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Aquatic Ecosystem Health & Management

ISSN: 1463-4988 (Print) 1539-4077 (Online) Journal homepage: http://www.tandfonline.com/loi/uaem20

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To cite this article: Erin S. McCallum, Julie R. Marentette, Claire Schiller, Shagun Jindal, Kyle Empringham, Susan Marsh-Rollo, Harri Pettitt-Wade, Marten A. Koops, Aaron T. Fisk & Sigal Balshine (2017) Diet and foraging of Round Goby (Neogobius melanostomus) in a contaminated harbour, Aquatic Ecosystem Health & Management, 20:3, 252-264, DOI: 10.1080/14634988.2016.1254468

To link to this article: http://dx.doi.org/10.1080/14634988.2016.1254468

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Diet and foraging of Round Goby (*Neogobius melanostomus*) in a contaminated harbour

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*Anthropogenic pollution and the introduction of invasive species are two contributing factors to ecosystem degradation. Although Hamilton Harbour (Ontario, Canada), a highly impacted ecosystem, is well-studied, the diet, trophic position, and foraging behaviour of the invasive Round Goby (*Neogobius melanostomus*) in this area is not well understood. In this study, we compared digestive tract contents, foraging behaviour, and stable isotope values of Round Goby from sites of low and high sediment contamination in Hamilton Harbour. We also assessed prey availability by conducting sediment invertebrate abundance analyses at these sites. Regardless of site, Chironomids, Cladocerans, Copepods and Dreissenids were the most common food items found in Round Goby digestive tracts, and females always had heavier gut contents compared to males. Fish from the high contamination site consumed fewer prey items, had lower gut fullness scores, and fed at a lower trophic level based on lower $\delta^{13}C$ and $\delta^{15}N$ values. Our results suggest that Round Goby living in highly contaminated areas are feeding less than Round Goby from areas of lower contamination, but that these diet differences do not reflect differences in prey availability. Fish from the high contamination site also typically moved more slowly while foraging. Taken together, these results provide an analysis of the main prey items of Round Goby in Hamilton Harbour, and demonstrate how polluted environments can impact diet, trophic position, and foraging of an introduced fish species.*

Keywords: stable isotopes, invasive species, trophic position, remediation, Area of Concern, Hamilton Harbour

Introduction

Freshwater habitat degradation is often caused by human activities such as pollution or invasive species introductions (Strayer and Dudgeon, 2010). Hamilton Harbour (Ontario, Canada)–the western-most embayment of Lake Ontario–is a highly impacted ecosystem and an

Aquatic Ecosystem Health & Management, 20(3):252–264, 2017. Copyright © 2017 Crown Copyright. ISSN: 1463-4988 print / 1539-4077 online
DOI: 10.1080/14634988.2016.1254468

International Joint Commission Area of Concern that has been undergoing remediation for the past 30 years (Hamilton Harbour Remedial Action Plan, 1992; IJC, 1999). Remediation efforts in the Harbour have resulted in significant social, economic, and environmental improvements (Hall et al., 2006). One important ongoing remediation goal is the restoration of fish and wildlife populations. Urban runoff, wastewater effluent discharge and combined sewer overflows, as well as historical inputs from industrial steel processing, have resulted in habitat degradation and decline in fish populations in Hamilton Harbour (Poulton, 1987; Hamilton Harbour Remedial Action Plan, 1992; Curran et al., 2000). Many fish species in the Harbour have been observed with morphological abnormalities, and fish consumption advisories have been issued for 21 different species due to high concentrations of PCBs, mercury, and pesticides in fish tissues (Hamilton Harbour Remedial Action Plan, 1992; MOECC, 2015). Increased nutrient inputs have made the Harbour eutrophic, which along with water quality fluctuations are thought to contribute to fish population declines (Minns et al., 1994; Hiriart-Baer et al., 2009). Hamilton Harbour's invertebrate community has similarly been degraded by pollution and poor water quality (Dermott and Bonnell, 2010). Both fish and invertebrates have begun to recover with remediation, but still do not meet delisting goals (Dermott and Bonnell, 2010; Brousseau and Randall, 2008). In addition, fish and invertebrate populations have been challenged by repeated introductions of invasive species, such as the Common Carp (*Cyprinus carpio*), and Zebra and Quagga Mussels (*Dreissena polymorpha* and *D. rostriformis bugensis*; Holeck et al., 2004). Invasive species introductions are of special concern when an ecosystem is unstable, as they impose an extra stressor for native species already experiencing poor conditions (Strayer, 2010).

