Signatures of contamination in invasive round gobies (Neogobius melanostomus): A double strike for ecosystem health?

Julie R. Marentette [a,*], Krista L. Gooderham [a], Mark E. McMaster [b], Tania Ng [c], Joanne L. Parrott [b], Joanna Y. Wilson [c], Chris M. Wood [c], Sigal Balshine [a]

[a] Department of Psychology, Neuroscience and Behaviour, McMaster University, Hamilton, ON, Canada L8S 4K1
[b] National Water Research Institute, Burlington, ON, Canada L7R 4A6
[c] Department of Biology, McMaster University, Hamilton, ON, Canada L8S 4KI

ABSTRACT

The invasive round goby has a recognised role in transferring contaminants through foodwebs, but little work has been done on contaminant impacts on round gobies themselves. Here we present the first case study of contaminant biomarkers and subpopulation structure variation in round gobies, in relation to habitat contamination, within a Canadian Area of Concern, Hamilton Harbour. Copper and cadmium were elevated in livers of fish from contaminated habitats. Although catch abundances were similar across sites, fish were smaller, a greater proportion of fish were female and more males were in reproductive condition in contaminated sites. Fish from contaminated areas showed more fin loss. Males from contaminated sites showed intersex gonads and genitalia. Ethoxyresorufin-o-deethylase (EROD) activity was higher in fish collected near polycyclic aromatic hydrocarbon (PAH)-rich sediments. The results indicate that contaminants impact the characteristics of round goby populations, which could affect ecosystems beyond toxicant biomagnification. This study also confirms that round gobies can be abundant in polluted habitats, which may draw predators—facilitating mobilisation of contaminants in foodwebs.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

The aquatic biotas of the Laurentian Great Lakes have been shaped by a succession of invading species, one of which is the round goby (Neogobius melanostomus). This small benthic fish from Europe invaded all five Great Lakes by 1998 (Mills et al., 2003), possibly aided by multiple introduction events in ship ballast water (Jude, 1997). The rapid range expansion of the round goby and its explosive population growth were facilitated by its ability to specialise on dreissenid mussels (Dreissena polymorpha and D. rostriformis bugensis), to outcompete native species for food and shelter, and to spawn several times each breeding season (Corkum et al., 2004). The round goby is now a dominant part of the biomass in many areas of the lower Great Lakes (Johnson et al., 2005) and its influence continues to expand as round gobies invade more inland waterways (e.g., Poos et al., 2010). These fish are now key players in Great Lakes aquatic ecology, both as an integral and important part of the diets of piscivorous fishes, reptiles and waterbirds (Mills et al., 2003; Somers et al., 2003;

King et al., 2006), and as a competitor and potential causal agent in the decline of native benthic fishes such as darters, logperch and sculpins (Jude et al., 1995; Dubs and Corkum, 1996; Janssen and Jude, 2001; Lauer et al., 2004; Balshine et al., 2005).

The benthivorous diet of round gobies with its emphasis on dreissenids, and the role of gobies as prey for many species, has additional implications. As first suggested by Jude et al. (1995), the round goby is one of the few fish in the Great Lakes capable of eating the abundant dreissenid mussels, which are filter-feeding invertebrates known to bioconcentrate contaminants in their tissues. This means that persistent contaminants may have found a new route for bioaccumulation in higher trophic levels of the Great Lakes (Morrison et al., 2000). Kwon et al. (2006) found evidence for biomagnification of PCBs (polychlorinated biphenyls) in simplified foodwebs across several sites in Lake Erie, although this has not been found in all studies (Hanari et al., 2004). Round gobies may be contributing to the biomagnification of other organic contaminants like perfluoroctanesulfonate (PFOS; Kannan et al., 2005), and metallic toxicants as well. A diet of round gobies, for example, might be responsible for the sustained mercury loads in smallmouth bass in Lake Erie even as sediment mercury contamination declines (Hogan et al., 2007).

Round gobies have the potential to impact local ecosystems due to contaminant transfer, but what effects do contaminants

* Corresponding author. Fax: +1 905 529 6225.
E-mail addresses: marentjr@mcmaster.ca, marentette.j@gmail.com (J.R. Marentette).

have on round gobies? As a pollution-tolerant species (Pinchuk et al., 2003), round gobies may be able to substantially colonise heavily polluted areas of the Great Lakes in greater numbers than native fishes, and possibly attract or support predator populations in these regions. One such area is Hamilton Harbour in western Lake Ontario, Canada. This 2150 ha embayment is a Canadian International Joint Commission (IJC) Area of Concern (International Joint Commission, 1999) with a long history of contamination and habitat modification, stemming from both local steel mills and local urban settlements that have discharged sewage, agricultural, road and other effluents (Hamilton Harbour RAP, 1992). The Harbour is the location of Randle Reef, a polycyclic aromatic hydrocarbon (PAH)-rich coal tar deposit ranked as the second most contaminated aquatic location in Canada (Hamilton Harbour RAP, 1992). Remedial efforts have made progress in recent years toward cleaning the Harbour (Hall et al., 2006), but many contaminants continue to persist at problematic levels. Pollutants of primary concern ("A list" contaminants) include organic contaminants of industrial origin (PCBs and PAHs), and metals such as arsenic, cadmium, lead, iron and zinc (Hamilton Harbour RAP, 2003). Nonetheless, Hamilton Harbour continues to support a speciose ecosystem (fishes, waterfowl and terrestrial life) fed by a local wetland serving as an important fish and avian breeding ground (Cootes Paradise Marsh; Hamilton Harbour RAP, 1992). It continues to serve as a resource for human waterfront activities, including sportfishing and boating, although consumption of Harbour fish is regulated (Hamilton Harbour RAP, 2008).

Round gobies were first discovered in Hamilton Harbour in 1999 (Hamilton Harbour RAP, 2003), making this one of the last areas of the Great Lakes to be invaded. Within two years of the initial report, round gobies were well established in the embayment (Young et al., 2010). Since their invasion round gobies have become an important prey item for Harbour predators, including the abundant avian piscivore, the double-crested cormorant *Phalacrocorax auritus* (Somers et al., 2003). If contaminant exposure affects round goby populations in highly impacted areas of Hamilton Harbour (through bioconcentration and bioaccumulation, or through changes in abundance or vulnerability), then it follows that predators consuming round gobies in those areas should also be affected.

The aim of this study was to examine the consequences of living in contaminated areas using the round goby population of Hamilton Harbour as a model system. This fish's limited adult mobility (Ray and Corkum, 2001) suggests that known field conditions at fish collection sites should be reflected in biomarkers of contaminant exposure. We contrasted several toxicant biomarkers (7-ethoxyresorufin-o-deethylase, or EROD, activity, incidence of intersex, and morphological abnormalities), and direct measurements of contaminants (concentrations of copper, nickel and cadmium in body tissues) of round gobies collected from two heavily contaminated (PCB-, metal- and PAH-rich), and two relatively less contaminated areas of Hamilton Harbour. We also compared subpopulation characteristics (body size, age, abundance as catch per unit effort or CPUE and sex ratio) between the two highly contaminated sites and the two cleaner sites.

2. Materials and methods

2.1. Site selection and collection of fish

Round gobies were collected during and after the breeding season (MacInnis and Corkum, 2000a) from four nearshore sites in Hamilton Harbour (Fig. 1) between May 3 and October 26 in 2006 (n=846), April 19 and October 23 in 2007 (n=695) and April 23 and October 24 in 2008 (n=643). In the summer months, the nearshore or littoral zone (< 7 m depth) represents the oxygenated portion of the Harbour (Hamilton Harbour RAP, 2003). Shallow warm waters in rocky
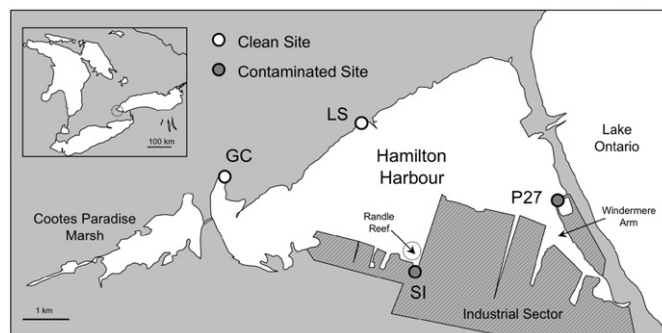
Fig. 1. Collection sites within Hamilton Harbour, Canada (43°17′N, 79°49′W) identified as a low impact or clean site (open circles): Grindstone Creek (GC) and LaSalle Park Marina (LS); or a high impact, contaminated site (grey circles): Pier 27 (P27) and Sherman Inlet (SI). Hamilton Harbour is shown in relation to the Laurentian Great Lakes (inset). Scales of maps are indicated.

