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Alternative reproductive tactics, an overlooked source of life history variation in the invasive Round Goby

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Abstract

Alternative reproductive tactics (ARTs) can generate considerable within species life history variation, but are often overlooked. Here, we use the invasive Round Goby (*Neogobius melanostomus*) to address a number of ecological and evolutionary questions about ARTs. Making use of a 12-year, multi-site, Laurentian Great Lakes dataset, we show that guarder males were twice as common as sneakers males, but that non-reproductive males were the most common tactic. The ratio of guarder to sneaker males did not vary spatially despite a wide range of resource densities across sites. Guarders and sneakers spanned similar age ranges, suggesting the ARTs are non-sequentially expressed. Based on short term (gut contents) diet analyses, both reproductive tactics consumed fewer types of food and tended to consume fewer items overall when compared to non-reproductive males. Long term (isotope) diet analyses showed that guarder males fed at a higher trophic level (higher $\delta^{15}N$) and had a broader isotopic niche. Our results show that ARTs are an important aspect of this invasive species' breeding system and should be accounted for when assessing and managing populations.

Keywords: sneaker, guarder, population, stable isotopes, Gobiidae, Laurentian Great Lakes

Introduction

The Round Goby (*Neogobius melanostomus*) is a highly successful invasive species that has established new populations in both Europe and North America (Corkum et al. 2004; Kornis et al. 2012). They have had a detrimental impact in invaded areas by causing the decline of native species via competition and predation (Janssen and Jude 2001; Lauer et al. 2004; Steinhart et al. 2004) and creating new vectors for pollutant and disease transfer to higher trophic levels (Kwon et al. 2006; Hebert et al. 2014). Despite nearly three decades of focused research on this species, several important aspects of their reproductive ecology remain largely unstudied. In particular, their expression of alternative reproductive tactics (ARTs).

ARTs are mainly observed in males (Oliveira et al. 2008; Taborsky and Brockmann 2010), and occur whenever two or more discrete morphological, physiological, and/or behavioural morphs exist within a sex. These morphs approach reproduction in alternative ways and commonly occur as a ‘conventional territorial’ or ‘guarder’ male tactic and a ‘parasitic’ or ‘sneaker’ male tactic. ARTs are found in phylogenetically disparate animal taxa, and can be both a cause and consequence of sexual selection (Oliveira et al. 2008; Taborsky and Brockmann 2010). Conventional males are typically larger and invest in secondary sexual traits that allow them to secure mates through male-male competition and/or via female choice. In contrast, parasitic males are typically smaller, avoid physical competition, do not court females, and invest heavily in traits that will improve the probability of fertilization either through sexual coercion or sperm competition. ARTs can provide a significant source of life history variation within a given species and are well documented in certain fishes such as salmonids and sunfishes (Gross 1984; Oliveira et al. 2008). For example, in Bluegill Sunfish (*Lepomis macrochirus*), males adopt one of two distinct developmental trajectories early in life. Some males, called guarders, delay maturation until ~7 years of age, grow larger, build nests, court females, and care for offspring. Other males, called sneakers, mature at ~2 years and use stealthy tactics to sneak into nests and cuckold caring males. But later, as they grow larger, they adopt female mimic tactics to gain access to spawning events (Gross 1982; Neff and Gross 2001). In general, interactions between individuals adopting different tactics are competitive, with the sneaker tactic stealing paternity from the guarder tactic, thereby adding complexity to the mating systems of many species (Taborsky and Brockmann 2010).

Marentette et al. (2009) and Bleeker et al. (2017) provided the first descriptions of Round Goby ARTs in North American and European populations, respectively. Both studies found bimodal variation in male morphology consistent with a conventional guarder and a parasitic sneaker. Guarder males are larger, darker, have wider heads with swollen cheek pads (a secondary sexual characteristic), and higher levels of plasma 11-ketotestosterone (Figure 1). In contrast, sneaker males are smaller, with more female-like mottled colouration, they invest more in their testes mass relative to their body mass, and have more sperm cells per ejaculate volume (Marentette et al. 2009; Figure 1). To date, most studies on Round Goby biology have ignored the presence of ARTs. In 2009 the first empirical study of ARTs in Round Goby was published and since then only 14 of the 333 (~4%) articles published on Round Goby have mentioned, considered, or accounted for the existence of ARTs (see literature review, Supplementary Materials). Additionally, unlike larger-bodied salmonids for which ARTs have been well characterized, Round Goby are a small-bodied benthic fish that exhibit very different life history strategies, which could impact the occurrence and frequency of ARTs. Thus, they provide a rich, but yet unexplored, system in which to study the evolution of ARTs.

In this study, we addressed a number of key ecological and evolutionary questions about ARTs using the Round Goby. First, ARTs are typically studied in a single exemplar population at a single time point, but sexual competition—a driving force in the evolution of ARTs—can vary across time and space (Taborsky and Brockmann 2010; Monroe et al. 2016). We used a large multi-year, multi-site dataset from Hamilton Harbor in the Laurentian Great Lakes to ascertain how Round Goby catch abundance and male tactic ratios differ across time and among sites with different resources (substrates and habitat types). We predicted that sites offering more complex microhabitats for taking shelter and building nests (i.e., rock and boulder substrates) would have a greater proportion of guarder males relative to sneaker males because competition for nesting sites would decrease in such areas. Second, we investigated age and growth differences between the male tactics by analyzing saccular otoliths. The mechanisms responsible for tactic adoption (e.g., genetic polymorphism, environmental determination, developmental threshold) is not known for Round Goby and is currently only known for a handful of species. Bleeker et al. (2017) suggested that Round Goby first adopt the sneaker tactic and then sequentially switch to the guarder tactic once a specific body size threshold is surpassed. These authors based this suggestion on the fact that they observed little overlap in body sizes between guarder and sneaker males in their study populations. However, several *non-sequential* mechanisms (e.g. a genetic polymorphism or a developmental threshold) could also lead to little overlap in body sizes between male tactics and by aging both tactics we can better resolve such mechanisms. Third, we investigated variation in diet among the male morphs by using gut contents and stable isotope analyses (δ^{15}N and δ^{13}C) to measure trophic position and isotopic niche. If male tactics differ in their diet and isotopic niches, then this information could be incorporated in models of resource-use and population growth. Few studies have examined how

ARTs vary with respect to their adult diet (but see Cogliati et al. 2015; Félix et al. 2016). Because guarder males provide parental care and are confined to a nest (Corkum et al. 1998), we predicted that they would have fewer food items in their digestive tracts and that they would also be less specific about what they would consume (Smith and Wooton 1995). We therefore also expected guarder males to have a broader isotopic niche relative to sneaker and non-reproductive males.

