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**Behavioural responses of the Round Goby, *Neogobius
melanostomus*, to signals from conspecifics**

by

Stan Yavno

A Thesis
Submitted to the Faculty of Graduate Studies
through Biological Sciences
in Partial Fulfillment of the Requirements
for the Degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada
2010

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Behavioural responses of the Round Goby, *Neogobius melanostomus*, to signals
from conspecifics

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DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, as follows:

In all cases, the author performed the key ideas, experimental designs, data collection, interpretation and analyses. Chapters 2 and 3 are co-authored by my advisor, Dr. Lynda D. Corkum, who supported my research financially, provided guidance during the experimental design phase and feedback in the preparation of both manuscripts. Chapter 1 was prepared as a manuscript for submission to *Behaviour*. Chapter 2 has been published in *Behaviour*. Chapter 3 has been prepared as a manuscript for submission to *Naturwissenschaften*.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from the co-author to include the above materials in my thesis. I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

II. Declaration of Previous Publication

This thesis includes one original papers that has been previously published in a peer-reviewed journal, as follows:

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Chapter 2	Yavno S & Corkum LD (2010) Reproductive female round gobies (<i>Neogobius melanostomus</i>) are attracted to visual male models at a nest rather than to olfactory stimuli in urine of reproductive males. <i>Behaviour</i> 147:121-132	Published

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ABSTRACT

There are several modalities through which fish can communicate, but oftentimes they rely primarily on chemical and visual signaling. The Round Goby (*Neogobius melanostomus*) is a prolific invader of the Laurentian Great Lakes. My goal was to examine the behavioural responses of this species to signals from conspecifics. Using behavioural assays, I found that visual signals (i.e. nuptial colouration) rather than chemical signals (i.e. urine) from males were attractive to reproductive females. I also examine the attraction of juveniles to eggs odours; individuals significantly preferred conspecific to heterospecific odours. Lastly, I compare the allometric relationships between pectoral fins and body length in males and females, which may contribute to the reproductive success of an individual. My research improves our understanding of the behavioural ecology of an invasive species of fish.

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CHAPTER 1

Introduction

The use of chemical and visual senses in fish: a review

Abstract

Communication is a behaviour that exchanges information between two individuals. While this can be accomplished using five different signalling modalities, environmental constraints limit fish to use either chemical and/or visual signals. Alone, these two modalities can facilitate predator recognition, foraging and social dominance. Chemical signals, in the form of pheromones or kairomones, are released into the environment through faeces, gills or urine, whereas visual signals vary in complexity and involve shape and size morphology. In this review, I examine how the combined factors affect anti-predator, courtship, foraging, homing, and species recognition behaviours that are fundamental in the behavioural ecology of fish. Chemical and visual components studies are summarized in 23 separate fish families, encompassing 45 species, and their effects on the exhibited behaviours that were observed. Behaviours were studied when the ability to use olfaction and/or vision is either absent or present, or when observing odours and/or visual cues being used by specific species or groups. Overall, chemical and visual signals had neutral or positive effects. When used in combination, these two modalities facilitate the completion of fundamental behaviours, such as mate attraction, in several different fish species.

Introduction

Communication, a universal behaviour responsible for most of the social structure found in animals (Oliveira et al., 1998), is the exchange of information between

one signaller to one receiver (Bradbury & Vehrencamp 1998). Among fishes, this is accomplished using acoustic, chemical, electrical, mechanical and/or visual signalling (Bailey et al., 2007; Hill 1969; Salazar & Stoddard 2009; Wright et al., 2005; Yavno & Corkum 2010). Due to various constraints, fish use some of these signalling modalities less frequently than others. Mechanical signalling requires physical contact for signal transmission; acoustic signalling is prone to heavy degradation with increasing distance; and, electrical signalling requires individuals to possess a specialized organ capable of creating an electrical discharge (Bradbury & Vehrencamp 1998). Alternatively, chemical signalling in the form of olfaction (Belanger & Corkum 2009) and visual signalling such as colouration (Osorio & Vorobyev 2008) are common methods of sending information. These two methods enable individual fish to locate food (Burks & Lodge 2002), recognize predators (Hall & Suboski 1995), establish territories (Meunier et al., 2009), navigate through their surroundings (Wisenden & Dye 2009), maintain a social dominance (Barata et al., 2007) and identify possible mates (Yavno & Corkum 2010).

Chemical/olfactory communication was the first signalling modality to develop and be identified in fish (Chidester 1924; Bradbury & Vehrencamp 1998). Species use semiochemicals called pheromones (Stacey et al., 2003), usually in the form of steroids (Corkum et al., 2008), which can significantly affect fish behaviour (Barata et al., 2007; Gammon et al., 2005; Johnson et al., 2009; Marentette & Corkum 2008; Poling et al., 2001; Sorensen et al., 1988; Sorensen

& Stacey 1999). Meanwhile, communication between heterospecific fishes involves the use of compounds called kairomones (Wyatt 2003). Most of these steroidal odourants are released into the environment through faeces (Brown et al., 1995), gills (Barata et al., 2007) or urine (Liley 1982), and are detected by the olfactory sensory neurons in the olfactory epithelium (Firestein 2001). However, some aquatic environments can diminish the transmission of the chemical signals and/or decrease the sensitivity of individuals towards them (Burks & Lodge 2002; Heuschele & Candolin 2007). Therefore, fish may choose to communicate through the use of a second signalling modality (i.e. visual), increasing the chance that information is sent and received (Cardé & Baker 1984).

Colour, courtship displays, as well as shape and size morphology are all examples of visual signalling (Oliveira et al., 1998; Suk & Choe 2002). They range in complexity, duration and intensity, and must be performed under sufficient levels of ambient light (Brandbury & Vehrencamp 1998). Males are often the conspicuous sex (Maan et al., 2006; Sargent et al., 1998), using vivid visual signals to attract discriminatory females (Endler & Houde 1995; Trivers 1972) that choose mates who provide the highest amount of direct (e.g. decreased parasites) and indirect (e.g. better genes) benefits (Sargent et al., 1998). For example, visual signals from rock-dwelling *mbuna* cichlids stimulated females to choose conspecifics over heterospecifics (Jordan et al., 2003). Seehausen et al. (1998) also describe how female mate choice in two *Haplochromis* cichlid species is based solely on body coloration.

Previous reviews of fish communication have focused on general chemical signalling (Solomon 1977; Sorensen 1996; Sorensen & Stacey 1999), even though studies have shown that many species of fish also rely heavily on vision (Davis & Olla 1995; Gonçalves et al., 2002; Plath & Tobler 2007; Ueda et al., 1998; Yavno & Corkum 2010). In fact, large numbers of fish use chemical signals in combination with visual signals, (neutrally or positively) facilitating fundamental behaviours such as anti-predation, courtship, foraging, homing, and species recognition (Table 1.1). In this paper, I examine how the combined use of chemical and visual senses affects fish behaviour, and I outline how these two sensory modalities vary among different fish families.

Anti-predatory

Fish receiving advanced warnings that predators are nearby have the opportunity to quickly inspect predators before engaging in evasive behaviours such as dashing (Yunker et al., 1999; Pellegrini et al., 2010; Wisenden et al., 2010). Injured Cyprinids (minnows) release chemical alarm pheromones (i.e. Schreckstoff) into the water (Pfeiffer et al., 1985) from cells found in their skin (Chivers & Smith 1998). However, water transports these chemical signals at a much slower rate than visual signals (Bradbury & Vehrencamp 1998), and while chemical cues alone are often sufficient in alerting individuals to danger (e.g. Pellegrini et al., 2010), fish that use two signalling modalities significantly decrease their overall predation risk (Brown et al., 2000). This is better

demonstrated in two members of the family Cyprinidae: Fathead Minnow (*Pimephales promelas*) and Finescale Dace (*Chrosomus neogaeus*). Both species display significantly higher levels of anti-predatory behaviour after exposure to a combination of predator odours from conditioned water and the physical presence of a predator, than to chemical cues alone (Brown et al., 1997; Brown & Cowan 2000).

Interestingly, alarm cues are not entirely species specific; heterospecific fish eavesdrop by detecting and reacting to alarm cues used exclusively between conspecifics. Field studies conducted by Mathis & Smith (1992) using chemical alarm cues from a common heterospecific induced anti-predatory behaviours in *P. promelas*, while cues from an unfamiliar tropical heterospecific did not (Mathis & Smith 1993a). Later, laboratory studies by Wisenden et al. (2010) described how Glowing Tetras (*Hemigrammus erythrozonus*) engage in anti-predatory inspection behaviours after having been exposed to realistic models of a predator. Those fish that have been pre-exposed to heterospecific alarm cues (obtained from Blacknose Shiner, *Notropis heterolepis*) exhibited even higher numbers of predator inspections. This reiterates the notion that using two different signals, even when one is detected through a form of eavesdropping (*sensu* Oliveira et al., 1998), may benefit individuals through reduced predation (Brown et al., 2000).

Shoaling fish frequently engage in anti-predatory behaviours, such as darting and shoal tightening, when exposed to high predation levels (Lima & Dill

1990; Brown & Godin 1999a). Using combined chemical and visual signals, Poeciliids, such as Mosquitofish (*Gambusia affinis*) and Sheepshead Swordtail (*Xiphophorus birchmanni*), have been observed tightening their shoals following exposure to conditioned water and the physical presence of predators (Smith & Belk 2001; Coleman & Rosenthal 2006). Because predators naturally release chemical cues that contain information regarding their diets (Brown et al., 1995), “shoalers” that are in the vicinity of these predators may be warned of danger (Mathis & Smith 1993b). Predators also easily manipulate visual signals by altering their own behaviour (Brown & Godin 1999b) so that prey seeking visual information regarding the attack motivation of a predator should engage in risky visual inspections (Dugatkin 1992; Murphy & Pitcher 1997). Nevertheless, in many behavioural studies (Utne & Bacchi 1997; Utne-Palm 2001; McCormick & Manassa 2008), the physical presence of a predator is almost always the visual component to successfully induce anti-predatory behaviour (Table 1.1).

Finally, chemical and visual signals can induce anti-predatory behaviours in juvenile and adult life stages. While adult Northern Pike (*Esox lucius*) are fish predators (Brown & Smith 1995), juveniles face high levels of predation (Lehtiniemi 2005). Lehtiniemi (2005) demonstrates how juvenile Northern Pike seek refuge following the detection of conditioned water from predatory Yellow Perch (*Perca flavescens*). Moreover, juveniles who have been exposed to visual cues spend significantly more time in refuge than fish that do not receive any alarm cues. Other examples include juvenile Atlantic Salmon (*Salmo salar*) that

take a significantly longer time to resume foraging following exposure to combined chemical (i.e. conspecific odours) and visual (i.e. a novel object) alarm signals, than individuals exposed to control stream water (Kim et al., 2009).

Courtship and Species Recognition

Chemical and visual signals are important components of mate assessment (courtship). While signals are costly for males to produce, they are honest indicators of quality (Grafen 1990) and enhance the ability of conspicuous males to attract choosy females (Trivers 1972; Andersson 1994). Using courtship displays and pheromones, male fish can facilitate sex recognition and provide information regarding their own physical condition to females (Jordan et al., 2003; Gammon et al., 2005; Meunier et al., 2009). For example, in the mating system of Threespine Stickleback (*Gasterosteus aculeatus*), mate choice is based on individual chemical and visual signals (Bakker & Milinski 1993; Frommen et al., 2007), but few studies have examined how combined signals are used by this species. Waas & Colgan (1992) examined if both sexes of Threespine Sticklebacks could distinguish between displaying and non-displaying males. Through the use of olfaction, males and females could recognize displaying males, indicating that some reproductive displays involve the simultaneous use of visual and chemical signals.

Similarly, signaling has been studied in gobiids (Lugli et al., 1995; Suk & Choe 2002; Pampoulie et al., 2004) and the simultaneous use of multiple signals

has recently been reported. Round Goby (*Neogobius melanostomus*) males nest in cavities (Wickett & Corkum 1998; MacInnis & Corkum 2000) and actively court females through the use of olfactory and visual signals (Meunier et al., 2009). Yavno & Corkum (2010) tested different combinations of chemical (i.e. urine) and visual (i.e. colour) signals to understand which were more attractive to reproductive females. Reproductive female Round Goby spent a significantly longer period of time near darker coloured males than mottled coloured males (regardless of the urine type used), indicating that visual signals are more important than chemical signals in attracting females to a nest. However, since chemical signals alone are attractive to Round Goby (Gammon et al., 2005; Marentette & Corkum 2008), the lack of a combined effect from chemical and visual signals may be attributed to insufficient pheromone concentrations (Yavno & Corkum 2010).

Species discrimination (especially within Cyprinodontidae and Poeciliidae) is accomplished through the use of olfaction and vision. Kodric-Brown & Strecker (2001) studied isolation mechanisms in two cyprinodontiid species. Using conditioned water as the chemical stimulus and the physical presence of fish as a visual signal, Maya (*Cyprinodon maya*) and Thicklip Pupfish (*Cyprinodon labiosus*) strongly preferred conspecifics to heterospecifics. *Cyprinodon maya* appeared to use both olfaction and vision to discriminate between the two species, whereas *C. labiosus* used only odours. In poeciliids, some species within the same genera are so closely related that they require combinations of

signals even more complex than olfaction and vision. For example, male Sailfin Molly (*Poecilia latipinna*) that live in close sympatry to Amazon Molly (*Poecilia formosa*) cannot differentiate between females from either species using combined visual and chemical signals, and instead rely on obtaining additional information from tactile signals (Aspbury et al., 2010). Meanwhile, sex discrimination does not require a third signal. Discrimination between male and female Shortfin Molly (*Poecilia mexicana*) can be accomplished using vision with little reliance on additional olfactory signals (Plath & Tobler 2007). Examples of Cichliidae and Pomacentriidae species that also use combinations of chemical and visual signals for species recognition are summarized in Table 1.1.

