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The roles of individual variation and female choice in round goby (*Neogobius melanostomus*) acoustic signalling

by
Meghan Donovan

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

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The roles of individual variation and female choice in round goby (*Neogobius melanostomus*) acoustic signalling

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August 17, 2015

DECLARATION OF ORIGINALITY

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ABSTRACT

Acoustic signalling in teleost fishes serves a variety of communicatory purposes, mostly centered around aggression and courtship. There is strong evidence that courtship calls are used by many fishes to locate mates, coordinate spawning, and for species recognition, but the possibility that these calls also act as honest signals is not yet highly explored. Calls produced by the male round goby, *Neogobius melanostomus*, were analyzed for dominant frequency, interpulse interval, duration and number of pulses in the call. Call characteristics were then analyzed for relationships to body morphometrics of total length, head width, total weight, and gonadosomatic index. Strong interactive relationships between male body traits and individual call characteristics were found. Females were shown to have a preference for longer interpulse interval, suggesting that calls are capable of honestly signalling male body size. These findings suggest that acoustic honest signalling as well as individual discrimination exists in this species.

DEDICATION

I dedicate this thesis to my family and friends for all of their love, support and understanding.

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Chapter I

REVIEW ON SEXUALLY SELECTIVE BIOACOUSTICS IN THE GOBIIDAE

Thesis Objectives

This thesis aims to determine if acoustic signalling in the round goby functions as an honest signal. For this purpose, I define honest signalling as a communicatory signal where some measurable aspect of male condition is related to the signals reliability and where females are able to accurately judge and have a preference for signal variability. The first portion of the study, presented in Chapter 2, examines the correlation between the male body characteristics of head width, total length, total weight and gonadosomatic index and the call characteristics of call duration, dominant frequency and average interpulse interval. If any of these call characteristics are strongly correlated to male body size, round goby courtship calls may act as reliable predictors of male body size. The second portion of this thesis, Chapter 3, focuses on female call preference, to determine if the relationship present between male body measures and call characteristics is a true honest signal. Confirming female preference is critical to defining these courtship calls as honest signals.

Sensory Systems in an Aquatic Environment

The underwater environment is a complex system, which heavily influences signal transmission and detection, therefore, communicating with conspecifics poses a challenge for inhabitants in such environments. One of the barriers that must be overcome is the challenge of communicating with conspecifics. To do this, many species will combine several types of sensory displays simultaneously (Saunders et al., 2010; Smith and van

Staaen, 2009; Thaker et al., 2006). While multimodal signals can be perceived as being redundant, the transference of the same information through several sensory systems assures that the receiver will perceive the sender's communication accurately (Partan and Marler, 2005). Redundant multimodal signals become increasingly important in habitats where the information communicated might be misinterpreted. For instance, visual displays are often limited to very short distances due to poor visibility, olfaction is complicated by highly variable water flow and auditory cues can be masked by the surrounding biotic and abiotic acoustic environment. Of these three common underwater signalling modalities, bioacoustic signals can often be transmitted the furthest, increasing the chances that the sender's information will reach a desired target. In many species, these acoustic displays are low in frequency (Ladich, 2014), allowing the sound to travel a great distance without attenuating in ideal conditions but causing propagation difficulties in shallow water (Rogers and Cox, 1988). Communicating with low frequency sounds can also create issues with sound reception and localization as the wavelength of the sound is larger than the distance between the ears of the fish (Popper and Fay, 1973) and yet the majority of fish communication calls are in the low frequency range (Ladich, 2014).

Underwater Sound Propagation

The basic physics of how sound travels underwater are important to understanding the reception and production of sound in the aquatic environment. Many differences exist between sound transmission through air compared to water. The substantial increase in speed from 332 m/s in air to 1484 m/s in water greatly increases the wavelengths of sounds underwater. Attenuation is the loss of energy as the sound propagates due to

effects like absorption and scattering. Low frequencies can travel great distances with very little attenuation in an unbounded medium, however higher frequency sounds tend to travel only very short distances. Absorption of sound also increases with increasing water temperature and salinity. This lack of attenuation means that the background noise caused by biotic and abiotic factors is immense, increasing the overall noise levels by up to 30 dB (Rogers and Cox, 1988). This increase in sound intensity becomes incredibly important when considering that communicatory sounds will potentially be masked, preventing the fish from hearing the sound of interest (Fay and Popper, 2012). As sound passes across a thermocline or halocline, the speed at which the sound wave is propagating will change, causing refraction of the sound wave. Similarly, when a sound wave encounters the surface it will both refract and reflect the sound. The substrate that the sound wave encounters will also affect how it travels; harder surfaces will reflect sound better than soft surfaces (Nedelec et al., 2015). As the angle of incidence increases, more sound will be reflected than refracted, until refraction becomes undetectable. This effect becomes important in shallow water where much of the energy of the sound wave is refracted instead of traveling to the potential receiver and can greatly complicate the use of sound as a communication signal in shallow waters.