habitats (rich in crevices) are where male round gobies typically establish nesting territories from which they court females and guard eggs (Charlebois et al., 1997). Sites were selected from known distributions of PAHs, metals and PCBs in harbour sediments (Hamilton Harbour RAP, 1992, 2003; Zeman, 2009), as these are the dominant contaminants of concern in this water body. Two cleaner sites were located along the relatively less impacted northern shore of the Harbour, at the mouth of Grindstone Creek (GC; 43°17′21″N, 79°53′13″W) and at LaSalle Park Marina (LS; 43°18′1″N, 79°50′47″W). Two highly contaminated sites were located along the southern and eastern shores, near a region of heavy steel industry discharges and historical coal tar dumping known as Randle Reef (Sherman Inlet, or SI; 43°17′3″N, 79°47′33″W; Murphy, 2000) and a confined, dredged-sediment disposal facility next to a high-use shipping channel known as Windermere Arm (Pier 27, or P27; 43°17′3″N, 79°47′33″W). Based on sediment contaminant databases generated between 1975 and 1989, sites near both SI (467 ng/g) and P27 (638 ng/g) have higher concentrations of PCBs than either GC or LS (30 and 124 ng/g), and both SI (1400–1470 μg/g) and P27 (20–67 ng/g) have higher concentrations of PAHs than GC and LS (< 17 ng/g; Zeman, 2009). PAH and PCB concentrations in the Harbour have remained stable from 1990 to 2004 (Zeman, 2009). Three sites had boulder and cobble cover extending to approximately 3 m from shore to a sand/silt substrate bottom, while one site (GC) had a mud substrate with few hard structures. All sites were similar in water clarity with typical Secchi depths > 1 m except for GC where the waters were consistently turbid (mean ± SE Secchi depth 20 ± 1.3 cm; Chi square test, χ^2_3=61.3, $p < 0.0001$). Sites were also similar in water temperature ($F_{7,151}$=0.6, p=0.6) and in dissolved oxygen within years ($F_{7,143}$=7.9, $p < 0.0001$, site $F_{3,143}$=2.7, p=0.05; Tukey HSD, $p > 0.05$ for all).

Round gobies were collected with minnow traps baited with 30 g of frozen corn, and set for 24 h in < 1 m of water. Fish were either processed on-site or transported back to the laboratory for processing within two days of capture. Round gobies processed for rapid collection of blood and tissue samples were placed in an ice-water bath followed by cervical severance; otherwise, fish were euthanized in a solution of benzocaine.

2.2. Site catch characteristics and morphological measurements

Sex was determined by an examination of the urogenital papilla (Miller, 1984) and confirmed by dissection. Fish could be assigned one of five reproductive states, as follows: gravid or reproductive female (RF, females with gonadosomatic index (GSI; see below) > 8%); adult but non-reproductive female (NRF, females with GSI < 8%); parental male (PM; males with a swollen urogenital papilla, black body colouration, enlarged head, developed testes and accessory glands); sneaker male (SM, males with a swollen urogenital papilla, a female-typical brown body colouration and narrow head, and developed testes); and adult but non-reproductive male (NRM, males with a small flat urogenital papilla and undeveloped testes and accessory glands, GSI ≤ 1%) (Marentette et al., 2009; Young et al., 2010). The standard length (SL), total length (TL), width of the head (across the posterior orbital edge; a wider head representing one of the secondary sex characteristics of reproductive parental males; Marentette et al., 2009) and length of the urogenital papilla (tip to anterior insertion at the posterior edge of the anus) were measured with calipers to 0.1 mm. Fish total mass, as well as the mass of the liver and gonads (accessory glands in males recorded separately) were recorded to 0.001 g. Fulton's condition factor K was calculated as total mass: $TL^3 \times 100$. The gonadosomatic index (GSI) was calculated as gonad mass:somatic mass × 100%, where somatic mass=total mass − gonad mass. Similarly, the hepatosomatic index (HSI) was calculated as liver mass:somatic mass × 100%, where somatic mass=total mass − liver mass. In males, the accessory gland somatic index (AGSI) was calculated as the mass of the organ:somatic

mass × 100%. Indices such as HSI and GSI are recognised to inadequately account for allometric differences across fish of varying size (Tomkins and Simmons, 2002), but are very commonly used and thus reported to facilitate comparisons with other studies.

In 2007, digital photographs were taken of the ventral surface of 197 round gobies, representing approximately 10 fish per reproductive category from all four Hamilton Harbour sites. The length and width (at the midpoint of the length) of the urogenital papilla as well as the length and area of the pelvic suction disc (fused pelvic fins characteristic of gobiid fishes) were determined in mm or mm^2 using the programme ImageJ (Wayne Rasband, National Institutes of Health, USA, available at http://rsb.info.nih.gov/ij). The width:length ratio of the urogenital papilla has been used in other gobiids to track male-to-female sex change (Carlisle et al., 2000).

2.3. Body burdens of copper, nickel and cadmium

Eight size-matched males from three sites (LS, P27 and SI; $n=24$) were obtained on September 12 and 26, 2008 and processed to quantify copper (Cu), nickel (Ni) and cadmium (Cd). Too few females and too few fish from GC were collected to be included in this study. Fish were euthanized in benzocaine and the liver, gills and gut removed (gut cut longitudinally to remove contents), rinsed in 0.9% NaCl solution (analytical grade, Sigma-Aldrich, prepared in NANOpure II water, Sybron/Barnstead, Massachusetts, USA) to remove surface bound metals, and placed on ice. Tissue was weighed and digested in 3–5 volumes of 1 or 2 N trace-metal grade HNO$_3$ (Fisher Scientific) at 65 °C for two days. Samples were shaken on the first day of digestion. The tissue digest was diluted to 1% HNO$_3$ for Cd, Cu and Ni analyses by a graphite furnace atomic absorption spectrometer (Varian Spectra AA-20 with graphite tube atomizer [GTA-110], Mulgrave, Australia). Standards from Fisher Scientific (Toronto, Canada) were used for calibration in every 40 samples. Certified reference material, TM15 (National Water Research Institute, Burlington, Canada) was analysed for validation in each run. Recovery of metals was within 20%. A maximum of 5% difference in metal concentrations between duplicates of samples was accepted. Tissue metal concentrations were expressed in $\mu g\,g^{-1}$ wet weight. To determine the importance of waterborne (ratios < 1) versus dietary (ratios > 1) routes of metal exposure, the gut:gill ratio was calculated in each fish. LS fish had larger livers than fish from P27 or SI (effect of site $F_{2,18}=7.9$, $p=0.003$).

2.4. EROD activity

Livers were collected from freshly euthanized animals up to two days after capture between May 22 and September 11 in 2007 ($n=179$), and on the day of capture between June 16 and July 14 in 2008 ($n=35$). The samples were individually wrapped in aluminium foil and stored at $-80\,°C$ until processing. Each liver was then thawed, bisected and one half of the organ was homogenised in buffer (50 mM Tris HCl, 0.15 M KCl; 4 mL g^{-1} tissue) at 4 °C. Liver homogenates were then centrifuged for 10 min at 750 g and 10 min at 12,000 g, at 4 °C (Sorvall Legend RT centrifuge). The supernatant S9 fraction was drawn off and the protein content of the S9 fraction (in $\mu g\,mL^{-1}$) was determined with 0.5 μL of sample, taken in duplicate, for a bicinchoninic acid protein assay adapted for a 96-well plate (Pierce Kit 23225, Thermo Scientific; Smith et al. (1985)). The remainder was stored at $-80\,°C$ until assayed for EROD activity (Kennedy et al., 1993) with Gen5 data analysis software and a Synergy microplate reader (BioTek Instruments, Inc.). EROD activity was calculated as pmol resorufin min^{-1} mg^{-1} of liver protein. In 2007, fish held in the laboratory for one to two days had lower EROD activity than fish processed on the day of collection ($F_{2,176}=7.7$, $p=0.0006$). Hence only fish processed on day 0 were used in further analyses. In this study, SI males were smaller than males of other sites, but females did not differ in TL (site × sex $F_{3,128}=4.3$, $p=0.007$, Tukey HSD, $p < 0.05$ and $p > 0.05$, respectively).

2.5. Gonad histology

The gonads of a subset of 6–31 reproductive males (PM and SM tactics) and 7–18 females per site were collected from the four sites in 2007. Gonad samples were preserved in 10% neutral buffered formalin for 24 h immediately after dissection from freshly euthanized fish, then placed in 70% ethanol for long-term storage (approximately nine months elapsed before processing). A randomly selected gonad half (either the left or right ovary in females, or the left or right lobes of the testes and accessory glands in males) was embedded in paraffin (Fisher Scientific) and sectioned at either 4 μm (males) or 6 μm (females) using Leica RM2155 and RM2235 microtomes. Embedded ovary samples were first soaked in glycerine and warm water, followed by immersion in ice, before sectioning to prevent loss of sample. Sections from the middle of the gonad sample were stained with hematoxylin and eosin (Leica Autostainer XL). Longitudinal sections from three areas of the testis were obtained per male, spaced at least 30 μm apart. Sections of all three areas per male were scanned using a light microscope at 100 × power to identify oocytes, indicating the presence of intersex. One section of each female ovary was digitally photographed at low magnification

(2.5 × or 5 ×) on a Leica DMR microscope with a Qimaging Qcam (Fast 1394 model) and the images stitched together to create a composite image of the entire section (Northern Eclipse 7.0, Empix Imaging, Inc.). The single stitched image was analysed using ImageJ to determine (a) the number of nucleated oocytes of one of three main categories (primary oocytes, early vitellogenic ova, late vitellogenic ova), and (b) the area of at least 10 nucleated cells in each category (mm^2).