Methods

Study 1: Does the relative abundance of male tactics differ across time and space?

The Round Goby population in Hamilton Harbour (ON, Canada; 43ºN, 70ºW) has been intensively monitored since 2002. Full details on the annual sampling regime and sites can be found in McCallum et al. (2014) and Young et al. (2010). Briefly, every two weeks from May to October, baited minnow traps were deployed at ~1m depth at four sites in Hamilton Harbour. For every fish collected, we measured: total length (snout to tail fin), standard length (snout to caudal peduncle), and papilla length (tip to base) using calipers accurate to the nearest 0.01cm. We measured the whole body, testes, and seminal vesicles (male accessory gland organs, which together with the testes make the gonad) masses using a digital balance accurate to the nearest 0.001 g (Ohaus Adventurer Pro). We used total gonad weight (testes plus seminal vesicles) to calculate gonadosomatic index (GSI: 100 x [gonad mass (g)]/ [body mass– gonad mass (g)]).

Males were identified as reproductive or non-reproductive based on whether their GSIs exceeded 1% (Marentette et al. 2009; Zeyl et al. 2014). Because male ARTs in Round Goby had not yet been recognized in the early years of the monitoring study, we used a linear discriminant analysis (LDA) to retroactively assign ARTs to reproductive males based on their morphological data. We focused on males collected in 2006 and onwards as this was the first year when both testes and seminal vesicles were measured separately during dissections. We then applied the LDA to the reproductive males to classify them as either guarder males or sneaker males, training the LDA on a sub-sample of fish between 2007-2010 (N = 280 reproductive males: 171 guarder males and 109 sneaker males that were previously described in Marentette et al. 2009). The LDA used three measures to assign a tactic to each reproductive male in the full dataset: 1) standard length (to the nearest 0.1 cm), 2) testes somatic index (testes mass as percent of total body mass), and 3) the seminal vesicle to testes mass ratio (Figure 2 B, C, D). The LDA assigned a tactic to each reproductive male when it was at least 80% confident, otherwise it labelled the males as 'unknown'. The trained LDA agreed with the male tactic assignments of the training dataset (2007-2010) 89% of the time and assigned tactics to 93% of all reproductive males in the whole dataset. Thus, our final dataset consisted of 2973 non-reproductive males and 1132 reproductive males (including 686 guarder males, 369 sneaker males, and 77 unknowns; unknowns were excluded from further analysis).

We performed all statistical analyses in R (version 3.4.3; R Core Team, 2017). First, we investigated whether the relative abundance of guarder males versus sneaker males changed across the round goby breeding season and differed between four distinct habitat types (mud, sand, cobble, boulder, see Young et al. (2010). For each sampling day of every year's breeding season, we recorded the total number of reproductive males (if any) that were captured (i.e. guarder males + sneaker males), as well as the proportion that were guarder males. The breeding season ends each year around Julian day 250 (in early September), when reproductive males become very scarce in the population (see Figure 2A). We fit a generalized linear mixed effects model (GLMM, lme4 package, Bates et al. 2015) assuming a binomial error distribution and included the proportion of guarder males as the response variable. Each data point was also weighted by the total number of reproductive males caught on that day at each site. We included Julian date (scaled, i.e. divided by its standard deviation) and field site (i.e. habitat type) as predictor variables and year as a random intercept. Second, we investigated whether the relative abundance of guarder males versus sneaker males changed across years. We fit a generalized linear model (GLM) assuming a quasi-binomial error distribution (suitable for overdispersed proportion data, Kabacoff 2011) and included the proportion of guarder males found within the reproductive male population at each site (pooled for each year) as the response variable. We also included field site and year as predictor variables.

Study 2: Does age and growth differ between the male ARTs?

We collected 113 Round Goby for aging (as well as diet and stable isotope analyses, detailed below) from Fifty Point Conservation Area, Hamilton (Ontario, Canada; 43°13' N, 79°37' W) on two sampling occasions in 2016 (May 18 and June 29, 2016). Following fish capture methods described in McCallum et al. (2017a), we sampled fish using minnow traps baited with corn held in a nylon pouch to prevent fish from consuming the bait. Round Goby forage most actively at dusk (Carman et al. 2006); accordingly, we set traps at dusk, tied off at shore, at a depth of ~1 m, and collected them ~3 hours later (i.e., when the sun had completely set). We sorted the male Round Goby collected into three categories: guarder male, sneaker male and non-reproductive male, based on external morphology. We dissected all male fish on site, confirmed their tactics by examining their gonads, recording all morphological measures detailed above in Study 1, extracted their saccular otoliths, and performed additional measures for specific diet and stable isotope analyses, detailed below.

We had one sagittal otolith per male (from 17 guarder males, 19 sneaker males, and 20 non-reproductive males) aged by an experienced otolith reader who had no prior information about the individual fish or their morphology. The fish we selected for aging were not a random sample of the population but were selected to evenly cover the complete range of body sizes sampled for each male type. Otoliths were cleaned, immersed in water, and viewed under transmitted light at 80x magnification with a Leica GZ6 stereomicroscope. Annuli of the otoliths were enumerated from the core to the edge, and the width of the first growth increment was measured (in µm) from the core to the first annulus. A single age estimate and width measurement was obtained for each fish.