Feeding, Foraging and Homing

The ability of fish to navigate and capture prey is critical for the growth, reproduction and survival of all species (Groves et al., 1968; Grant and Brown 1998; Gardiner & Atema 2007). Because conditions are often variable, fish may rely on the use of multiple sensory modalities (Meager et al., 2005). Salmoniids are known to use odours and vision independently to migrate from oceans to spawn in their natal streams (e.g. Hasler et al., 1958; Lorz & Horthcote 1965; Jahn 1969). Little is known if these two modalities are used simultaneously during homing. Field studies have been conducted to determine if Chinook Salmon (*Oncorhynchus tshawytscha*), Chum Salmon (*Oncorhynchus keta*) and Cutthroat Trout (*Oncorhynchus clarkii*) could return to their own natal areas

without the use of olfaction and/or vision. *Oncorhynchus clarkii*, *O. keta* and *O. tshawytscha* had their olfactory and visual abilities eliminated by Yano and Nakamura (1992), Jahn (1969) and Groves et al. (1968) (respectively). Both olfaction and vision were important in *O. keta* and *O. clarkii* to successfully lead them to their natal areas. *Oncorhynchus tshawytscha*, on the other hand, were unaffected by vision loss and returned to their natal areas using only olfaction. Variable results, from similarly designed homing studies, were found within Cichlidae, Clupeidae, Gadidae, and Scorpaenidae (Table 1.1).

Chemical and olfactory signals are equally as important in feeding and foraging behaviours as they are in homing. Several species use prey odours and sight to localize their prey. Two examples were identified by Batty & Hoyt (1995), who studied the use of olfaction and vision with respect to feeding in closely related families of fish, Pleuronectidae and Soleidae. Under normal and infrared light, European Plaice (*Pleuronectes platessa*) and Common Sole (*Solea solea*) were exposed to odours from prey. Both species exhibited higher biting rates when odours were present. However, *P. platessa* required normal light to attack and *S. solea* was capable of biting under infrared light. Another similar fish, Tongue Sole (*Cynoglossus semilaevis*, Cynoglossidae), required light, but not prey odours to feed (Wang & Ma 2009).

The combined use of chemical and visual signals while feeding or foraging has also been documented in Carcharhiniformes, Characiformes, Gadiformes, Gasterosteiformes, Perciformes, and Siluriformes (Table 1.1). Studies of primitive

families (i.e. Triakidae, Characidae, Trichomycteridae, Gadidae) were more likely to observe limited effects from visual signals on behaviour (Meager et al., 2005; Tesser & Portella 2006; Gardiner & Atema 2007; Webster et al., 2007). In contrast, observed behaviours in advanced families (i.e. Gasterosteidae, Percichthyidae, Percidae) were less likely to be affected by chemical signals (Liang et al., 1998; Spotte et al., 2001; Mikheev et al., 2006). This is expected since olfactory signalling was the first and simplest modality to develop in fish (Bradbury & Vehrencamp 1998), so species that are less developed would be expected to rely more on chemical rather than visual signals.

Conclusion

In fish, chemical and visual senses are often important in mediating anti-predator and courtship behaviours in aquatic systems (Bradbury & Vehrencamp 1998; Sorensen & Stacey 1999; Wyatt 2003; Barata et al., 2007; Meunier et al., 2009). Signals are not necessarily species specific (Mathis & Smith 1992; Jordan et al., 2003; Wisenden et al., 2010) and studies have been conducted on numerous life stages (i.e. Lehtiniemi 2005). Olfaction and vision enhance predator detection (Yunker et al., 1999; Wisenden et al., 2010), yet some species rely on one modality over the other (Smith & Belk 2001; Coleman & Rosenthal 2006). Signals, while often costly to produce, can be honest indicators of quality (Frommen et al., 2007; Yavno & Corkum 2010) and assist in facilitating species recognition (Kodric-Brown & Strecker 2001). Multiple sensory modalities can

assist fish in homing (Groves et al., 1968; Jahn 1969; Yano & Nakamura 1992; Meager et al., 2005) when the environment is variable, or help to localize prey (Batty & Hoyt 1995). Most of the components of chemical and visual signals have either positive or neutral effects on fish behaviours, with no consistent patterns (Table 1.1). However, the methods in which they are used is often dependent on the species or family in question.

Objectives and Study species

The Round Goby has been a benthic invader of the Laurentian Great Lakes since 1990 (Jude et al., 1992). A broad diet and aggressive reproductive strategy have contributed to the successful establishment of this species (Charlebois et al., 1997; MacInnis & Corkum 2000; Corkum et al., 2004). Males are colonial breeders, occupying a single cavity or nest (Wickett & Corkum 1998) and are capable of spawning with up to 15 females during one season (MacInnis & Corkum 2000). Both sexes can detect pheromones (Liley 1982; Murphy et al., 2001; Belanger et al., 2006), while males use chemical signals to attract females (Arbuckle et al., 2005; Gammon et al., 2005), before selectively allowing individual females to enter a nest for spawning (Meunier et al., 2009). It may be possible to use pheromone traps to control the spread of this fish (i.e. Johnson et al., 2009), but we need to first understand how Round Goby respond to conspecific signalling. Also, because this species uses more than one

communication modality (discussed earlier), it may be more advantageous to bait traps with both chemical and visual signals.

The objectives of my thesis are to determine how Round Goby respond (behaviourally) to signals from conspecifics. Because males possess steroids that attract females, I test if mate attraction is based solely on these pheromones or if other characteristics are involved (Chapter 2). Secondly, because Round Goby are egg predators (Charlebois et al., 1997), I examine if juveniles prefer odours from conspecific eggs to those of heterospecifics. This may be indicative of a cannibalistic behaviour, often occurring in colonial settings as a means of regulating a population (Hunter & Kimbrell 1980). Finally, physiological traits (i.e. pectoral fin size) are important in parental care (Meunier et al., 2009) and may affect the reproductive success. In Chapter 4, I examine the allometric relationship between body length and pectoral fin size, and compare them between males and females. These studies are the first to describe the direct behavioural responses of Round Goby to various types of chemical and/or visual signals, and help us to better understand the role of intraspecific communication in this invasive fish.

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Table 1.1 Summary of behavioural responses exhibited by fish following exposure to a combination of a chemical and visual stimulus. Families are listed from primitive to advanced following Nelson (2004), while genus and species are listed alphabetically within families. Each chemical or visual component that was tested had either a positive (+), neutral (0) or negative (–) effect on the behaviour exhibited by each species. Studies reviewed were conducted either in the field (F), laboratory (L) or both (FL).

Family	Species	Chemical component	Visual component	Observed behaviour	Reference
Triakidae	<i>Mustelus canis</i>	Water washing of prey (+)	Ability to use vision (0)	Feeding (Odour discrimination)	Gardiner & Atema 2007 ^L
Clupeidae	<i>Alosa sapidissima</i>	Ability to use olfaction (+)	Ability to use vision (0)	Homing (Return to natal areas)	Dodson & Leggett 1974 ^F
Cyprinidae	<i>Brachydanio rerio</i>	Alarm pheromone from a conspecific (+)	Physical manifestation of red light (+)	Anti-predator (Refugia)	Hall & Suboski 1995 ^L
	<i>Chrosomus neogaeus</i>	Conditioned water from a predator (+)	Physical presence of a predator (+)	Anti-predator (Predator inspections)	Brown & Cowan 2000 ^L
	<i>Pimephales promelas</i>	Alarm cue from a conspecific (+)	Physical presence of a predator (0)	Anti-predator (Predator inspections)	Pellegrini et al., 2010 ^L
	—	Conditioned water from a predator (+)	Physical presence of a predator (+)	Anti-predator (Dashing and immobility)	Brown et al., 1997 ^F
	—	Alarm pheromone from a conspecific (+)	Physical manifestation of red light (+)	Anti-predator (Dashing, disorganized swimming)	Yunker et al., 1999 ^L
Characidae	<i>Rutilus rutilus</i>	Water containing live a conspecific (+)	Physical presence of a conspecific (+)	Anti-predator (Tighter shoaling)	Hemmings 1966 ^L
	<i>Hemigrammus erythrozonus</i>	Alarm cue from a heterospecific (+)	Dummy model of a predator (+)	Anti-predator (Increased distance from predator)	Wisenden et al., 2010 ^{FL}
	—	Conditioned water from a predator (+)	Physical presence of a predator (+)	Anti-predator (Predator inspections)	Brown & Godin 1999 ^L ; Brown & Magnavacca 2003 ^L
	<i>Piaractus mesopotamicus</i>	Odour from live prey (+)	Physical presence of prey (0)	Foraging (Differential prey ingestion rates)	Tesser & Portella 2006 ^L
Trichomycteridae	<i>Vandellia cirrhosa</i>	Various amino acid solutions (0)	Physical presence of prey (+)	Foraging (Differential attack rates)	Spotte et al., 2001 ^L
Pimelodidae	<i>Pseudoplatystoma corruscans</i>	Water extract of conspecific skin (+)	Physical presence of food (+)	Anti-predator (Latency to attack)	Giaquinto & Hoffmann 2010 ^L

Salmonidae	<i>Oncorhynchus clarkii</i>	Ability to use olfaction (+)	Ability to use vision (+)	Homing (Return to natal areas)	Jahn 1969 ^F
	<i>Oncorhynchus keta</i>	Ability to use olfaction (+)	Ability to use vision (+)	Homing (Variable movement patterns)	Yano & Nakamura 1992 ^F
	<i>Oncorhynchus nerka</i>	Odour from stream water (+)	Ability to use vision (+)	Homing (Return to natal areas)	Ueda et al., 1998 ^F
	<i>Oncorhynchus tshawytscha</i>	Ability to use olfaction (+)	Ability to use vision (0)	Homing (Return to natal areas)	Groves et al., 1968 ^F
	<i>Salmo salar</i>	Water extract of conspecific skin (+)	Physical presence of a predator (+)	Anti-predator (Increased latency to resume foraging)	Kim et al., 2009 ^L
Esocidae	<i>Esox lucius</i>	Conditioned water from a predator (+)	Physical presence of a predator (0)	Anti-predator (Decreased attack rate)	Lehtiniemi 2005 ^L
Gadidae	<i>Gadus morhua</i>	Odour from live prey (+)	Different levels of turbidity (0)	Foraging (Differential predation rates)	Meager et al., 2005 ^L
	<i>Theragra chalcogramma</i>	Water extract of prey (0)	Different gradients of light (+)	Homing (Increased aggregation)	Davis & Olla 1995 ^L
Mugilidae	<i>Chelon labrosus</i>	Water containing live conspecific (+)	Physical presence of a predator (+)	Anti-predator (Tighter shoaling)	Hemmings 1966 ^L
Cyprinodontidae	<i>Cyprinodon maya</i>	Conditioned water from a heterospecific (+)	Physical presence of a heterospecific (+)	Species recognition (Preference towards conspecifics)	Kodric-Brown & Strecker 2001 ^L
	<i>Cyprinodon labiosus</i>	Conditioned water from a heterospecific (+)	Physical presence of a heterospecific (-)	Species recognition (Preference towards conspecifics)	Kodric-Brown & Strecker 2001 ^L
Poeciliidae	<i>Gambusia affinis</i>	Conditioned water from a predator (0)	Posture of predator (+)	Anti-predator (Predator avoidance and inspections)	Smith & Belk 2001 ^L
	<i>Poecilia mexicana</i>	Pheromone from a conspecific (0)	Physical presence of a conspecific (+)	Species recognition (Sex discrimination)	Plath & Tobler 2007 ^L
	<i>Poecilia latipinna</i>	Water containing live <i>Poecilia</i> spp. (0)	Physical presence of a <i>Poecilia</i> spp. (0)	Species recognition (Preference towards conspecifics)	Aspbury et al., 2010 ^L
	<i>Poecilia reticulata</i>	Water extract of conspecific skin (+)	Dummy model of a predator (+)	Anti-predator (Increased distance from predator)	Brown & Godin 1999 ^{FL}
	<i>Xiphophorus birchmanni</i>	Conditioned water from a predator (+)	Physical presence of a predator (0)	Anti-predator (Tighter shoaling)	Coleman & Rosenthal 2006 ^L
	<i>Xiphophorus nigrensis</i>	Water containing live <i>Xiphophorus</i> spp. (+)	Physical presence of <i>Xiphophorus</i> spp. (0)	Species recognition (Preference towards conspecific males)	Caprona & Ryan 1990 ^L
	<i>Xiphophorus pygmaeus</i>	Water containing live <i>Xiphophorus</i> spp. (0)	Physical presence of <i>Xiphophorus</i> spp. (0)	Species recognition (Preference towards conspecific males)	Caprona & Ryan 1990 ^L

