Behav Ecol Sociobiol (2010) 64:1947–1958
DOI 10.1007/s00265-010-1005-1

ORIGINAL PAPER

Impact of contaminant exposure on resource contests in an invasive fish

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Received: 3 February 2010 / Revised: 9 April 2010 / Accepted: 16 June 2010 / Published online: 13 July 2010

Abstract There is increasing concern for the disruptive effects seen in aquatic species exposed to environmental contaminants. However, few studies have investigated the impact of such contaminants on the behavior of individuals living in exposed waters. Contaminant exposure can affect animal populations by disrupting behaviors including feeding, locomotion, and mating. In this study, we examined how living in an ecosystem polluted by combinations of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and heavy metals (arsenic, cadmium, iron, lead, zinc) impacts contest behavior in the round goby (*Neogobius melanostomus*). Fish collected from heavily contaminated and cleaner sites in Lake Ontario were subjected to a resource contest to determine the effect of these contaminants on aggression and the establishment of dominance hierarchies, which in turn influence access to food, shelter, and mating opportunities. Dominance establishment (a clear resource winner) was less obvious among fish from the contaminated site compared to the more stable hierarchies that formed between pairs of fish from the clean site. Pairs of fish from the contaminated site performed more assessment displays compared to fish from clean sites. These results suggest that the costs of living in an environment under exposure can shape behavioral repertoires. The altered conflict resolution strategies of contaminated fish may reflect impaired cognitive function, sensory perception, and/or higher metabolic load associated with aggression. This study provides support for the utilization of quantifiable behavioral differences as ecologically relevant measures of contaminant exposure.

Keywords Round goby · Intra-specific competition · Aggression · Contest structure · Aquatic pollutants · *Neogobius melanostomus*

Introduction

Competitive ability and aggressiveness determine an individual's success at acquiring food, nesting sites, mates, and high social rank (Manning and Dawkins 1992). Aggressive interactions between individuals competing for resources often result in the emergence of a dominance hierarchy (Chase et al. 2003). There are several factors that can influence the level of aggression an individual displays and whether an individual emerges dominant after a competitive interaction. Variation in aggressiveness may depend on energy stores (Adams et al. 1995), sex (Magurran and Maciás Garcia 2000), age (Blanchard et al. 1988), familiarity of opponent (Husak and Fox 2003), current rank and previous agonistic encounters (Goessmann et al. 2000), circulating levels of steroid hormones (Soma 2006), and exposure to a variety of environmental toxicants (reviewed in Scott and Sloman 2004). Studies of a diverse range of taxa have explored the effects of toxicants on social behaviors, including agonistic behaviors often associated with hierarchy formation (rodents, Ogilvie and Martin 1982; birds, MacLellan et al. 1997; fish, Scott and Sloman 2004). This paper examines the impact of inhabiting a contaminated environment on agonistic behaviors, using a benthic fish now common in the Laurentian Great Lakes.

Communicated by: C. St. Mary

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Laboratory studies using trace and heavy metals as well as numerous organic pollutants have found that these contaminants can disrupt social behaviors (Atchison et al. 1987; Jones and Reynolds 1997; Scott and Sloman 2004; Clotfelter and Rodriguez 2006). There are several physiological mechanisms that may be the cause of the observed behavioral changes. Contaminant-mediated alterations in sensory (in particular olfactory), endocrine, metabolic, and neurological systems may all modify behavior (reviewed in Weis et al. 2001a; Peakall et al. 2002; Scott and Sloman 2004). For example, disruption to olfactory senses (Sloman et al. 2003; Sloman 2007) or neurological dysfunction (Smith et al. 1995; Weis et al. 2001a; Peakall et al. 2002) could alter decision-making processes, such as when and whether to initiate aggression when faced with a competitor. Toxicant-induced metabolic loading can reduce available energy (Al-Akel and Shamsi 1996) or oxygen (Witeska et al. 2006; Barbieri 2009) available for executing locomotion in agonistic encounters.

More specifically, particular contaminants have been shown to affect the outcome of competitive interactions. In rainbow trout (*Oncorhynchus mykiss*), an 8-week exposure to dietary copper (721 mg/kg) reduced the occurrence of agonistic interactions among fish (Campbell et al. 2005) and a 24-h waterborne exposure to cadmium (2 mg/l) decreased a fish's competitive ability when contesting an unexposed opponent (Sloman et al. 2003). Reduced aggressiveness observed in these cadmium-exposed salmonid fish resulted in faster hierarchy formation than fish not exposed to metal toxicants (Sloman et al. 2003). In fish, exposure to some metal toxicants (34 μg/l of copper for 24 h or exposure to a zinc (99–124 μg/l) and cadmium (21–40 μg/l) mixture for 15 days) can also increase the frequency of aggressive behaviors (Atchison et al. 1987). Though behavioral and physiological impacts of individual metal and organic pollutants have been studied extensively, these studies have been conducted in controlled laboratory environments with fish experimentally exposed to a single contaminant. Investigation into the effects of naturally occurring cocktails of contaminants on fish behavior, and broader applications to species conservation, is extremely limited (Weis et al. 2001a).