To investigate early life growth rates, we compared the widths of the first otolith growth increment (from the otolith core to the first annulus) between guarder males, sneaker males, and non-reproductive males. Otolith growth is often used as a proxy for somatic growth (Campana and Neilson 1985). We fit a LM, including increment width as the response variable and male tactic as the predictor. Next, to investigate relative ages, sizes, and growth rates, we compared fish size-at-age between guarder males, sneaker males, and non-reproductive males. We fit a LM, including standard length as the response variable, and age, male tactic, and their interaction as predictors.

Study 3: Does diet and isotopic niche differ between the male ARTs?

As mentioned above, we collected 113 Round Goby for diet and stable isotope analyses. While dissecting the males on-site (see above), we removed the digestive tract from the esophagus to the anus for each fish. The digestive tract contents were then removed and preserved in 95% ethanol for later diet identification with microscopy. We analyzed the gut contents of a random subset of 55 of these 113 fish (19 guarder, 20 sneaker males, and 16 non-reproductive males) under a dissecting scope at 2x magnification (Leica 151 MZ75). Items in the gut were counted and identified down to the lowest possible taxonomic group. We fit two linear models, one to food item abundance (the number of distinct food items found in each gut, count), and the other to food item richness (the number of different taxonomic groups in each gut, count), after log-transformation. We included male tactic, sampling month, and standard length as predictors.

From each of the 113 sampled males, we collected liver tissue and a section of the dorsal axial muscle. We stored the tissue samples individually and froze them at -20°C for later analyses (21 muscle samples spoiled in a freezer malfunction, leaving $N = 92$ for muscle isotope analyses). On each sampling trip, we also collected dreissenid mussels to serve as baseline primary consumers in the stable isotope analyses. We shucked the mussels and froze them individually at -20°C for later analyses. Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were determined following standard procedures outlined in Pettitt-Wade et al. (2015), see Supplementary Materials for further details including accuracy and precision data. Primary consumers did not vary between samplings in $\delta^{15}N$ (LM: $N = 34$, est. ± se = -0.22 ± 0.11, $t = -2.02$ $p > 0.05$, Table 1), but those collected in June had more positive $\delta^{13}C$ than those collected in May (LM: $N = 34$, est. ± se = 1.70 ± 0.11, $t = 15.91$, $p < 0.0001$). For each tissue (muscle and liver), we fit a LM with $\delta^{13}C$ or $\delta^{15}N$ as the response variables, and male tactic, month, and standard length as predictors.

To compare isotopic niche size and niche overlap among the male tactics, we constructed $\delta^{13}C$ and $\delta^{15}N$ bi-plots and standard ellipse areas (SEA) (R-SIBER package; Jackson et al. (2011)). SEAs measure mean isotope variability and represent 40% of the spread in the data.

When sample sizes are < 30, as in our sample, standard ellipses are corrected to provide an unbiased estimate by removing an additional degree of freedom (Jackson et al. 2011). We calculated the probability of one tactic having a smaller isotopic niche than another by comparing credible intervals for niche size produced from multiple Bayesian iterations of the corrected standard ellipse area (10^5 posterior draws, also provides adjustment for sample size differences; see Jackson et al. (2011) for details). The probability of isotopic niche size differences ranged from zero (no difference) to one, and niche size was considered to be significantly different at a probability > 0.95 (Jackson et al. 2011; Pettitt-Wade et al. 2015).

Ethical note

All methods for collecting and handling Round Goby were approved by McMaster University's Animal Research Ethics Board (Animal Utilization Protocols. 17-12-45; 13-12-51; 10-11-70; 06-10-61; 03-09-54) and adhere to the standards of the Canadian Council on Animal Care.

Results

Study 1: Does the relative abundance of male tactics differ across time and space?

The proportion of the male population that was reproductive (i.e., guarders and sneakers) was highest at the end of April and then steadily declined until the end of the breeding season (early September) when the male population became dominated by non-reproductive individuals (Figure 2A). We caught 57 ± 25 guarder males (mean ± SD; range = 19 - 107) and 31 ± 14 (range = 14 – 60) sneaker males per year across the four sites. Guarder males comprised 67 ± 17.5% of all the reproductive males sampled from each site per year. See Supplementary

Materials Table S1 for more information on catch abundance for reproductive males at our field sites. The proportion of guarder males within the reproductive male population during the breeding season did not vary with either Julian date (Generalized linear mixed effects model, GLMM, est. ± se = -0.054 ± 0.076, z = -0.72, $p = 0.47$) or between habitat types (all pair-wise contrasts were at least: est. ± se = -0.28 ± 0.19, z = -1.48, $p = 0.14$). Furthermore, the proportion of guarder males in the reproductive male population neither increased nor decreased across the twelve years of population monitoring (Generalized linear model, GLM, est. ± se = -0.0032 ± 0.023, $t_{42} = -0.14$, $p = 0.89$).

Study 2: Does age and growth differ between the male ARTs?

The mean ages of guarder, sneaker, and non-reproductive males were 1.8 ± 1.0, 1.4 ± 0.7, and 1.7 ± 0.7 (mean ± SD), respectively (ranges = 1-4, 1-3, and 1-3). Age strongly correlated with body length in non-reproductive males (Linear model, LM, est. ± se = 0.61 ± 0.13, $t_{50} = 4.60$, $p < 0.0001$) but not in sneaker males (est. ± se = 0.15 ± 0.12, $t_{50} = 1.28$, $p = 0.21$, 3A) nor in guarder males (est. ± se = 0.068 ± 0.089, $t_{50} = 0.76$, $p = 0.45$). Sneaker males were smaller than guarder males (est. ± se = -1.43 ± 0.36, $t_{50} = -3.98$, $p < 0.001$, Figure 3A) and were also smaller than non-reproductive males (est. ± se = -1.55 ± 0.34, $t_{50} = -4.55$, $p < 0.0001$) but non-reproductive males and guarder males did not differ in body size (est. ± se = -0.12 ± 0.35, $t_{50} = -0.35$, $p = 0.73$). Though sneaker males were smaller than guarder males on average, there was still some overlap in body size between the tactics. The saccular otolith analyses showed that sneaker males had smaller growth increments initially (before the first annuli) than guarder males (LM, est. ± se = -1.54 ± 0.55, $t_{53} = -2.81$, $p = 0.007$) and non-reproductive males (est. ± se = -1.15 ± 0.53, $t_{53} = -2.19$, $p = 0.03$). Guarder males and non-reproductive males did not differ significantly in the widths of the first growth increments (est. ± se = 0.39 ± 0.54, $t_{53} = 0.72$, $p = 0.48$, Figure 3A). Our data also suggest that following one year of age, the two alternative male reproductive tactics had similar somatic growth rates (interaction term, est. ± se = -0.08 ± 0.15, $t_{50} = -0.56$, $p = 0.58$, Figure 3B).

