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# Behavioural discrimination of conspecific call types and its potential use in control strategies of the invasive round goby (*Neogobius melanostomus*)

by Bianca Carmela Mancini

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 © 2010 Bianca Carmela Mancini

# Behavioural discrimination of conspecific call types and its potential use in control strategies of the invasive round goby (*Neogobius melanostomus*)

by

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### Declaration of Co-Authorship

I hereby declare that this thesis incorporates material that is the result of joint research undertaken in collaboration with my supervisor Dr. Dennis Higgs, who co-authored the second chapter of my thesis. As primary researcher, the key ideas, experimental designs, data analysis, and interpretation, were performed by myself, however, Dr. Higgs was indispensable in the direction of proper development of techniques, date acquisition and statistical analyses. Contributions by Dominique Seremack, an undergraduate student, were through an honours thesis which involved data collection and analyses of the male playback experiments found in chapter two.

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 each of the co-author(s) 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, completed during my registration as a graduate student at the University of Windsor.

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#### Abstract

Vocalisations are integral to the workings of social communication in fish. In round gobies (*Neogobius melanostomus*) males produce acoustic signals for mate attraction.

This thesis investigates the functions of round goby acoustic signals and explores the use of acoustics in developing a trapping system that may help in controlling this invasive species. Round gobies were tested in three different experiments to measure behavioural responsiveness. For female playback experiments, goby attractiveness differed between call types. The differential responses suggest calls have different functions in goby courtship. In male playback experiments, there was an effect of stimulus on calls emitted but no difference with male size. Finally, round gobies were significantly more attracted to traps coupled with a conspecific call, suggesting a useful avenue for goby control. Understanding communication tactics in gobies aids in understanding information processing in fish and may prove essential when assessing control strategies in this highly invasive species.

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## Chapter 1: General Introduction

#### I. Evidence for the importance of sound in aquatic environments

Communication modalities, be they visual, olfactory, acoustic, electrical reception, or tactile signals, all play a role in allowing an organism to interact with its environment. For any modality to be effective at relaying information, the signal type must match the transmission properties of the medium and environment (Roger & Cox, 1988). As such, certain modes of communication are more advantageous and effective depending on the constraints imposed by the habitat. This is particularly true in aquatic environments due to changes in signal transmission in the aqueous environment. Sound is one of many modalities used to communicate underwater. Sound maintains its transmission rate and integrity through a variety of conditions, such as different distances and depths, varying light intensity, and turbidity (Roger & Cox, 1988), and it is thought to be less expensive to produce than other modalities, such as visual or olfactory signals (McKibben & Bass, 1998).

#### I.i: Attenuation and propagation

Aquatic environments can be classified into specific habitat types based on parameters such as depth, current speed, and available light. Each habitat type (e.g. shallow waters, coastal areas, deep ocean, pelagic zones, fast flowing rivers) can affect acoustic signal transmission with different levels of attenuation. Attenuation occurs when sound waves are reflected, scattered, or refracted by the habitat thereby decreasing

their sound level: this may result in the receiver detecting false information or no information at all. Even with these potential drawbacks, acoustic communication remains an important stimulus for long-range transmission of information in underwater environments (Tavolga, 1977).

Inversely related to attenuation is the propagation of sound. Propagation must be greater than attenuation for sound to be an effective form of communication. Just as certain habitat parameters can increase attenuation, others can aid signal transmission through propagation. Salt water and freshwater environments also differ in the manner in which they affect the ability of sound to propagate. Salt water, being more dense, attenuates sound by 1 dB at 100 km (for sound at 500 Hz), whereas freshwater does not begin to attenuate a signal at 500 Hz by 1 dB until 10,000 km (Rogers & Cox, 1988). In an underwater medium, this would suggest that freshwater species will transmit sounds much further through their environment, although underwater depth and physical obstructions will greatly affect propagation as well (see below).

#### Noise

Ambient noise exists in all environments whether the environment is aquatic or terrestrial. Noise, in general, is any sound that is not needed or wanted by the receiver. Underwater environments have a wide variety of sound, such as man-made noise (ships and industrial work), biological sounds (marine mammals, snapping shrimp, etc.), and naturally occurring sounds (rain, wind, waves, earthquakes, volcanoes, etc.) (Richardson et al., 1995). Ambient noise is highest in the shallow coastal areas inhabited by the

majority of fish species (Rogers & Cox, 1988). Noise can affect hearing by masking intended signals and can confuse the receiver about the location of the sound source.

*I.ii: Underwater sound propagation* 

#### Reflection

Reflection occurs when sound waves bounce off the surface interface, seafloor, or any object in their path towards the intended receiver; this can distort the signal and give the receiver false information. Some boundaries are better conductors of sound waves than others and allow for a greater amount of the sound wave to be reflected. The surface interface is one such reflector and propels sound off the air-water boundary; sound absorption is minimal, making the surface border an effective reflector for long distance transmission (Rogers & Cox, 1988). This contrasts with the reflection properties of the bottom substrate, which often absorbs more of the sound than what is reflected; therefore, the substrate may be better suited for short distance propagation.

#### Scattering

Sound waves are scattered by coming into contact with objects such as fish, human-made structures and disturbances in the water (e.g. the influence of ships or other vessels). Scattering disrupts the original path and propels sound waves off the object in many directions; this often causes the sound wave to travel in a direction previously not intended (Richardson et al., 1995). Different substrates can also scatter sound waves and limit the transmission ability. To avoid this, fish should position themselves at the highest point on the substrate to limit the scattering effect.

#### Refraction

When sound travels through different media having a different sound speed, sound waves will bend or refract. The speed of sound in each medium will determine the direction in which the wave is bent (Rogers & Cox, 1988). If the second medium has a higher transmission rate (speed of sound), the sound waves will be bent back towards the original medium and refracted in the direction of the interface. If, however, the second medium has a slower sound speed, then the signal will bend away from the interface and further into the second medium (Rogers & Cox, 1988).

Temperature, salinity, and pressure affect long range propagation of sound. As pressure increases, with greater depth, the speed of sound increases (Mann, 2006). In addition, as temperature increases (e.g. when approaching the surface) the speed of sound also increases. Propagation may be maximal in the area known as the SOFAR (Sound Fixing and Ranging) channel. This occurs at a depth of approximately 1,000 m (Rogers & Cox, 1988). At this depth, both parameters are in balance with each other, neither overriding the other, and both existing at high enough levels that they have positive effects on sound propagation.

#### *I.iii:* Nearfield and farfield

The nearfield and farfield are determined by the 'type' of sound a receiver can detect and correlate with the distance from the sound source. The nearfield is comprised of two parts that make up the sound heard by the receiver: a pressure component and a displacement component (Richardson et al., 1995). The pressure component refers to the

'true sound' where movement of the particle velocity is only due to fluid compression by the source itself. The displacement component refers to the 'flow' where the particle velocity of the sound wave can be felt by the recipient. The farfield does not have a displacement (or flow) component as it is solely based on the pressure component (Richardson et al., 1995). With respect to discussing fish sound production and hearing in teleosts, it is important to make a distinction between these two fields as it is thought that only hearing specialists (or fish with special adaptations) can hear in the farfield (Mann, 2006).

#### II. Introduction to fish vocalisations

Acoustic signals are a primary form of communication in many aquatic animals (Bass & McKibben, 2003; reviewed in: Ladich, 2004). Vocalizations can be an ideal way to signal to conspecifics and heterospecifics and can convey distress, warn intruders and protect territories, attract mates, and potentially assess the qualities of neighbours (Winn et al., 1964; Myrberg et al., 1986; Ladich, 1997; Amorim et al., 2003). Fish produce different sounds in different behavioural contexts, although not all of these behavioural actions are well understood in most fish, as particular sounds have not yet been associated with a function in many cases (reviewed in: Bass & McKibben, 2003). Fish produce agonistic behaviour calls, advertisement calls (reviewed in: Ladich, 1997) and, to a lesser extent, when foraging for food (Amorim & Hawkins, 2000). This review focuses on the significance of acoustic signals in fish by highlighting the mechanisms and structures of fish sounds, and behavioural studies which demonstrate the many ways fish use this modality in social communication.

II.i: Mechanisms of sound production

The swimbladder

Fish produce sounds using a variety of different mechanisms; the best studied of which is the swimbladder (reviewed in: Tavolga, 1977). The swimbladder is a gas-filled sac, originally evolved as a mechanism to control buoyancy (Tavolga, 1977), and in multiple species, the swimbladder has been co-opted to also function in sound production (Tavolga, 1977; Bass & McKibben, 2003). While functioning in slightly different ways between species, in general, swimbladder vocalisations typically involve sonic muscles causing vibrations in the swimbladder, leading to sonic emissions, much like beating a drum leads to sound in air (Tavolga, 1977). Sonic muscles can be attached to the swimbladder in a variety of ways. Some sonic muscles are found laterally, as in the grey gurnards (Eutrigla gurnarrdus) and Mormyridae (Amorim et al., 2004; Crawford et al. 1986). When these striated muscles contract, they create sounds by resonating the swimbladder (reviewed in: Tavolga, 1977). In other species, such as pigfish (Congiopodus leucopaecilus), striated muscles run between the vertebral intercentra and the back border of the pectoral girdle (Packard, 1960). The muscles contract simultaneously and produce a drum like sound through the swimbladder, as found in oyster toadfish (*Opsanus tau*) and midshipman (*Porichthys notatus*) (Bass & McKibben, 2003).

Structures other than sonic muscles can also have the same vibrating effect on the swimbladder. The mechanism of an 'elastic-spring', created by a thin bone firmly attached to the anterior dorsal wall of the swimbladder, vibrates and transfers this motion

to the air-filled sac (reviewed in: Tavolga, 1977). When the muscles around the anterior dorsal wall contract, the motion moves the anterior section of the swimbladder and this vibration produces the sound (reviewed in: Tavolga, 1977).

#### Rubbing and knocking of bones

A second common mechanism of sound production in teleosts is the rubbing of bones together. When rubbed together the bones produce either short wide-band pulses or burst-like sounds. The stridulation can be produced by the rubbing of pectoral spines in the sockets of the shoulder girdle as seen in many catfishes (Siloridae, Ladich, 2004), by rubbing pectoral spines against each other as in croaking gouramis (Ladich, 2004), or by smaller bones interacting with parts of the appendicular skeleton (reviewed in: Ladich, 1997). The use of bones as sound generation devices tends to generate higher dominant frequencies than the swimbladder (Ladich, 1999) and tend to result in pulsatile sound sources (Ladich, 2004).