This study investigated the impacts of exposure to naturally occurring levels and combinations of contaminants found in Lake Ontario by comparing the aggression and competitive ability of round gobies (*Neogobius melanostomus*) collected from highly contaminated and relatively uncontaminated sites. The round goby is a bottom-dwelling fish native to the Caspian region of Europe. It has invaded the Laurentian Great Lakes, likely through transfer in ship ballast water (Jude et al. 1992). A subset of the invaded population inhabits Hamilton Harbour, an International Joint Commission (IJC) Area of Concern located in western Lake Ontario. Hamilton Harbour is surrounded by two steel producers and a wastewater treatment plant and receives the city of Hamilton's combined sewer overflow and urban runoff, each source contributing to the current state of water quality concern (International Joint Commission (IJC) 1999; Hamilton Harbour Remedial Action Plan (RAP) 2003, Fig. 1). Small home ranges and high site fidelity (Wolfe and Marsden 1998; Ray and Corkum 2001) mean that populations of round gobies near the steelmills in Hamilton Harbour will be chronically exposed (through sediments, diet, and water) to a combination of polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs), and other polychlorinated compounds, as well as numerous metals such as arsenic, cadmium, iron, lead, and zinc (IJC 1999; Marentette et al. 2010). Additionally, the diet of round gobies includes zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena rostriformis bugensis*, Jude et al. 1995), which are filter-feeding dreissenids likely responsible for the significant bioaccumulation of aquatic toxicants such as PCBs (Bruner et al. 1994). Round gobies have been proposed to be potential vectors for transferring contaminants and may have a role in accelerating the movement of contaminants up trophic levels (Jude et al. 1995; Morrison et al. 2000). Toxicant-induced behavioral changes may accelerate or decelerate the round goby's capacity to biotransfer contaminants.

The round goby is known to be an aggressive species; its behavior has been previously studied and high levels of inter-specific aggressiveness (Jude et al. 1995; Dubs and Corkum 1996; Balshine et al. 2005; Savino et al. 2007), forceful defense of nests (Wickett and Corkum 1998; MacInnis and Corkum 2000), a role in the displacement and decline of other native benthic species (Jude et al. 1995; Janssen and Jude 2001; Lauer et al. 2004), and consumption of eggs of native fish (Chotkowski and Marsden 1999; Steinhart et al. 2004) have been documented. In contrast to studies that use controlled levels of single contaminants on chemically naive fish, we explored the influence of long-term exposure to contaminant mixtures on aggressive behaviors in fish collected from habitats within the original distribution and source of industrial contamination. Resource competitions were initiated between two size-matched, non-reproductive male gobies, and instances of aggressive behaviors were contrasted between individuals from heavily contaminated and relatively cleaner sites in Hamilton Harbour. We paired fish from the same site to test for differences in hierarchy formation between contaminated and clean fish. We predicted that, if contaminant exposure in general reduces the competitive capacity of round gobies, pairs from the contaminated sites would have fewer aggressive interactions than pairs from clean sites, resulting in a more rapid hierarchy formation.

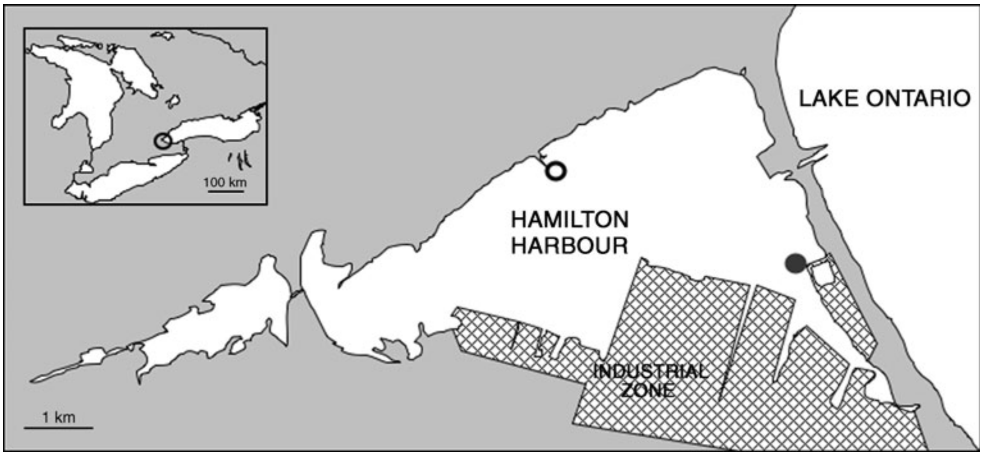


Fig. 1 Clean (*open circle*) and contaminated (*filled circle*) collection sites in Hamilton Harbour, Lake Ontario, Canada. *Hatched shading* represents the industrial shoreline. Steel mills and associated industry occur in this area in addition to wastewater treatment plants and combined sewer overflow facilities

Additionally, we predicted that when paired with a fish from a clean site, contaminated fish would be (1) less aggressive, (2) less likely to initiate a confrontation, and (3) spend less time in the contested resource (shelter).