2.6. Statistical analyses

Data were log- or arcsine-square-root-transformed where necessary to meet parametric test requirements. Data from GC were excluded from analyses when too few fish were collected. Morphological data were examined as one-, two- or three-factor ANOVAs or ANCOVAs with a combination of sex, reproductive status, site and/or year as independent variables, and where applicable, log TL or log somatic mass (total mass − mass of organ of interest) as the covariate. Non-significant factor-covariate interaction terms were removed from ANCOVA models. Significant differences between groups were determined with *post-hoc* Tukey Honestly Significant Difference (HSD) tests. In cases where transformation was not successful in satisfying parametric test requirements, non-parametric Kruskal Wallis (KW) tests were used, and differences among groups identified using non-parametric post-hoc tests (Zar, 1999). Statistics for which non-parametric tests were performed were reported as medians and ranges. Non-parametric correlations were examined with the Spearman rho (r_s) test. Ratio data (for sexes, for reproductive to non-reproductive individuals, and for parental and sneaker male tactics) were analysed for departure from a 1:1 ratio with chi square (χ^2) tests, and were examined for differences among sites and/or years with heterogeneity chi square tests. Site or year differences among sets of ratios were identified by sequentially removing the largest or smallest site or year value in a set, and then recalculating the heterogeneity chi square on the remaining ratios until no pairwise differences are achieved (a technique recommended by Zar, 1999).

2.7. Ethical note

All animal handling methods met criteria for McMaster University Animal Research Ethics Board (AREB) (AUPs #03-09-54 and 06-10-61) according to standards of the Canadian Council on Animal Care.

3. Results

3.1. Evidence of contaminant burdens (Ni, Cu, Cd)

The livers of fish from one of the contaminated sites (P27) contained higher levels of Cd than those found in fish from the cleaner site (LS, $F_{liver\ 2,21}=5.2$, $p=0.02$; Fig. 2a); gill and gut concentrations of Cd did not vary among sites ($F_{gill\ 2,21}=2.2$, $p=0.13$, $F_{gut\ 2,21}=1.1$, $p=0.4$). The livers but not the gills or the gut of fish from the other contaminated site (SI) had higher levels of Cu compared to fish from the clean site ($F_{liver\ 2,21}=4.2$, $p=0.03$, $F_{gill\ 2,21}=3.6$, $p=0.04$, $F_{gut\ 2,21}=1.4$, $p=0.3$; Fig. 2b). Ni concentrations in fish tissues did not vary across sites ($F_{liver\ 2,21}=2.8$, $p=0.08$, $F_{gill\ 2,21}=0.2$, $p=0.8$, $F_{gut\ 2,21}=2.7$, $p=0.09$; Fig. 2c). The gut:gill ratio of Ni and Cd were similar across sites ($p > 0.1$; pooled mean ± SE of 1.17 ± 0.12 for Ni and 12.75 ± 2.19 for Cd); for Cu, the gut:gill ratio was higher for P27 males (0.78 ± 0.14) than for SI males (0.37 ± 0.07, $F_{2,21}=3.6$, $p=0.043$), but LS males did not differ from either (0.58 ± 0.11). Confidence intervals (95%) generated for the gut:gill ratio indicated that Cd was acquired primarily through the gut in all sites, Cu was acquired primarily through the gills, but only in fish from two of three sites (LS and SI), and neither route of entry predominated for Ni acquisition in fish tissues.

3.2. Subpopulation variation related to site contamination

Catch rates at the four sites were not related to the degree of site contamination and varied across years for which data were available (Table 1). Fish from the clean sites, GC and LS, were longer and heavier than fish from the contaminated sites, P27 or SI, and male round gobies were both longer and heavier than
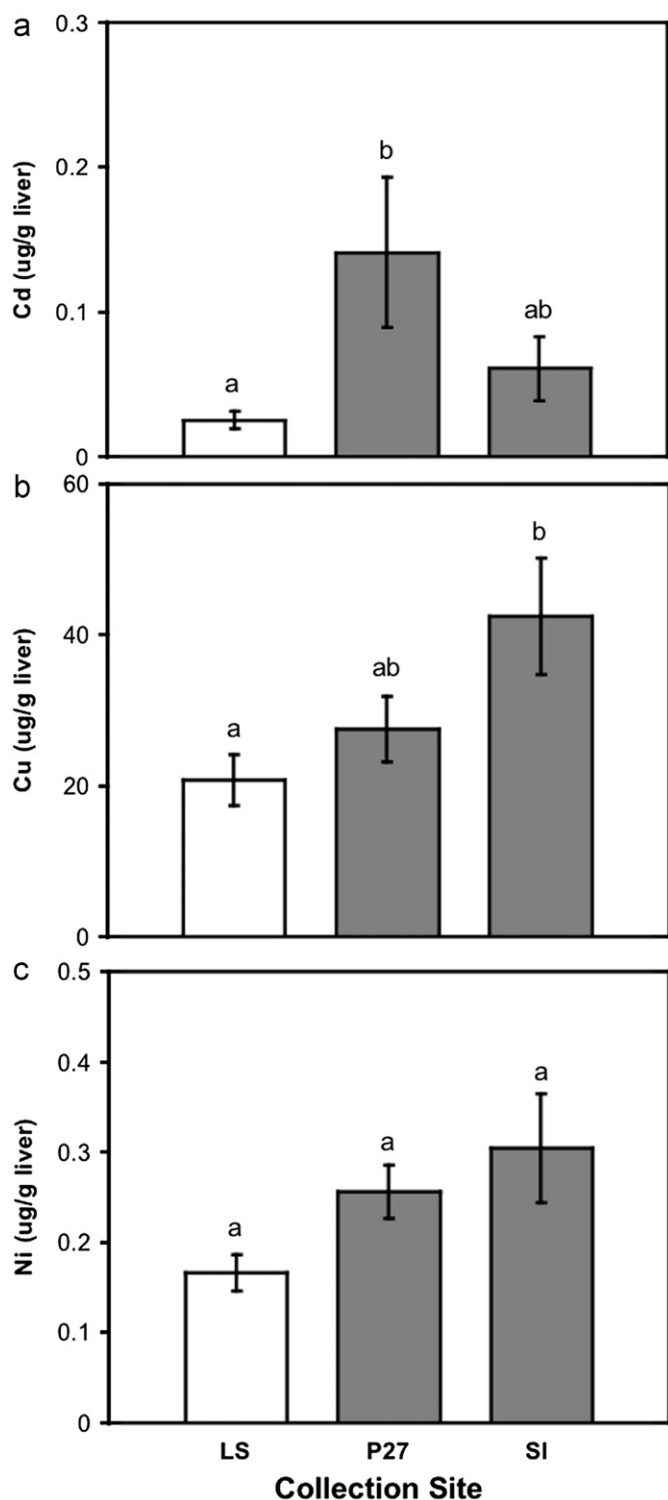
Fig. 2. Body burdens of contaminants in round gobies from clean and contaminated sites. White bars=clean site. Grey bars=contaminated site. (a) Mean ± SE liver Cd burdens for male round gobies ($n=8$). (b) Mean ± SE liver Cu burdens for male round gobies ($n=8$). (c) Mean ± SE liver Ni burdens for male round gobies ($n=8$). Letters represent differences among sites (Tukey HSD, $p < 0.05$).

Table 1
Median and range of catch rates per unit effort (round gobies per minnow trap) across years at clean (GC, LS) and contaminated (P27, SI) sites. Letters indicate significant differences among sites within a year (KW tests, $p < 0.05$; post-hoc non-parametric tests, $p < 0.05$).

Site	2007	2008	Total
GC	0 (0–7)[a] $n=62$	0 (0–6)[a] $n=16$	0 (0–7)[a] $n=78$
LS	4 (0–16)[b] $n=126$	4 (0–35)[b] $n=78$	4 (0–35)[b] $n=204$
P27	4 (0–19)[b] $n=77$	3 (0–28)[c] $n=72$	3 (0–28)[c] $n=149$
SI	2 (0–25)[c] $n=81$	5 (0–19)[b] $n=47$	3 (0–25)[c] $n=128$

