

# 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, multisite Laurentian Great Lakes data set, we show that the guarder male tactic was twice as common as the sneaker male tactic but that nonreproductive males were the most common morph. 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 that the ARTs are nonsequentially 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 with nonreproductive 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.

**Résumé :** Si différentes tactiques de reproduction peuvent se traduire par des variations considérables du cycle biologique au sein d'une même espèce, dans bien des cas, elles ne sont pas prises en considération. Nous utilisons le gobie à taches noires (*Neogobius melanostomus*), une espèce envahissante, pour examiner différentes questions relatives à l'écologie et l'évolution touchant aux différentes tactiques de reproduction. À la lumière d'un ensemble de données de 12 ans couvrant de multiples sites dans la région des Grands Lacs laurentiens, nous démontrons que les mâles gardiens étaient deux fois plus répandus que les mâles furtifs, mais que les mâles non reproducteurs représentaient la tactique la plus répandue. Le rapport des mâles gardiens et furtifs ne variait pas dans l'espace, malgré une grande fourchette de densité des ressources entre les sites. Les fourchettes d'âge des mâles gardiens et furtifs étaient semblables, donnant à penser que les différentes tactiques de reproduction ne s'expriment pas séquentiellement. À la lumière d'analyses des régimes alimentaires à court terme (contenus stomacaux), les individus adoptant les deux tactiques de reproduction consommaient moins de types d'aliments et avaient tendance à consommer moins d'articles en général que les mâles non reproducteurs. Des analyses des régimes alimentaires sur le long terme (isotopiques) montrent que les mâles gardiens s'alimentaient à un niveau trophique plus élevé ( $\delta^{15}$ N plus grands) et occupaient une niche isotopique plus large. Nos résultats montrent que l'adoption de différentes tactiques de reproduction est un aspect important du système de reproduction de cette espèce envahissante qui devrait être intégré à l'évaluation et la gestion des populations. [Traduit par la Rédaction]

## 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

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**Fig. 1.** Photograph showing examples of the two male alternative reproductive tactics (ARTs) in round goby. Inset shows anterior or facial characteristics of the two male tactics. Photographs were taken by Hossein Mehdi. [Colour online.]



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) 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 through either 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  $\sim$ 2 years and use stealthy tactics to sneak into nests and cuckold caring males. But later, as the sneakers 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 (Fig. 1). In contrast, sneaker males are smaller, have a more mottled colouration, invest more in their testes mass relative to their body mass, and have more sperm cells per ejaculate volume (Marentette et al. 2009; Fig. 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<sup>1</sup>). 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, yet unexplored, system in which to study the evolution of ARTs.

In this study, we address 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, multiyear, multisite data set from Hamilton Harbour in the Laurentian Great Lakes to ascertain how round goby catch abundance and male tactic ratios differ across time and 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, and developmental threshold) are not known for round goby and are currently only known for a handful of species. Bleeker et al. (2017) suggested that round goby first adopt the sneaker tactic and then 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 nonsequential mechanisms (e.g., genetic polymorphism and 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 ( $\delta^{15}N$  and  $\delta^{13}C$ ) 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 Wootton 1995). We therefore also expected guarder males to have a broader isotopic niche relative to sneaker and nonreproductive males.

<sup>1</sup>Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2018-0340.

**Fig. 2.** (A) Proportion of the round goby male population that are nonreproductive 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 morph over time, with the ribbons indicating a 95% confidence interval around the mean. (B–D) Histograms of the variables used by the linear discriminant analysis (LDA) to classify males into guarder males or sneaker males. [Colour online.]



### Methods

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

The round goby population in Hamilton Harbour (Ontario, Canada; 43°17'N, 79°49'W) has been monitored intensively 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 2 weeks from May to October, baited minnow traps were deployed at ~1 m 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 0.01 cm. We measured the masses of the whole body, testes, and seminal vesicles (male accessory gland organs, which together with the testes make the gonad) using a digital balance accurate to the nearest 0.001 g (Ohaus Adventurer Pro). We used total gonad mass (testes mass + seminal vesicles mass) to calculate gonadosomatic index (GSI = 100 × [gonad mass (g)]/[body mass (g) – gonad mass (g)]).