The Round Goby (*Neogobius melanostomus*) is an invasive species that poses a challenge to Hamilton Harbour ecosystem health and remediation. Originating in the Ponto-Caspian area of Europe, Round Goby are a benthic fish that were introduced to the Laurentian Great Lakes via ship ballast discharge (Jude et al., 1992). Round Goby are extremely successful invaders; they spread quickly throughout all five Great Lakes and continue to invade the surrounding streams and tributaries (Poos et al., 2010). Deterministic back-calculations show that Round Goby likely arrived in Hamilton Harbour in 1994–1995 and had reached establishment densities by 1998–1999 (Vélez-Espino et al., 2010). They were first observed in the Harbour in 1999 (Balshine et al., 2005). Round Goby have had negative impacts on native species for several reasons. As an aggressively territorial species, Round Goby outcompete native fish for food and shelter (Balshine et al., 2005; Bergstrom and Mensinger, 2009). Round Goby aggression has been linked to population declines of native benthic species such as the Johnny Darter (*Etheostoma nigrum*) and Mottled Sculpin (*Cottus bairdii*) (Janssen and Jude, 2001; Lauer et al., 2004). Round Goby have also been implicated in declines in invertebrate quantity and species richness in the Great Lakes (Kuhns and Berg, 1999; Lederer et al., 2008). Finally, because they may consume contaminated benthic organisms or have constant physical contact with contaminated environments, Round Goby may also play a role in contaminant cycling, facilitating transfer of pollutants to higher trophic levels through their diet (Charlebois et al., 2001). This has been recorded for polychlorinated biphenyls (Kwon et al., 2006), perfluorinated compounds (Kannan et al., 2005) and Type E Botulism (Hebert et al., 2014).

Many of the negative impacts exerted by Round Goby result from their foraging and diet. Understanding the feeding ecology of an invasive species like the Round Goby can inform ecosystem managers of potential paths for further environmental disruption. To date, diet studies of Round Goby in the Great Lakes have revealed a generalist benthic feeder with a diet composed of invertebrates, especially Chironomids, Cladocerans and Dreissenids (Johnson et al., 2008). Studies have also shown an ontogenetic shift in diet to foraging on Dreissenid mussels, at approximately 6.0 cm standard length, with larger fish more easily and readily consuming mollusks (Ray and Corkum, 1997). Additionally, because Round Goby can tolerate a wide range of ecological conditions, they can be found in both pristine and degraded areas such as industrial harbours (Roche et al., 2013; McCallum et al., 2014). Indeed, in Hamilton Harbour, Round Goby are equally abundant at sites of high and low sediment contamination (Marentette et al., 2010; McCallum et al., 2014). However,

there is little knowledge of their diet in this well-studied ecosystem, even though Round Goby have been identified as an abundant and central species in the Hamilton Harbour food web (Hossain et al., 2012). The use of diet analyses partnered with stable isotope analyses can provide detailed information on the trophic position of this invasive species (Vander Zanden et al., 1997).

To address how contaminated environments affect Round Goby diet, trophic position, and foraging behaviour, we compared fish from an area of relatively low sediment contaminant burdens (La Salle) and another one of extremely high sediment contaminant burdens (Pier 15, near Randale Reef) in Hamilton Harbour. We quantified gut fullness, identified prey items in gut contents, and assessed prey availability from sediment samples. Based on Round Goby diet studies from other Great Lakes locations (Barton et al., 2005; Lederer et al., 2008; Johnson et al., 2008), and invertebrate prey abundance in the Harbour (Dermott and Bonnell, 2010), we predicted that Round Goby would mainly consume Chironomids, Copepods, Cladocerans and Dreissenids. Second, we expected to observe the same ontogenetic diet shift reported in other studies with more Dreissenid mussels found in larger individuals (Ray and Corkum, 1997). Third, because exposure to toxicants have been shown to decrease general activity, food consumption, and prey capture in fishes (Kasumyan, 2001; Weis et al., 2001; Candelmo et al., 2010), and because fewer organisms might be present in contaminated sediment (Beasley and Kneale, 2002), we predicted that fish from the low contamination site would have fuller guts and more prey items than fish from the high contamination site. We would expect to see this reflected in the stable isotope values, where fish from the low contamination site would have higher trophic position. We also examined feeding behaviour in the laboratory, and predicted that fish from the high contamination site would approach food more slowly and have lower foraging rates relative to fish from the low contamination site (Marentette et al., 2010).

Methods

Round Goby were collected from two sites in Hamilton Harbour (Figure 1): Pier 15 (43°16′ N, 79°50′ W) and La Salle (43°18′ N, 79°50′ W). Both sites are embayments with a rocky substrate and underlying sand and silt. Across collection years, both sites had similar mean water clarity, dissolved oxygen, temperature, and pH (Supplementary Table 1); however, they differ in the degree of sediment contamination. Sites were selected based on established sediment contamination studies (Hamilton Harbour Remedial Action Plan, 1992; Zeman, 2009). The high contamination site (Pier 15) has a long history of sediment contamination resulting from close proximity to Randale Reef, an area of historic coal tar deposits with high concentrations of