Uses of Sound in Fish

Several types of calls are produced by fishes, most notably alarm, agonistic and courtship calls (Smith, 1992; Bass and McKibben, 2003; Van Staaden and Smith, 2011). Typically, fish produce alarm calls when being handled or when the fish encounters a predator (Smith, 1992). Alarm calls are those that elicit a response by conspecifics, while distress calls are meant to dissuade the predator from attacking (Smith, 1992). The

Atlantic cod (*Gadus morhua*) will produce grunting calls when chased by a novel object or by a predator and will continue to grunt when cornered and immobile (Brawn, 1961). In the cod, the cues may be used as a warning to nearby conspecifics or as a means of attracting a secondary predator, potentially allowing escape (Smith, 1992). A more clear alarm signal exists in the longspine squirrelfish (*Holocentrus rufus*) where an encounter with a predator will cause the squirrelfish to produce a staccato call, which either elicits retreat or mobbing behaviour by nearby conspecifics (Winn et al., 1964).

Many fish species are known to produce calls under agonistic contexts, especially when competing for food or mates. Competition for food stimulates the grey gurnard, *Eutrigla gurnardus*, to produce knocking sounds when grasping food and grunting sounds when doing frontal displays to competitors (Amorim et al., 2004). Growling and other sounds produced by gurnards may act as keep-away signals to nearby competitors (Amorim and Hawkins, 2000; Amorim et al., 2004). Acoustic signalling is also often used by territory holding males in both an agonistic context as well as in courtship. Parental male plainfin midshipmen, *Porichthys notatus*, have two call types, the grunt used in agonistic displays and the hum used in attracting mates (McKibben and Bass, 1998). Hums will elicit a phonotactic response from females, but grunts and modified calls that do not closely resemble a hum receive no response from females (Bass and McKibben, 2003).

Perhaps the most well documented uses of acoustic signalling in fish are courtship calls. These calls are used by monogamous pairs (Boyle and Tricas, 2011), group spawners (Rowe and Hutchings, 2003) and territory holders when attracting mates (Amorim et al., 2011). Courtship calls are used to locate mates (McKibben and Bass, 1998), ensure correct species recognition (Rollo and Higgs, 2008) and potentially even

act as honest signals of mate quality (Amorim et al., 2013a). In species like the burbot, *Lota lota*, which spawns under the cover of sea-ice, acoustic signalling seems to be critically important to the timing of gamete release (Cott et al., 2014). Calls are likely very important for species that nest under objects where the caller may be hard to locate (Lugli et al., 1997). Many fish calls may serve multiple purposes and have simply yet to be examined.

Sound Production in Fish

There is an exceptionally diverse variety of fish that are known to produce sounds as a means of communication. Fish and Mowbray (1970) provided extensive documentation on 36 families of Osteichthyes fish with sound producing members and one species of Chondrichthyes. Several more soniferous families have been discovered since the publication of this compendium. Over 700 species of fish are now known to produce sound (Kaatz, 2002; Luczkovich et al., 2008) and there are many bioacoustic sounds attributed to fish that have yet to be identified (Anderson et al., 2008). In the cases of the families examined by Fish and Mowbray (1970), all species documented were producing agonistic calls in response to electric or mechanical stimulus. Fish will also produce these calls under agonistic and aggressive interactions, such as when a predator or other intruder has entered their territory (Ladich, 1997). Many of these sound producing families also contain species that produce courtship sounds. Courtship sounds are typically only produced by males, whereas aggressive and agonistic calls are commonly produced by both sexes (Ladich, 1997). Several species have separate calls that they produce in agonistic and courtship contexts (Table 1.1). However, the characterization of these sound types may vary between researchers, leaving the simple

Table 1.1

Table 1.1. Sound production across families. The qualitative descriptions of fish sounds across 42 families of soniferous fishes. Most calls are described similarly and many families contain species that produce distinct calls for courtship and agonistic displays.

