# ALTERNATIVE REPRODUCTIVE TACTICS

# AND FISH TRAPPING BIASES

# ALTERNATIVE REPRODUCTIVE TACTIC BEHAVIOUR AND WITHIN GEAR-TYPE TRAPPING BIAS OF THE INVASIVE ROUND GOBY, *NEOGOBIUS MELANOSTOMUS*

By

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TITLE: Alternative Reproductive Tactic Behaviour and Within Gear-Type Trapping Bias of the Invasive Round Goby, *Neogobius melanostomus*

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## ABSTRACT

Alternative reproductive tactics (ARTs), occur where members of one sex of a species have two or more strategies of obtaining fertilization. The tactics differ in behavioural approaches to reproduction, but also commonly differ in physiological and morphological traits. The round goby, *Neogobius melanostomus*, is a globally invasive species with male ARTs. How behaviour may influence invasiveness in round goby has been of recent interest, but researchers have not considered the role or impact that male ARTs may have. I compared guarder and sneaker male round goby, assessing differences in their boldness, activity, exploration, sociality, aggression, startle responses, and dispersal tendencies. Sneaker males were more bold, active, and explorative while guarder males were more aggressive. In addition, I studied whether more guarders or sneakers are caught in a population survey study and how variation in a common round goby trapping method, minnow traps, may create catch biases. Two commonly employed minnow trap models include a black vinyl plastic coated metal trap (black traps) and a galvanized metal steel trap (silver traps). I investigated whether these black and silver traps and baited (corn) and unbaited traps differ in terms of the numbers, ART ratios, and sizes of round goby captured. I found silver traps captured 1.7 times more round goby than black traps, while baited traps captured 3.4 times more round goby than unbaited traps. Baited traps captured larger round goby and tended to capture more guarders than unbaited traps. I also found black traps captured larger males, but there was no difference in the size of females captured. Taken together my results indicate that care needs to be applied when making estimates of round goby populations in terms of the types of individuals present and the trapping method used.

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## THESIS ORGANIZATION AND FORMAT

This thesis is organized into four chapters. **Chapter 1** details pertinent background theory that will help more fully understand and appreciate the empirical studies in the thesis. This first chapter also introduces my study species, study sites, and research aims. **Chapter 2** is a manuscript, soon to be submitted for publication, that describes a study where I assessed how non-reproductive behaviour differed between round goby males adopting alternative reproductive tactics. **Chapter 3** is a manuscript assessing efficiency and selectivity of round goby capture by two different models of minnow traps, and it too will soon be submitted for publication. **Chapter 4** summarizes the findings of Chapters 2 and 3 and offers avenues for future research that build upon the findings in this thesis.

**CHAPTER 1:** The invasive round goby, behavioural variation between alternative reproductive tactics, and fisheries gear selectivity & efficiency.

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**CHAPTER 2:** Non-reproductive behavioural differences between male alternative reproductive tactics in the invasive round goby.

**Authors**: Caitlyn Synyshyn, Alexandra E. Green-Pucella, Sigal Blashine

**Publication**: We plan to submit this manuscript to the journal Animal Behaviour

**Comments**: This manuscript was based on the work conducted by CS with the help of AEG and under the supervision of SB.

**CHAPTER 3:** Within-gear variation impacts population estimates in an invasive fish.

**Authors**: Caitlyn Synyshyn, Sigal Balshine

**Publication**: We plan to submit this manuscript to The Canadian Journal of Fisheries and Aquatic Sciences

**Comments**: This manuscript was based on the work conducted by CS under the supervision of SB.

**CHAPTER 4**: Discussion and future directions

Author: Caitlyn Synyshyn

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# CHAPTER 1

## THE INVASIVE ROUND GOBY, BEHAVIOURAL VARIATION BETWEEN ALTERNATIVE REPRODUCTIVE TACTICS, AND FISHERIES GEAR SELECTIVITY & EFFICIENCY

### *Introduction*

Alternative reproductive tactics (ARTs) are alternative ways for members of the same sex of a species to achieve reproduction, arising when there is an evolutionary advantage to employing a different tactic (Taborsky and Brockmann 2010). ARTs are present in many diverse taxa including birds, fish, insects, and crustaceans (Gross 1982, Starks 1998, Bachman and Widemo 1999, Moczek and Emlen 2000, Johnson and Brockmann 2012, Han and Jablonski 2016). Most of the research on ARTs has focused around how morphological, physiological, and mating based behavioural traits differ among tactics. Only a handful of studies have sought to understand whether the individuals employing these tactics also differ in their *non-reproductive* behaviours (Han and Jablonski 2019, Wilson and Kelly 2019). Such behavioural differences induced by ARTs need to be considered as behavioural variation could increase population persistence (Conner and White 1999), and may even have implications for managing an invasive species (Chapple et al. 2012). To address the first aim of my thesis, I used the invasive round goby, *Neogobius melanostomus,* to explore how guarder and sneaker male alternative reproductive tactics differ in boldness, exploration, activity, sociality, aggression, and dispersal behavioural tendencies.

Research on fish ARTs or any aspect of fish biology in the wild requires that researchers sample fish underwater or bring the fish to the surface for study. A number of trapping gears and different fish capture methods have been developed to effectively catch many fish species. These include common gear such as seine nets, gill nets, fishing lines, and electrofishers, which can be used to capture fish for recreation, consumption, and/or research. Gear type bias—generally referred to as selectivity—exists, where each gear type is more likely to catch one size range, sex, and even individuals with specific behaviours (Diaz Pauli et al. 2015). Such biases need to be, and generally are, considered when estimating population and community compositions of fishes (Huse et al. 2000, Stergiou and Erzini 2002, Diana et al. 2006, Diaz Pauli et al. 2015). For example, in a study assessing how round goby size varies across gear types, minnow traps were found to capture smaller goby on average (80.6 mm) compared to trotlines (103.9 mm) and gill nets (100.7 mm).

While researchers are generally aware of and try to avoid the "among gear type bias", there can also be substantial variation within a single gear type. In contrast to the "among gear type bias", the "within gear type bias" is not well researched and subsequently often ignored. Minnow traps are an extremely common gear type used to capture small bodied fish, such as round goby, and come in a variety of materials and designs (ex. fabric, metal, rectangular, cylindrical). Two of the most common minnow trap models are black vinyl-coated metal (black) traps and galvanized steel (silver) traps. These traps could have their own set of biases and treating them interchangeably or comparing data using different trap types could lead to erroneous conclusions, which could be harmful especially in the context of invasive species research and management. The second aim of my thesis was to investigate the degree of "within gear bias" in two commonly used minnow trap models with respect to round goby catch rates and population parameters.

To address the second aim of my thesis I compared the catch rates, sex ratios, ratio of alternative reproductive tactics, and size of round goby caught in black versus silver minnow traps. Additionally, I assessed how baiting the traps with corn and leaving them unbaited affects the above parameters. In the remainder of this introductory chapter, I introduce our current theoretical understanding of alternative reproductive tactics, discuss the underlying predictions about differences in their behaviour in the context of invasion, and delve into how these concepts relate to the invasive round goby. I also discuss various gear types and methods used to capture fish, provide details about the sampling sites used for collection, and introduce the study species—the round goby—used for the research in this thesis. I end the chapter by clearly re-stating the aims of my thesis and explaining the structure of the thesis.

## *1.1 Alternative reproductive tactics*

There is a great diversity in the types of alternative reproductive tactics that exist, such as a colony foundresses and colony adopters in female paper wasps, *Polistes dominulus* (Starks 1998), signalling and non-signalling males in water striders, *Gerri gracilicornis* (Han and Jablonski 2016), territorial males, satellite males, and faeder (female mimic) males in ruff, *Philomachus pugnax* (Bachman and Widemo 1999), and nest guarding, nest sneaking, and female mimicking males in bluegill sunfish, *Lepomis macrochirus* (Gross 1982). ARTs evolve under high levels of sexual competition, where discontinuous selection occurs on traits that act to maximize an individual's fitness (Brockmann 2001). For example, taurus scarab beetles, *Onthophagus taurus*, exhibit discontinuity in the horn sizes of males. Female scarab beetles create tunnels in which they will lay their eggs. Males surpassing a size threshold develop large horns used to competitively defend the entrances of these tunnels, whereas males that do not surpass the size threshold remain small and hornless (Moczek and Emlen 2000). Small hornless males forgo tunnel defense, instead sneaking past defending males and through tunnels to gain access to females (Moczek and Emlen 2000). Males with the smallest horn sizes had the highest maneuverability in tunnels (Moczek and Emlen 2000). Horn size in male scarab beetles represents disruptive selection because intermediate horn sizes are not beneficial in either tunnel defense nor in tunnel sneaking behaviours and thus individuals with intermediate horn sizes will suffer from low reproductive success (Moczek and Emlen 2000).

Alternative reproductive tactics can be a genetically polymorphic trait, where an individuals' tactic is genetically predetermined by different alleles of the same gene (Gross 1996), such as in male ruff (Lank et al. 1995) and pygmy swordtails, *Xiphophorus multilineatus* (Zimmerer and Kallman 1989). Genetically polymorphic tactics can be subject to frequency-dependent selection, where fitness is dependent on the relative frequency of each tactic in a population (Gross 1996). As the frequency of one tactic increases relative to the other tactic, the more common tactic loses fitness while the rarer other tactic gains fitness (Dominey 1984, Gross 1996). At an intersection point, these tactics can exist in an evolutionary stable state frequency, where the average lifetime fitness of each tactic is equal (Figure 1.1, Dominey 1984, Gross 1996). However, the ART an individual adopts may not be entirely genetically determined but may also depend on the environmental conditions that individual experiences, aptly referred to as condition-dependent selection (Gross 1996). Under condition-dependence, the tactics do not necessarily have equal fitness. A ‘primary’ tactic may have higher fitness than a ‘secondary’ tactic, but an individual is required to pass a certain condition, such as a size threshold for example, to successfully adopt the primary tactic (Dominey 1984, Gross 1996). If early life conditions are poor and results in little growth, an individual may not be able to reach a specific threshold (such as size), and may instead maximize its fitness by adopting a secondary tactic (Gross 1996). In scarab beetles, males grow large horns only if they surpass a size threshold, which is highly linked to having more food resources during the larval stage (Emlen 1994). Such reproductive tactics may be fixed for life or expressed sequentially. Often individuals begin as a sneaking tactic when small, and once large enough, transition into a guarding tactic (Taborsky et al. 2008). Alternatively, the males employing the sneaking tactic can remain small and transition into a female mimic (satellite male, a second type of a parasitic tactic), such as observed in male bluegill sunfish (Gross 1982).

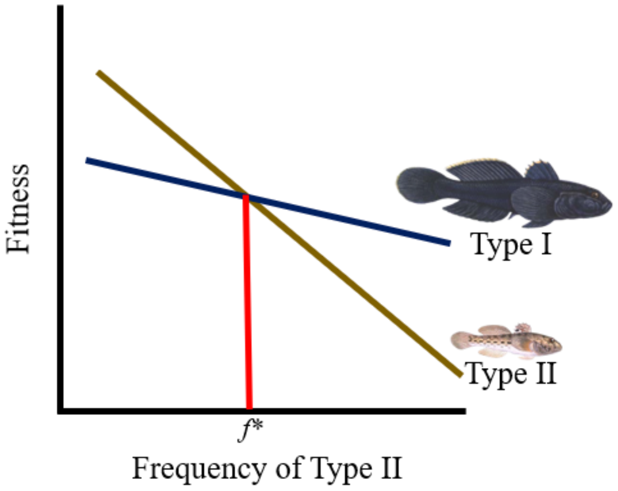

**Figure 1.1.** The relationship of two phenotypes under frequency dependent selection. In the context of alternative reproductive tactics, Type I might represent the conventional large nest guarding territorial males (guarders), while Type II might represent small nest parasitizing sneaker males (sneakers). As the frequency of sneakers increases, the fitness of guarders slightly decreases because more sneakers males exploit their nests and decrease their paternity. However, in sneaker males, as the frequency of sneakers increases, fitness sharply declines due to extremely high levels of competition and/or low availability of guarder male nests to exploit. The intersection of these two lines represents equal fitness for both guarder and sneaker males, where the frequency of sneakers is *f** and the frequency of guarder is 1 – *f**. An evolutionary stable strategy (ESS) can exist in the population, driving the frequency of sneaker males to *f**. Figure adapted from (Gross 1996).

Alternative reproductive tactics are especially common among many fish species (Gross 1984, Taborsky 2001). ARTs are thought to have evolved more often in fish because many fishes have external fertilization, allowing easier access by non-courting, extrapair males during copulatory events (Taborsky 2001). Another reason why ARTs are thought to have evolved more frequently in fishes is that paternal care is common, and there is likely to be a great benefit to employing a tactic that exploits the parental efforts of others and avoids the high costs of paternal care. Finally, ARTs are also thought to be common among fishes based on their continuous growth, where an individual will keep growing larger throughout its lifespan (Taborsky 2001). Continuous growth creates a much larger imbalance between large and small males in competition for females or for resources that females need. Hence small sneaking males may be selected as a "best of a bad job" (Dawkins 1980) option when competing directly with much larger males is futile.

Common alternative tactics in male fish include nest guarding males, nest sneaking males, and satellite (female mimic) males (Gross 1982). Guarder males seek and defend territory, court females, and sometimes provide parental care. Sneaker and satellite males both exploit the parental investment of guarder males by accessing their nest either through speed or female mimicry, releasing sperm during copulation, and leaving without contributing any parental care (Gross 1982; Taborsky 2001). These different reproductive tactics have associated life history trade-offs to maximize fitness (Taborsky and Brockmann 2010). Sneaker males often reach maturity earlier and at a much smaller size compared to guarder males (Taborsky and Brockmann 2010), potentially exhibiting a faster pace-of-life syndrome (Réale et al. 2010). Individuals that exhibit fast pace-of-life syndromes may also exhibit associated behavioural traits such as high activity levels and boldness (Réale et al. 2010).

### *1.2 Animal behaviour, alternative reproductive tactics, and invasion*

Variation in the behaviour of animals was once thought of as 'noise', but recently scientists have started asking not just how animals behave, but whether this behaviour is consistent over time and contexts (Sih et al. 2004a, 2004b). Consistent behaviour or animal personalities arise when individuals exhibit different behavioural tendencies from one another, and these different behaviours are consistent over the course of time (Sih et al. 2004a, 2004b). When two or more behavioural traits are consistently correlated, they form what is known as a behavioural syndrome (Sih et al. 2004a, 2004b). If an individual exhibits a high degree of boldness in one context, such as feeding, that same individual might show a similar degree of boldness under mating contexts, for example. If boldness is consistent across time, and context, scientists might claim that this individual has a bold personality. Five general personality traits that are usually assessed in animal behaviour studies include activity, boldness, exploration, aggression, and sociality. Taken together, these five main traits and associated syndromes are used to form a basic personality of an individual.

Researchers have recently been interested in the adaptive advantage animal personalities can serve in the context of invasive species (Holway and Suarez 1999, Chapple et al. 2012, Carere and Gherardi 2013). Invasion success may be dependent on certain personalities that are well suited to handle the challenges associated with dispersal and survival in a novel habitat. For example, high boldness, activity, aggression, and low sociality form a fast pace-of-life syndrome (Juette et al. 2014). Individuals exhibiting this fast pace-of-life syndrome may be better adapted for success on invasion fronts (Fraser et al. 2001). Additionally, behavioural differences among individuals in a population may increase the likelihood of persistence of a population (Morozov et al. 2013). In three copepod populations, *Calanus finmarchicus, Calanus glacialis,* and *Calanus euxinus,* the inclusion of feeding behaviour in population models dampened predator-prey cycles, leading to enhanced population persistence (Morozov et al. 2013). Certain behavioural traits and behavioural variation may also facilitate dispersal into novel habitats and contribute to success in invaded habitats.

In mosquitofish, *Gambusia affinis,* asocial individuals were found to disperse further than their social counterparts, potentially to distance themselves from conspecifics (Cote et al. 2010; Gonzalez-Bernal et al. 2014). Exploration is also regarded as a beneficial trait for range expansion. Great tits, *Parus major,* who were more exploratory in novel environments also dispersed the furthest and had high-dispersing offspring (Dingemanse et al. 2003). The tendency to explore is often associated with high levels of boldness (Wilson and Godin 2009, Cote et al. 2010). Exploring and spreading out into a novel habitat is risky, requiring individuals to have a certain degree of risk-taking or bold behaviours. Trinidad killifish, *Rivulus hartii,* that were more bold and explorative in a novel tank assay dispersed further when placed in a fragmented habitat in the field (Fraser et al. 2001). Aggression has been shown to couple with and facilitate dispersal in Passerine birds (Duckworth and Badyaev 2007; Duckworth 2008). Individuals on the leading edges of range expansion are generally more aggressive than individuals from established populations (Duckworth 2008) and native species (Dubs and Corkum 1996; Savino et al. 2007; Chucholl et al. 2008). Dispersal also requires a certain degree of activity, where more active individuals are also more likely to disperse (O'Riain et al. 1996). Understanding individual behavioural traits and between individual behavioural variation can help researchers model how behaviour might influence population persistence and invasion success (Morozov et al. 2013).

### *1.3 The invasive round goby*

The round goby, *Neogobius melanostomus*, is a benthic fish species native to the Ponto-Caspian region of Eastern Europe (Kornis et al. 2012). The round goby has invaded North America, and more recently, Western Europe, having various detrimental impacts in invaded ecosystems in North America. Highly aggressive (Dubs and Corkum 1996, Balshine et al. 2005) and tolerant of high conspecific densities (Ray and Corkum 2001, Pennuto et al. 2012), round goby out-compete native benthic species such as logperch, *Percina caprodes,* slimy sculpin, *Cottus cognatus,* spoonhead sculpin, *Cottus ricei,* and mottled sculpin, *Cottus bairdi,* for resources such as food and territory (Dubs and Corkum 1996, Balshine et al. 2005, Bergstrom and Mensinger 2009). The round goby has been incorporated into the food webs of invaded ecosystems. They predate on the eggs and fry of many predatory species, including walleye, *Sander vitreus*, lake trout, *Salvelinus namacyush*, and smallmouth bass, *Micropterus dolomieu* (Chotkowski and Marsden 1999, Steinhart et al. 2004, Roseman et al. 2006), potentially impacting the recruitment of these ecologically important species. Additionally, the diet of many piscivorous species have shifted to include round goby (Dietrich et al. 2006, Kornis et al. 2012). The inclusion of round goby in native species' diets can be problematic for two reasons. First, round goby are vectors of contaminant bioaccumulation in native predatory species such as smallmouth bass (Kwon et al. 2006) and of diseases such as botulism (Yule et al. 2006). Second, round goby appear to persist in environments with relatively high levels of contamination, which may act as a trap, luring predators in search of rich food sources into these highly contaminated environments (Marentette et al. 2010). Due to the widespread invasion success and associated detrimental impacts of the round goby (Corkum et al. 2004, Kornis et al. 2012), they have been the subject of many scientific studies seeking to understand what behavioural (Groen et al. 2012, Myles-Gonzalez et al. 2015, Thorlacius et al. 2015) and life history factors contribute to their invasiveness (Gutowsky and Fox 2012, Brandner et al. 2013, Masson et al. 2018). Round goby on the frontiers of spreading populations (invasion fronts) exhibit higher activity and boldness levels and are less social compared to goby from established areas (Myles-Gonzalez et al. 2015, Thorlacius et al. 2015). In an invasion front population found in the Trent-Severn Waterway (Ontario, Canada), female round goby exhibit higher investment in reproduction and earlier maturation (Masson et al. 2018), and are smaller than females from established populations (Brownscombe and Fox 2012, Masson et al. 2018). However, in an invasive front population in the Danube River (Europe), larger females with low reproductive investment were observed (Brandner et al. 2013).

One interesting but usually overlooked aspect of round goby biology is the presence of male alternative reproductive tactics (Figure 1.2). Guarder male round goby build nests, attract females, and care for their eggs and young (Kwon et al. 2006). These nest defending males are externally identified through secondary sexual characteristics such as dark, often black body colouration, which attracts females (Yavno and Corkum 2010), enlarged cheek pads, and turgid urogenital papillae (Marentette et al. 2009, Bleeker et al. 2017). These reproductive males have enlarged testes, but also show investment in a secondary gonadal structure, accessory glands (Marentette et al. 2009, Bleeker et al. 2017). A second male morph also exists among round goby, these are reproductively mature males but they do not exhibit black colouration or wide cheeks (Marentette et al. 2009, Bleeker et al. 2017). These males often are smaller, remain a light brown mottled colour, and have turgid papillae that are longer relative to their body length (Marentette et al. 2009, Bleeker et al. 2017). While these secondary males have larger testes, they invest very little into accessory glands, which produce mucins that are spread on the nest by guarder males (Marentette et al. 2009, Bleeker et al. 2017). These smaller mottled but reproductive males are often referred to as sneaker males in the round goby.

