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Behavior as biomarker? Laboratory versus field movement in round goby (*Neogobius melanostomus*) from highly contaminated habitats

Julie R. Marentette · Stephanie Tong · Grace Wang · Natalie M. Sopinka · Matthew D. Taves · Marten A. Koops · Sigal Balshine

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Abstract Changes in animal movement (frequency or speed of locomotion) following exposure to a toxicant are frequently considered a biomarker of contaminant exposure and are some of the most widely reported behavioral results in toxicological literature. However, the ecological consequences of such behavioral changes, such as effects on toxicant transfer in foodwebs, are far less well understood, complicated in part by the short-term nature of laboratory experiments and the lack of complementary field studies where the nature of toxicant exposure is more complex. Here we examine whether naturally exposed individuals of the round goby, a benthic, site-loyal fish, move in a manner similar to conspecifics from less contaminated habitats. In the laboratory, round goby from a relatively cleaner site showed greater activity and exploration than goby from two highly contaminated sites. Male fish were more active than females but the site effects were similar in both sexes. In contrast to laboratory findings, a field mark-recapture study of 881 round goby showed that fish from the cleaner site did not move greater distances or exhibit shorter residence times within the site than round goby from highly contaminated sites. Our results indicate that while behavioral changes in the laboratory may be one of several useful diagnostics of toxicant exposure of wild-exposed animals, they do not necessarily translate readily into measurable differences in a natural context. Thus, the potential fitness consequences of toxicant exposure based on behavioral changes need to be assessed carefully.

Keywords Locomotion · Activity level · Lake Ontario · Mark-recapture · Behavioral ecotoxicology

J. R. Marentette (✉) · S. Tong · G. Wang · N. M. Sopinka · M. D. Taves · S. Balshine
Department of Psychology, Neuroscience and Behavior, McMaster University, Hamilton, ON L8S 4K1, Canada
e-mail: marentjr@mcmaster.ca

M. A. Koops
Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, ON, Canada

Introduction

Movement is a fundamental component of many behaviors and the qualities of locomotion (frequency of movements, velocity, and diel or seasonal patterns of activity) can affect success in foraging, finding mates and avoiding predators (Dingle and Holyoak 2001). Increased movement can also increase encounter rates with or visibility to predators (Werner and Anholt 1993; Martel and Dill 1995). Higher levels of activity are often correlated with increased exploration, aggression and boldness (Sih et al. 2004) and these behaviors, measured in laboratory tests, are commonly used to predict long-term and large-scale movements, such as dispersal, in the field (Trinidad killifish, *Rivulus hartii*, Fraser et al. 2001; great tits, *Parus major*, Dingemanse et al. 2003; bullhead, *Cottus perifretus*, Kobler et al. 2009; reviewed in Cote et al. 2010).

A very wide range of contaminants are known to affect locomotion in animals, especially in fishes, and as a consequence changes in activity or movement quality are some of the most widely measured behavioral biomarkers of contaminant exposure (Little and Finger 1990; Bayley 2002). Exposed individuals may decrease activity (rainbow trout, *Oncorhynchus mykiss*, and aluminum; Allin and Wilson 1999, rainbow trout and copper; Campbell et al. 2002), increase activity (e.g., stickleback, *Gasterosteus aculeatus*, and EE_2; Bell 2004), induce a change in circadian activity patterns (carp, *Cyprinus carpio*, exposed to

PCBs and TBT; Schmidt et al. 2005), or affect the location of activity (goldfish, *Carassius auratus*, and carbofuran; Bretaud et al. 2002). Toxicants to which animals are exposed may have neurotoxic effects (e.g., methylmercury or many pesticides), producing cognitive or motor deficits that can reduce or increase movement (Brewer et al. 2001; Bretaud et al. 2002). High doses of contaminants can also increase the metabolic burden of the body (e.g., to repair toxicant damage), depleting energy reserves and reducing body condition (Campbell et al. 2002). Certain substances reduce the oxygen-carrying capacity of the blood, or the oxygen intake of the gills through structural or physiological damage (Allin and Wilson 1999; Schmidt et al. 2005). This can affect the amount of locomotion produced.

Despite the array of evidence that contaminants can affect behavior, the fitness effects of sublethal behavioral impacts remain unclear for wild populations (Heinz 1989; Peakall 1996; Peakall et al. 2002). In fact, studies of behavioral impacts on naturally exposed individuals, or tests for consequences of exposures in more natural settings are rarely performed (see Weis et al. 2001; Grue et al. 2002; Breckels and Neff 2010 and Candelmo et al. 2010 for notable examples). The results of acute laboratory exposures do not always capture the consequences of the chronic exposures common in the wild. For example, exposed animals that show initial behavioral impacts may recover to baseline activity (i.e., acclimate) over longer time periods (Schmidt et al. 2005). Laboratory studies also typically examine impacts of only one toxicant at a time, while exposure to multiple toxicants is the rule, not the exception, in the field.