any other site (effect of site $F_{3,190}=3.8$, $p=0.01$, Fig. 3b). Round goby investment in liver tissue did not consistently vary with site, only with reproductive status (effect of status $F_{4,2101}=48.7$, $p < 0.0001$; NRFs > NRMs > PMs > SMs and RFs; Tables 2 and 3). There was also no clear effect of site on reproductive investment (Tables 2 and 3). Only SM fish showed a decrease in gonad size in contaminated areas, in both ANCOVA models of testes investment and GSI values; SMs from both contaminated sites of SI and P27 had smaller testes than SMs from LS, and accessory glands of SMs from SI were also smaller than those of LS.
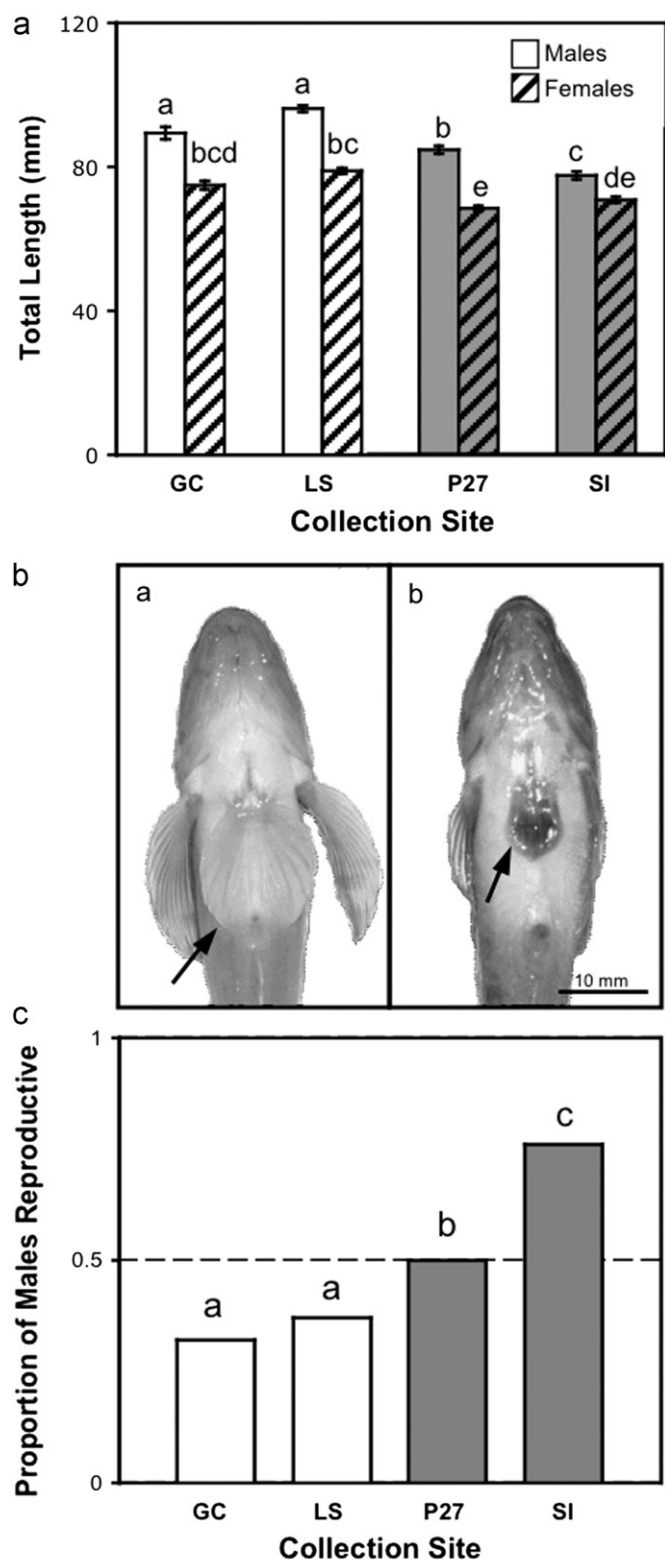
Three of the sites showed a male-biased catch (χ^2 tests, all $p < 0.001$), while catches from one of the contaminated sites, SI were not significantly different from a 1:1 male:female sex ratio (across years $\chi^2=1.96$, $p=0.16$). This site, SI, also had the highest ratio of reproductive to non-reproductive (R:NR) males, followed by the other contaminated site, P27, and then the two cleaner sites GC and LS both had the lowest ratio of R:NR males across years (pairwise χ^2 tests, $p < 0.0001$; Fig. 3c). Ratios of parental to sneaker males (PM:SM) varied among sites. GC exhibited no bias in the ratio ($\chi^2=2.1$, $p=0.15$) while LS and P27 both exhibited strongly PM-biased ratios ($\chi^2=15.9$ and 17.5, $p < 0.0001$). SI had a strikingly strong SM-biased ratio in 2006–2007 ($\chi^2=12.8$ and 11.2, $p < 0.001$) but an unbiased ratio in 2008 ($\chi^2=0.01$, $p=0.91$).

3.3. EROD activity

The effect of fish collection site on EROD activity varied marginally with reproductive status ($F_{15,120}=8.3$, $p < 0.0001$; site x status $F_{9,120}=1.8$, $p=0.07$; Table 4). Within each status, site effects were only apparent for NRF fish (Tukey HSD, $p < 0.05$) where NRFs from SI had higher activity than NRFs from any other site. Within each site, NRM fish consistently had the highest EROD activity, and RF fish the lowest (NRMs > RFs, Tukey HSD, $p < 0.05$). PM and SM males had equivalent EROD activities and were pooled into one category, RM (reproductive males; $t_{33}=0.2$, $p=0.8$). When considering reproductive status alone, NRM fish had the greatest EROD activity, and NRF fish had higher activity than RFs ($F_{3,131}=20.9$, $p < 0.0001$).

3.4. Reproductive impacts and indicators of endocrine disruption

Male and female round gobies had different width:length ratios of the urogenital papilla, with males having longer, narrower papillae and thus smaller ratios than females ($F_{1,183}=89.7$, $p < 0.0001$; Fig. 4a). The width:length ratio did not vary with reproductive status or site in females ($F_{4,71}=1.3$, $p=0.27$). Among reproductive males (PM and SM fish together), LS fish had smaller, more male-typical length:width papilla ratios

same-site females (effect of sex × site $F_{3,2047}=8.2$ and $F_{3,2174}=8.2$, p's < 0.0001; Fig. 3a and Table 2). The digital photographs revealed that the fused pelvic fins of males and females from one of the contaminated sites, SI, were typically damaged, covering a smaller area of the ventral surface than in fish from

than fish from either contaminated site (P27 or SI), but all reproductive males regardless of site still had smaller ratios than females ($F_{3,148}=45.9$, $p<0.0001$, Fig. 4a). There was no correlation of GSI with the degree of male papilla feminisation (papilla ratio) in either clean ($r_s=0.3$, $p=0.9$) or contaminated-site ($r_s=0.16$, $p=0.23$) reproductive males.

No intersex was detected in males collected from the two clean sites GC ($n=6$), LS ($n=22$), or from fish from one of the contaminated sites, P27 ($n=26$). Four of 31 males (12.9% of males) from SI showed the presence of oocytes. Of these, two still showed production of spermatozoa; two did not show development of seminiferous lobules. One male possessed a few developed vitellogenic ova both distally and medially placed within the testis (Fig. 4b); three males had primary oocytes scattered throughout the testis sections, with or without spermatogenesis evident (Fig. 4c and 4d).

Female gamete size did not differ across sites for any of the three stages of oocyte development: primary oocyte (effect of site $F_{3,49}=2.4$, $p=0.08$); early vitellogenic ova (effect of site $F_{3,48}=0.6$, $p=0.62$); or late vitellogenic ova (effect of site $F_{3,34}=0.9$, $p=0.45$), nor did gonad investment vary across sites in these fish (effect of site $F_{3,47}=2.1$, $p=0.11$). Females from one of the contaminated sites (SI) had more primary oocytes than females from one of the clean sites, LS (KW, $H=8.1$, $p=0.02$) but no differences were observed across females from different sites in the numbers of early vitellogenic (KW, $H=0.6$, $p=0.73$) or late vitellogenic ova (KW, $H=4.4$, $p=0.11$).

4. Discussion

4.1. Subpopulation variation related to site contamination

The smaller mean fish sizes observed in contaminated areas relative to clean areas could be attributed to either (a) an inhibition of growth and/or (b) higher mortality and thus fewer older fish in contaminated sites (Newman and Clements, 2008). A previous study elsewhere in the Great Lakes has reported a maximum age of two years for males and three years for females (MacInnis and Corkum, 2000b). Establishing the ages of individuals and their growth rates from otolith annuli will be a crucial next step to understanding the shift in body size in gobies of Hamilton Harbour's contaminated regions.

A male-biased catch is commonly reported for round gobies in nearshore areas (Corkum et al., 2004; Shemonaev and Kirilenko, 2009; Young et al., 2010; but see Kovtun 1980; Bergstrom et al. 2008). This is despite the fact that males are thought to experience high mortality after each breeding season (Charlebois et al., 1997; MacInnis and Corkum, 2000b; Pinchuk et al., 2003). Interestingly, a female-biased sex ratio in round gobies has been reported in the Detroit River, another Area of Concern (MacInnis and Corkum, 2000b). The absence of male bias in the nearshore of most highly contaminated region in Hamilton Harbour (Sherman Inlet) could be due to higher mortality rates for male versus female fish. Endocrine disruption of larval or juvenile sexual development could also yield a higher than normal percentage of female hatched fry by feminisation of males in a critical window (Van Aerle et al., 2002). However, round goby larvae are expected to disperse over very great areas (Hayden and Miner, 2009), so it is possible but unlikely that the lack of sex bias is due to an increased production of female offspring in Sherman Inlet, since larvae from all over the Harbour may settle in the area. In addition, little is known about the range of values expected for parental to sneaker ratios in round goby populations. The increased prevalence of sneaker males relative to parental males in the highly polluted site of Sherman Inlet could be due to

Fig. 3. Characteristics of round goby catches at different sampling sites. White bars=clean site. Grey bars=contaminated site. Open bars=male data and hatched bars=female data where indicated. (a) Differences in mean ± SE total length (cm) for male and female round gobies across sites. Letters represent significant differences among sites and sexes (Tukey HSD, $p<0.05$). (b) Photographs of the ventral anterior aspect of a round goby with a normal pelvic disc (panel a) and an eroded disc characteristic of many fish from contaminated areas (panel b). Arrows indicate the disc, and the scale of the photographs is given in mm. (c) The proportion of all adult males found to be in reproductive condition (parental or sneaker male) across sites, pooled across years. Letters indicate significant differences between sites (Chi square, $p<0.05$).

