian clams exposed to hexavalent chromium [Cr(VI)] (27). Bright and Ellis (28) described vacuolation of digestive cells and fragmentation-phase tubules in *Macoma carlottensis* collected from Howe Sound, British Columbia, a fjord that was subjected to massive deposition of copper-mine tailings. Tubular dilation of digestive gland in this study is similar to those observed in the

Asian clam by Teh et al. (26). No vacuolation of digestive cells and fragmentation-phase tubules were detected in this study. Only one lesion, DMA, correlated significantly with Pb. The organic contaminants, especially PCBs, in the Sydney Harbour sediments correlated better with the observed lesions.

Clams from station 3, the station with the highest sediment PCBs and the second highest sediment PAHs, had the highest ACP activity and the second highest activities of ATP, glycogen, and P450 in their digestive gland. Enhanced staining of ACP was observed in the three sampling stations located at the mouth of Muggah Creek (stations 1, 2, and 3), whereas enhanced staining of GGT, ATP, and PAS were more frequently observed at stations 3, 4, and 5. Overall, increased enzyme and antibody activities in the digestive gland were more commonly observed in the treatment groups than in the controls. Among the enzymes observed in this study, ACP was the only enzyme that showed significant positive correlation between its activities and both the concentrations of inorganic and organic contaminants. Station 7 clams had decreased ACP and ATP intensities, probably caused by the high number of digestive gland tubular cell necrosis. Decreased ACP activity was also observed in the station 6 clams, which had the third lowest tubular cell necrosis, though sediments from this site had the highest PCBs concentration among all the stations outside of the Muggah Creek area. Teh et al. (26) observed a decrease of ACP in the digestive diverticula of the Asian clams exposed to water contaminated by high levels of trace metal. They theorized that the inhibition of ACP by heavy metals occurs either through the interaction between metal and sulfhydryl groups or by displacement of an essential metal cofactor of the enzyme. More research is needed to understand the mechanism of organic and inorganic contaminant impacts on enzyme activities in clams.

Of the 48 clams used for histochemical observation, only 11 had alterations of P450

reactions. It is possible that the salmon CYP1A antibody used in this study did not react specifically to P450 protein of *M. balthica*. Further study using techniques such as Western blot analysis may be needed to verify the specificity of salmon CYP1A in *M. balthica*. If P450 is to be used as a biomarker for assessing contaminants effects on *M. balthica*, additional research should be focused on the cytochrome P450 protein of this species.

Neoplastic changes or histopathologic alterations of gonad cells have been observed in shellfish collected from contaminated areas or exposed to contaminated sediments in the laboratory (14,16,19,21,24–36). The observed lesions ranged from dilation of reproductive follicles in mussel, *Mytilus edulis* (33), to gonadal neoplasms in soft-shell clam, *Mya arenaria* (21), and the American oyster, *Crassostrea virginica*. Peters and Lehtonen (16) observed neoplasms of possible germ cell origin in *M. balthica* collected from a contaminated area in the Gulf of Riga. No neoplastic lesions were observed in the exposed clams in this study. However, the frequent occurrence of digestive cell, germ cell, and oval cell necroses and tissue inflammation may be an early indication of chronic alterations in the digestive gland and reproductive disorder caused by the sediment contaminants.

Clams from station 13, one of the four stations located at the mouth of Muggah Creek, had relatively lower numbers of tissue lesions and less alteration of enzyme and antibody activities than clams from other stations. Though sediment from station 13 had the third highest concentration of PAHs, it also had the highest concentration of organic carbon. The highest organic carbon content in this sediment may influence the bioavailabilities of the organic and inorganic contaminants which, in turn, reduce the effects of toxicity to the exposed clams (37–41).

The relationship observed between sediment PCBs and the alterations of cells and tissues in digestive gland and female gonad of the exposed clams is difficult to explain, especially when the sediment PCBs concentration

Table 5. Means of male gonad lesions in unexposed, control, reference, and treated clams.

Station	No.	GMA	GINF	GCN	SCN	MPG	Intersex	Total
Unexposed	9	0.22	0.00	0.22	0.11	0.33	0.00	0.88
Control	8	0.50	0.00	0.38	0.00	0.13	0.00	1.01
Reference	11	0.18	0.00	0.45	0.00	0.00	0.00	0.63
1	7	0.43	0.00	0.14	0.00	0.14	0.00	0.71
2	8	0.50	0.38	0.38	0.00	0.50	0.00	1.76
3	9	0.33	0.33	0.44	0.00	0.00	0.00	1.10
4	9	0.22	0.00	0.11	0.00	0.00	0.00	0.33
5	7	0.29	0.14	0.14	0.14	0.00	0.00	0.71
6	8	0.13	0.00	0.38	0.00	0.25	0.00	0.76
7	5	0.20	0.00	0.80	0.00	0.00	0.60	1.60
13	8	0.25	0.00	0.25	0.00	0.00	0.00	0.50
23	4	0.00	0.00	0.25	0.00	0.00	0.00	0.25
26	5	0.00	0.00	0.20	0.20	0.00	0.00	0.40

MPG, male primordial germ cell.