Here we examine how long-term exposure to pollutants impacts movement in a population of round goby, *Neogobius melanostomus*, living in a highly contaminated Canadian harbour (Hamilton Harbour) in Lake Ontario, one of the Laurentian Great Lakes (Fig. 1). The round goby is a small fish invasive in North America (Jude et al. 1995), with a benthivorous diet comprising largely invasive dreissenid mussels, prey known to accumulate persistent toxicants (Jude 1997). It has become an important prey species for many piscivores in higher trophic levels and is known as a pollution-tolerant species (Pinchuk et al. 2003) with high site fidelity (Ray and Corkum 2001). For these reasons, the round goby has been identified as an important vector for contaminant mobilization in Great Lakes foodwebs (e.g., Kwon et al. 2006; Hogan et al. 2007; Jude et al. 2010). Any behavioral changes, following contaminant exposure, that impair survival could affect the rate of this mobilization locally (Marentette et al. 2010).

Round goby are thought to have invaded our study site, Hamilton Harbour, a 2,150 ha embayment on the western tip of Lake Ontario, over a decade ago (Young et al. 2010; Vélez-Espino et al. 2010). Hamilton Harbour is a Canadian

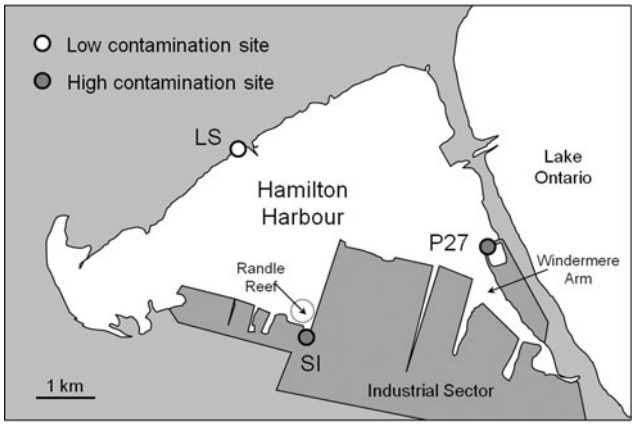

Fig. 1 Map of Hamilton Harbour with the round goby collection sites indicated as a site of low contamination (LaSalle Park, or LS; white circle) or one of high contamination (P27 and SI; *gray circle*). The site known as Pier 27 (P27) is located near a channel called Windermere Arm, a region known to be contaminated with PCBs and many metals; the site known as Sherman Inlet (SI) is located at Pier 15 near a coal tar dump rich in PAHs and metals known as Randle Reef (Hamilton Harbour RAP 2003; Zeman 2009)

Area of Concern designated by the International Joint Commission (International Joint Commission 1999) due to a long history of contamination and degradation by urban and industrial sources, primarily steel mills (Hamilton Harbour Remedial Action Plan (RAP) 1992; Murphy 2000). Contaminants known to be at problematic levels in the Harbour ("A list" contaminants) are polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and metals including arsenic, cadmium, lead, iron, mercury and zinc, and the Harbour is also the recipient of discharges from four urban wastewater treatment plants (Hamilton Harbour Remedial Action Plan (RAP) 2003). PAHs (Goncalves et al. 2008; Gravato and Guilhermino 2009), PCBs (Nakayama et al. 2005; Schmidt et al. 2005), mercury (Zhou and Weis 1998), cadmium (Honda et al. 2008), as well as complex combinations of contaminants (Triebskorn et al. 1997; Breckels and Neff 2010) can all affect locomotion.

We examined differences in movement between round goby collected from areas of known high contamination versus low contamination. Round goby collected from these areas have previously been shown to differ in contaminant biomarkers related to the level of relative contaminant exposure (see "Methods"). We investigated round goby activity level as they explored a novel environment in a laboratory behavioral assay. We also performed a mark-recapture study of round goby in the field, in areas of both high and low sediment contamination, to examine differences in residence times and distances moved between capture events. Based on the majority of studies examining contaminant effects on locomotion (Little and Finger 1990; Bayley 2002), we predicted that

round goby from highly contaminated areas would show reduced activity, and thus also have lowered exploration relative to fish from cleaner areas. We also predicted that activity level in the laboratory would correlate positively with home range size and dispersal capacity in the field (Cote et al. 2010). Specifically, we predicted that round goby in highly contaminated areas would move less than round goby in less contaminated areas.