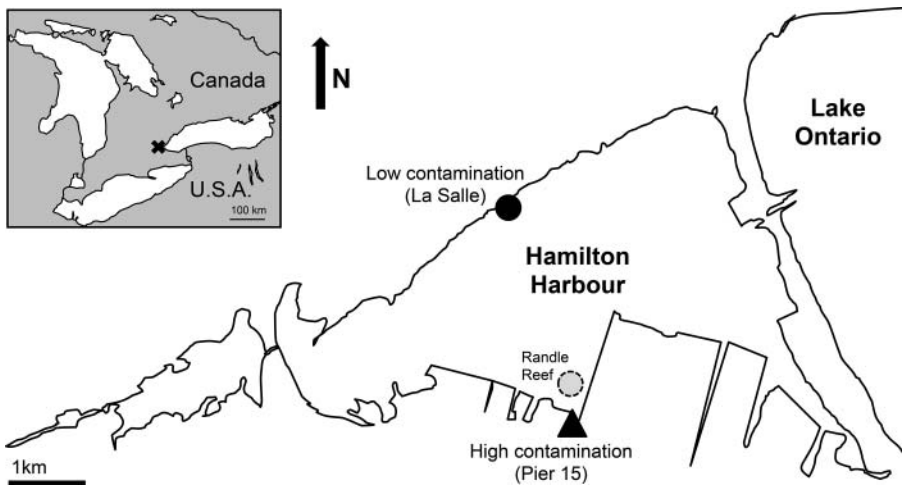

Figure 1. Hamilton Harbour map indicating the low contamination site (La Salle – black circle) and high contamination site (Pier 15 – black triangle). Randale Reef (grey circle), a historic coal tar deposit, is also marked.

polycyclic aromatic hydrocarbons (Hamilton Harbour Remedial Action Plan, 1992; Zeman, 2009). Previous work has shown that total PAHs and total PCBs were higher at the high contamination site, as were sediment concentrations of arsenic, chromium, cobalt, copper, iron, lead, mercury, vanadium, and zinc, exceeding provincial guidelines for probable effect levels (PELs; Milani and Grapentine, 2006; Zeman, 2009), compared to our low contamination sampling site, La Salle. Moreover, Round Goby collected from this high contamination site have higher tissue burdens of copper and cadmium, evidence of fin erosion, higher levels of EROD expression, and males with high vitellogenin levels, feminized external genitalia and higher levels of intersex when compared to fish from the site with lower contamination (Bowley et al., 2010; Marentette et al., 2010).

Diet and sediment analyses of benthic organisms

A total of 213 fish were collected from La Salle ($N = 145$) and Pier 15 ($N = 68$) between 24 June and 26 July 2010 (see Supplementary Table 2 for detailed fish demographics, basic gut contents). Minnow traps were set 1.5 h before sunset (7:30 pm), and collected 1.5 h after sunset (10:30 pm), as Round Goby are most actively feeding during crepuscular periods (Johnson et al., 2008). Traps were baited with frozen corn enclosed in a nylon bag to ensure no bait was eaten. Fish were euthanized immediately by immersion in a 0.025% benzocaine solution (Sigma Aldrich) and preserved in a 70% ethanol solution, with an incision made in the abdominal cavity to permit ethanol to rapidly penetrate the body wall. In the laboratory, fish were measured with calipers to the nearest 0.01 cm for standard length (SL). The total body mass, liver mass and gonad mass were measured to the nearest 0.001 g using a digital balance (Acculab Vicon). The gut was removed from esophagus to anus, weighed, and then visually rated on a five-point gut fullness scale (adopted from Puvanendran and Brown, 2002). On this scale, 0 = 0% fullness; 1 = 25% fullness; 2 = 50% fullness; 3 = 75% fullness; 4 = 100% fullness. The gut contents were then removed, weighed and the mass of the empty gut was also measured. Gut contents were preserved in 70% ethanol and stored in scintillation vials. All vials were visually inspected for the presence of Dreissenids by an observer who was blind to sampling site. The gut contents of 50 randomly selected fish (counterbalancing for site and sex) were examined under a dissecting scope at 2x magnification (Leica MZ75). Items in the gut were counted and identified by taxonomic group.

Sediment samples were collected at La Salle and Pier 15 on 24 June and 10 July 2012. Three samples were collected at each location within 1 m of the shore, 10 m apart, and placed in a 500 ml glass container and preserved with 70% ethanol. In the laboratory, samples were passed through a stack of mesh sieves of 1 mm, 250 μm and 63 μm sizes. Sorted samples were examined under a Luxo KFM magnifier (120 V, 220 W, 60 Hz) and a stereo microscope at 0.63x–2.5x magnification (Leica MZ75). Samples were sorted and organisms were identified to lowest possible taxonomic grouping. Each sediment sample was placed in a glass dish, dried in an oven (Lab-Line L-C Oven) at 105°C for 24 h, and then cooled for 5–6 h. A top-loading balance (Mettler Toledo, AB204-S/FACT) was used to take the mass of the sample, which was then transferred into a graduated cylinder to record volume.

Stable isotope analyses

Between 1 June and 30 July in both 2012 and 2013, 119 Round Goby were collected from La Salle and Pier 15 for stable isotope analyses ($N =$ 52 fish in 2012 and $N = 67$ fish in 2013; Supplementary Table 2). Additionally, in 2012, we collected 15 Dreissenids from La Salle and 20 Dreissenids from Pier 15 to serve as baseline primary consumers in the stable isotope analyses. Round Goby were collected using minnow traps as above, but deployed for 24 h. Upon retrieval, fish were euthanized by ice bath immersion followed by cerebral concussion and spinal severance before being transported on ice to the laboratory. Fish were measured with calipers to the nearest 0.01 cm for standard length (SL), and the total body mass, liver mass and gonad mass were measured to the nearest 0.001 g using a digital balance (Acculab Vicon). Then a muscle (dorsal axial) section was taken from each fish, which was placed in a glass scintillation vial, and frozen at −20°C. Dreissenids were transported live to the