Pharyngeal teeth exist as bony plates on both the dorsal and ventral surfaces of the pharynx. Primarily found in species that use their pharyngeal teeth to masticate hard food items, these teeth, when rubbed together, also produce sounds (reviewed in: Bass & McKibben, 2003). The grinding of pharyngeal teeth produces a burst-like sound in nature, which has lower energy than sounds produced by the swimbladder. These sounds, however, can have sections that reach up to several kHz (Ballantyne & Colgen, 1978). While some pharyngeal sounds may be a by-product of feeding (Ladich & Popper, 2001), they are also well documented to be used for actual sound generation in communication contests (Ladich & Popper, 2001).

#### Novel mechanisms

A novel mechanism of sound production has recently been discovered in damselfish (Parmentier et al., 2007). Amphiprion, comprised of 27 species, have all been found to use vocal communication, but the exact mechanisms of how their sounds are produced have not been elucidated until recently. Throughout this group, variation in tooth arrangement appears to give each species a unique vocalization (Parmentier et al., 2006). One species of damselfish, the clownfish (Amphiprion clarkii), produces a sound that is usually higher in frequency than sounds produced with a swimbladder. The sounds are created when the fish quickly lowers the hyoid apparatus while closing the mouth at the same time (Parmentier et al., 2007). A ligament attached to the hyoid bar and the internal section of the mandible pulls the mouth closed. The collision of the teeth as the mouth closes produces the sound; here the jaw is suggested to be "the sound radiator" (Parmentier et al., 2007). Clownfish are known to produce 'chirps' and 'pop' sounds when trying to attract mates or when confronted with possible danger. These sounds can be in trains (1-8 pulses) and average a frequency between 450-800 Hz (Parmentier et al., 2007).

#### II.ii: Structure of a fish call

Fish calls have different components that can be used to classify and distinguish one call from another. These different components include the length of the call, the intercall duration, the pulse duration, and the interpulse duration (the time in between each pulse in a call) which makes up the temporal envelope (Kihslinger & Klimley, 2002). Fish sounds are pulsed and broadband, or tonal, and vary over a range of

frequencies (Kihslinger & Klimley, 2002). It has been suggested that there is not as much uniformity when describing fish sounds as is seen in terrestrial animals and birds (Kihslinger & Klimley, 2002). Nonetheless, temporal, frequency, and amplitude features are part of every call. Bout duration encompasses all the sounds within a series of vocalisation and can consist of more than one sound type (Kihslinger & Klimley, 2002).

A bout can be subdivided into individual units. These units are a string of sounds within one type of vocalisation and are (Lindstörm & Lugli, 2000); units are classified by Winn (1964) as unbroken segments that can be heard or seen in a spectrogram. Units can be further broken down into pulses. The pulse number refers to the amount of pulses in each unit (Kihslinger & Klimley, 2002), which can vary between call types of a given species or between species, making pulse number a distinguishing characteristic (Myrberg et al., 1986). The silence between the end of one pulse and the beginning of the next is termed interpulse interval (Mann & Lobel, 1997). It has been suggested that this quiet time, 'or off-time', may be the feature that holds the most information when comparing different calls and species identification (reviewed in: Kihslinger & Klimley, 2002).

Finally, when describing a call, frequency modulation (changes in frequency), fundamental frequency (natural frequency), and the frequency range are all commonly used to quantify call characteristics of fishes (Kihslinger & Klimley, 2002). Frequency is often affected by body size and therefore may be involved in female mate choice (Amorim & Vasconcelos, 2008; Amorim et al., 2008); however, this size effect is not evident in all fish species. Frequency may be an important feature for honest signalling in

those species where frequency is negatively correlated with body size. Females could assess a male's quality during the breeding season; many parental and fitness qualities have been associated with male body size, such as foraging abilities, territory and nest guarding (Malavasi et al., 2003; Colleye et al., 2009).

#### III. Interspecific difference in calls from different groups

Fish sounds are generally classified into: tonal sounds, drumming sounds, and complex sounds (a combination of tonal and drums), and can be harmonic or non-harmonic in nature (reviewed in: Bass & McKibben, 2003). Tonal sounds have a high pulse repetition rate which results in the pulses blending together to form a sinusoidal-like waveform (Lugli et al., 1997). The reproductive call of the Arno goby (*Padogobius nigricans*) is characterised as being tonal, lasting 300-400ms, and close in structure to a pure sine wave (Lugli et al., 1997). Drumming sounds, characterized as repeating pulse trains with a lower pulse repetition rate than that of tonal sounds where each pulse is distinct (Lugli et al., 1997), make up the reproductive call of the Panzarolo goby (*Knipowitschia punctatissima*) (Lugli et al., 1997). Complex sounds are a combination of tonal and drum vocalisations (Lugli et al., 1997; Amorim & Neves, 2007). Male Common gobies (*Padogobius martensii*) switch between drumming and complex sounds during different reproductive stages (Lugli et al., 1997).

Fish sounds have been further classified into call types associated with specific species (examples being grunts, growls, knocks, hums, moans, and boatwhistles)

(Kasumyan, 2008). While call structure can be defined in discrete categories (as shown

above), many fish species can produce more than one call type (Myrberg & Spires, 1972). The precise function of many calls remains unclear at this time. Nonetheless, researchers have started comparing similar call types in different species to gain insight on the behaviours associated with the sounds.

The two best studied species are the plainfin midshipman (*Porichthys notatus*) and the toadfish (Opsanus spp.) where each produces a distinguishing call (reviewed in: Bass & McKibben, 2003). Hums, suggested as advertisement calls of the plainfin midshipman, are long in duration and are unmodulated (Bass & McKibben, 2003). Hums are harmonic in structure, reaching up to 700 Hz in some individuals, and have a fundamental frequency between 90-100 Hz (Bass & McKibben, 2003). These sounds can last minutes to an hour in duration (Brantley & Bass, 1994). Only nesting male midshipman are known to produce this sound during the breeding season (reviewed in: Bass & McKibben, 2003). Toadfish produce a specific complex sound named the boatwhistle (reviewed in: Bass & McKibben, 2003). This advertisement call is comparable to the hum. It is characterised by being a shorter (500 ms), multi-harmonic call with two segments (reviewed in: Tavolga, 1977). The first segment or note, as some call it, is a single grunt that leads to the second note, which begins with a grunt then switches to a multi-harmonic 'hoot' (Bass & McKibben, 2003). The initial grunt can be followed by another single grunt and the second segment is often followed by a grunthoot combination (reviewed in: Tavolga, 1977).

The two main types of behaviour classifications associated with sound production are mating and defence (reviewed in: Bass & McKibben, 2003). Mating sounds are

diverse and are associated throughout the many mating sequences, such as advertisement, courtship, pre-spawning, and spawning (Myrberg et al., 1986; Lugli et al., 1996a,b; McKibben & Bass, 1998; Amorim & Neves, 2007). Advertisement calls tend to be long in duration and are repeated to form call trains (reviewed in: Bass & McKibben, 2003). The boatwhistle produced by the toadfish is a prime example (reviewed in: Tavolga, 1977). Advertisement calls should be long on duration as they act as a beacon for females to follow (Fay, 2005). Often breeding areas are densely filled with competing males and a female needs a continuous signal to follow and locate the source (Rollo & Higgs, 2008). Courtship sounds signal the presence of a male which is ready to mate (Bass & McKibben, 2003). These sounds are often produced when a male is alone in his nest; once a female enters his territory, courtship sounds may change as he approaches a female (Amorim & Neves, 2007). A similar change in sound type with reproductive displays has been shown in damselfish. Damselfish produce different variations of a chirp sound (brief multi-pulse broadband) during courtship; the 3-pulsed chirp sound is produced while mature males perform a courtship display (termed the dip) (Myrberg & Spires, 1972). Males perform the dip to display their nuptial colouration to females and this swimming motion has been shown to lead the prospective female back to the nest (Myrberg & Spires, 1972). Further work on damselfish chirp sounds have shown four temporal structures and frequency modulation differ between species, those being, call duration, pulse number, pulse duration, pulse interval, and dominant frequency (Kihslinger & Klimley, 2002).

Pre-spawning sounds have been shown to accompany other communication modes, such as visual displays (Lugli et al., 1997; Lindström & Lugli, 2000). Male green

damselfish (*Abudefduf abdominalis*) try to lead females to their nest by performing a zigzag swimming pattern; sounds are always accompanied by the visual display and continue into the nest (Maruska et al., 2007). The Canestrini's goby (*Pomatoschistus canestrinii*) moves its head rapidly in conjunction with a thump sound while outside the nest with a female but then quickly moves inside and continues to call until spawning occurs (Malavasi et al., 2009). Some fish species also produce sounds as spawning signals (Lugli et al., 1995). Broadcast spawning often accompanies acoustic cues to ensure gametes are released simultaneously. Two coral reef fish, the hamlet (*Hypoplectrus unicolor*) and the striped parrotfish (*Scams iserti*), both produce sound during gamete release which are different from their courtship signals (Lobel, 1992).

Aggressive sounds are typically short in duration which potentially serves to gain the attention of the receiver (Ladich, 1997; Sebastianutto et al., 2008). Fish produce agonistic sound in response to intruders, during male-male interactions, to assert dominance, and when frightened (reviewed in: Ladich, 1997). Dominant male midshipman produce growls while building nests in the breeding months (Brantley & Bass, 1994). Mormyrids also produce growls during the beginning of the mating season, as such; growls may be used to assert dominance and create territories prior to actively courting females (Crawford et al., 1997). Grunt trains are thought to be produced by males during agonistic behaviour (McKibben & Bass, 1998; Bass & McKibben, 2003). Several species produce this type of vocalisation, including the well-studied plainfin midshipman (*Porichthys notatus*) and the toadfish (*Opsanus tau*) (reviewed in: Bass & McKibben, 2003). Only dominant males have been observed making this call during the breeding season (reviewed in: Bass & McKibben, 2003).

#### IV. Evidence that temporal or amplitude modulation matters

To better understand the importance and meaning behind the difference in temporal structures, behavioural experiments are essential. Playback experiments allow researchers to assess the behaviour of fish and to link this to a particular function (Tavolga, 1977). Playback studies can compare the reaction of a fish to intra-or interspecific communications. Different damselfish species (*Eupomacentrus partitus*, *E. planiforns*, and *E.leucostictus*) can discriminate between conspecific vocalisations; as well, it was also shown that *E. partitus* was able to discriminate wild conspecific calls better than unedited conspecific laboratory calls (Myrberg & Spires, 1972). While these calls differ in many aspects, the most important difference appears to be the pulse interval, with the 'off-time' being the most sensitive segment to changes in the calls (Myrberg & Spires, 1972).