Methods

Study areas and collection of fish

Fish used for both laboratory and field studies came from one area of low contamination, LaSalle Park (LS hereafter) and two areas of very high contamination, Pier 27 (P27) and the mouth of Sherman Inlet in Pier 15 (SI), all within Hamilton Harbour (Fig. 1). Regions of high and low contamination were based on sediment distribution patterns of multiple contaminants (Hamilton Harbour Remedial Action Plan (RAP) 1992; Hamilton Harbour Remedial Action Plan (RAP) 2003; Zeman 2009). Compared to round goby from areas of low contamination, round goby collected from highly contaminated areas were smaller, had a higher frequency of intersex and vitellogenin production in males (indicating endocrine disruption), higher body burdens of copper and cadmium, greater hepatic EROD activity indicating exposure to AhR-binding contaminants such as PAHs, and altered aggressive behavior (Bowley et al. 2010; Marentette et al. 2010; Sopinka et al. 2010). Sites were similar in water parameters such as turbidity, oxygen concentration and temperature (Marentette et al. 2010), and all three sites have substrates comprising a sand, cobble and boulder mix. Rugosity, or habitat complexity, was measured using a chain method (Saleh 1993); a 3 m (L1) chain was laid out over four different trap locations at each site at 1 m depth, and the resulting horizontal chain length measured (L2), to produce a dimensionless rugosity measure of L1/L2. One high contamination site, SI, had higher rugosity (mean 2.67) than either LS or P27 (means both 1.86; $F_{2,9} = 13.9$, $P = 0.002$).

Fish were collected using baited minnow traps set for 24 h, up to 7 m from shore in <1 m of water, and transported back to the laboratory within 4 h of capture. Once in the laboratory, fish were housed by site and sex in 60 L aquaria filled with dechlorinated tap water, equipped with an external box filter, two airstones, 2 cm of aquarium gravel, and 15-cm-long sections of PVC piping as shelter, and allowed to acclimate for 2–7 days. Fish were fed once daily ad libitum with Nutrafin Staple fish flakes, except on the day of testing.

Laboratory behavioral assay

Round goby ($N = 198$) were collected between 16 May and 25 July 2008 from three locations described above (LS, P27 and SI) and housed in sex- and site-specific 60 L holding tanks (Marentette et al. 2011). Fish were held under a shifted 16L:8D light cycle, with the dark phase between 1200 and 2000 h, to facilitate behavioral observations performed in the nocturnal phase under red light (when round goby are most active; Dubs and Corkum 1996; Diana et al. 2006). Fish were divided into 66 groups of three fish, or 11 groups for each sex and site combination. We tested the fish in triads because a pilot study indicated that round goby were more active in triads than when tested alone (mean increase of 2.0 non-social movements/min, 95% CI of 0.8—3.1 movements/min, $N = 81$ fish).

Fish exploration and activity were measured in a large, segmented arena (2.5 m long × 0.75 m wide, divided lengthwise into five chambers 0.50 m long, 0.75 m wide, 0.15 m deep; Fig. 2a). The chambers were separated by removable acrylic dividers, each with a doorway (25 cm long, 15 cm high) in the middle. All five chambers were equipped with three acrylic shelters, white sand 1 cm deep and one external box filter. Fish of the same sex and site were placed in one end chamber of the arena in triads, but fish in the triad were not matched in size to facilitate fish identification by the observer. Each group was given a unique ID. The observer was blind to the sex and collection site of the fish. Consecutively tested groups were started from alternating ends of the arena, and water was thoroughly mixed between trials to eliminate odor gradients or cues from previous groups. Water within the experimental apparatus was changed once daily.

Each group was allowed to acclimate for 30 min in the first chamber, with the entrance to the second chamber blocked by a removable divider. An observer was positioned behind a blind 1 m away from the testing arena. During the last 15 min of the acclimation period, the observer recorded all behaviors exhibited by each fish for 5 min for 60 of 66 groups, 10 of each sex and site combination. The order of fish observation (by size rank) was randomized for each group. Behaviors were counted and grouped by function: horizontal locomotion, exploration, and substrate-oriented behaviors and expressed as a rate per minute (Table 1). Social behaviors such as bites and chases were enumerated separately from these non-social movements. Following the acclimation period, the observer removed the divider blocking the entrance to the second chamber, and recorded all entries to chambers made by each fish during the 30 min test period, for all 66 groups. The time to begin exploration was defined as the time elapsed (in seconds) until the fish exited the start chamber.