Gasterosteidae	<i>Gasterosteus aculeatus</i>	Conditioned water from a predator (+)	Physical presence of a predator (+)	Anti-predator (Decreased swimming rate)	Lehtiniemi 2005 ^L
	_____	Filtered water extract of macerated prey (+)	Different levels of turbidity (0)	Foraging (Prey capture rates)	Webster et al., 2007 ^L
	_____	Water containing live conspecific (+)	Physical presence of a conspecific (+)	Courtship (Mate attraction)	Waas & Colgan 1992 ^L
Scorpaenidae	<i>Sebastes inermis</i>	Odour from stream water (+)	Ability to use vision (0)	Homing (Ability to reach original habitat)	Mitamura et al., 2005 ^F
Percichthyidae	<i>Siniperca chuatsi</i>	Odour from live prey (0)	Physical presence of prey (+)	Feeding (Ability to capture prey)	Liang et al., 1998 ^L
Percidae	<i>Perca fluviatilis</i>	Water containing live predator (+)	Physical presence of a predator (+)	Foraging (Prey intake rates)	Mikheev et al., 2006 ^L
Cichlidae	<i>Amatitlania siquia</i>	Conditioned water from a conspecific (+)	Physical presence of a conspecific (+)	Homing (Orientation towards home tank)	Wisenden & Dye 2009 ^L
	<i>Oreochromis mossambicus</i>	Urine from males (+)	Body size of males (+)	Species recognition (Hierarchical male dominance)	Barata et al., 2007 ^L
	_____	Urine from females (+)	Physical presence of females (+)	Courtship (Mate attraction)	Almeida et al., 2005 ^L
Pomacentridae	<i>Oreochromis niloticus</i>	Odour from live conspecific (0)	Physical presence of conspecific (+)	Courtship (Undulation frequencies)	Castro et al., 2009 ^L
	<i>Amphiprion ocellaris</i>	Water containing live conspecific (0)	Physical presence of a conspecific (+)	Species recognition (Conspecific recognition)	Brolund et al., 2003 ^L
	<i>Chromis chromis</i>	Water containing live conspecific (+)	Physical presence of a conspecific (0)	Anti-predator (Tighter shoaling)	Hemmings 1966 ^L
Blenniidae	<i>Salaria pavo</i>	Pheromone from males (0)	Physical presence of a male (+)	Courtship (Mate attraction)	Gonçalves et al., 2002 ^L
Gobiidae	<i>Asterropteryx semipunctatus</i>	Water extract of conspecific skin (+)	Physical presence of a predator (+)	Anti-predator (Decreased movement)	McCormick & Manassa 2008 ^L
	<i>Gobiusculus flavescens</i>	Water containing live predator (+)	Physical presence of a predator (+)	Anti-predator (Predator avoidance)	Utne-Palm 2001 ^L ; Utne & Bacchi 1997 ^L
	<i>Lythrypnus dalli</i>	Odour from live male (+)	Physical presence of a male (0)	Courtship (Reproductive suppression)	Lorenzi et al., 2006 ^L
	<i>Neogobius melanostomus</i>	Urine from males (0)	Dummy models of males (+)	Courtship (Mate attraction)	Yavno & Corkum 2010 ^L
	_____	Odour from nest-guarding male (+)	Courtship behaviour at nest entrance (+)	Courtship (Mate attraction)	Meunier et al., 2009 ^L
Pleuronectidae	<i>Pleuronectes platessa</i>	Odour from live prey (+)	Ability to use vision (+)	Feeding (Biting rates)	Batty & Hoyt 1995 ^L
Soleidae	<i>Solea solea</i>	Odour from live prey (+)	Ability to use vision (0)	Feeding (Biting rates)	Batty & Hoyt 1995 ^L
Cynoglossidae	<i>Cynoglossus semilaevis</i>	Odour from live prey (0)	Ability to use vision (+)	Feeding (Attack rates)	Wang & Ma 2009 ^L

CHAPTER 2

Reproductive female round gobies (*Neogobius melanostomus*) are attracted to visual male models at a nest rather than to olfactory stimuli in urine of reproductive males

Abstract

Fish are known to communicate in many ways and commonly use olfactory and visual signals. When round goby (*Neogobius melanostomus*) males become reproductive, they change from mottled grey to black and release sex steroids in their urine. In this study, we conducted a laboratory experiment to determine if reproductive female round gobies were attracted to a combination of olfactory (urine) and visual (silicone models) stimuli, representing reproductive and non-reproductive male round gobies. Females spent significantly more time at a nest with a black reproductive male model compared with a mottled non-reproductive male model. Neither urine type nor the interaction between model type and urine affected the time spent by reproductive females at a nest. Knowledge of the reproductive habits of the round goby may enable researchers to develop a method of species control for this invasive fish by manipulating its breeding habits.

Introduction

Fish respond to stimuli in many ways, but rely predominately on vision and olfaction to reproduce (Liley & Stacey, 1983). These two signalling modalities convey messages between conspecifics to attract mates, initiate courtship and spawn; and, depending on the breeding system, to defend fertilized eggs and offspring. Male nuptial colouration is designed to both attract females and deter male competitors (Kodric-Brown, 1990). Additionally, several species release

sexual scents via their urine, which elicit significant behavioural and/or physiological responses in conspecifics (Colombo et al., 1982; Almeida et al., 2005; Appelt & Sorensen, 2007). Sex pheromones play an important role in species recognition, mate recognition and mate assessment (Wyatt, 2003; Johansson & Jones, 2007).

The round goby (Gobiidae: *Neogobius melanostomus*), a bottom-dwelling nuisance fish, entered the Laurentian Great Lakes from Eurasia via ballast water (Jude et al., 1992). The success of the invasive round goby in becoming established in new areas is due in part to its broad diet, repetitive annual spawning, and male parental care (Corkum et al., 2004). This species uses a polygynous mating system in which many reproductive females deposit eggs in the nests of a single male (MacInnis & Corkum, 2000). Parental males are black; whereas non-reproductive males and females are mottled, mimicking colours of bottom substrates (Miller, 1984; Wickett & Corkum, 1998). Washings from reproductive male round gobies initiate a strong behavioural response in reproductive females with observable changes in time spent near the source of the male odour (Gammon et al., 2005). Recently, urine has been shown to be the main excretion route for sex pheromones in the round goby (Kereliuk, 2009). Round gobies pose a threat by feeding on eggs of native fishes (Steinhart et al., 2004), transferring contaminants up the food chain (Jude et al., 1995), out-competing other species (Jude et al., 1995; Dubs & Corkum, 1996) and by contributing to the bycatch in nets of commercial fishers (Corkum et al., 2004).

Knowledge of the reproductive habits of the round goby may enable researchers to develop a method of species control by manipulating its breeding habits.

In this study, we tested the relative strengths of visual (models) versus olfactory (urine) stimuli in attracting female round gobies in a laboratory flume. We expected that (1) reproductive females should exhibit a stronger attraction to urine collected from reproductive males than to urine obtained from non-reproductive males; and (2) a reproductive (black) model that represents a parental male should be more attractive to gravid females compared with a non-reproductive (mottled) model, resulting in the movement of the female to a nest.

Materials and methods

Animals

Round gobies were collected by angling along shoreline areas of the Detroit River at Windsor, ON (42°20'N, 82°56'W) and Lake Erie at Leamington, ON (42°03'N, 82°36'W) from May to August (2007, 2008) and May (2009). Because fish captured were not injured and quickly acclimated to holding tanks (feeding immediately and actively swimming), we concluded that angling did not influence subsequent behaviour of the fish.

Round gobies were sexed by the shape of the genital papilla — broad in females and pointed in males (Miller, 1984). Reproductive status was confirmed after experimental trials by sacrificing the fish and examining and weighing the gonads. In the lab, reproductive and non-reproductive males and females were

held in separate holding tanks with a flow-through system, air stone, and gravel. Reproductive females were used in experiments within 7 days of capture; urine was obtained from males 24 h after capture. Fish were fed daily with Nutrafin® fish flakes, and held under a 16/8 h light/dark cycle with water temperature $18 \pm 1^\circ\text{C}$. These holding conditions were based on previous studies (e.g., Gammon et al., 2005).

In females, the mass of the ovaries was expressed as a percentage of total body mass, the gonadosomatic index, GSI. A value of 8% or higher was taken as an indication of reproductive status; i.e., the body cavities of these females were filled with ripe eggs (Gammon et al., 2005). The GSI values (mean \pm SE) for all reproductive females used in our experiments were $11.46 \pm 0.061\%$. Additionally, the reproductive females had round eggs with a well defined yolk centre. There was no significant difference ($t_{40} = 1.249$, $p = 0.219$) in mean (SE) GSI of reproductive females collected from the Detroit River ($11.96 \pm 0.57\%$, $N = 21$) and Lake Erie at Leamington ($10.97 \pm 0.54\%$, $N = 21$) nor in the size (total length) of reproductive females ($t_{40} = 0.085$, $p = 0.932$) between the two populations (Detroit River: 9.31 ± 0.27 cm, $N = 21$; Leamington: 9.28 ± 0.29 cm, $N = 21$).

Collection of male urine

To obtain sufficient amounts of urine from males, we initially anaesthetized the males with clove oil, and used dental floss to tie their papillae for 4 h to prevent

urination. Urine was extracted from reproductive and non-reproductive male round gobies using a syringe (25 gauge needle); samples (including dechlorinated control water) were stored at -20°C until needed. In other studies, male round gobies were designated as reproductive if the GSI value was $\geq 1.38\%$ and non-reproductive if the GSI value was $\leq 0.4\%$; i.e., gonads were transparent and miniscule in size (Belanger et al., 2006). In our study, we obtained urine from many males but only selected urine samples for experiments from gonadally developed (reproductive) and gonadally regressed (non-reproductive) males. There was a significant difference ($t_{22} = 16.29$, $p < 0.0001$) between the mean \pm SE GSI values for reproductive ($2.02 \pm 0.194\%$) and non-reproductive ($0.22 \pm 0.037\%$) males from which we obtained urine.

Preparation of models

To prepare the round goby male models, we mixed USG® regular dental plaster to create a mould of the gobies. A non-toxic, odourless silicone compound called Oomoo 30® (Smooth-on Plastics, Easton, PA, USA) was used to cast the two round goby models. The compound was tinted black while still in the liquid (un-set) phase to represent the reproductive male model. After the removal of the set silicone, the non-reproductive male model was painted with tinted Oomoo 30® to create a mottled appearance. The models were designed to represent reproductive and non-reproductive males in both total length, 15 cm, and head width, 3 cm (Figure 2.1). The mean \pm SE total length (TL) of specimens used for

urine extraction was 13.53 ± 0.20 cm (reproductive male) and 12.89 ± 0.24 cm (non-reproductive male).

Laboratory experiment

We used a 2 (model) \times 3 (urine) factorial ANOVA design to test if reproductive female round gobies were attracted to either olfactory or visual stimuli from males. Using both reproductive and non-reproductive male models, we conducted trials with reproductive urine (N = 7), non-reproductive urine (N = 7) and a control, i.e., dechlorinated water (N = 7); i.e., 42 reproductive females were used. All 42 trials (3 urine types \times 2 male models \times 7 replicates) were randomized and fish were used only once. All experiments were conducted between 09 : 00 and 18 : 00 h.

A silicone model (either reproductive or non-reproductive male) was placed in a plastic shelter (16 \times 11.5 \times 5 cm) with opaque walls and transparent roof at one end of the flow-through flume (1 m \times 30 cm \times 30 cm) containing 20 l of dechlorinated, aerated water (Figure 2.2). Water flow in and out of the metre-long tank was controlled with a Gilmont® 6.5 mm industrial flow meter (Gilmont Instruments, Racine, WI, USA) and ranged from 40 to 45 ml/min. Valves at the opposite end of the flume were set so that water was removed at the same rate as it entered. A reproductive female was placed in a shelter at one end of the flume. After a 1-h acclimation period, a designated urine type was injected into the tube entering the flume behind the shelter containing the model at the

opposite end of the tank (Figure 2.2). The two shelters contained small holes along their respective rear walls that allowed water to pass through, preventing the build up of stagnant water. Water temperature in the flume was maintained at $18 \pm 1^{\circ}\text{C}$, a temperature at which round gobies are known to reproduce (Charlebois et al., 1997).

Trials were conducted under fluorescent lights, consisting of a 1-h acclimation period (where dechlorinated water flowed into the flume), and a 15-min stimulus period in which 0.2 ml (the maximum obtained) of urine from reproductive or non-reproductive males or dechlorinated water (control) entered the flume. An opaque gate located 50 cm from the odour source, which kept the females from visual contact with the model, was lifted immediately following an injection of urine or control water into the flume. The final concentration of urine and control water in the flume was 0.00001%.

The criterion for female mate choice was the total time spent in the area at and along the sides or back of the nest. Because our earlier studies on spawning behaviour with live parental males (Meunier et al., 2009), showed that females and males appeared to evaluate the status of each other before the female entered the nest, we selected time spent at the nest occupied by a model as the most appropriate surrogate for mate choice. Each trial was videotaped using a colour camera (Hitachi VKC-370) positioned above the flume. Trials were simultaneously recorded on DVD. The activity of the fish was analyzed using

FishTracker software (Shen, 2005).

Results

Observations obtained from the video images of the reproductive females showed that females moved from one end of the flume to the other by swimming along the bottom and along the side walls of the tank. The dependent variable, time spent at the nest, included the total time at the nest entrance and between the front of the nest and the back of the flume where the nest was positioned.

Results of the ANOVA test showed that model type (visual signal) had a significant effect on the length of time females spent at the shelter (nest) at the opposite end of the flume ($F_{1,41} = 7.957$; $p = 0.008$). In contrast, neither urine type ($F_{2,41} = 1.677$; $p = 0.201$) nor the interaction term of model \times urine type ($F_{2,41} = 0.0753$; $p = 0.928$) had any effect on the time spent by the female at the nest.

Overall, reproductive females spent the most time at a nest when it was occupied by a parental (black) male model, regardless of the chemical stimuli (urine from either reproductive or non-reproductive male or control water; Figure 2.3).