Males were identified as reproductive or nonreproductive 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 2006 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 or sneaker males, training the LDA on a subsample of fish between 2007 and 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 data set: (i) standard length (to the nearest 0.1 cm), (ii) testes somatic index (testes mass as percentage of total body mass), and (*iii*) the seminal vesicle mass to testes mass ratio (Figs. 2B, 2C, and 2D). 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 data set (2007–2010) 89% of the time and assigned tactics to 93% of all reproductive males in the whole data set. Thus, our final data set consisted of 2973 nonreproductive males and 1132 reproductive males (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 throughout the round goby breeding season and differed among four distinct habitat types (mud, sand, cobble, and 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 Fig. 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 quasibinomial 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 in a later section) from Fifty Point Conservation Area, Hamilton (Ontario, Canada; 43°13'N, 79°37'W), on two sampling occasions in 2016 (18 May and 29 June). Following fish capture methods described by 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); therefore, we set traps at dusk, tied them off at shore at a depth of  $\sim 1$  m, and collected them  $\sim$ 3 h later (i.e., when the sun had completely set). We sorted the male round goby that were collected into three categories based on external morphology: guarder male, sneaker male, and nonreproductive male. We dissected all male fish onsite, confirmed their tactics by examining their gonads, recorded all morphological measures (detailed in the previous section on Study 1), extracted their saccular otoliths, and performed additional measures for specific diet and stable isotope analyses (detailed in a later section).

We had one saccular otolith per male (from 17 guarder males, 19 sneaker males, and 20 nonreproductive males) aged by an experienced otolith reader who had no prior information about the individual fish or its morphology. The fish that 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 80× 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  $\mu$ m) 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) among guarder males, sneaker males, and nonreproductive males. Otolith growth is often used as a proxy for somatic growth (Campana and Neilson 1985). We fit a linear model (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 among guarder males, sneaker males, and nonreproductive males. We fit an 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 previously, we collected 113 round goby for diet and stable isotope analyses. While dissecting the males on-site (see previous section), we removed the digestive tract (from the esophagus to the anus) from 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, and 16 nonreproductive males) under a dissecting scope at 2× magnification (Leica 151 MZ75). Items in the gut were counted and identified down to the lowest possible taxonomic group. We fit two LMs after log-transformation: 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). 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

**Table 1.** Summary of sample size (N), standard length (SL, cm),  $\delta^{13}$ C, and  $\delta^{15}$ N values for round goby collected in 2016 from Lake Ontario for stable isotope and isotopic niche analyses.

-	-	-		
	Ν	SL (cm)	δ <sup>13</sup> C	$\delta^{15}N$
Baseline bivalves	34	NA	-23.48 (0.92)	10.06 (0.34)
Liver				
Nonreproductive	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				
Nonreproductive	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)
NY 1 17 1 1		(1.6D) N		

Note: Values are shown as mean (± SD). NA, not available.

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 by Pettitt-Wade et al. (2015) (see Supplementary Materials for further details, including accuracy and precision data<sup>1</sup>). Primary consumers did not vary in  $\delta^{15}$ N among samplings (LM: *N* = 34, estimate (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 an LM with  $\delta^{13}$ C or  $\delta^{15}$ N as the response variable and male tactic, month, and standard length as the predictors.

To compare isotopic niche size and niche overlap between the male tactics, we constructed  $\delta^{13}C$  and  $\delta^{15}N$  biplots and standard ellipse areas (SEAs) (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<sup>5</sup> posterior draws, also provided 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).

### 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 nonreproductive individuals (Fig. 2A). We caught 57 ± 25 guarder males (mean  $\pm$  SD; range = 19–107) and 31  $\pm$  14 (range = 14–60) sneaker males per year across the four sites. Guarder males comprised  $67\% \pm 17.5\%$  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<sup>1</sup>). The proportion of guarder males within the reproductive male population during the breeding season did not vary with either Julian date (GLMM, est.  $\pm$  SE = -0.054  $\pm$  0.076, z = -0.72, p = 0.47) or habitat type (all pairwise contrasts were at least est. ±  $SE = -0.28 \pm 0.19$ , z = -1.48, p = 0.14). Furthermore, the proportion of guarder males in the reproductive male population neither

Fig. 3. (A) Otolith growth in the first year of life, plotted by reproductive tactic. Boxplots show median, interquartile range (box), and maximum and minimum values (whiskers), excluding outliers. 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* < 0.05; \*\*, *p* < 0.01. [Colour online.]