laboratory, where they were shucked to remove their shells. Dreissenids were placed in individual glass scintillation vials and frozen at −20°C until stable isotope analyses. Frozen tissue samples were freeze-dried and ground to homogeneity using a mortar and pestle. Dreissenid tissues were then lipid extracted using Solvent Distillation with 2x agitation of tissue in 2:1 chloroform:methanol solution at 85°F for 24 h, solvent decanted and then sample air-dried. Round Goby muscle tissues were not lipid extracted because they have a low C:N ratio (<3.5). Individual samples were then weighed into tin cups (5 mm × 9 mm). Samples and standards were then run for δ^{13}C and δ^{15}N, C% and N%, using a Delta V IRMS (Thermo Electron Corporation, Waltham, Massachusetts, USA) equipped with an elemental analyzer (Costech, Santa Clarita, California, USA). The abundance of carbon and nitrogen stable isotopes within samples was expressed in delta notation (relative to standard materials) and calculated using the following equation:

$$\delta X\ (‰) = [R_{sample}/R_{standard} - 1]\ \times\ 1000$$

where R is the ratio of nitrogen (^{15}N/^{14}N) or carbon (^{13}C/^{12}C) isotopes. Pee Dee Belemnite (PDB) carbonate and atmospheric nitrogen were standard reference materials. To assess repeatability every 10th sample was run in triplicate. Precision of analysis from internally run standards run every 12th sample was 0.15‰ for δ^{15}N, and 0.1 and 0.08‰ for δ^{13}C (internal fish muscle standard and NIST bovine muscle 8414, $N = 30$). Accuracy based on the difference between standards run internally and certified NIST standards (2 year average ± SE) was 0.03 and 0.02 for δ^{13}C (NIST 8542, $N = 97$ and 8573, $N = 96$, respectively), and 0.03, 0.10 and 0.17‰ for δ^{15}N (NIST 8573, 8549 and 8548, respectively, $N = 118$–120). The following equation was used to estimate the effect of sampling site on fish trophic position:

$$\textit{Trophic position} = [(\delta^{15}N_{fish} - mean\ \delta^{15}N_{mussel})/3.4] + 2$$

Where 3.4 is the diet tissue discrimination factor for δ^{15}N and represents the change in δ^{15}N for each trophic position, assuming that Dreissenids, as a filter feeder, occupy a trophic position of 2 (Post, 2002).

Foraging behaviour experiment

Fish for this experiment ($N = 45$; Supplementary Table 2) were collected between 3 September and 24 October 2008, as described above. Fish were transported live to McMaster University and placed in 60 l laboratory stock tanks (60 × 45 × 30 cm) for 48 h in sex and site matched groups. The stock tanks contained ~2.0 cm of aquarium gravel substrate, and a static renewal filter. Fish were fed Nutrafin© fish flakes ad libitum, daily. Experimental tanks (60 l) were similarly set up but were divided in half with a removable, opaque acrylic barrier. One half contained a PVC half-cylinder shelter, while the other half contained a food stimulus placed there before a trial started (the side with the shelter was counter-balanced across the trials). Water temperature in both experimental and stock tanks was maintained at 20–22°C. Experimental foraging trials began by removing a fish from the stock tank, placing it on the side of the experimental tank with the shelter for a 48 h habituation period. Fish were not fed during this habituation period. Before the foraging trial, commercially-available lumpfish eggs were placed on a 6 cm petri dish in the empty half of the experimental tank. For every 5 g of fish mass, 2.5 g of eggs were provided. Foraging observations by an observer blind to sex and collection site began when the opaque barrier was removed and the fish on the shelter side was followed continuously for 15 min. We recorded the time the fish spent on each side of the tank, the time taken to enter the food compartment, and the time taken to until the first feed. All subsequent feeds were also recorded. Since Round Goby are more active during dusk and night (Johnson et al., 2008), the trials were conducted during the dark phase of the light cycle using red lights. Following each trial, the fish was removed from the experimental tanks, euthanized with a benzocaine solution, dissected, and measured with calipers and a scale as above.

Statistical analyses

All statistical analyses were performed using R (version 3.1.2, R Core Team, 2014). In all analyses, site and sex were included as fixed factors. Gut

content mass was log transformed to meet model assumptions and analyzed using an ANCOVA, where body mass was included as a covariate. Our gut fullness index was analyzed using an ordinal regression. Abundance of items in the guts was analyzed using a negative binomial regression for count data, with standard length as a continuous covariate. Taxon richness in gut samples was assessed using an ANOVA. The effect of fish size (standard length) on the probability of Dreissenids being present in gut contents was analyzed using a logistic regression, with standard length as a continuous predictor. Pearson's chi-square tests were used to test the effect of site and sex on Dreissenid mussel presence or absence in the gut. A linear mixed effects model was used to assess Round Goby and Dreissenid $\delta^{13}C$ and $\delta^{15}N$, with sampling year included as a random effect. Additionally, for the $\delta^{15}N$ and trophic position models, the variance was weighted by site due to uneven variances between sampling sites. For the sediment samples, taxon richness and item abundance were scaled by sample volume before analysis. The effect of site on taxon richness and item abundance was assessed using T-tests for estimating model parameters, and permutation tests of the same models to extract accurate p-values, using 10,000 random permutations of the data. Three measures were used to quantify foraging behaviour: latency to enter the food compartment, latency to first feed, and total feeds during the trial. Latency to enter the food compartment and latency to the first feed were analyzed using ANOVA on log-transformed values. Total feeds were analyzed using a generalized linear model assuming a quasi-Poisson error distribution appropriate for count data.