Table 2
Morphological measures of round gobies at clean (GC, LS) and contaminated (P27, SI) sites, by sex and reproductive status. Reported values are means (SE; TL and mass) or medians and ranges (indices). Letters indicate significant differences among group means (log-transformed data, Tukey HSD, $p < 0.05$) or medians (non-parametric post-hoc comparisons, $p < 0.05$). TL=total length, K=mass:$TL^3 \times 100$, HSI=hepatosomatic index, GSI=gonadosomatic index, AGSI=accessory gland somatic index (male only). M=all males, F=all females. PM=parental males, SM=sneaker males, NRM=non-reproductive males, RF=reproductive females, NRF=non-reproductive females.

	Sites	n	TL (mm)	Mass (g)	Fulton's K	HSI (%)	GSI (%)	AGSI (%)
M	GC	126	89.4 (1.7)[a]	10.3 (0.5)[a]	11.5 (0.3)[a]	2.4 (0–10.6)[a]	0.2 (0–10.1)[a]	0.1 (0–1.5)[a]
	LS	613	96.2 (1.1)[b]	14.4 (0.4)[b]	13.5 (0.2)[b]	2.5 (0.1–8.4)[a]	0.1 (0–14.3)[a]	0.2 (0–6.4)[ab]
	P27	342	84.7 (1.1)[a]	10.0 (0.4)[a]	11.5 (0.2)[a]	2.2 (0–7.0)[b]	0.2 (0–8.0)[b]	0.0 (0–6.9)[b]
	SI	278	77.7 (1.1)[c]	7.6 (0.4)[c]	10.4 (0.2)[c]	2.0 (0–5.2)[c]	0.3 (0–7.8)[c]	0.2 (0–2.0)[c]
F	GC	73	74.9 (1.2)[a]	5.8 (0.3)[ab]	9.5 (0.2)[a]	2.2 (0.1–10.9)[a]	4.5 (0.3–19.7)[a]	
	LS	288	78.9 (0.8)[a]	7.0 (0.2)[a]	10.8 (0.1)[b]	2.5 (0–20.7)[a]	1.9 (0.1–23.2)[b]	
	P27	218	68.5 (0.7)[b]	4.7 (0.2)[c]	9.1 (0.1)[a]	2.1 (0–6.1)[b]	3.9 (0–23.3)[a]	
	SI	246	71.0 (0.8)[b]	5.2 (0.2)[bc]	9.4 (0.1)[a]	1.8 (0–6.2)[b]	4.3 (0–28.8)[a]	
PM	GC	24	96.4 (2.5)[ab]	12.8 (1.0)[ab]	13.3 (0.3)[abc]	2.4 (0.2–6.0)	1.1 (0.2–2.1)	0.6 (0–1.5)
	LS	149	111.9 (1.7)[c]	22.9 (1.0)[c]	15.9 (0.4)[a]	2.7 (0.5–6.9)	1.2 (0–7.5)	0.4 (0–2.3)
	P27	119	99.4 (1.6)[a]	15.4 (0.9)[a]	14.0 (0.3)[b]	2.6 (0.3–5.2)	1.2 (0–3.8)	0.5 (0–2.9)
	SI	83	92.3 (2.0)[b]	12.4 (1.0)[b]	12.8 (0.4)[c]	2.4 (0–5.1)	1.3 (0.1–4.7)	0.4 (0–1.2)
SM	GC	15	77.6 (4.6)[a]	5.5 (1.0)	9.4 (0.7)	1.8 (0–10.6)	5.2 (0–10.1)[a]	0.4 (0–1.3)[a]
	LS	88	68.6 (1.4)[ab]	4.7 (0.3)	9.2 (0.2)	1.1 (0.2–6.1)	4.7 (0.1–14.4)[a]	0.3 (0–6.4)[a]
	P27	63	64.5 (1.8)[b]	4.3 (0.5)	8.8 (0.3)	1.2 (0–6.6)	4.1 (0.1–13.6)[b]	0.3 (0–6.9)[b]
	SI	134	67.2 (1.0)[ab]	4.4 (0.2)	8.8 (0.2)	1.4 (0–5.2)	3.3 (0.1–7.8)[c]	0.2 (0–2.0)[c]
NRM	GC	87	89.6 (2.0)[a]	10.4 (0.6)[a]	11.4 (0.3)[a]	2.6 (0.3–8.0)[a]	0.1 (0–0.7)[a]	0.0 (0–0.3)[a]
	LS	376	96.4 (0.9)[b]	13.7 (0.4)[b]	13.5 (0.2)[b]	2.7 (0.1–8.4)[a]	0.1 (0–0.7)[a]	0.0 (0–0.8)[b]
	P27	160	81.8 (1.3)[c]	8.2 (0.4)[c]	10.7 (0.2)[a]	2.4 (0.1–7.0)[b]	0.1 (0–0.9)[a]	0.0 (0–0.7)[b]
	SI	61	81.0 (2.0)[c]	8.0 (0.6)[ac]	10.8 (0.4)[a]	2.7 (0.2–5)[a]	0.1 (0–1.0)[b]	0.0 (0–0.3)[a]
RF	GC	23	73.7 (2.3)[ab]	5.6 (0.5) [ab]	9.7 (0.4)[ab]	1.3 (0.1–10.9)	12.4 (8.5–19.7)[a]	
	LS	54	80.4 (2.0)[a]	7.3 (0.5)[a]	11.3 (0.3)[a]	1.6 (0–5.0)	12.8 (8.1–23.2)[a]	
	P27	60	68.9 (1.3)[b]	5.0 (0.3)[b]	9.7 (0.3)[b]	1.4 (0–5.2)	17.3 (8.1–23.3)[b]	
	SI	74	70.6 (1.4)[b]	5.2 (0.4)[b]	9.4 (0.2)[b]	0.9 (0–5.0)	12.6 (8.1–28.8)[a]	
NRF	GC	50	75.4 (1.4)[ab]	5.8 (0.3)[ab]	9.4 (0.2)[a]	2.7 (0.5–5.8)[a]	1.9 (0.3–7.8)[ab]	
	LS	234	78.6 (0.8)[a]	7.0 (0.2)[a]	10.7 (0.2)[b]	2.6 (0–20.8)[a]	1.2 (0.1–7.9)[a]	
	P27	158	68.3 (0.9)[c]	4.5 (0.2)[c]	8.9 (0.6)[a]	2.3 (0–6.1)[b]	2.1 (0–7.9)[ab]	
	SI	172	71.1 (0.9)[bc]	5.3 (0.2)[bc]	9.4 (0.2)[a]	2.2 (0–6.2)[b]	2.5 (0–8.0)[b]	

Table 3
Results of ANCOVA models (effect of site) on liver and gonad investment in round gobies of different reproductive states. Models were run on log-transformed data with log somatic mass as covariate; post-hoc differences were identified with Tukey HSD tests (letters). PM=parental males, SM=sneaker males, NRM=non-reproductive males, RF=reproductive females, NRF=non-reproductive females. Clean sites: GC, LS. Contaminated sites: P27, SI.

	PM	SM	NRM	RF	NRF
Liver	$F_{3,369}=1.4$ $p=0.25$	$F_{3,293}=1.5$ $p=0.21$	$F_{3,678}=0.63$ $p=0.60$	$F_{3,197}=0.74$ $p=0.53$	$F_{3,608}=0.50$ $p=0.68$
Gonad	$F_{3,369}=1.2$ $p=0.32$	$F_{3,294}=9.0$ $p < 0.0001$[a]	$F_{3,673}=9.6$ $p < 0.0001$[b]	$F_{3,206}=4.6$ $p=0.004$[c]	$F_{3,605}=1.7$ $p=0.17$
Accessory gland	$F_{3,369}=1.8$ $p=0.14$	$F_{3,294}=3.7$ $p=0.01$[d]	$F_{3,673}=5.5$ $p=0.0009$[e]		

[a] LS > P27, SI.
[b] SI > P27, LS; GC > LS.
[c] P27 > SI.
[d] LS > SI.
[e] All > LS.

differential migration or mortality of the two male tactics (perhaps, an increased attraction of sneaker males to areas with many females), or an increased tendency of reproductively maturing males in contaminated areas to take on the sneaker tactic. A potential role for endocrine disruption during this sexual maturation phase and tactic adoption phase would be an interesting avenue of future research.

Reproductive investment in round gobies was not compromised in habitats with contamination. Lower gonadal investment has been reported in many species collected from contaminated areas, but the opposite can also occur (Schlenk et al., 2008). For example, elevated reproductive investment has been observed in populations of mummichog (*Fundulus heteroclitus*) that demonstrate tolerance to habitat contamination (Weis, 2002). The lack of a change in gonad size with contaminant exposure, and a higher proportion of reproductive individuals in contaminated areas may mean that round gobies are relatively unimpaired by exposure to contaminants in terms of reproductive investment. Alternatively, the long breeding season and repetitive spawning habits of the round goby may mean that individuals are not at the same point of the reproductive cycle when sampled, and this asynchrony, or antagonistic effects by different contaminants (Schlenk et al., 2008),

masks small but otherwise real changes in gonad investment and reproductive capacity.