Methods

Animal collection and site description

Non-reproductive male round gobies (*N. melanostomus*) were collected from 22 August to 24 October 2008. Using minnow traps, baited with 30 g of frozen corn kernels, and set for 24 h, fish were collected from two sites: (1) LaSalle Park Marina (43°18′03 N, 79°50′45 W), on the north shore of Hamilton Harbour, Lake Ontario was designated as the clean site and (2) Pier 27 (43°16′53 N, 79°47′32 W) located on the eastern shore was designated as the contaminated site (Fig. 1). In contrast to LaSalle Park Marina, Pier 27 is located near steel and iron industrial activities, a combined disposal facility with dredged sediments from the Harbour's industrial areas and effluents from a wastewater treatment plant, expected to be a primary source of PCBs (Fig. 1, Zeman and Patterson 2003; Hamilton Harbour Remedial Action Plan (RAP) 2008). Compared to other areas in Hamilton Harbour, sediment concentrations of PCBs near Pier 27 are two to three times higher (1,270 ng/g, Hamilton Harbour Remedial Action Plan (RAP) 2008; Zeman 2009), and the levels of PCBs detected in water samples are also significantly higher (<25.85 ng/l, Hamilton Harbour Remedial Action Plan (RAP) 2009). Tissues of fish collected from Pier 27 also contain elevated PCB concentrations compared to LaSalle Park Marina and other Harbour areas (fillet of carp, brown bullhead, largemouth bass; Hamilton Harbour Remedial Action Plan (RAP) 2008; livers of round goby, Holly Hynes/Greg Slater, unpublished data). PCBs are not the only contaminant class known to differ in sediment concentrations between the two sites; sediment loadings of PAHs (20–67 μg/g), iron (76–100 μg/g), lead (301–450 μg/g), and copper (151–225 μg/g) have all been reported to be higher at Pier 27 than at LaSalle Park Marina (Hamilton Harbour Remedial Action Plan (RAP) 1992).

Fish were sexed in the field based on examination of the urogenital papilla, which is pointed in males and blunted in females (Miller 1984). Initially, reproductive status was determined by traits including papilla length, head width, and male nuptial coloration (Miller 1984; Marentette et al. 2009). Fish were brought back to the laboratory facilities at McMaster University's Department of Psychology, Neuroscience and Behaviour, sorted, and housed by collection site for 3–5 days in 66-l holding tanks (61.0 cm long× 30.5 cm wide×35.6 cm high) under a reversed 16:8 h light/dark cycle. Behavior was not affected by the reversed light schedule (personal observations). Water temperatures were maintained between 22°C and 24°C. Fish were fed food flakes (Nutrafin Basix Staple food) ad libitum once daily, up until the day of behavioral trials. In total, this experiment utilized a total of 60 non-reproductive male fish (standard length 51.9–100.9 mm, mean ± SE = 76.1 ± 0.2 mm; body mass 3.3–28.1 g, mean ± SE = 11.2 ± 0.7 g). Any fish collected but not applicable for use in this study (i.e., female or reproductive males) were used in other ongoing studies.

Behavioral observations

Thirty behavioral trials (10 clean pairs, 10 contaminated pairs, 10 mixed pairs) were conducted in experimental tanks (61.0 cm long×30.5 cm wide×35.6 cm high) that were partitioned into three sections using opaque dividers. An opaque acrylic shelter (15.2 cm long×15.2 cm wide× 5.1 cm high) was provided in each end chamber and in the central middle section of each experimental tank. Each fish was uniquely marked prior to behavioral observations with non-toxic acrylic paint (see Wolfe and Marsden 1998) on the anterior, lateral region of the body, directly in front of the first dorsal fin, to facilitate fish identification. Each individual fish was then placed in one of the end chambers in size-matched pairs (with a mean size difference between pairs of 2.4 mm, range=0.1–8.8 mm). Pairs were given 24 h to recover from handling and marking.

To control for diel effects and to simulate diurnal conditions (when round gobies spend most time in their shelters and motivation to sequester shelter would presumably be highest, Dubs and Corkum 1996), trials were conducted during the light phase of the light/dark cycle between 12:30 and 17:30. Each trial was recorded on a mini DV videotape using a Sony Digital Handycam camcorder (model DCR-VX2000 NTSC). Trials began with the removal of the two outer shelters and the opaque partitions. Each individual was then observed for 10 min and then again for 5 min 3 h later. The fish that initiated aggression and the time of initiation were both noted. All behaviors were scored (Table 1) and time spent in the remaining, central shelter was recorded. Once a trial ended, total length, standard length, and body mass were recorded and the liver and gonads removed and weighed. A calculation of gonadosomatic index (GSI, total gonad mass/total body mass−total gonad mass × 100%) was calculated and used to confirm the non-reproductive state of each fish ($GSI<<1\%$, Marentette and Corkum 2008). A calculation of hepatosomatic index (HSI, total liver mass/total body mass−total liver mass × 100%) was also calculated.

Behavioral analyses

Aggressive behaviors could be easily divided into two categories: (a) assessment behaviors (parallel fin displays, mouth gapes, mouth fights; see Table 1) and (b) pursuit aggression (bites, chases, displacements; Table 1). Assessment behaviors, such as parallel fin displays, function to assess an opponent's size and strength, without using excessive locomotory behaviors (Parker 1974; Maynard Smith 1982). During the most common assessment behavior, a parallel fin display, fish face each other with all fins erect and thrust toward each other without physical contact. Forward movement pushes water current against an opponent's lateral lines. The lateral line organ detects disturbances in water movement (Dijkgraaf 1962) and so the body size of a fish will determine the amount of water disturbance created (Moyle and Cech 2000). Pursuit aggressions are also common contest behaviors among gobiid fishes (Kroon et al. 2000; Kangas and Lindström 2001; Whiteman and Côté 2004; Forrester et al. 2006; Magnhagen 2006; Amorim and Neves 2008), whereby a fish explicitly causes its opponent to move away. An aggressive interaction (or bout) was defined as the consecutive performance of two or more aggressive acts by one fish towards another. Following a series of aggressive acts, two or more non-aggressive acts signaled the end of the bout. Intensity of aggressive behaviors was calculated based on the equation: Intensity = 3× (mouth fight + bite) + 2 × (mouth gape + chase) + 1× (parallel fin display + displacement). Non-aggressive behaviors (mainly isolated swimming, see Table 1) also occurred frequently during the trials and these movements were recorded and tallied. Three measures of shelter monopolization were utilized: (a) time of first shelter entry, (b) total number of shelter entries, and (c) total time spent in the shelter. Winners were identified as the fish that spent the most time in the shelter during the second focal watch.