Results

Diet and sediment analyses for benthic invertebrates

Of the 213 fish collected for basic diet analyses, three were excluded from further analyses due to poor preservation. Round Goby at the high contamination site (Pier 15) tended to have lighter gut content mass than fish from the low contamination site (La Salle), but this difference did not reach significance (ANCOVA, $F_{Site}(1, 206) = 3.46$, $p = 0.064$), and female Round Goby had heavier gut content mass than males at both sites ($F_{Sex}(1, 206) = 12.26$, $p = 0.00057$; Figure 2a). Fish from the high contamination site also had lower gut fullness scores than fish from the low contamination site (ordinal regression, $Z_{Site} = -2.79$, $p = 0.0053$; Figure 2b). Again, females had higher gut fullness scores than did males (ordinal Regression, $Z_{Sex} = -2.35$, $p = 0.019$). Not surprisingly, gut fullness scores and gut content mass were positively correlated (Spearman's rank correlation: $\rho = 0.16$, $p = 0.018$). Larger fish were more likely to have Dreissenid mussels present in their gut contents compared to smaller fish (Logistic regression: estimate (±SE): 0.47 (±0.11), $N = 213$, $Z = 4.37$, $p < 0.0001$). The

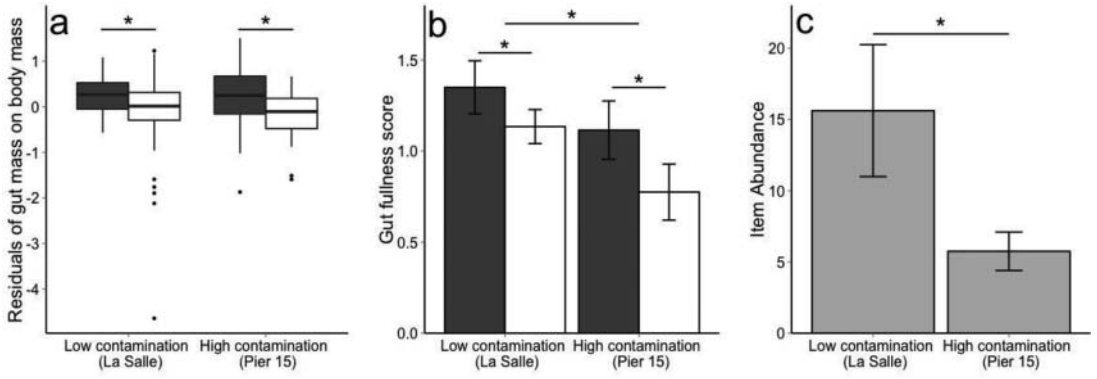

Figure 2. (a) Residuals of gut content mass on body mass plotted by sampling site and sex (females: dark grey, males: white). Box hinges represent the first and third quartile, whiskers show 1.5 x inter-quartile range from hinges, and points show outliers. (b) Average gut fullness scores (as rated from 0–4) plotted by sampling site and sex (females: dark grey, males: white). (c) Average prey item abundance found in guts plotted by site, with sexes combined. In all panels * indicates $p < 0.05$, error bars indicate ± SE.

smallest fish to consume a Dreissenid mussel was 5.80 cm standard length. Of fish over 5.80 cm standard length, 26% of them had consumed Dreissenids. There was no effect of sex (33% females, 24% males: Pearson's Chi-square: $\chi^2 = 1.38$, $p = 0.24$) or collection site (25% La Salle, 28% Pier 15: Pearson's Chi-square: $\chi^2 = 0.030$, $p = 0.86$) on the total number of Dreissenids consumed.

Across the 50 fish sampled for detailed gut content analyses, we identified 13 different types of items. Item richness in the gut samples ranged from 0–12 (mean ± SD: 3.48 ± 2.21). Chironomids, Cladocerans and Copepods were the most common items in the gut samples, and were identified in 74%, 56% and 46% of the samples, respectively. The distribution of the ten most common types of items in the guts is plotted in Figure 3, and a detailed summary can be found in Supplementary Table 3. There was no effect of sampling site or sex on item richness in the gut samples (ANOVA: $F_{Site}(1, 47) = 0.10$, $p = 0.75$; $F_{Sex}(1, 47) = 0.58$, $p = 0.45$). Item abundance in the gut samples ranged from 0–95 items per gut, with an average of 11 items being identified per gut sample. Fish scales were the most abundant item, but resulted from many scales being found in only a few gut samples. Copepods and Chironomids were the next most abundant items, with a total of 136 and 115 being counted across all the samples, respectively. On average, fish from the high contamination site had fewer items in their guts than fish from the low contamination site (Negative Binomial Regression: estimate (±SE): −0.83 (±0.36), $N = 50$, $Z = -2.31$, $p = 0.021$), and there was no effect of body size (estimate (±SE): −0.099 (±0.11), $N = 50$, $Z = 0.84$, $p = 0.40$) or sex on prey item abundance in the guts (estimate (±SE): 0.041 (±0.31), $N = 50$, $Z = 0.13$, $p = 0.90$).