Results of Duncan's post-hoc test showed a significant difference in time spent by reproductive females at the nest between treatments with the reproductive black male model with urine from reproductive males (628 ± 29 s) and the non-reproductive mottled model with urine from non-reproductive males (320 ± 104 s), $p = 0.017$. Also, there was a significant difference between the time that reproductive females spent at the nest with a black male model with reproductive

male urine and the non-reproductive male mottled model with control water (303 ± 64 s), $p = 0.014$. There was no correlation between the GSI values of the reproductive females and time spent at the nest ($r = -0.179$, $p = 0.256$) nor between female size (total length) and time spent at the nest ($r = 0.150$, $p = 0.342$).

Discussion

This study showed that the round goby model type (a visual signal) was more effective than urine type (an olfactory signal) in attracting conspecific reproductive females to a nest in a laboratory flume. Specifically, reproductive females spent more time at a nest when it was occupied by a black round goby model than a mottled one. Not all black round gobies are reproductive, but reproductive parental, nest-holding males have black nuptial colouration (MacInnis & Corkum, 2000; Marentette & Corkum, 2008; Marentette et al., 2009). Parental males are black throughout the breeding season from early May until the end of the summer. Sexual selection tends to favour conspicuous colouration; i.e., in our study, a black or contrasting colour if the male leaves the nest, whereas a mottled pattern favours crypsis with bottom substrates (cf., Endler, 1991).

Marentette et al. (2009) present morphological evidence, supporting the existence of male alternative reproductive tactics in the round goby. The parental dark male morph with secondary sexual traits have larger investment in

accessory glands and elevated 11-ketotestosterone levels compared with the parasitic light morph that invests more in testes mass and sperm volume (Marentette et al., 2009). Black parental male morphs defend nests during courtship, spawning and development of gametes, unlike the lighter mottled morph that may sneak fertilizations or non-reproductive mottled males that may temporarily occupy shelters (Meunier et al., 2009; Corkum, personal observations).

The mean time spent by a reproductive female at a nest with a reproductive male model was longer in the presence of reproductive than non-reproductive male or control urine, but differences were not significant. Why was the response of the reproductive females to a nest not significantly enhanced in the presence of urine from reproductive males? Previously, Arbuckle et al. (2005) identified a suite of steroids that are synthesized in the testes of sexually mature male round gobies as well as the presence of steroid producing cells in the testes. Recently, Katare (University of Windsor, unpublished data) reported an unknown sulphated conjugate of 11-oxo-etiocholanolone in round goby male urine. Thus, at least one of the steroids synthesized in the testes is released into the environment via urine. In other studies, we have shown that reproductive females spent more time in the far half of a flow-through tank when washings from reproductive rather than non-reproductive male round gobies were introduced (Gammon et al., 2005), but not when offered a choice of blended synthesized steroids known to occur in the testes of reproductive males (Corkum

et al., 2008). Both studies (Gammon et al., 2005; Corkum et al., 2008) conducted under the same environmental conditions (clear water, same temperature) as ours, lacked males or fish models. Interestingly, Kereliuk et al. (2009) recently showed that reproductive female round gobies were attracted to high-performance liquid chromatography fractions of conditioned water (which includes urine) from gonadotropin releasing hormone (GnRH)-injected reproductive males; GnRH is known to increase the release of steroids. The lack of a significant response by reproductive females to male urine in our study may be explained because the males were not treated with GnRH, there was variation in steroid concentrations among reproductive males and/or because the key steroid was not present in sufficient concentrations to initiate a response. Moreover, male stimuli (vision and odour) may result in differential responses by reproductive females given their distance to a nest. We showed that the presence of a visual signal (i.e., male nuptial colouration) is attractive to reproductive females when they are near a nest.

Time spent at the nest by females is assumed to be a good predictor of mating preference (Meunier et al., 2009). Colouration has been shown to influence mate choice in several fishes, including threespined sticklebacks, *Gasterosteus aculeatus* (Baube et al., 1995); guppies, *Poecilia reticulata* (Houde & Endler, 1990); tailspot wrasse, *Hailchoeres melanurus* (Kuwamura et al., 2000); bluegills, *Lepomis macrochirus* (Cogliati, 2009) and others. In contrast to vibrant colours of other fishes, cavity nesting parental males such as mottled

sculpins, *Cottus bairdii*; upland bullies, *Gobiomorphus breviceps*; fathead minnows, *Pimephales promelas* and round gobies are typically black (Page & Burr, 1991; McDowall, 2001), presumably to blend in with dark interior of the nest to avoid predation. A nest-holding male protrudes its head from the cavity and briefly exits the nest (Corkum, personal observation), enabling the male to be visible to approaching females.

In the present study, females swam to stimuli by moving along the bottom of the flume and at the junction of the floor and walls of the flume. Such thigmotactic behaviour is typical of many bottom-dwelling species (i.e., sea lamprey, crayfish) and is advantageous in avoiding predators (Alberstadt et al., 1995; Vrieze & Sorensen, 2001). Round gobies lack a swim bladder and although they are able to enter the water column briefly and 'flit' from one spot to another, all but the early larval stages are benthic (Hensler & Jude, 2007).

Animals have an array of signalling modalities (acoustic, hydrodynamic, pheromonal, visual); however, the main sense organ used depends on the medium in which the signal is transmitted. Reproductive females may process multiple signals when approaching nest-holding parental males and signal strength of a given stimulus may vary with distance from the nest. Once the female is near the nest, nest entry decisions may be determined by colour (as shown in this study), and sounds (Rollo et al., 2007). Also, behavioural displays (pectoral or tail fin fanning) by the male could be detected by the lateral line of females (Meunier et al., 2009). Owing to the parental investment provided by

nest-holding round gobies, mutual mate choice that is known to occur in other resource-based mating systems (Kraak & Bakker, 1998) also may occur in this species. Courtship behavioural displays and responses between males and females need to be explored more fully to understand the reproductive habits of this species.

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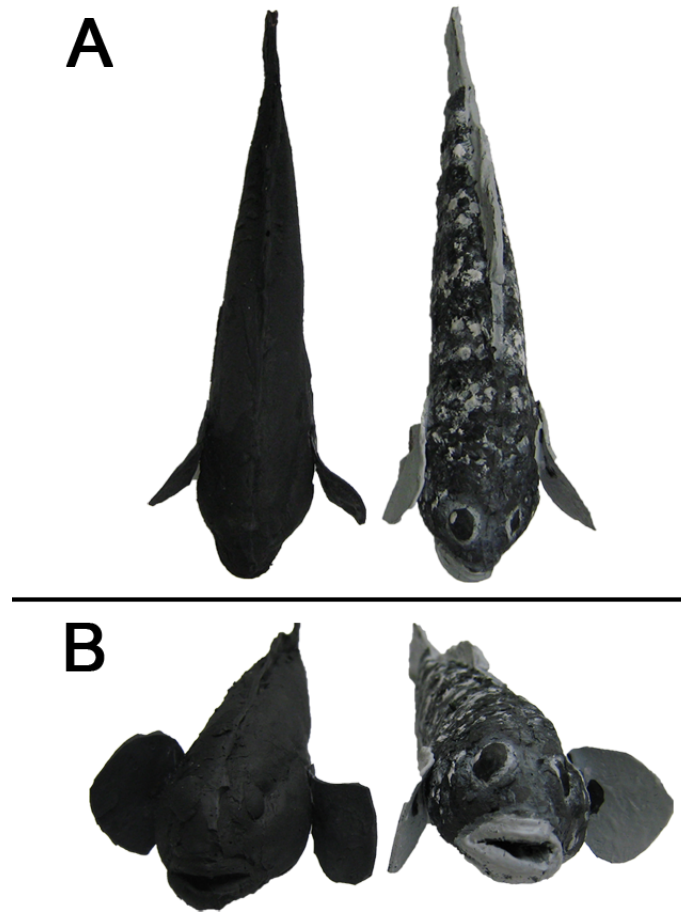


Figure 2.1 Dorsal (A) and anterior (B) views of reproductive (black) male and non-reproductive mottled male round goby are presented.

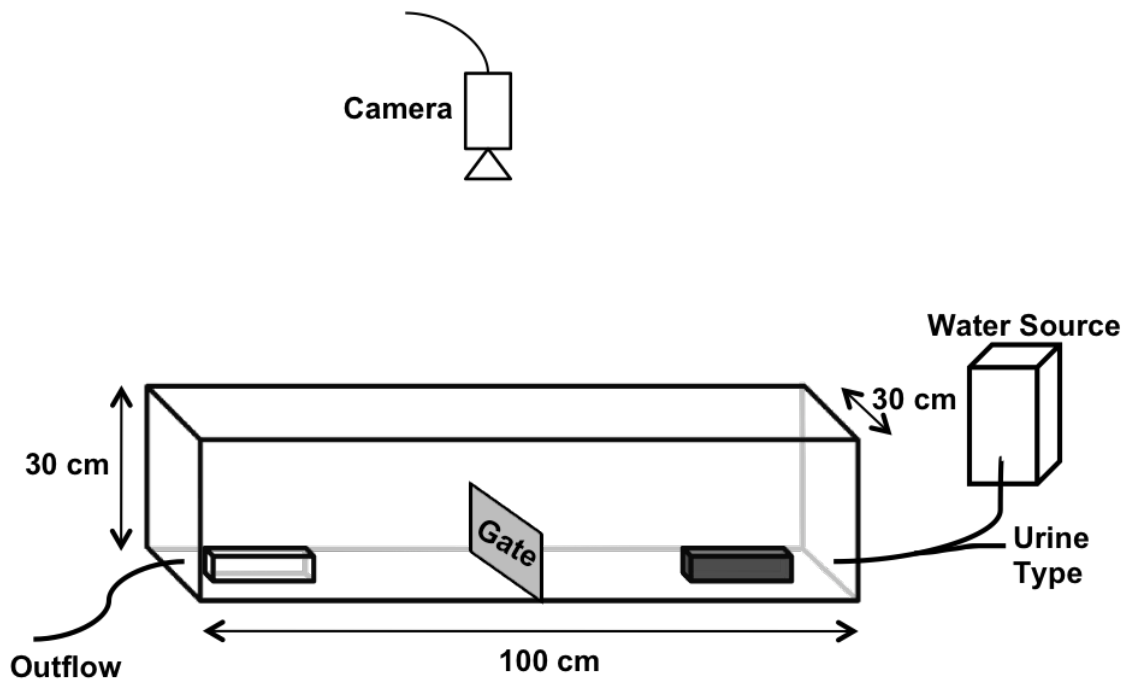


Figure 2.2 Sketch of flume indicates the relative positions of the holding shelter for the reproductive female and the shaded shelter for the male model. Urine type (reproductive, non-reproductive or control water) was added at the end of the nest where male models were positioned.

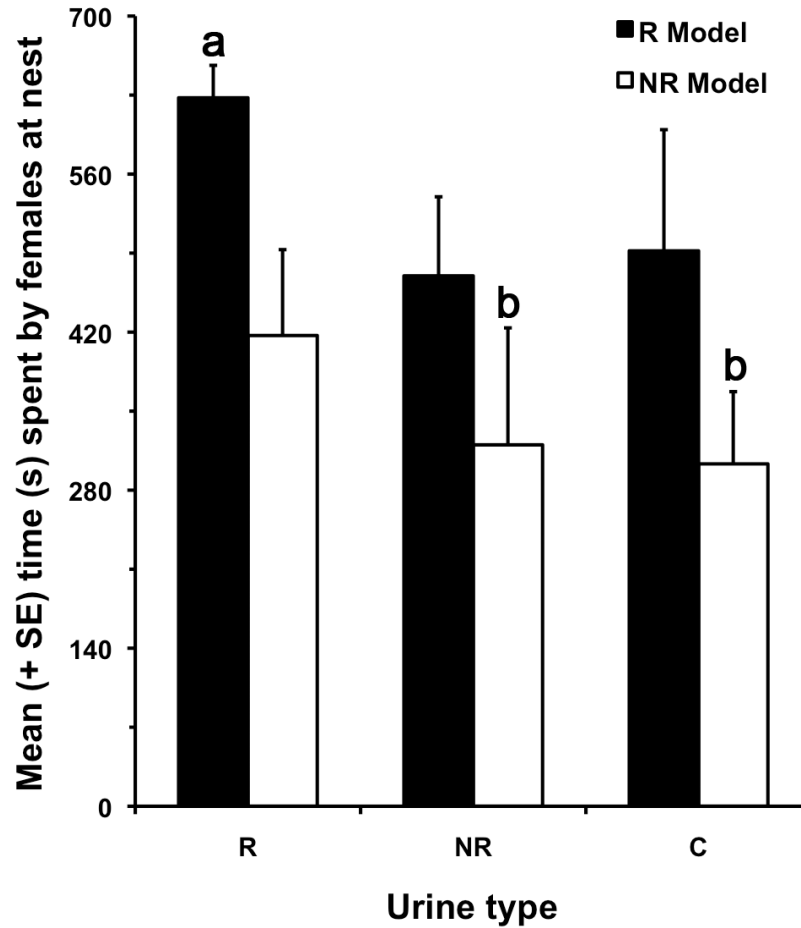


Figure 2.3 Mean + SE time (s) spent by reproductive females at the nest with the fish model at the far end of the flume. Black bars represent cases with reproductive (black) males; open bars represent non-reproductive mottled males. Urine was obtained from reproductive males (R), non-reproductive males (NR) and control (dechlorinated water). The letters 'a' and 'b' represent significant differences in responses by reproductive females at the nest.

