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The cortisol stress response in male round goby (*Neogobius melanostomus*): effects of living in polluted environments?

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Abstract Acute exposure to contaminants frequently induces stress, but prolonged exposures, such as those experienced by individuals in wild populations, can impair the capacity to mount a stress response. Using the round goby (*Neogobius melanostomus*), a small benthic fish and potential sentinel species for habitat contamination in the Great Lakes, we explored the impacts of living in highly polluted areas. Round goby were collected from highly contaminated and less contaminated areas of Hamilton Harbour (a well-known site of polycyclic aromatic hydrocarbon (PAH) and heavy metal contamination). The cortisol stress responses of fish from sites of high and low contamination were compared using an EIA assay on blood collected (n=112) either prior to or 0, 10, 30, 60, 240 min and 24 h following the application of a 4-min confined air exposure stressor. Plasma cortisol levels were elevated at 10 and 30 min post-stressor (100.3 and 87.5 ng/mL), and returned to levels similar to baseline (22.3 ng/mL) 1 h after the stressor. In contrast to predictions, round goby from areas of high and low contamination had similar cortisol levels at all time-points. We also monitored stress responses immediately after a chasing stressor (n=19). During the chasing stressor, contaminated-site round goby exhausted faster than individuals from a less contaminated site, although they had similar levels of plasma cortisol (23.5 versus 20.9 ng/mL) and lactate (2.53 versus 1.98 mmol/L). Our results indicate that not all fishes may demonstrate impaired stress responses, even in highly contaminated habitats; however, such animals may still have increased vulnerability to predation.

Keywords Environmental contamination · Sentinel species · Hamilton Harbour · In situ exposure · Biomarker · Behaviour

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Introduction

The disruption of endocrine function is one major avenue by which sublethal contaminant exposure can perturb organism health (Kime 1998). Evaluating the severity of endocrine disruption has become an important tool in the development of biomarkers of pollutant exposure in wild populations (Hutchinson et al. 2006). Although the focus of much recent research has been towards impacts on reproduction and associated sex steroids (Tyler et al. 1998), contaminant interference in other endocrine systems such as those of thyroid hormones (Eales and Brown 2005) and glucocorticoid stress hormones (Hontela 2005; Vijayan et al. 2005) are also of growing interest.

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Glucocorticoid hormones are released when a stress response, common to all vertebrates, is mounted following exposure to a stressor and loss of organism homeostasis (Wendelaar Bonga 1997). The glucocorticoid stress response may be affected by toxicants in two ways. First, toxicant exposure itself may act as a stressor, activating the hypothalamo-pituitary-interrenal (HPI) axis. Acute exposures of fishes to a range of contaminants have demonstrated such responses in the laboratory (e.g., PAHs, Levitan and Taylor 1979; crude oil, Thomas et al. 1980; Thomas and Rice 1987; mercuric compounds, Bleau et al. 1996; cadmium, Hontela et al. 1996). Elevated cortisol levels in the short-term can provide protection against toxic effects (Bury et al. 1998; DeBoeck et al. 2003). However, prolonged cortisol elevation can have many negative effects, such as immunosuppression, reduced reproductive investment or production of sex steroids and reduced growth (Barton et al. 1987; Pickering 1989; Pickering and Pottinger 1989; Carragher and Sumpter 1990; Gregory and Wood 1999). The second way that toxicants may act is to directly impact the HPI axis itself (e.g., mercuric compounds, Kirubagaran and Joy 1991; endosulfan, Leblond et al. 2001; *o,p'*-DDD, a metabolite of DDT, Benguira et al. 2002). Interrenal cells may produce cytochrome P450 enzymes that, in metabolizing toxicants, produce reactive oxygen species that create oxidative cellular damage, also impairing cortisol production (Hontela 1997).

The effects of prolonged exposures to toxicants on cortisol production have been examined in both the laboratory, and in wild populations. Many studies have indicated HPI axis exhaustion as a common phenomenon-prolonged cortisol elevation eventually creates negative feedback on the HPI axis, down-regulating receptors and causing atrophy of cells (e.g., pituitary corticotrophs; Basu et al. 2002). In general, individuals in more contaminated field locations produce smaller quantities of plasma cortisol following a standardized stressor such as confinement than individuals from cleaner locations (reviewed by Hontela 1997 and Hontela 1998; in yellow perch, *Perca flavescens*: Laflamme et al. 2000; Levesque et al. 2003; Gravel et al. 2005; northern pike, *Esox lucius:* Hontela et al. 1992; Hontela et al. 1997; white sucker, *Catostomus commersoni*: McMaster et al. 1994; brown trout, *Salmo trutta:* Norris 1999). Some studies have shown alterations to other stages of the stress response of field-exposed fish such as liver glycogen levels, blood glucose and lactate (European perch, *Perca fluviatilis*: Forlin et al. 1995; Andersson et al. 1988). These field results have been paralleled in the laboratory—a few long-term studies have also demonstrated HPI axis exhaustion or other alterations of the stress response (in walleye, *Sander vitreus* exposed to methylmercury, Friedmann et al. 1996; rainbow trout, *Oncorhynchus mykiss*, exposed to copper, Gagnon et al. 2006; whitefish, *Coregonus lavaretus*, exposed to bleached kraft mill effluent (BKME), Lappivaara 2001). The consequences of an inability to mount a proper glucocorticoid stress response may be severe (Hontela 1998); for example, fish may have a reduced capacity to survive winter conditions (Odermatt et al. 2006), or respond appropriately to predators (Sigismondi and Weber 1988).