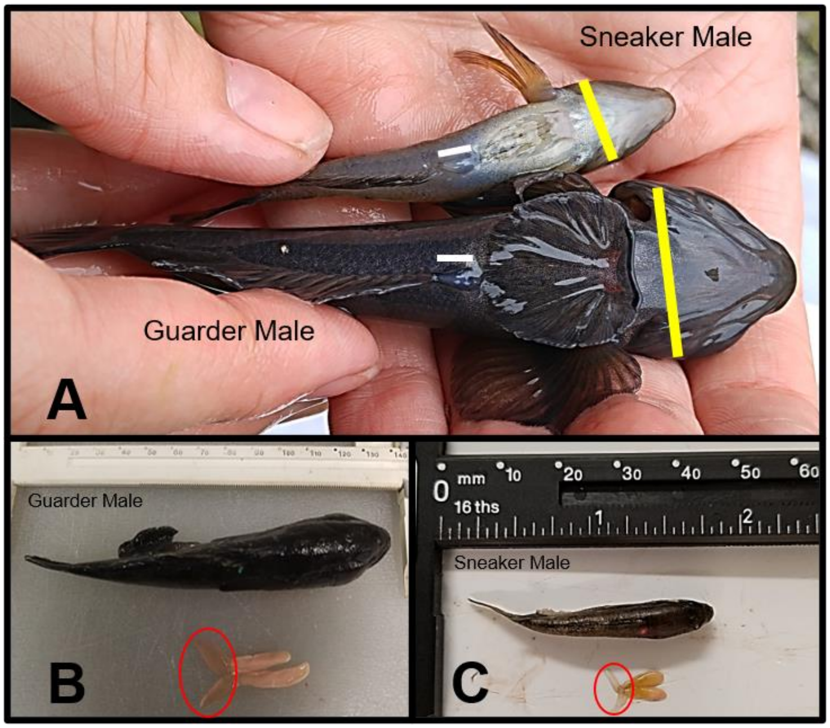

**Figure 1.2.** Various morphological differences between guarder male and sneaker male round goby. (A) Guarder males can be differentiated externally from sneaker males through their dark to black colouration, enlarged head width (see yellow lines), and sneaker males will often have a turgid and very long papilla relative to body length (see white lines). (B) Internally, guarder males can be distinguished by having both large testes and large accessory glands (red circle) while (C) sneaker males have large testes but invest much less in the accessory glands (red circle).

Although there is ample evidence that alternative reproductive tactics exist in the round goby, many questions about these ARTs remain unanswered. First, there is uncertainty as to how the tactic an individual adopts is determined, and whether tactics in the round goby remain fixed for life or are flexible. Guarder and sneaker males have a large overlap in age and size, with guarders ranging in age from 1–4 years and 5.0–11.9 cm in body length and sneaker males ranging from <1–3 years in age and 3.6–9.5 cm in body length (Bose et al. 2018). Guarders tended to have faster early somatic growth, which when considering the overlap in age and sizes, suggests that ARTs may become fixed early in ontogeny (Bose et al. 2018). It remains unclear whether ARTs in round goby result from genetic polymorphism or a condition dependent state (Bose et al. 2018). If a large proportion of reproductive males display intermediate traits between guarder and sneaker tactics during the breeding season, then it might suggest males are capable of switching between the tactics (Bleeker et al. 2017). However intermediate males only accounted for 9% of captured reproductive male round goby from a population in the Netherlands, suggesting tactics are fixed, at least at the beginning of the breeding season (Bleeker et al. 2017). Lastly, behavioural observations of nest interactions by sneaker males have never been achieved in the laboratory or the field. Hence it remains unclear whether sneaker males mainly perform streak spawning behaviour, where they quickly enter a nest while a guarding male and female are copulating and release sperm in an attempt to fertilize some eggs, or if instead sneaker males act as satellites, mimicking females and tricking guarder males into allowing access into their nest. It is also possible that males employ both sneaking and female mimicry. The sneaking strategy, where sneaker males enter guarder male nests to spawn, has been observed by few researchers but has not been well described or documented (MacInnis and Corkum 2000, Marentette and Corkum 2008). Regardless, understanding the life history and behavioural variation association with each tactic can provide insight into why the round goby is such a successful invasive species.

### *1.4 Fisheries gear types and capture methods*

Capturing fish for recreation, commercial, management, or research purposes requires the use of specialized equipment referred to as fisheries gear. Various gear types exist, and the best specific gear type to use usually depends on the target species of interest. Fisheries gear types fall under two broad categories, passive gear and active gear. Passive gear types are set up and remain immobile in the water, requiring fish to encounter the gear to become trapped. As such, passive gears may select for species or individuals that have higher levels of activity, boldness, and/or exploration (Härkönen et al. 2014; Diaz Pauli et al. 2015). In contrast, active gear requires humans to maneuver and manipulate the gear, capturing any fish in the general vicinity. Electrofishing is an example of an active gear type, where a current is induced into the water through an anode pole held by a person. Electrofishing temporarily stuns fish, causing the fish to float up near the waters surface and towards the anode, where they can be more easily netted and captured. Species without a swim bladder, like the round goby, are stunned but do not float, decreasing capture chances, especially in environments with rock and boulder substrates where fish without swim bladders can fall between crevices (Polačik et al. 2008). Gill nets are an example of a passive gear type and are usually set perpendicularly to the shore and left suspended in the water column by floats. Gill nets are constructed with flexible meshing, where fish are captured by becoming wedged in the mesh, caught on protrusions such as gills, or become entangled in the mesh (Hubert and Pope 2012). If a fish is too large, they will bounce off the net and if a fish is too small, they swim through the mesh entirely. Fyke nets are yet another passive gear type and are extensively used to sample benthic and pelagic fish species; these nets work by resting on the sediment with large mesh funnels coming off the central trap that guide fish through a series of cylindrical enclosures. Each gear type comes with its own set of biases, or selectivity, in the types of species and individuals they are most likely to catch. These biases are generally considered and even controlled for by fisheries scientists (Huse et al. 2000, Stergiou and Erzini 2002, Diana et al. 2006, Diaz Pauli et al. 2015).

One extremely common passive gear type used to capture near-shore small-bodied fish are minnow traps. Minnow traps work by having two funnels that guide a fish into a semi-enclosed chamber (Figure 1.3). Escape from the trap is possible but challenging; once inside the trap the fish must precisely locate the small entry holes to exit (Figure 1.3). Minnow traps are an attractive gear type as they require little human investment to set, are relatively inexpensive to purchase and maintain (Johnson et al. 2005), and can be used in a variety of aquatic environments and sediments. While minnow traps can have lower catch rates compared to other gear types (Johnson et al. 2005), they remain highly popular based on these previously mentioned attributes. Depending on the study, minnow traps can be supplemented with a variety of bait types or they can be left unbaited. The addition of bait can greatly increase catch efficiency (Stone 2005, Diana et al. 2006). However, baited minnow traps can capture larger fish compared to unbaited minnow traps, suggesting that bait can induce selectivity on fish size (Diana et al. 2006).

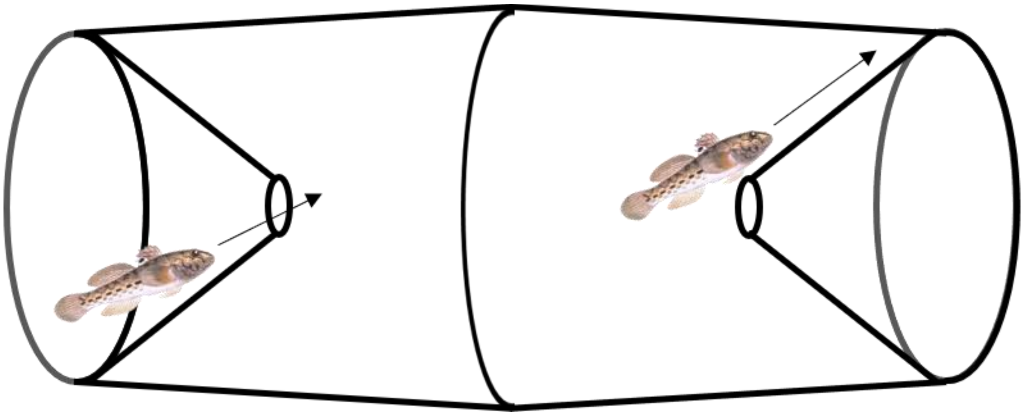

**Figure 1.3.** A diagram indicating how minnow traps work to capture small-bodied fish. Fish that encounter minnow traps are guided by a funnel into a semi-enclosed chamber (left arrow). Once inside the chamber, funnels act to guide fish away from the entrance holes (right arrow).

Minnow traps are readily available for purchase by recreational fishing shops, and as such, a variety of companies manufacture minnow traps, leading to diversity in designs (Figure 1.4). The two most common minnow trap models available for purchase include black vinyl-coated metal minnow traps and exposed galvanized steel minnow traps, hereafter referred to as black and silver minnow traps, respectively. While the difference in colour is obvious, they might at first glance look to have similar construction (Figure 1.4a & 1.4b), however black traps have a thicker mesh, a shorter funnel length, and a larger entry hole diameter compared to silver traps (see Chapter 3, Table 3.1). Silver traps are more reflective and may act to attract fish, or conversely, ward fish away. Differences in the entry hole diameter could select for fish of differing sizes. Hole diameter and funnel length may influence the ability of a trap to retain a fish once captured. Bias between the trap types have already been identified in three-spined stickleback, *Gasterosteus aculeatus*, and nine-spined stickleback, *Pungitius pungitius*, populations, where silver traps had higher catch rates in both species (Merilä et al. 2013, Budria et al. 2015). Additionally, silver traps were found to have higher retention rates than black traps (Budria et al. 2015). Researchers may be using black and silver traps interchangeably without even considering whether traps have the same catch efficiencies and selectivity for their species of interest. This can inadvertently introduce bias when comparing long term data within a population, or the parameters between populations, and when assessing community compositions. Unlike across gear biases, the within gear biases and variation in catch efficiency and selectivity has not been well appreciated or studied.

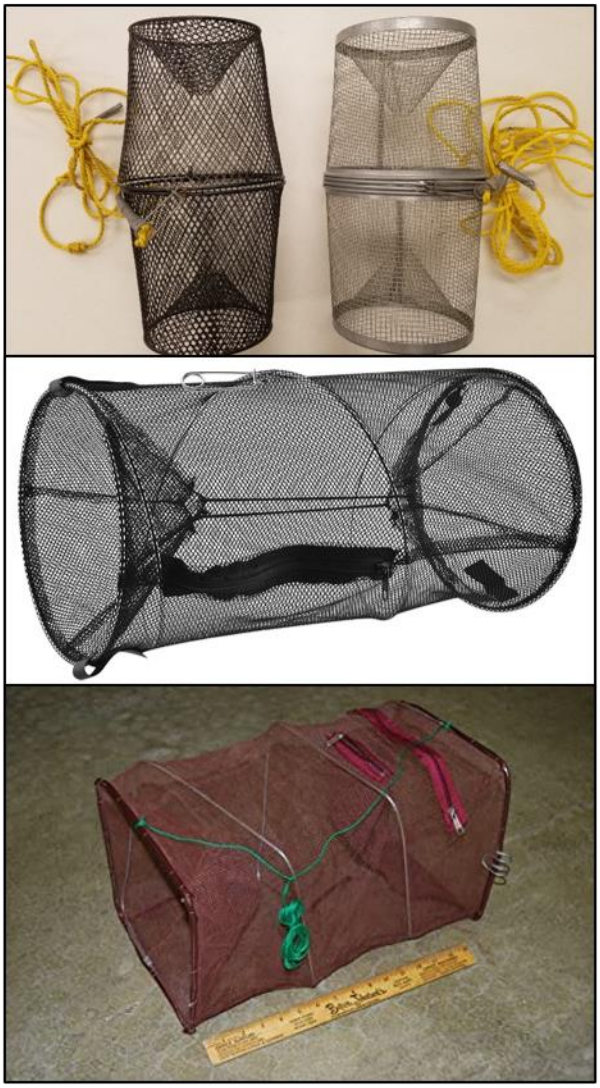

**Figure 1.4.** An example of the variation that can exist within a popular passive fisheries sampling gear, minnow traps.

### *1.5 Study sites in Hamilton Harbour*

Hamilton Harbor—formerly known as Burlington Bay—and the connected Cootes Paradise Marsh represent the western most point of Lake Ontario. Anthropogenic stressors such as industrialization, pollution, and overexploitation have caused Hamilton Harbour to be designated as one of 43 Areas of Concern across the Great Lakes by the International

Joint Commission (Great Lakes Water Quality Agreement, 2012). A Remedial Action Plan was devised for Hamilton Harbour in 1987, aiming to reverse degradation, reduce stressors, and restore ecological health (Hamilton Harbour Remedial Action Plan, 2014). While the index of biotic integrity and proportion of piscivore biomass have both increased since 1998, tolerant and non-native species still dominate the fish biomass in Hamilton Harbour (Boston et al. 2016). Round goby are both a tolerant and invasive (non-native) species that arrived in North America in the 1990's through accidental transport in transoceanic ship ballast water (Jude et al. 1992), and likely arrived in Hamilton Harbour in 1994 or 1995 (Vélez-Espino et al. 2010). By 2002, round goby were highly abundant in Hamilton Harbour (Balshine et al. 2005, Young et al. 2010) and the ratio of guarder males to sneaker males was consistent between 2006 and 2017 (McCallum et al. 2019). All round goby captured for study in this thesis were collected from six sites around the Hamilton Harbour: Desjardins Canal (DC), Grindstone Creek (GC), LaSalle Marina (LS), Fisherman's Pier (FP), Pier 27 (P27), and Sherman's Inlet (SI), plus an additional site located east of Hamilton Harbour: Fifty Point Conservation Area (50P; Figure 1.5). FP, P27, and SI are located near the industrial sector and contain higher levels of contaminants compared to DC, GC, and LS (Burniston et al. 2016; Milani et al. 2017). Although Hamilton Harbour is a contaminated ecosystem (Marvin et al. 1993, Pozza et al. 2004), my studies do not focus on the topic of contamination.

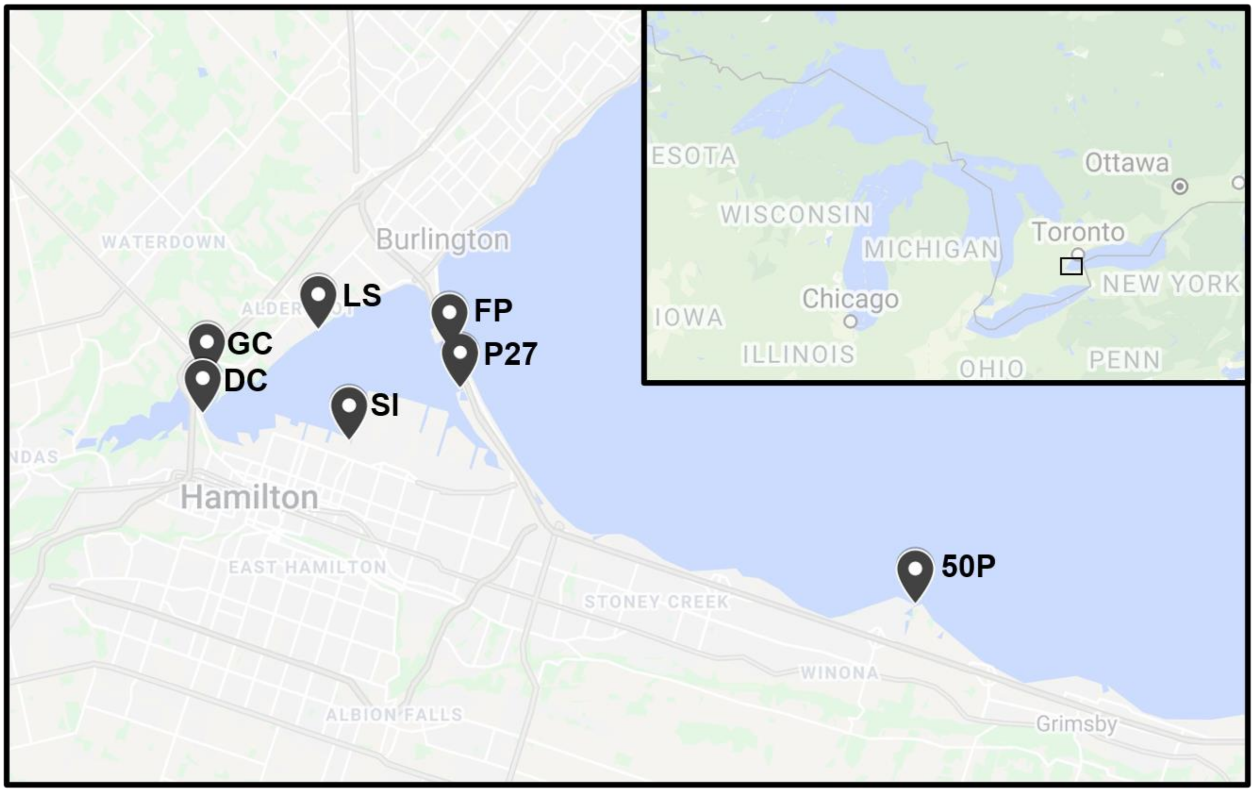

**Figure 1.5**. All the round goby collection locations used in this thesis came from six sites around the Hamilton Harbour and one in Stoney Creek, Ontario, Canada. Sites include: Desjardins Canal (DC; 43.277984, -79.888725), Grindstone Creek (GC; 43.286629, -79.886802), LaSalle Marina (LS; 43.300212, -79.846016), Fisherman's Pier (FP; 43.296320, -79.796384), Pier 27 (P27; 43.284453, -79.791594), and Sherman's Inlet (SI; 43.270107, -79.833852), and Fifty Point Conservation Area (50P; 43.226133, -79.622640;

### *1.6 Thesis Aims and Structure of the Thesis*

In this thesis, I aimed to fill in knowledge gaps first with respect to non-reproductive behavioural differences of round goby male alternative reproductive tactics, and second about how variation within a common fisheries gear type, minnow traps, can influence the estimations of round goby population parameters. In Chapter 2, I analyzed and compared guarder and sneaker males on six behavioural traits: activity, exploration, boldness, sociality, aggression, and dispersal tendency. In Chapter 3, I assessed if and how round goby catch rates, sex ratios, alternative reproductive tactic ratios, and size differ between black vs. silver and baited vs. unbaited minnow traps. In Chapter 4, I conclude my thesis with a general discussion of my results found in Chapters 2 and 3, and I indicate possible avenues for future research. Taken together, my MSc studies will help expand our knowledge of round goby behaviour and shed light on best trapping methods, furthering our understanding of among individual behavioural variation and how gear type can influence population estimates.

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# CHAPTER 2

## NON-REPRODUCTIVE BEHAVIOURAL DIFFERENCES BETWEEN MALE ALTERNATIVE REPRODUCTIVE TACTICS IN THE INVASIVE ROUND GOBY

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## Abstract

Alternative reproductive tactics (ARTs) exist where two or more different strategies to achieve reproductive exists in one sex of a species. Variation among ARTs in morphological, physiological, and mating based behavioural traits are generally well understood, however, whether variation also exists in behaviour outside of the mating context is poorly regarded. Here, we used the round goby, *Neogobius melanostomus*, a globally prolific invasive species where males exhibit ARTs, to address this knowledge gap. We compared non-mating behaviour of sneaker and guarder males in terms of boldness, exploration, activity, aggression, sociality, and dispersal. Sneaker males were found to be more active, explorative, bold, and less aggressive compared to guarder males. We also explored correlations among behaviours and found that in guarder males many behavioural traits were correlated (i.e. activity and exploration) but in sneaker males, the only behavioural traits that were correlated was a negative relationship between exploration and sociality. This information could help inform fisheries managers of which tactic is most likely to spread. Additionally, by employing capture techniques that target individuals with certain behavioural traits, managers might potentially dampen the probability that an invasive population will spread and persist.