Study 3: Does diet and isotopic niche differ between the male ARTs?

Chironomids, *Driessena*, and fish scales were the most abundant food items (by count) in Round Goby digestive tracts (Figure 4A). Compared to non-reproductive males, sneaker males had fewer food items in their digestive tracts (LM, est. ± se = -0.74 ± 0.30, $t_{50} = -2.44$, $p = 0.02$, Figure 4B) and a lower richness (count of taxonomic groups) of food types in their digestive tracts (LM, est. ± se = -0.96 ± 0.47, $t_{50} = -2.02$, $p = 0.049$, Figure 4C). Guarder males did not differ from non-reproductive males or from sneaker males in terms of the number of food in their digestive tracts (est. ± se = -0.40 ± 0.24, $t_{50} = -1.67$, $p = 0.10$; est. ± se = 0.34 ± 0.32, $t_{50} = 1.03$, $p = 0.31$), but guarders had fewer different food items (lower richness) when compared to non-reproductive males (est. ± se = -0.86 ± 0.37, $t_{50} = -2.33$, $p = 0.02$). Guarder males and sneaker males did not differ in terms of food item richness (est. ± se = -0.09 ± 0.52, $t_{50} = -0.18$, $p = 0.86$). Fish size did not predict the abundance or richness of food items in Round Goby digestive tracts (all $p > 0.05$). We found more food items in Round Goby digestive tracts in June compared to in May (0.79 ± 0.19, $t_{50} = 4.02$, $p = 0.0002$) and a greater richness of food items in June compared to May (0.99 ± 0.31, $t_{50} = 3.19$, $p = 0.003$).

Both guarder and sneaker males had higher liver δ^{15}N than non-reproductive males (LM, est. ± se = 0.13 ±0.02, $t_{108} = 7.06$, $p < 0.0001$; est. ± se = 0.08 ± 0.21, $t_{108} = 3.72$, $p = 0.0003$), and guarder males also had higher liver δ^{15}N than sneaker males (est. ± se = 0.044 ± 0.022, t_{108} =

-2.04, $p = 0.044$; Figure 5A). There were no differences in liver $\delta^{13}C$ between male tactics (LM, $N = 113$, all contrasts, $p > 0.05$; Figure 5A), nor did we find tactic differences in muscle tissue $\delta^{15}N$ ($N = 92$, all contrasts, $p > 0.1$, Figure 5B). However, guarder males had more negative muscle $\delta^{13}C$ values when compared to non-reproductive males (LM, est ± s.e. = -0.51 ± 0.20, $t_{87} = -2.51$, $p = 0.014$, Figure 5B, Table 1). Guarder males did not differ from sneaker males in muscle $\delta^{13}C$ (est ± s.e. = 0.25 ± 0.25, $t_{87} = 1.00$, $p = 0.32$), nor did non-reproductive males differ from sneaker males (est ± s.e. = -0.26 ± 0.25, $t_{87} = -1.06$, $p = 0.29$). In all isotope analyses, fish size and collection month did not predict $\delta^{13}C$ and $\delta^{15}N$ (all contrasts, $p > 0.05$).

In liver tissue, guarder males had a larger isotopic niche (standard ellipse area) than both sneaker and non-reproductive males (contrasts in Table 2, Figure 5A, B, Supplementary Figure S1). In muscle tissue, guarder males had the broadest isotopic niche, followed by non-reproductive, and then sneaker males (Table 2). There was a higher degree of isotopic niche overlap (i.e., ellipse overlap) among the male tactics in muscle tissue samples when compared to niches estimated from liver tissue samples (Figure 5A, B, Table 2).

Discussion

Study 1: Male tactics remain stable across the breeding season, across years, and across habitats

The proportion of the Round Goby male population that was reproductive (i.e. guarder and sneaker males) was greatest early in the season (April – June) and then steadily declined until September. On average, guarder males were more abundant than sneaker males and comprised ~ 67% of the reproductive male population. The relative abundance of guarder males versus sneaker males was stable over the breeding season and over sampling years. Non-reproductive males dominated the male population; even during the early breeding season, the majority of the male population is comprised of adult individuals in a non-reproductive state. Long term, multi-site studies of tactic ratios, such as this, are rare, but can inform how environmental variation affects tactic pay-offs and therefore their expected ratios (Taborsky and Brockmann 2010)

We expected to find more guarder males in complex habitats (i.e., cobble, boulder) because such areas offer more suitable resources for nesting, reproduction, and parental care (Corkum et al. 1998). However, we found no differences in the proportion of guarder males across our four habitat types. It is possible that habitat variation indeed had little effect on male tactic ratios, or it is also possible that our study was not powerful enough to detect these differences. Round Goby reproductive behaviour has been scantly observed in the wild, and there is still much to be learned about how guarder, sneaker, and non-reproductive males use habitats and resources. In other species, resource abundance and habitat type are known to affect male tactic ratios. For exmaple, smaller "jacks" in Sockeye Salmon (*Oncorhynchus nerka*) are more abundant in streams with undercut banks that aid with sneaking behaviour when compared to lake or river habitats (DeFillipo et al. 2018). Also, for *Telmatochromis vittatus* in environments with high shell (i.e. nesting resource) density, males are more likely to adopt an alternative "pirate male" tactic that monopolizes multiple nests over the more traditional "nesting male" tactic (Ota et al. 2012). Finally, male *Sancassania berlesei* mites that adopt a "fighter" tactic are better able to outcompete rival "scrambler" males in complex habitats. Naturally aggressive fighter males use the terrain to corner or trap the benign scrambler males (Lukasik et al. 2006).