The unique call of the toadfish, the boatwhistle, was determined to be an advertisement call for males to attract gravid females through playback experiments with gravid females, non-reproductive females, and males (reviewed in: Bass & McKibben, 2003). Gravid females are most likely to swim towards a speaker playing the boatwhistle call than are the other two groups (reviewed in: Bass & McKibben, 2003). This call not only functions as an advertisement call but could contain information for males in the area, as males also approached the playing speaker (reviewed in: Bass & McKibben, 2003). Male midshipman hums were also found to attract gravid females during similar playback studies (McKibben &Bass, 1998).

Knowing the difference between calls allows the receiver to identify the source, especially at long distances where the sender is out of visual range. Hawkins and Rasmussen (1978) demonstrate this by classifying the differences between haddock (*Melanogrammus aeglefish*) and cod (*Gadus morhua*). These two economically important fish both produce knocks and grunts; however, the haddock have fewer pulses, and the calls are shorter in duration than those of the cod. As well, the duration of a call can be a distinct characteristic between certain species, as Ladich (1997) describes, *Pimelodus pictus* displays a stress call 10 times longer than *P. blochii*. Being able to distinguish ones species from congeners saves the receiver energy by not wasting time investigating a signal that was perhaps intercepted by mistake and reduces the chance of hybridization (Kihslinger & Klimley, 2002).

Modulation of the temporal envelope (see above) has been tested to determine its overall effects in vocal recognition (McKibben & Bass, 2001a). Temporal envelope modulation may be significant as a whole when distinguishing between different call types of one species (McKibben & Bass, 2001b). Midshipman modify the drumming sounds to produce their three call types as well (Brantley & Bass, 1994). The duration of the sound decreases as the midshipman change from hums to growls to grunts (>1 min, ms-min, ms, respectively). Each call type also has a unique fundamental frequency (Brantley & Bass, 1994). Midshipman grunts and hums are comprised of different temporal structures and are thought to have different functions. Continuous tones (hums) are more effective at attracting reproductive females than pulsed sounds (grunts) during playback studies (McKibben & Bass, 1998; reviewed in: Bass &McKibben, 2003).

Call duration may be an indicator of fitness (Lindström & Lugli, 2000). Calling is metabolically expensive and muscle fatigue can lead to a decrease in calling rate (Lindström & Lugli, 2000). The sand goby (*Pomatoschistus minutes*) have been shown to adjust its calling rate and extend the 'off-time' between calls to lessen the strain on tired muscles (Lindström & Lugli, 2000). However, this is at a cost as female painted gobies have been shown to prefer higher calling rates and a mating preference for males with increased call rates (Amorim & Neves, 2007).

#### V. Future direction overall

Though our knowledge of encoding temporal structure in fish is still in its infancy, approaches taken by other researchers can provide us with clues on the aspects likely to be important in fish communication. It is clear that females use the temporal structures not only to recognize potential mates but also to detect a preferred male but it remains unclear how different conspecific calls affect attraction of females and responses of males in species with a broader sound repertoire. It is also unclear how different conspecific call types affect the ability of fish to localize conspecifics from a distance. Examination of responses of free-swimming fish to different call types will allow the beginning of an understanding of how these sounds propagate and how they are processed by the intended receiver. In this way, we can best determine how fish decipher relevant information from complex calls and how the aquatic features have driven evolution of acoustic signals in this speciose group.

The goals of the present study are twofold. The first is to investigate the affect of conspecific vocalisations on the behaviour of round gobies. It is known the round goby

shows higher phonotactic responses to conspecific calls when compared to heterospecific calls and can distinguish between conspecific and congeners (Rollo & Higgs, 2008). To date, it is unclear if round gobies can distinguish between different signals within their own repertoire. Male round gobies are known to produce a reproductive call during the breeding season to attract females (Rollo et al., 2007). Round gobies are also very territorial and have demonstrated an aggressive display that may accompany a sound as well. Here we present different reproductive calls and possible aggressive sounds to determine the behavioural attraction of females. To further investigate the round goby repertoire, we test males during playback trials to look at the affects different conspecific sounds have on male behaviour. By presenting conspecific sounds, we can quantify the temporal structures and frequency components in the male vocalisations to determine the level of natural variation found within the male sounds. These two experiments will demonstrate if signal recognition is capable in the round goby and potentially link sounds with a behavioural function, adding to our understanding of information transfer by means of acoustic communication.

The second goal of this thesis is to develop an acoustic trapping device for this invasive species. My objectives are to incorporate the sound which shows the highest level of phonotaxis in the first sections and test that sound in a trapping device. The trap will first be tested in a laboratory environment and then be tested in the field.

Although these studies focus on different aspects initially (that being behavioural ecology and applied conservation), the overall goal is to gain knowledge of the round goby to better understand the biology of this invasive fish. As well our study will add to

further understanding of the role of acoustics in communication in fish and be incorporated into an applied aspect of control strategies of invasive species.

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# Chapter 2:

Behavioural discrimination of conspecific call types and its potential use in control strategies of the invasive round goby (*Neogobius melanostomus*)

#### Introduction

Acoustic signals in fishes represent one of many modalities used to communicate with hetero-and conspecifics. Although not as well studied as in mammals (Richardson et al., 1995; Wyman et al., 2008), birds (Byers & Kroodsma, 2009), and amphibians (Ryan & Wilczynski, 1991), research in fish vocalisations highlights the importance of communication strategies in an aquatic medium (reviewed in: Popper & Schilt, 2008). Sound is not impeded in low light environments such as murky or deep waters where visual signal information would be lost and is more directional than chemical cues, which can be greatly impacted by currents (Fay & Popper, 2000). Because of this, sound can travel great distances between a sender and receiver (Rogers & Cox, 1988) while maintaining directional information about the sound source (Fay & Popper, 2000; Popper & Schilt, 2008). Transmission distance may be the most advantageous component to acoustic communication. The use of advertisement calls in long distance signalling sets acoustic communication apart from other aquatic signalling modalities (reviewed in: Ladich, 2004).

Fish use acoustic signalling in both reproductive and agonistic contexts (reviewed in: Bass & McKibben, 2003). Males produce advertisement calls to attract females (Myrberg et al., 1986; Lugli et al., 1996a,b; McKibben & Bass, 1998; Amorim & Neves, 2007) and also emit aggressive sounds during male-male competition or territorial defence (Winn et al., 1964; Ladich, 1997; Amorim et al., 2003). Tilapia (*Oreochromis mossambius*) will continue to emit courtship sounds after having courted with a female to ward off territorial attacks; this may also allow nearby conspecifics to eavesdrop and gather information about neighbouring dominant males (Amorim et al., 2003). The

streaked gurnard (*Trigloporus lastoviza*) increases its chance of acquiring limited food sources by producing agonistic vocalisations (Amorim & Hawkins, 2000; Amorim et al., 2004); whereas, the topmouth minnow (*Pseudorasbora* parva) produces sounds during feeding to potentially advertise the presence of food (Scholz & Ladich, 2006).

Signals need distinct characteristics to allow a receiver the ability to discriminate variations within a call (Amorim & Vasconcelos, 2008). Changing the characteristics of a call can change the signal content (Amorim et al., 2006) and temporal coding can be changed to vary a call and perhaps increase information content (Wysocki & Ladich, 2002). Temporal structures play an important role in acoustic communication in many fish species (mormyrids, batrachoids, damselfish, gobiids - Bass & McKibben, 2003). Temporal patterns, frequency range, amplitude levels, and distribution of energy, may all be important for signal identification (Ladich, 2004) and species (Myrberg & Spires, 1972; Lugli et al., 1997; McKibben & Bass, 2001a,b; Rollo & Higgs, 2008) and individual recognition (Myrberg & Riggio, 1985). For example, three groups of Mediterranean gobies can be identified by similarities in their calls; larger sized species produced both tonal and grunt sounds unlike smaller species which only produce grunts and larger species produce grunts with a lower duration than smaller species (Malavasi et al., 2008). Overall, acoustic signalling may provide vital information used by conspecifics to assess a sender's location, fighting ability, sex, readiness to mate, body size, and individual identity (Amorim et al., 2003; de Jong et al., 2007; Colleye et al., 2009).

Phonotaxis, through playback experiments, has proven that fish directionalize sound and potentially use information in the signals to assess the sender (reviewed in:

Tavolga, 1977; Lugli et al., 1996a; McKibben & Bass, 1998; Rollo & Higgs, 2008).

Coral reef pomacentrid larvae use acoustic cues from reefs to locate habitats after the pelagic phase (Tolimier et al., 2004; Wright et al., 2010). Fishes from the families

Pomacentridae, Apogonidae, Lethrinidae, and Gobiidae, orient towards high-frequency (570-2000 Hz) reef noises as compared to low-frequency (<570 Hz) sounds or no sound at all (Simpson et al., 2008). Localisation is vital in the success of mating in many fish species where males produce advertisement calls to attract females (Crawford et al., 1986; Myrberg et al., 1986; reviewed in: Bass & McKibben, 2003). Females can locate a preferred male by positive phonotaxis. Without acoustic cues, females might suffer a diminished capacity to hone in on the position of a desired mate. This concept is well studied in the reproductive tactics of the toadfish (Batrachoididae) (reviewed in: Bass & McKibben, 2003; Amorim et al., 2006, 2008a); all male oyster toadfish (*Opsanus tau*) produce acoustic cues however only mature males can produce the boatwhistle advertisement call to attract females to their nest (Maruska & Mensinger, 2009).

In the present study, I investigate the behavioural responses of the round goby to known conspecific sounds. Round gobies (*Neogobius melanostomus*), originating from the Ponto –Caspian Sea, were first discovered in the St. Clair River in 1990 (Jude et al., 1992) and quickly invaded the Laurentian Great Lakes (Charlebois et al., 2001). Round gobies are prolific breeders, mating several times in a season and have an extensive range in diet (Jude et al., 1992; reviewed in: Charlebois et al., 2001); this has aided their spread into North American waterways (Charlebois et al., 2001). The round goby has had extensive effects on native populations in the Great Lakes (Jude et al., 1995); their territorial nature has depleted the sculpin (*Cottus bairdi*) population (Janssen & Jude,

2001) and has caused great concern to species of commercial importance (as cited in Corkum et al., 2004). Male round gobies are known to produce reproductive calls (Rollo et al., 2007; Speares, 2007); other sounds have been documented though have not been fully described (Meunier et al., 2009). The mechanisms used to produce these sounds are unknown at this time; nonetheless, round gobies do show clear directional responses to conspecifics calls (Rollo et al., 2007) and also have the ability to differentiate goby calls from other sounds (Rollo & Higgs, 2008).

In the current study, three different experiments explore the behavioural response of female and male round gobies to conspecific acoustic emissions. Females were used in choice experiments to determine attractiveness to four male sounds; two reproductive calls, a possible aggressive sound and a vocalisation recorded in lab while a male was exposed to a female (stimulus 4). Male playback trials focused on male-male interactions to determine if males are more likely to respond to neighbouring advertisement calls or possible agonistic cues. Data from these studies were used in the final experiment to design a preliminary acoustic trap. Determining the acoustic cue which exhibited the highest phonotactic response can aid in developing a trapping system to decrease the threat of their spread further into the Great Lakes systems.