We examined the stress response of the round goby (*Neogobius melanostomus*), a small benthic fish invasive to North America, exposed in situ to naturally-occurring levels of contaminants in Hamilton Harbour, Ontario, Canada. We chose the round goby as this fish may be a useful sentinel species for habitat contaminant exposure (Marentette et al. 2010). It is tolerant of a wide variety of environmental conditions (Pinchuk et al. 2003), is abundant, benthic and molluscivorous, and thus exposed to contaminants in both the sediment and via a dietary route (Jude et al. 1995) and is philopatric, so it reflects the conditions of the collection site (Ray and Corkum 2001; Marentette et al. 2012). Hamilton Harbour, located in western Lake Ontario, is part of the round goby North American invasive range. This harbour was designated as one of the 43 Areas of Concern in the Laurentian Great Lakes by the International Joint Commission (IJC) due to high levels of pollutants originating both from wastewater treatment plants and historical coal tar contamination from the local steel mills (Hamilton Harbour Remedial Action Plan 1992; IJC 1999). Hamilton Harbour contains both areas of low and high pollution, where the concentrations of contaminants in water and sediment exceed federal and provincial water quality standards and areas where the sediment contains extremely high levels of compounds such as polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs) and heavy metals (including zinc, cadmium, arsenic, lead and iron; Zeman 2009). Round goby are abundant throughout Hamilton Harbour and are thought to have entered the ecosystem in 1996 (Vélez-Espino et al. 2010).

We compared the cortisol stress responses of round goby collected from a low contamination site and a highly contaminated site in Hamilton Harbour, Lake Ontario (Fig. 1) to 1) a standardized 4-min emersion (air exposure) stressor, to generate a stress response

curve, and 2) a chase stressor which more closely mimicked a natural predatory stressor. Round goby were collected from two sites, or subpopulations, that have been previously demonstrated to differ in a variety biomarkers of contaminant exposure such as vitellogenin expression, body size, intersex, fin erosion, and hepatic EROD activity (Bowley et al. 2010; Marentette et al. 2010). Given a hypothesis that exposure to a wide variety of contaminants causes HPI axis exhaustion, we predicted that round goby from our contaminated site, like fish from other contaminated habitats (reviewed in Hontela et al. 1997) would mount a smaller cortisol response, show altered levels of plasma lactate and recover to baseline cortisol levels more quickly than round goby from our low contamination site.

Material and methods

Experiment 1: cortisol stress response

Between September 2 and October 22, 2009, after the breeding season, a total of 112 male round goby were collected from Hamilton Harbour, Ontario, Canada. Round goby catches in our study area are typically male-biased (Young et al. 2010), so we focused our study on male fish as not enough females were collected for a female comparison study. Fish were caught in minnow traps baited with 30 g of corn and set for 24 h at a depth of 1 m. Fish were sexed based on their urogenital papilla shape (Marentette et al. 2010) and transported back to the laboratory at McMaster University in large aerated plastic bins. Fifty-six round goby were collected from La Salle Park in Burlington, Ontario (low contamination site; 43° 18′N, 79°50′W), and 56 were collected from the mouth of Sherman Inlet in Hamilton, Ontario (43°16′N, 79°50 W; Fig. 1), a highly contaminated site near Randle Reef, with concentrations exceeding provincial and national safety guidelines of sediment PAHs, PCBs, arsenic, cadmium, mercury, iron, copper, nickel, zinc and lead (Hamilton Harbour RAP 2003; Zeman 2009).

In the laboratory, round goby were individually housed in 20 L tanks containing an airstone and a black opaque PVC tube for shelter, with dechlorinated municipal tap water. Tanks were covered with black, opaque plastic in order to eliminate external visual stimuli. Water temperature was maintained at 21±1 °C under a 16 L:8D light cycle (with light from 07:00 to 23:00). Test water had a slightly alkaline mean pH of 7.77 (± 0.06), a mean dissolved oxygen content of 8.51±0.05 mg/L and an ammonia concentration of 0 mg/L. The fish were allowed to acclimate for 24 h before the experiment began and they were not fed during this period. A maximum of 24 h acclimation was chosen to ensure that as little contaminant depuration as possible from these fish occurred. Each of the fish was measured, and randomly assigned to either the baseline control group (blood taken without air exposure stress) or to one of six experimental groups in which blood was sampled after air exposure (either 0 min, 10 min, 30 min, 1 h, 4 h, or 24 h after air exposure). There were eight fish, or replicates, for each of the seven groups and two collection sites. Air exposure stress was always applied between 16:30 and 17:30 to control for any diel effects. To apply this air exposure stressor, fish were quickly caught with a hand net and held for 4 min in a dry suspended net at the edge of the tank. They were then either killed by a blow to the head, followed by immediate blood collection (0 min) or returned to the tank until the appropriate time frame had elapsed (see below) before sacrifice and blood collection. As collection was terminal, only one blood sample was taken per fish. Blood was collected via caudal severance within 2 min. Thus, fish in the control and 0 min treatments had blood collected immediately after capture or the application of the stressor, while the individuals assigned to the 10, 30 min, 1, 4 and 24 h treatments were returned to their tanks to recover following the emersion until the assigned collection time. Fish carcasses were then dissected to obtain morphological measurements (see below).

Experiment 2: chasing stressor

Between October 7 and 21, 2009, 19 male round goby were collected from the same two sites as in experiment 1 (11 from the low contamination site and 8 from the high contamination site). Few round goby were available from field collections during this experiment, and so cortisol levels for only one time-point post-stressor (0 min) were evaluated. Round goby captured were brought back to the laboratory as above and were individually housed in 10 L tanks covered with black, opaque plastic on all sides, Water was maintained between 20 °C and 23 °C and each tank contained one airstone and a semi-cylindrical tube as shelter.