Study 2: ARTs are unlikely to be sequentially expressed in Round Goby

Our saccular otolith aging showed that guarder and sneaker males overlapped in age (guarder males age: 1 – 4 years, sneaker males age: 1 – 3 years) and that at any given age guarder males were larger than sneaker males. Therefore, a population-specific body size threshold is unlikely to drive a transition between tactics, as had been previously suggested by Bleeker et al. (2017). Our findings indicate that back-calculating age based on body size alone, a technique commonly used in Round Goby research (e.g, MacInnis and Corkum 2000; Sokołowska and Fey 2011; Huo et al. 2014; Duan et al. 2016), will be inaccurate if males are measured without identifying their tactic, as sneaker and guarder males could be the same size but different ages or vice versa. We also found that sneaker males had less growth before their first annuli when compared to guarder males. It is possible that Round Goby that are born later or grow more slowly in the first year of life adopt a sneaker tactic, while the other males adopt a guarder tactic, but this requires more research. Alonzo et al. (2000) found that in Mediterranean Wrasse (*Symphodus ocellatus*) sneaker and satellite males had lower growth in their first year of life when compared to guarding and non-reproductive males. Our data suggests that Round Goby express each male tactic non-sequentially (i.e., males do not necessarily transition from sneakers to guarders). We suggest the "birthdate effect" may underlie tactic adoption in this species (Taborsky 1998) and future studies should explicitly test this idea.

Study 3a: Sneaker and guarder males fed less than non-reproductive males

Based on stomach contents, sneaker males fed the least, non-reproductive males fed the most, and guarder males were intermediate and not statistically different from the other two morphs. Both guarder and sneaker males had lower food item richness in their digestive tracts when compared to non-reproductive males. Taken together, it appears that guarders and sneakers have more limited feeding during the reproductive season. We had expected guarder males to feed the least overall, but have greater variety in the items they consumed (i.e., opportunistic feeding) because they are spatially restricted while nesting and tending offspring (e.g., Cogliati et al. 2015; Félix et al. 2016). It is possible that we did not see a significant difference between sneaker and guarder males because our sampling method, minnow traps, may have collected guarder males directly before or after they had taken up nests and were no longer providing care. However, only guarders and sneakers were found with completely empty digestive tracts, consistent with the idea of restricted feeding for both reproductive tactics.

The types of food items found in the digestive tracts were similar to other studies of Round Goby diet from elsewhere in the Laurentian Great Lakes (e.g., Carman et al. 2006; Raby et al. 2010; Duncan et al. 2011; Brush et al. 2012; McCallum et al. 2017a; Pothoven 2018). The food items identified were similar across the tactics in our study—with the exception of fish scales. Sixty-eight percent of the guarder males had fish scales in their digestive tracts compared to only 31% and 15% for non-reproductive and sneaker males, respectively (Figure 4A). Round Goby display high levels of intraspecific aggression over shelter resources and it is possible that guarders ingest competitor scales while fighting for nesting sites or in the collection traps (Groen et al. 2012; McCallum et al. 2017b). Alternatively, Round Goby may scavenge on carcasses or passively acquire scales while feeding in the benthos (Polačik et al. 2015).

Study 3b: Guarder males have larger isotopic niches, indicating opportunistic foraging

Liver $\delta^{15}N$ values suggested that guarder males fed at the highest trophic position, followed by sneakers and then non-reproductive males, but no such pattern was revealed with muscle $\delta^{15}N$ analyses. There were no or minor differences in $\delta^{13}C$ between reproductive morphs in both tissues, which was not surprising given that the fish were collected in the same location. Isotopes in liver and muscle are indicative of feeding over different time scales (days to weeks, weeks to months, respectively; Trudel et al. 2010), and round goby migrate offshore in the winter (Kornis et al. 2012). The similar muscle isotope values could indicate a common over-wintering diet with limited options or limited variation in isotopes between items in the deeper habitat, followed by a tactic specific shift with the onset of reproductive behaviour. Additionally, starvation stress has also been shown to increase $\delta^{15}N$ (Smith et al. 2013; Bowes et al. 2014), which could explain higher levels in the guarder and sneaker males because they consumed fewer prey items.

Isotopic niches were generally consistent with stomach contents and mean isotope values, indicating that guarder males had an overall broader diet than sneaker males. This was a consistent pattern in both tissues, although the difference was smaller for muscle. These results further support that guarders, who are restricted spatially—although the extent of that restriction has not been quantified in Round Goby—are opportunistic feeders. The small isotopic niche of the sneakers was consistent between muscle and liver and indicates that these males have more selective or restricted diets (e.g., perhaps due to smaller gape widths). The smaller sneaker isotopic niche could also indicate consistent diet among individuals but on a wide variety of items (i.e., low variability between sneakers); however, stomach contents suggest they consume a smaller range of diet items. Isotopic niche overlap between ART morphs was smallest in liver, showing some partitioning of resources during the breeding season. Sneakers and guarders had the greatest overlap, while the low overlap with non-reproductive males suggested that the fish utilize a different prey base depending on the reproductive season. Overlap was much greater between all three tactics in muscle, reaffirming that there is likely a common diet during the non-breeding season. Generally, the stable isotope values and isotopic niche sizes were similar to those reported recently for Round Goby from a study in Lake Ontario that did not account for ARTs (Mumby et al. 2017). These findings, when combined with the gut content results, demonstrate that reproductive tactics can influence feeding ecology. The implications of variation in feeding ecology among male ARTs for round goby adults and offspring needs further study. It would be beneficial to quantify fine-scale movement among tactics in relation to feeding and breeding, potentially with acoustic telemetry studies, to understand seasonal shifts in diet.