4.2. Body burdens of contaminants

Metal distributions in round goby tissues varied with fish collection site and related to sediment metal contamination data (Zeman, 2009). In these reports, cadmium levels were higher in sediments sampled near Pier 27 (17.1 µg/g) than in Sherman Inlet (4.3 µg/g), but sites near LaSalle were not measured. Copper levels were higher in sediments near Sherman Inlet (in the range 110–197 µg/g) than near Pier 27 (102 µg/g) but were similar to LaSalle (113 µg/g). Nickel sediment deposits were distributed across the Harbour in the range 16–75 µg/g, above the lowest

effect level but below the severe effect level according to provincial sediment quality guidelines; interestingly, sediment nickel concentrations at LaSalle (53 µg/g) exceeded those at Pier 27 (41 µg/g) and Sherman Inlet (32 µg/g; Zeman, 2009). In round gobies, cadmium levels were highest in livers of Pier 27 males, and copper levels were highest in livers of Sherman Inlet males. However, liver burdens of nickel did not vary significantly among sites. The gut:gill ratio indicates a predominantly dietary route of entry for cadmium throughout the Harbour, and predominantly waterborne route of entry for copper. Cadmium is one of the "A list" contaminants for Hamilton Harbour (i.e., a contaminant of primary concern for remediation) and these results point to a potential role for round gobies in contaminated areas mobilising cadmium to piscivores in the region, including sportfishes that may be consumed by humans.
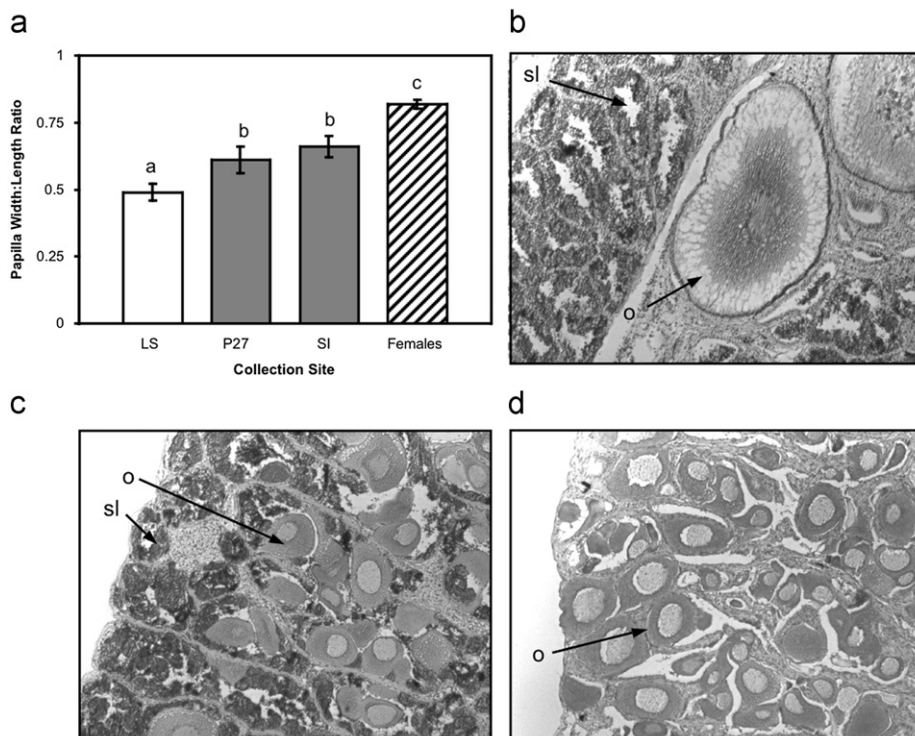
4.3. EROD activity as indicator of PAH exposure

Increases in EROD activity are expected with exposure to contaminants that bind the aryl hydrocarbon receptors (AhR) in the liver, which commonly include PAHs, coplanar PCBs and other planar halogenated aromatic hydrocarbons (PHAHs). The magnitude of EROD activity for round gobies in Hamilton Harbour is greater than that seen for brown bullheads from this same region (Arcand-Hoy and Metcalfe, 1999) or centrarchids from contaminated streams in the southern USA (Brammell et al., 2004). This may reflect not only species-specific differences but also the limited mobility of the round goby leading to a chronic high level of exposure to PAHs and PHAHs. PAHs are likely to be rapidly metabolised and cleared by the body (Arcand-Hoy and Metcalfe, 1999) and so measurements of PAHs or their metabolites may not

Table 4
Mean $\pm$ SE EROD activity (pmol min^{-1} mg^{-1}) for round gobies of different sexes and reproductive states. Fish from four Hamilton Harbour sites were collected in 2007 and 2008. RM=reproductive male (parental and sneaker males), RF=reproductive female, NRM=non-reproductive male, NRF=non-reproductive female. Letters indicate significant differences among groups (Tukey HSD, $p < 0.05$).

Site	RM	RF	NRM	NRF
GC	664.7 (354.7) $n=3$	50.5 (12.8) $n=2$	1821.7 (390.9) $n=6$	176.7 (54.9)[a] $n=4$
LS	166.8 (42.9) $n=10$	79.8 (25.6) $n=3$	3053.3 (624.8) $n=8$	742.1 (409.0)[a] $n=17$
P27	404.3 (170.2) $n=10$	139.9 (61.1) $n=6$	2223.1 (788.9) $n=13$	777.0 (307.1)[a] $n=11$
SI	611.1 (244.9) $n=13$	384.2 (97.2) $n=6$	4933.8 (1438.8) $n=9$	2605.2 (738.7)[b] $n=15$

Fig. 4. Intersex in male round gobies. White bars=clean site. Grey bars=contaminated site. Open bars=male data and hatched bars=female data where indicated. Microphotographs were taken at 100× magnification. (a) Mean $\pm$ SE ratios of the urogenital papilla width:length in reproducing males from three sites compared to a pooled sample of females from all sites. Letters represent significant differences among sites (Tukey HSD, $p < 0.05$). Papillae of males from contaminated sites were more female-like in their ratios than males of a clean site. (b) Male round goby testis from SI showing vitellogenic oocytes (o) and seminiferous lobules (sl). (c) Male round goby testis from SI showing the presence of both primary oocytes (o) and seminiferous lobules (sl). (d) Male round goby testis from SI showing presence of primary oocytes (o) but no developed seminiferous lobules.

adequately reflect PAH exposure. Although we did not measure the presence of PAH metabolites in our samples, the loss of EROD activity after even one night in clean laboratory water would point to a very quick clearance of the inducing substance, again supporting PAH exposure as the likely causal agent of patterns of EROD induction in our study.

EROD patterns are known to be modulated by 17β-estradiol or E_2 (Navas and Segner, 2001; Elskus, 2004), and so it is not surprising that male round gobies had higher EROD activity levels than did females, nor that non-reproductive individuals of both sexes had higher activities than reproductive individuals. Further studies exploring differences in underlying endocrine patterns are likely to parallel EROD patterns.

4.4. Endocrine disruption?

Low rates of intersex, present only at one contaminated site (SI), do indicate that some degree of early exposure to endocrine disrupting compounds (EDCs) may be occurring (Van Aerle et al., 2002). Naturally occurring rates of spontaneous, non-contaminant-related intersex are not known for this species. The very rare presence of vitellogenic follicles also indicates that male round gobies in contaminated sites are likely to be producing vitellogenin, another common biomarker of endocrine disruption that was not examined in this study. The intersex rates described in this study appear to be much lower than those reported for other species in Hamilton Harbour, such as white perch (Morone americana) where levels of intersex reach over 50% (Kavanaugh et al., 2004). This may be because larval round gobies hatch in different locations from the larvae of other species (many of which spawn in the nearby wetland of Cootes Paradise Marsh), and thus are exposed to different EDCs, or because round gobies have a different thresholds for EDC-determined intersex development.

More female-like urogenital papillae occurred in reproducing males of both contaminated sites, similar to what has been found in sand gobies (Pomatoschistus minutus) exposed to EDCs (Kirby et al., 2003). The papilla width:length ratio appears to be a more sensitive indicator of round goby endocrine disruption than histologic scoring of oocytes in testes. The simplicity of this measure means that papilla shape would be an efficient metric to track in other studies where endocrine disruption of round gobies is of interest or suspected.

4.5. Summary

Round gobies appear to be site-faithful enough to reliably differ in their indicators of contaminant exposure, reflecting the conditions of their site of capture. For this reason, round gobies may be a potentially useful bioindicator species throughout their global range, in the Great Lakes and beyond. Understanding round goby movements within and between clean and contaminated areas will be critical to understand the gradients formed by clean and contaminated fish in terms of home range, and thus the gradient of their possible impacts.