Statistical analyses

All statistical analyses were performed using the program JMP (version 5.0.1, 2001; SAS Institute Inc., Cary, NC, USA) on a Macintosh computer. The data were not normally distributed nor could they be transformed; hence, non-parametric tests were employed throughout. Kruskal–Wallis, Wilcoxon rank-sum, and Fisher's exact tests were used when analyzing aggressive and non-aggressive behaviors, measures of shelter monopolization, number of bouts, duration of fight, and dominance hierarchy establishment between individuals and between collection sites. Frequency and intensity of behaviors (see Table 1) and measures of shelter monopolization were analyzed per fish ($N=60$). In trials with clean pairs or contaminated pairs, time of first shelter entry could only be obtained for 29 fish as 11 fish did not enter the shelter at all during the first focal watch. Number of bouts, duration of bout, and dominance hierarchy establishment were analyzed per pair of fish. One clean pair of fish did not execute an aggressive interaction/bout (as defined above) and winner–loser assignment could not be determined (as described above) for two pairs of contaminated fish. A size difference ratio was calculated for each pair of fish ($N=30$) using the formula, total length (millimeter) of the small fish/total length (millimeter) of the large fish. Different letters on graphs denote differences between groups measured at a $P<0.05$ level.

Ethical note

No fish were injured as a result of this experiment. Marking did not affect fish behavior. All behavioral trials were carefully monitored and fish did not appear to be in distress. Trials would have been stopped if interacting fish appeared to be in distress or if any signs of physical harm were observed. Following each trial, both fish were sacrificed to provide physiological data and confirm non-reproductive status. To ensure rapid death, an overdose of benzocaine (Sigma Aldrich Canada Ltd., Oakville, Canada) was used to

Table 1 Ethogram for *Neogobius melanostomus*

Behavior		Description
Aggressive	Mouth fight (MF)	Jaw of focal fish makes contact with the jaw of another fish. Jaws are interlocked and fish push back and forth
	Parallel fin display (PFD)	Both focal fish and opponent face each other (approximately 5cm away from each other), with all fins erect. Often displayed with a curve-like body shape (like the letter "C")
	Mouth gape (MG)	Focal fish flares out its opercula and lower jaw cavity, gaping mouth in direction of another fish
	Bite (Bt)	Focal fish rapidly approaches another fish and opens and closes its mouth making contact on the other fish's body
	Chase (Ch)	Focal fish rapidly approaches another fish and the other fish swims rapidly away (see Flee)
	Displace from shelter (Ds-SH)	Focal fish occupying a shelter forces intruding fish to leave shelter
	Displace (Ds)	Focal fish passes by or approaches another fish slowly, causing the other fish to swim rapidly away (see Flee)
Submissive	Bury (Bu)	Focal fish vigorously wiggles its body into the substrate, partially or completely hiding its body
	Hide (Hi)	Focal fish inserts its body into an available crevice
	Flee (Fl)	Focal fish makes a quick movement away from another fish
Locomotor	Swim (Sw)	Sustained locomotion in the water column using all fins
	Hop (H)	Smooth locomotion on substrate apparently driven by pectoral fins. A forward or sideways distance of movement less than one body length
	Dart (D)	A spontaneous, rapid swim along the substrate not directed at anything
	Glass swim (GS)	Focal fish orients towards the side of the tank and repeatedly swims up and down
	Glass touch (GT)	Focal fish orients towards the side of the tank and approaches surface quickly making contact with glass once followed by a still posture (including Prop, see below)
	Prop (Pr)	Focal fish is still with the anterior of body arched upward and raised slightly above substrate. Can occur with or without a vertical fin display (see VFD below)
Maintenance	Churn (Cr)	Rapid opercular and mouth movements as though fish is chewing something in its mouth
	Dig (Dg)	Focal fish picks up an object in mouth and moves it to a different location
	Scrape (Sc)	Focal fish very quickly scrapes its side or ventral surface against a surface
	Yawn (Y)	Big slow stretch of the mouth
	Feed (Fe)	Focal fish orients towards food and opens its mouth to take in particles
	Shelter (Str)	Entire body length of focal fish enters shelter
	Vertical fin display (VFD)[a]	Focal fish raises first dorsal fin exposing the black spot
Reproductive	Bark (Bk)[a]	Focal male holds body rigid while quivering caudal fin and waving pectoral fins. Involves the forceful expulsion of water/air from the buccal cavity
	Loop (L)	In one continuous motion, focal male emerges whole body from shelter, turns entire body length 180° and re-enters shelter
	Pop (Pp)	Head of focal male (any portion of head pectoral fins and forward) quickly appears in shelter opening and protrudes outward
	Ram (R)[a]	Focal fish holds body rigid and strikes the body of another fish
	Spawn (Sp)	Female focal fish repetitively rubs belly oriented towards a specific substrate in order to deposit eggs
	Pseudo-spawn (Ps-Sp)	Female focal fish repetitively rubs belly oriented towards a specific substrate without depositing eggs
	Laying of sperm trails (ST)	In the absence of female, focal male rubs belly and erect urogenital papilla on shelter or substrate
Parental care	Fan (Fn)	Focal fish moves pectoral fins over or toward eggs, undulating entire body
	Mouthing (M)	Focal fish makes contact between mouth and eggs, apparently tasting the eggs
	Egg feeding (EF)	Behavior that looks like mouthing but eggs are removed