Conclusions

Few studies on the invasive Round Goby have accounted for or acknowledged variation that can be caused by the presence of ARTs (see Supplementary Materials for literature review). Future work on male ARTs in Round Goby and other fish species should focus on describing the behavioural repertoire of each male tactic, particularly that of sneaker males, and assessing the extent of paternity loss due to competition between and within the tactics. It would be worth considering how management techniques might exploit the unique characteristics of reproducing individuals of this invasive species (Corkum and Belanger 2007). For example, it was recently shown that Round Goby guarder and sneaker males were less often caught in a novel auditory trap than were non-reproductive males and females (Isabella-Valenzi and Higgs 2016). Additionally, models that have been used to examine management strategies of Round Goby do not include ARTs, but these models should because life history will influence responses to management actions (e.g., Vélez-Espino et al. 2010; N'Guyen et al. 2018). We have shown that male ARTs in Round Goby differ greatly in their life history strategies, their population demographics, and also their diet and foraging. We emphasize the importance of considering male ARTs when studying the biology and management of invasive and native fishes.

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Table 1: Summary of sample size (*N*), standard length (SL, cm), δ^{13}C, and δ^{15}N values for Round Goby collected in 2016 from Lake Ontario for stable isotope and isotopic niche analyses. Values are shown as mean (± s.d.). NA = not available.

	N	**SL, cm**	**δ^{13}C**	**δ^{15}N**
Baseline bivalves	34	NA	-23.48 (0.92)	10.06 (0.34)
LIVER				
Non-reproductive	37	8.41 (1.46)	-22.08 (0.93)	12.70 (0.93)
Guarder	43	8.88 (1.18)	-22.59 (1.32)	14.47 (1.44)
Sneaker	33	6.82 (0.88)	-21.77 (1.18)	13.63 (0.89)
MUSCLE				
Non-reproductive	36	8.36 (1.46)	-20.74 (0.79)	13.92 (1.16)
Guarder	33	8.47 (0.88)	-21.27 (1.00)	13.82 (0.78)
Sneaker	23	7.17 (0.75)	-20.99 (0.71)	13.97 (0.56)

Table 2: Isotopic niche metrics among round goby male tactics collected in 2016. Niche size (Bayesian Standard Ellipse Area, SEA$_B$) was calculated from 10^5 Bayesian iterations of $\delta^{13}C$ and $\delta^{15}N$ bi-plot ellipses and is shown here with upper-lower 95% credible intervals in parentheses. The probability of niche size difference is shown as the probability that ellipse A is larger than ellipse B, with higher probabilities indicating a higher likelihood of size difference and vice versa. *indicates ellipse size difference is considered significant (> 0.95 and < 0.05 if ellipse B is > A). Niche overlap area (permille squared, ‰^{2}) gives the area of overlap between two ellipses, while overlap percentage gives the percentage area overlapping of the total area occupied by both ellipses. All calculations done with SIBER 2.1.3 in R.

	Liver	**Muscle**
	Isotopic niche size, SEA$_B$	
Non-reproductive	2.61 (1.90 – 3.68)	1.94 (1.40 – 2.73)
Guarder	5.84 (4.31 – 7.90)	2.86 (2.92 – 4.05)
Sneaker	1.68 (1.22 – 2.44)	0.76 (0.51 – 1.19)
	Probability of niche size difference (ellipse A > ellipse B)	
Guarder vs sneaker	1.00*	1.00*
Guarder vs non-reproductive	0.99*	0.95*
Sneaker vs. non-reproductive	0.04*	0.00*
	Niche overlap area ‰^{2} (overlap %)	
Guarder vs sneaker	2.06 (30%)	1.27 (49%)
Guarder vs non-reproductive	0.67 (8%)	1.58 (40%)
Sneaker vs. non-reproductive	0.82 (16%)	1.17 (38%)

Figure 1: Photograph showing examples of the two male alternative reproductive tactics in Round Goby. Inset shows anterior/facial characteristics of the two male tactics. Photographs were taken by Hossein Mehdi.