All chasing treatments were conducted between 13:00 and 15:00 to control for any diel effects. The experimenter was blind to the site of collection for each fish. Chased fish

Fig. 1 Map of Hamilton harbour illustrating varying levels of PAHs in surface sediment and our two study sites (Zeman 2009)

were introduced individually to a 93×55×12 cm elliptical chasing arena with water to a depth of 5 cm, in a 15×7×6 cm opaque plastic start box. A video camera recorded each of the chasing trials. The arena bottom was a grid of 10×7 cm rectangles to assist in scale and distance calculations (see below). To begin each chasing trail, fish were encouraged to leave the start box by a quick, gentle pinch administered to the caudal peduncle using a pair of padded forceps. This pinch initiated the fish's first escape response. After the fish left the start box, each fish was chased with a hand net (6×4 cm) until they reached exhaustion. Exhaustion was defined as the point at which the fish could be scooped up into the net without struggle. The time from the escape response to exhaustion was recorded as the time to exhaustion. Fish were immediately killed by a blow to the head and blood was then collected within 90 s as described below and each fish was dissected. In order to accurately measure the first escape response distance, videotapes of the chases were digitally converted into Windows Media Player 11 (Microsoft Corporation, 2006) files. Windows Movie Maker 5.1 (Microsoft Corporation, 2007) was then used to isolate the necessary frames (the first with the goby in start box before movement, and the second after its response to being pinched). The program ImageJ 1.42q (National Institutes of Health) was employed to measure the distance that the fish travelled (cm) after being pinched.

Blood collection and dissection

Individual blood samples were collected via caudal severance using 20 μL microcapillary tubes, and then centrifuged for 10 min at 14,500 rpm (8000 G). The plasma was stored at −80 °C until analysis. Each goby carcass was measured for external morphology (total body mass to 0.001 g, and total length to the nearest 0.1 mm). Gonads and livers were also removed and measured to the nearest 0.001 g. The gonadosomatic index (GSI) was calculated by dividing total testes (gonad) weight by the fish's somatic mass (total mass−gonad mass) × 100. Hepatosomatic index (HSI) was calculated using the same formula where liver mass was divided by the fish's total mass minus the liver mass. Fulton's condition factor K was calculated as the mass of the fish (g) divided by the cube of the fish's total length (cm^3) × 100.

Cortisol and lactate analysis

Cortisol concentrations in plasma were determined using an enzyme-linked immunosorbent assay (EIA; Pradelles et al. 1990) Cortisol Kit (Cayman Chemical, Inc., Ann Arbor, MI, USA; Catalogue No: 582121). Samples were not pooled. Samples were run in duplicates and plates were left to incubate at 4 °C overnight. After incubation, plates were developed using Ellman's reagent, covered with aluminum foil to allow development in the dark and placed on an orbital shaker in a dark room to develop for at least 1.5 h (maximum: 3 h, mean: 2.5 h). Plates were read at a single wavelength of 405 nm. The ratio of absorbance for a sample or standard well to that of the maximum binding well fell between 20 % and 80 % in each plate. The mean±SE % CV (coefficient of variation) for sample duplicates was 7.9±0.5 %. For fish in Experiment 2, plasma lactate was evaluated with a Lactate Pro

Blood Lactate test meter (Arkray, Inc., Edina, MN, USA; Saunders et al. 2005) as an indicator of anaerobic metabolic processes following exhaustive exercise.

Statistical analysis

All statistical analyses were performed using the computer package JMP 8.0.2 (SAS Institute Inc. 2009). All data were tested for normality and transformations performed whenever necessary; if transformations could not normalize the data, non-parametric statistics were used. A two-factor ANOVA or ANCOVA was used to evaluate differences in plasma cortisol concentrations across time-points, between treatments and between sites of high and low contamination. Covariates such as log-transformed GSI, HSI, total length and condition factor were tested in ANCOVA models, and removed as they did not reveal significance. Post-hoc pair-wise differences among groups were established, where necessary, using Tukey's HSD tests, and differences relative to the baseline or control group were established with Dunnett's tests. Student's *t*-tests and the normal approximation to the Wilcoxon rank-sum tests were used to compare morphological measurements between fish from the two sites. Wilcoxon rank-sum tests (normal approximation) were used to compare fish from sites of high and low contamination in times to exhaustion and first escape responses during the chasing stressor. Correlations were performed using Pearson's r (parametric), or Spearman's rho (r_s; non-parametric) where needed.

Ethical note

Handling methods for the fish used in this study were approved by the McMaster Animal Research Ethics Board (AREB, AUP # 06-10-61) and are in accord with animal care guidelines from the Canadian Council of Animal Care.

Results

Morphological differences

Round goby from the low contamination site were longer, heavier and in better condition than round goby from the more highly contaminated site (Table 1). Round goby from the low contamination site also had greater relative liver investment, as measured by the hepatosomatic index or HSI, but smaller gonad investment, as measured by the gonadosomatic index or GSI, than fish from the more contaminated site (Table 1).

Experiment 1: cortisol stress response

Following an air exposure stressor, round goby mounted a cortisol response with plasma concentrations that peaked at 10 and 30 min (means±SE of 100.3± 1.52 ng/mL and 87.5±13.0 ng/mL respectively) following the stressor, and returned to levels comparable to baseline (22.3±4.2 ng/mL) by 1 h following the stressor (ANOVA on log cortisol concentrations, $F_{13,98}=2.9$, $p=0.002$; effect of time-point $F_{6,98}=5.86$, $p<0.0001$, Tukey HSD test, $p<0.05$; Fig. 2). Among fish from the low contamination site, plasma cortisol levels peaked above baseline at 10 min post-stressor (Dunnett's test, $p<0.05$), but among fish from the highly contaminated site, plasma cortisol levels had a slightly longer peak, from 10 to 30 min post-stressor, relative to baseline conditions. However, round goby from the two sites did not differ in cortisol levels overall (effect of site $F_{1,\ 98}=0.001$, $p=0.97$) or at any one timepoint after exposure to the stressor (site-timepoint interaction $F_{6,98}=0.34$, $p=0.91$; Tukey HSD tests, $p>0.05$).

Male round goby GSI correlated negatively with plasma cortisol ($r=-0.24$, $p=0.01$). Using an ANCOVA analysis with log-transformed GSI as a covariate to account for gonad size differences in males did not change the plasma cortisol peak across or within sites (which remained at 10–30 min post-stressor), nor reveal differences between sites at any timepoint, although overall plasma cortisol levels were higher in this model in fish from the highly contaminated site SI than fish from LS (effect of site $F_{1,103}=4.6$, $p=0.034$). No other body measurements (body size, condition, and HSI) correlated with cortisol concentrations.