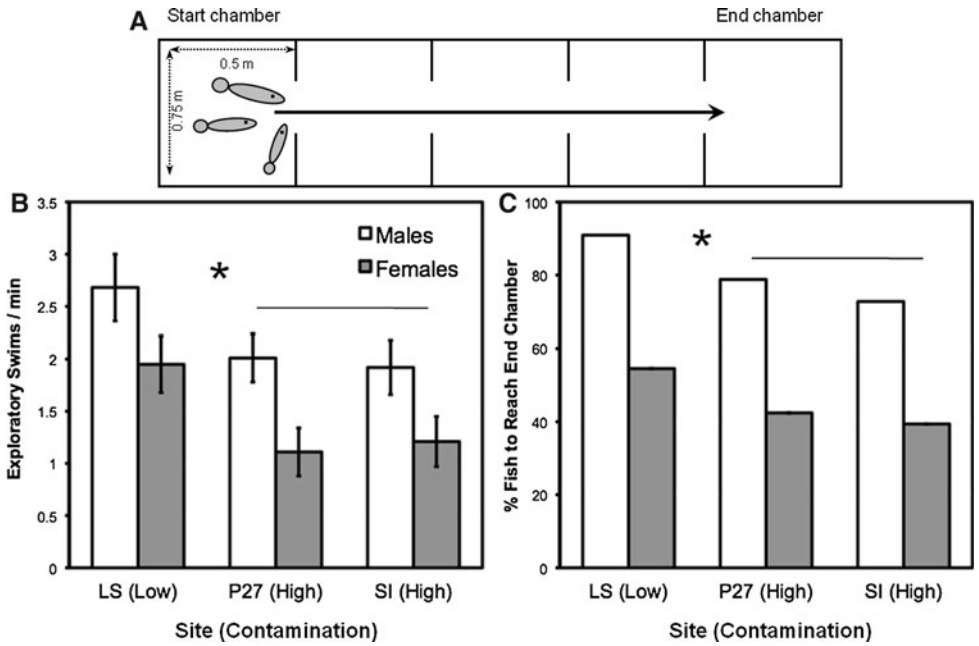

Fig. 2 **a** Top view of chambered arena used to test round goby for activity and exploration. Fish were introduced to either end of the arena, which became the Start chamber. **b** The mean ± SE rate of exploratory swims/min exhibited by males (*white bars*) and females (*gray bars*) from areas of low and high contamination. **c** More fish from low contamination sites reached the last chamber of the arena, versus fish from high contamination sites. More males (*white bars*) than females (*gray bars*) reached the last chamber as well. Linear contrasts revealed significant differences between fish from the low contamination site LS, and the two high contamination sites P27 and SI ($P < 0.05$), indicated with an asterisk (*). A *line* over the *bars* of P27 and SI indicates there were no significant differences between these sites ($P > 0.05$)

Table 1 Categories of movement classification for round goby laboratory behavioral assays

Category	Behavior	Description
Horizontal locomotion	Hop	Fish movement of ≤1 body length
	Swim	Sustained horizontal movements in water column of >1 body length
	Dart	Rapid swim of >1 body length
Exploration	Swim	Sustained, repeated, frequently vertical movements in water column with mouth oriented at perimeter of aquarium
Social interactions	Bite or chase	Fish rapidly approaches another, with (Bite) or without (Chase) opening and closing its mouth on the body of the other
	Bitten, chased or displaced	Reciprocal of above. A fish rapidly departs from the approach of another. In the case of displacement, the approaching individual moves slowly and does not appear to initiate a Bite or a Chase

Each fish was assigned a number of chamber switches, where fish that never left the start chamber were assigned a 0. Fish were also classed as to whether they moved through all five chambers during the test phase (yes or no). At the end of the test period, all tested fish were euthanized with an overdose of Benzocaine and their body size (total length, to 0.1 mm, and total mass, to 0.001 g) measured during dissection. Body condition was evaluated as Fulton's condition factor K (total mass:total length^3 × 100). The presence of eroded or damaged ventral fins, found most frequently in round goby from highly contaminated sites (see Marentette et al. 2010), was scored as present or absent.

Field mark-recapture study

Adult ($N = 867$) and juvenile ($N = 14$) round goby were tagged and released in five cohorts at the low contamination site LS and the high contamination sites P27 and SI between May 5 and August 21 2009 (Marentette et al. 2011). Six traps were set at each site along the shore at 6 m intervals. All fish collected in these traps between (a) May 5–8 ($N = 65$ fish), (b) June 2 and 5 ($N = 148$), (c) June 23–25 ($N = 208$), (d) July 22–24 ($N = 142$), and (e) August 18–21 ($N = 319$), were sexed and measured to obtain total length and total mass. Fish were then given four markings in any of 12 body locations (495 unique codes for each of two

colors, orange and green) along the dorsolateral aspect of the body with a subcutaneous injection of VIE (Visible Implant Elastomer, Northwest Marine Technologies, Inc.) and allowed 5–15 min to recover before being released at the location of capture.

From May 5 to November 6 2009, weekly sampling was performed for recaptures. Sites were also sampled every other week from May 5 to November 3 2010. When VIE-tagged individuals were recaptured, they were identified, re-measured for length and mass, and the distance they had moved and the number of days elapsed since the previous capture was calculated. Occasionally tagged fish were recovered in traps not part of the mark-recapture study. Distances moved by these fish were calculated by measuring the distance along the shoreline to the trap from which the fish was last caught. At the end of the study, each recaptured fish was assigned a maximum distance moved and a maximum known residence time (the days elapsed between first and last capture). From these, fish were assigned a travelling rate in m/week, which was calculated as the maximum distance moved by the fish divided by the number of weeks, with weekly units calculated as residence time in days/7.