CHAPTER 3

Round Goby (*Neogobius melanostomus*) attraction to conspecific and heterospecific egg odours

Abstract

The Round Goby (*Neogobius melanostomus*), a fish invader, owes its success to its parental care, colonial breeding habits and broad diet. Parental males guard and maintain fertilized eggs, but may exhibit filial cannibalism when costs of care exceed benefits. Field observations show that whenever parental males leave nests to chase intruders, juvenile Round Goby enter nests to consume eggs. Thus, egg odours may be attractants and cannibalism could be adaptive for species with high site fidelity or high population densities. I hypothesized that chemical cues released by fertilized eggs of conspecifics and heterospecifics are equally attractive to Round Goby. Using a lab flume, I tested if juvenile Round Goby (either those provided or withheld from receiving food) showed an increased preference to washings of conspecific (Lake Erie) eggs compared with washings of heterospecific, Rainbow Trout (*Oncorhynchus mykiss*), (hatchery) eggs. I also examined preference between egg washings vs. lake water. Fed juvenile Round Goby spent significantly more time ($t_{1,11} = 2.11$; $p = 0.05$) near washings of conspecific egg odours compared with control lake water, but preferred control lake water significantly more ($t_{1,11} = -3.10$; $p = 0.01$) than washings of heterospecific egg odours. Also, there was a significant difference in time spent by fed ($t_{1,11} = 2.19$, $p = 0.05$), but not food withheld ($t_{1,11} = -0.023$, $p = 0.98$), fish towards conspecific rather than heterospecific egg odours when stimuli were presented simultaneously. The mean time spent near Round Goby egg odours was 1.5 times that spent near Rainbow Trout egg odours. Our findings

show that conspecific egg odours attract fed juveniles, and that there is a potential to lure fish to odours traps as a means of control.

Introduction

In fish, different strategies for feeding and reproduction often lead to different types of egg cannibalism (Smith and Reay 1991). If there is a decrease in food availability or an increase in population density, cannibalistic behaviours may become more prevalent, and fish may begin to prey on conspecifics and their eggs (i.e. non-kin intercohort cannibalism) (Wootton 1971; Smith and Reay 1991; Elgar and Crepsi 1992). Other fish, incur high energetic costs due to parental care and engage in filial egg cannibalism to improve their ability to reproduce in the future (Sargent 1992; Klug et al. 2006; Chin-Baarstad et al. 2009). In order to increase the probability their offspring will survive, some fishes guard their nests (Takegaki 2000; Cheney 2008) since eggs are often palatable to predators (Acha et al. 2002). Still, eggs are often lost, especially to conspecifics, when individuals are in close proximity to each other or in a colony (Valdés et al. 1987; Slotte et al. 2006; Cheney 2008; Meunier et al. 2009). Furthermore, when eggs are lost to conspecifics due to cannibalism, there is an overall reduction in intraspecific competition (Kinzler et al. 2009), which is a useful strategy for an invasive species when the population density is high.

The Round Goby (*Neogobius melanostomus*), a colonial breeding fish that successfully invaded the Laurentian Great Lakes, has a broad diet that includes

dreissenids, invertebrates and fish eggs (Charlebois et al. 1997; Corkum et al. 2004). In the laboratory, Round Goby have been known to consume eggs of conspecifics (Meunier et al. 2009) and heterospecifics such as Rainbow Trout (*Oncorhynchus mykiss*) (Chotkowski and Marsden 1999; Fitzsimons et al. 2006). Organisms that prey upon aquatic eggs often detect the chemical cues that are naturally given off during egg development (Mirza and Chivers 2002; Ferrari and Targett 2003). Field observations in western Lake Erie reveal that Round Goby feed on conspecific eggs deposited in nests (Wickett and Corkum 1998). Therefore, the Round Goby is believed to be an opportunist predator, attracted to the chemical cues released by fertilized conspecific and heterospecific eggs. Previous studies have demonstrated that Round Goby are attracted, albeit not significantly, to food odours from co-occurring heterospecific species of the Great Lakes (Sreedharan et al. 2009).

In this study, I examined the behavioural responses of juvenile Round Goby (fed and food deprived), a life stage capable of exhibiting non-kin intercohort cannibalism, to water washings of fertilized eggs from conspecifics (*N. melanostomus*), heterospecifics (*O. mykiss*) and control lake water. I expect that (1) fish will engage in opportunistic feeding behaviour, and would therefore exhibit a higher attraction to washings of eggs than to control lake water. (2) Juvenile Round Goby, having been previously exposed in their colonies to odours from conspecific eggs, will be more attracted to washings of conspecific rather

than heterospecific eggs. Moreover, this preference should be even more pronounced in starved rather than fed fish.

Materials and methods

Experimental animals and fish eggs

Juvenile Round Goby were collected using a seine net along the Detroit River at Windsor, ON (42°18' N, 83°04' W) from June to August and November (2009). The net was 9.1 m long x 1.8 m deep (mesh size = 6.4 mm) and had a 1.8 m long x 1.8 m deep (mesh size = 3.2 mm) bag. Fish were brought back to the laboratory holding facility and placed randomly into flow-through equipped tanks under a constant 16:8 h light-dark cycle, 22°C temperature, and fed daily with Nutrafin® flakes.

Seven sets of artificial nests were built allowing us to collect fertilized Round Goby eggs. Each set was composed of five polyvinyl chloride (PVC) cylindrical tubes (7.5 cm diameter, 29 cm length) secured together through two plastic plates. One end of the PVC tubes was sealed, while the other end had a removable cap with a circular opening (3 cm diameter) for fish to enter (Figure 3.1). I inserted an acetate sheet into each PVC tube to act as a substrate for egg deposition, allowing us to remove any eggs from the tube without damaging them. Nests were deployed on June 5th, 2009 at a depth of 7-8 m on the north shore of the central basin of Lake Erie at Eriean, ON (42°15' N, 81°54' W), and retrieved June 29th, 2009. Acetate sheets containing developing Round Goby

eggs (eggs with clearly visible embryos) were removed from the artificial nests. The eggs, deposited in a single layer on the surface of sheet, were gently removed from the surface of the sheet and transferred to 50 mL sterile Cellstar[®] test tubes containing fresh lake water. Tubes were immediately frozen on site in dry ice, thereby killing the embryos but maintaining the integrity of the egg membrane. Chemical stimuli that have previously been frozen have still been known to elicit behavioural responses in Round Goby (Yavno and Corkum 2010). Fertilized eggs of Rainbow Trout, a species that can be found in the same waterways as the Round Goby, were obtained from the Ringwood Fish Culture Station (Stouffville, ON) and also frozen until needed.

Water washings of eggs

In the lab, I selected 5 random Round Goby egg samples collected from different nests. Similar to the protocol of Mirza and Chivers (2002), 4 g of eggs were removed from each sample, pooled together (20 g total), and placed in 2 L of lake water collected where nests were deployed. The eggs were aerated in the water for 15 min, after which the supernatant was poured off into 50 mL aliquots to be used for stimuli. All aliquots were stored at -20°C until needed. I similarly placed 20 g of Rainbow Trout eggs in 2 L of lake water, and aerated the eggs for 15 min. The supernatant was also poured off into 50 mL aliquots to be used for stimuli, and stored at -20°C. Lake water was selected as a control and a carrier for egg odours since the developing eggs were collected from (and frozen in) lake water

in the field. Control lake water was poured into 50 mL aliquots and stored at -20°C until needed.

Behavioural experiments

Tests were conducted to examine the attraction of Round Goby juveniles to randomly paired chemical stimuli from eggs of conspecifics (RG; n = 12), heterospecifics (RT; n = 12) and control water (CNT; n = 12). Also, I conducted trials using fish that were fed, and those withheld from receiving food for 36 hours, to examine Round Goby attraction to paired chemical stimuli from eggs of conspecifics (food provided, n=12; food withheld, n=12) and heterospecifics (food provided, n=12; food withheld, n=12). Fish were sacrificed at the end of each trial to determine the mass of their gonads, expressed as a percentage of total body mass (the gonadosomatic index; GSI). With respect to Round Goby, GSI values of less than 1.3% in males and 8% in females are indications of non-reproductive status (Belanger et al. 2006). Fish had a mean (\pm SE) total length 7.31 ± 0.11 cm, with equal numbers of males (n = 24; GSI = $0.17 \pm 0.04\%$) and females (n = 24; GSI = $2.02 \pm 0.41\%$). Fish were used in trials only once, and within 7 days of capture. Each trial was performed in a 1 m long flow-through flume, with an inflow valve on each side and one outflow valve located on the bottom in the center. I randomized the pairing of stimuli for each treatment, along with the end of the flume in which odours were released. Trials consisted of a 1 h acclimation period with dechlorinated water flowing, followed by a 15 min stimulus period (based on

dye trials). Stimuli were introduced directly into tubing carrying dechlorinated water over the course of the stimulus period. Fish were held in the center quarter of the flume (acclimation area) by transparent gates during the acclimation period. Immediately following the introduction of stimuli, the gates were simultaneously lifted using a remote pulley system, limiting any physical disturbance to the fish. Fish were observed for the amount of time spent in left and/or right three eighths of the flume (stimulus areas) using a video camera (Hitachi VKC-370) mounted above the flume (Figure 3.2), and simultaneously record onto DVD for analysis using FishTracker software (Shen 2005).

Results

Data were $\text{Log}(x + 1.1)$ transformed and analyzed using a paired t -test. I found that juvenile Round Goby spent significantly more time ($t_{1,11} = 2.11$; $p = 0.05$) on the side of the flume containing stimuli from Round Goby eggs ($285 \pm 66.8\text{s}$) vs. control lake water ($89.25 \pm 26.3\text{s}$), and significantly preferred ($t_{1,11} = -3.10$; $p = 0.01$) control lake water ($394.75 \pm 52.41\text{s}$) vs. Rainbow Trout stimuli ($201.5 \pm 31.33\text{s}$). Also, fed juveniles showed a significant preference ($t_{1,11} = 2.19$, $p = 0.05$) towards stimuli from Round Goby eggs vs. Rainbow Trout stimuli (Figure 3.2). The mean time spent associated with the Round Goby egg odours ($310.08 \pm 44.33\text{s}$) was 1.5 times that spent with Rainbow Trout egg odours ($204.16 \pm 49.27\text{s}$). Interestingly, the fish that had food withheld did not show any significant preference ($t_{1,11} = -0.023$, $p = 0.98$) towards either Round Goby

(132.33 ± 36.22s) or Rainbow Trout stimuli (food withheld, 187.33 ± 57.58s) (Figure 3.4).

Discussion

Egg cannibalism, a process that regulates fish populations (Hunter and Kimbrell 1980), becomes more frequent when the density of a population increases (Elgar and Crepsi 1992). In a colony, dense numbers of fish not only limit the amount of food available (Alexander 1974; Tyler 1995), but also tend to overwhelm individuals engaged in nest defense (Cheney 2008). Therefore, individuals have more opportunities to prey on the eggs of nearby conspecifics (Pájaro et al. 2007). Our findings indicate that juvenile Round Goby are significantly attracted to odours of conspecific (285 ± 66.8s) over control lake water (89.25 ± 26.3s), while significantly preferring control lake water (394.75 ± 52.41s) to odours of heterospecific eggs (201.5 ± 31.33s). Also, juveniles prefer odours of conspecific eggs (310.08 ± 44.33s) to those of heterospecifics (204.16 ± 49.27s).

Since the size of a fish limits its ability to capture larger prey (DeVries et al. 1998; Grabowska et al. 2009), eggs are often lost to small juvenile fish that sneak into nests (Ferrari and Targett 2003; Meunier et al. 2009). The diet of juvenile Round Goby, such as those used in our study, includes invertebrates and fish eggs (Wickett and Corkum 1998; Fitzsimons et al. 2006), whereas larger Round Goby (total length > 8 cm) feed predominantly on *Dreissena* spp. (Kovtun et al. 1974; Ray and Corkum 1997). Because of their body size, many fish are

limited to feeding on smaller prey (Deudero and Morales-Nin 2001). Juvenile fish, in particular, will feed exclusively on small eggs, invertebrates and zooplankton (Dittman et al. 1998; Foote and Brown 1998), which are easier to catch, handle and consume (Nunn et al. 2007). Also, in many cases, those fish will use a specialized sensory modality to assist them in the detection of prey. For example, Dittman et al. (1998) discovered that two species of sculpin (*Cottus aleuticus* and *Cottus cognatus*) detect salmonid eggs using only chemical cues (odours) released by the eggs, and not visual cues. The fish in our study also appear to be capable of detecting eggs based on chemical cues alone.

Round Goby that were provided food were significantly attracted to conspecific eggs over lake water using egg odours alone; there were no visual cues to indicate the presence of conspecific eggs in the flume. Meanwhile, juveniles that had food withheld did not prefer conspecific egg odours to heterospecific egg odours. Because these fish were captured in November, as opposed to the fed juveniles that were captured between June and August, previous exposure to odours of conspecific eggs may not have occurred. During the reproductive season, parental male Round Goby engage in fanning behaviour to remove debris and promote oxygenation of eggs (Meunier et al. 2009). Thus, males pump water out of their nests at a rate of 36.7 mL/s (Meunier 2009), exposing juveniles in the surrounding colony to conspecific egg odours. Exposure to heterospecific egg odour, such as those of Rainbow Trout, also occurs less

frequently and juveniles may have had more exposure to conspecific egg odours in the field compared with heterospecific egg odours.