Experiment 2: chasing stressor

Round goby from the less contaminated site took longer to reach exhaustion while being chased (median 138 s) than round goby from the highly contaminated site (median 77 s; $Z=-2.94$, $p=0.003$; Fig. 3a). There were no site differences in the distance travelled in the fish's first escape response ($Z=0.37$, $p=0.71$). Plasma lactate concentrations did not differ between fish from different sites (mean±SE, clean site = 1.98±

Table 1 Mean ± standard error (total length, total mass and K) or median and range (HSI and GSI) of morphological measurements for round goby from sites of low and high contamination in Hamilton Harbour. *HSI* hepatosomatic index. *GSI* gonadosomatic index. *K* Fulton's condition factor K. See text for calculations of indices. Statistical tests are given as *t*-tests for parametric data and the normal approximation to the Wilcoxon test for nonparametric data (*Z*)

Measurement	Low contamination n=56	High contamination n=56	Statistical difference
Total length (mm)	106.5±1.7	97.7±2.1	t_{129}=3.54, p=0.0006
Total mass (g)	17.64±0.88	13.22±0.74	t_{129}=4.07, p<0.0001
HSI	3.7 (0.7–6.2)	3.1 (1.1–5.5)	Z=2.64, p=0.008
GSI	0.1 (0–3.2)	0.25 (0–1.9)	Z=6.31, p<0.0001
K	2.5±0.1	1.8±0.1	t_{129}=4.26, p<0.0001

0.24 mmol/L, contaminated site = 2.53±0.24 mmol/L; t_{17}=1.3, p=0.22).

At 0 min post-stressor, chased round goby had mounted a slightly smaller cortisol stress response than round goby that had been subjected to air exposure (ANOVA on log cortisol concentrations, effect of stressor type $F_{1,32}$=4.93, p=0.03; Fig. 3b). However, there was no site difference in the cortisol levels across the different stressors (effect of site $F_{1,32}$=0.31, p=0.58; interaction of site-stressor type $F_{1,32}$=0.89, p=0.35). Chased round goby from the low contamination site thus had similar cortisol levels (20.9±4.8 ng/mL) to fish from the high contamination site (23.5±6.8 ng/mL). While the duration of the chasing stressor (median 118 s, range 64–291) was generally shorter than the air exposure (240 s), there was no correlation between cortisol concentration produced and the duration of chasing stressor (r_s=−0.08, p=0.75, n=19). GSI did

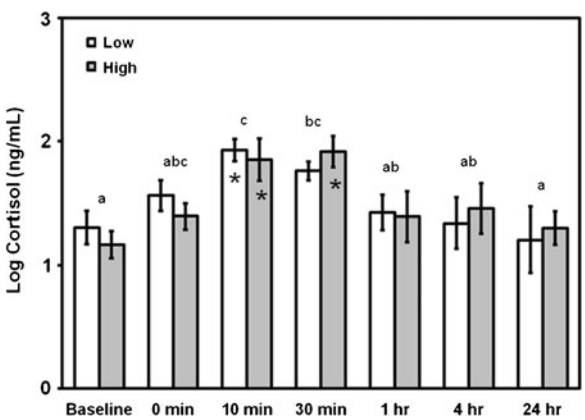

Fig. 2 Log-transformed mean plasma cortisol concentrations (± SE) at baseline and various timepoints following fish exposure to an air exposure stressor. *Bars* indicate cortisol concentrations for round goby from sites of low (*white*) and high (*grey*) contamination. *Letters* indicate significant differences among timepoints (Tukey HSD tests, p<0.05). *Asterisks* indicate significant elevation from baseline cortisol levels within each site (Dunnett's tests, p<0.05)

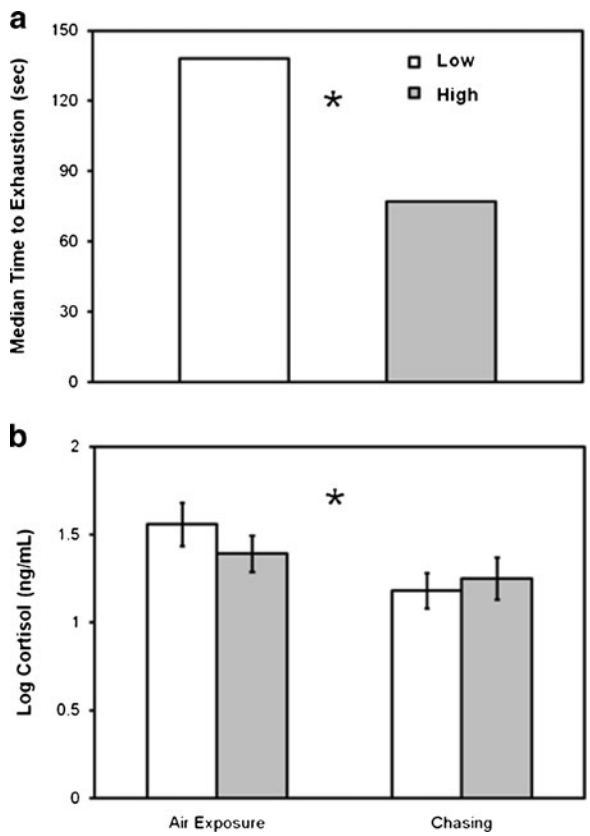

Fig. 3 **a** Median times to exhaustion during the chasing stressor for round goby from sites of low (*white*) and high (*grey*) contamination. An *asterisk* indicates significant differences between sites. **b** Log-transformed mean plasma cortisol concentrations (± SE) at 0 min post-stressor for both the air exposure and chasing treatments. An *asterisk* indicates significant differences between treatments

not significantly covary with plasma cortisol in this second experiment. Plasma cortisol also did not correlate with plasma lactate (r_s=−0.40, p=0.10).