## Material and methods

# Study species

All round gobies were caught in the Detroit River on the Canadian border either by seining or angling from May to October 2007 and 2008. The fish were housed at the University of Windsor's animal quarters facility and all animal care procedures were

followed as approved by the University of Windsor Animal Care Committee. Gobies were kept in aerated tanks with carbon filtration systems. The temperature was kept at  $20^{\circ}\text{C}$  ( $\pm 1^{\circ}\text{C}$ ) to mimic the river water temperatures during the summer months, and photoperiod was set to a 12:12 light cycle. The gobies were fed Nutrifin fish flakes (Hagen Pet Foods Inc.) and were held a minimum of a week before being tested to acclimate to the lab conditions.

## Sound types

Gobies were presented with four different conspecific sound types. Stimuli 1 and 2 are reproductive in nature as males were observed actively guarding nests when producing the vocalisations. The stimulus 1 call was recorded in the wild in Lake Michigan by John Janssen (University of Wisconsin). Stimulus 1 has a fundamental frequency of 180 Hz and is characterised by having long pulse trains (Rollo et al., 2007) with most of the energy occurring below 400 Hz (Figure 1, A-B). The sound is broadband and pulse trains contain an average of eight pulses per train. Pulse train duration is an average of 0.07 s and the 'off-time' between the pulse trains is around 0.25 s. Stimulus 2 was recorded in the Higgs lab at the University of Windsor and has a fundamental frequency of 160 Hz (Figure 1, C-D). Broad-band pulse trains are again evident; however, the interpulse train duration is smaller than those in stimulus 1. Most of the energy of the call is found below 350 Hz. The stimulus 3 sound, (acquired from John Janssen and Greg Andraso) consist of one group of pulses with a fundamental frequency of 102 Hz with most of the energy occurring below 400 Hz (Figure 1, E-F). This sound may be a by-product of the goby hitting its tail on the substrate as it is accompanied by a flipping motion where the goby quickly twisted its body. The function of stimulus 3 may

communicate aggression; however this is still unclear. Stimulus 4 is a potential novel vocalisation and has no known function. Recorded in the Higgs lab at the University of Windsor, stimulus 4 was first observed while a male patrolled the area around his nest but was not actively guarding his nest as there were no eggs present. It is clear stimulus 4 is a vocalisation and not a by-product from body movement because the sound was associated with the male moving his mouth. Stimulus 4 contains fewer pulse trains and greater interpulse train intervals (averaging 0.75 s between each pulse train) than either stimulus 1 or 2. The fundamental frequency of stimulus 4 is approximately 118 Hz with most of the energy occurring below 400 Hz; pulse amplitude is largest during the final pulse train of the call (Figure 1, G-H). Sound production mechanisms for all sounds are unknown at this time.

# Female choice experiment

Female choice trials consisted of four types of sound treatments (stimulus 1, stimulus 2, stimulus 3, and stimulus 4) and a control silent treatment. Females were put into groups of five and one group was tested per week to ensure all five females in a given group received the same acclimation conditions and sound treatment on a given day. This was done to decrease the chance that a female's response to sound was affected by environmental conditions. A total of 32 females were tested however only 12 females were exposed to the each of the five treatments so only these 12 are analysed here. At the beginning of a new group, treatment order was selected at random. Trials lasted a total of 10 minutes with the sound alternating one minute on and one minute off for the duration. Two underwater speakers (UW-30 Electo-Voice, 30W, Lubell Labs Inc. Columbus OH, USA) were in the tank at all times; an active speaker playing the sound and a silent

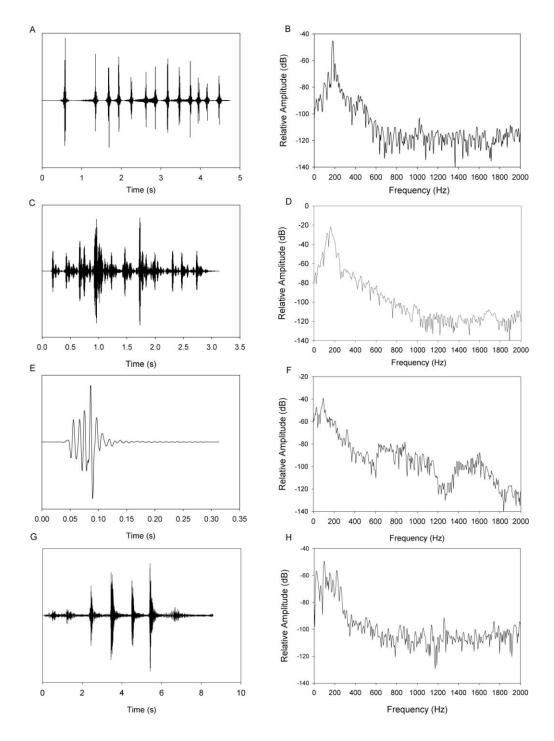


Figure 1: Oscillograms (left) and power spectra (right) of the Stimulus 1 (A-B), Stimulus 2 (C-D), Stimulus 3 (E-F), and Stimulus 4 (G-H) sounds produced by male round gobies. Power spectra are summed across the entire sound duration.

Janssen and Flip sounds received from John Janssen (University of Wisconsin) and Greg Andraso (Gannon University).

speaker, which served as a control. The active speaker was chosen at random by drawing a piece of paper from a container before every trial. The sound was played from a Durabrand CD player (model CD-566, Lenoxx Electronic Corp, N.J., USA) which passed through a Phase Linear amplifier (model UPA 424CS, Audiovox Electronic Corp, N.Y., USA) to the speaker. The amplifier was powered by a MotoMaster Eliminator car battery (part# 10-2500-4). Background and sound levels were recorded by a hydrophone (model 902, Inter Ocean Systems, CA, USA) prior to the beginning of each trial and all sounds were played at 150 dB re 1µPa at the starting point of the fish (see below). The responses of fish during all trials were recorded for further analysis by a Sony digital Handycam Recorder (model DCR-TRV27) or an EverSecure video camera (SX-800-HR).

Trials took place in a 1020 L rectangular fibreglass tank which was filled to a depth of 33 cm with dechlorinated tap water (Figure 2). Water was kept at  $20^{\circ}$ C ( $\pm 1^{\circ}$ C) to match that of the holding tanks. Two underwater speakers, 26 cm apart, were hung at one end of the tank. A mesh net bisected the tank at 144.78 cm from the speakers; the experimental area kept the fish in view during recordings and allowed the sound to travel through the rest of the tank to reduce reverberations at the starting zone. An arbitrary line at 114.3 cm from the speakers denoted the 'positive zone' for all experiments.

Females were given a minimum of two hours to acclimate to the tank water in a separate plastic container prior to all treatments. Females were tested individually and were given an additional five minutes in the experimental tank before the start of the trial to allow for adjustments to the new environment. Female length was measured to keep track of each individual for further analyses. To start a trial females were placed at the back of the experimental area, 144 cm from the speaker; however, if the fish moved into

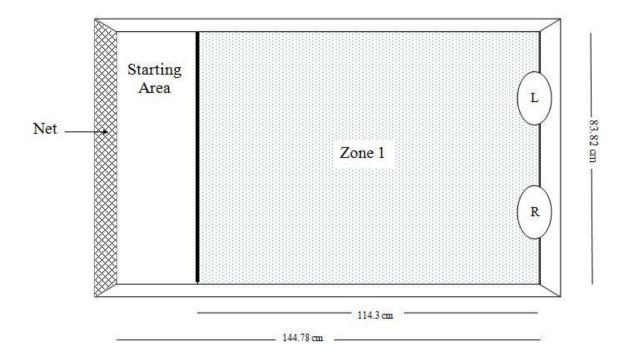


Figure 2: Experimental tank for female choice trials. Bold line denotes beginning of positive area (Zone 1). Females were placed against the net for the acclimation period and start of trial; however gobies could move anywhere within the 'Starting Area' before the trail began. L= left speaker, R= right speaker. A video recorder was suspended over the tank to monitor goby movement.

the 'positive' zone (denoted Zone 1) before the start of the trial, the fish was removed and used later in the day. The positive zone area was chosen to allow a clear distinction between actively approaching the sound source and arbitrarily swimming in the tank.

Female responsiveness was quantified as the percent that swam into Zone 1, the length of time spent in Zone 1, minimum distance approach to the active speaker, swimming speed, swimming path and mean path angle. Females were only exposed to one treatment sound per day.

# Statistical analysis

Logistic regression (SPSS Statistics 17.0, Chicago, IL, USA) was used to analyse the percent response rate of females to the different treatments. A positive response occurred when a goby swam into Zone 1. Repeated measures ANOVA was used to analyse all other tests as all females were exposed to each sound type. All gobies who were exposed to all five treatments (n=12) were used in repeated measures ANOVA analyses to quantify time spent in Zone 1, minimum distance approached to sound source, swimming speed, swimming path (distance ratio) and mean path angle. If a female did not elicit a positive response during the percent response rate analysis, a value of zero was given for time spent in Zone 1. Swimming speed, swimming path and angle were first determined using Fish Tracker software (obtained from Dr. Lynda Corkum and developed by Pauline Shen, University of Windsor), which monitored the movement of the females during the trials. The distance ratio, an estimate of directionality, (Speares, 2007) was calculated as follows:

Distance Ratio = <u>potential distance travelled – distance from speaker</u> total distance travelled Where potential distance travelled is the distance from the starting line to the speaker (144.78 cm), distance from speaker is the distance the fish stopped in front of the speaker, and total distance travelled is the sum of all swimming during the trial, as calculated in Fish Tracker, until the fish stopped at the speaker of choice. Calculation of the mean path angle accompanied the distance ratio values; a path angle equalling 0° represented a perfect linear line and an angle of 90° denoted no movement towards the signal source. *Male vocalisations to playback* 

# Recordings

To ascertain the effect of different sound presentations on goby vocalisations and the effect of body size on temporal structures, playback experiments were conducted on a series of male gobies in the lab. Males ranged in size from 14.9 g to 42.1 g. Experiments took place in a glass tank (38 X 85 cm) filled to a depth of 27 cm; temperature was  $20^{\circ}$  C and water was treated as was the water in the holding tanks. An UW-30 Underwater speaker was placed in the tank to present the sounds to the male gobies. The speaker was connected to an Alesis RA 300 amplifier (Alesis Studio Electronics, Santa Monica CA, USA) which was connected to a laptop computer (Hewlit-Packard Palo Alto, CA, USA). All sounds were presented to the gobies at an output amplitude of 150 dB re  $1\mu$ Pa at the speaker.