| Family | Agonistic call | Uncharacterized sound | Courtship call | Reference |
|----------------|---------------------------|---------------------------------|---|--|
| Elopidae | thumps | | | Fish and Mowbray (1970) |
| Albulidae | thumps | | | Fish and Mowbray (1970) |
| Clupeidae | thumps and knocks | | | Fish and Mowbray (1970) |
| Arridae | sobs, yelps, grunts | | | Fish and Mowbray (1970) |
| Anguillidae | clucking and knocks | | | Fish and Mowbray (1970) |
| Gadidae | thumps | | grunts and hums | Fish and Mowbray (1970); Cott et al. (2014) |
| Syngnathidae | | snaps and clicks during feeding | | Fish and Mowbray (1970) |
| Holocentridae | grunts | | | Fish and Mowbray (1970) |
| Centropomidae | thumps and knocks | | | Fish and Mowbray (1970) |
| Serranidae | thumps | | pulses and a tonal sweep | Fish and Mowbray (1970); Lobel (1992) |
| Lutjanidae | thumps | | | Fish and Mowbray (1970) |
| Pomatomidae | thumps and knocks | | | Fish and Mowbray (1970) |
| Carangidae | thumps and knocks | | | Fish and Mowbray (1970) |
| Gerridae | weak knocks | | | Fish and Mowbray (1970) |
| Pomadasyidae | grunts | | | Fish and Mowbray (1970) |
| Sciaenidae | knocks and croaks | | drumming | Fish and Mowbray (1970); Connaughton et al. (2002) |
| Mullidae | thumps and knocks | | | Fish and Mowbray (1970) |
| Sparidae | thumps and knocks | | | Fish and Mowbray (1970) |
| Kyphosidae | thumps and knocks | | | Fish and Mowbray (1970) |
| Ephippidae | thumps, knocks and grunts | | | Fish and Mowbray (1970) |
| Chaetodontidae | thumps and knocks; pulses | | | Fish and Mowbray (1970); Boyle and Tricas (2011) |
| Pomacentridae | thumps, knocks and clicks | | clicks | Fish and Mowbray (1970); Parmentier et al. (2010) |
| Labridae | thumps and knocks | | pulses | Fish and Mowbray (1970); Boyle and Cox (2009) |
| Scaridae | clicks and knocks | | group spawning, individual sounds indiscernible | Fish and Mowbray (1970); Lobel (1992) |

| | | | | |
|-----------------|--|---|-------------------------|---|
| Acanthuridae | Knocks and grunts | | | Fish and Mowbray (1970) |
| Triglidae | growls, grunts and barks | | | Fish and Mowbray (1970); Amorim et al. (2004) |
| Cottidae | growls | | | Fish and Mowbray (1970) |
| Dactylopteridae | barks | | | Fish and Mowbray (1970) |
| Sphyaenidae | | thumps and knocks with swift movement | | Fish and Mowbray (1970) |
| Polynemidae | knocks | | | Fish and Mowbray (1970) |
| Balistidae | grunts, knocks, scrapes, thumps and clicks | | | Fish and Mowbray (1970) |
| Ostraciidae | groans, clicks and scrapes | | | Fish and Mowbray (1970) |
| Tetraodontidae | | "erks" with inflation | | Fish and Mowbray (1970) |
| Diodontidae | | whines with inflation | | Fish and Mowbray (1970) |
| Molidae | grunts | high-pitched groan when lifted from the water | | Fish and Mowbray (1970) |
| Batrachoididae | grunts | | boatwhistle | Fish and Mowbray (1970); Amorim et al. (2011) |
| Blenniidae | | | grunt | DeJong et al. (2007) |
| Cichlidae | | | pulses | Maruska et al. (2012) |
| Percidae | | | knocks, purrs and drums | Speares and Johnston (2011) |
| Mormyridae | | | grunts and moans | Crawford et al. (1997) |
| Doradidae | grunts and growls | | pulse | Kaatz and Stewart (2012) |
| Auchenipteridae | grunts and growls | | pulse | Kaatz and Stewart (2012) |

description of the sound up for debate (Parmentier et al., 2006). While the majority of fish sounds are categorized as pulsed sounds resembling grunts, thumps or knocks, there is a huge amount of variation from species to species, and in many cases calls are species specific (Luczkovich et al., 2008).

Sound Production in the Gobiidae

The ability to produce sound has been documented across many taxa of fish, with one of the most intensively studied families being the Gobiidae. As these fish lack specialized structures that aid in hearing, they can be thought of as hearing generalists (Popper and Fay, 2011); the actual amount of sound detection abilities in these fish is debated as the group is exceptionally diverse. Currently, the family contains some 1950 species across 210 genera (Nelson, 2006) of which only 23 species are documented as sound producers (Lugli et al., 1997; Parmentier et al., 2013).