Discussion

Round goby from both highly and less contaminated areas of Hamilton Harbour mounted stress responses after an air exposure stressor, with cortisol peaks generated 10–30 min after the cessation of the stressor. Compared to fish from the more contaminated location, fish from the less contaminated habitat took longer to exhaust during chasing. No effect of site was observed on cortisol levels produced after chasing, or the distance travelled in the first escape response. Round goby released more cortisol in response to air exposure than to chasing, although this may be the result of longer stressor duration.

No evidence of HPI axis exhaustion in round goby

Our prediction that round goby collected from contaminated habitats would have an impaired ability to mount a stress response was not supported. This result is surprising for several reasons. First, our contaminated site is burdened with large quantities of PAH-rich coal tar (an area known as Randle Reef; Hamilton Harbour RAP 1992). PAHs are known agonists of aryl hydrocarbon receptors in the liver and interrenal cells, inducing the production of cytochrome P450 enzymes, frequently measured as EROD activity, that dampens cortisol stress responses (Aluru and Vijayan 2006), possibly due to increased cortisol metabolism and thus rapid clearance from the body (Wilson et al. 1998). Second, there is sometimes a general negative relationship between gonad size or androgen concentrations (e.g., 11-ketotestosterone, a dominant androgen in fishes) and cortisol (Pickering 1989; Pottinger et al. 1996). Round goby collected from our contaminated site had relatively larger gonads, although the fish were collected after the end of the breeding season, had regressed testes and were no longer producing sperm. We might have predicted lower cortisol levels in fish from our contaminated site on the basis of gonad size alone, but did not observe this result.

Why did we not see a reduction in stress response capacity in round goby in contaminated areas? The phenomenon of HPI axis exhaustion may simply not occur in this resilient fish. While a plethora of field studies have shown impaired stress responses, most of these have been conducted on yellow perch or northern pike (Lockhart et al. 1972; Hontela et al. 1992, 1995, 1997; Brodeur et al. 1997; Girard et al. 1998; Laflamme et al. 2000; Levesque et al. 2003; Gravel et al. 2005). In addition, in our study we examined only male stress responses. It is possible that female round goby stress responses would have presented a different picture—Gendron and others (1997), for example, found evidence of HPI axis exhaustion only in female salamanders, *Necturus maculosus*. It is also possible that the round goby from our contaminated site may have evolved tolerance to high levels of contaminant exposure (Weis 2002) and an adaptation to those chemical stressors could leave HPI axis fully functional (Pratap and Wendelaar Bonga 1990; Wendelaar Bonga 1997). We did not perform histological examination of the cellular structures along the HPI axis. Further studies on this topic would shed light on whether the cortisol-producing pathways were truly affected.

Handling and recovery may have influenced the results of our study. We permitted round goby from both clean and contaminated sites to recover from the stress of capture for 24 h in clean laboratory water before the application of a controlled air exposure. This is in contrast to some field studies that used the stress of capture as the inducer of cortisol stress responses, thus collecting blood samples on-site (e.g., Hontela et al. 1992, 1995, 1997), and allowed recovery to baseline in the field as well (e.g., Laflamme et al. 2000). It is possible that differences in cortisol between sites would have been apparent if we used such a study design, as other factors in the field (e.g., water chemistry) may play a prominent role. Our use of 24-h recovery may not have been long enough to get a true baseline cortisol measure; Jardine and others (1996) found that even three days in the laboratory was not long enough for white suckers (*Catostomus commersoni*) to recover from the stress of capture, although site differences in cortisol were still apparent at the end of that time. Alternatively, our recovery period may have been too long, allowing too much depuration of contaminants from fish to occur. Previous work on the round goby have demonstrated that 24 h in clean laboratory water is enough to eliminate hepatic EROD activity differences among sites (Marentette et al. 2010), indicating that contaminants like PAHs may be quickly cleared metabolically by the system, thus

reducing the stressor load on the fish. However, it is unlikely that 24 or 48 h in clean laboratory water is enough to both depurate all contaminants from the fish, even PAHs (Niimi and Palazzo 1986), and allow the HPI axis to recover fully in its cortisol-mounting capacity. Persistent contaminants like mercury and PCBs, both found in our contaminated site, will likely depurate much more slowly than rapidly lost contaminants such as PAHs, maintaining impacts on the HPI axis for long periods (Lockhart et al. 1972). Our study is one of several to examine stress response impairments in field-exposed animals with recovery in the laboratory, where depuration is likely to have occurred to some extent. Andersson et al. (1988) allowed BKME-exposed fish of multiple species recovery from capture stress in wooden bins for 3 days, and continued to find differences among sites in carbohydrate metabolism (glucose, lactate) as a secondary aspect of the stress response. Tilapia (*Oreochromis mossambicus*) subjected to a single injection of *o,p'*-DDD required over 100 days to recover full stress response capacity in the laboratory (Ilan and Yaron 1983), and northern pike transplanted from a mercury-contaminated lake to a cleaner lake demonstrated only partial HPI axis recovery after 1 year of residence (Lockhart et al. 1972).

Cortisol as a biomarker for habitat contamination?

Round goby in our study had a mean baseline plasma cortisol concentration of 22.3 ng/mL, and peak cortisol concentrations (10 min post-stressor) of up to 100.3 ng/mL. This represents a rapid increase of approximately 350 % (i.e., 4.5×) in plasma cortisol that dissipated after 1 h had elapsed. We utilized an air exposure stressor for experiment 1. Most fishes appear to reach peak cortisol levels later than this, after 30 min to 1 h (Wendelaar Bonga 1997; Barton 2002), and the differences between baseline and peak cortisol concentrations, while varying across species, tend to be much higher in other studies (reviewed in Barton 2002). It is possible that the stressor used in this study, a combination of air exposure coupled with net confinement, was not strong enough to induce a large, long stress response in the round goby. Round goby may be tolerant of hypoxia (Cross and Rawding 2009) potentially making air exposure a fairly weak stressor for this species. Other studies have found variation in cortisol differences depending on which type of stressor was employed—site effects were seen with some stressors but not others. Norris and others (1999), for example, were able to detect differences in brown trout collected from different areas of the Eagle River in Colorado, U.S.A., only with an intense confinement stressor, not a mild one. By varying types of handling stressors, McMaster and others (1994) obtained very different endocrine profiles of white sucker collected from the same site; the pattern of differences in fish from clean and contaminated sites varied with the stressor. In tilapia exposed to PCBs, differences in HPI axis functioning relative to controls were only detected with acute, but not prolonged, stress (Quabius et al. 1997). Utilizing a greater diversity of stressors may reveal site-related differences in the ability of the round goby to mount a cortisol response.