Statistical analyses

Data were normalized by log or arcsine square root transformations when possible; otherwise, rank-based statistical tests were used. Spearman's rho non-parametric correlations were used to determine relationships between continuous variables. Binary data (yes/no classifications, such as the proportion of fish to move through all five chambers) were examined with nominal logistic regression where possible, followed by likelihood ratio tests to establish differences among sites or sexes; otherwise comparisons were performed with Chi-square tests. Where individual fish were tested as part of a triad, linear mixed models (using the residual maximum likelihood method) were used to examine behavioral data using Sex and Site as main factors and Group ID specified as a random effect, nested within Site and Sex. Sex-Site interaction terms were not significant ($P > 0.1$) and were removed from models. Covariates (log-transformed total length) were incorporated into the models but removed when they did not turn out to be significant. The number of days spent in the laboratory did not correlate with any behavioral data and were not incorporated in models. Orthogonal linear contrasts were used to calculate post hoc significant differences among sites: low contamination (LS) versus high contamination (P27 and SI), and also to detect any differences between P27 and SI. All data analysis was performed using the program JMP 9 (SAS, 2010). Effect sizes were calculated as Cohen's d (δ/σ, where $\delta = \sqrt{}$ (sum of squares/N)) with $\alpha = 0.05$. Power and sample size estimates for comparing two proportions were calculated against a null hypothesis of H_0: $p_1 = p_2$ and $\alpha = 0.05$.

Results

Laboratory behavioral assay

Acclimation phase

Fish from the low contamination site (LS) showed more horizontal locomotion and more exploratory swimming (Fig. 2b) than fish from the two high contamination sites (P27 and SI; Table 2). Irrespective of site, males showed greater exploration than females (Fig. 2b), but sexes showed similar rates of horizontal locomotion (Table 2).

Test phase

Fish from the low contamination site LS began to explore sooner than fish the high contamination sites P27 and SI, and males begin exploring earlier than females (Table 2). More fish from the low contamination site than the highly contaminated sites reached all five chambers (fish from P27 and SI pooled), as did more males than females (Fig. 2c; Table 2). Over the entire test period, fish from the low contamination site LS made a greater number of chamber switches than fish from either contaminated site, and males made more switches than females (Table 2). Across all fish, a higher rate of exploration in the acclimation phase correlated with a quicker start to begin exploration ($r_s = -0.22$, $P = 0.01$, $N = 44$), a greater number of chamber switches ($r_s = 0.45$, $P < 0.0001$, $N = 60$) and farther chambers reached (i.e., distances travelled; $r_s = 0.51$, $P < 0.0001$, $N = 60$) during the test phase.

Physical differences among sites

Fish from the low contamination site LS were larger than fish from the two highly contaminated sites, P27 and SI, and male fish were larger than females (Table 2). These size differences are similar to findings from the same sites in previous years (Marentette et al. 2010). Fish total length was a significant covariate in models of horizontal locomotion (i.e., small fish move more), time to start exploration, and the number of chamber switches, but not exploratory swimming (Table 2). Fish total length also did not correlate with the furthest chamber distance reached ($r_s = 0.10$, $P = 0.16$, $N = 180$).

Body condition as measured by Fulton's K did not vary with fish collection site or sex. Eroded ventral fins were only found on fish from highly contaminated sites (observed in

Table 2 Summary of statistical results (general linear models and logistic regression) for the laboratory behavioral assay

Behavior	Effect of sex		Effect of site		Effect of TL
Acclimation phase					
Horizontal locomotion	$F_{1,56} = 1.27$, $P = 0.27$	NS	$F_{2,56} = 3.67$, $P = 0.03$	LS > P27, SI $F_{1,56} = 7.26$, $P = 0.01$	$F_{1,119} = 28.9$, $P < 0.0001$
Exploration	$F_{1,56} = 6.51$, $P = 0.01$	M > F	$F_{2,56} = 3.09$, $P = 0.05$	LS > P27, SI $F_{1,56} = 6.15$, $P = 0.02$	$F_{1,119} = 0.01$, $P = 0.93$
Test phase					
Time to start exploring	$F_{1,57} = 7.98$, $P = 0.007$	M < F	$F_{2,57} = 3.14$, $P = 0.05$	LS < P27, SI $F_{1,57} = 5.59$, $P = 0.02$	$F_{1,84} = 14.5$, $P = 0.0003$
Moved to all five chambers	$\chi^2_1 = 25.5$, $P < 0.0001$	M > F	$\chi^2_1 = 4.2$, $P = 0.04$*	Low > high*	$\chi^2_1 = 0.01$, $P = 0.91$
# chamber switches	$F_{1,62} = 15.88$, $P = 0.0002$	M > F	$F_{2,62} = 3.31$, $P = 0.04$	LS > P27, SI $F_{1,62} = 6.58$, $P = 0.01$	$F_{1,131} = 6.21$, $P = 0.01$
Physical differences					
Total length	$F_{1,194} = 18.1$, $P < 0.0001$	M > F	$F_{2,194} = 7.87$, $P = 0.0005$	LS < P27, SI $F_{1,194} = 15.4$, $P = 0.0001$	
Fulton's K	$F_{1,194} = 0.13$, $P = 0.72$	NS	$F_{2,194} = 0.76$, $P = 0.47$	NS	