We identified 18 different prey items in the sediment samples. Item richness in the sediment samples ranged from 9–16 item types (mean ± SD: 12.9 ± 2.8). Ostracods, Copepods, and gastropod shells were the three most common types found in the sediment samples, all being found in 100% of samples. Nematodes and Cladocerans were the next most common item types, being identified in 90% of the samples. See Supplementary Table 3 for a detailed summary of sediment analyses. There was no effect of sampling site on item richness ($t = 1.27$, $N = 10$, permutation $p = 0.10$), on item abundance ($t = 0.0036$, $N = 10$, permutation $p = 0.53$) in our sediment samples.

Stable isotope analyses

Male and female Round Goby had similar δ^{15}N (Linear mixed effects model: estimate (±SE): −0.07(±0.23), $N = 119$, $t = -0.32$, $p = 0.76$) and δ^{13}C values (estimate (±SE): −0.06 (±0.28), $N = 119$, $t = -0.23$ $p = 0.81$), and we therefore pooled the data from both sexes and compared these to the baseline values from Dreissenids of the same sites. Dreissenids had lower δ^{15}N values than Round Goby at both sampling sites (estimate (±SE): −4.91 (±0.20), $N = 156$, $t = -24.08$ $p < 0.001$; Figure 4a). Both Round Goby and Zebra Mussels had lower δ^{15}N values at the high contamination site than the low contamination site (3.3 and 1.4% difference, respectively; estimate (±SE): −2.77 (±0.22), $N = 156$, $t = -12.36$ $p < 0.001$: Figure 4a). Dreissenids had higher

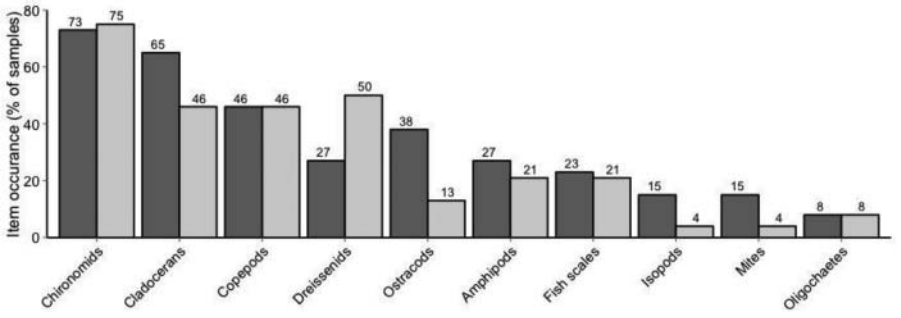

Figure 3. Occurrence of the top-ten prey items in Round Goby gut samples plotted by site, where darker bars = low contamination site (La Salle), and lighter bars = high contamination site (Pier 15).

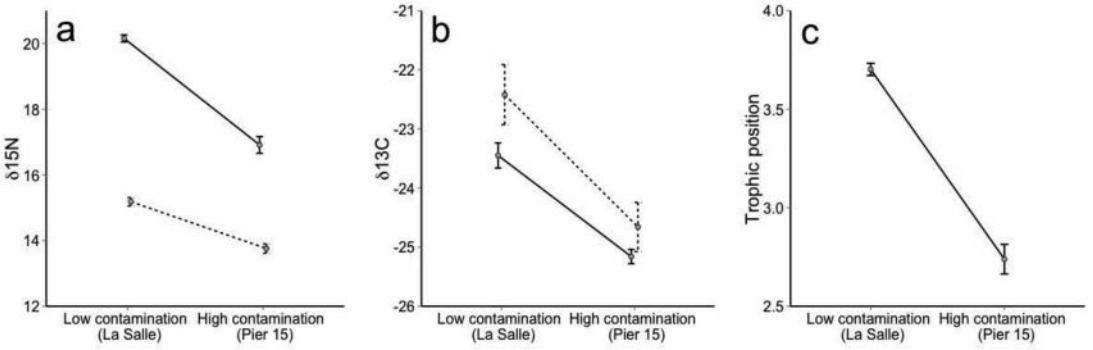

Figure 4. (a) Average δ^{15}N (%) plotted by sampling site and species. (b) Average δ^{13}C (%) plotted by sampling site and species. (c) Average trophic position for Round Goby plotted by sampling site. In all panels: Round Goby = solid line, Dreissenids = dashed line, error bars indicate ± SE.

(less negative) δ^{13}C values than did Round Goby at both sampling sites (estimate (±SE): 0.88 (±0.29), $N = 156$, $t = 3.06$, $p = 0.0026$; Figure 4b). Both Round Goby and Dreissenids had lower (more negative) δ^{13}C values at the high contamination site compared to the low contamination site (1.8% and 2.4% lower, respectively; estimate (±SE): −1.87 (±0.23), $N = 156$, $t = -8.17$, $p < 0.001$; Figure 4b). Round Goby from the high contamination site had a lower trophic position (estimate (±SE): −0.96 (±0.081), $N = 119$, $t = 60.30$, $p < 0.001$; Figure 4c), and trophic position did not differ between the sexes (estimate (±SE): −0.04 (±0.061), $N = 119$, $t = -0.61$, $p = 0.54$).