**Keywords**: sneaker, guarder, life history, invasion, Great Lakes, *Neogobius melanostomus*, teleost fishes

## Introduction

Alternative reproductive tactics (ARTs) are a taxonomically widespread phenomenon where two divergent reproductive tactics arise because there is an evolutionary advantage to employing a different route to achieve fertilization (Taborsky et al. 2008). ARTs can be controlled by genetics, or they can be condition-dependent, where the tactic an individual adopts is driven by some environmental cue or by some ‘switch point.’ For example, in scarab beetles, *Onthophagus acuminatus*, males develop into the resource holding tactic once a certain larval stage size threshold is met (Emlen 1994). The way in which the different alternative tactics approach reproduction is quite varied among species. Resource holding, large horned, scarab beetle males guard tunnel entrances that lead to females (Emlen 1997, Moczek and Emlen 2000), while small non-horned males, well adapted to digging and navigating tunnels, bypass these guarding males to access females inside the tunnels (Emlen 1997, Moczek and Emlen 2000). In male water striders, *Gerris gracilicornis*, a signalling male straddles a female’s back and produces courtship ripples that also attract predators, coercing the female into rapid mating to avoid predation from hunting fish that attack from underneath (Han and Jablonski 2019). In contrast, the non-signalling tactic also straddles the female’s back, but produces no ripples and instead waits for the female to expose her genitalia to induce mating (Han and Jablonski 2019). While morphological and even physiological differences between tactics have been well studied (Bass 1992, Gross 1996, Emlen 1997, Moczek and Emlen 2000, Sinervo et al. 2000), there is little known about non-reproductive behaviours, those that occur outside the mating context, and how these might differ between males adopting alternative tactics.

Certain behavioural traits may be more common or more beneficial for one reproductive tactic over the other. For example, in species where the conventional tactic defends a resource (guarders) and a second male tactic sneaks copulations and/or parasitizes parental care (sneakers), such as in the scarab beetle example above (Emlen 1997, Moczek and Emlen 2000) and in other species such as bluegill sunfish, *Lepomis macrochirus* (Gross 1991), aggression may be necessary for the resource guarding tactic to protect against intrusion by the parasitizing tactic. In the black goby, *Gobius niger*, and grass goby, *Zosteriesessor ophiocephalus*, guarder males provide parental care to their offspring and increase their aggression levels in the presence of sneaker males that exploit this parental care (Scaggiante et al. 2005). However, such aggressive tendencies would likely be disadvantageous for sneaker males that want to avoid detection by guarder males and are unlikely to win contests because of their commonly smaller size. Moreover, behavioural traits can be correlated across both mating and non-mating contexts, forming what is known as a behavioural syndrome (Sih et al. 2004). For example, in male water striders activity levels were correlated across both mating and non-mating contexts, males that made more movements in a general context also made more mating attempts (Han and Jablonski 2019). The number of mating attempts (activity) was negatively correlated with latency to emerge from a shelter (cautiousness) in the coercing signalling male water strider morph, but a slight positive correlation was observed in males adopting the non-signalling non-coercive male tactic (Han and Jablonski 2019). If a reproductive strategy requires one tactic to be opportunistic, then behavioural flexibility may be beneficial for that tactic, not to have consistent behaviour but instead behave in a plastic or flexible manner depending on the environment it finds itself in, and as such, behavioural syndromes may be weak or absent all together. This could lead to a situation where behavioural syndromes may not be the same for males employing each of the two tactics.

Individual behavioural variation within a population can have important implications for persistence, especially in unstable environmental conditions (Dingemanse et al. 2004, Dingemanse and Réale 2005). In great tits, *Parus major*, adult males that were more explorative had higher survival rates after food plentiful winters, but had lower survival rates after food scare winters, with the opposite trend observed in females (Dingemanse et al. 2004, Dingemanse and Réale 2005). ARTs may contribute to consistent behavioural variation in a population, and as such, the associated behavioural differences between them could influence the likelihood of persistence for that population. This may be an important consideration when managers want to conserve an at-risk population or mitigate damage by an invasive population.

The round goby, *Neogobius melanostomus*, is a globally invasive species with male alternative reproductive tactics, making it an ideal study species to understand non-mating behavioural differences associated with two male reproductive tactics, nest guarding and nest sneaking males, in the context of invasion. Round goby are highly prolific benthic fish that are thought to have been introduced into the Laurentian Great Lakes through ballast water expelled by transoceanic ships arriving from the Black Sea (Jude et al. 1992). Round goby are considered an ecologically disruptive species as they outcompete native benthic fish species for habitat and resources (Dubs and Corkum 1996; Savino et al. 2007; Bergstrom and Mensinger 2009), predate on native fish eggs (Chotkowski and Marsden

1999, Steinhart et al. 2004, Roseman et al. 2006)). In addition, round goby accumulate and transfer contaminants up the food chain. They acquire contaminants through consumption of zebra mussels, *Dreissena polymorpha* (Kwon et al. 2006) and then in turn are consumed by piscivorous fish species such as smallmouth bass, *Micropterus dolomieu*, burbot, *Lota lota*, lake trout, *Salvelinus namacyush*, and lake whitefish, *Coregonus clupeaformis* (Steinhart et al. 2004; Kornis et al. 2012) as well as by waterfowl such as double-crested cormorants, *Phalacrocorax auritus* (Johnson et al. 2010; Kornis et al. 2012). Currently, round goby are spreading into previously uninvaded habitats in Western Europe and North America. Behaviour and behavioural syndromes associated with each round goby ART may in part dictate which individuals are most prone to spreading into new habitats.

There have been a number of studies on round goby behaviour, especially in the context of secondary range expansion (Groen et al. 2012, Myles-Gonzalez et al. 2015, Thorlacius et al. 2015); however, most of these studies focus on non-reproductive males. To date, only one study (an unpublished thesis) has performed a comprehensive examination of behavioural differences between guarder and sneaker round goby, however in that study the sneaker male sample size was small (n=7) and the reproductive tactics were not fully confirmed by dissection (Nguyen-dang 2017). In the current study, we complete a behavioural scan assessing aggression, sociality, boldness, activity, exploration, and dispersal tendencies in a larger sample (~30) of guarder and sneaker male round goby and confirmed reproductive status with dissections. Our objective was to elucidate any non-reproductive behavioural differences between sneaker and guarder male round goby. We also explored whether there were correlations of behavioural traits observed either in sneaker and/or guarder males. Since sneaker males are likely more opportunistic at gaining reproductive opportunities compared to guarder males, males adopting this tactic may benefit more from having behavioural flexibility. This assumption led us to predict that sneakers would show weak or no behavioural syndromes compared to guarder males.

## METHODS

### *Fish collection*

Round goby were caught using baited (~25g of frozen corn) and unbaited silver (Gee's galvanized brand) and black (Eagle Claw brand) minnow traps between May and July 2019. Fish were caught in these traps across four locations in Hamilton Harbour (LaSalle Marina: 43.300463, -79.846205; Fisherman's Pier: 43.296562, -79.796471, Pier 27: 43.284425, -79.791556, Pier 15: 43.270072, -79.833856) and at Fifty Point Conservation Area (43.226029, -79.622445). Traps were placed approximately 10 meters apart and retrieved after 24 hours. Any captured round goby were sexed via visual inspection of the urogenital papilla (sex was later confirmed by dissections; see below). Following the methods outlined in Marentette et al. (2009), males with flat papillae were designated as non-reproductive males (NRM) while those with a turgid papilla were classified as reproductive. In the field males with a turgid papilla were further qualitatively differentiated into either guarder males (GM) or sneaker males (SM) based their body color, and a visual assessment of head size: fish that were dark or black with a large head width were classified as GM and fish with narrow heads, that were generally smaller and mottled in colour, were designated as SM. Once fish were ran through behavioural experiments (see below), their reproductive status and tactic was finally confirmed with quantitative external measures and dissections. Males were held individually in flow through containers and transported live within an aerated cooler to McMaster University for behavioural experimentation. The remainder of round goby caught were humanely euthanized using an overdosed benzocaine and water mixture. Any native species captured were promptly returned to the waterbody.

Immediately upon arrival to McMaster, round goby were tagged with subdermal injections of non-toxic acrylic paint (Wolfe and Marsden 1998), weighed (to nearest 0.001g) and allowed to recover in aerated containers. Round goby were then transferred to 75L laboratory tactic specific holding tanks, containing ~1 cm thick layer of gravel and four–six PVC pipes as shelter. Tanks were filtered, aerated, and maintained on a 14:10 h light-dark schedule. Water changes were completed every two weeks. Fish were fasted as they were held in the lab for short periods, and generally fish do not feed during the first few days after being brought into the lab. Fish were held in these tactic specific holding tanks until behavioural experimentation.

### *Experimental tank set up*

Behavioural tendencies were assessed in one of four identical tri-divided 150 L (92 x 45 x 38cm) filtered and aerated experimental tanks. We used six consecutive behavioural assays that were administered in the following order: exit test, exploration, sociality, aggression, activity, startle response, and dispersal tendency. We ran the behavioural assays in this set order to minimize potential carryover effects and to standardize the comparison of sneaker and guarder males. At the study outset, we were also uncertain whether we would obtain a large enough sample of each morph to statistically account for all the permutations a randomized order would create (Bell 2013).

The experimental tanks in which the behavioural assays were conducted were divided into sections using both transparent and opaque acrylic barriers (Supplemental Figure 2.1). To remove glare and interference from neighbouring tanks, experimental tanks were lined with contact paper on the 45 cm wide sides and on the bottom of the tank. Tanks contained no substrate. An opaque curtain was set up between the experimenter and tanks to minimize fish disturbance during trials. Before trials commenced, the filters and airstones were removed from the tanks. Assays were recorded from above each tank using Sony video cameras mounted on tripods. Videos were imported into BORIS video scoring software (Friard and Gamba 2016) and all videos within a behavioural assay were scored by the same researcher.

### *Exit Test and Exploration Assay*

A focal round goby from one of the holding tanks was guided into an opaque tube-shaped black refuge and transferred in this refuge to the middle section of one of the four tri-divided experimental tanks. The fish remained submerged in water inside the refuge through the entire transfer process to minimize stress. The fish was allowed one hour to habituate and recover from capture and transport. After habituation, a door on one end of the refuge was remotely lifted via a pulley system, allowing the focal fish to exit from the refuge (Figure 2.2a). The focal fish was given 15 minutes to independently exit the refuge, after which the entire refuge was remotely lifted from the closed end via a pulley system, thus forcing the fish to leave the refuge if it had not done so already or had returned. Whether or not the fish willingly left the shelter was recorded, as well as time taken to leave the shelter if the fish left of its own volition. The duration of time the fish spent with their heads partially emerged from the shelter was also recorded. Once the refuge was remotely lifted out, the fish was given 30 minutes to freely explore the middle chamber of the novel experimental tank that had a 4x4 grid of 10 cm x 10 cm squares marked on the bottom (Figure 2.2d). The number of unique squares the focal goby visited after 15 and 30 minutes were recorded as the tendency to explore a novel environment (Jones and Godin 2010). Further, as an assessment of risk taking, the proportion of time spent in the middle four grid squares was also recorded.

### *Activity Assay*

Activity was measured during the exploration trial. The total amount of time spent making any movement was recorded for the 30-minute-long trial.

### *Sociality Assay*

Sociality was measured following methods developed and validated in Capelle et al. (2015). Before the trials started, we placed a small tank containing a halved flowerpot that could be used as a refuge in each of the end chambers. In one of these two small tanks (chosen by the flip of a coin), three male conspecifics were also added. Immediately following the exploration/activity trial, two opaque barriers concealing the two end chambers were remotely removed via a pulley system, allowing the focal fish in the middle to see the two end chambers (Figure 2.2g). We monitored where the focal fish spent its time within the central chamber over a 30-minute trial where it could see both end chambers. The center focal fish chamber was divided into three zones for this assay: (1) a social zone, closest to the three stimulus fish, (2) a neutral central zone in the middle, and (3) an asocial zone, furthest from the stimulus fish. The total amount of time spent in the social zone nearest to the stimulus fish was taken as an indication of sociality, with a higher duration indicating a greater tendency to seek social interactions. We also recorded any aggressive acts (ramming and parallel displays) made by the focal fish during this trial.

### *Aggression Assay*

Once the sociality assay was completed, two opaque barriers were remotely dropped concealing the two end chambers. One of these barriers (chosen by the flip of a coin) had a 30.5 cm square mirror affixed to the barriers surface (Figure 2.2j). The mirror introduced a perfectly size matched ‘competitor conspecific’. Aggressive behaviours towards the mirror image of the 'competitor' were recorded for 30 minutes. The number of aggressive acts (ramming and parallel displays) by the focal individual was used as a measure of aggressive tendency.

### *Startle Response Assay*

Following the aggression trial, two opaque barriers were remotely lowered via a pully system, one to cover the mirror and the other to balance the disturbance caused by the lowering barrier on the other side of the tank. Movement of the focal fish was then recorded for 15 minutes, after which a marble was remotely rolled through a PVC tube fixed to the top of the tank and dropped into the tank (Figure 2.2l). The fish's response to the marble drop (freeze, dart and freeze, or no response) and the latency to first movement (seconds) post marble drop were recorded. We also calculated the time spent moving for 15 minutes after the startle and compared this to the time spent moving before the marble drop.

### *Dispersal Assay*

Dispersal was assessed in a 2.5m long by 0.75m wide acrylic dispersal chamber. The chamber was divided into three laneways that allowed us to run three separate round goby simultaneously. Each laneway contained light sand substrate approximately 1.5 cm deep. Four alternating wall barriers protruded from the sides at 45 cm, 65 cm, 130 cm and

150 cm from the starting area (Figure 2.3a). Two additional canyon-like barriers, each 21 cm long, were placed at 90 cm and 170 cm from the starting area (Figure 2.3b). Flow was created in each laneway using 940gph submersible pumps. The start position of the fish was at the opposite end from the flow such that fish were swimming against the artificial current. Individual round goby were collected from holding tanks and transferred into one of the three laneways, enclosed in submerged refuges identical to those used in the exit test assay. The refuges were connected to a pully system so that the door and refuges could be remotely lifted independently as in the exit test assay above. Fish were given 30 minutes to habituate following the transfer, after which the door to the refuge was remotely lifted. Fish were given 15 minutes to freely exit the refuge before the entire refuge was remotely removed from the dispersal chamber. After removal of the refuge, fish were given 1.5 hours to disperse and move freely in the laneway. Each trial was recorded from overhead using a GoPro videorecorder. Whether fish reached the end, the time taken to reach the end, total number of laps completed (defined as fully going from one end section to the other, regardless of flow direction), and crosses made between zones were all recorded.

### *Morphological Differences*

After completing the dispersal assay, round goby were immediately euthanized in an overdosed benzocaine-water solution. The following measurements were collected for each fish: total length, standard length, papilla length, head width, body width, total mass, liver mass, seminal vesicle mass, and total gonad mass. Length measures were taken with callipers accurate to the nearest millimeter. Mass was taken in grams using an Ohaus Adventurer Pro digital scale, accurate to three decimal places. Males designated as guarders (GM) were black or extremely dark, had wide heads, and were generally larger (~ > 5.0 cm SL) (Marentette et al. 2009, McCallum et al. 2019). Males classified as sneakers (SM) were lightly mottled, had narrow heads, were typically smaller (~ < 5.0 cm SL), and had a long papilla relative to their body size (Marentette et al. 2009, McCallum et al. 2019). A variety of indices were calculated from the collected measurements. Condition was calculated using Fulton's Body Condition (Total mass (g)) / (Standard length (cm) ^ 3 x 100%) and Hepatosomatic index (HSI) (Liver mass (g)) / (Total mass (g) – Liver mass (g) x 100%). Gonadosomatic index (GSI) (Total gonad mass (g)) / (Total mass (g) – Total gonad mass (g) x 100%) and seminal vesicle (accessory gland) mass were used to further confirm the alternative reproductive tactic. Sneakers usually have large GSIs and small accessory glands while guarders usually have relatively smaller GSIs and larger accessory glands (Marentette et al. 2009, McCallum et al. 2019). Sneaker males were classified as such if they exhibited the usual visual characteristics (narrow head, pale or mottled body colour, small bodied, and a large papilla relative to standard length) and had a GSI of greater than 2% (Marentette et al. 2009; Young et al. 2010). Guarder males were classified as such if they exhibited the usual visual guarder male characteristics (wide head, dark or black coloured body, large seminal vesical mass, larger bodied) and had a GSI of greater than 1% (Marentette et al. 2009; Young et al. 2010).

*Statistical Analysis*

All statistical analyses were performed using R version 3.6.1 (R Core Team 2020). Morphological comparisons were performed using linear models. A PCA was conducted on morphological measures to better visualize the disparity between sneaker and guarder male round goby. Behavioural response variables were modelled as a function of status (guarder or sneaker) using univariate linear mixed effects models (LMMs), generalized linear mixed effects models (GLMMs), or Wilcox-Rank-Sum tests if parametric assumptions could not be met. (G)LMM's were performed using the (g)lmer function in the package LmerTest (Kuznetsova et al. 2017). Negative binomial models were performed using the glmer.nb function in the package lme4 (Bates et al. 2015). Random effects included capture site, Julian date, total days in lab since capture, and experimental tank/lane. The side in which the fish started on was included as random effect for the sociality trial. Data were assessed visually for normality using quantile-quantile plots and homoscedasticity through residual-versus-fitted plots. Arcsine-square root or $\log_{10}$ transformations were performed where required and noted for each model. All assays within a behaviour were standardized to the nearest minute rounded down. This resulted in the following assay durations: 29 minutes in the exploration/activity assay, 25 minutes in the social assay, 27 minutes in the aggression assay, 14 minutes in the startle assay, and 165 minutes in the dispersal assay. Fish excluded from analyses and the resulting sample sizes for each analysis/comparison are provided in Supplemental Table 2.1.

Correlation matrices across behaviours were calculated and analysed for guarder and sneaker males separately using the rcorr function in the Hmisc package (Harrell and

Dupont 2020). All correlations were analyzed using Pearson's r-squared after checking for normality. Each matrix consisted of the following behaviours: number of grid squares explored in 29 minutes (Exploration Assay), activity over the 29 minutes (Activity Assay), duration of time spent near the conspecifics (Sociality Assay) and the aggressive acts performed during the 25 minute sociality assay (Aggression Assay), latency to move after the drop of the marble simulating a predator attack (Startle Assay), and time taken to reach the end of the dispersal lane (Dispersal Assay). Observation numbers for each comparison are provided in Supplemental Table 2.2.

Significance for morphological and behavioural tests were assessed at $\alpha < 0.05$ after adjusting for multiple comparisons using the Benjamini-Hochberg method with a false discovery rate of 0.05 (Benjamini and Hochberg 1995). Original and adjusted p-values are provided throughout. Correlation analyses were exploratory and therefore the adjustment of p-values to maintain type I error rate was not considered.

### *Ethical Note*

Animal housing, handling and study protocols were approved by the McMaster Animal Research Ethics Board (Animal Utilization Protocol 17-45-12) and adhered to the guidelines of the Canadian Council for Animal Care. Fish were marked with a non-toxic acrylic paint injected just beneath the skin. This marking method did not cause any apparent long-term distress to the fish. Fish were monitored closely after injection and while in the holding tanks for any signs of injury or stress but non occurred.

## RESULTS

### *1.0 Morphological Differences Between Guarder and Sneaker Males*

Guarder and sneaker males differed significantly on a wide variety of morphological metrics (Table 2.1). The PCA conducted on the morphological metrics grouped by reproductive status indicates a clear separation in PC scores (Figure 2.1). Sneaker males had larger gonadal investment (GSI) and papilla length to body length ratios while guarder males had larger head width to body width ratios, liver investment (HSI), seminal vesicle (accessory glands) to gonad ratios, body mass, and standard length. Guarder males also had slightly larger body condition values.