increased nor decreased across the 12 years of population monitoring (GLM, est.  $\pm$  SE = -0.0032  $\pm$  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 nonreproductive males were  $1.8 \pm 1.0$ ,  $1.4 \pm 0.7$ , and  $1.7 \pm 0.7$  years (mean  $\pm$  SD), respectively (ranges = 1-4, 1-3, and 1-3 years, respectively). Age strongly correlated with body length in nonreproductive males (LM, est. ± SE = 0.61  $\pm$  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.  $\pm$  SE = 0.068  $\pm$  0.089,  $t_{50}$  = 0.76, p = 0.45). Sneaker males were smaller than both guarder males (est.  $\pm$  SE = -1.43  $\pm$  0.36,  $t_{50}$  = -3.98, p < 0.001; Fig. 3A) and nonreproductive males (est.  $\pm$  SE =  $-1.55 \pm 0.34$ ,  $t_{50} = -4.55$ , p < 0.0001), but nonreproductive males and guarder males did not differ in body size (est.  $\pm$  SE = -0.12  $\pm$ 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 initial growth increments (before the first annulus) than those of both guarder males (LM, est.  $\pm$  SE = -1.54  $\pm$  0.55,  $t_{53}$  = -2.81, p = 0.007) and nonreproductive males (est.  $\pm$  SE = -1.15  $\pm$  0.53,  $t_{53}$  = -2.19, p = 0.03). Guarder males and nonreproductive males did not differ significantly in the widths of the first growth increments (est.  $\pm$  SE = 0.39  $\pm$  0.54,  $t_{53}$  = 0.72, p = 0.48; Fig. 3A). Our data also suggest that following 1 year of age, the two male ARTs had similar somatic growth rates (interaction term, est.  $\pm$  SE = -0.08  $\pm$  0.15,  $t_{50}$  = -0.56, p = 0.58; Fig. 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 (Fig. 4A). Compared with nonreproductive males, sneaker males had fewer food items (LM, est.  $\pm$  SE = -0.74  $\pm$  0.30,  $t_{50}$  = -2.44, p = 0.02; Fig. 4B) and a lower richness (count of taxonomic groups) of food types in their digestive tracts (LM, est.  $\pm$  SE = -0.96  $\pm$  0.47,  $t_{50}$  = -2.02, p = 0.049; Fig. 4C). Guarder males did not differ from nonreproductive males or sneaker males in terms of the number of food items in their digestive tracts (est.  $\pm$  SE = -0.40  $\pm$  0.24,  $t_{50}$  = -1.67, p = 0.10; est.  $\pm$  SE = 0.34  $\pm$  0.32,  $t_{50} = 1.03$ , p = 0.31; respectively), but guarders consumed fewer different food items (lower richness) when compared with nonreproductive males (est.  $\pm$  SE = -0.86  $\pm$ 0.37,  $t_{50} = -2.33$ , p = 0.02). Guarder and sneaker males did not differ in terms of food item richness (est.  $\pm$  SE = -0.09  $\pm$  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 the month of June compared with May (0.79  $\pm$  0.19,  $t_{50}$  = 4.02, p = 0.0002) and a greater richness of food items in June compared with May (0.99  $\pm$  $0.31, t_{50} = 3.19, p = 0.003).$ 

Both guarder and sneaker males had higher liver  $\delta^{15}N$  than nonreproductive males (LM, est.  $\pm$  SE = 0.13  $\pm$  0.02,  $t_{108}$  = 7.06, p < 0.0001; est. ± SE = 0.08 ± 0.21,  $t_{108}$  = 3.72, p = 0.0003; respectively), and guarder males also had higher liver  $\delta^{\rm 15}N$  than sneaker males (est.  $\pm$  SE = 0.044  $\pm$  0.022,  $t_{108}$  = -2.04, p = 0.044; Fig. 5A). There were no differences in liver  $\delta^{13}C$  between male tactics (LM, N = 113, all contrasts, p > 0.05; Fig. 5A), nor did we find tactic differences in muscle tissue  $\delta^{15}N$  (N = 92, all contrasts, p > 0.1; Fig. 5B). However, guarder males had more negative muscle  $\delta^{13}$ C values when compared with nonreproductive males (LM, est. ± SE =  $-0.51 \pm 0.20$ ,  $t_{87} = -2.51$ , p = 0.014; Fig. 5B; Table 1). Guarder males did not differ from sneaker males in muscle  $\delta^{13} C$  (est. ± SE = 0.25  $\pm$  0.25,  $t_{87}$  = 1.00, p = 0.32), nor did nonreproductive males differ from sneaker males (est.  $\pm$  SE = -0.26  $\pm$  0.25,  $t_{87}$  = -1.06, p = 0.29). In all isotope analyses, fish size and collection month did not predict  $\delta^{13}$ C or  $\delta^{15}$ N (all contrasts, p > 0.05).

In liver tissue, guarder males had a larger isotopic niche (SEA) than both sneaker and nonreproductive males (contrasts in Table 2; Figs. 5A and 5B; also refer to Supplementary Materials Fig. S1<sup>1</sup>). In muscle tissue, guarder males had the broadest isotopic niche, followed by nonreproductive males, and then sneaker males (Table 2). There was a higher degree of isotopic niche overlap (i.e., ellipse overlap) among the male morphs in muscle tissue samples when compared with niches estimated from liver tissue samples (Figs. 5A and 5B; Table 2).