Foraging behaviour experiment

Fifteen of the tested fish were excluded because they did not move during the 15-min trial. Compared to fish from low contamination site, high contamination site fish tended to enter the food compartment later (ANOVA: F(1, 27) = 3.47, $p = 0.07$; Supplementary Figure 1a), and tended to take longer to make their first feeding attempt (F(1, 27) = 3.01, $p = 0.094$; Supplementary Figure 1b), however, these effects did not reach statistical significance. Total feeding strikes taken did not differ between fish from the different sites (Quasi-Poisson generalized linear model: estimate (standard error): −0.018 (0.46), $t = -0.039$, $p = 0.97$; Supplementary Figure 1c). There was no effect of sex on foraging behaviour (effect of sex, all comparisons $p > 0.10$)

Discussion

We found that Chironomids, Cladocerans, Copepods and Dreissenids were the most abundant diet items in Round Goby from Hamilton Harbour, and this fits well with results from other Round Goby diet studies from coastal areas of Lake Michigan and Lake Huron (Barton et al., 2005; Lederer et al., 2008; Cooper et al., 2009), and from eastern Lake Ontario (Johnson et al., 2008; Brush et al., 2012). In our study, 26% of the fish consumed Dreissenids: all fish that ate Dreissenids were larger than 5.8 cm (standard length), supporting the size-dependent diet shift that has been documented in the past (Ray and Corkum, 1997). Many Round Goby in our study did not consume any Dreissenids possibly because Hamilton Harbour is one of the few shallow areas of the Laurentian Great Lakes that has not been heavily invaded by Dreissenids (Gerlofsma et al., 2007). Additionally, Round Goby have been shown to prefer other invertebrate prey types over Dreissenids in laboratory experiments (Diggins et al., 2002), and fish fed exclusively Dreissenids had reduced growth (Coulter et al., 2011). Given the abundance of other prey items consumed by the fish in our study, and the potential cost of consuming only Dreissenids, Round Goby may be favouring other invertebrates that are easier to handle (Brush et al., 2012). We also found a sex difference in Round Goby diet, where females had fuller and heavier digestive tracts. While female Round Goby are not restricted to any specific territory and can continue feeding throughout the breeding season, males defend a territory and offspring

(Corkum et al., 1998). Paternal care is energetically costly for males and restricts their foraging opportunities during the breeding season (Bose et al., 2014). Similar sex differences in gut fullness have been reported in Round Goby from their invasive range in Europe and in other goby species with male-only parental care (Salgado et al., 2004; Brandner et al., 2013).

Round Goby were consuming benthic prey items that were abundant in their environment. Our sediment analyses revealed Chironomids, cladocera, Copepods, ostracods, Dreissenids and Gastropods to be the most abundant prey items available across sites. Our results confirm findings from earlier, detailed analyses of Hamilton Harbour sediments across multiple years by Gerlofsma et al. (2007) and Dermott and Bonnell (2010) showing these invertebrate groups to be very abundant in the Harbour. Some notable potential prey items that were present at high frequencies in the sediment, but were *not* present or common in the digestive tracts, included nematodes, Turbellaria, bryozoan statoblasts, and oligochaetes (e.g. oligochaetes were present in 67% of sediment samples versus 8% of gut samples). These items all tend to be soft bodied compared to other prey items that were observed, and thus could have been digested before identification. Alternatively, Round Goby may avoid these prey items in favour of other prey that may be easier to handle or find. Lastly, if these prey items are patchy in the environment, then it is possible that we would need an even more intensive sampling study to capture the complete range of the prey items consumed by Round Goby at each site.

Round Goby from our high contamination site had fewer prey items in their guts and lower gut fullness scores compared to fish from our low contamination site. These findings are not a result of lower prey availability at the high contamination site. In contrast to our predictions, and in contrast to findings of previous studies (Beasley and Kneale, 2002), benthic invertebrate abundance and diversity were not lower at the site with high contamination (Pier 15). Fewer prey items in the guts and lower gut fullness scores at the high contamination site also do not appear to be caused by Round Goby being more selective of the types of prey items consumed, as we found similar prey item richness in the guts and in the sediment samples from both sites. Moreover, the top-five types of prey in the guts were similar between Round Goby from each site. Our observations of lower gut fullness scores at the high contamination site could be the result of more direct effects of contaminants on foraging behaviour. We observed that fish from the high contamination site tended to initiate feeding more slowly. Though this trend did not reach statistical significance, previous studies have shown that Round Goby from this high contamination site had decreased activity levels (Marentette et al., 2012). Moreover, exposure to contaminants such as polycyclic aromatic hydrocarbons and metals like those documented at our high contamination site are known to decrease activity in other fish species (Kasumyan, 2001; Weis et al., 2001; Candelmo et al., 2010) and in Round Goby (Leonard et al., 2014).