Although HPI axis exhaustion is proposed to be a general phenomenon in contaminant-exposed populations (Hontela 1998), in this study the cortisol stress response did not prove to be a bioindicator of contaminant exposure of round goby. Biomarkers, particularly when examined individually, need to be evaluated carefully for their utility in separating individuals on the basis of habitat contamination (Norris 2000). It does not appear that the physiological apparatus, the HPI axis, of round goby in even highly contaminated habitats is necessarily impaired. Cortisol may not be a useful biomarker of contaminant exposure in this sentinel species. However, our study revealed site differences in time to exhaustion while being chased that could impact the ability of round goby to successfully evade predators. Round goby from the contaminated site tired nearly twice as quickly as round goby from a cleaner site. The lower endurance observed in contaminated goby corresponds with findings from other studies that reveal a variety of contaminants impair swimming capacity in fishes (as reviewed in Scott and Sloman 2004; McKenzie et al. 2007) which may be due to altered metabolism, muscle physiology, decreased lipid and glycogen storages or fin erosion (Wicks et al. 2002; Hopkins et al. 2003; Barbieri 2007; Marentette et al. 2010). We did not find a difference, however, in the anaerobic metabolic capacity (measured as plasma lactate) of round goby between clean and contaminated sites. Whatever the cause, the impaired ability of round goby to avoid predation in areas of contamination may impact the ways in which these fish act as a conduit for contaminants biomagnifying in foodwebs (Marentette et al. 2010). Understanding the ways in which sublethal contaminant impacts on behaviour and physiology can manifest as changes in vulnerability to predation, and

thus the survival of individuals in afflicted populations, remains an important direction of future research on the ecological consequences of pollution.

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References

Aluru N, Vijayan MM (2006) Aryl hydrocarbon receptor activation impairs cortisol release to stress in rainbow trout by disrupting the rate-limiting steps in steroidogenesis. Endocrinology 147:1895–1903

Andersson T, Forlin L, Hardig J, Larsson A (1988) Physiological disturbances in fish living in coastal water polluted with bleached kraft pulp mill effluents. Can J Fish Aquat Sci 45:1525–1536

Barbieri E (2007) The use of active metabolism and swimming activity to evaluate the toxicity of dodecil benzene sodium sulfonate (LAS-C12) on the *Mugil platanus* (mullet) according to the temperature and salinity. Water Environ Res Estados Unidos 79:707–719

Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr Comp Biol 42:517–525

Barton BA, Schreck CB, Barton LD (1987) Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Dis Aquat Org 2:173–185

Basu N, Kennedy CJ, Hodson PV, Iwama GK (2002) Altered stress responses in rainbow trout following a dietary administration of cortisol and beta-napthoflavone. Fish Physiol Biochem 25:131–140

Benguira S, Leblond VS, Weber J-P, Hontela A (2002) Loss of capacity to elevate plasma cortisol in rainbow trout (*Oncorhynchus mykiss*) treated with a single injection of *o, p'*-dichlorodiphenyldichloroethane. Environ Toxicol Chem 21:1753–1756

Bleau H, Daniel C, Chevalier G, van Tra H, Hontela A (1996) Effects of acute exposure to mercury chloride and methylmercury on plasma cortisol, T3, T4, glucose and liver glycogen in rainbow trout (*Oncorhynchus*). Aquat Toxicol 34:221–235

Bowley L, Alam F, Marentette JR, Balshine S, Wilson JY (2010) Characterization of vitellogenin gene expression in round goby *Neogobius melanostomus* using a quantitative PCR assay. Environ Toxicol Chem 29:2751–2760

Brodeur JC, Sherwood G, Rasmussen JB, Hontela A (1997) Impaired cortisol secretion in yellow perch (*Perca flavescens*) from lakes contaminated by heavy metals: in vivo and in vitro assessment. Can J Fish Aquat Sci 54:2752–2758

Bury NR, Jie L, Flik G, Lock RAC, Wendelaar Bonga SE (1998) Cortisol protects against copper induced necrosis and promotes apoptosis in fish gill chloride cells in vitro. Aquat Toxicol 40:193–202

Carragher JF, Sumpter JP (1990) The effect of cortisol on the secretion of sex steroids from cultured ovarian follicles of rainbow trout. Gen Comp Endocrinol 77:403–407

Cross EE, Rawding RS (2009) Acute thermal tolerance in the round goby, *Apollonia melanostoma* (*Neogobius melanostomus*). J Therm Biol 34:85–92

DeBoeck G, DeWachter B, Vlaeminck A, Blust R (2003) Effect of cortisol treatment and/or sublethal copper exposure on copper uptake and heat shock protein levels in common carp, *Cyprinus carpio*. Environ Toxicol Chem 22:1122–1126

Eales JG, Brown SB (2005) Thyroid hormones. In: Mommsen TP, Moon TW (eds) Environmental toxicology. Biochemistry and molecular biology of fishes, vol. 6. Elsevier, New York, pp 397–412

Forlin L, Andersson T, Balk L, Larsson A (1995) Biochemical and physiological effects in fish exposed to bleached kraft mill effluents. Ecotoxicol Environ Saf 30:164–170

Friedmann AS, Watzin MC, Brinck-Johnsen T, Leite JC (1996) Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquat Toxicol 35:265–278