Reproductive behaviors are also described in Meunier et al. (2009). Non-aggressive behaviors (submissive, locomotor, maintenance) were observed in the laboratory

[a] Indicate behaviors that can be demonstrated in an aggressive manner, in addition to being primarily and more frequently used in a maintenance or reproductive context

euthanize fish. Death was verified prior to dissection by the absence of ventilation. All research conformed to the protocols approved by the Animal Research Ethics Board of McMaster University (AUP # 06-10-61) and met the Canadian Council for Animal Care guidelines. This research was conducted with the permission and cooperation of the Hamilton Port Authority and the Royal Botanical Gardens.

Results

Contest structure

Round goby resource contests were made up of distinct bouts of aggression that contained two types of aggressive acts: assessment displays, performed largely in a stationary position, and pursuit aggression, which were acts with a significant locomotor component (described in detail in Table 1). Assessment behaviors accounted for a greater proportion of all aggressive behaviors (Wilcoxon rank-sum test, $Z=-3.10$, $N=60$, $P=0.002$). Of all the assessment displays, parallel fin displays, in which the fish face each other with all fins erect, were the most common (median number of parallel fin displays/contest—2.0±0.5, Kruskal–Wallis test, $H=42.07$, $N=60$, $P<0.0001$).

Do contaminated fish contest a resource as aggressively as clean fish?

Clean pairs of fish initiated aggression sooner (Wilcoxon rank-sum test, $Z=2.12$, $N=20$, $P=0.03$; Fig. 2a) and performed fewer acts per bout ($Z=-2.49$, $N=19$, $P=0.01$; Fig. 2b) compared to pairs of fish from contaminated areas. However, overall, the numbers of aggressive bouts per contest and contest duration did not vary between clean and contaminated pairs of fish (bout number: $Z=1.51$, $N=19$, $P=0.13$; contest duration: $Z=-1.37$, $N=19$, $P=0.17$). Intensity of contest aggression did not differ between clean and contaminated pairs ($Z=0.81$, $N=40$, $P=0.42$).

Do contaminated fish contest resources in the same way as clean fish?

Contests between clean pairs began with assessment behaviors 70% of the time while contests between contaminated pairs began with assessment behaviors only 40% of the time, but this difference was not significant (Fisher's exact test, $N=20$, $P=0.37$). Over the entire course of the contest, contaminated fish performed far more assessment displays (Wilcoxon rank-sum test, $Z=2.49$, $N=40$, $P=0.01$; Fig. 2c) but not more pursuit aggression ($Z=0.71$, $N=40$, $P=0.47$; Fig. 2d).

Do contests between clean pairs and contaminated pairs progress in the same way?

The first bout of aggression was typically longest (4.5±1.5 acts) and was the only bout that reliably involved reciprocation of aggressive behaviors between the two fish. Subsequent bouts were mainly unidirectional and involved mainly pursuit aggression by one fish toward its opponent. During the first aggressive bout, contaminated pairs performed more overall aggression (pursuit and assessment combined: $\text{median number of acts} \pm \text{SE} = 8 \pm 2.2$) compared with clean pairs (2.5±1.3, Kruskal–Wallis test, $H=20.11$, $N_{\text{first bout}}=20$, $N_{\text{subsequent bouts}}=20$, $P=0.0002$; Fig. 3). However, in subsequent bouts, clean and contaminated fish performed similar numbers of aggressive behaviors (Fig. 3).

Do contaminated fish play by different contest rules?

Resource contests were considered to be resolved when one fish dominated the shelter and the winner status was assigned to the fish that monopolized the shelter. Contest resolution (and the formation of a dominance hierarchy) occurred more quickly in clean pairs and winner status appeared to be more stable over time in clean pairs. After the first focal watch, a winner could be assigned in 9/10 of the clean pairs and 8/10 of the contaminated pairs. In the second focal watch (3 h later), clear resource winners were obvious in 100% of clean trial pairs and 80% of contaminated trials. Winner status assignment remained 78% unchanged between focal watches in clean pairs, while only 25% of the contaminated winners in the first focal watch held on to the resource by the end of the second focal watch (Fisher's exact test, $N=20$, $P=0.04$). Winner fish from clean sites also tended to spend more time in the shelter ($\text{median time in shelter (s)} \pm \text{SE} = 161 \pm 113$) compared to winners from contaminated sites (26±116 s, Wilcoxon rank-sum test, $Z=-1.79$, $N=18$, $P=0.07$).

Do contaminants influence shelter monopolization?

No measures of shelter monopolization varied between clean and contaminated fish (time to first entry: $Z=-0.33$, $N=29$, $P=0.74$; time in shelter: $Z=-0.86$, $N=40$, $P=0.39$), although clean fish tended to make more shelter entries than contaminated fish ($Z=-1.84$, $N=40$, $P=0.06$).

Do clean fish win when placed in a resource contest against contaminated fish?