After a 20 minute acclimation period, a male was exposed to one of three conspecific sound types (from the above study): stimulus 1 (reproductive call), stimulus 3 (potential aggressive sound), and stimulus 4 (unknown sound). Stimulus 2 was not used in the male playback study because the female choice trials were on going and the attracted nature of the reproductive vocalisation was not yet determined; as well, stimulus

1 has been used as a reproductive cue in past experiments (Rollo & Higgs, 2008). All males (n=6) were exposed to each sound type a total of three times. Trials began with the sound playing for one minute followed by 1.5 minutes of silence and finished with one minute of sound. Male responses were recorded for a total of 20 minutes at the end of the trial. This protocol was repeated an additional two times for each sound type giving a five minute rest period between each trial repeat. The order of the three sound types was chosen at random and males were only exposed to one sound per day.

## Analysis of goby calls

Vocal responses to the playbacks were recorded by a Reson TC 4032 hydrophone (Reson Inc. USA) attached to a solid state recorder (model PMD670, Marantz Inc.).

Background noise was removed after recording with the noise reduction setting in Adobe Audition (Adobe Systems Inc. USA). A 1 s sample of the recording before sound was played was used to capture the noise reduction profile and then applied to the entire recording to filter this "noise" from the recording with 4096 points in the FFT profile.

Four sound structures were assessed: number of calls per trial, call duration, intercall interval, and fundamental frequency. For this experiment, a call was categorized as any pulse (sound) separated from the previous or next pulse (sound) by one second or more.

A pulse was characterized as any sound (peak) equal to or above an arbitrary amplitude of -30 dB on Adobe Audition. An intercall interval is the silent time or off time between each call which must be a length of one second or greater. Interpulse interval is the time between each pulse; this is measured from the last peak at -30 dB or above to the next pulse at -30 dB. The fundamental frequency is the first frequency with the most energy

(usually the first dominant frequency after 60 Hz [electrical noise]) produced for a given call.

## Statistical analysis

All body size and call structure analyses were done using Linear Regression (SPSS Inc.). Separate regressions were run to quantify if body size affected fundamental frequency, call duration, and number of calls produced by males when exposed to each of the sound types. Repeated measures ANOVA (SPSS Inc.) were run to quantify differences of each sound structure with respect to stimuli 1, 3, and 4. Missing values were generated by the missing values application in SPSS.

#### Acoustic trap

# Design and set up

The potential use of acoustic cues to control behaviours in the round goby was also assessed. A black, plastic bucket (30.5 x 34.3 x 25.4 cm) was converted into an acoustic trap (Figure 3). There were two circular openings (4.5 cm diameter) on each of the four sides made from PVC piping; holes were fit with elbow shape PVC pipe (2.54 cm diameter) that pointed up inside the trap. The outer openings were close to the bottom of the trap for ease of entrance and the interior holes were 5 cm above the bottom (Figure 3). This made it more challenging for the gobies to exit once inside because, as benthic dwelling fish, they would swim to the bottom of the trap. An UW-30 underwater acoustic speaker rested on three rods 17.78 cm above the bottom (Figure 3). The top of the trap had a clear plexiglass window to make the inside visible during trials. The speaker cable exited through a small hole in the lid and attached to a portable Phase Linear car stereo

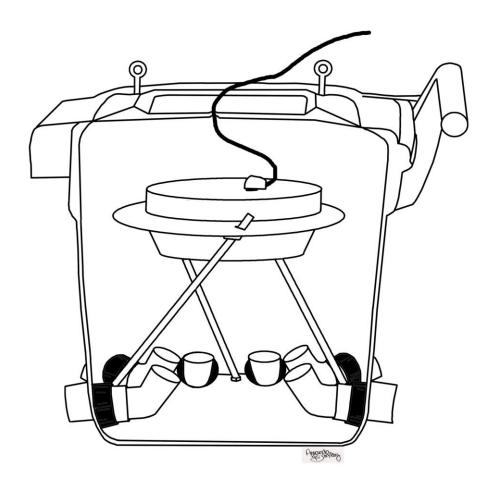


Figure 3: Schematic diagram of the acoustic trap showing the exterior and interior workings. Drawing was produced by Amanda Barkley (University of Windsor).

amplifier. A MotoMaster Eliminator car battery powered the amplifier and a Durabrand CD player, attached to the amplifier, played the sound files.

## Experimental procedures

Laboratory studies took place during the summers of 2006-2008 and were run in the behaviour tank described above. Stimulus 2 was used as the acoustic cue as females showed the highest phonotactic response to this sound over all other conspecific sounds (Experiment 1). Background noise was measured using a hydrophone (model 902, Inter Ocean Systems, San Diego, CA, USA) and sound was played 10dB above ambient noise (118-123 Hz) to ensure fish could clearly hear the vocalisations.

Trap trials mimicked the same protocol set up for all sound equipment as in the female choice trials described above. Each trial contained ten gobies. Gobies were held in a plastic holding tank, containing the same water as in the experimental tank, for a two hour acclimation period prior to the trials. After the acclimation time, the gobies were moved into the experimental tank (Figure 4) for an additional five minutes and kept behind a plexiglass divider to keep them separate from the trap before the start of the trial. Just before the sound started playing, the divider was removed. All trials were 10 minutes in length with the sound alternating one minute on followed by one minute of silence. No sound was present for silent treatments and lasted 10 minutes as in the other sound treatment trials. During this time, a Sony digital Handycam Recorder (model DCR-TRV27) placed over the tank recorded the trials for further analysis. A plastic circle (71.12 cm diameter) was placed around the trap to indicate a positive zone which was used to count the number of approaches to the trap.

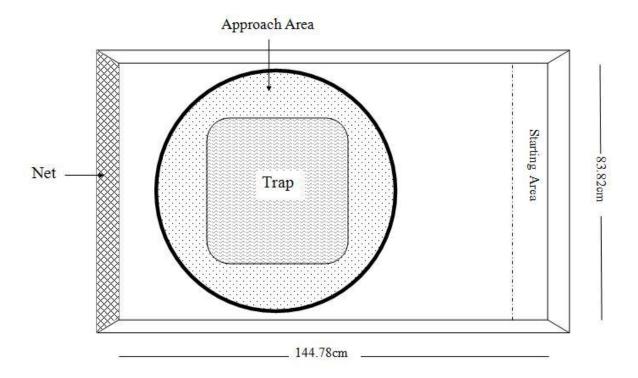


Figure 4: Experimental tank for lab acoustic trap trials. Dash line denotes plexiglass barrier which kept gobies away from trap while acclimating in the tank; it was removed at the start of a trial. Black circle denotes the approach area around trap. Gobies were free to swim in and out of the approach area at will for the length of the 10 minute trial. A video recorder was suspended above the tank to monitor all trials.

## Statistical analysis

Videos of all trials were reviewed; the number of gobies that entered the trap and the total number of approaches made by the gobies to the trap was quantified for percent response. An approach was counted when a goby crossed into a designated circle surrounding the trap. If a goby left the defined area, any re-entry was counted as a separate approach. The number trapped, time at first approach, number of approaches at five and ten minutes and total number of approaches were recorded and analysed using an independent t-test (SPSS Inc.).

#### Results

# Female choice experiment

Overall when exposed to stimulus 2, females entered the positive zone significantly more often than during any other conspecific sound or silent trial (p = 0.045, Logistic Regression) (Table 1). Stimulus 2 elicited a 92% response rate while only 50% responded to the control (Figure 5). Stimuli 1 and 3 also showed similar patterns to stimulus 2, with both having a response rate of 75%; however, neither showed a significance difference from silent trials (p = 0.213). The Stimulus 4 sound showed no difference from control trials (p = 0.410) and attained a 67% response rate.

Females spent the most time in Zone 1 when exposed to the stimulus 3, with an average of 219.08 seconds in the positive area compared to an average of 95.42 seconds during control trials (F = 5.587, p = 0.038; repeated measures ANOVA) (Figure 6). Stimulus 2 elicited the second longest response with an average of 160.75 seconds and was significantly different from the control (F = 4.750, p = 0.05; repeated measures ANOVA). Stimuli 1 and 4 did not show any difference from the control trials (F = 0.551,

Table 1: Logistical regression comparing the response rate of females during sound trials to the response rate of control trials. Positive responses are calculated by the percentage of trials where the female crossed into Zone 1 (see Figure 2 for tank diagram). B-b coefficient, S.E – standard error; df - degrees of freedom; Exp(B) – odds ratio

		В	S.E.	df	Sig.	Exp(B)
Step 1 <sup>a</sup>	Treatment			4	.321	
	Stimulus 1	.693	.842	1	.410	2.000
	Stimulus 2	2.398	1.193	1	.045	11.000
	Stimulus 3	1.099	.882	1	.213	3.000
	Stimulus 4	1.099	.882	1	.213	3.000
	Constant	1.058	.328	1	.001	2.880

a. Variable(s) entered on step 1: Treatment.

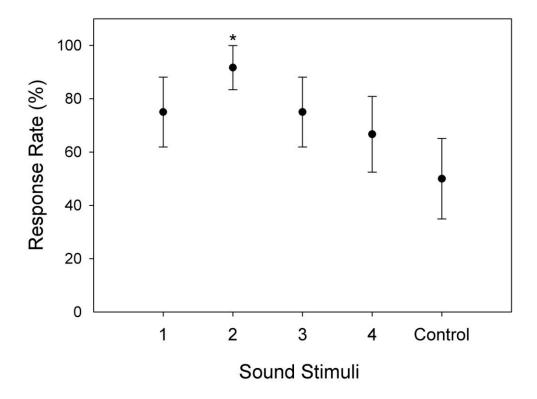


Figure 5: Mean ( $\pm$  S.E.) percent response rate of female round gobies to four conspecific sounds and control trials. Response rate is calculated by the percentage of trials where females entered the positive area, denoted Zone 1 (see Figure 2 for tank diagram). An \* indicates significance from control.

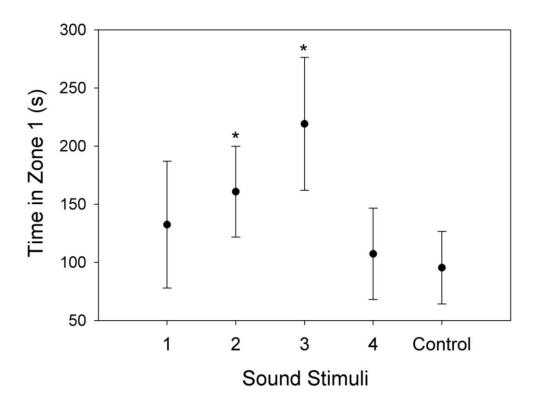


Figure 6: Mean (±S.E.) total time (s) spent by female round gobies in Zone 1 per sound type. Total time calculated over a 10 minute trial. Stimuli 2 and 3 affected the duration in Zone 1. An \* indicates significance from control.

p = 0.473; F = 0.062, p = 0.808, respectively; repeated measures ANOVA) with an average time 137.42 and 107.33 seconds, respectively, in the positive zone.