Gagnon A, Jumarie C, Hontela A (2006) Effects of Cu on plasma cortisol and cortisol secretion by adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol 78:59–65

Gendron AD, Bishop CA, Fortin R, Hontela A (1997) In vivo testing of the functional integrity of the corticosterone-producing axis in mudpuppy (Amphibia) exposed to chlorinated hydrocarbons in the wild. Environ Toxicol Chem 16:1694–1706

Girard C, Brodeur JC, Hontela A (1998) Responsiveness of the interrenal tissue of yellow perch (*Perca flavescens*) from contaminated sites to an ACTH challenge test in vivo. Can J Fish Aquat Sci 55:438–450

Gravel A, Campbell PGC, Hontela A (2005) Disruption of the hypothalamo-pituitary-interrenal axis in 1+ yellow perch (*Perca flavescens*) chronically exposed to metals in the environment. Can J Fish Aquat Sci 62:982–990

Gregory TR, Wood CM (1999) The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. Physiol Biochem Zool 72:286–295

Hamilton Harbour Remedial Action Plan (RAP) (1992) Hamilton Harbour Stage 1 Report: environmental conditions and problem definition. Burlington, Ontario

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

Hontela A (1997) Endocrine and physiological responses of fish to xenobiotics: role of glucocortisoid hormones. Rev Toxicol 1:1–46

Hontela A (1998) Interrenal dysfunction in fish from contaminated sites: in vivo and in vitro assessment. Environ Toxicol Chem 17:44–48

Hontela A (2005) Adrenal toxicology: environmental pollutants and the HPI axis. In: Mommsen TP, Moon TW (eds) Environmental toxicology. Biochemistry and molecular biology of fishes, vol. 6. Elsevier, New York, pp 331–364

Hontela A, Rasmussen JB, Audet C, Chevalier G (1992) Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs and mercury. Arch Environ Contam Toxicol 22:278–283

Hontela A, Dumont P, Duclos D, Fortin R (1995) Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence River. Environ Toxicol Chem 14:725–731

Hontela A, Daniel C, Ricard AC (1996) Effects of acute and subacute exposures to cadmium on the interrenal and thyroid function in rainbow trout, *Oncorhynchus mykiss*. Aquat Toxicol 35:171–182

Hontela A, Daniel C, Rasmussen JB (1997) Structural and functional impairment of the hypothalamo-pituitary-interrenal axis in fish exposed to bleached kraft mill effluent in the St Maurice River, Québec. Ecotoxicology 6:1–12

Hopkins WA, Snodgrass JW, Staub BP, Jackson BP, Congdon JD (2003) Altered swimming performance of a benthic fish (*Erimyzon sucetta*) exposed to contaminated sediments. Arch Environ Contam Toxicol 44:383–389

Hutchinson TH, Ankly GT, Segner H, Tyler CR (2006) Screening and testing for endocrine disruption in fish—biomarkers as "signposts", not "traffic lights", in risk assessment. Environ Health Perspect 114(s1):106–114

Ilan Z, Yaron Z (1983) Interference of *o, p'*-DDD with interrenal function and cortisol metabolism in Saratherodon aureus (Steindachner). J Fish Biol 22:657–669

International Joint Commission (IJC) (1999) Hamilton Harbour Area of Concern Status Assessment. Windsor, Ontario

Jardine JJ, Van Der Kraak GJ, Munkittrick KR (1996) Capture and confinement stress in white sucker exposed to bleached kraft pulp mill effluent. Ecotoxicol Environ Saf 33:287–298

Jude DJ, 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. SPB Academic Publishing, Amsterdam, pp 447–460

Kime DE (1998) Endocrine disruption in fish. Kluwer, Norwell

Kirubagaran R, Joy KP (1991) Changes in the adrenocortical-pituitary activity in the catfish, *Clarias batrachus* (L.), after mercury treatment. Ecotoxicol Environ Saf 22:36–44

Laflamme J-S, Couillard Y, Campbell PGC, Hontela A (2000) Interrenal metallothionein and cortisol secretion in relation to Cd, Cu and Zn exposure in yellow perch, *Perca flavescens*, from Abitibi lakes. Can J Fish Aquat Sci 57:1692–1700

Lappivaara J (2001) Effects of acute handling stress on whitefish *Coregonus lavaretus* after prolonged exposure to biologically treated and untreated bleached kraft mill effluent. Arch Environ Contam Toxicol 41:55–64

Leblond VS, Bisson M, Hontela A (2001) Inhibition of cortisol secretion in dispersed head kidney cells of rainbow trout (*Oncorhynchus mykiss*) by endosulfan, an organochlorine pesticide. Gen Comp Endocrinol 121:48–56

Levesque HM, Dorval J, Hontela A, Van Der Kraak GJ, Campbell PGC (2003) Hormonal, morphological and physiological responses of yellow perch (*Perca flavescens*) to chronic environmental metal exposures. J Toxicol Environ Health A 66:657–676

Levitan WM, Taylor MH (1979) Physiology of salinity-dependent naphthalene toxicity in *Fundulus heteroclitus*. J Fish Res Board Can 36:615–620

Lockhart WL, Uthe JF, Kenney AR, Mehrle PM (1972) Methylmercury in northern pike (*Esox lucius*): distribution, elimination and some biochemical characteristics of contaminated fish. J Fish Res Board Can 29:1519–1523

Marentette JR, Gooderham KL, McMaster ME, Ng T, Parrott JL, Wilson JY, Wood CM, Balshine S (2010) Signatures of contamination in invasive round gobies (*Neogobius melanostomus*): a double strike for ecosystem health. Ecotoxicol Environ Saf 73:1755–1764

Marentette JR, Wang G, Tong S, Sopinka NM, Taves MD, Koops MA, Balshine S (2012) Laboratory and field evidence of sex-biased movement in the invasive round goby. Behav Ecol Sociobiol 21:1003–1012