We found that Round Goby had higher δ^{15}N and higher δ^{13}C at the low contamination site, and similar trends were also observed in the Dreissenids. Round Goby and Dreissenids at both sites had very similar δ^{13}C values, which suggests similar carbon sources. Round Goby had much higher δ^{15}N than Dreissenids at both sites, as would be expected given their higher position in the ecosystem. When δ^{15}N was used to calculate tropic position, we found that Round Goby from the low contamination site had a trophic-position estimate of 3.5, while Round Goby from the high contamination site had a much lower trophic-position estimate of ~2.5. This difference does *not* match the gut content findings, and would suggest that Round Goby at the low contamination site are feeding on different items than Round Goby from the high contamination site. This may be a result of the isotope values reflecting differences in feeding over longer time periods compared to the "snap-shot" nature of stomach contents. However, a trophic position estimate of ~2.5 at the high contamination site would suggest Round Goby are partially feeding on primary production. This is highly unlikely given the results of our study and other published diet studies (Barton et al., 2005; Lederer et al., 2008; Cooper et al., 2009; Brush et al., 2012). This trophic value for Round Goby may also be driven by the trophic position assigned to our baseline Dreissenids that was taken from an established average in the literature (Post, 2002). As there is always variation around this average, it is possible that the Dreissenids in Hamilton Harbour occupy a higher trophic position than the literature average, which would also increase the trophic position of Round Goby. It is

not possible to address the above issues without additional stable isotope analyses, and future work will use multiple sampling spatially and temporally (Syväranta et al., 2006).

The difference in stable isotope values observed between sites for both Dreissenids and Round Goby likely stem from proximity to point sources of nitrogen input, such as wastewater treatment plant effluent (Carey and Migliaccio, 2009). The low contamination site is located slightly closer (~3.5 km) to a wastewater treatment effluent source than the high contamination site, ~5.1 km. Interestingly, when compared with stable isotope values obtained for Round Goby elsewhere in the Great Lakes (Barton et al., 2005; Brush et al., 2012, Pettit-Wade et al., 2015), Round Goby in Hamilton Harbour possess very high δ^{15}N, consistent with previous stable isotope values reported for the food web of Hamilton Harbour (Ryman, 2009). Even Round Goby measured directly outside the Harbour entrance in Lake Ontario had lower δ^{15}N than those within the Harbour (Pettitt-Wade et al., 2016), suggesting that proximity to sources of nutrient input can influence stable isotope values even at small scales (< a few kms). The eutrophic nature of Hamilton Harbour (Hiriart-Baer et al., 2009) is likely due to multiple nitrogen-rich wastewater effluent sources and combined sewer overflow input across the Harbour (Hamilton Harbour Remedial Action Plan, 1992). Yet, as our isotope measurements suggest, eutrophication within Hamilton Harbour is likely to be heterogeneous and centered on sites of high nutrient input. We therefore stress the importance of accounting for proximity to environmental sources of nitrogen and carbon when measuring stable isotopes.

Conclusions

In this first diet and foraging analysis of Round Goby from a highly contaminated ecosystem, we show that fish from a contaminated site consumed fewer prey, had emptier digestive tracts, and occupied a lower trophic position. These results were not driven by prey availability, and instead may be related to foraging behaviour of fish exposed to contaminants. As remediation goals for Hamilton Harbour include improving aquatic biodiversity, our results indicate that the abundant Round Goby could negatively impact (via predation pressure) the invertebrate community in Hamilton Harbour, with similar impacts elsewhere in the Great Lakes (Kuhns and Berg, 1999; Lederer et al., 2008). We show that benthic organisms comprise a large portion of the Round Goby diet. Because these benthic organisms, especially Dreissenids, accumulate toxicants and chemicals from the sediments and the water column (Reynoldson, 1987), this fish in a key position in the food web may be crucial in mobilizing contaminants to higher trophic levels (Hossain et al., 2012). Hence, future research assessing contaminant burdens in invertebrates and Dreissenids from highly contaminated sites, and their possible transfer to Round Goby and larger predators is a necessary step to identify contaminant transfer in the Hamilton Harbour ecosystem. Stable isotope analyses will certainly continue to be an important tool for understanding these trophic relationships and monitoring eutrophication in the aquatic community of Hamilton Harbour. Our study demonstrates how diet, trophic and foraging analyses can provide a rich understanding of the aquatic community in Hamilton Harbour, and shows that the abundant and invasive Round Goby can be used as an indicator of ecosystem health.

Acknowledgements

We would like to thank M. Baxter and the Hamilton Port Authority for permissions, A. Hussey for help with stable isotope analyses, R. Dermott for advice on sediment analysis protocols, B. Bolker and J. Dushoff for assistance with statistical analyses, A. Bose for helpful comments on the manuscript, and many previous students that have helped with field work for this project: N. Sopinka, J. Young, P. Capelle, R. Caldwell, O. Petric, G. Fortin, N. Berlin, and C. Lau.

Funding

This project was supported by NSERC PGS scholarships (to E. McCallum and J. Marentette), NSERC Discovery Grants and Canada Research Chair Programs (to S. Balshine and A. Fisk), and funding from Fisheries and Oceans, Canada (to S. Balshine).

Supplemental material

Supplemental data for this article can be accessed on the publisher's website.

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