Females swam significantly closer to the sound source during exposure to stimulus 2 when compared to control trials (F = 7.899, p = 0.017, repeated measures ANOVA); stimuli 1, 3, and 4 did not display this trend (p > 0.05, repeated measures ANOVA) (Figure 7). On average, when hearing stimulus 2, the gobies swam closest to the speaker, with a distance of 14.0 cm away from the sound source. Stimuli 1 and 3 elicited similar responses with a distance of 49.8 cm and 42.9 cm, respectively. Females stayed further away from the speaker when hearing stimulus 4, only approaching the speakers a distance of 67.8 cm and stayed furthest from the sound source during silent trials, with an average distance of 70.3 cm from the speakers.

Distance ratio showed gobies swam in a more straight line towards the sound source upon hearing stimuli 2 or 3 when compared to silent trials (F = 13.160, p = 0.004; F = 5.322, p = 0.042, respectively; repeated measures ANOVA) (Figure 8). Stimulus 2 elicited the largest distance ratio at 0.82 and stimulus 3 attained 0.67. Stimulus 1 was not significantly different from the control trials at  $\alpha$ =0.05 level (F = 3.318, p = 0.096); however, stimulus 1 elicited a distance ratio of 0.71. Stimulus 4 showed no difference from the control (F = 0.566, p = 0.468) with a distance ratio of 0.51. Silent trials attained the lowest distance ratio at 0.38 showing the most zigzag pattern while swimming. Stimuli 1 and 2 were significantly different from the control trials (F = 5.968, P = 0.033; F = 17.811, P = 0.001, respectively, repeated measures ANOVA) in terms of mean path angle (Figure 9). Stimulus 2 also elicited the smallest mean path angle at 18.50° and was followed by stimulus 1 with an angle of 20.96°. Stimulus 3 elicited a mean path angle of

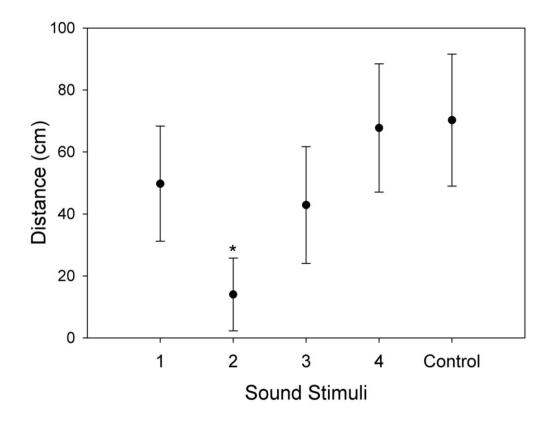


Figure 7: Mean (±S.E.) distance (cm) of closest approach of female round gobies to the speaker during a 10 minute trial when exposed to each of the five treatments (Stimulus 1, Stimulus 2, Stimulus 3, Stimulus 4, and Control). A distance of zero indicates that the goby swam under the speaker. Approach to speaker was affected by stimulus 2. An \* indicates significance from control.

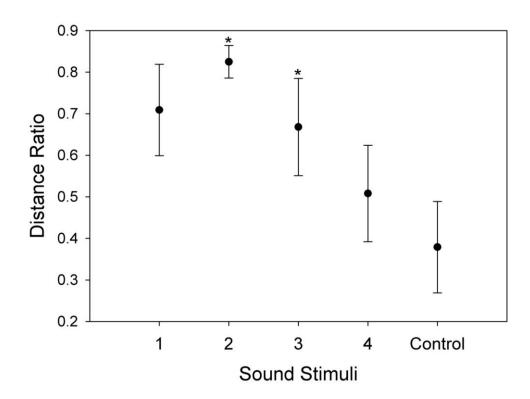


Figure 8: Mean (±S.E.) distance ratio displayed by female round gobies when approaching the speaker during sound and control trials. Paths were tracked by monitoring software from beginning of the trial until the goby reached the speaker or the trial ended. Stimuli 2 and 3 were significantly different from control trials. An \* indicates significance from control.

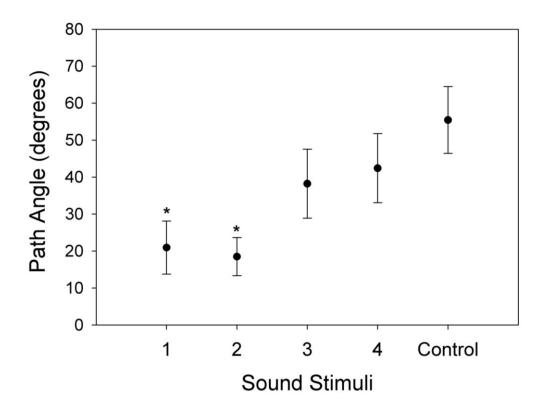


Figure 9: Mean (±S.E.) path angle (degrees) of first approach of female round gobies to the speaker during sound and control trials. Paths analyzed for angle direction were the same as those used in distance ratio calculations (Figure 12). Stimuli 1 and 2 were significantly different from control trials. An \* indicates significance from control.

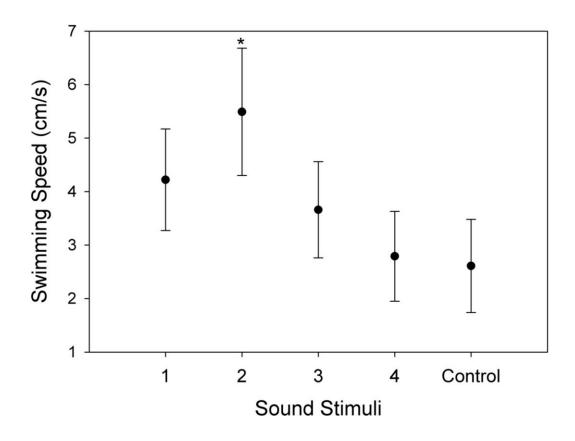


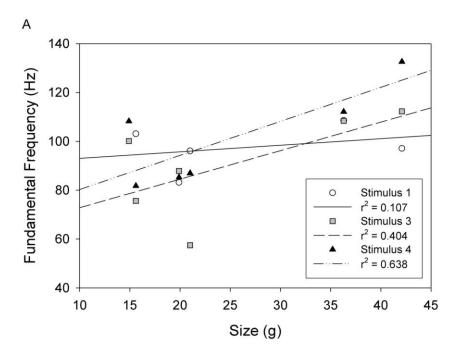
Figure 10: Mean (±S.E.) swimming speed (cm/s) of female round gobies when approaching the speaker during sound and control trials. Stimulus 2 did increase swimming speed as compared to the control trials. An \* indicates significantly different from control.

 $38.23^{\circ}$  but did not differ significantly from silent trials (F = 2.397, p = 0.150). When exposed to the stimulus 4 sound, mean path angles did not differ at all from the silent trials (F = 0.636, p = 0.442). Stimulus 4 elicited a mean path angle of  $42.43^{\circ}$  and control trials produced the highest mean path angle at  $55.47^{\circ}$  when swimming towards a silent sound source.

During the sound trials, stimulus 2 was the only sound type which affected swimming speed. Stimulus 2 showed the highest mean swimming speed at 5.61 cm/s and was significantly different from the control (F = 4.633, p = 0.05, repeated measures ANOVA) (Figure 10). All other sound types did not show a significant difference when compared to the control trials (p > 0.05, repeated measures ANOVA). Stimulus 1 elicited an average speed of 4.22 cm/s and stimulus 3 attained 3.66 cm/s as an average swimming rate. Gobies swam an average speed of 2.79 cm/s during stimulus 4 trials and silent trials produced an average speed of 2.61 cm/s.

## Male playback experiments

All males produced acoustic emissions during all playback trials. Fundamental frequency of emitted sounds did not show a significant correlation with body size for any of the sound types presented (p > 0.05, Regression) (Figure 11A). Stimulus 4 showed a positive trend between fundamental frequency and body size (p = 0.057) but was not statistically significant. When collapsed across body size, presentation of different call types had no effect on the average fundamental frequency of sound emissions (p > 0.05, repeated measures ANOVA) (Figure 11B). The stimulus 4 sound playback yielded the



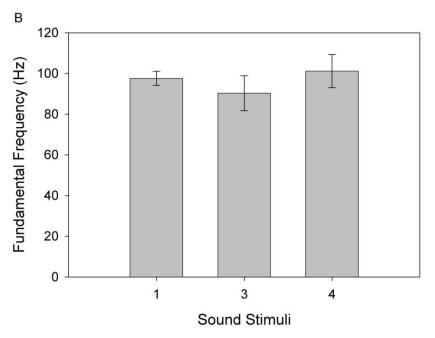
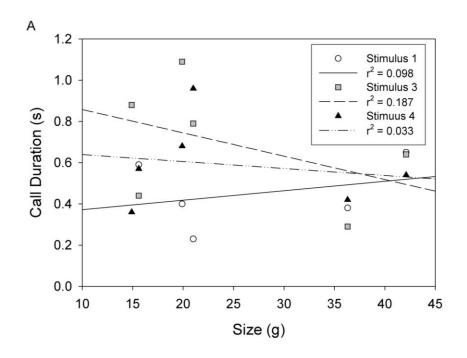


Figure 11: A) Mean fundamental frequency (Hz) as a function of size of emitted sounds from male round gobies [Stimulus 1-n=5, ∘; Stimulus 3-n=6, □; Stimulus 4-n=6, ▲]. B) Mean (±S.E.) fundamental frequency (Hz) of emitted sounds of male gobies, collapsed across size, when exposed to three call types used in the current study. Stimulus 1 had n=5 because of death as stated in text.



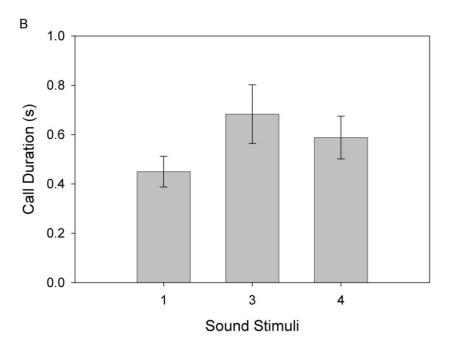


Figure 12: A) Mean call duration (s) as a function of size of emitted sounds from male round gobies [Stimulus 1-n=5, ∘; Stimulus 3-n=6, □; Stimulus 4-n=6, ▲]. B) Mean (±S.E.) call duration (s) of emitted sounds of male gobies, collapsed across size, when exposed to three call types. See Fig.11 for n values.