**Table 2.1.** Comparison of round goby guarder males (GM, n = 25) to sneaker males (SM, n = 40) on a variety of morphological metrics. Round goby were collected from Lake Ontario, Canada.

| | μ | | Statistics | | |
|---|---|---|---|---|---|
| Measure | GM | SM | t | *p* | $p_{adj}$ |
| Standard Length | 7.80cm | 4.60cm | 11.90 | <0.001 | <0.001 |
| Total Mass | 13.62g | 2.35g | 13.20 | <0.001 | <0.001 |
| Head Width to Body Width | 1.26 | 1.05 | 8.64 | <0.001 | <0.001 |
| Body Condition | 2.63% | 2.29% | 3.28 | 0.002 | 0.01 |
| Hepatosomatic Index | 2.13% | 0.70% | 9.14 | <0.001 | <0.001 |
| Papilla to Body Length | 0.06 | 0.09 | -6.70 | <0.001 | <0.001 |
| Seminal Vesicle to Gonad Mass | 0.37 | 0.08 | 11.21 | <0.001 | <0.001 |
| Gonadosomatic Index | 2.32% | 4.63% | -7.53 | <0.001 | <0.001 |

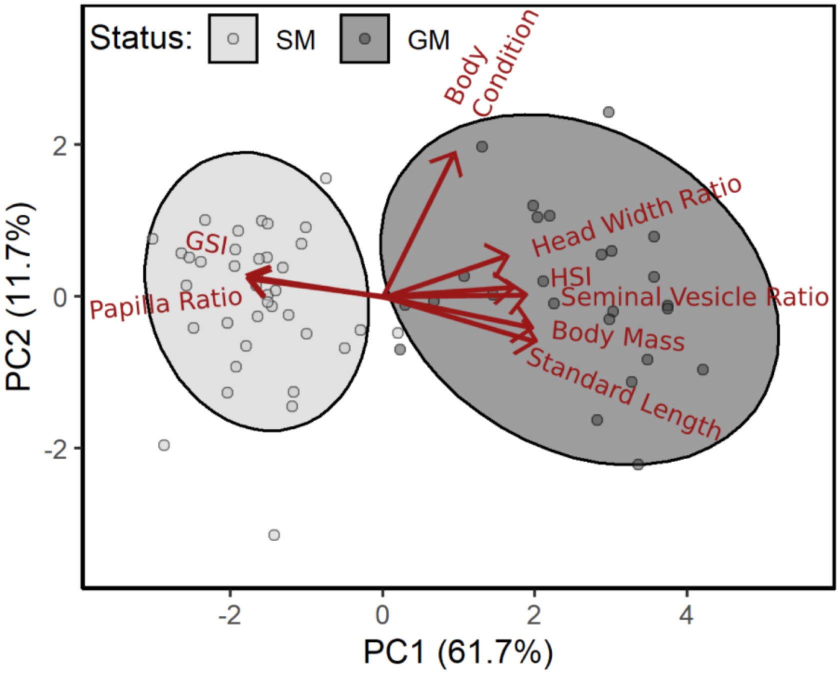

**Figure 2.1**. Principal component analysis (PCA) of round goby sneaker male (SM) and guarder male (GM) morphological traits. Together, the PC1 and PC2 axes account for 73.4% of the total variance. Ellipses indicate the 95% confidence intervals for the SM (in lighter grey) and GM (in darker grey) groups. Red arrows represent the loading and direction of each morphological variable.

### *2.0 Behavioural Differences Between Guarder and Sneaker Males*

### 2.1 *Exit Test*

Guarder males partially emerged (with just their heads) from the shelter and remained in this position for longer durations than sneaker males ($\log_{10}$-Linear Mixed Model, LMM, est. ± se = 0.76 ± 0.26, t = 2.92, *p* = 0.005, $p_{adj}$ = 0.01, Figure 2.2b). However, sneaker males were 92.7% more likely to exit the shelter completely compared to guarder males (Binomial Generalized Liner Mixed Model, GLMM, est. ± se = 2.53 ±

1.08, z = 2.34, $p = 0.02$, $p_{adj} = 0.03$, Figure 2.2c). Eight out of 25 guarder males and three out of 40 sneaker males never left the refuge on their own accord. Of the fish that did exit the shelter, there was no observed difference in the latency to exit between guarder and sneaker males (Wilcox-rank-sum test, est. W = 279, $p = 0.52$, $p_{adj} = 0.61$).

### 2.2 *Exploration and Activity*

Sneaker males explored more unique grid squares than guarder males after both 15 minutes (arcsine-square root-LMM, est. ± se = 0.39 ± 0.10, t = 3.77, $p < 0.001$, $p_{adj} = 0.002$) and 29 minutes (Wilcox-rank-sum test, W = 189, $p = 0.02$, $p_{adj} = 0.03$, Figure 2.2e). Sneaker males also spent more time in the center of the tank, averaging 78 seconds in the open center while guarder males only averaged 26 seconds in this more exposed central area of the tank (Wilcox-rank-sum, W = 179, $p = 0.02$, $p_{adj} = 0.03$). Sneaker males were more active than guarder males (LMM, est. ± se = 178 ± 80.0, t = 2.22, $p = 0.03$, $p_{adj} = 0.046$, Figure 2.2f).

### 2.3 *Sociality and Aggression*

Guarder and sneaker males did not differ in the time spent near conspecifics (LMM, est. ± se = 16.78 ± 89.4, t = 0.19, $p = 0.85$, $p_{adj} = 0.89$, Figure 2.2h). Both morphs spent more time near conspecifics but guarder males performed more aggressive acts towards conspecifics during the sociality assay compared to sneaker males (Negative Binomial-GLMM, est. ± se = 1.48 ± 0.44, t = 3.35, $p < 0.001$, $p_{adj} = 0.002$, Figure 2.2i). However, guarder and sneaker males did not differ in the number of aggressive acts performed towards the mirror (Negative Binomial-GLMM, est. ± se = -0.02 ± 0.75, z = -0.03, $p$ = 0.98, $p_{adj}$ = 0.98, Figure 2.2k) and both morphs performed fewer aggressive acts towards a mirror image than they did to live conspecifics during the sociality trial (Wilcoxon Signed-ranks test; V = 35, $p$ = 0.02, $p_{adj}$ = 0.03).

### 2.4 *Response to a Simulated Predator*

In response to the marble drop (a simulated predator strike), six guarders and two sneakers darted away while 15 guarders and 20 sneakers froze. No fish continued to swim normally. A similar number of guarder and sneaker males never resumed to move after the marble drop (Binomial-GLM, est. ± se = 0.69 ± 0.92, z = 0.75, $p$ = 0.45, $p_{adj}$ = 0.56). Of the fish that did move post marble drop (83% of guarders and 91% of sneakers), sneaker males were quicker to first move after the initial startle response compared to guarder males (LMM, est. ± se = -150.1 ± 61.6, t = -2.44, $p$ = 0.02, $p_{adj}$ = 0.03, Figure 2.2m). However, guarder and sneaker males did not differ in the duration of time spent moving before versus after the marble drop (LMM, est. ± se = -12.2 ± 22.4, t = -0.54, $p$ = 0.59, $p_{adj}$ = 0.67, Figure 2.2n).

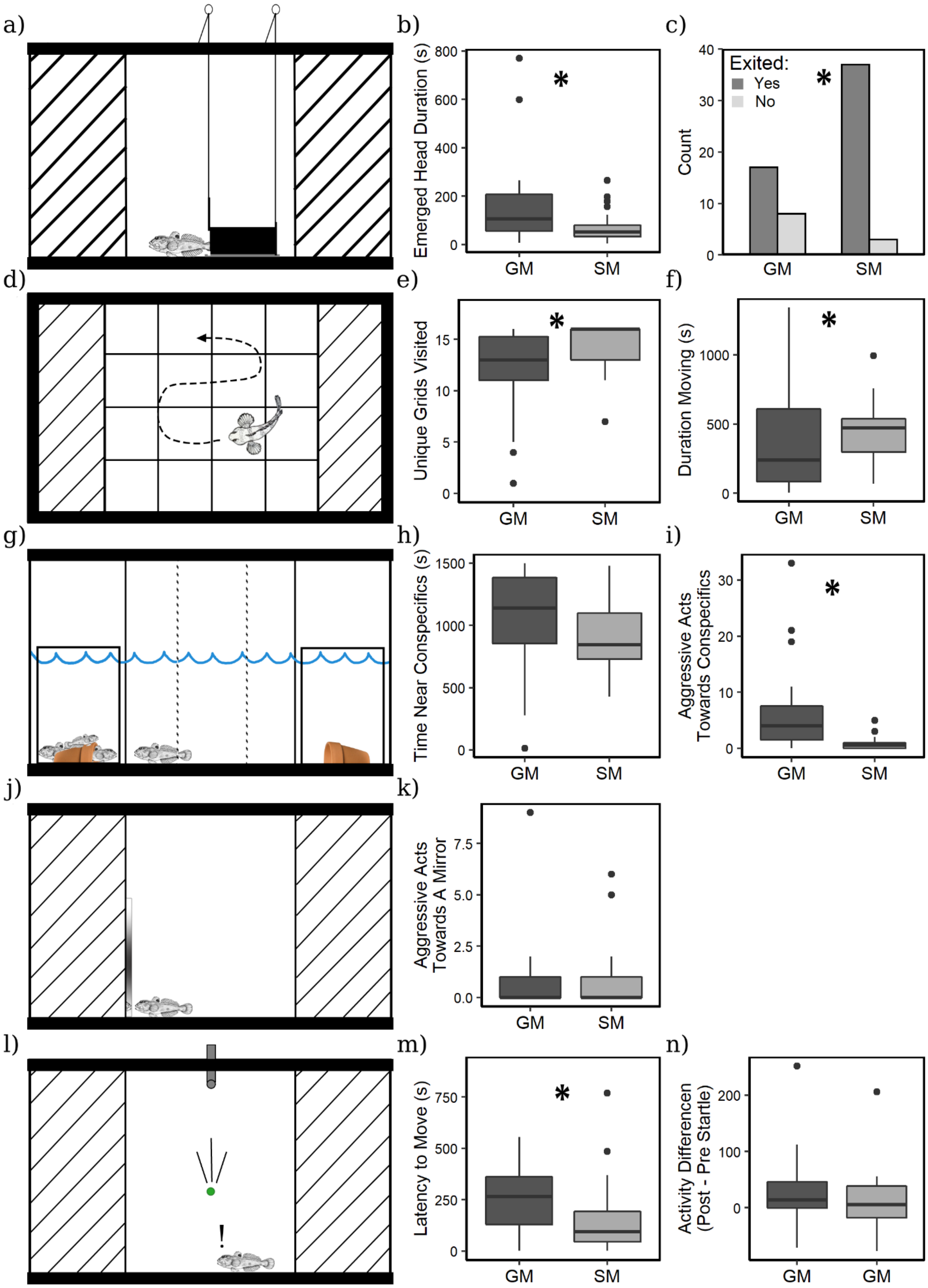
a)
b)
Emerged Head Duration (s)
800
600
400
200
0
*
GM
SM
c)
Exited:
■ Yes
■ No
*
Count
40
30
20
10
0
GM
SM
d)
e)
Unique Grids Visited
15
10
5
0
*
GM
SM
f)
Duration Moving (s)
1000
500
0
*
GM
SM
g)
h)
Time Near Conspecifics (s)
1500
1000
500
0
GM
SM
i)
Aggressive Acts
Towards Conspecifics
30
20
10
0
*
GM
SM
j)
k)
Aggressive Acts
Towards A Mirror
7.5
5.0
2.5
0.0
GM
SM
l)
!
m)
Latency to Move (s)
750
500
250
0
*
GM
SM
n)
Activity Differencen
(Post - Pre Startle)
200
100
0
GM
GM

**Figure 2.2**. Illustrations of the experimental tank set ups for the various behavioural assays employed and corresponding results. Each row shows the assay and associated behavioural results comparing round goby guarder males (GM) and sneaker males (SM): a) the refuge exit test, b) the average duration spent with head partially emerged from the refuge, and c) the number of fish that did and did not exit by status; d) the exploration assay, e) the number of unique grid squares visited, and f) activity; g) the sociality assay, h) the time spent near conspecifics (max 1500 seconds), and i) the average number of aggressive (ramming and parallel displays) acts toward conspecifics; j) the mirror aggression assay, k) the number of aggressive acts made towards a mirror, and l) the startle assay, m) the latency to move after the marble drop, and n) the difference in time spent moving before and after the marble drop. Boxplots indicate median and whiskers extend to the furthest datapoint within 1.5x the interquartile range. Individual points indicate values falling outside this range. * $p < 0.05$.

### 2.5 *Dispersal Tendency*

About one third of the fish (11 out of 31 guarder males and 13 out of 34 sneaker males) reached the end of the dispersal chamber (Binomial-GLMM: est. ± se = 0.16 ± 0.57, t = 0.28, $p = 0.78$, $p_{adj} = 0.85$). Guarder males took on average 1249 seconds to reach the end chamber while sneaker males took on average 1476 seconds, but this difference was not significant (LMM, est. ± se = -422 ± 404, t = -1.05, $p = 0.31$, $p_{adj} = 0.40$, Figure 2.3c). Sneaker males did not differ from guarder males in the total number of laps completed (Negative Binomial-GLMM; est. ± se = 0.36 ± 0.27, t = 1.35, $p = 0.18$, $p_{adj} = 0.24$, Figure 2.3d), however, sneaker males crossed between more zones than did guarder males (sqrt-LMM, est. ± se = 2.34 ± 0.66, t = 3.56, $p < 0.001$, $p_{adj} = 0.002$, Figure 2.3e).

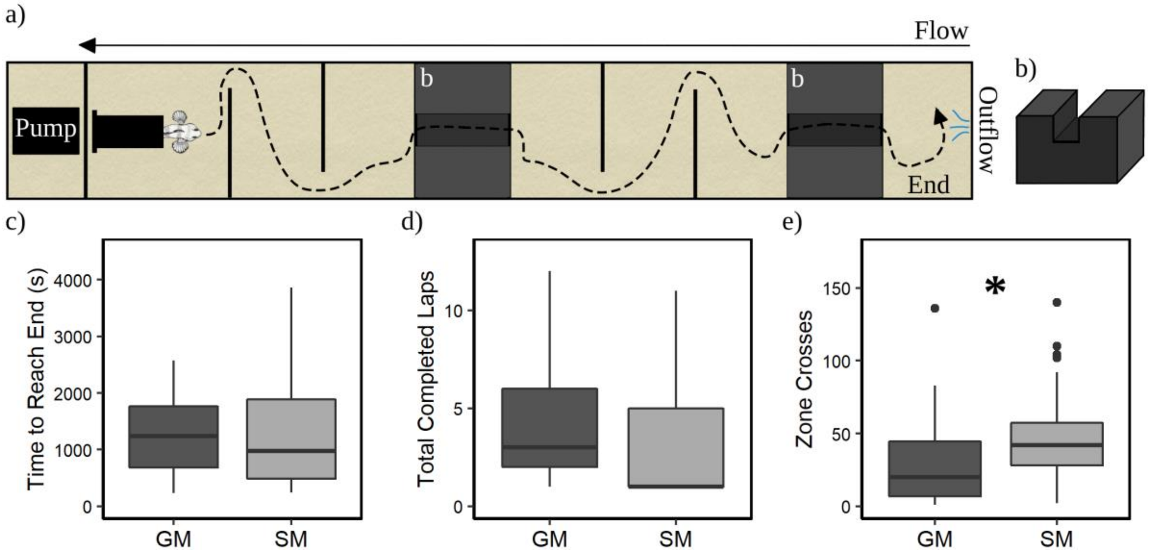

**Figure 2.3.** Illustration of the a) dispersal assay laneway used to assess dispersal tendency differences between round goby guarder males (GM) and sneaker males (SM). The dispersal tank contained three identical lanes side-by-side. Vertical lines indicate wall barriers that extended ¾ the way across the lane and b) canyon-type barriers were added to elevate goby closer to the surface to increase perceived risk. Results include c) the average time taken to reach the end after exiting the refuge (this graph only includes goby that reached the end), d) the average number of completed laps (travelling from one end to the other), and e) the average number of crosses made between barriers within the dispersal arena. Boxplots indicate median and whiskers extend to the furthest datapoint within 1.5x the interquartile range. Individual points indicate values falling outside this range. * $p < 0.05$.

### *3.0 Behavioural Correlations Across Assays*

We conducted correlational analyses on guarder and sneaker male behaviours and uncovered five behavioural correlations in guarder males (Figure 2.4a) and only one behavioural correlation in sneaker males (Figure 2.4b). In guarder males, there was a negative correlation between activity and the startle response ($r = -0.61$, $p = 0.002$), and between exploration and the startle response ($r = -0.47$, $p = 0.03$), where fish that were more active or more explorative were also quicker to move again after the marble drop. Activity was positively correlated with exploration in guarder males (*r* = 0.57, *p* = 0.004). A negative correlation was observed between sociality and dispersal, guarders that were less social dispersed more quickly (*r* = -0.94, *p* = 0.005). Finally, a positive correlation was observed between sociality and aggression in guarder males (*r* = 0.56, *p* = 0.01). One correlation in sneaker males was significant: a negative correlation between exploration and sociality, fish that were more social were less explorative (*r* = -0.46, *p* = 0.03).

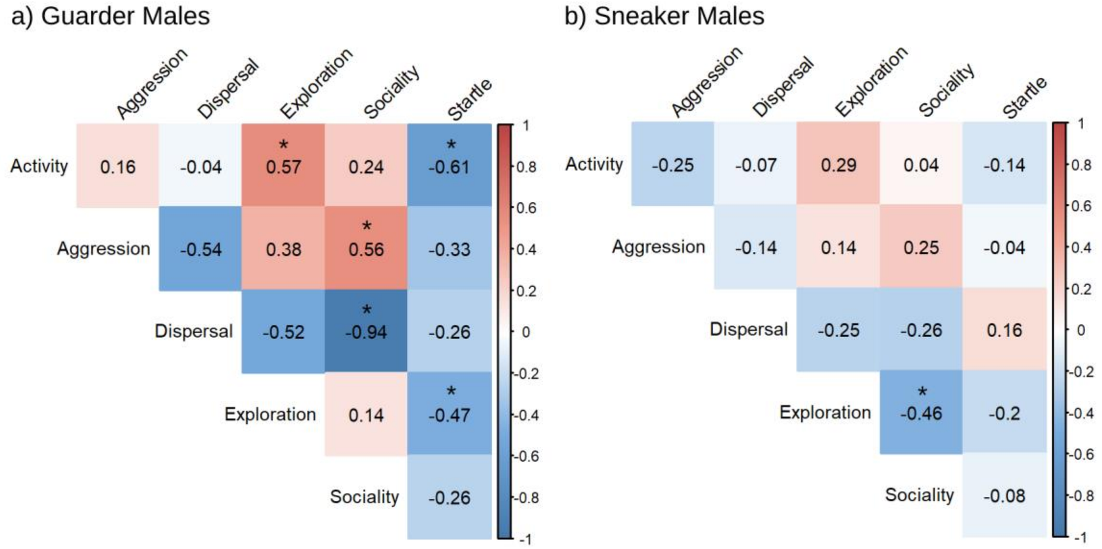

**Figure 2.4**. Correlation matrix of behavioural tendencies for round goby a) guarder males and b) sneaker males. Red indicates positive Pearson's r-squared values while blue indicates negative Pearson's r-squared values for each correlation. Colour saturation indicates the strength of the correlation, and the Pearson's r-squared values are given inside each square. * $p < 0.05$.

## DISCUSSION

### *1.0 Round Goby Male Tactics Have Clear Morphological Differences*

Sneaker and guarder male round goby exhibit vastly different morphologies. Guarder males had larger seminal vesicles. Seminal vesicles are thought to aid in the production of pheromones, acting as an attractant for females (Jasra et al. 2007), which likely aids guarder males more so than sneaker males. Additionally, seminal vesicles may also aid in sperm storage (Jasra et al. 2007, Marentette et al. 2009). Sneaker males had a larger average gonadosomatic index. These patterns are consistent with past research conducted on round goby male tactics (Marentette et al. 2009, Bleeker et al. 2017, McCallum et al. 2019). Sneaker males are consistently under sperm competition as they require the presence of a guarder male to reproduce, and invest more into their gonads as a result (Parker and Ball 2005, Marentette et al. 2009). Guarders were in better condition than sneakers, having higher Fulton's body condition and hepatosomatic index scores. This was surprising as we expected guarder males to be in worse condition than sneaker males as a result of parental investment associated with nest guarding and offspring care. It is possible sneaker males have lower condition scores because they are smaller and thus outcompeted for food by larger conspecifics such as guarder males.