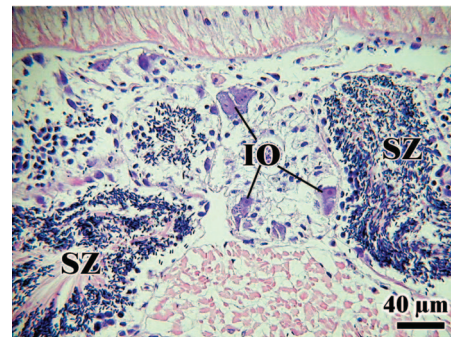


Figure 8. Intersex gonad in *M. balthica* exposed to station 7 sediments. SZ, spermatozoon; IO, immature oocyte.

was < 2 mg/kg. In fact, as demonstrated in Figure 5, there was no clear relationship between these two variables in sediments with < 2 mg/kg PCBs. It is possible that the relationship may be masked by the integrated effects of the mixtures of inorganic and organic contaminants in the Sydney Harbour sediments. Figure 5 indicates that, when the concentration of sediment PCBs increases to 3 mg/kg, this relationship becomes much more apparent. Furthermore, there were likely individual variations in sensitivity and uptake of contaminants within treatment. It is, therefore, important that future body burden analysis focus on individual organisms instead of pooled samples.

Most of the histopathologic studies on shellfish focused on female gonads. Recent studies by Teh and associates (26,27) showed mild or moderate necrosis of spermatogonia and moderate to severe granulomatous

inflammation in testis of the Asian clam *P. amurensis*. SCN and GCN, tissue inflammation (GINF), and macrophage aggregation (GMA) were observed in the exposed males in this study. However, because some of these lesions, especially GCN, SCN, and GMA, were also detected in the unexposed and control males, it is difficult to associate the occurrence of these lesions in the exposed clams with sediment contaminants.

Overall, the results of this study showed that digestive cell and germ cell necroses and inflammations in both digestive gland and gonad, especially the female gonad, were the most common histopathologic lesions detected in *M. balthica* exposed to Sydney Harbour sediments. At least one digestive gland lesion (TDA) and one female gonadal lesion (OCN) correlated significantly with the organic sediment contaminants. A lesser degree of correlation existed between these

lesions and the inorganic sediment contaminants. Among the histochemical biomarkers used in this study, only the activity of acid phosphatase was correlated with the concentration of sediment heavy metals, PAHs, and PCBs. Although no significant differences for ATP, GGT, PAS, and P450 were observed in this study, in general, they were more active in the clams exposed to the test sediments than the control. The observed responses of these histopathologic and histochemical biomarkers were a likely indication of chronic biologic effects induced by exposure to a mixture of inorganic and organic contaminants in the sediments. In addition to showing early indication of environmental stress on the health of the test organisms, they can also be used to localize the target tissues and organs of sediment contaminants in the affected individuals. When both biomarkers are used together, they are effective tools for detecting chronic biologic effects of environmental contaminants.

The exposure conditions used in our histopathologic and histochemical evaluation were identical to that used in the U.S. EPA bioaccumulation test method to quantify