### Discussion

#### Study 1: Male tactics remain stable across breeding season, years, and 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  $\sim$  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. Nonreproductive males dominated the male population; even during the early breeding season, the majority of the male population is comprised of adult individuals in a nonreproductive state. Long-term, multisite studies of tactic ratios such as this

**Fig. 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 male tactic. (C) Food item richness in round goby digestive tracts, plotted by male tactic. Boxplots show median, interquartile range (box), and maximum and minimum values (whiskers). Raw data points are overlaid on the boxplot. \*, p < 0.05. [Colour online.]



**Fig. 5.** Biplots of  $\delta^{13}$ C and  $\delta^{15}$ N with standard ellipse areas (SEAs) from round goby (A) liver and (B) muscle tissue. Standard ellipses capture 40% of the spread in the data. Graphs and ellipses were generated using ggplot2 in R. [Colour online.]



are rare but can inform how environmental variation affects tactic payoffs and, therefore, their expected ratios (Taborsky and Brockmann 2010).

We expected to find more guarder males in complex habitats (i.e., cobble and 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 the four habitat types. It is indeed possible that habitat variation 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 nonreproductive males use habitats and resources. In other species, resource abundance and hab**Table 2.** Isotopic niche metrics among round goby male tactics collected in 2016.

	Liver	Muscle
Isotopic niche size, ‰ <sup>2</sup> , SEA <sub>B</sub>		
Nonreproductive	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 differe	nce (ellipse A > e	llipse B)
Guarder versus sneaker	1.00*	1.00*
Guarder versus nonreproductive	0.99*	0.95*
Sneaker versus nonreproductive	0.04*	0.00*
Niche overlap area, ‰ <sup>2</sup> (overlap	9 %)	
Guarder versus sneaker	2.06 (30%)	1.27 (49%)
Guarder versus nonreproductive	0.67 (8%)	1.58 (40%)
Sneaker versus nonreproductive	0.82 (16%)	1.17 (38%)

Note: Niche size (‰<sup>2</sup>, per mille squared; SEA<sub>B</sub>, Bayesian standard ellipse area) was calculated from 10<sup>5</sup> Bayesian iterations of  $\delta^{13}$ C and  $\delta^{15}$ N biplot 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. An asterisk (\*) indicates that ellipse size difference is considered significant (>0.95 and <0.05 if ellipse B is >A). Niche overlap area 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 were done with SIBER 2.1.3 in R.

itat type are known to affect male tactic ratios. For example, smaller "jacks" in sockeye salmon (*Oncorhynchus nerka*) are more abundant in streams with undercut banks that aid with sneaking behaviour when compared with lake or river habitats (DeFilippo 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 (1-4 and 1-3 years, respectively) 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 backcalculating 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 with 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 with guarder and nonreproductive males. Our data suggest that round goby express each male tactic nonsequentially (i.e., males do not necessarily transition from sneakers to guarders). We suggest that 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 nonreproductive males

Based on stomach contents, sneaker males fed the least, nonreproductive 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 with nonreproductive 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 those of 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. 2017*a*; Pothoven 2018). The food items identified were similar across the morphs in our study, with the exception of fish scales. Sixty-eight percent of the guarder males had fish scales in their digestive tracts, compared with only 31% and 15% for nonreproductive and sneaker males, respectively (Fig. 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. 2017*b*). 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 nonreproductive males, but no such pattern was revealed with muscle  $\delta^{15}N$ analyses. There were no or minor differences in  $\delta^{13}C$  among 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 and 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 overwintering diet with limited options or limited variation in isotopes among items in the deeper habitat, followed by a tactic-specific shift with the onset of reproductive behaviour. Additionally, starvation stress has 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 among sneakers); however, stomach contents suggest that they consume a smaller range of diet items. Isotopic niche overlap among 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 nonreproductive males suggested that the fish utilize a different prey base depending on the reproductive season. Overlap was much greater among all three morphs in muscle, reaffirming that there is likely a common diet during the nonbreeding season. Generally, the stable isotope values and isotopic niche sizes were similar to those reported recently for round goby in Lake Ontario from a study that did not account for ARTs (Mumby et al. 2018). These findings, when combined with the gut content results, demonstrate that reproductive tactics can influence feeding ecology. The implications of variation in feeding ecology between male ARTs for round goby adults and offspring needs further study. It would be beneficial to quantify fine-scale movement between 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<sup>1</sup>). 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 nonreproductive 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; however, they 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, population demographics, 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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