### *2.0 Sneaker and Guarder Males have Different Behavioural Tendencies*

Guarder and sneaker males differed in terms of exploration, activity, boldness, and aggression. Sneaker males were more likely to exit a refuge into a novel tank compared to guarders (92% of sneakers exited vs 68% of guarders). Sneaker males also were more active (had more zone crosses) and explored more of the novel tank than guarder males. Exploration may increase probabilities of locating a nest with a spawning females while guarder males territoriality may inhibit explorative behaviour (Dubs and Corkum 1996, MacInnis and Corkum 2000). Exploration of a novel habitat generally requires some degree of activity and exploration-activity behavioural syndromes have been observed in fish before (Cote et al. 2010). While we observed a positive association between exploration and activity in guarder males, the correlation between these behaviours in sneaker males did not reach significance.

Not only did sneaker males explore more of the novel tank than did guarders males, they also spent more time in the central portion of the tank and were also quicker to begin moving after the startle. These metrics are common measures of boldness (Krause and Godin 1994, Aspbury et al. 2010, Thorlacius et al. 2015) and suggest that sneakers are less risk adverse. Structure, such as tank walls, provide fish with more security than do open spaces, such as the center of a tank (Burns 2008, Magnhagen et al. 2014). To achieve reproductive success, and exploit guarder males' courtship and paternal care, sneaker males require daring and bold and sneak into a nest and stealing fertilization (Gross 1982; Taborsky 2001). Such behaviour has risks as defensive response from much larger guarder males may result in injury or mortality (Gross and Charnov 1980, Magnhagen 1995, Scaggiante et al. 2005). When analyzing the response to a simulated predator strike, another common boldness assay, we

We found guarder males were more aggressive than sneaker males. Resource guarding males often display aggressive nest defence behaviours (Gross 1991, Scaggiante et al. 2005). In contests where there is a disparity in size between competitors, the larger individual often has the competitive advantage (Rowland 1989, Stammler and Corkum 2005). We used conspecifics of varying sizes during the sociality assay. Guarder males may have been more aggressive simply because some conspecifics were smaller than focal guarders, and therefore the costs of initiating an aggressive contest against them would be lower based on increased likelihood of a successful outcome. In contrast, sneakers, being smaller in size, may have faced higher costs from engaging aggressively with any of the conspecifics. Significantly less aggressive acts were made towards the mirror, and no difference in aggression between the tactics was observed during this assay. Mirror assays are an attractive method of studying aggression as they present the focal individual with a perfectly size matched 'competitor', an important factor as a difference in mass of just 3% reliably predicts victory for the larger individual in round goby (Stammler and Corkum 2005). Presenting this size matched 'competitor' likely dissuaded sneaker and guarder males from aggressive tendencies as the probability for victory was lessened. There is some ambiguity on whether mirrors assays are a valid test of aggression in fish (Desjardins and Fernald 2010, Balzarini et al. 2014). Some argue that mirror assays do not always capture the full complexity of dyadic aggressive interactions (Balzarini et al. 2014), including in round goby (McCallum et al. 2017), however others have found fish to have similar aggressive behaviours between mirrors and conspecific competitors (Desjardins and Fernald 2010). In other species, such as the guppy, *Poecilia reticulata*, mirror tests may be a better measure of sociality than aggression (Cattelan et al. 2017). This ambiguity may partially explain why we observed differences in aggressive tendencies between the two assays.

We found no differences in either sociality or dispersal tendencies between sneaker and guarder males. Both guarder and sneaker males spent the majority of the trial time near the conspecifics. Past research has indicated a preference for associating with larger groups of conspecifics in non-reproductive male round goby (Capelle et al. 2015). Round goby often live in highly dense populations which may necessitate a higher tolerance towards conspecifics, even among the territorial guarder males (Ray and Corkum 2001, Pennuto et al. 2012). There is evidence to suggest large round goby males (>70mm) seasonally migrate into tributaries during the spring season to spawn (Blair et al. 2019). Additionally, larger body size has been correlated with dispersal tendency in round goby (Thorlacius et al. 2015). While we found guarder males reached the end of the dispersal assay faster than sneaker males (1249 seconds versus 1476 seconds, respectively), this comparison was not significant. However, it is possible that because only a small number of goby reached the end of the dispersal assay ($n_{sneaker}$ = 13 & $n_{guarder}$ = 11), the conclusion of no differences between tactics in terms of their dispersal tendency could be overturned with additional research. Further, other experiments assessing non-reproductive male round goby dispersal have used arenas greater than four meters long (Myles-Gonzalez et al. 2015, Thorlacius et al. 2015). Due to space constraints, it was not possible for us to use a longer testing arena and so it is possible that our dispersal arena was not long enough (2.5m) to adequately track dispersal tendencies.

### *3.0 More behaviours are correlated in guarder males compared to sneaker males*

Guarder males exhibited five behavioural correlations while sneaker males only exhibited one set of behavioural correlations. In Wellington tree weta, *Hemideina crassidens*, males that adopted a sneaking tactic displayed a behavioural syndrome, while guarding and flexible males (ie. those that can adopt either a sneaking or guarding tactic) displayed no such behavioural syndromes (Wilson and Kelly 2019). In the water strider *G. gracilicornis*, signalling tactic males (that coerce females into reproduction) had a behavioural syndrome where increased mating attempts were associated with lower cautiousness (Han and Jablonski 2019). However, nonsignaling tactic males (that wait for females to expose genitals) showed no behavioural syndrome, likely because their reproductive tactic is characterized by an opportunistic sit-and-wait strategy where nonsignaling males do not actively search in open risky areas for mates to coerce (Han and Jablonski 2019). Round goby reproductive behaviour is not well understood as it is challenging to observe these fish in the field or to induce their breeding in laboratory environments (Marentette et al. 2009). It is not known whether the sneaking tactic is fixed for life. If sneaking males can adopt the guarding tactic, or switch between the tactics, then more behavioural flexibility, and by extension, weak or no behavioural syndromes may be expected, such as the case in Wellington tree weta (Wilson and Kelly 2019). The one correlation we observed in sneaker males was a positive association between exploration and sociality. This result has previously been found in round goby, where males designated as ‘small parental males’ were found to have high levels of exploration, boldness, risk-taking, and sociality (Nguyen-dang 2017). We found five different behavioural correlations in round goby guarder males. First, activity and exploration were positively correlated. These two behaviours are commonly correlated across many different species such as eastern chipmunks, *Tamias striatus* (Martin and Réale 2008), rock pool prawns, *Palaemon elegans* (Chapman et al. 2013), fairy-wrens, *Malurus cyaneus* (Hall et al. 2015), and delicate skinks, *Lampropholis delicata* (Moule et al. 2016). We also found that activity and startle responses were negatively correlated; fish that were more active were quicker to move after a startle (marble drop). Exploration and startle responses were also negatively correlated. High boldness, activity, and exploration are commonly correlated behaviours across different fish species (Wilson and Godin 2009, Cote et al. 2010), but interestingly these correlations were not observed in a previous study of non-reproductive male goby (Thorlacius et al. 2015). Lastly, we observed a strong negative correlation between sociality and dispersal and a strong positive correlation between sociality and aggression. Asocial behaviours have been correlated with higher dispersal tendencies in invasive mosquitofish, *Gambusia affinis* (Cote et al. 2010) and in common lizards, *Lacerta vivipara* (Cote and Clobert 2007). The positive correlation between sociality and aggression in guarder males is unsurprising as aggression is viewed as form of interaction with conspecifics.

Round goby guarder and sneaker males differ in size, where the average guarder male standard length was 7.8 cm and the average sneaker male standard length was 4.6 cm in this study. Some behaviours, such as exploration and boldness, can be size dependent, where smaller individuals differ consistently in their behaviour from larger individuals (Dowling and Godin 2002, Ness et al. 2004, Maillet et al. 2015, Mayer et al. 2016). However, not every species exhibits this size dependent behaviour (Harris et al. 2010, Wilson et al. 2010), and even within a species, some behaviours are size dependent while others are not (Kelleher et al. 2017). For example, in southern corroboree frogs, *Pseudophryne corroboree*, body size was not associated with activity or boldness, but was strongly associated with exploration (Kelleher et al. 2017). In round goby, length has been correlated with behaviours such as activity and exit time, but not with exploration and dispersal (Marentette et al. 2012). The size disparity between sneaker and guarder males makes our certainty about whether some of the observed differences are due to tactic or size challenging to tease apart as the two measures are confounded. Additionally, smaller individuals in the round goby consume more food relative to their weight compared to larger individuals (Lee and Johnson 2005). Sneakers, being smaller in size, may move more and take more risks as a means of finding food and keeping up with higher foraging demands (Krause et al. 1998, Dowling and Godin 2002, Brown and Braithwaite 2004). Indeed, w

### *4.0 Future directions and significance*

While guarder and sneaker male round goby differ in size, there is an overlap in standard lengths. Generally, that overlap falls between ~5.0–7.0 cm, but could be as high as 5.0–9.5 cm (Bose et al. 2018). To tease apart whether behavioural differences are due to reproductive tactic or due to size differences, we recommend that future behavioural analyses be conducted using similarly size sneaker and guarder males, falling within the ~5.0–7.0 cm range. Alternatively, small, and large non-reproductive males could also be studied to explore the extent to which these behavioural differences are related to size alone and not tactic.

Our results indicate that researchers should 1) perform behavioural assessments of each tactic in species with alternative reproductive tactics to deepen our understanding of how behavioural variation and reproductive variation interact, and 2) be cognisant of potential behavioural variation induced by alternative reproductive tactics, especially in invasive populations. In the round goby, it is often challenging to distinguish guarder males from sneaker males when both tactics are in a non-reproductive state. Therefore, behavioural assessments performed on only non-reproductive males may miss important variation tied to ARTs if the behavioural tendencies remain consistent between reproductive and non-reproductive states. Future research should focus on testing whether guarder and sneaker males remain consistent in their behaviour between reproductive and non-reproductive states. As the round goby is an invasive fish, understanding if one tactic is more prevalent in spreading populations may be advantageous, and we recommend that researchers pay careful attention to the ratios of guarder to sneaker males on an invasion front and compare the ratio to that found in the originating established population. Using the knowledge that different fish capture techniques may target individuals with certain behaviours (Diaz Pauli et al. 2015), fisheries and management techniques can then be optimized to target individuals on invasion fronts with specific behavioural and life history traits, potentially mitigating spread of invasive populations.

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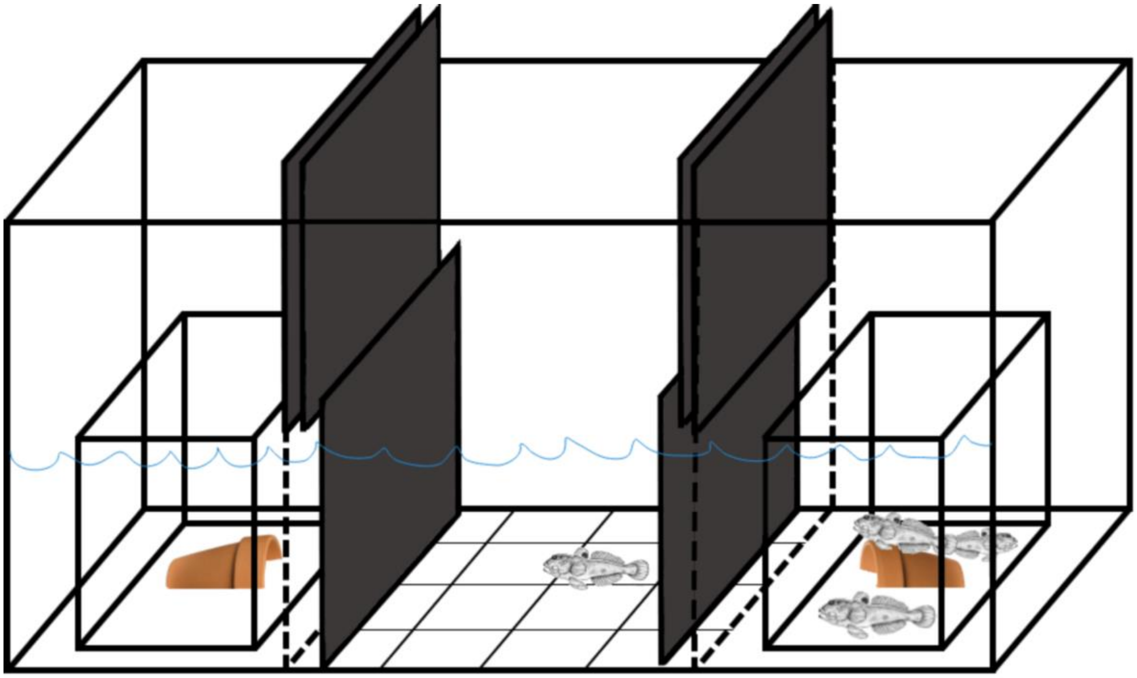
## Supplemental Materials



**Supplemental Figure 2.1**. Experimental tank set up for assessing behavioural tendency differences between guarder and sneaker round goby. The two end sections contained one 2.5 gallon tank with a flow pot half each. One 2.5 gallon tank contained an additional three conspecifics. The separated tanks allowed for visual cues while limiting olfactory cues. Opaque barriers were held on strings and connected to a pully system behind curtains, allowing for the remote lifting or lowering of barriers with minimal disturbance to the focal individual.

**Supplemental Table 2.1.** Number of sneaker and guarder males ran for each behavioural assay and any associated exclusions.

| Assay | Exclusions | Reason | Final Counts |
|---|---|---|---|
| **Overall** | 5 | Unknown Reproductive Status | 27 GM, 40 SM |
| **Exit Test** | | | |
| SM | 0 | NA | 40 |
| GM | 2 | Experimental Set-up Error (Excluded from rest of assays) | 25 |
| **Exploration** | | | |
| SM | 15 | Escaped Focal Area (Excluded from rest of assays) | 25 |
| GM | 1 | Experimental Set-up Error (Excluded from rest of assays) | 24 |
| **Sociality** | | | |
| SM | 3 | Escaped Focal Area (Excluded from rest of assays) | 22 |
| GM | 5 | Escaped Focal Area (1; Excluded from rest of assays), Never Moved/Switched Zones (4) | 19 |
| **Aggression** | | | |
| SM | 4 | Never Moved/Switched Zones | 18 |
| GM | 9 | Never Moved/Switched Zones | 15 |
| **Startle** | | | |
| SM | 1 | Escaped Focal Area | 21 |
| GM | 1 | Experimental Set-up Error | 22 |
| **Overall** | 4 | Unknown Status | 38 GM, 42 SM |
| **Dispersal** | | | |
| SM | 8 | Escaped Lane (6), Conspecific Entered Lane (2) | 34 |
| GM | 7 | Escaped Lane (1), Conspecific Entered Lane (4), Never Moved (1), Experimental Set-up Error (1) | 31 |

**Supplemental Table 2.2.** Number of observations for each pair of behavioural correlations in sneaker male and guarder male round goby.

| Guarder Males | | | | | | |
|---|---|---|---|---|---|---|
| | Activity | Aggression | Dispersal | Exploration | Sociality | Startle |
| Activity | - | 23 | 7 | 24 | 19 | 22 |
| Aggression | - | - | 7 | 23 | 19 | 22 |
| Dispersal | - | - | - | 7 | 6 | 7 |
| Exploration | - | - | - | - | 19 | 22 |
| Sociality | - | - | - | - | - | 18 |
| Startle | - | - | - | - | - | - |
| **Sneaker Males** | | | | | | |
| | Activity | Aggression | Dispersal | Exploration | Sociality | Startle |
| Activity | - | 23 | 6 | 25 | 22 | 22 |
| Aggression | - | - | 6 | 23 | 22 | 22 |
| Dispersal | - | - | - | 6 | 6 | 6 |
| Exploration | - | - | - | - | 22 | 22 |
| Sociality | - | - | - | - | - | 21 |
| Startle | - | - | - | - | - | - |

# CHAPTER 3

## WITHIN-GEAR VARIATION IMPACTS POPULATION ESTIMATES IN AN INVASIVE FISH

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## Abstract

Different fisheries capture gear each have their own biases in terms of catch rates, species, body sizes, sexes, and even behavioural types that are most likely to be caught. Although the *between gear-type variation* is well accepted, biases stemming from *within gear-type variation* remain largely unexplored. Minnow traps are a common fisheries gear available in slightly different sizes and materials, however these different trap models are typically used interchangeably, and trap type is rarely reported in publications. In this study we investigated if trap model might influence the number or type of fish caught, making use of two popular types of minnow traps (vinyl-coated metal *black traps* and galvanized metal *silver traps*). We compared the effectiveness of black traps to silver traps for catching the round goby, an invasive species in North America and more recently, Western Europe. Additionally, we assessed how baiting minnow traps (corn) compares to leaving minnow traps unbaited in terms of catch efficiency and selectivity. We found that silver traps, captured ~2x more round goby than black traps and baited traps captured ~3.5x more round goby than unbaited traps. Neither trap nor bait had a significant impact on the sex ratio (1.3 males for every 1 female) or on the operational sex ratio caught (1 male to 1 female) or on the ratio of the two male reproductive morphs (1.3 guarder males to every 1 sneaker male). Baited traps captured larger fish overall and tended to catch more guarders. Black traps also captured larger males compared to the silver traps. Our study confirms the need to carefully consider and control the gear type, model and bait type employed when sampling.

**Keywords:** gear bias, round goby, Great Lakes, *Neogobious melanostomus,* alternative reproductive tactics, fisheries, minnow trap, selectivity

## Introduction

Fisheries scientists and resource managers have at their disposal an assortment of gear types to sample and estimate fish population size and status. However, each gear type may have a selectivity for particular fish species, sizes, sexes, or even behavioural/personality types; so the use of different gear types can produce different or biased population or community estimates (Huse et al. 2000; Stergiou and Erzini 2002; Ruetz et al. 2007). For example, passive gear (e.g. trap nets and gill nets) require that fish actively encounter the gear to be captured, which may inadvertently select for individuals or species that are more active, bold, or exploratory (Härkönen et al. 2014; Diaz Pauli et al. 2015). In contrast, active gear, which requires active human manipulation for capture (e.g. seine netting or electrofishing), is less likely to create such biases for a particular sex, size range, and behavioural temperaments. While scientists are generally aware of the biases induced *among* gear types, biases *within* a gear type have not been well-studied or considered. In this study, we examined the degree of bias that can be generated when using two slightly different varieties of the same passive gear type: minnow traps.

Despite the potential biases described above with passive gear, it remains a popular choice for many organizations and researchers because of the ease and speed of deployment, reduced need for man/woman power, and associated low costs. One of the most popular passive gear types are minnow traps, a small meshed two-sided net that is widely available and are manufactured by a variety of companies. Minnow traps can be made of a fabric or metal mesh, and metal traps may or may not have a vinyl coating. The most common minnow trap models are galvanized silver coloured metal traps and black vinyl coated metal traps, hereafter referred to as silver and black traps, respectively (Figure 3.1). While appearing similar in construction, these silver and black traps do differ in a variety of measures, such as entry hole diameter and mesh thickness (see Table 3.1). Three previous studies have shown that silver traps had higher catch rates compared to black traps (when capturing three-spined stickleback, *Gasterosteus aculeatus*, Merilä et al. 2013; nine-spined stickleback, *Pungitius pungitius*, Budria et al. 2015; pumpkinseed sunfish, *Lepomis gibbosus*, creek chub, *Semotilus atromaculatus*, and white suckers, *Catostomus commersonii* (Paradis et al. 2012).