* Both SI and P27 fish pooled

NS not significant, *TL* total length, *M* males, *F* females, *LS* low contamination site; P27 and SI = high contamination sites

$N = 3$, or 5% of fish from P27; $N = 25$, or 38% of fish from SI; ($\chi^2_2 = 49.4$, $P < 0.0001$). SI fish with eroded fins showed significantly less horizontal movement than SI fish with normal fins ($F_{1,58} = 4.5$, $P = 0.037$), but similar levels of exploratory swimming ($F_{1,58} = 0.4$, $P = 0.85$) in the acclimation phase. In the test phase, however, SI fish with eroded fins started exploring at similar times ($F_{1,46} = 0.01$, $P = 0.94$), were just as likely to move through all five chambers ($\chi^2_1 = 2.4$, $P = 0.12$), and in fact tended to make more chamber switches than SI fish with normal fins in the test phase ($F_{1,58} = 3.7$, $P = 0.06$).

Field mark-recapture study

Of the 881 round goby tagged in 2009, 167 or 19% were recaptured the same year. This represents 21.1% of fish at LS ($N = 66$ of 311), 14.5% of fish at P27 ($N = 41$ of 283) and 20.9% of fish at SI ($N = 60$ of 227). This recapture percentage did not differ across sites ($\chi^2_2 = 4.6$, $P = 0.10$) or between sexes ($\chi^2_1 = 0.1$, $P = 0.80$), and to simplify analyses, data from both P27 and SI were pooled into one high-contamination dataset. Most recaptured fish were only recaptured once (113; 67.7%) although some fish were recaptured up to six times ($N = 3$). Across all sites, the earlier fish were tagged in the study, the longer their known residence times were ($r_s = -0.29$, $P = 0.0001$, $N = 167$) and the further they travelled ($r_s = -0.29$, $P = 0.0002$); travelling rates in m/week, however, were not related to date of tagging ($r_s = -0.11$, $P = 0.16$).

Recaptured fish in 2009 were seen anywhere from 1 to 168 days after their initial capture (Table 3). Most recaptured fish (112; 67.1%) were only recaptured at the trap of their original capture, and were therefore assigned a movement distance of 0 m. The proportion of fish that did move between traps (designated as movers) was similar between sexes and sites (Table 3). When considering only movers, there were no differences in distances travelled per week between sites or sexes (Table 3).

Thirteen tagged round goby were recaptured in 2010, the second year of the study. There were no differences in the proportion of movers (2009–2010) between highly and less contaminated sites, or between males and females (Table 3). There were also no site differences in distances travelled per week along the nearshore (Table 3). Males ($N = 9$), however, travelled further than females ($N = 4$; Table 3). After restricting the data only to males, there were no differences in distances travelled per week between fish from high ($N = 3$) and low contamination sites ($N = 6$; Table 3).

Physical differences among sites

As found for fish used in the laboratory experiment described above, and field data from other years

Table 3 Results of the field mark-recapture study

Time	Measurement	Sex		Site contamination	
		Males	Females	Low	High*
2009 (all fish)	*N*	106	61	66	101
	P(movers)	**0.33**	**0.33**	**0.27**	**0.36**
		$\chi^2 = 0.001$, $P = 0.98$		**$\chi^2 = 1.6$, $P = 0.21$**	
2009 (movers)	*N*	35	20	18	37
	Distance (m)	6 (3–18)	6 (3–18)	6 (3–18)	6 (3–12)
	Days elapsed	43 (1–134)	66 (1–168)	53.5 (1–168)	50 (1–134)
	Rate (m/week)	**1.0 (0.15–12)**	**0.60 (0.17–6)**	**0.76 (0.33–6)**	**0.67 (0.15–12)**
		$Z = 1.2$, $P = 0.22$		**$Z = 0.4$, $P = 0.68$**	
2010	*N*	9	4	9	4
	P(movers)	**0.89**	**0.5**	**0.78**	**0.75**
		$\chi^2 = 2.4$, $P = 0.12$		**$\chi^2 = 0.01$, $P = 0.91$**	
	Distance (m)	6 (0–12)	1.5 (0–6)	6 (0–12)	6 (0–12)
	Days	290 (233–386)	322 (282–386)	293 (271–386)	313.5 (233–386)
	Rate (m/week)	**0.15 (0–0.35)**	**0.04 (0–0.11)**	**0.15 (0–0.29)**	**0.13 (0–0.35)**
		$Z = 2.3$, $P = 0.02$		**$Z = 0.1$, $P = 0.94$**	
				$Z_{males} = 0.4$, $P = 0.70$	