No, when paired against a contaminated fish, clean fish won only 3 out of the 10 contests (Fisher's exact test, $N=10$ contests, $P=0.18$). When clean fish were paired with a contaminated fish, clean fish initiated the aggression 70% of

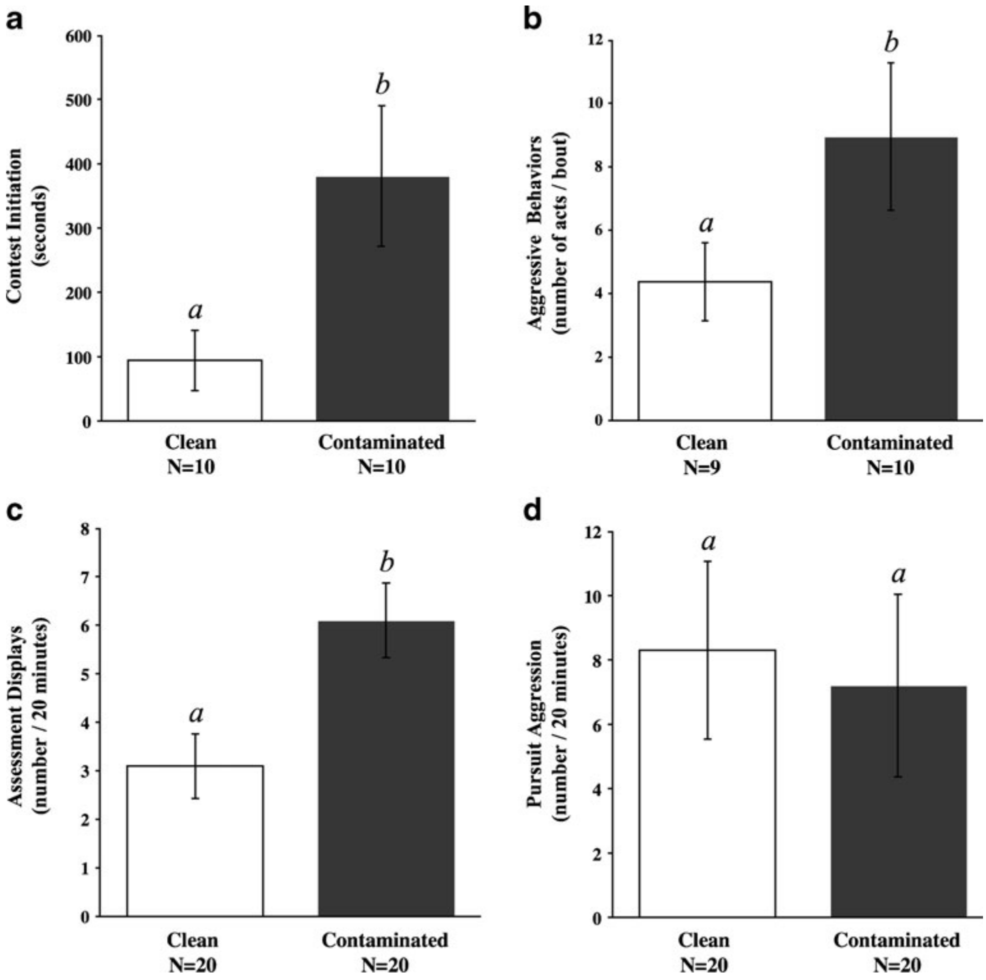


Fig. 2 **a** Time of contest initiation (mean ± SE, in seconds) for contests between clean pairs of fish (*open bar*) and contaminated pairs of fish (*filled bar*). **b** Mean number (±SE) of aggressive acts per bout for clean and contaminated fish. **c** Assessment displays (mean ± SE) displayed by clean and contaminated fish. **d** Pursuit aggression (mean ± SE) displayed by clean and contaminated fish. Letters indicate significant differences between groups ($P<0.05$)

time ($N=10$, $P=0.18$). In these mixed pairs, the clean fish did not show an earlier time of first shelter entry, spend overall more time in the shelter, or enter the shelter a greater number of times than their contaminated opponents (all Ps>0.37). Similarly, clean fish did not perform more pursuit aggression or assessment displays than their contaminated partners (all Ps>0.52). Mixed pairs performed assessment displays at a similar frequency to contaminated pairs and these assessment rates were significantly greater than those observed in clean pairs (Kruskal–Wallis test, $K=9.05$, $N=60$, $P=0.01$; Fig. 4a). No differences were observed in the frequency of pursuit aggression or the number of acts per bout across pairings (pursuit aggression: $H=0.86$, $N=60$, $P=0.65$; Fig. 4b; number of acts per bout: $H=2.73$, $N=29$, $P=0.10$).

Does gonad or liver investment differ between clean and contaminated fish?