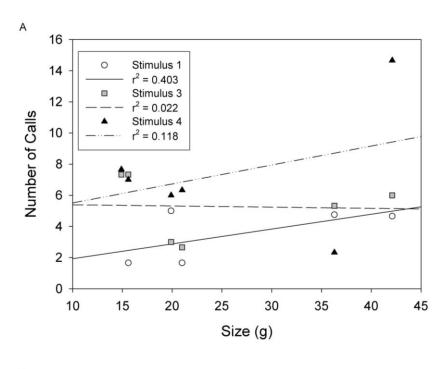
highest frequency at 101.2 Hz, followed by stimulus 1 at 97.6 Hz and stimulus 3 at 90.3 Hz.

The duration of calls produced during playback experiments did not differ significantly as a result of male size (p > 0.05, Regression) (Figure 12A). Stimuli 3 and 4 showed a negative relationship between the length of a call and the increase in size; while stimulus 1 showed a positive relationship between these variables. No significant difference was found between presented sound types (Stimuli 1, 3, and 4) with respect to call duration (p > 0.05, repeated measures ANOVA) (Figure 12B). Overall, stimulus 3 elicited the longest calls averaging 0.68 s, followed by stimulus 4 at 0.59 s and stimulus 1 with 0.45 s in duration.

Body size did not significantly affect the number of calls produced during playback experiments (p > 0.05, Regression) (Figure 13A). Stimuli 1 and 4 showed a positive relationship between male size and number of calls, with stimulus 3 showing only a slight negative relationship. Sound type showed trends of having an effect on the number of vocal emissions (Figure 13B); Stimulus 4 elicited an average of 7.3 calls during playback trials yet stimulus 1 only elicited an average of 3.5 calls (p = 0.078, repeated measure ANOVA). Stimulus 3 did not differ in vocal emissions when compared to stimuli 1 or 4, with an average production of 5.3 calls during a trial.

## Acoustic trap

Number of gobies which entered the trap showed no difference between sound and silent trials. When presented with an acoustic trap playing stimulus 2, gobies approached the trap more quickly than during silent trials (p = 0.021, t-test) (Figure 14).



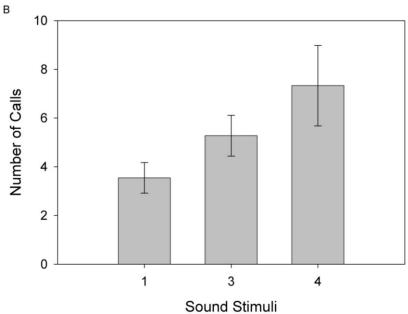


Figure 13: A) Mean number of calls produced by round goby males in relation to body size [Stimulus 1-n=5, ○; Stimulus 3-n=6, □; Stimulus 4-n=6, ▲]. B) Mean (±S.E.) of the number of emitted calls, collapsed across size, when exposed to three call types. See Fig. 11 for n values.

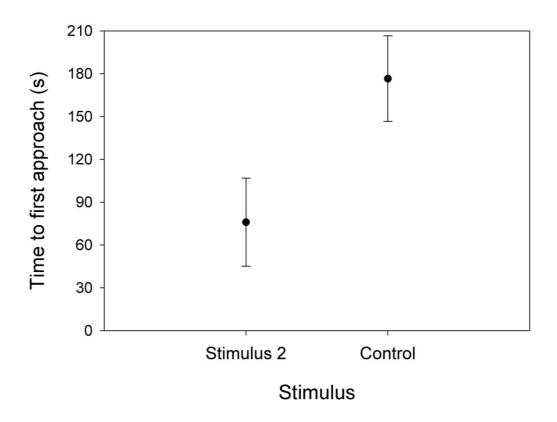


Figure 14: Mean (±S.E.) time (s) until the first approach by a round goby to the trap during 20 minute trials. Gobies approached the trap significantly more quickly during sound trials then control trials.

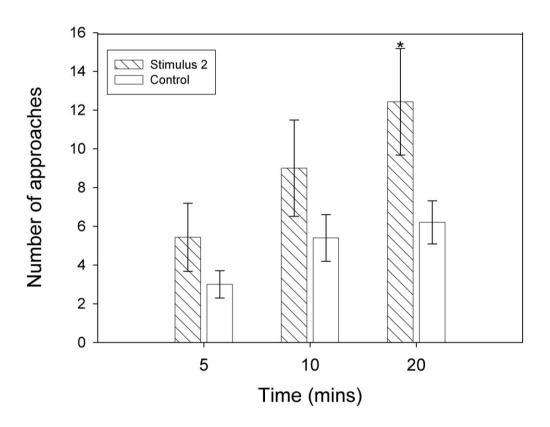


Figure 15: Mean (±S.E.) of the total number of approaches made by round gobies to the trap at 5, 10, and 20 minutes during 20 minute trials. Round gobies approached the playing trap significantly more often than the silent trap over the total 20 minute trial. An \* indicates significance from control.

On average, gobies approached the trap within 72.00 seconds from the beginning of stimulus 2 trials and 176.60 seconds during control trials.

Total number of approaches was also quantified and overall, gobies approached the playing trap significantly more often than the control trap during 20 minute trials (p = 0.049, t-test) (Figure 15). Approach number was further divided into approaches within the first five minutes and the ten minute halfway point to further quantify attraction timing. Number of approaches within the first five and ten minutes did not show any difference between playing and control trials (p = 0.147; p = 0.14, respectively; t-test); however, on average gobies approached the trap more often during stimulus 2 trials than in control trials by the end of the 20 minute trial period (Figure 15).

#### Discussion

# Female choice experiment

Overall, females had a higher response rate, spent more time in the positive zone, swam closer to the sound source, and had a more linear swimming path when exposed to stimulus 2. This suggests stimulus 2 may elicit a greater phonotactic response than all other conspecific sounds and silent trials. These results show round goby females can directionalize a sound source and demonstrate that sounds affect female behaviour.

Conspecific cues showed different levels of attraction compared to the control trials, which suggests that different vocalisations carry different communication functions.

Rollo and Higgs (2008) saw similar behavioural responses in the round goby when playing conspecifics compared to either a heterospecific tone burst or white noise, where a higher number of gobies showed a positive response when hearing a conspecific call.

Similar findings were shown in the sand goby, *Padogobius martensii*, when presented with either a tonal reproductive call, white noise, or silent treatment; female sand gobies responded significantly more often to a tonal sound over white noise or silent trials (Lugli et al., 1995). The current study shows stimuli 1 and 3 do attract female gobies at a similar rate, but possibly for different reasons. The structure of the stimulus 1 call is long and repetitive, allowing a female to localise a male's territory. The stimulus 3 sound is a single, short pulse and may be better suited to gain attention of an intruder.

The phonotactic response elicited by the stimulus 1 call, a known reproductive call (Rollo et al., 2007), was not as strong as seen in stimulus 2, suggesting certain advertisement calls are more attractive than others. There has been some discussion that individuals will respond best to sounds from within their population, rather than conspecific calls from other populations (Amorim et al., 2008b). This may be the case with our findings: stimulus 2 was recorded from a Detroit River population goby, whereas stimulus 1 was produced by a goby in Lake Michigan. Many species of fish produce more than one reproductive sound (reviewed in: Bass & McKibben, 2003; Finstad & Nordeide, 2004; Amorim & Neves, 2007, 2008; Amorim et al., 2008a,b). Male sand gobies also produce different courtship calls, a broad-band pulse sound and a drumming sound (similar to stimulus 4); the drumming sound is only produced in certain reproductive contexts, such as when a female is close to the nest (Lugli et al., 1995). Females showed more positive phonotactic responses to the drumming cues than to the silent trials (Lugli et al., 1995); however, the tonal and drumming sounds were not tested against each other. Male painted gobies also produce two different courtship sounds depending on whether the male is alone in the nest or outside courting a female (thumps)

or displaying by a female (drums) (Amorim & Neves, 2007). Drums were never produced without a female present, suggesting the drum sounds are used in face to face (short range) interactions, whereas the thump sound is produced to attract females to the nest (Amorim & Neves, 2007).

The stimulus 3 sound, in the current study, did elicit positive phonotactic responses and shows potential as an attractant sound but not to the same extent as stimulus 2. One reason is stimulus 3 may play a role in male-male interactions more so than female attraction; the sound is potentially a by-product of the caudal fin hitting the substrate during the flipping motion (Andraso et al., submitted). Females may approach the nest of territorial males but keep a distance from aggressive male-male interactions so as to not become injured. By observing aggressive exchanges between two males, females may gain information about the individuals which may have an effect on mate choice. Female cichlids (*Pundamilia nyererei*) from Lake Victoria have been shown to approach males during aggressive displays to potentially eavesdrop on agonistic communications (Verzijden et al., 2010). However, some males of other species also produce aggressive acts towards females (Crawford et al., 1986); this may explain why the females in the present study stayed further away from the sound source during the trials.

Females showed no preference towards stimulus 4, suggesting this vocalisation is not an important attractive cue. Nonetheless, the stimulus 4 sound may still have a function in reproductive vocalisations. There is increasing evidence which suggests acoustic communication has different roles in the many stages of mating besides attraction and courtship but also in pre-spawning and spawning (Lobel, 1992; Lugli et al.,

1995, 1996a). Male gobies have been shown to change acoustic cues in reference to a female's position in the territory (Lindström & Lugli, 2000). Stimulus 4 may be produced in the last stages of courtship when a female is in front of a male's nest; furthermore, visual cues may accompany this sound for proper information transfer as the male was seen moving his head up and down and swimming in and out of his nest quickly during recording (pers. observation). *Padogobius martensii* produces both acoustic and visual modalities when a female is in his nest; common visual cues include head raising and covering the opercula of the mate (Lugli et al., 1995). The less frequent drum sounds by the male painted goby were only produced when a male was outside his nest displaying erect fins and making a quivering motion towards the female (Amorim & Neves, 2007), suggesting the drum sounds accompany select visual cues. These examples suggest that some species of gobiids do use multi-modal signalling in courtship communication. If stimulus 4 is part of a multimodal signal, playback trials would have to accompany both visual and acoustic parameters to assess the attractiveness of the sound.