Number of recaptures (*N*), proportion of fish that moved between traps, and median (range) values for the absolute distances moved, in meters (m), the number of days elapsed between first and last captures, and distance moved per week, categorized by round goby sex, collection site, and year of study. Tests for bolded statistics are reported as Wilcoxon rank-sum tests (*Z*) or Chi-square comparisons (χ^2). * Data for P27 and SI fish pooled. LS = low contamination site, P27 and SI = high contamination sites. Mover = a fish that moved traps between first and last observations (distance >0 m)

* Data for P27 and SI fish pooled

(Marentette et al. 2010), round goby from the low contamination site LS were larger than those from the high contamination sites P27 or SI, and males were larger than females (site $F_{2,863} = 46.0$, $P < 0.0001$, sex $F_{1,863} = 32.4$, $P < 0.0001$). The total length of the fish when first caught and tagged did not correlate with the absolute distance moved, in m ($r_s = 0.06$, $P = 0.40$) or with the travelling rate, m/week ($r_s = 0.05$, $P = 0.5$).

Power and effect size of sex and site contamination

The amount of exploration exhibited in the acclimation phase of the laboratory experiment predicted how far fish travelled in the test phase. The effect sizes of fish sex ($F_{1,57} = 6.62$, $P = 0.01$; Cohen's $d = 0.165$) and site contamination (with P27 and SI fish pooled; $F_{1,57} = 6.25$, $P = 0.02$; Cohen's $d = 0.159$) on exploration behavior were similar. However, when we investigated the proportion of fish to reach all five chambers (Table 2), the ability to distinguish the sexes ($N = 198$; power of 0.999) was stronger than for fish from sites of high and low contamination (power 0.525; Fig. 3). This laboratory pattern was

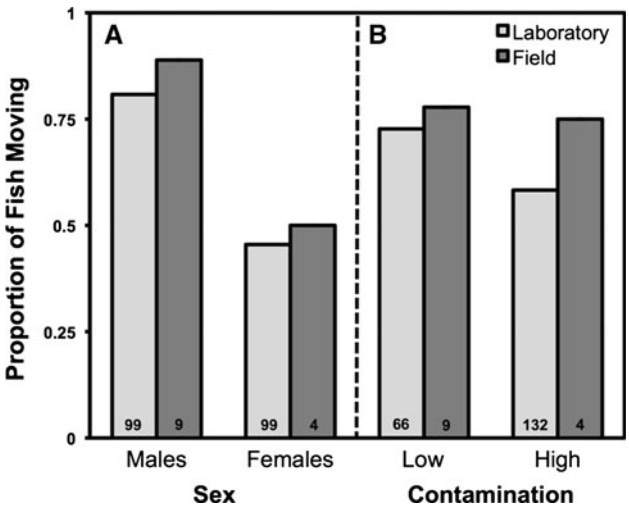

Fig. 3 A comparison of the proportion of fish moving in both the laboratory assay (*light grey*: defined as the proportion of fish moving through all five chambers of the novel environment) and the field mark-recapture study (*black*: defined as the proportion of fish moving between traps between the last sighting in 2009 and recapture in 2010). Numbers at the base of bars indicate the sample size. **a** A comparison by fish sex. **b** A comparison by site contamination level, either low (site LS) or high (P27 and SI)

closely paralleled by the proportion of fish moving between traps from 2009 to 2010 in the field mark-recapture study ($N = 13$, Fig. 3). With so few fish recovered, however, power was greatly reduced for detecting differences in fish propensity to move across years for both sex (power of 0.247) and habitat contamination (0.022).

Discussion

A change in activity has been frequently observed in animals exposed in the laboratory to a wide variety of contaminants. Round goby from two highly contaminated sites show less exploration and a reduced tendency to move in the laboratory compared to fish from a lower contamination site; however we did not observe site differences in field movement. Sex also played an important role: male round goby were more explorative and had a greater propensity to move than females, and also moved further in the field between years than did females. Greater male movement (exploration, home range size and between-year dispersal) is expected in this polygamous species, where males are also larger and grow faster than females, and may therefore precede females in invasion fronts (Marentette et al. 2011). Site effects were consistent within each sex.