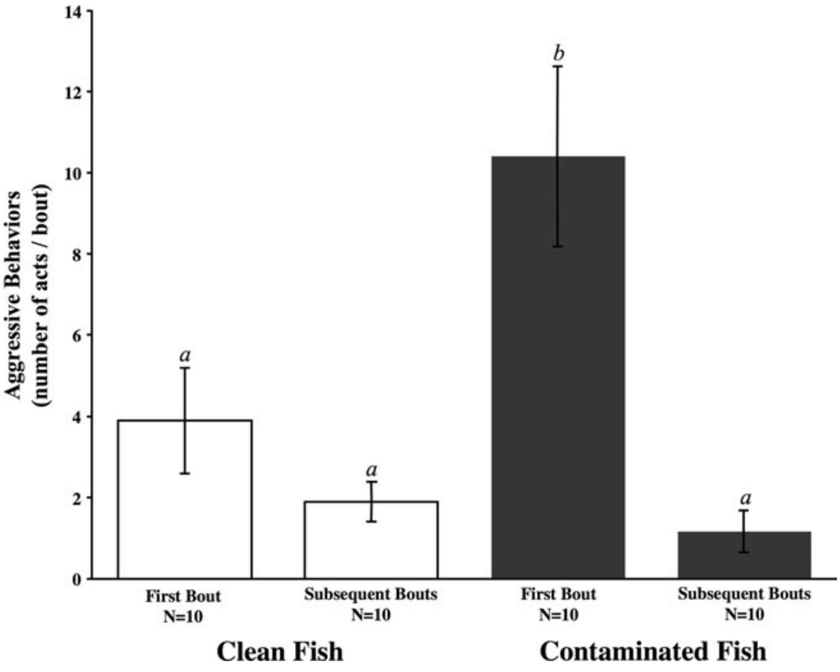
Fish from contaminated areas had lower GSI (Wilcoxon rank-sum test, $Z=-2.05$, $N=60$, $P=0.04$) and lower HSI ($Z=-2.30$, $N=60$, $P=0.02$) compared to fish collected from clean areas.

Discussion

Pairs of round gobies collected from areas with significant aquatic pollution took longer to initiate aggression, tended to have longer aggressive bouts, and performed more assessment behaviors than fish collected from clean areas. Contaminated fish performed more assessment behaviors and a stable hierarchy was formed in only 25% of trials compared to 78% of contests between clean fish. Despite these clear differences, when a clean and contaminated fish were paired together, clean fish did not appear to have an advantage, winning the resource (shelter) in only 30% of the trials.

Generally, three factors have the greatest influence on contest progression: (1) difference in contestant strength (sometimes called resource holding power), (2) differences between contestants in motivation to win or benefit from winning (called the subjective resource value), and (3) contestants' intrinsic aggressiveness (Hurd 2006). In our study, opponents were size-matched to minimize variation in resource holding potential, and both contestants were provided with a shelter prior to the contest to minimize

Fig. 3 Temporal comparison of assessment displays and pursuit aggression (summed mean ± SE) observed during the first aggressive bout versus subsequent bouts



differences in the value of the contested resource (shelter). The observation that fish from contaminated areas fared well in contests with fish from clean areas suggests that exposure to cocktails of contaminants does not affect the intrinsic aggressive tendencies of round gobies in Hamilton Harbour.

Contaminant-related impairment of cognitive systems could have resulted in the longer aggressive bouts and the increased performance of assessment behaviors observed in contaminated fish. Sloman and Wilson (2006) highlight studies where exposure to metal (lead, mercury) and organic contaminants (aromatic hydrocarbons) impairs spatial learning and the execution of conditioned avoidance responses. If sensory mechanisms are also disrupted by contaminants in round gobies, then this could potentially explain the increased time contaminated fish spent evaluating opponents. In fish, lateral line mechanoreceptors, located on the body surface, function to detect movements and size of prey or predators (Dijkgraaf 1962). Round gobies use lateral line sensors to perceive size differences between themselves and another fish (Jude et al. 1995; Stammler and Corkum 2005). Lateral line detection is impaired by cobalt and antibiotics and sensitized by 24-h exposure to the pesticide dichlorodiphenyltrichloroethane (DDT, 0.1–0.3 ppm, Blaxter and Ten Hallers-Tjabbes 1992). Copper, a metal found at high concentrations in the contaminated site's sediment, causes lateral line tissue damage and disorganization of neuromast cells in the lateral line sensors of zebrafish (*Danio rerio*, 50 μM exposure for up to 2 h, Hernández et al. 2006). Impaired function of these organs could lead to an inaccurate interpretation about an opponent's size or strength and necessitate the use of repetitive assessment displays. In addition to the lateral line, round gobies use their olfactory bulbs to identify the sex and status of a conspecific, by pheromone release (Gammon et al. 2005; Belanger et al. 2006, but see Marentette and Corkum 2008). In other fish species, cell damage and death in the olfactory system is induced by exposure to metals and can result in disrupted communication between olfactory tissues and the brain (Blaxter and Ten Hallers-Tjabbes 1992; Scott and Sloman 2004). Incorrect processing of pheromone signals may have also accounted for greater assessment in contaminated fish and the lack of stability in social hierarchy formation.

Differences in the number of acts per bout could be reflected by differences in metabolic rate due to contaminant burdens. Metabolic processes generate the energy required to execute behavior, but there is also a metabolic load of detoxifying contaminants. This load may impose an energetic constraint on the extent of behavioral activity a fish can perform (Weis et al. 2001a; Scott and Sloman 2004). In Japanese medaka (*Oryzias latipes*), compared to unexposed fish, schooling behaviors were impaired in fish subjected to a 24-h consumption of a 25-μg/g PCB-laced diet (Nakayama et al. 2005). The metabolism of PCBs and metals largely occurs in the liver (Sipes and Schnellmann 1987), an organ that also functions to breakdown stored glycogen stores and release glucose (Marshall and Hughes 1980). In this study, round gobies collected from contaminated sites had lower HSI. Liver size has been found to increase in rainbow trout after a 3-month laboratory exposure to 4 μg/l of cadmium (Lowe-Jinde and Niimi