Although Rainbow Trout are found in the same waterways as Round Goby (Kelch et al. 2006), and juvenile Round Goby consume eggs of Rainbow Trout in the laboratory (Fitzsimmons et al. 2006), few studies have demonstrated a direct attraction of Round Goby to odours from Rainbow Trout. Sreedharan et al. (2009) lured Round Goby to minnow traps using food odours, and while they did not test Round Goby eggs, they did find that traps baited with Rainbow Trout eggs attracted the fewest numbers of fish. In our study, heterospecific (Rainbow Trout) egg odours did not significantly attract juveniles over other odours (control lake water), indicating that juveniles may not completely associate odours from heterospecific (Rainbow Trout) eggs with food. When simultaneously comparing both types of egg odours, fish favoured conspecific over heterospecific egg odours. Some studies have shown that components from water-hardened salmonid eggs are not fully soluble in water, and therefore olfactory cues may not be easily detectible (Hemming and Buddington 1983). In our study, odours were collected using fresh eggs, which emit strong olfactory cues that attract predators (Mirza and Chivers 2002). Moreover, the increased variability observed in the treatment containing both types of fresh egg odours could be explained by interference created from the strong olfactory cues being released, thereby making it difficult for juvenile fish to distinguish one type of odour from another.

While it is clear that Round Goby respond to odours of fertilized fish eggs, we do not know what specific components found in eggs elicit these behaviours. Eggs of some aquatic species contain proteins that are detected by predators (Ferrari and Targett 2003); other eggs may contain steroidal compounds (Lucas et al. 1979). This study showed that odours from conspecific eggs attract juvenile Round Goby, indicating the presence of one or more chemoattractants being released by fertilized Round Goby eggs. Ultimately, there is a potential to use the attractants found in Round Goby eggs as a means to lure juveniles to traps, and thereby control the spread of this invasive fish.

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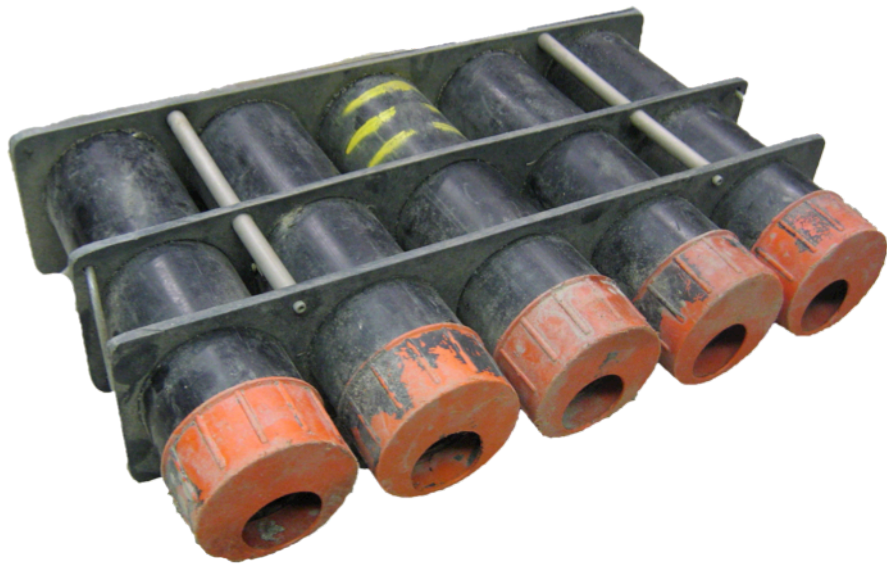


Figure 3.1 Example of one set of artificial nests built to collect fertilized Round Goby eggs. Each set contained five nests composed of polyvinyl chloride (PVC) cylindrical tubes (7.5 cm diameter, 29 cm length). One end of the tube was sealed, while the other end had a removable cap with a circular opening (3 cm diameter) for fish to enter.

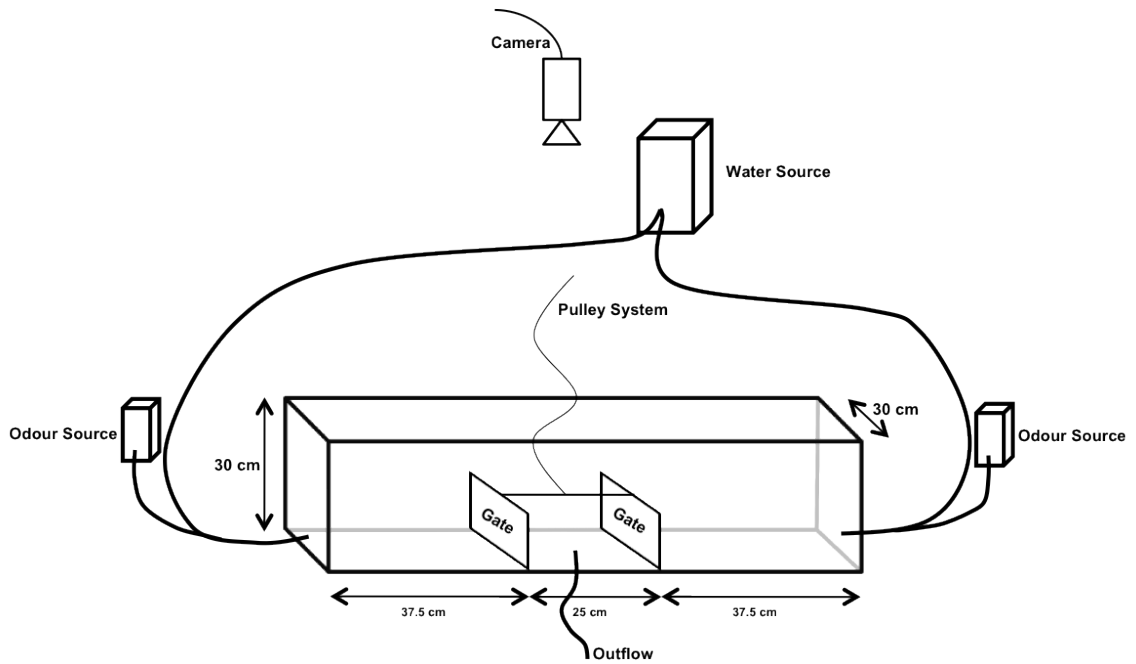


Figure 3.2 Sketch of flume used in behavioural experiments. Paired stimulus odours (conspecific eggs, heterospecific eggs or control water) were introduced at the ends of the flume into tubing carrying dechlorinated water.

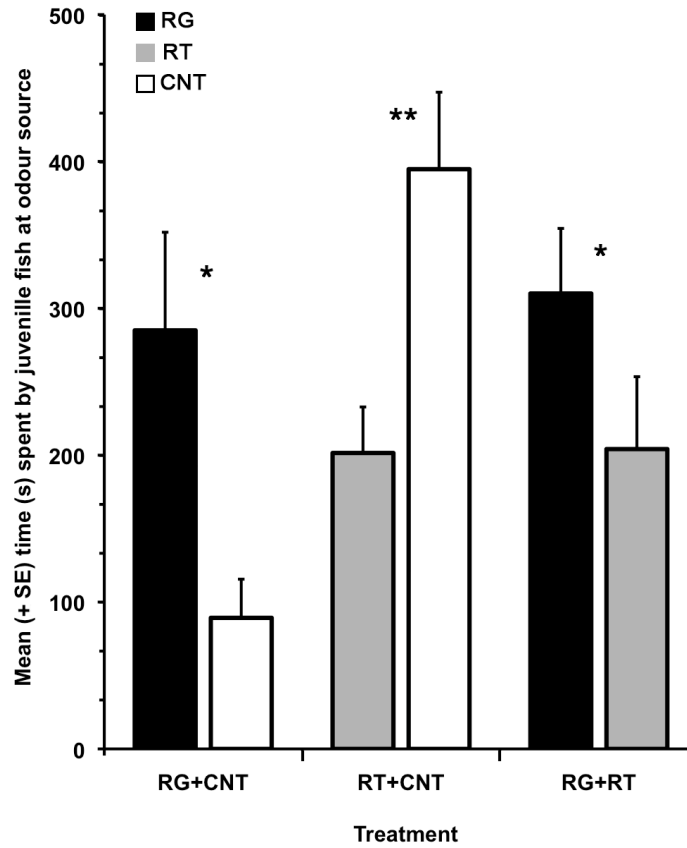


Figure 3.3 Mean (+ standard error) time (seconds) spent by juvenile fish in the area of the flume containing egg odour stimuli during each paired treatment. Black bars represent conspecific (Round Goby; RG) egg odour stimuli; grey bars represent heterospecific (Rainbow Trout; RT) egg odour stimuli; open bars represent control (CNT) lake water stimuli. Asterisks indicate significant differences between preferences towards odour stimuli (*, $p = 0.05$; **, $p = 0.01$).

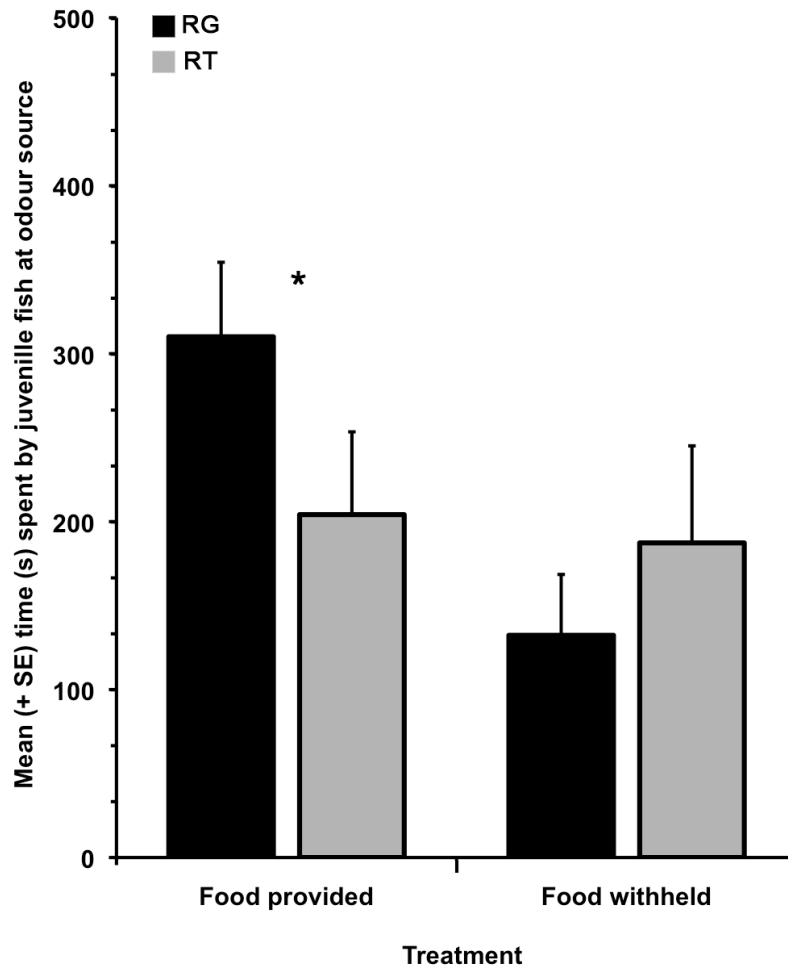


Figure 3.4 Mean (+ standard error) time (seconds) spent by juvenile fish, which were either provided or withheld from receiving food, in the area of the flume containing egg odour stimuli during each paired treatment. Black bars represent conspecific (Round Goby; RG) egg odour stimuli; grey bars represent heterospecific (Rainbow Trout; RT) egg odour stimuli. Asterisks indicate significant differences between preferences towards odour stimuli (*, $p = 0.05$).

CHAPTER 4

Allometric relationships in the secondary sexual characteristics of Round Goby (*Neogobius melanostomus*)

Abstract

The Round Goby, a prolific invader of the Laurentian Great Lakes, is a species that exhibits uniparental care. Males use pectoral fins to aerate egg clutches and help prevent intruders from entering their nests. Although the morphology of Round Goby has been described, little is known about the differences in the relationships between surface area of pectoral fins and body length for males and females. I hypothesized that males will exhibit proportionately larger pectoral fins than females and that reproductive (i.e. nest guarding) males will exhibit proportionately larger fins than non-reproductive males. Individuals that do not engage in egg care or nest defense, such as females and non-reproductive males, would not gain any reproductive benefits from having larger fins. Using digital measurements, I examined if relationships existed between the pectoral fins (used during nest defense and egg care) and total body length in males and females. In general, males ($r^2 = 0.75$, $p < 0.001$, $n = 43$) and females ($r^2 = 0.28$, $p = 0.005$, $n = 26$) had a significant positive association between total body length and total surface area of pectoral fins. However, males exhibited a stronger allometric relationship than females, given that the male slope of the line between variables was 1.8 times greater than the slope for females. Moreover, reproductive males ($r^2 = 0.78$, $p < 0.001$, $n = 31$) and non-reproductive males ($r^2 = 0.34$, $p = 0.047$, $n = 12$) had significant positive associations between the total body length and the total surface area of pectoral fins. Reproductive males exhibited a stronger allometric relationship than non-reproductive males (given by

a 1.67 times greater slope). These results suggest that reproductive (spawning males) males might increase their chances of reproductive success by investing more in their external reproductive morphology than non-reproductive males.