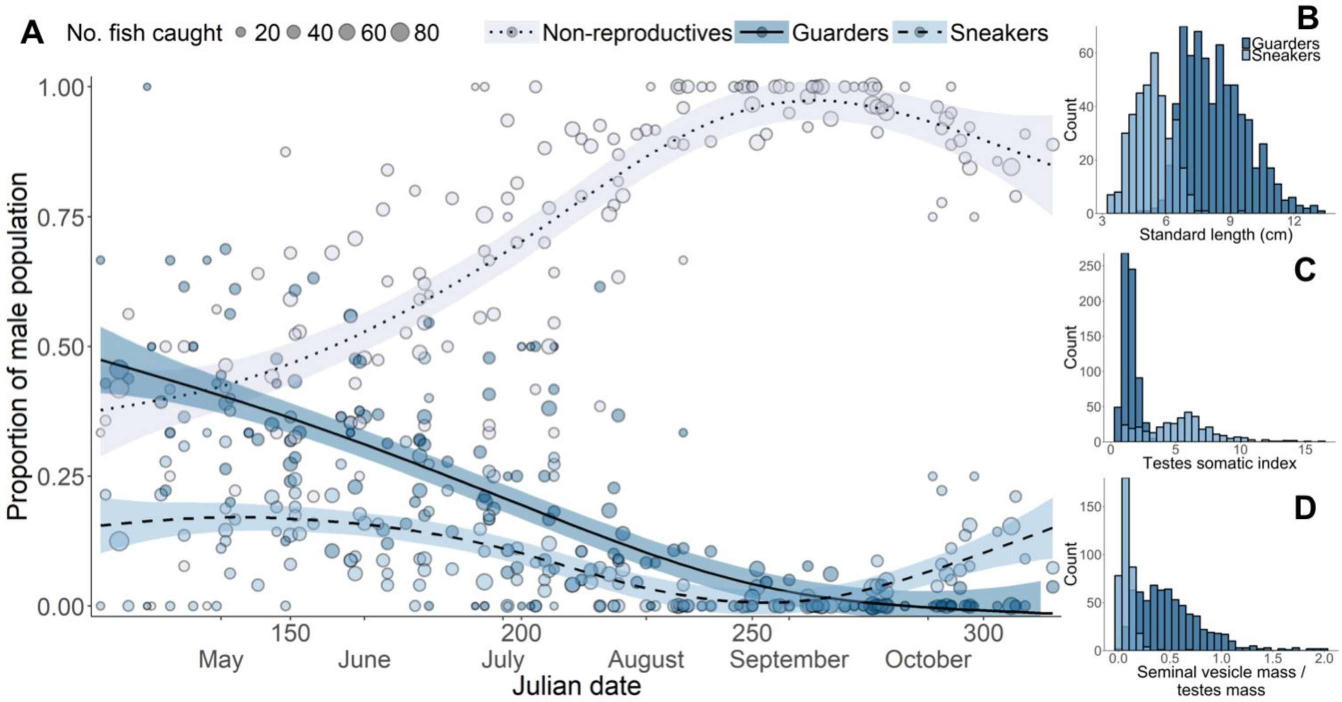

Figure 2: (A) Proportion of the Round Goby male population that are non-reproductive males (light blue), guarder males (dark blue), and sneaker males (intermediate blue), plotted by Julian date. Data are from long term population monitoring in Hamilton Harbour (2006-2017) pooled across the four field sites. Lines show the mean proportion for each tactic over time, with the ribbons indicating 95% confidence interval around the mean. **(B-D)** panels show histograms of the variables used by the LDA to classify males into guarder males or sneaker males.

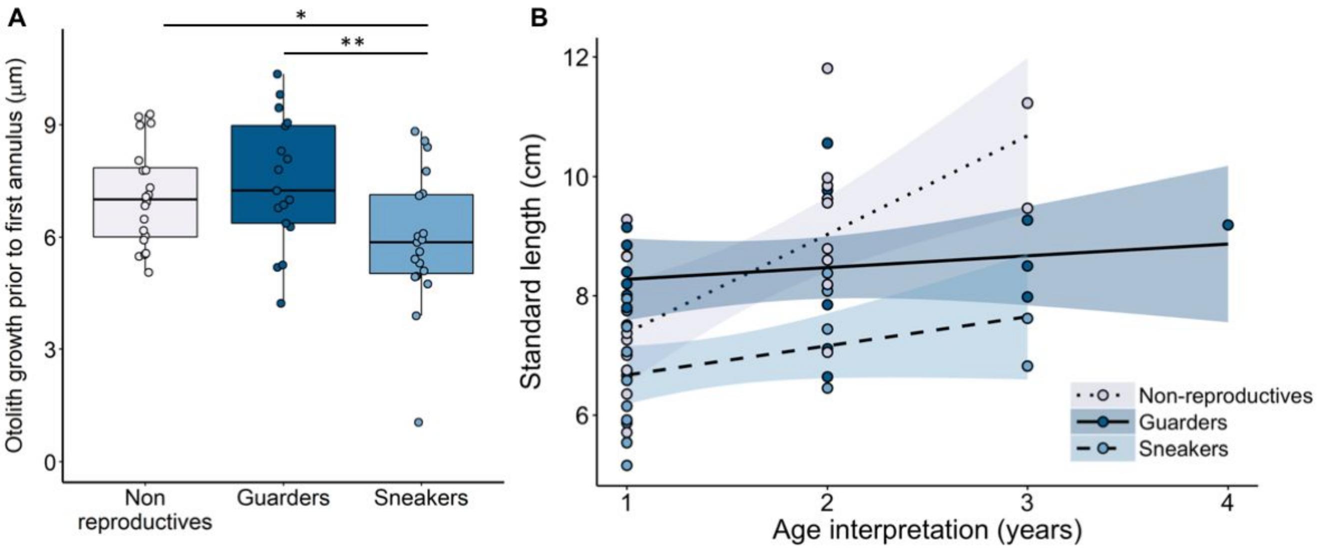

Figure 3: (A) Otolith growth in the first year of life, plotted by reproductive tactic. Boxplots show median, inter-quartile range (box), and maximum and minimum values excluding outliers (whiskers), raw data points are overlaid on the boxplot. **(B)** Size-at-age relationship for Round Goby males plotted by reproductive tactic. Lines indicate the mean size-at-age, ribbons show the 95% confidence interval around the mean, and raw data points are overlaid on the plot. * $p < .05$, ** $p < .01$.