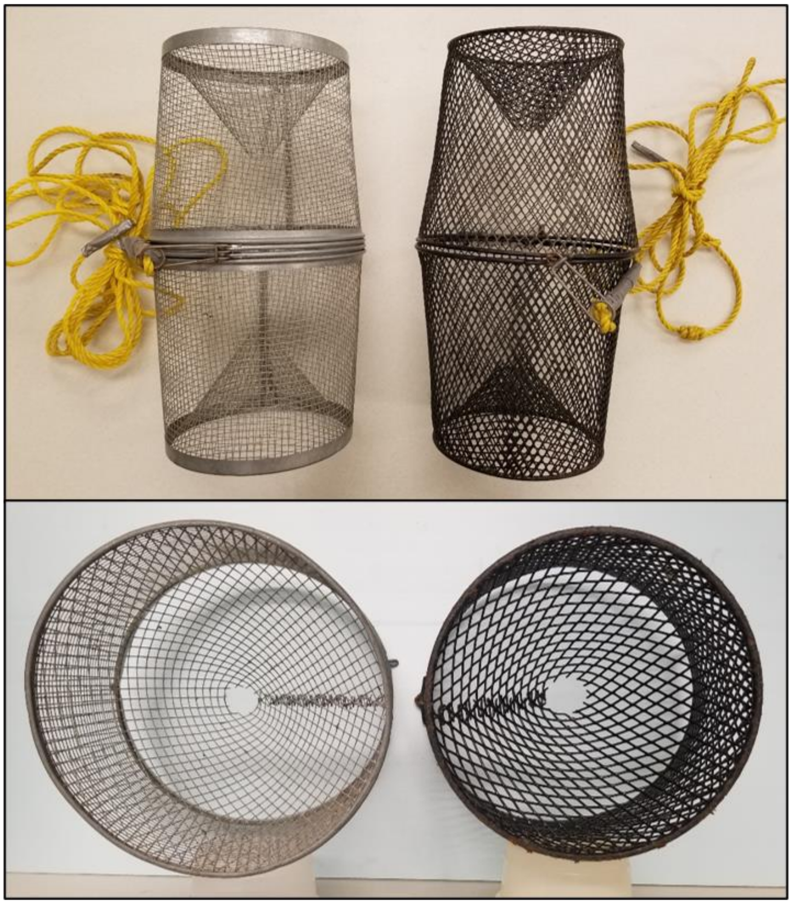

**Figure 3.1**. Two popular minnow trap models, Gee's Galvenized (silver, left) and Eagle Claw (black, right) commonly sold in retail shops in North America. Aside from colour differences, silver traps have a smaller entry hole and longer funnel length. Black traps have a thicker mesh lining and a smaller base diameter.

Although silver traps appear more effective than black traps, there is not a clear understanding as to why this phenomenon occurs. It might be a result of differences in visual saliency of the trap relating to the reflectivity of the exposed galvanized steel and/or a result of the silver traps having a slightly longer funnel and shape that promotes greater fish retention compared to black traps (Merilä et al. 2013). Indeed, three-spined stickleback escaped less often from silver traps compared to black traps (Paradis et al. 2012, Budria et al. 2015). Trap colour can have an important role in attracting animals, influencing the number of individuals captured. For example, more male velvet bean caterpillar moths, *Anticarsia gemmatalism,* and fall armyworm moths, *Spodoptera frugiperda,* were captured in multicoloured traps (that were green, yellow, and white) compared to traps that were monocoloured (just green) (Mitchell et al. 1989). Different coloured glow sticks (green, yellow, red, pink, and blue) were assessed for their efficiency and selectivity for capturing Centrarchid fish larvae, and researchers found green and blue glow-sticks captured the most larvae while red and pink glow-sticks captured the least (Marchetti et al. 2004). Understanding what trap attributes act to best attract a species could have important management implications, especially in the context of invasive species.

**Table 3.1.** A comparison of various measurements between Gee's Galvanized (silver) and Eagle Claw (black) minnow traps.

| Measurement | Black Traps | Silver Traps |
|---|---|---|
| Total mass | 614g | 609g |
| Mesh wire thickness | 1.7mm | 0.8mm |
| Mesh hole diameter | 7.6mm | 5.8mm |
| Funnel length | 85mm | 115mm |
| Entry hole diameter | 21mm | 17mm |
| Base diameter | 167mm | 183mm |
| Middle diameter | 212mm | 212mm |

The round goby, *Neogobius melanostomus,* is an invasive species frequently monitored using minnow traps (Diana et al. 2006, Marentette et al. 2009, Young et al. 2010, Bose et al. 2018). Round goby are a highly prolific benthic fish that was likely introduced into the Laurentian Great Lakes through ballast water from transoceanic ships (Jude et al. 1992). Currently, round goby are spreading into previously uninvaded habitats in Western Europe and North America, making them a global subject of research and management concern (Corkum et al. 2004). As such, understanding how different trap models may bias round goby catch is critical for accurate data comparisons across North America and with other continents. One less appreciated aspect of round goby biology is the presence of male alternative reproductive tactics (ARTs); territorial guarder males and parasitic sneaker males both exist in the same population (Marentette et al. 2009, Bleeker et al. 2017, Bose et al. 2018). These males differ in their external morphology, guarder males are generally larger than sneaker males (Marentette et al. 2009, Bleeker et al. 2017, Bose et al. 2018), and also differ in their behavioural tendencies, sneaker males are more active, explorative, and bold (Chapter 2). Based on these morphological and behavioural differences, trap types might also capture the alternative tactics in different ratios. In this study we experimentally tested whether silver or black minnow traps catch more round goby, differ in selectivity of the types of round goby captured (sex, ART, and size), and attempt to untangle why such capture differences might occur.

To investigate this question we quantified the number, sex, reproductive status, alternative reproductive tactic, and size of round goby captured in black (Eagle Claw brand) versus silver (Gee’s Galvanized brand) minnow traps over a two-year period. As methods around the use of bait also can vary across jurisdictions, government agencies, and research projects we also assessed the effect of baiting traps (using corn) in both silver and black traps on catch statistics mentioned above. We predicted that baited traps would have higher catch rates purely because food is an incentive to enter a trap. We also predicted that silver traps would capture more fish than black traps because other studies based on other species have found similar results (Paradis et al. 2012, Merilä et al. 2013, Budria et al. 2015). As the silver traps have a smaller entry hole compared to the black traps (Table 3.1), we predicted that silver traps would both catch smaller fish, such as sneaker males, and retain more fish compared to black traps. Lastly, to better understand if silver traps are more attractive because of their colour or reflectivity, or because of their shape, we spray-painted silver traps black and compared their catch to unaltered silver traps (see Figure 3.1a). If the colour/reflectivity is the major driver of catch performance, then we would expect to find black spray-painted traps to capture significantly less round goby. Alternatively, if the shape of the funnel and size of the entry hole is the driving factor in better performance of silver traps, than we would expect to see no difference in catch rates across true silver and silver traps spray-painted black.

## Methods

### *Fish collection*

Minnow traps were set at six locations around Hamilton Harbour, Ontario, Canada, an area of concern under the Great Lakes Water Quality Agreement. Locations included Desjardins Canal (DC; 43.277984, -79.888725), Grindstone Creek (GC; 43.286629, -79.886802), LaSalle Marina (LS; 43.300212, -79.846016), Fisherman's Pier (FP; 43.296320, -79.796384), Pier 27 (P27; 43.284453, -79.791594), and Sherman's Inlet (SI; 43.270107, -79.833852). At each site, a set of four silvers traps (Gee's galvanized brand)

and six black traps (Eagle Claw brand) were set on 18 separate dates roughly every two weeks between August–November 2018 and between April–November 2019.

Lake Ontario experienced an extreme high-water level event in the summer of 2019, resulting in inaccessibility to two sites, DC and GC, between May to July 2019. While access to our regular GS site was impossible, we set traps 304 meters to the northwest from the original inaccessible GC location in July 2019. At each collection site, two silver traps (1S and 2S) and two black traps (1 and 2) were left unbaited, and two silver traps (AS and BS) and four black traps (A, B, C, D) were filled with 25g of corn. The traps were set in a specific order at all sites and consistent between all sampling dates: 2S, BS, 2, D, C, B, A, 1, AS, 1S, with the exception of LS, where the order was: 2S, 1S, BS, AS, 2, 1, D, C, B, A due to private property. The numbers of different traps types and set order used were partly dictated by the fact this study was tagged onto a long-term  round goby monitoring project in Hamilton Harbour that has been ongoing since 2002 (Vélez-Espino et al. 2010, Young et al. 2010, McCallum et al. 2014, 2019). Traps at each location were attached to long ropes and were thrown from the shore where they were left at ~1 m depth and ~5 m from shore for approximately 24 hours.

Upon retrieval, fish were identified to the species and each trap was processed separately. The sex of any caught round goby was determined by examining their external urogenital papilla and recorded per individual trap. Female papillae are short and broad while male papillae are thin and triangular (Marentette et al. 2009). All round goby caught were then euthanized with an overdosed benzocaine-water mixture and placed in bags labelled with the collection date, site, and trap. Bags were placed on ice and then transported to McMaster University for dissection. Any native species captured were recorded and released back into the water.

In addition, to explore whether colour/reflectivity attracts more fish, a trio of silver traps (all baited) were set at each site every two weeks from June 30th 2020 to August 26th, 2020. These included a silver trap spray-painted black, a silver trap spray-painted clear, and an unaltered silver minnow trap (Figure 3.3a). We used Plasti Dip® to coat the traps as this brand has been used for various applications in past aquatic studies with no deleterious effects to fish (Herke and Moring 1999, Cooke and Philipp 2004).

### *Dissections*

In the laboratory, the following measurements were collected for each fish: standard and total length, papilla length, head width, body width, total mass, liver mass, total gonad mass, and seminal vesicle mass in the case of males. Morphological body measures were taken with calipers accurate to the nearest millimeter. Body and organ masses were taken in grams using an Ohaus Adventurer Pro digital scale, accurate to three decimal places. A variety of indices were calculated from the collected measurements and used to determine reproductive status and condition. Condition was calculated using Fulton's Body Condition (Total mass (g)) / (Standard length (cm) ^ 3 x 100) and Hepatosomatic index (HSI) (Liver mass (g)) / (Total mass (g) – Liver mass (g) x 100). Gonadosomatic index (GSI) (Total gonad mass (g)) / (Total mass (g) – Total gonad mass (g) x 100) was used to confirm reproductive status. Seminal vesicles, or accessory glands, were used to further confirm alternative reproductive tactic in male round goby; sneakers usually have small accessory glands while and guarders usually have larger accessory glands (Marentette et al. 2009). Sneaker males were classified as such if they were small, had narrow heads, and very large papillae and gonads relative to their body size, and had a GSI of greater than 2% (Marentette et al. 2009; Young et al. 2010). Guarder males were classified as such if they had a wide head, were very dark or black in colour, had large seminal vesical mass, and had a GSI of greater than 1%. Females were classified as reproductive if they had a GSI greater than 8% (Marentette and Corkum 2008).

*Statistical Analysis*

All statistical analyses were performed using R (Version. 3.6.1, R Core Team, 2019), and a significance of $\alpha < 0.05$ was used for all tests. Total catch was analyzed using a multivariate mixed effects model constructed using the glmmTMB function from the package 'glmmTMB' with the family 'nbiom2'. When one fish is caught this often can attract others to the trap. To analyze the probability that one trap type was more likely to capture groups of fish vs capture a single fish, we used a glmmTMB model with a binomial distribution (Group Catch = Yes or No). These models included bait (Yes vs No), trap type (Black vs Silver), site, and year as predictor variables, with interactions between bait type and site, trap type and site, and bait type and trap type. Month and individual trap ID within each site were included as random effects in each model. Catch abundance among the trio of spray-painted silver minnow traps was analyzed with glmmTMB using the 'nbinom2' family. Trap type (black-spray painted, clear spray-painted, and unaltered silver), site, and year, with an interaction between trap type and site were included as predictors. Trap type within site was included as a random effect. Proportion of males to females captured, and the operational sex ratio (reproductive males:reproductive females) were analyzed using glmmTMB with a binomial distribution, as was the proportion of guarder males to sneaker males. Trap type and bait type were included as predictors, as well as a bait type by trap type interaction. Random effects included month, site, and trap within site. Each glmmTMB model was visually assessed for fit by plotting simulated residuals using the DHARMa package. No significant deviations were detected. Finally, the difference in standard length was $\log_{10}$ transformed and analyzed using a multivariate linear mixed effects model, lmer, from the 'lmer' package with sex, year, trap type, and bait type as predictors. Interactions included bait type by trap type, sex by trap type, and sex by bait type. Random effects included month, site, and trap within each site. Interactions were retained in all models regardless of significance. Assumptions of normality and homoscedasticity were visually assessed using quantile-quantile and scale-location plots. Overall effects for all models were assessed with the Anova function from the 'car' package using type three sums-of-squares. Further analysis to calculate estimates and statistics was done using the 'emmeans' package. Analysis of sex and ART ratios by trap type and bait type were done using a one-way chi-squared test assuming even proportions.

## Results

### *1.0 Catch Differences*

### *1.1 Trap Type and Bait Types*

Silver traps caught 1.7 times more round goby than black traps (Negative Binomial Generalized Linear Mixed Model, GLMM, $t = 4.82$, $p < 0.001$, Figure 3.2a) and baited traps caught 3.4 times more round goby than unbaited traps (Negative Binomial GLMM, $t = 10.3$, $p < 0.001$, Figure 3.2b). There was a significant trap type by site interaction ($\chi^2 = 32.7$, $p < 0.001$); we found silver traps outperformed black traps at all sites except at P27 an GC. We observed no difference at P27 and we observed the opposite pattern from all other sites in GC, where black traps tended to outperform silver traps, but this did not quite reach significance ($p = 0.05$, Table 3.2, Figure 3.2c). We also found a significant bait type by site interaction on catch ($\chi^2 = 20.9$, $p < 0.001$). Baited traps captured more round goby in all sites, however the difference between baited and unbaited traps was less pronounced in GC and P27 (Table 3.2).

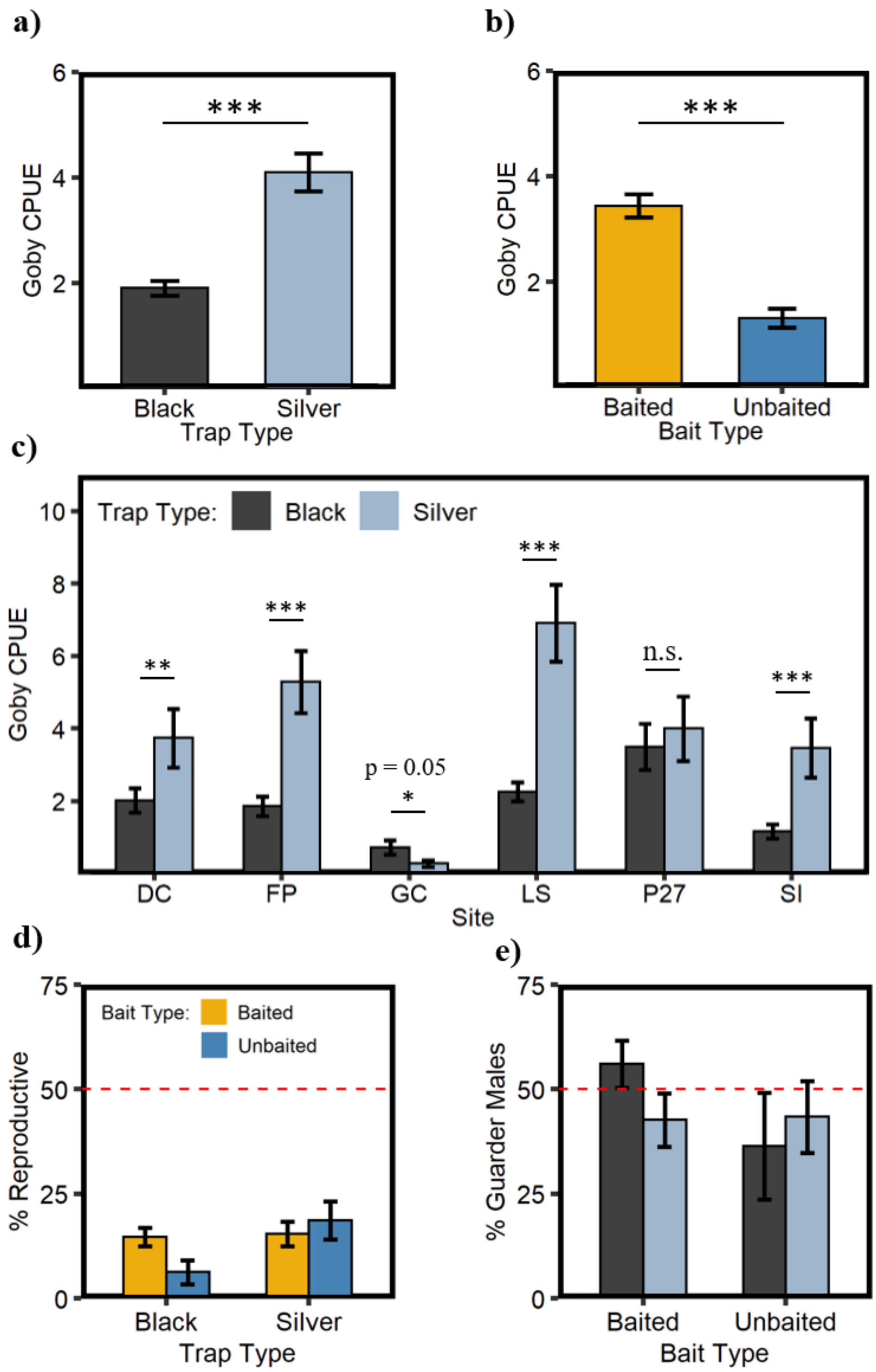
a)
Goby CPUE
6
4
2
***
Black
Silver
Trap Type
b)
Goby CPUE
6
4
2
***
Baited
Unbaited
Bait Type
c)
Trap Type: Black Silver
Goby CPUE
10
8
6
4
2
**
***
p = 0.05
*
***
n.s.
***
DC
FP
GC
LS
P27
SI
Site
d)
Bait Type: Baited Unbaited
% Reproductive
75
50
25
0
Black
Silver
Trap Type
e)
% Guarder Males
75
50
25
0
Baited
Unbaited
Bait Type

**Figure 3.2.** a) Catch per unit effort (CPUE) ± SE of round goby caught in black and silver minnow traps, b) CPUE ± SE of round goby caught in baited and unbaited minnow traps, and c) CPUE ± SE round goby caught per black and silver minnow traps separated by site within Hamilton Harbour, ON, Canada. Of the traps that captured round goby, d) the proportion of the total male catch that was made up of reproductive males (guarders & sneakers combined) by trap type and bait type and e) the proportion of the total reproductive male catch that was made up of guarder males by trap type and bait type. Error bars represent mean ± SE. *p < 0.05, **p < 0.01, ***p < 0.001.

**Table 3.2.** Overall mean ± standard error number of round goby caught per minnow trap (CPUE) made of black vinyl coated (Black) and of galvanized steel (Silver) and the mean ± standard error number of round goby caught in minnow traps baited with ~25g of corn (Baited) versus minnow traps left with no bait (Unbaited).

| | Trap Type | | Statistics | |
|---|---|---|---|---|
| | Black | Silver | t | *p* |
| Mean catch by site | | | | |
| DC | 2.02 ± 0.34 (n = 95) | 3.74 ± 0.81 (n = 50) | -2.70 | **0.007** |
| FP | 1.86 ± 0.26 (n = 109) | 5.30 ± 0.86 (n = 61) | -5.77 | **< 0.001** |
| GC | 0.73 ± 0.19 (n = 95) | 0.28 ± 0.09 (n = 46) | 1.93 | 0.05 |
| LS | 2.27 ± 0.26 (n = 113) | 6.92 ± 0.26 (n = 59) | -4.87 | **< 0.001** |
| P27 | 3.49 ± 0.64 (n = 91) | 4.00 ± 0.89 (n = 54) | -0.53 | 0.60 |
| SI | 1.17 ± 0.19 (n = 107) | 3.47 ± 0.82 (n = 62) | -4.15 | **< 0.001** |
| | **Bait Type** | | **Statistics** | |
| | Baited | Unbaited | t | *p* |
| Mean catch by site | | | | |
| DC | 3.65 ± 0.52 (n = 94) | 0.71 ± 0.20 (n = 51) | 6.22 | **< 0.001** |
| FP | 4.21 ± 0.52 (n = 109) | 1.10 ± 0.34 (n = 61) | 6.77 | **< 0.001** |
| GC | 0.77 ± 0.19 (n = 92) | 0.22 ± 0.10 (n = 49) | 2.57 | **0.01** |
| LS | 4.72 ± 0.62 (n = 111) | 2.30 ± 0.44 (n = 61) | 3.78 | **< 0.001** |
| P27 | 4.23 ± 0.64 (n = 92) | 2.74 ± 0.87 (n = 53) | 2.23 | **0.03** |
| SI | 2.77 ± 0.50 (n = 107) | 0.71 ± 0.21 (n = 62) | 5.10 | **< 0.001** |

Bold values indicate significant differences (*p* < 0.05). DC: Desjardin's Canal, GC: Grindstone Creek, LS: LaSalle Marina, FP: Fisherman's Pier, P27: Pier 27, SI: Sherman's Inlet.