Phylogenetic Links Between Sound Production

Where the Gobiidae is such an expansive and widely distributed group, the taxonomic links between members are still under some consideration. Many studies use different names for some call producing species, most notably *Padogobius martensii* and *P. bonelli*, which appear to be the same species (Nocita and Vanni, 2001). Very detailed work has been done to attempt to organize this clade using morphological and genetic analyses (Thacker and Roje, 2011). While the phylogenetics of the Gobiidae is still unclear, there appears to be no evolutionary patterns in call structure with some species using pulse trains while other use simple grunts, regardless of phylogenetic position (Figure 1.1). There are also clear differences in call structure between species, allowing

Figure 1.1

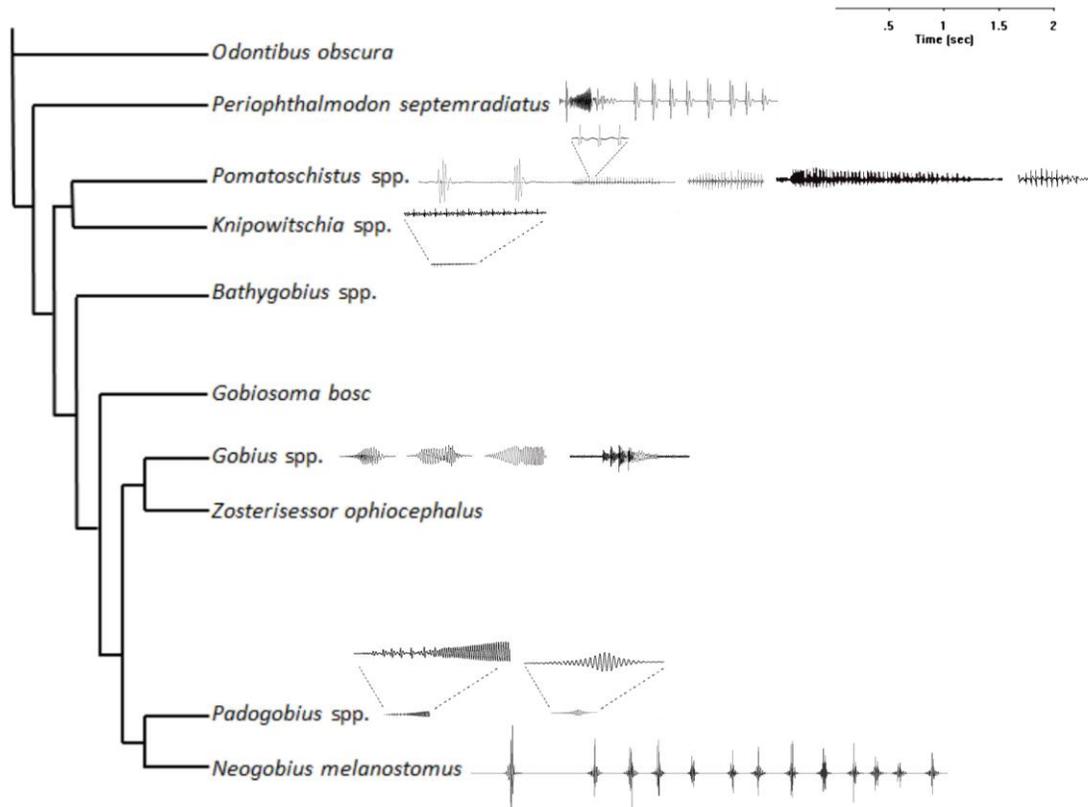


Figure 1.1. Phylogenetic tree of soniferous gobies. All oscillograms are scaled to the same time frame. Individual calls represent different species, from left to right in each genus where species are not labeled, calls belong to *Pomatoschistus*: *P. pictus*, *P. canestrinii*, *P. marmoratus*, *P. minutus*; *Knipowitschia*: *K. panizzae*; *Gobius*: *G. niger*, *G. cobitis*, *G. paganellus*, *G. cruentatus*; *Padogobius*: *P. martensii*, *P. nigricans*. This phylogeny was modified from Thacker and Roje (2011), to include soniferous species in the Gobiidae. Oscillograms were used with permission from Drs. S. Malavasi, C. Amorim, and G. Polgar.

for species recognition when examining the calls. The soniferous species in this family are widely distributed across the clade, and yet, more ancestral species like the mudskipper, *Periophthalmodon septemradiatus*, also produce sound (Polgar et al., 2011). This suggests that acoustic signalling may be far more common in this family than the few species already described.