The contaminant body burdens in particular have worrisome implications for the remediation of Hamilton Harbour. As more piscivores like double-crested cormorants switch their diets to include an increasing proportion of round gobies (Somers et al., 2003), the risk of accumulating contaminants increases. This will impact the ability to deregulate sportfish consumption guidelines for species that are known to consume round gobies (e.g., largemouth bass, yellow perch, walleye). As PCBs are a primary contaminant of concern in Hamilton Harbour (Hamilton Harbour RAP, 2008), quantifying both PCB body burdens and

biomagnification of PCBs in round gobies and their predators in this region is an important area of future research. PAHs are readily metabolised and excreted (Arcand-Hoy and Metcalfe, 1999) and are not expected to bioaccumulate in organisms, including round goby predators. Nonetheless, predators consuming round gobies with high levels of PAHs will experience pulses of exposure to this carcinogenic class of contaminants (Balch et al., 1995), and if predators are drawn to areas with PAHs as a result of round goby prey, the predators themselves may become exposed to coal tar deposits and suffer the effects of these direct exposures in addition to consequences of consuming contaminated prey.

Contaminant-modulated shifts in round goby populations (e.g. altered sex ratios, size and age structure, dispersal and reproductive rates) could also alter round goby vulnerability to specific predators, as well as their dietary composition. Foraging preferences of round gobies, such as rates of consumption of dreissenid mussels, chironomids, ostracods and other fishes vary with round goby size (Jude et al., 1995; Walsh et al., 2007). Body size is also likely to affect which piscivorous species prefer them as prey, or which size classes of predators may eat round gobies (e.g. Dietrich et al. (2006)). Contaminant exposure can also make fish more vulnerable behaviourally to predation (reviewed in Sloman and Wilson, 2006). Any or all of these interacting factors could modify the structure of foodwebs, and thus the flow of energy and contaminants throughout such foodwebs, on a local scale. This potential modification warrants future studies.

Round gobies are known to be tolerant of pollution (Pinchuk et al., 2003). Have the heavily contaminated seas of Europe pre-armed this resilient species with adaptations to cope with such environments? Isolated populations living in contaminated areas can and do adapt to contaminant levels that are fatal to conspecifics from other regions (Nacci et al., 2002). One characteristic taken to indicate such adaptation is the loss or reduction of a cytochrome P450 1A (CYP1A; EROD) response, since CYP1A or AhR-mediated interactions are often key to the toxicity of many compounds (Nacci et al., 1999; Brammell et al., 2004; Van Veld and Nacci, 2008). Our data indicate that round gobies in Hamilton Harbour can mount a CYP1A response, but it is not possible to determine, at this stage, whether adaption to Hamilton's PAH-laden sediments has occurred.

The presence of abundant round goby populations in highly contaminated areas is likely to attract predators, or at least support higher numbers of predators than could exist in those regions otherwise. This may facilitate, or possibly accelerate, contaminant transfer in a way that operates in tandem with the role round gobies have in contaminant transfer because of their diet of dreissenid mussels. Round gobies are found throughout the Great Lakes and more inland waters (Poos et al., 2010), so similar situations may arise in other IJC Areas of Concern where contaminant bioaccumulation is a remedial priority. For these reasons, the possibility that pollution-tolerant round gobies can draw or sustain more predators in contaminated areas is a question, with many implications for conservation and remediation, which is worthy of future exploration.

5. Conclusions

Round gobies from different areas of Hamilton Harbour, a Canadian Area of Concern, exhibited signs of contaminant exposure that are similar to known sediment distributions of PAHs, metals and PCBs in the embayment. Fish in the heavily PAH- and metal-contaminated area of Sherman Inlet (SI), near Randle Reef, showed elevated copper burdens and higher EROD activity, consistent with PAH exposure, while fish from Pier 27

(P27), near the heavily PCB- and metal-contaminated area of Windermere Arm, show elevated cadmium body burdens. Fish in both contaminated sites were smaller, had higher metal body burdens and signs of endocrine disruption not observed in fish from cleaner areas of the Harbour. The multiple lines of evidence indicate that there is likely a causal link between contaminant exposure, and shifts in the morphological and physiological characteristics of the fish inhabiting these locations. The impacts that such population-level changes will have on round goby vulnerability to predation, and thus the mobilisation of persistent contaminants from round gobies to piscivorous predators, are important future directions for population-level studies in this system.

Acknowledgments

References

Arcand-Hoy, L.D., Metcalfe, C.D., 1999. Biomarkers of exposure of brown bullheads (*Ameiurus nebulosus*) to contaminants in the lower Great Lakes, North America. Environ. Toxicol. Chem. 18 (4), 740–749.

Balch, G.C., Metcalfe, C.D., Huestis, S.Y., 1995. Identification of potential fish carcinogens in sediment from Hamilton Harbour, Ontario, Canada. Environ. Toxicol. Chem. 14, 79–91.

Balshine, S., Verma, A., Chant, V., Theysmeyer, T., 2005. Competitive interactions between round gobies and logperch. J. Great Lakes Res. 31, 68–77.

Bergstrom, M.A., Evrard, L.M., Mensinger, A.F., 2008. Distribution, abundance and range of the round goby, *Apollonia melanostoma*, in the Duluth-Superior Harbor and St. Louis River Estuary 1998–2004. J. Great Lakes Res. 34, 535–543.

Brammell, B.F., Price, D.J., Birge, W.J., Elskus, A.A., 2004. Apparent lack of CYP1A response to high PCB body burdens in fish from a chronically contaminated PCB site. Mar. Environ. Res. 58, 251–255.

Carlisle, S.L., Marxer-Miller, S.K., Canario, A.V.M., Oliveira, R.F., Carneiro, L., Grober, M.S., 2000. Effects of 11-ketotestosterone on genital papilla morphology in the sex changing fish *Lythrypnus dalli*. J. Fish Biol. 57, 445–456.

Charlebois, P.M., Marsden, J.E., Goettel, R.G., Wolfe, R.K., Jude, D.J., Rudnicka, S., 1997. The round goby, *Neogobius melanostomus* (Pallas), a review of European and North American literature. Illinois-Indiana Sea Grant Program and Illinois Natural History Survey, Champaign. INHS Special Publication Number 20.

Corkum, L.D., Sapota, M.R., Skora, K.E., 2004. The round goby, *Neogobius melanostomus*, a fish invader on both sides of the Atlantic Ocean. Biol. Invasions 6, 173–181.

Dietrich, J.P., Morrison, B.J., Hoyle, J.A., 2006. Alternative ecological pathways in the eastern Lake Ontario food web—round goby in the diet of Lake Trout. J. Great Lakes Res. 32, 395–400.

Dubs, D.O.L., Corkum, L.D., 1996. Behavioral interactions between round gobies (*Neogobius melanostomus*) and mottled sculpins (*Cottus bairdi*). J. Great Lakes Res. 22, 838–844.

Elskus, A.A., 2004. Estradiol and estriol suppress CYP1A expression in rainbow trout primary hepatocytes. Mar. Environ. Res. 58, 463–467.

Hall, J.D., O'Connor, K., Ranieri, J., 2006. Progress toward delisting a Great Lakes Area of Concern: the role of integrated research and monitoring in the Hamilton Harbour Remedial Action Plan. Environ. Monit. Assess 113, 227–243.

Hamilton Harbour Remedial Action Plan (RAP), 1992. Hamilton Harbour Stage 1 Report: Environmental Conditions And Problem Definition. Burlington, Ontario, Canada.

Hamilton Harbour Remedial Action Plan (RAP), 2003. Remedial Action Plan for Hamilton Harbour: Stage 2 Update 2002. Burlington, Ontario, Canada.

Hamilton Harbour Remedial Action Plan (RAP), 2008. An Assessment Of Polychlorinated Biphenyls (PCBs) in the Hamilton Harbour Area Of Concern (Aoc) in Support Of The Beneficial Use Impairment (BUI): Restrictions on Fish and Wildlife Consumption. Burlington, Ontario, Canada.

Hanari, N., Kannan, K., Horii, Y., Taniyasu, S., Yamashita, N., Jude, D.J., Berg, M.G., 2004. Polychlorinated naphthalenes and polychlorinated biphenyls in benthic organisms of a Great Lakes food chain. Arch. Environ. Contam. Toxicol. 47, 84–93.

Hayden, T.A., Miner, J.G., 2009. Rapid dispersal and establishment of a benthic Ponto-Caspian goby in Lake Erie: diel vertical migration of early juvenile round goby. Biol. Invasions 11 (8), 1767–1776.

Hogan, L.S., Marschall, E., Folt, C., Stein, R.A., 2007. How non-native species in Lake Erie influence trophic transfer of mercury and lead to top predators. J. Great Lakes Res. 33, 46–61.

International Joint Commission, 1999. Hamilton Harbour Area of Concern Status Assessment. Windsor, Ontario, Canada.

Janssen, J., Jude, D.J., 2001. Recruitment failure of mottled sculpin *Cottus bairdi* in Calumet Harbour, Southern Lake Michigan, induced by the newly introduced round goby *Neogobius melanostomus*. J. Great Lakes Res. 27, 319–328.

Johnson, T.B., Allen, M., Corkum, L.D., Lee, V.A., 2005. Comparison of methods needed to estimate population size of round gobies (*Neogobius melanostomus*) in western Lake Erie. J. Great Lakes Res. 31, 78–86.

Jude, D.J., 1997. Round gobies: cyberfish of the third millennium. Great Lakes Res. Rev. 3, 27–34.