## Male playback experiments

Fundamental frequency of recorded sounds was not a strong indicator of body size in round gobies. Male sound emissions showed trends of a positive relationship between body size and fundamental frequency, which is contrary to most findings in the literature where fundamental frequency shows a negative correlation with body size (Crawford et al., 1997; Bass & McKibben, 2003; Malavasi et al., 2003; de Jong et al., 2007; Amorin et al., 2008b). Frequency has been correlated with body size in many species such as gouramis (Ladich et al., 1992), mormyrids (Crawford et al., 1997) and

weakfish (Cynoscion regalis) (Connaughton et al., 2000). In the damselfish (Dascyllus albisella), size effect on frequency did not differ between species but did for individuals (Mann & Lobel, 1998), suggesting females could assess the size of males through acoustic communication. Female damselfish prefer low frequency acoustic cues over high frequency ones during playback studies, and low frequency emissions were associated with increased courtship rates in males (Maruska et al., 2007). Myrberg et al. (1986) showed a negative relationship between the peak frequency of a sound and body size in damselfish (Eupomacentrus partitus). As well, sound peak frequency was found to be negatively correlated with male body size in five species of cichlids (Pseudotropheus spp.) (Simões et al., 2008). All of these examples suggest frequency may be an accurate predictor in individual recognition and females may be able to assess a male's body size by the frequency of his calls in some species. That this was not found in the round goby may indicate that fundamental frequency is not an honest indicator of male size in this species or that our sample size was not adequate to discern this relationship. All sound types would benefit from larger sample sizes to verify this trend in the round goby as the importance of fundamental frequency in individual recognition is found in other species (Mann & Lobel, 1998). As well, we are certain that the sounds recorded and analysed are indeed vocalisations produced by the males and not simply by-product sounds made from male movement in the tank. Sound emissions were linked to mouth movement by the males through video assessment.

Call duration and number of calls produced are among many temporal aspects discussed in the literature with respect to individual recognition (Myrberg & Riggio, 1985), signal recognition (reviewed in: Bass & McKibben, 2003), and species recognition

(Verzijden et al., 2010). In the present study, body size and sound stimuli were not effective predictors of call duration in the round goby. This suggests call duration may not be an important temporal structure for individual or signal recognition in the round goby. Larger males, in theory, would produce longer calls as body size is an indicator of fitness (Lindström & Lugli, 2000). Similar to our findings, Malavasi et al. (2003) did not find a correlation between body size and call duration in male grass gobies; call duration did not differ between larger parental males and alternative tactic, smaller sneaker males. Lindström and Lugli (2000) suggest call duration is associated with the cost of sound production and, in turn, the physiological constraints of the task; male sand gobies were reported to increase the interpulse interval (or 'off-time') and shorten burst lengths to rest fatigued muscles. Manipulation in burst length suggests some variation in sound duration is common, and as a result may not be a good indicator for individual or signal recognition (Lindström & Lugli, 2000). Conversely, call duration may be an important characteristic in species recognition. Mean body size was an effective predictor of sound duration between different species of Mediterranean gobies (Malavasi et al., 2008). Morphological constraints on sound production mechanisms may affect call duration more so than that of size variation within a species. Size variation within a species may be too small to show significant effect on sound duration, whereas size variation between species is more vast and can thus be used to distinguish heterospecifics.

Call production rate in round gobies may aid females in locating a male's nest rather than identifying a particular individual. Number of sound emissions by other gobiid males has been shown to correlate with his location in his territory (Lugli et al., 1995); the common goby and panzarolo goby (*Knipowitschia punctatissima*) produced

the highest number of calls when in their nest. This may guide a female to the nest location within the territory. We did not see this trend, however, as the males were alone in the tanks during playback trials. Sound production rate may also affect mating success; male painted gobies increased their courtship rate as they increased the frequency of acoustic signalling (Amorim & Neves, 2007). In addition, males who produce more calls may signal a higher level of fitness as sound production is costly; females may choose males with greater courtship intensity which is an indicator of higher genetic quality and parental care capabilities (Malavasi et al., 2003; Amorim & Neves, 2007).

There are many advantageous reasons for a male to listen, respond (call back), or approach another signalling conspecific male; information gained may indicate location of nearby breeding sites and food sources, and may aid in assessing the qualities of neighbouring males (Bass & McKibben, 2003). Sneaker males benefit from eavesdropping on territorial, parental males to gain access to spawning females (Amorim et al., 2003). Males can gather information about the fighting ability of aggressors before a fight escalates (Ladich, 1997; Amorim & Neves, 2008). Fighting is costly; therefore, males should only engage in aggressive acts if there is something to gain, such as a mate, nest site, or food resources (Ladich, 1997). Mozambique tilapia (Oreochromis mossambicus) males will emit agonistic sounds to retain their social status and dominance (Amorim et al., 2003); this suggests males should respond to other male vocalisations to possibly remind nearby males of their presence and status. Certain call characteristics may send honest signals, as frequency has been correlated with body size in some species (reviewed in: Ladich, 1997). Sound pressure level and production rate may, however, be better indicators of fighting ability over frequency: Ladich (1997)

reports smaller males that produce more intense sounds often 'win' against larger males, even though body size would presume otherwise, especially if there are no visual cues present. In the current study, male gobies emitted sounds continuously during all playback trials and no body size correlation was found, suggesting round goby call rate may correlate with wiliness to defend a territory.

### Acoustic trap

Although no significant difference was found between number of gobies which entered the trap during sound and silent trials, the acoustic trapping device developed here did show a significant increase in the number of approaches made by the gobies over silent trails. This suggests that the use of acoustic cues alone shows promise in development of a selective trapping device. Male-male fighting and the flip display were also observed during a few trials where the sound was playing (pers. observation); however, no recording device was present to confirm the possible sound associated with the flipping motion.

Preliminary field trials showed similar success as lab trials with limited occurrences of gobies entering the trap but a potential different between the number of gobies attracted to the trap during sound trials compared to silent trials. One reason may be the effects of different environments on the attraction of the stimuli. Popper and Schilt (2008) suggest that results obtained at one site may not hold true against different backgrounds; therefore, transmission properties of sound frequency are dependent on the environment (Rogers & Cox, 1998). Round gobies are primarily found in shallow waters, an environment which greatly affects sound transmission. Replicating natural

environments in a laboratory setting can prove to be difficult; thus, the roles of sound transmission and dynamics in the natural environment must be taken into account when performing laboratory experiments. Further lab trials may be improved with the addition of a rocky substrate and other shelters to be more comparable with their natural habitat. Moreover, this would further eliminate any question that the gobies were attracted to the trap because it was the only form of shelter available.

Sensory modalities, such as vision, olfaction, acoustics, electroreception, and pressure, have been shown to modify fish behaviour (Popper & Carlson, 1998). However, not all fish can detect all modalities and the range of detection for particular stimuli is often species specific (Popper & Carlson, 1998; Bullen & Carlson, 2003). Thus far, light has been the main modality tested for use in manipulating fish behaviour. Doherty (1987) used light traps to assess the abundance of larval fish. The use of sound, on the other hand, allows the manipulation and control of fish behaviour from further distances than do light traps.

Sound as a tool to control or manipulate the behaviour of fish has had limited success to date. Primary areas of study have concentrated on industrial projects such as dams, locks, water intake areas, and hydrodynamic flows (Popper & Schilt, 2008). Before acoustics can be used in behaviour control programs, a thorough understanding of the behavioural responses in the target species is needed. Acoustic cues, primarily high-frequency sounds, have shown some success and have been tested to manipulate and reroute swimming paths of fish away from hydropower plant turbines (Nestler et al., 1992; reviewed in: Popper & Carlson, 1998). Nestler et al. (1992) tested high-frequency sound on behaviour responses in blueback herrings (*Alosa aestivalis*) and found high-

frequency sounds deterred the herrings from entering turbine areas. Devices which emit high-frequency sound could be used as non-physical barriers to stop or impede certain species from entering into non native streams. The behavioural responses of fish to sound must be well understood before any acoustic devices can be used to control fish behaviour. If sound can repel fish from certain potentially dangerous areas like turbine intake bays, then can sound also be used to draw fish into a particular area? We know female round gobies show positive phonotactic responses to male courtship calls (Rollo et al., 2007; Rollo & Higgs, 2008); we may be able to manipulate this behaviour to attract and trap round gobies. The main purpose of trapping this highly invasive species is to reduce the population numbers and potentially decrease the chance of gobies spreading into new habitats. As well, non-physical barriers are essential to not disturb native species and the natural habitat.

#### **Conclusions**

Sound is an important modality in the goby communication system in their social communities. Sound has been shown to play an important role in their mating strategies, from advertisement and courtship calls, to possibly pre-spawning courtship calls. Males are extremely territorial and sound production rate may be an indicator of their fighting ability, as it indicates their motivation to defend their territories and nests. With additional playback experiments, we can better understand the temporal characteristics that are most important in their call structure and use this to further develop an acoustic control program.

Round gobies are able to distinguish between conspecific and heterospecific calls (Rollo & Higgs, 2008). Female round gobies do show preference to certain male calls over others (Rollo et al., 2007); this suggests that information encoded within the calls may indicate individual characteristics of sender. The round goby may have different variations of the reproductive vocalisations and these variations may affect the level of attraction. Here, we have potential found an aggressive vocalisation produced by males as males responded more intensely to stimulus 4 then females. To further investigate these findings, stimuli 1 and 2 could be tested against each other to explore how the degree of variation within reproductive calls affects the intensity of female attraction. Stimulus 4 should be presented to females with visual cues, as was seen during recording and further tested with males in a resident-intruder type study.

As fundamental frequency was not a good indicator of body size in the round goby, dominant frequency of emitted sounds may be a better indicator of body size as this has been tested in other gobiids. Call production rate may be an effective predictor of signal recognition in the round goby, as different call types show trends of eliciting longer call rates than others. Other temporal structures to investigate would include sound pressure level and interpulse interval. Both of these have been shown to correlate with production rate, and as such they may be better indicators of fitness.

Since round gobies live in dense colonies, it is important for females to be able to locate preferred males. Only a few fish species have been shown to distinguish individuals; however, this ability is common in other taxa, such as mammals, birds, and

insects. As such, further investigations regarding individual recognition in fish are warranted.

The above studies demonstrate the importance of acoustic communication in the social communities of the round goby. Females showed a higher preference towards certain male calls over others by the degree of phonotaxis elicited by each vocalisation. Males produced sound when presented with conspecific emissions. All of these behaviours suggest that round gobies will change their behaviour depending on the acoustic signal presented. These results do suggest different call types possess different information which gobies can interpret. Therefore, sound as a modality to control their behaviour should be further investigated to be incorporated into a trapping device.

In recent years, round gobies have had a serious effect on native fish populations in the Great Lakes. This has impacted several aspects of this resource, such as fisheries, recreational fishing, and the overall ecosystem of the habitat. Control programs are needed to decrease the threat of this invasive species entering new waterways. Here, we have shown the use of acoustics may be advantageous when designing a trapping device, as round gobies approach an acoustic trap more often than a silent trap. Acoustics are an important modality to manipulate goby behaviour, as sound transmits well in turbid, low-light environments. Acoustic traps are also a good form of non-physical barriers and are species specific.

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