Introduction

Organisms exhibit physiological differences in the size and proportions of their traits (Gould 1966). These differences, referred to as allometric relationships, may improve the reproductive success of an individual. In fish, traits such as large fins have been shown to benefit males during territory defense (O'Connor et al. 1999), courting females (Suk and Choe 2002), and caring for young (Meunier et al. 2009). Large or elaborate fins give the impression that an individual has an overall larger body size (MacLaren and Daniska 2008) and in the case of parental males, allow them to better control the conditions inside their nests, such as the flow of water (Meunier 2009), which may improve the overall reproductive success of males.

Few details are known about the specific morphological differences in fish fins (Bakker and Mundwiler 1999), especially in species that exhibit parental care. Sexual selection only accounts for a few of the size differences observed (Smith et al. 2002), while different growth patterns in males and females are believed to account for the rest. For example, Threespine Stickleback (*Gasterosteus aculeatus*) males experience an increased growth rate in their fins during the breeding season (Guderley and Foley 1990). In other species of fish,

males are simply bigger than females: territorial male Bluegills (*Lepomis macrochirus*) grow bigger than females of the same age (Spotte 2007).

Furthermore, some invasive fish exhibit morphological plasticity, and are capable of adapting their fin morphology to better suit their environment (Bhagat et al. 2006).

First discovered in Lake St. Clair in 1990, the Round Goby (*Neogobius melanostomus*) is now found throughout the Laurentian Great Lakes (Jude et al. 1992). Males can nest in any enclosed cavity (Wickett and Corkum 1998) and provide sole parental care for their offspring. Using their pectoral fins, males block the nest opening from intruders and remove metabolic wastes from the nest by circulating fresh water to the developing embryos (Meunier et al. 2009). Females spawn with some males more than others, and are capable of spawning with several males during the reproductive season (MacInnis and Corkum 2000). While Round Goby morphology has been described (Charlebois et al. 1997), data are lacking on the morphological relationships that exist between sexes.

Moreover, males invest into internal morphological structures such as testes and accessory glands (Marentette et al. 2009), but there have not been any similar observations with respect to external characteristics. Males and females possess several different fins on their bodies, but I elected to analyze only pectoral fins due to their obvious involvement during nest defense and egg care (Meunier et al. 2009). I hypothesize that (1) males will exhibit larger fin morphology than females at any given total body length, since males rely heavily on their fins

during parental care and females do not. Also, (2) reproductive (i.e. nest guarding) males rather than non-reproductive fish (i.e. fish not presently using fins for nest defense or egg care) will exhibit larger fin morphology at any given total body length.

Materials and methods

Experimental animals

Round Goby were collected by angling along shoreline areas of Lake Erie at Leamington, ON (42°03' N, 82°36' W) from May to August (2008) and June (2009). Fish were brought back to the laboratory holding facility and placed randomly into flow-through equipped tanks under a constant 16:8 h light-dark cycle, $20 \pm 2^{\circ}\text{C}$ temperature, and fed daily with Nutrafin[®] flakes. All fish used in this study were adults and were previously used in behavioural trials within 7 days of capture. Fish were sexed by the shape of the genital papillae, pointed in males and broad in females (Miller 1984). Fish were sacrificed, after their use in other experimental trials, to confirm their reproductive status (Table 4.1) by examining and weighing the gonads, i.e. small and transparent in non-reproductive fish. Total body length measurements were taken for each fish and the pectoral fins were removed and preserved in 70% ethanol for morphometric analysis.

Secondary Sexual Characteristic Assessment

To estimate the differences in secondary sexual characteristics of Round Goby, preserved fins were photographed using a digital camera (Sony HDR SR8).

Under standardized light conditions, fins were opened to their maximum and laid out on a flat surface. All fins were photographed from above at a fixed height (75 cm). The total surface area (mm^2) and circularity were determined from the digital images using NIH Image analysis software ImageJ[®] (<http://rsb.info.nih.gov/nih-image/>). Circularity is expressed as a percentage, where a value of '1' indicates the fin is in the shape of a perfect circle (fin with a narrow base) and any values approaching '0' indicating a lopsided shape (fin with a broader base) (Figure 4.2). Values for surface area and circularity were regressed against total body length in an ANCOVA, and a comparison of intercepts and slopes of lines was conducted.

Results

I tested for differences in the slopes of the lines, between the total body length of each fish and the total surface area of pectoral fins of males and females (Figure 4.1A), which were significantly different from each other (ANCOVA, $F_{1,65} = 4.65$, $p = 0.03$). Males, reproductive and non-reproductive, ($y = 0.96x - 6.93$, $r^2 = 0.75$, $p < 0.001$, $n = 43$) and females ($y = 0.53x - 2.25$, $r^2 = 0.28$, $p = 0.005$, $n = 26$) had significant positive associations between these values. The slope of the relationship between variables for males (0.96) was 1.8 times greater than the

slope for females (0.53), indicating that males had proportionally larger pectoral fins than females at any given total body length. The mean (\pm SE) pectoral fin surface area for males and females was $611.85 \pm 41.57 \text{ mm}^2$ and $271.44 \pm 30.34 \text{ mm}^2$, respectively.

Reproductive males (RM; $y = 0.94x - 6.45$, $r^2 = 0.78$, $p < 0.001$, $n = 31$) and non-reproductive males (NRM; $y = 0.57x - 2.67$, $r^2 = 0.34$, $p = 0.047$, $n = 12$) had significant positive associations between the total surface area of pectoral fins and total body length (Figure 4.1C). The intercepts of the lines were significantly different from each other (ANCOVA, $F_{1,40} = 4.37$, $p = 0.04$), indicating that the surface area of RM ($678.68 \pm 50.95 \text{ mm}^2$) was larger than NRM ($439.20 \pm 34.89 \text{ mm}^2$).

There was a significant negative association between the total body length and the circularity of the pectoral fins for reproductive and non-reproductive males combined, ($y = -0.008x + 0.96$, $r^2 = -0.19$, $p = 0.003$, $n = 43$), but not females ($y = -0.006x + 0.92$, $r^2 = 0.12$, $p = 0.08$, $n = 26$) (Figure 4.1B). There was no significant difference in either the slopes (ANCOVA, $F_{1,65} = 0.05$, $p = 0.81$) or intercepts (ANCOVA, $F_{1,66} = 2.57$, $p = 0.11$) between lines. The mean (\pm SE) circularity for male and female pectoral fins was $0.85 \pm 0.007 \%$ and $0.87 \pm 0.004 \%$ respectively. In contrast, there was a significant relation between the total body length and the circularity of the pectoral fins for RM ($y = -0.008x + 0.96$, $r^2 = 0.18$, $p = 0.017$, $n = 31$), but not NRM ($y = -0.004x + 0.91$, $r^2 = 0.07$, $p = 0.41$, $n = 12$) (Figure 4.1D). However there was no significant difference between slopes

(ANCOVA, $F_{1,39} = 0.16$, $p = 0.68$) or intercepts (ANCOVA, $F_{1,40} = 0.07$, $p = 0.79$) of the lines. The mean (\pm SE) circularity for RM and NRM pectoral fins was 0.85 ± 0.007 % and 0.86 ± 0.007 % respectively.

Discussion

Males of several gobiid species provide sole parental care to their offspring (Takegaki 2000; Meunier et al. 2009). Their nests often occupy enclosed cavities (Miller 1984) where stagnant water may cause metabolic wastes to accumulate and oxygen levels to decrease (Lissåker et al. 2003), threatening offspring survival. As a result, spawning males use their fins to fan inside the nest, thereby circulating water and providing adequate nutrients to the developing eggs and larvae (Jones and Reynolds 1999; Wickett and Corkum 1998). Round Goby have been described using their pectoral fins to fan their egg clutches (Meunier et al. 2009). Since larger fins are capable of moving larger volumes of water (Bakker and Mundwiler 1999), reproductive (i.e. nesting) males should benefit the most from having larger fins. Our findings indicate that while all Round Goby (i.e. males and females) have significant allometric relationships between pectoral fin surface area and total body length, male pectoral fins were generally 1.8 times larger than female pectoral fins at any given body length. Furthermore, RM pectoral fins were 1.67 times larger than NRM pectoral fins for any given total body length.

Larger secondary sexual characteristics, such as fins, may improve the overall reproductive success of fish that guard eggs (Guderley and Foley 1990; Naesje et al. 1988; Westley et al. 2008). Only recently have specific Round Goby external morphological investments been described. Marentette et al. (2009) describe two distinct male Round Goby morphs: one incorporating larger physical traits (i.e. significantly larger total body length and body mass), suggesting individuals engage parental male tactics, while the other morph is physically smaller, with characteristics suggestive of a sneaker male tactic. Moreover, parental (RM) gobiids invest more in their gonads than NRM (Belanger et al. 2006) and have higher levels of plasma testosterone (Marentette et al. 2009). Our findings suggest that parental males also invest more than NRM into their external reproductive morphology through stronger allometric relations (i.e. between pectoral fin size and total body length).

There was a significant negative relation between the percent circularity of RM pectoral fins and total body length, resulting in the base of the fin becoming increasingly broader and lopsided (Figure 4.2). Round Goby males engaged in nest guarding use their pectoral fins to block the nest opening from intruders (Meunier et al. 2009). Since pectoral fins that are wider would help to block a larger portion of the nest entrance, males with wider fins could increase their reproductive success by decreasing number of offspring lost to intruding predators. In most fish, pectoral fins also have the second largest surface area

behind the caudal fin (Tucker et al. 2002), and in Round Goby, both fins are used by nest guarding males during egg care (Meunier et al. 2009).

In this study, caudal fins for each fish were not preserved along with the pectoral fins, so there were no measurements to compare the allometric relationships between the surface area of the caudal fin and total body length. Since caudal fins are used to create the strongest water currents out of the nest (Meunier 2009), any relationship between caudal fin morphology and total body length may be just as significant as those relationships observed in our study. In fact, caudal fins are often important for more than just egg care: their size can influence female mating preferences (Basolo 1991) and in some cases affect the survival individuals (Tucker et al. 2002). Nevertheless, secondary sexual traits linked to reproductive morphology in RM Round Goby (i.e. the sex that provides sole parental care) have stronger allometric relationships than in NRM. Males are thereby increasing their chances of reproductive success, not only by invest energy in their reproductive organs, but also into their external reproductive morphology.

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Table 4.1 Sample sizes, means and associated standard error of the gonadosomatic index (GSI) of male (reproductive, non-reproductive) and female Round Goby.

Sex	<i>N</i>	GSI	
		Mean	SE
Females	26	11.26	0.43
Males			
Reproductive	31	0.13	0.027
Non-reproductive	12	1.97	0.11

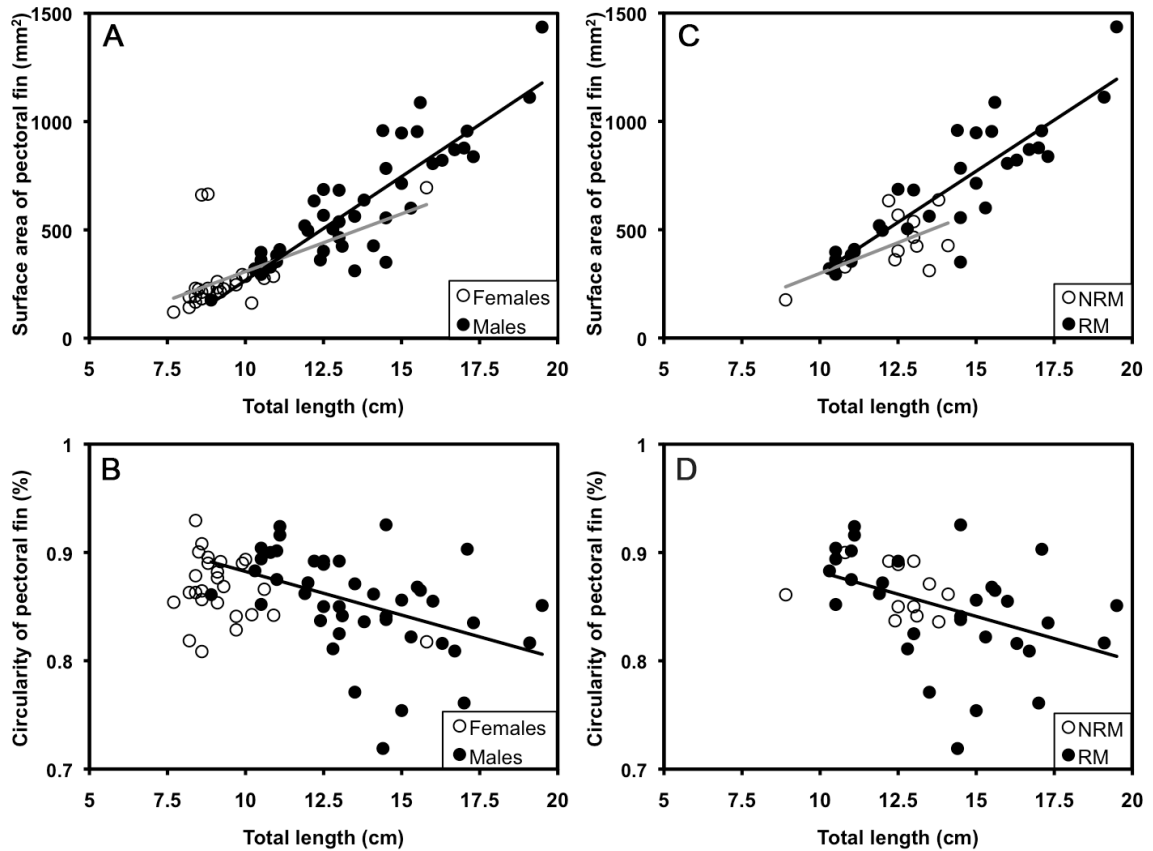


Figure 4.1 A) Plot of total body length (cm) vs. surface area (mm²) of pectoral fins for males (closed circles) and females (open circles). B) Plot of total body length (cm) vs. circularity (%) of pectoral fins for males (closed circles) and females (open circles). C) Plot of total body length (cm) vs. surface area (mm²) of pectoral fins for reproductive (RM, closed circles) and non-reproductive (NRM, open circles) males. D) Plot of total body length (cm) vs. circularity (%) of pectoral fins for reproductive (RM, closed circles) and non-reproductive (NRM, open circles) males.