### *1.2 Spray Painted and Unaltered Silver Traps*

Black spray-painted, clear spray-painted, and unaltered silver traps did not differ in their catch rates overall ($\chi^2 = 4.91$, p = 0.09; Figure 3.3b). Over these four sampling dates, there was no trap type by site interaction ($\chi^2 = 3.34$, p = 0.97).

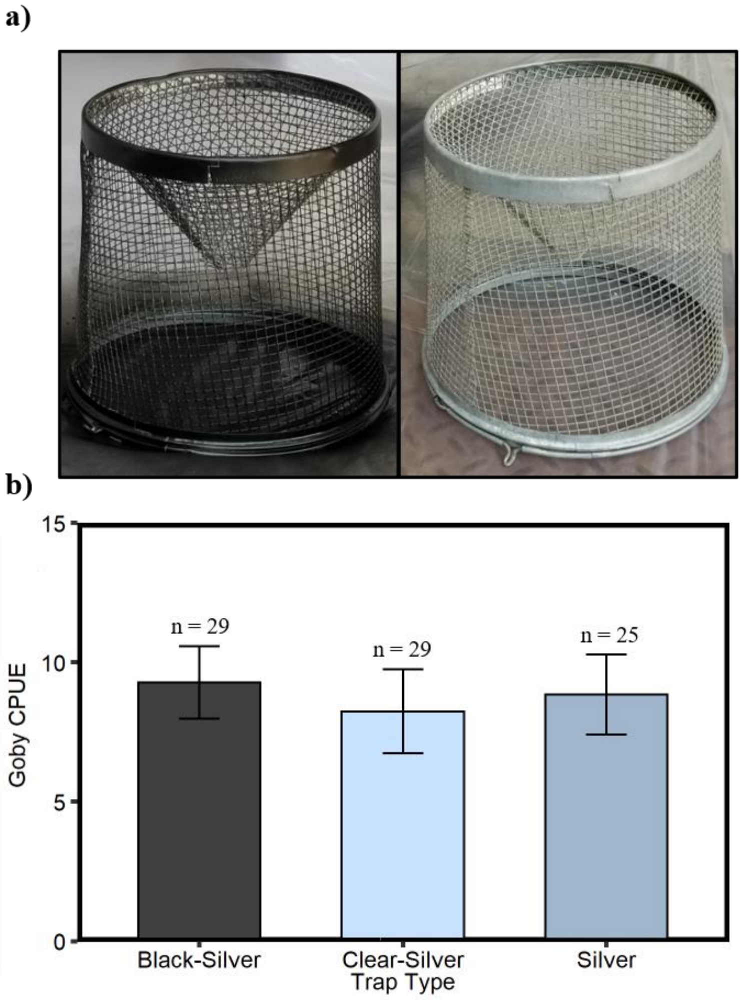

**Figure 3.3.** a) Manipulation of trap colour for the silver Gee's galvanized minnow traps. The picture on the left is a black spray-painted previously silver trap and the picture on the right is an unaltered silver trap. A third of the silver traps were also painted with clear spray-paint that did not alter the appearance of the trap (not pictured). b) There were no significant differences in round goby catch per unit effort between black spray-painted silver traps (black), clear spray-painted silver traps (light blue), and unaltered silver traps (grey).

### *1.3 Group Capture versus Single Fish Capture Events*

Silver traps were approximately twice as likely to capture round goby in groups compared to black traps, but this comparison did not quite reach significance (binomial-GLM, t = 1.88, *p* = 0.06). Baited traps were 2.7 times more likely to capture groups of round goby compared to unbaited traps (binomial-GLM, t = 3.52, *p* < 0.001). At one site, LS, round goby were more likely to captured in groups compared to all other sites (Table 3.3).

**Table 3.3.** Odds of capturing round goby in groups for each site compared to the overall average probability of capturing round goby in groups across all sites.

| Site | Odds Ratio | t | *P* |
|---|---|---|---|
| DC | 1.08 | 0.26 | 0.83 |
| FP | 1.26 | 0.86 | 0.58 |
| GC | 0.42 | -2.05 | 0.12 |
| LS | 2.58 | 3.38 | **0.004** |
| P27 | 0.95 | -0.22 | 0.83 |
| SI | 0.71 | -1.25 | 0.42 |

Bold values indicate significant differences (*p* < 0.05). DC: Desjardin's Canal, GC: Grindstone Creek, LS: LaSalle Marina, FP: Fisherman's Pier, P27: Pier 27, SI: Sherman's Inlet.

## *2.0 Population Ratios and Size Differences*

### *2.1 Sex and Reproductive Ratios*

Our overall catch was biased towards males: 1401 males were captured and only 1090 females were captured (1.3 males for every 1 female; $\chi^2 = 38.8$, p < 0.001). Neither trap type nor bait type influenced the ratio of males to females captured (binomial-GLMM,

$t_{trap}$ = -1.36, p = 0.17, $t_{bait}$ = 1.20, p = 0.23), nor were there interactions between trap type and bait type on the ratio of males to females captured ($\chi^2$ = 2.43, p = 0.12). Of the 1401 male round goby captured, 1024 were non-reproductive (73.1%), 205 were guarder males (14.8%), 153 were sneaker males (11.0%), and 19 could not be distinguished (1.4%). There was no difference in the ratio of reproductive to non-reproductive males captured between either trap types (binomial-GLMM, t = 0.20, p = 0.84) or bait types (binomial-GLMM, t = -1.08, p = 0.28; Figure 3.2d). No significant trap type by bait type interaction was observed ($\chi^2$ = 1.56, p = 0.21, Figure 3.2d). The ART ratio overall was biased towards guarder males (57.3%; $\chi^2$ = 7.55, p = 0.006), however, trap type did not influence the ratio of guarder males to sneaker males (binomial-GLMM, t = 1.06, p = 0.29). Baited traps were 1.8 times more likely to capture guarder males than sneaker males, a pattern that did not quite reach significance (binomial-GLMM, t = 1.80, p = 0.07, Figure 3.2e). There was no trap type by bait type interaction on the ratio of guarder to sneaker males captured ($\chi^2$ = 0.99 p = 0.32, Figure 1f). Of the 1090 females captured, 666 were non-reproductive (61.1%), 351 were reproductive (32.2%), and 73 could not be categorized as reproductive or non-reproductive due to a freezer malfunction or being dead upon capture (6.7%). The operational sex ratio was nearly 1:1 (358 reproductive males to 351 reproductive females; $\chi^2$ = 0.07, p = 0.79). The operational sex ratio did not differ with trap type (binomial-GLMM, t = 0.93, p = 0.35) nor with bait type (binomial-GLMM, t = 0.32, p = 0.75), and there was no trap type by bait type interaction ($\chi^2$ = 0.25 p = 0.62).

### *2.2 Size Differences*

Baited traps captured round goby that were 1.06 cm larger on average than those captured in unbaited traps ($\log_{10}$-LMER, $t = 4.25$, $p < 0.001$, Figure 3.4a). Silver and black traps captured similar sized fish ($\log_{10}$-LMER, $t = 0.86$, $p = 0.39$, Figure 3.4b). When analyzing whether trap or bait were selecting for differently sized fish within the sexes, we found no bait type by sex interaction ($\chi^2 = 1.64$, $p = 0.20$, Figure 3.4c). We did however find a significant trap type by sex interaction on the size of round goby captured ($\chi^2 = 8.07$, $p = 0.004$, Figure 3.4d); males captured in silver traps were smaller than those captured in black traps ($\log_{10}$-LMER, $t = -2.24$, $p = 0.03$) but there was no difference in the size of females captured between silver and black traps ($\log_{10}$-LMER, $t = 0.69$, $p = 0.49$, Figure 3.4d).

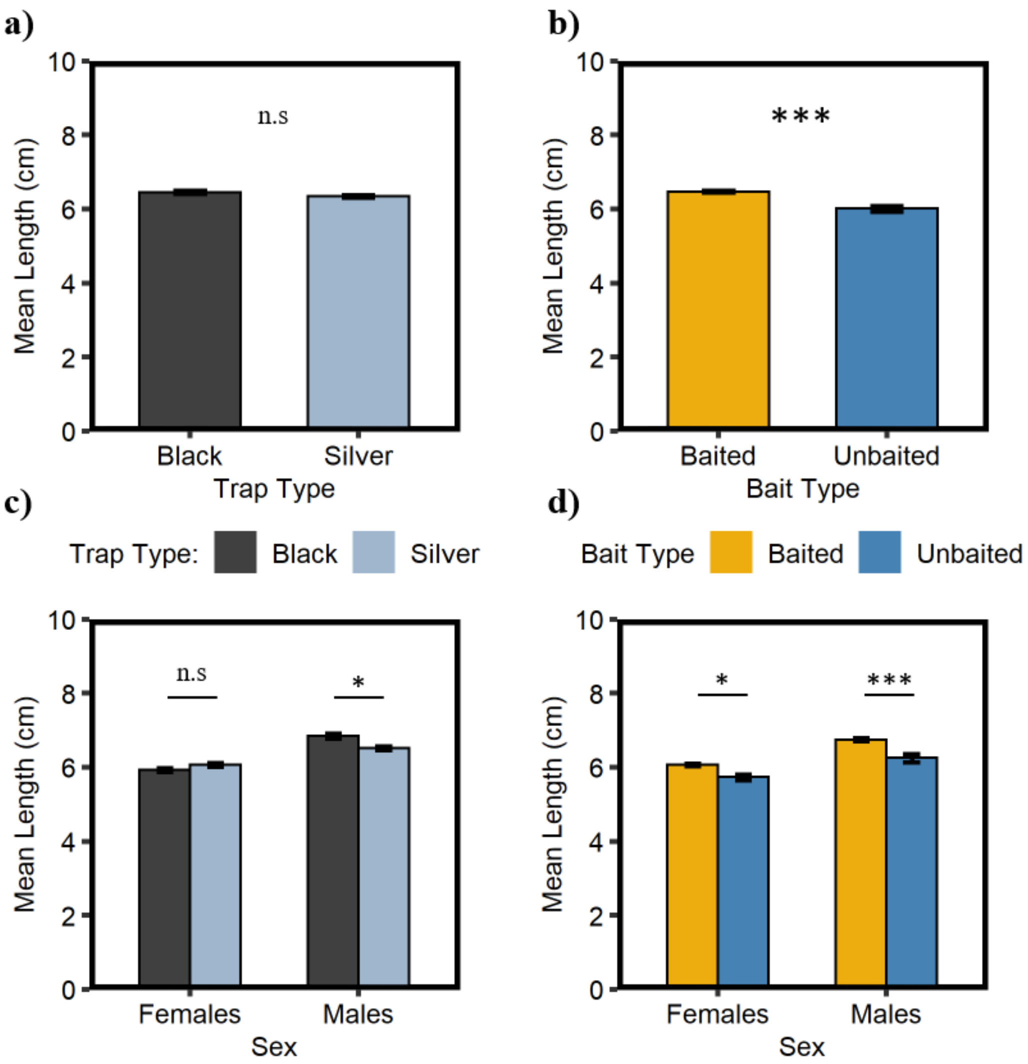

**Figure 3.4.** a) Overall standard length (cm) of round goby captured in black and silver minnow traps and b) in baited and unbaited minnow traps. b) Standard length (cm) for males and females captured in black and silver minnow traps and d) in baited and unbaited minnow traps. Error bars represent mean ± SE. *p < 0.05, **p < 0.01, ***p < 0.001.

## Discussion

Silver traps vastly outperform black traps when targeting round goby, in line with our predictions. These results confirm observations in studies targeting other species, such as three- and nine-spined stickleback, creek chub, and white sucker (Paradis et al. 2012, Merilä et al. 2013, Budria et al. 2015). To understand whether this silver > black trap result was due to the colour/reflective properties of silver traps, we spray-painted a set of silver traps black, and painted another set clear to serve as a control. We then set traps out at each site in a three-way design (a black painted, a clear painted, and an original silver trap). Despite our prediction that black spray-painted traps would capture the least amount of round goby, surprisingly we found that all traps performed equally well. Therefore, the higher round goby catch efficiency of silver traps appears to be more related to the shape of the trap and its impact on retention rather than attraction to reflectivity of the silver traps. In a trap retention study comparing minnow trap models, the probability that three-spined stickleback remained in the trap after three hours was ~70% for silver traps but only ~15% for black traps (Budria et al. 2015). Silver traps have a smaller entry hole, longer entry funnel, and thinner mesh thickness compared to black traps (see Table 3.1). A smaller entry hole may facilitate entry but make potential escape once in the trap more challenging. The thinner mesh size of silver traps might also make the entry hole harder to perceive as the contrast between the wire and the background environment may be lower (see Figure 3.1). We had intended to run laboratory studies with round goby to compare the retention of silver and black traps, however due to unforeseen circumstances relating to the 2020 pandemic, we were unable to do so.

Although silver traps outperform black traps overall, they were not more effective in all the sites sampled. In one site, grindstone creek (GC), black traps marginally outperformed silver traps. Grindstone creek is characterized by extremely high turbidity and muddy substrate (having a secchi depth average of 31 cm). Round goby prefer rocky habitats, but can be found in soft substrates in lower numbers (Young et al. 2010, Kornis et al. 2012). If round goby are attracted to silver traps based on reflective properties, then in highly turbid water, these properties are likely diminished, leading to lower catch rates. However, our spray-painted trap results suggest that reflectivity is not necessarily an attractant for crepuscular/nocturnal round goby. Additionally, turbidity might make it challenging for fish to locate the holes to escape, and this challenge might be greater for black traps, leading to higher retention and potentially explaining why black traps preformed better in GC. At a second site, pier 27 (P27), silver and black traps did not have significantly different catch rates. P27 is adjacent to a large, dense, nesting site for herring gulls, *Larus argentatus*, ring-billed gulls *Larus delawarensis*, and double-crested cormorants, *Phalacrocorax auritus* (Quinn et al. 1996), all of which predate on round goby (Somers et al. 2003, Corkum et al. 2004, Johnson et al. 2010). It is possible that the minnow traps were perceived as a place of refuge and were in fact appealing under conditions of high predation risk. Interestingly, Gee's galvanized silver minnow traps captured less red-bellied dace, *Chrosomus eos*, under predation risk compared to no risk of predation (Dupuch et al. 2011). However, we noted that the black traps at P27 caught more round goby than black traps at all other sites. Assuming round goby are more able to escape black minnow traps like three-spined stickleback (Budria et al. 2015), then round goby may choose to remain in the black traps as refuge from the diving birds to avoid predation. This could possibly explain why silver and black traps perform similarly at P27.

We also assessed whether trap type and bait influenced the likelihood of capturing groups of goby and found that silver traps tended to capture round goby in groups more than black traps, however this comparison was not significant. Round goby are a social species and can live at high densities (Capelle et al. 2015). Silver traps, having thinner mesh, may enable conspecifics to more readily perceive other fish caught inside the trap, enticing investigating fish to enter the trap. Round goby males are more social than females (Capelle et al. 2015), and given that the silver traps tended towards capturing more groups of fish, we may have expected that silver traps would be more selective for males. However, no differences in sex ratios were observed across the trap types. Baited traps captured more groups of round goby, a result that is unsurprising given that bait likely entices more fish to interact with the traps, thus increasing the likelihood of capturing more than one fish. We expected P27 would have the highest rates of group catches if fish were using minnow traps as refuge. However, we found LaSalle Marina (LS) was the only site where round goby were more likely to be captured in groups compared to the other sites. We are not sure why these site differences occur, but LS, being a marina, is under higher anthropological influence and round goby appear to thrive in impacted areas (Mehdi et al. submitted, Marentette et al. 2010, McCallum et al. 2014, Bose et al. 2018).

Black and silver traps did not differ in the ratio of guarder males to sneaker males captured. We expected that black traps, having larger entry holes, would capture larger round goby, and thus more guarder males compared to silver traps. Although the odds of capturing a guarder male in black traps was not statistically different from the odds of capturing a guarder male in silver traps, we find it important to note that 53.5% of the reproductive males captured in *black* traps were guarder males, whereas only 42.9% of the reproductive males captured in *silver* traps were guarder males. We also found that baited traps tended to capture more guarder males than unbaited traps. We did not expect bait would influence the proportion of guarder to sneaker males captured as fish often forgo feeding during reproductive periods, however reproductive fasting has not been confirmed in male round goby (Kornis et al. 2012). If male goby were interested in the bait, sneaker males may be more motivated to escape the corn filled trap as their smaller mouth gape may exclude them from effectively consuming our corn bait. Gape limitation, coupled with a potential for sneakers to escape more readily based on their smaller sizes, may explain the tendency of baited traps capturing more guarder males.

We found evidence that bait and trap type influence the size of fish captured. Baited traps captured larger fish than unbaited traps, which has been found in a previous study assessing round goby capture methods (Diana et al. 2006). Contrary to our predictions, we found the average size of round goby captured (males and females combined) did not differ between the black and silver traps. However, we did find black traps captured larger *male* round goby than did the silver traps, but we observed no body size differences between the trap types for females. Female round goby are smaller than males on average (Marentette et al. 2010, Kornis et al. 2012). The smaller entry hole diameter of silver traps may not have resulted in size differences across trap types for females because the average female may easily fit through the holes on both trap types.

In summary, we show that silver traps captured substantially more round goby than black traps, but not in sites of high predation or high turbidity, and that adding corn as bait increases the catch rate for both silver and black traps. We found no difference in catch when silver traps were painted black, suggesting that it is the shape and not the reflectivity or colour of a trap that results in higher capture rates (and possibly retention). We also show there are some differences in the types of individuals captured across different trap models within the same gear type; black traps captured larger males than silver traps and baited traps captured larger individuals overall. While we did not find any significant differences in the ratio of males to females captured, we did find that baited traps tended to have higher guarder to sneaker ratio compared to unbaited traps. These results reinforce the need for researchers and conservation organizations to take caution when comparing data using different minnow trap models, and to be consistent in their use of gear and bait within a single study or when comparing across studies. Additionally, inconsistent use and lack of reporting of which trap types were used by different organizations, in different areas of the world, or across years makes comparing results difficult if not impossible. Researchers and resource managers are strongly urged to be more explicit in describing the type of gear, minnow trap, and bait used. Taken together, our results highlight how not having this trap model information can reduce catch estimate reliability, efficiency, and result in inaccuracies or erroneous conclusions, and hamper comparative work between organizations, researchers, and jurisdictions. Such inaccuracies could have important impacts on fisheries management policies and research that need to be considered.

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# CHAPTER 4

## DISCUSSION AND FUTURE DIRECTIONS

### *Summary*

The aims of my thesis were to 1) understand if any non-reproductive behavioural differences exist between the male alternative reproductive tactics in the invasive round goby (Chapter 2) and 2) assess if two common minnow trap models differed in round goby catch efficiencies, sizes captured, sex ratios, and the ratios of the two alternative reproductive tactics (Chapter 3). In this final chapter, I summarize my findings that address these questions, discuss the broader implications of my research, and suggest potential avenues of future research that would build upon the findings in my thesis.

### *4.1 Are there non-reproductive behavioural differences between round goby guarder and sneaker male alternative reproductive tactics?*

To date, behavioural research on round goby has mainly focused on males (either non-reproductive or reproductive without tactic included) (Sopinka et al. 2010, Capelle et al. 2015, Myles-Gonzalez et al. 2015, Thorlacius et al. 2015, McCallum et al. 2017), and to a smaller extent, on females (Marentette et al. 2011, Capelle et al. 2015, McCallum et al. 2017, Nguyen-dang 2017) and juveniles (Nguyen-dang 2017). Only two other studies have investigated the behaviour of reproductive male round goby (Marentette et al. 2011, Nguyen-dang 2017). In one of these previous studies, the researchers were primarily focused on range expansion (Nguyen-dang 2017), and although the sneaker male sample size was small ($n_{sneaker}$ = 7, $n_{guarder}$ = 59), these researchers found that sneaker males were more bold, explorative, and social (Nguyen-dang 2017). My own research partially confirms these results; using a larger sample, I too show sneaker males to be bolder ($n_{sneaker}$ = 21, $n_{guarder}$ = 22) and more explorative ($n_{sneaker}$ = 25, $n_{guarder}$ = 24) compared to guarder males, however, I did not find evidence that the tactics differ in their sociality tendencies ($n_{sneaker}$ = 22, $n_{guarder}$ = 19). Nguyen-dang (2017) quantified social tendencies through the preference/avoidance of a mirror (interestingly, a mirror was used to stimulate aggression in my study), whereas I used a group of three live conspecifics. Using live conspecifics, I found both guarder and sneaker males to strongly prefer associating with conspecifics. Interestingly, in the second study assessing reproductive male round goby behaviour, guarder and sneaker males were found to have similar exploration rates (Marentette et al. 2011). However, in the Marentette et al. (2011) study, exploration was quantified as sustained vertical swimming movements (casting) while in my study it was quantified as the number of horizontal unique grid squares visited, which may explain the observed disparity in results between the Marentette et al 2011 study and my own. As sneakers must find guarder male nests to exploit, sneakers might require higher levels of activity and exploration to locate appropriate nests. I also found sneaker males were more active and less aggressive than guarder males. I found no evidence for dispersal differences between guarder and sneaker males, a result that supports a previous study looking at lab based movement and dispersal (Marentette et al. 2011). However, using a year-long field-based mark-recapture study, Marentette et al. 2011 recaptured guarder males (n = 5) six meters from their release site while sneaker males (n = 4) were recaptured from their release site.