Jude, D.J., Janssen, J., Crawford, G., 1995. Ecology, distribution and impact of the newly introduced round and tubenose gobies on the biota of the St. Clair and Detroit Rivers. In: Munawar, M., Edsall, T., Leach, J. (Eds.), The Lake Huron Ecosystem: Ecology, Fisheries And Management. Ecovision world monograph series S.P.B. Academic Publishing, Amsterdam, The Netherlands, pp. 447–460.

Kannan, K., Tao, L., Sinclair, E., Pastva, S.D., Jude, D.J., Giesy, J.P., 2005. Pefluorinated compounds in aquatic organisms at various trophic levels in a Great Lakes food chain. Arch. Environ. Contam. Toxicol. 48, 559–566.

Kavanaugh, R.J., Balch, G.C., Kiparissis, Y., Niimi, A.J., Sherry, J., Tinson, C., Metcalfe, C.D., 2004. Endocrine disruption and altered gonadal development in white perch (*Morone americana*) from the lower Great Lakes region. Environ. Health Perspect. 112 (8), 898–902.

Kennedy, S.W., Lorenzen, A., James, C.A., Collins, B.T., 1993. Ethoxyresorufin-O-deethylase and porphyrin analysis in chicken embryo hepatocyte cultures with a fluorescence multiwell plate reader. Anal. Biochem. 211, 102–112.

King, R.B., Ray, J.M., Stanford, K.M., 2006. Gorging on gobies: beneficial effects of alien prey on a threatened vertebrate. Can. J. Zool. 84, 108–115.

Kirby, M.F., Bignell, J., Brown, E., Craft, J.A., Davies, I., Dyer, R.A., Feist, S.W., Jones, G., Matthiessen, P., Megginson, C., Robertson, F.E., Robinson, C., 2003. The presence of morphologically intermediate papilla syndrome in the United Kingdom populations of sand goby (*Pomatoschistus* spp.): endocrine disruption? Environ. Toxicol. Chem. 22 (2) 239–251.

Kovtun, I., 1980. Significance of the sex ratio in the spawning population of the round goby, *Neogobius melanostomus*, in relation to year-class strength in the Sea of Azov. J. Ichthyol. 19, 161–163.

Kwon, T.-D., Fisher, S.W., Kim, W.G., Hwang, H., Kim, J.-E., 2006. Trophic transfer and biotransformation of polychlorinated biphenyls in zebra mussel, round goby, and smallmouth bass in Lake Erie, USA. Environ. Toxicol. Chem. 25, 1068–1078.

Lauer, T.E., Allen, P.J., McComish, T.S., 2004. Changes in mottled sculpin and Johnny darter trawl catches after the appearance of round gobies in the Indiana waters of Lake Michigan. Trans. Am. Fish. Soc. 133, 185–189.

MacInnis, A.J., Corkum, L.D., 2000a. Fecundity and reproductive season of the round goby *Neogobius melanostomus* in the upper Detroit River. Trans. Am. Fish. Soc. 129, 136–144.

MacInnis, A.J., Corkum, L.D., 2000b. Age and growth of round goby *Neogobius melanostomus* in the upper Detroit River. Trans. Am. Fish. Soc. 129, 852–858.

Marentette, J.R., Fitzpatrick, J.L., Berger, R.G., Balshine, S., 2009. Multiple male reproductive morphs in the invasive round goby (*Apollonia melanostoma*). J. Great Lakes Res. 35, 302–308.

Miller, P.J., 1984. The tokology of gobioid fishes. In: Potts, G.W., Wootton, R.J. (Eds.), Fish Reproduction: Strategies And Tactics. Academic Press, New York, pp. 119–153.

Mills, E.L., Casselman, J.M., Dermott, R., Fitzsimons, J.D., Gal, G., Holeck, K.T., Hoyle, J.A., Johannsson, O.E., Lantry, B.F., Makarewicz, J.C., Millard, E.S., Munawar, I.F., Munawar, M., O'Gorman, R., Owens, R.W., Rudstam, L.G., Schaner, T., Stewart, T.J., 2003. Lake Ontario: food web dynamics in a changing ecosystem (1970–2000). Can. J. Fish. Aquat. Sci. 60, 471–490.

Morrison, H.A., Whittle, D.M., Haffner, G.D., 2000. The relative importance of species invasions and sediment disturbance in regulating chemical dynamics in western Lake Erie. Ecol. Modelling 125, 279–294.

Murphy, T., 2000. Coal tar in Hamilton Harbour—attempts at biological restoration guidelines. Environ. Toxicol. 15, 484–495.

Nacci, D., Coiro, L., Champlin, D., Jayaraman, S., McKinney, R., Gleason, T.R., Munns Jr., W.R., Specker, J.L., Cooper, K.R., 1999. Adaptations of wild populations of the estuarine fish *Fundulus heteroclitus* to persistent environmental contaminants. Mar. Biol. 134, 9–17.

Nacci, D.E., Gleason, T.R., Munns Jr., W.R., 2002. Evolutionary and ecological effects of multi-generational exposures to anthropogenic stressors. Human Ecol. Risk Assess. 8 (1), 91–97.

Navas, J.M., Segner, H., 2001. Estrogen-mediated suppression of cytochrome P4501A (CYP1A) expression in rainbow trout hepatocytes: role of estrogen receptor. Chemico-Biol. Interact. 138, 285–298.

Newman, M.C., Clements, W.H., 2008. Ecotoxicology: A Comprehensive Treatment. CRC Press, Boca Raton, Florida, U.S.A.

Pinchuk, V.I., Vasil'eva, E.K., Vasil'ev, V.P., Miller, P.J., 2003. *Neogobius melanostomus* (Pallas, 1814). In: Miller, P.J. (Ed.), The Freshwater Fishes of Europe. AULA-Verlag, pp. 293–345.

Poos, M., Dextrase, A.J., Schwalb, A.N., Ackerman, J.D., 2010. Secondary invasion of the round goby into high diversity Great Lakes tributaries and species at risk hotspots: potential new concerns for endangered freshwater species. Biol. Invasions 12, 1269–1284.

Ray, W.J., Corkum, L.D., 2001. Habitat and site affinity of the round goby. J. Great Lakes Res. 27, 329–334.

Schlenk, D., Handy, R., Steinert, S., Depledge, M.H., Benson, W., 2008. Biomarkers. In: Di Giulio, R.T., Hinton, T.E. (Eds.), The Toxicology of Fishes. CRC Press, Boca Raton, FL, USA, pp. 683–731.

Shemonaev, E.V., Kirilenko, E.V., 2009. Some features of biology of the round goby *Neogobius melanostomus* (Perciformes, Gobiidae) in waters of Kuibyshev Reservoir. J. Ichthyol. 49, 454–459.

Sloman, K.A., Wilson, R.W., 2006. Anthropogenic impacts upon behaviour and physiology. In: Sloman, K.A., Wilson, R.W., Balshine, S. (Eds.), Behaviour and Physiology of Fish: Volume 24. Fish Physiology. Elsevier Academic Press, San Diego, CA, USA, pp. 413–469.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150 (1), 76–85.

Somers, C.M., Lozer, M.N., Kjoss, V.A., Quinn, J.S., 2003. The invasive round goby (*Neogobius melanostomus*) in the diet of nestling double-crested cormorants (*Phalacrocorax auritus*) in Hamilton Harbour, Lake Ontario. J. Great Lakes Res. 29, 392–399.

Tomkins, J.L., Simmons, L.W., 2002. Measuring relative investment: a case study of testes investment in species with alternative male reproductive tactics. Anim. Behav. 63, 1009–1016.

Van Aerle, R., Pounds, N., Hutchinson, T.H., Maddix, S., Tyler, C.R., 2002. Window of sensitivity for the estrogenic effects of ethinylestradiol in early life-stages of fathead minnow, *Pimephales promelas*. Ecotoxicology 11, 423–434.

Van Veld, P.A., Nacci, D.E., 2008. Toxicity resistance. In: Di Giulio, R.T., Hinton, T.E. (Eds.), The Toxicology of Fishes. CRC Press, Boca Raton, FL, USA, pp. 597–641.

Walsh, M.G., Dittman, D.E., O'Gorman, R., 2007. Occurrence and food habits of the round goby in the profundal zone of southwestern Lake Ontario. J. Great Lakes Res. 33, 83–92.

Weis, J.S., 2002. Tolerance to environmental contaminants in the mummichog, *Fundulus heteroclitus*. Human Ecol. Risk Assess. 8, 933–953.

Young, J.A.M., Marentette, J.R., Gross, C., McDonald, J.I., Verma, A., Marsh-Rollo, S.E., Macdonald, P.D.M., Earn, D.J., Balshine, S., 2010. Demography and substrate affinity of the round goby (*Neogobius melanostomus*) in Hamilton Harbour. J. Great Lakes Res. 36, 115–122.

Zar, J.H., 1999. Biostatistical Analysis 4th ed. Prentice-Hall, Inc., Upper Saddle River, NJ, USA.

Zeman, A.J., 2009. Contaminated sediments in Hamilton Harbour: compilation and evaluation of sediment databases, publications and reports, 1975–2008. Environment Canada Water Science and Technology Directorate WSTD Contribution No. 09-263.