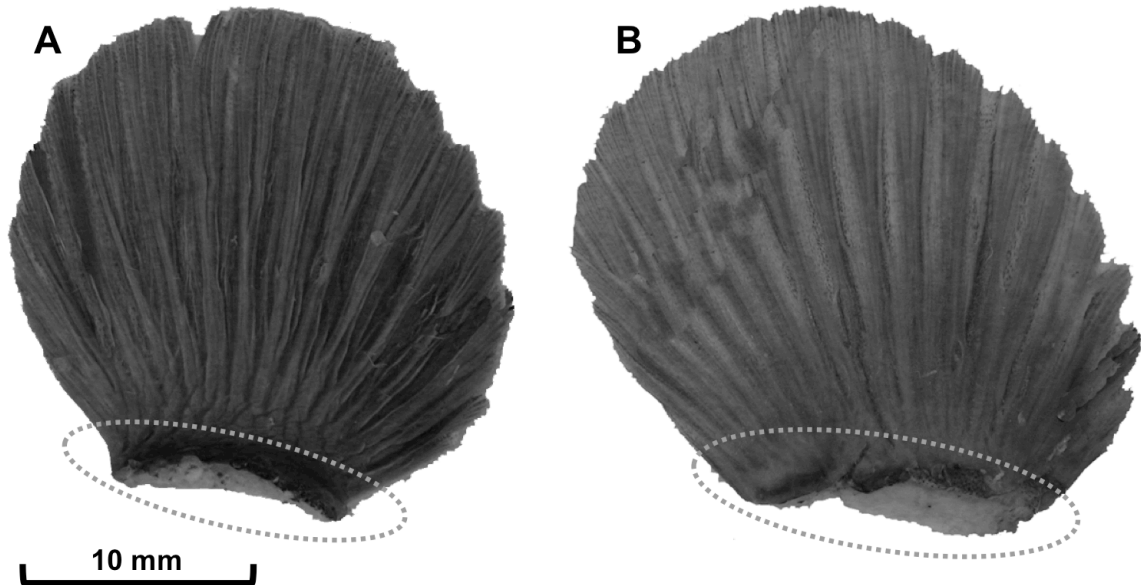


Figure 4.2 Comparison of non-reproductive (A) and reproductive (B) male fins. Reproductive male pectoral fins are broader (i.e. wider) at the base of the fin (within dotted elliptical area), resulting in fins that are lopsided and less circular than non-reproductive male fins.

CHAPTER 5
General Discussion

Since 1990, the Round Goby has been a prolific invader of the Laurentian Great Lakes and surrounding waterways (Jude et al., 1992), with several factors having contributed to their successful establishment: they feed primarily on invasive dreissenids (Charlebois et al., 1997; Corkum et al., 2004), multiple females can deposit eggs in a single nest (MacInnis & Corkum, 2000) and reproductive males aggressively defend their egg clutches (Meunier et al., 2009). In an effort to better understand their communication strategy, and as a possible means of controlling their spread (Corkum et al., 2008), researchers have begun to examine the use of pheromones by Round Goby. Through the use of electro-olfactogram and gill ventilation experiments, studies have demonstrated that Round Goby are capable of detecting steroids (Murphy et al., 2001; Belanger et al., 2006). More specifically, reproductive females respond strongly to water washings from reproductive males (Gammon et al., 2005) and to specific blends of steroids (Corkum et al., 2008).

The use of chemical and visual senses in fish behaviour has been studied in several taxa (Chapter 1), with the vast majority examining endemic species (e.g. Barata et al., 2007; Gardiner & Atema 2007; Kim et al., 2009; Wisenden et al., 2010). Few studies have detailed how signaling affects the behaviour of a non-indigenous fish, and how this can ultimately contribute to the spread of the species. The goal of my research was to determine which chemical and/or visual signals are the most attractive to Round Goby and investigate their effects on the behaviour of this species. My studies indicate that male colouration (visual

signals) and egg odours (chemical signals) are potent attractants of Round Goby (Chapter 2, 3). Moreover, the surface area and circularity of reproductive male pectoral fins, which are both significantly smaller in females and non-reproductive males (Chapter 4), may enhance reproductive success. Using combined chemical (urine) and visual (model) signalling, my findings indicate that reproductive females spend significantly more time in the vicinity of a nest containing a reproductive male model rather than a non-reproductive male model, regardless of the added chemical stimulus. Secondly, juvenile Round Goby are significantly attracted to odours of conspecific eggs over control lake water, and are 1.5 times more attracted to conspecific over heterospecific egg odours. Lastly, when comparing the positive allometric associations between fin morphology and body size in both sexes, males have significantly higher association than females.

Conspecific Signals

My research suggests that visual and chemical signals from conspecifics are attractive to reproductive female and juvenile Round Goby, respectively. Based on my results in Chapter 2 and 3, the physical characteristics of males (i.e. nuptial colouration) are significant visual attractants, while developing egg odours are significant chemical attractants. Nest-guarding Round Goby males have a black nuptial colouration (MacInnis & Corkum 2000; Marentette & Corkum 2008; Meunier et al., 2009). In many species, males possess conspicuous visual traits

(i.e. colouration and shape) (Kodric-Brown 1990; Sargent et al., 1998) that help discriminatory females to assess male quality (Trivers 1972), ultimately influencing female mate choice (Oliveira et al., 1998; Cogliati 2009).

In my first study, conducted under low turbidity, the dark pigmentation of a reproductive male is an obvious attractant of gravid females (Chapter 2).

Contrary to the expectations of my study, and because Round Goby can spawn at depths where visual signals are difficult to transmit (Bradbury & Vehrencamp 1998; Wickett & Corkum 1998), chemical signals should have been more effective in attracting females than actually observed. The decreased response to the chemical (urine) signals I observed is most likely attributed to insufficient levels of chemoattractants present in male urine.

Round Goby are capable of detecting various types of chemical stimuli (Murphy et al., 2001; Gammon et al., 2005), and the species is a known consumer of fish eggs (Wickett & Corkum 1998). I demonstrated that juvenile Round Goby spend significantly more time in the vicinity of odours from conspecific eggs over control lake water (Chapter 3). Furthermore, juveniles spend fifty percent more time in the vicinity of conspecific egg odours than heterospecific (Rainbow Trout) egg odours. Olfaction is important in locating food (Burks & Lodge 2002) and several studies have demonstrated that predators are attracted to olfactory cues released by eggs (Ferrari & Targett 2002; Mirza & Chivers 2002). Because Round Goby are colonial spawners (Charlebois et al., 1997), the availability of food may be low due to high population densities (Smith

& Reay 1991; Elgar & Crepsi 1992). Individuals are expected to engage in cannibalization, using olfactory cues dispersed by parental males (Meunier 2009; Meunier et al., 2009), by preying on nearby conspecific eggs. This form of cannibalization, termed non-kin intercohort cannibalism, may regulate the population of a species (Hunter and Kimbrell 1980). In other words, by consuming conspecific eggs, juvenile Round Goby are reducing future levels of intraspecific competition.

Allometry

Male Round Goby invest in internal morphological structures, such as testes and accessory glands (Marentette et al., 2009), however little is known about changes in the external morphology of this species. In Chapter 4, I demonstrated that allometric relationships exist between pectoral fin surface area and total body length in both Round Goby sexes. At any given body length, male pectoral fins had a surface area 1.8 times larger than the pectoral fins of females, while reproductive male pectoral fins were 1.67 times larger than non-reproductive male pectoral fins. A Round Goby nest may contain up to 10000 eggs (MacInnis & Corkum 2000), all of which require continuous care. The fin size of fish is often correlated with fanning efficiency (Bakker & Mundwiler 1999) and field and laboratory studies have demonstrated that in the Round Goby, only the male provides egg care by moving large volumes of water with their fins (Wickett & Corkum 1998; Meunier et al., 2009). Fanning with pectoral fins allows a male to

circulate water within the nest (Meunier 2009); this provides the eggs with a fresh supply of oxygen by preventing water from stagnating. Therefore, with respect to male Round Goby nest-guarders, larger fins may correlate with an increased reproductive success because fish can provide more oxygen to their eggs.

Also, males continually defend their nests by using pectoral fins to block the nest opening (Wickett & Corkum 1998; Meunier et al., 2009). I have shown that a negative relationship exists between the circularity pectoral fins and total body length in reproductive males (Chapter 4); fins become broader at the base (less circular) with increasing body length. Wider fins may allow males to block larger portions of the entrance to their nests, potentially decreasing the number of intrusions by juvenile Round Goby that are attracted to the odours released from fertilized eggs (Chapter 3). As a result, males with wider fins should have an increased reproductive success.

Summary and Significance

Using behavioural assays, I have identified two conspecific signals that affect the behaviour of Round Goby. Reproductive females are more affected by visual male characteristics rather than chemicals found in male urine, whereas conspecific egg odours affect the behaviours of juvenile fish significantly more than lake water or heterospecific egg odours. In both studies, fish respond by spending significantly more time in the vicinity of the above-described signals. While the behavioural responses of males were not tested, individuals appear to

possess physiological characteristics that enhance their reproductive success (Marentette et al., 2009; Yavno & Corkum 2010).

These findings are important to consider in developing tools to control the spread of this species into new areas. While continuing to spread into waterways adjacent to the Laurentian Great Lakes and the Mississippi River (Charlebois et al., 1997), the Round Goby poses a significant threat to the populations of native fishes (Steinhart et al., 2004). However, we may be able to take advantage of their behaviours by mimicking the signals used by this fish. Using field traps, baited with models of reproductive males and/or fertilized eggs, Round Goby could be actively caught. Removing juveniles may prevent future establishment of this species, while reproductive females may be removed to limit the number of potential mates available and also reduce the number of eggs laid during the reproductive season. Furthermore, my research helps us to better understand the behavioural ecology of invasive fish by examining how chemical and visual signals affect fish behaviour, before and after reproduction.

Future Directions

There are several opportunities to continue this research using both field and laboratory studies. First, it is important to determine why the urine used in my study did not affect the behaviour of females as strongly as visual signals (Chapter 2). Using conditioned water from males that had been injected with gonadotropin releasing hormone (GnRH), Kereliuk (2009) demonstrated that high

performance liquid chromatography (HPLC) fractions attract females. Because GnRH increases the release of steroids, we could determine the levels of steroids present in the urine of GnRH-injected males and compare them with urine from males caught in the wild. This may tell us if the volume of urine I obtained from wild-caught males contained sufficient quantities of pheromones. Also, Marentette et al. (2009) recently described the presence of two distinct types of male Round Goby: a 'light' morph with the characteristics of a sneaker, and a 'dark' morph, characteristic of a parental male with plasma containing significantly higher levels of a fish androgen. Behavioural assays could be conducted to compare female responses to urine from both male morphs, which would better indicate if female choice is based on the hormonal levels of males.

Round Goby are efficient predators of eggs, capable of not only consuming an entire nest full of native fish eggs (Charlebois et al., 1997; Steinhart et al., 2004), but also cannibalizing on the eggs of conspecifics (Wickett & Corkum 1998; Meunier et al., 2009). In Chapter 3, I provided evidence that fed juveniles are not only attracted to the odours of conspecific eggs, but they significantly prefer them to odours of heterospecific eggs. However, when fish are starved, they do not prefer one egg odour to the other. These fish in particular were caught outside of the reproductive season, thereby raising possibility that they have not been previously exposed to odours of fertilized conspecific eggs. This experiment should be redone, using fish that have been captured between June and August. Also, behavioural assays could be used to test how Round

Goby respond to other heterospecific egg stimuli. While Round Goby will feed on Rainbow Trout eggs in the laboratory (Fitzsimons et al., 2006), encounters with this species occur infrequently in the wild. It may be more prudent to test juvenile attraction to egg odours from heterospecific species that are more common, and thereby susceptible to egg predation by Round Goby (i.e. Smallmouth Bass, *Micropterus dolomieu*) (Steinhart et al., 2004).

Finally, the Round Goby is not the only species known to engage in egg predation. Roseman et al. (2006) documented predation of Walleye (*Sander vitreus*) eggs by thirteen different species, including Round Goby. Often times, analyzing the stomach contents reveals that the highest numbers of Walleye eggs were consumed by species from the same order (Wolfert et al., 1975; Roseman et al., 2006). While fish may opportunistically feed on eggs (Acha et al., 2002), we do not know if the eggs of all species are preyed upon equally. Are the eggs from any heterospecific species attractive to a fish predator, or is predation more likely to occur on conspecific eggs? To answer this question, studies could be conducted to test the behavioural responses of several different species of fish that are given a choice between either conspecific and heterospecific egg odours. Moreover, HPLC analyses could reveal the specific egg compounds that are attractive to predators, which in previous studies have possibly identified as complex proteins (Ferrari & Targett 2003). This may provide insight into the behavioural ecology of several fish species, including those that are considered to be non-indigenous. Ultimately, the goal of any future study should be to

determine how best to use chemical attractants, in combination with other signaling modalities (i.e. visual), to control the spread of invasive fish such as the Round Goby.

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