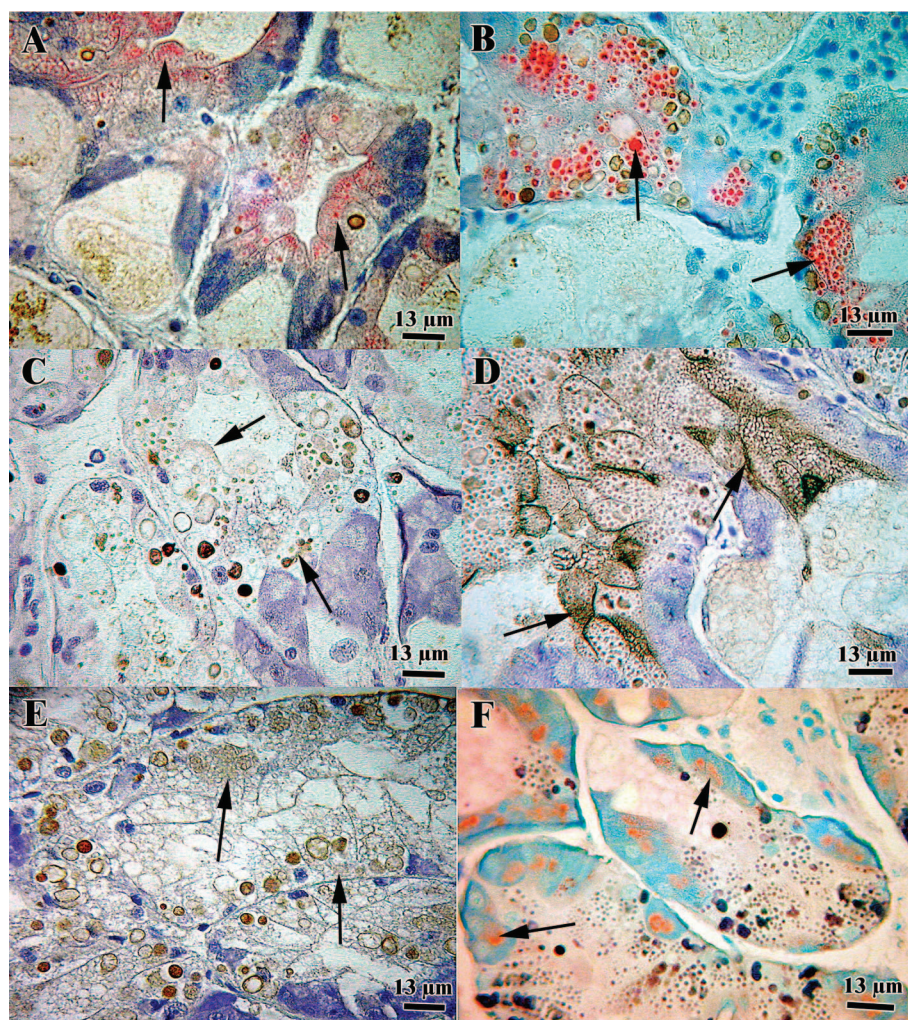


Figure 9. Alterations of the activities of enzyme and antibody in *M. balthica* in the control and Sydney Harbour sediments. (A) Normal ACP in digestive gland of control clams. (B) Enhanced ACP reaction in digestive gland of station 3 clams. (C) Decreased ATP reaction in digestive gland of station 7 clams. (D) Enhanced ATP reaction in digestive gland of station 4 clams. (E) Increased P450 staining in digestive gland of station 6 clams. (F) Enhanced GGT reaction in digestive gland of station 4 clams. The arrows indicate staining of enzyme and antibody reactions.

Table 6. Means of enzyme and antibody scores ($n = 4$) in control, reference, and treated clams.

Station	ACP	GGT	ATP	PAS	P450
Control	0.00	0.00	0.25	0.75	0.00
Reference	0.25	0.25	0.50	0.75	0.00
1	0.75	0.25	0.75	0.75	0.00
2	0.75	0.00	0.75	1.00	0.00
3	1.00	0.75	1.00	1.00	0.25
4	0.25	1.25	1.25	1.00	0.25
5	0.25	1.00	1.00	1.00	0.00
6	-0.25	0.25	0.50	0.00	1.00
7	-0.25	0.00	-0.50	0.50	0.25
13	0.25	0.25	0.75	1.00	0.00
23	0.00	0.50	0.25	1.00	0.00
26	0.25	0.50	1.00	1.50	1.00

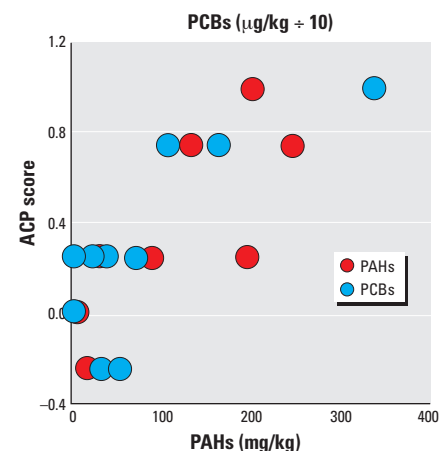


Figure 10. Relationship between means of ACP score in *M. balthica* exposed to Sydney Harbour sediments and the concentrations of PAHs (mg/kg; $r = 0.83$, $p = 0.003$) and PCBs ($\mu\text{g}/\text{kg} + 10$; $r = 0.77$, $p = 0.01$) in the sediments.

contaminant uptake in the tissue of *M. balthica*. The U.S. EPA method was designed to predict bioaccumulation of contaminants in individuals or uptake of contaminants through the food chain; it cannot be used for predicting the health of the test organisms. The suite of histopathologic and histochemical biomarkers identified in this study can be used to complement the U.S. EPA test method by expanding the potential of the test results to include identification and localization of chronic effects of the bioaccumulated contaminants in the test organisms. Although preliminary observations in this study suggest correlation between some of the selected biomarker assays and contaminant levels, regulatory acceptance of their general use has not been provided by this study. Logistical constraints of our field and laboratory programs limited the number of samples, particularly the histochemical data set, and thus constrained the power of our statistical analysis. Additional studies are currently being conducted to validate these biomarker responses against a suite of approved regulatory lethal and sublethal tests used by Environment Canada for the assessment of sediment contaminants.

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