Additionally, there is evidence that larger goby males (and most guarder males are large) will seasonally migrate into tributaries for spawning purposes (Blair et al. 2019). Thus, it is possible that guarding males primarily disperse before spawning occurs, and as round goby are more active during the night (Christoffersen et al. 2019), that they may disperse more readily during darker conditions. I conducted my studies in daytime and in daylight. Hence future studies assessing dispersal in the round goby should attempt to quantify dispersal in dark conditions and during early spring, before the breeding season begins, as these more ecologically salient conditions might better capture any possible tactic differences in dispersal and whether these might be season dependent. Taken together my results further elucidate non-reproductive behavioural differences between males adopting each of the alternative reproductive tactics, building on our knowledge of invasive round goby behaviour.

### *4.2 Is there evidence of differential behavioural syndromes between the round goby male alternative reproductive tactics?*

In Chapter 2, in addition to comparing non-reproductive behaviour between guarders and sneakers, I also explored whether activity, exploration, sociality, aggression, predation responses, and dispersal were correlated. Such correlations allowed me to explore if guarder and sneaker males show evidence of behavioural syndromes, and if these syndromes differ between male morphs. Thus far, only two studies have looked at behavioural correlations/syndromes in species with alternative reproductive tactics, one used the Wellington tree weta, *Hemideina crassidens* (Wilson and Kelly 2019) and the other focused on a water strider, *Gerris gracilicornis* (Han and Jablonski 2019). Males of the Wellington tree weta can mature either at the 8th, 9th, or 10th larval instar (insect development stages), where 8th instar males are smaller and adopt a sneaking approach to reproduction and 10th instar maturing males are large and adopt a guarding/fighting reproductive approach. The intermediate 9th instar males are a 'jack-of-all-trades', choosing to fight or sneak depending on the context (Wilson and Kelly 2019). The researchers studying these males found 8th instar males to be consistent in their behaviour across tests and exhibit a behavioural syndrome whereas 9th instar males did not show behavioural consistency, supporting their hypothesis that 9th instar males would exhibit more behavioural flexibility (Wilson and Kelly 2019). Interestingly, 10th instar males were more related to 9th instar males than 8th instar males in terms of behavioural variation, despite also exhibiting a consistent tactic (Wilson and Kelly 2019). In my study, I found that round goby guarder males had five different behavioural correlations (ie. Activity-Exploration, Activity-Predator Response, Aggression-Sociality, Dispersal-Sociality, and Predator Response-Exploration) while sneaker males only exhibited one behavioural correlation (Exploration-Sociality), which may suggest sneaker males do not exhibit the same behavioural syndromes as guarder males.

In other goby species such as black goby, *Gobius niger* (Jennings and Philipp 1992) and sand goby, *Pomatoschistus minutus* (Takegaki et al. 2012), sneaking males have been shown to switch to a guarding tactic under low levels of competition. It is unknown whether round goby sneaker males can assume a guarding male tactic under certain ecological conditions and if they exhibit the behavioural plasticity observed in the black and sand goby. Reproductive female round goby are most attracted to visual cues, specifically to the secondary sexual characteristics of black colouration in guarding males (Yavno and Corkum 2010). As such, if round goby sneaker males could flexibly move between alternative reproductive tactics, then they might be required to develop a black colouration and grow thicker heads/cheek pads to effectively attract females. If tactic flexibility occurs in a single breeding season, then one might expect to find males that exhibit intermediate traits between sneaker and guarder males (Bleeker et al. 2017). With tactic flexibility might come behavioural flexibility, such as the case in weta (Wilson and Kelly 2019). However, in a Netherlands population of round goby sampled during the breeding season, only 9% of reproductive males showed intermediate traits (cheek size, eye diameters, standard lengths, and gonad mass), which could suggest that males adopting sneaker tactics remain fixed at the beginning of the breeding season (Bleeker et al. 2017). Regardless, sneaker males may have to be more opportunistic than guarders in order to achieve reproductive success, and so it may pay for sneaker males to have flexible behaviours, which could explain the low number of correlated behaviours we observed in sneaker males. In contrast, guarder males have a seemingly more standardized approach to reproduction, where behavioural syndromes might be more likely.

In male water striders, males adopt one of two fixed tactics: a ‘signalling’ tactic, where the male jumps on a females back and creates ripples that attract predators, coercing the female into quickly mating to avoid predation, or a ‘non-signalling’ tactic, where the male still jumps on a females back but produces no ripples and waits for her to expose her genitalia instead (Han and Jablonski 2019). Signalling and non-signalling males were found to differ in their behavioural syndromes, signalling males made more mating attempts and were less cautious while non-signalling males exhibited no correlation between the frequency of mating attempts and their degree of cautiousness (Han and Jablonski 2019). When signalling and non-signalling male behavioural scores were combined, no overall correlation was observed between cautiousness and mating activity, but instead overall activity was positively associated with mating activity (Han and Jablonski 2019). Therefore, by ignoring variation associated with alternative reproductive tactics, researchers can reach starkly different conclusions about the types of behavioural syndromes, or even if they exist at all, within a population.

Future studies that aim to better understand alternative reproductive tactics in the round goby can aid in our interpretation of observed behaviors and behavioural syndromes in each male tactic. First, a better understanding of how the alternative reproductive tactics are determined in round goby and what type of strategy sneaker males adopt may help in understanding observed behavioural tendencies and syndromes—or the absence of these syndromes. For example, we do not know if round goby sneakers mainly perform streak spawning or primarily use female mimicry strategies. To address this question, eggs from a female could be stripped, partitioned, and artificially inseminated by sperm from both a sneaker male and a guarder male in a paired design. Fertilized eggs could then be separated from the guarding male, aerated until hatching (Meunier et al. 2009), but reared in identical conditions. Growth rates could then be determined early on in a subset of fry to understand if offspring sired by guarder and sneaker males differ in their early life growth rates when provided with the same opportunities to feed (Neff 2004). Further, some round goby fry could be reared to maturity, where reproductive investment can be triggered by lowering water temperatures to winter conditions and slowly raised back to simulate spring (Meunier et al. 2009). Whether fish sired by guarder males develop the guarder tactic and vice vera for sneaker males could then be assessed. This type of study would help inform us if alternative reproductive tactics in the round goby have a genetically polymorphic basis where tactic is inherited and passed on from fathers to sons. Alternatively, rearing conditions could also be altered during development of offspring to understand whether tactics are at all influenced by condition-dependence. Half of the offspring sired by the same guarder male could be subject to poor early ontogeny conditions, while the other half subject to better conditions. If the tactic a round goby male adopts is condition-dependent, then we might expect the offspring raised under the different conditions to exhibit different tactics, despite all being sired by a guarder male. To date, behavioural observations of round goby spawning behaviour has been limited, as they do not easily spawn in laboratory environments. However it has been achieved a number of times before through lowering and raising water temperatures (Meunier et al. 2009). A logical next step is to attempt to observe and quantify spawning behaviour with sneaker males present, which may help to understand whether a streaking or female mimic strategy is typically being employed.

Another recommended avenue of future research includes assessing the *consistency* of measured behaviours in guarder and sneaker male round goby. Whether behaviour remains consistent over time, between reproductive and non-reproductive states, or is flexible, can help better inform researchers about any seasonal influences that might occur on the behavioural structure of a population. Additionally, behavioural syndromes are ideally analyzed and interpreted by including both within- and between- individual variation in statistical models, requiring that individuals be measured at least twice for each behaviour (Dingemanse and Dochtermann 2013, Wilson and Kelly 2019). Variation in behavioural types can aid in the dispersal and establishment of an invasive species (Fogarty et al. 2011, Wolf and Weissing 2012). For example, asocial individuals were theoretically predicted to disperse first, followed by social individuals, which then increased the density of the population, inducing further dispersal by asocial individuals (Fogarty et al. 2011). The niche an individual occupies may also be influenced by their behaviour. If behaviours are consistent between reproductive and non-reproductive states, and consistently different between the tactics, then the tactics could potentially occupy different niches in an environment (Schirmer et al. 2020). Occupation of different niches within a single population can help populations persist in the face of environmental changes.

### *4.3 How does bait, and two common minnow trap models influence round goby capture efficiency and selectivity?*

My research indicates that silver traps capture significantly more round goby compared to black traps, and that baited traps outperform unbaited traps. Similar results on trap colour and bait have been found in past studies based different species (Paradis et al. 2012, Merilä et al. 2013, Budria et al. 2015). Reasons for the disparity in catch have been hypothesized to result from either visual attraction to the reflectivity of silver traps (Merilä et al. 2013) or based on silver traps having higher retention rates (Budria et al. 2015). In fact, after 3 hours, the probability that a captured three-spined stickleback remained in a trap was ~70% for silver traps, but was less than 20% in black traps (Budria et al. 2015). In my work, I went further and addressed whether the attraction of round goby to reflective properties played a role in silver traps having higher catch rates by spray-painting silver traps black. I expected that reflectivity would serve as an attractant to round goby and that the silver traps spray-painted black would capture much fewer round goby, but to my surprise, black spray-painted silver traps performed on par with unaltered silver traps. This lends further support to the hypothesis that silver traps have higher catch rates because their design better enables the retention of fish once captured. To confirm this is the case for round goby, future studies should assess round goby retention between black and silver traps.

Interestingly, I found black and silver traps performed similarly in a site subject to heavy avian predation, Pier 27 (P27), with black traps being almost as efficient at P27, which may suggest round goby use the black traps as a place of refuge under predation risk. Silver traps, having a thinner mesh, may not seem as favourable as a place of refuge. Interestingly, silver trap catch rates of the prey fish, redbelly dace, *Chrosomus eos*, also decreased in the presence of predators (Dupuch et al. 2011). Performing a lab-based experiment to understand if and how predation risk influences capture rates of round goby differently between black and silver traps might be an interesting avenue of future research.

I also found black traps tended to capture more round goby in a site characterized by high turbidity (low water clarity, Figure 4.1), Grindstone Creek (GC). If visual attraction to reflectivity is a driving factor in the greater performance of silver traps, it is sensible that performance would decrease in murky water. However, as silver traps that were spray-painted black did not perform differently from regular silver traps, this is unlikely to be the cause for the switch in trap performance (B>S) observed at GC. A complimentary study to further confirm whether round goby are attracted to reflective properties could be accomplished using a forced choice T/Y maze, where one arm is coated by a material mimicking that of black traps and the other coated with material mimicking that or silver traps, or by performing a trap retention study altering the turbidity of the water. Overall, my results indicate that silver traps perform better than black traps in most cases, but environmental factors such as water clarity and predation risk may change this relationship.

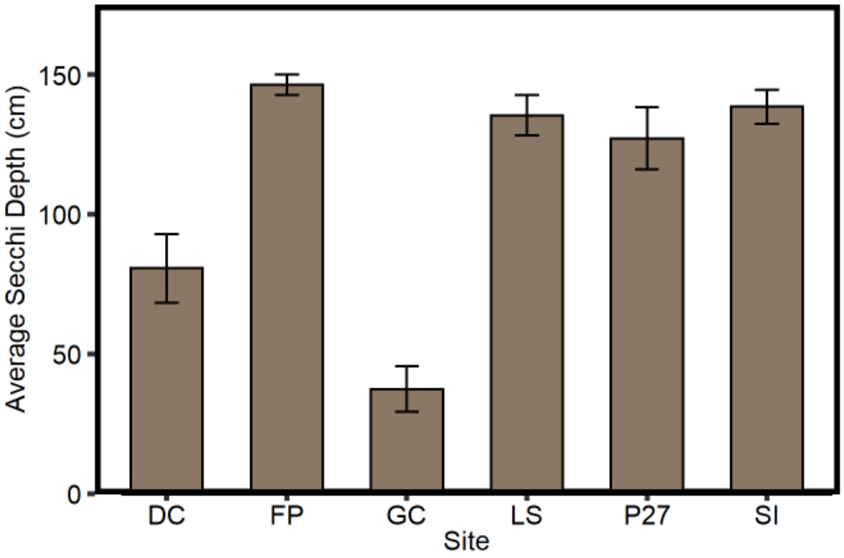

**Figure 4.1** The average secchi depth recorded at each of six sites sampled for round goby around the Hamilton Harbour in between August 2018 and November 2019. Error bars indicate mean ± SE. DC: Desjardin's Canal, GC: Grindstone Creek, LS: LaSalle Marina, FP: Fisherman's Pier, P27: Pier 27, SI: Sherman's Inlet.

### *4.4 Do trap type and bait type differ in their selectivity on population parameters?*

To my knowledge, no other study has assessed whether these different traps models give different estimations of various population parameters. In my study, I addressed whether the sex ratios, reproductive status ratios, ART ratios, and body sizes of round goby captured differ between black and silver traps, as well as between baited (corn) and unbaited traps. Past research on the Hamilton Harbour round goby population using black minnow traps baited with corn found the sex ratio to be heavily male biased, with approximately two males captured for every female (Young et al. 2010, McCallum et al. 2014). While I did find a male biased sex ratio during my sampling, it was biased to a lesser degree, averaging about 1.3 males for every female compared to the two to one ratio previously observed in the Hamilton Harbour population. Interestingly, I found no evidence that trap type or bait type influenced the observed sex ratio, so I am unsure as to why this difference exists (Figure 4.2). Alongside the sex ratio, I analyzed the operational sex ratio (OSR), which was roughly 1:1, and again as with the sex ratio, I found that the OSR was not influenced by either trap type or bait type. Among males, I assessed the effect of trap type and bait type on the ratio of reproductive to non-reproductive males and of guarders to sneakers captured. I found no effect of trap type, however I did find baited traps tended towards capturing a higher proportion of guarder males compared to unbaited traps, suggesting that the addition of bait may inflate the estimated ratio of guarder males to sneaker males. The tendency of bait to capture more guarder males was particularly surprising as we did not expect bait to have an effect as most reproductive males (guarders and sneakers) are expected to fast while reproductively active (Kornis et al. 2012). It is possible the guarder males we captured in our traps may not have been in an active nest defending state, but rather in a nest preparation state. In this case, guarder males may be more motivated by corn bait to gain reserves before fasting during parental care. Sneakers may not fast at all while reproductively active as they do not partake in any nest defense behaviours, and so they may not be as compelled to enter a trap. Additionally, sneakers may be unable to effectively consume the corn bait due to smaller mouths and associated smaller gape sizes, and so captured sneaker males may seek to exit traps more often than guarder males. Future studies can asses if baited trap attraction/retention varies with the size of round goby.

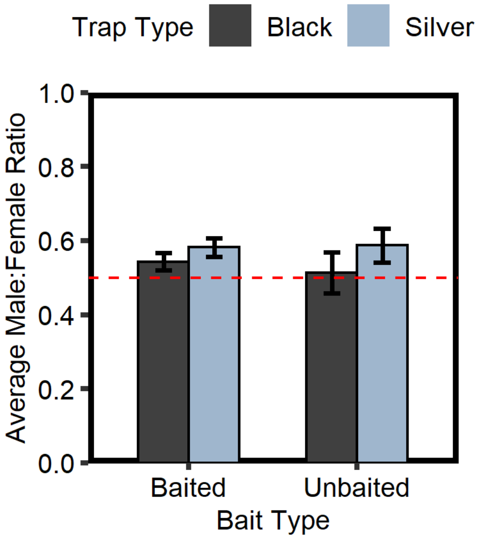

**Figure 4.2** The average ratio of males to females captured in silver, black, baited, or unbaited traps set in Hamilton Harbour, Canada. Error bars indicate mean ± SE.

Last, I assessed whether trap type and bait type influenced the size of round goby captured. Overall, traps baited with corn captured larger individuals than traps without corn. This conforms with the results from a past study where round goby captured in Gee's galvanized (silver) minnow traps baited with nightcrawlers, *Lumbricus terrestris*, were 7.9 cm long on average, whereas fish captured in unbaited traps were only 5.7 cm long on average (Diana et al. 2006). Many types of bait can be used to entice species to interact with passive gear, such as worms (Diana et al. 2006), cat food (Brandner et al. 2013), dog food (Litvak and Hansell 1990), fish food (Culp and Glozier 1989), corn, and even blue cheese (Merilä 2015). I am not aware of any previous studies that test the whether the type of bait used influences round goby catch efficiency in minnow traps. Knowing what bait type is most effective at capturing round goby could help researchers and managers better target round goby where populations are small and increase efficiency of round goby sampling in general. We found no evidence of a bait type by sex interaction on the size of fish captured, baited traps captured both larger male and larger female round goby. Interestingly, when we analyzed whether trap types were capturing males and females of differing sizes, we found black traps captured larger male round goby than did silver traps. We theorized this to be a result in the size dimorphism between the sexes interacting with the different entry hole diameters between black and silver traps, where males are larger than females and the entry hole of black traps are larger in diameter. These results indicate that silver and black traps may not be selecting for similarly sized males, which needs to be considered by researchers and fisheries managers when they want to describe a population or habitat. Although my findings pertain to round goby populations, similar issues are likely to be found in studies of other fish species or even other species that are trapped. Careful consideration of not only which gear type to use but also which gear variety or gear model (variation within a gear type) is paramount.

### *Conclusions*

Chapter 2 posed the question "Do round goby alternative reproductive tactics differ in their non-reproductive behavioural tendencies" – The answer to this question is **yes. I found that guarder and sneaker males differ in their behavioural tendencies, where sneaker males were more active, explorative, bold, and less aggressive than guarder males**. I also showed evidence of behavioural correlations between Activity-Exploration, Activity-Predator Response, Aggression-Sociality, Dispersal-Sociality, and Predator Response-Exploration in guarder males, and Activity-Exploration in sneaker males, suggesting that round goby form behavioural syndromes, and that these syndromes could differ between the two male tactics. This research adds to the small but growing body of literature exploring behavioural variation between alternative reproductive tactics. This knowledge can have applications for either conservation management, or for the mitigation of an invasive species. A better understanding of the life history and behavioural variation that may exist in round goby could help uncover why round goby are highly successful invaders and model their likelihood for persistence in novel habitats. In an applied aspect, understanding behavioural variation can aid in targeting specific individuals in a population (Garamszegi et al. 2009, Carter et al. 2012, Diaz Pauli et al. 2015). If managers aim to capture and remove guarding males from a population, and guarder males exhibit less explorative, active, and bold behaviours, than they may be best captured using active gear types. A complimentary avenue of research would be to assess the proportion of guarder males to sneaker males across different fisheries gear types and habitats.

In Chapter 3, I asked "Do two different minnow trap models and the addition of bait lead to differences in capture rates and population parameters in the round goby" – The answer to this question was also **yes. Silver and baited traps capture more round goby compared to black and unbaited traps, size selectivity exists, where males captured in black traps are larger than those found in silver traps and larger individuals were captured overall in baited traps, and baited traps tended to capture more guarder males.** My results indicate that fisheries researchers and managers must be cognisant of what minnow trap model is being used when performing assessments on round goby populations, and probably other fish species too. A black minnow trap is not equivalent to nor can be exchanged with a silver trap. Comparing data when studies have employed different trap types, or using a random assortment of trap types, is not recommended. As silver traps have a higher catch efficiency than black traps, which is further enhanced by the addition of bait, the combined use of silver traps with bait may be preferred configuration when the desire is to capture large amounts of round goby, or when attempting to target round goby in low density populations.

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