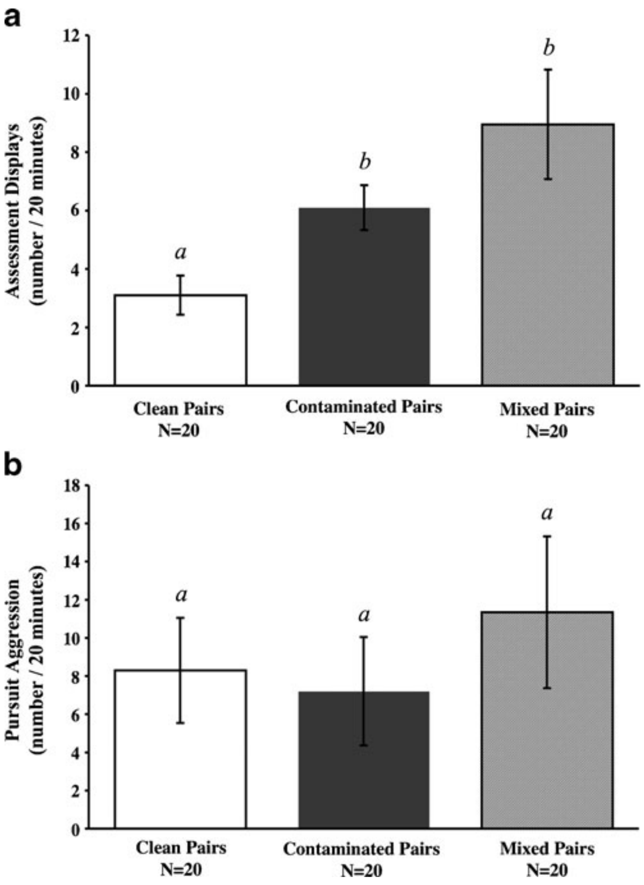


Fig. 4 **a** Comparison of assessment displays (mean ± SE) between clean pairs of fish, contaminated pairs of fish, and mixed pairs of fish (a clean fish contesting a contaminated fish). **b** Pursuit aggression (mean ± SE) between clean, contaminated, and mixed pairs of fish

1984). Brown et al. (2002) also detected increases in liver size after a 30-day exposure to diets laced with 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126, 126 ng/g). In contrast, in the freshwater tropical fish *Mystus nemurus*, juveniles exposed for 6 weeks to hydrogen sulfide (0.5 µg/l) demonstrated decreased liver size (Hoque et al. 1998). Relative to less contaminated sites, HSI was lowest in European eels (*Anguilla anguilla* L.) collected from the Meuse basin, a body of water with high concentrations of mercury, cadmium, zinc, nickel, arsenic, and chromium (Maes et al. 2005). The ecological consequences of contaminant exposure for round gobies remain somewhat unclear, and increased opponent assessment and longer aggressive bouts during resource contests may mean that individuals undergo increased energy expenditure. To further test this idea of contaminant burden influencing metabolism and energy allocation, future studies should quantify and contrast levels of enzymes associated with lipid, carbohydrate, protein, and lactate metabolism in clean and contaminated fish (e.g., Levesque et al. 2002; Rajotte and Couture 2002).

Contaminants may indeed have influenced round goby contests by altering their cognitive, perceptual, or metabolic capacities. Alternatively, the behavioral differences may have more to do with other ecological factors that differ between the sites. Aerial predator (over 2,000 double-crested cormorant *Phalacrocorax auritus*) nests are abundant near Pier 27 (Somers et al. 2007). Double-crested cormorants feed on round gobies (Somers et al. 2003), so it is possible that fish from that site in general are experienced with frequent predator attacks and learn to perform fewer chases and other locomotor movements that might attract the attention of avian predators (Martel and Dill 1995). If predation pressure is higher at the contaminated site, we would expect to catch fewer round gobies at the contaminated site than at the clean site, but catches per unit effort were equal (Marentette et al. 2010). We would also expect contaminated fish to spend more time in shelter than fish from the clean site, and yet they did not do so in this study. Areas near the contaminated site and clean site do not differ in biomasses of piscivorous fishes (Brousseau and Randall 2008), known predators of the round goby (Jude et al. 1995). Thus, fish from the contaminated site do not appear to be moving less in contests in order to avoid aerial or aquatic predators.

Behavioral differences in round gobies exposed to high levels of aquatic pollutants provide further evidence for toxicant-mediated changes in behavior. The prevalent use of low-intensity aggressive behaviors (assessment displays) may facilitate the tolerance of high-density populations (Holway et al. 1998; Tsutsui et al. 2000), particularly at contaminated sites. Coupled with physiological impairments that may also alter predator avoidance, prey capture, and dispersal, contaminated fish are likely to be preyed upon more frequently (Little et al. 1990; Weis et al. 2001a, b), quickening the rate at which contaminants transfer to organisms at higher tropic levels. There exists tremendous scope to investigate which physiological impairments drive the observed changes in behavior. This study's findings and current investigations on the impact of contaminant exposure on predator avoidance, foraging, and dispersal behaviors will broaden our understanding and awareness on the use of behaviors as effective biological indicators and monitoring tools for the detrimental effects of aquatic pollutants.

Acknowledgments We kindly thank C. Schiller for field assistance in 2008 and J. Wilson, S. Marsh-Rollo, and A. Reddon for helpful comments on the manuscript. This work was funded by a New Investigator and a New Opportunities award from the Canada Foundation for Innovation (CFI), Ontario Innovation Trust (OIT), Ontario Ministry of Research and Innovation, and by a Discovery and Research Tools Grant from the National Science and Engineering Council of Canada (NSERC) awarded to SB. NMS and JRM are funded by NSERC CGS awards.

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