Exploration of a novel environment and boldness in laboratory tests have been used successfully to predict movement and dispersal patterns in the field in many taxa (reviewed by Cote et al. 2010), despite the fact these occur over very different spatiotemporal scales and represent fundamentally different behaviors. Why did we not observe the reduction in travelling rate and the proportion of movers in the field that would correspond with the results of our laboratory assay of movement? It may be possible that behavioral differences in fish from clean and contaminated habitats only manifest in novel, stressful or changing environments, potentially including contaminant levels to which fish are acclimated (e.g., Breckels and Neff 2010). Another reason may be the very low numbers of fish recaptured in the field by the second year of the study (Fig. 3), which greatly reduced power. Yet another reason may be that our field study may have needed finer-scale spatial sampling in recaptures to reveal differences in movement, particularly given the high degree of site loyalty over time in round goby. The use of PIT tags or other similar technology, in conjunction with detection arrays, would allow more frequent sampling of individual positions and may resolve issues of both sample size and spatial resolution (e.g., Cookingham and Ruetz 2008). This resolution might be further strengthened if the same individuals could be observed in both the laboratory and the field (Kobler et al. 2009).

Why might contaminant exposure reduce fish activity? Round goby collected from contaminated areas often have demonstrated ventral fin damage or erosion (Marentette et al. 2010; this study), but fish with eroded fins did not explore or disperse differently in a novel environment than fish with normal fins. Round goby from sites of low and high contamination were in similar body condition; condition reflects body composition and energetic levels (Kaufman et al. 2007) and can be used as a contaminant biomarker (Schlenk et al. 2008). Other physiological mechanisms, such as neurotoxic effects, chronic stress, or endocrine disruption may have been the proximate cause of the reduced activity here observed here, but these factors have yet to be explored in detail in this system.

A major benefit of working with field-exposed individuals is that the animals have been exposed at realistic levels, routes of entry and temporal scales not easily replicated in the laboratory. Causal links between contaminant exposure and behavioral differences in our work are suggested by our data, but other habitat variables might contribute as well. Although all three sites were similar in water parameters such as pH, turbidity, oxygen and temperature (Marentette et al. 2010), one site (the highly contaminated site SI) was more rugose, or rocky, than the others. Attributing behavioral differences to habitat complexity would not, however, explain why fish from SI had similar levels of activity to fish from P27 (both contaminated sites), and lower levels of activity than the low contamination site LS. Round goby used in these studies were smaller, and thus possibly younger, at sites of high contamination than low contamination; however body size was rarely correlated to movement measured in the laboratory (when small fish actually move more) and did not correlate with movement in the field. The three sites may be subject to different predation regimes. Higher levels of predation risk may favour shy, less active individuals over bolder ones (Huntingford 1982; but see Brown et al. 2005). Differences in predation rates among sites are unfortunately not clear. The high contamination site P27 is located near a colony of double-crested cormorants (*Phalacrocorax auritus*) and other piscivorous birds; however, cormorants often forage many km from their nests, affecting sites throughout the harbour (Stapanian et al. 2002). In contrast, piscivorous fish are more prevalent within one km of the low contamination site LS than near the high contamination site P27; data on predator fish distributions near the high contamination site SI are not yet available (Brousseau and Randall 2008).

Much is known about the impacts of various classes of contaminants on animal behavior in the laboratory (Sloman and Wilson 2006), and behavior is frequently evaluated as one of a suite of biomarkers of exposure in controlled experiments. In these contexts, behavior is often posited as a potentially useful diagnostic tool for predicting impacts of contaminant exposures on populations, because behavior

conveniently integrates the whole-animal effects of multiple physiological processes, in ways that directly affect animal survival and reproduction. The challenge to this proposition is that considerably less is known about the ecological impact that contaminant-mediated behavioral changes have in natural settings (Grue et al. 2002). Similarly, behavior is a rarely explored biomarker of field exposures, where verification of biomarker predictions generated from laboratory studies must be made (Peakall 1996). Both of these areas are necessary research routes to pursue in the development of behavior as an ecotoxicological tool. In this paper, we were able to show that round goby collected from populations known to exhibit signs of contaminant exposure, also show differences in behavior that are not easily related to any other site-specific cause like habitat complexity or predation. Thus, we propose that activity level in the round goby, and other species, may be a useful biomarker of exposure to complex contaminant mixtures in the field in conjunction with other physiological biomarkers. We were not able to show, however, detectable movement differences across sites in the field. The absence of differences in field movement should not be interpreted to mean that there are no ecologically significant consequences for these fish in their natural habitat. It must be recognized that the pious hopes (Grue et al. 2002), or claims that all toxicant-induced behavioral changes are ecologically meaningful found so frequently in the toxicology literature, may not always be supported. The utility of behavioral metrics chosen in both laboratory and field, such as activity level, must be carefully evaluated. A more cautious inference is that establishing connections between contaminant exposure, laboratory observations, and population-level consequences is not a simple matter. These connections need to be pursued more vigorously in future endeavors if more accurate predictions of population-level consequences are to be generated.

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