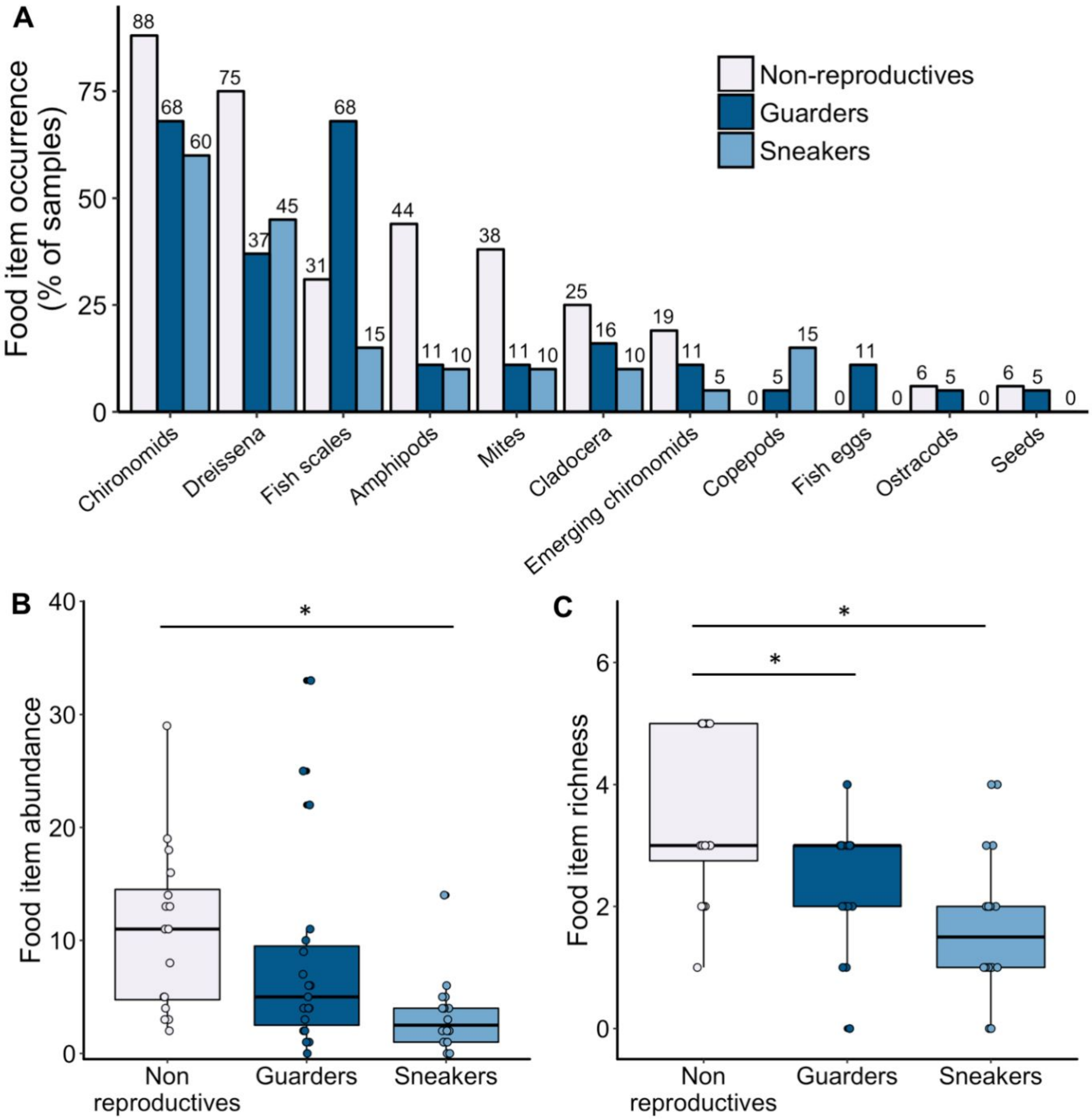

Figure 4: (A) Percent occurrence of food items in Round Goby digestive tracts, plotted by male tactic. (**B)** Food item abundance in Round Goby digestive tracts, plotted by a male tactic. (**C)** Food item richness in Round Goby digestive tracts, plotted by male tactic. Boxplots show median, inter-quartile range (box), and maximum and minimum values (whiskers), raw data points are overlaid on the boxplot. * $p < 0.05$.

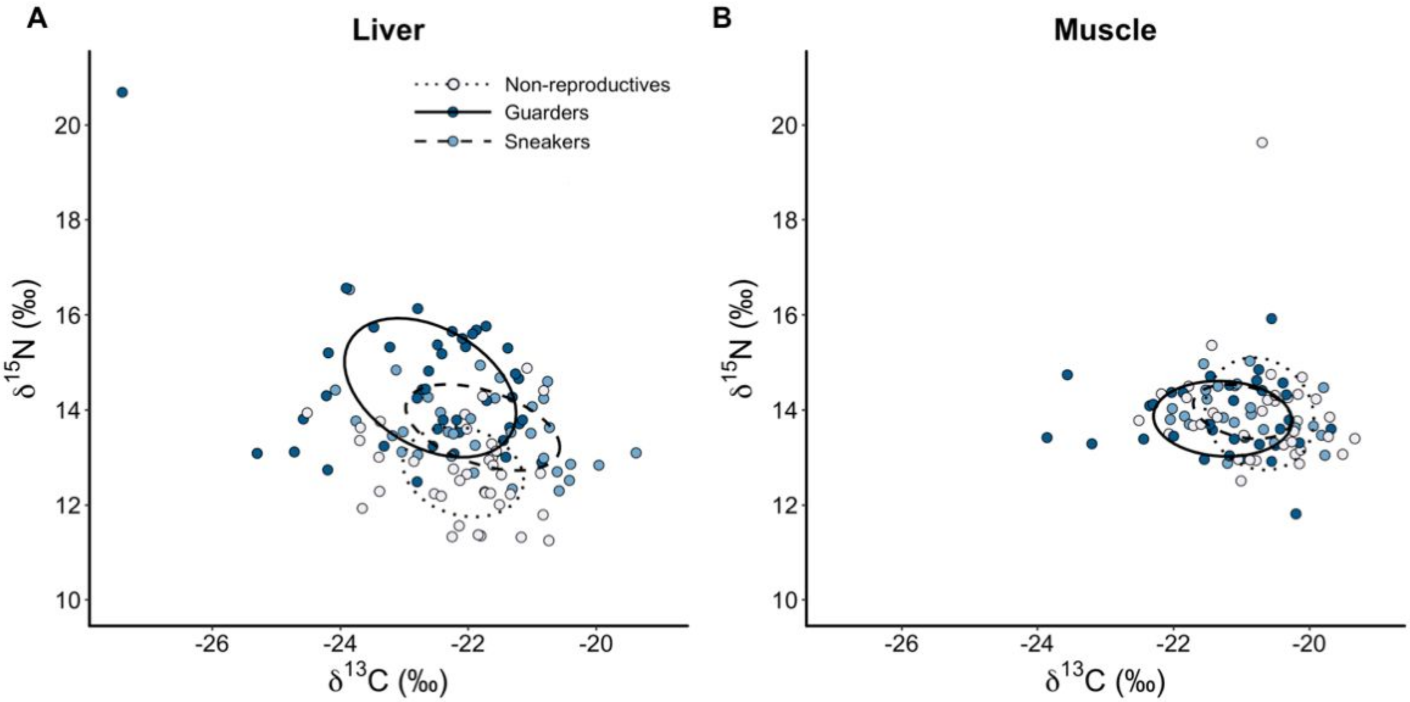

Figure 5: Bi-plots of $\delta^{13}C$ and $\delta^{15}N$ with standard ellipse areas from Round Goby **(A)** liver and **(B)** muscle tissue. Standard ellipses capture 40% of the spread in the data, graphs and ellipses are generated using ggplot2 in R.