McKenzie DJ, Garofalo E, Winter MJ, Ceradini S, Verweji F, Day N, Hayes R, van der Oost R, Butler PJ, Chipman JK, Taylor EW (2007) Complex physiological traits as biomarkers of the sub-lethal toxicological effects of pollutant exposure in fishes. Phil Trans R Soc Lond B 362:2043–2059

McMaster ME, Munkittrick KR, Luxon PL, van der Kraak GJ (1994) Impact of low-level sampling stress on interpretation of physiological responses of white sucker exposed to effluent from a bleached kraft pulp mill. Ecotoxicol Environ Saf 27:251–264

Niimi AJ, Palazzo V (1986) Biological half-lives of eight polycyclic aromatic hydrocarbons (PAHs) in rainbow trout (*Salmo gairdneri*). Water Res 20:503–507

Norris DO (1999) Impaired adrenocortical response to stress by brown trout, *Salmo trutta*, living in metal-contaminated waters of the Eagle River, Colorado. Gen Comp Endocrinol 113:1–8

Norris DO, Donahue S, Dores RM, Lee JK, Maldonado TA, Ruth T, Woodling JD (1999) Impaired adrenocortical response to stress by brown trout, *Salmo trutta*, living in metalcontaminated waters of the Eagle River, Colorado. Gen Comp Endocrinol 113:1–8

Norris DO (2000) Endocrine disruptors of the stress axis in natural populations: how can we tell? Am Zool 40:393–401

Odermatt A, Gumy C, Atanasov AG, Dzyakanchuk AA (2006) Disruption of glucocorticoid action by environmental chemicals: potential mechanisms and relevance. J Steroid Biochem Mol Biol 102:222–231

Pickering AD (1989) Environmental stress and the survival of brown trout, *Salmo trutta*. Freshw Biol 21:47–55

Pickering AD, Pottinger TG (1989) Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish Physiol Biochem 7:253–258

Pinchuk VI, Vasil'eva EK, Vasil'ev VP, Miller PJ (2003) *Neogobius melanstomus* (Pallas, 1814). In: Miller PJ (ed) The freshwater fishes of Europe. AULA-Verlag, pp 293–345

Pottinger TG, Carrick TR, Hughes SE, Balm PHM (1996) Testosterone, 11-ketotestosterone, and estradiol-17B modify baseline and stress-induced interrenal and corticotropic activity in trout. Gen Comp Endocrinol 104:284–295

Pradelles P, Grassi J, Maclouf J (1990) Enzyme immunoassays of eicosanoids using acetylcholinesterase. Methods Enzymol 187:24–34

Pratap HB, Wendelaar Bonga SE (1990) Effects of water-borne cadmium on plasma cortisol and glucose in the cichlid fish *Oreochromis mossambicus*. Comp Biochem Physiol 95C:313–317

Quabius ES, Balm PHM, Wendelaar Bonga SE (1997) Interrenal stress responsiveness of tilapia (*Oreochromis mossambicus*) is impaired by dietary exposure to PCB 126. Gen Comp Endocrinol 108:472–482

Ray WJ, Corkum LD (2001) Habitat and site affinity of the round goby. J Great Lakes Res 27:329–334

Saunders AC, Feldman HA, Correia CE, Weinstein DA (2005) Clinical evaluation of a portable lactate meter in type I glycogen storage disease. J Inherit Metab Dis 28:695–701

Scott GR, Sloman KA (2004) The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat Toxicol 68:369–392

Sigismondi LA, Weber LJ (1988) Changes in avoidance response time of juvenile Chinook salmon exposed to multiple acute handling stresses. Trans Am Fish Soc 117:196–201

Thomas RE, Rice SD (1987) Effect of water-soluble fraction of Cook Inlet crude oil on swimming performance and plasma cortisol in juvenile Coho salmon (*Oncorhynchus kisutch*). Comp Biochem Physiol 87C:177–180

Thomas P, Woodlin BR, Neff JM (1980) Biochemical responses of striped mullet *Mugil cephalus* to oil exposure I. Acute responses—interrenal activations and secondary stress responses. Mar Biol 59:141–149

Tyler CR, Jobling S, Sumpter JP (1998) Endocrine disruption in wildlife: a critical review of the evidence. Crit Rev Toxicol 28:319–361

Vélez-Espino LA, Koops MA, Balshine S (2010) Invasion dynamics of round goby (*Neogobius melanostomus*) in Hamilton Harbour, Lake Ontario. Biol Invasions 12:3861–3875

Vijayan MM, Prunet P, Boon AN (2005) Xenobiotic impact on corticosteroid signalling. In: Mommsen TP, Moon TW (eds) Environmental toxicology. Biochemistry and molecular biology of fishes, vol. 6. Elsevier, New York, pp 365–396

Weis JS (2002) Tolerance to environmental contaminants in the mummichog, *Fundulus heteroclitus*. Hum Ecol Risk Assess 8:933–953

Wendelaar Bonga SE (1997) The stress response in fish. Physiol Rev 77:591–625

Wicks BJ, Joensen R, Tang Q, Randall DJ (2002) Swimming and ammonia toxicity in salmonids: the effects of sublethal ammonia exposure on the swimming performance of coho salmon and the acute toxicity of ammonia in swimming and resting rainbow trout. Aquat Toxicol 59:55–69

Wilson JM, Vijayan MM, Kennedy CJ, Iwama GK, Moon TW (1998) B-naphthoflavone abolishes interrenal sensitivity to ACTH stimulation in rainbow trout. J Endocrinol 157:63–70

Young JAM, Marentette JR, Gross C, McDonald JI, Verma A, Marsh-Rollo SE, Macdonald PDM, Earn DJ, Balshine S (2010) Demography and substrate affinity of the round goby (*Neogobius melanostomus*) in Hamilton Harbour. J Great Lakes Res 36:115–122

Zeman AJ (2009) Contaminated sediments in Hamilton Harbour. Environment Canada Water Science and Technology Directorate (WSTD) Contribution No. 09-263