Call structure

Several terms are used by different researchers seemingly interchangeably when referring to certain sounds, such as drums, grunts and pulses. A call is usually referred to as “drumming” when grunts are repeated in a grunt or pulse train (Lugli et al., 1997). A grunt is defined as a single, low frequency pulse and a pulse is a short, continuous sound burst. The grunt is possibly the most commonly produced call by gobiids. Grunts are generally low in frequency, typically between 100 and 200 Hz for most species (Malavasi et al., 2008). Grunts may be produced singly, in pulse trains resembling a drumming sound or as part of complex sound that includes a grunt train and a tonal sound (Lugli et al., 1995). The tonal sound is a continuous waveform with a low range of frequency modulation. It is possible that the tonal sound is produced by reducing the inter-pulse duration to the point that the pulse train becomes a single tone (Malavasi et al., 2008). Depending on the method of sound production, some species should be incapable of making tonal sounds as these sounds would likely depend on rapid stridulation, something not all species are capable of (Stadler, 2002). Similar to the pulse train is another call described as a stutter where the call is weaker than individual grunts, but otherwise similar in structure (Stadler 2002). The weakness of this call is potentially caused by the alternation of body sides when the fish is ejecting water through the gills

where single grunts would have water ejected simultaneously from both sides (Stadler, 2002). This method of calling has only been described in one species thus far, the notchtongue goby, *Bathygobius curacao*, though may be found in other species of this genus (Tavolga, 1958; Stadler, 2002). Differentiation of call components may be important for species recognition when several species of gobies live sympatrically to avoid hybridization (Blair, 1958).

Seasonality and Nesting

Sound production is generally linked to courtship behaviour in the Gobiidae and as such occurs during the spawning seasons (Table 1.2). Typically, acoustic signals are paired with visual (Lugli et al., 1995) and olfactory stimuli (Kasurak et al., 2012). Visual displays vary by species, but often the male will actively fan his pectoral fins (Meunier et al., 2013), swim in and out of the nest and perform tail beats (Lugli et al., 1995). Calls are produced solely by males (Parmentier et al., 2013) and occur during aggressive, courtship and spawning interactions (Stadler, 2002). During the spawning season, male gobies guard nests from which they produce these calls (Rollo et al., 2007). Generally, these nests are simple cavities under rocks or shells. However, this resource may be limiting, causing males to be territorial for the best nesting sites (Lugli et al., 1992). The distance between nesting freshwater goby (*Padogobius martensii*) increases with male size and when females are not a limiting factor, larger males will take over larger nesting sites (Lugli et al., 1992). When deciding on a nesting site, male sand gobies (*Pomatoschistus minutus*) preferentially choose the nest cavity that looks larger from the outside (Lindström, 1992). Larger males have a tendency to win more fights, thus gaining better

Table 1.2

Table 1.2. Reproductive seasons of soniferous gobies. The reproductive season of 17 of the 23 known soniferous gobies. The taxonomy of *Padogobius bonelli* and *Padogobius martensii* is unclear however and these may be the same species.

| Species | Seasonality | Location | Reference |
|------------------------------------|----------------|-------------------|---------------------------|
| <i>Bathygobius fuscus</i> | June-September | Indo-Pacific | Zhang and Takemura 1989 |
| <i>Gobius cobitis</i> | March-June | Venice lagoon | Malavasi et al. 2008 |
| <i>Gobius cruentatus</i> | March-May | Mediterranean Sea | Sebastianutto et al. 2008 |
| <i>Gobius niger</i> | March-June | Venice lagoon | Malavasi et al. 2008 |
| <i>Gobius paganellus</i> | March-June | Venice lagoon | Malavasi et al. 2008 |
| <i>Knipowitschia panizzae</i> | March-June | Venice lagoon | Malavasi et al. 2008 |
| <i>Knipowitschia punctatissima</i> | March-May | Italy | Lugli et al. 1997 |
| | March-June | Venice lagoon | Malavasi et al. 2008 |
| <i>Neogobius melanostomus</i> | May-August | Detroit River | MacInnis and Corkum 2000 |
| <i>Odontobutis obscura</i> | May-July | Japan | Takemura 1984 |
| <i>Padogobius bonelli</i> | March-June | Venice lagoon | Malavasi et al. 2008 |
| <i>Padogobius martensii</i> | March-May | Italy | Lugli et al. 1997 |
| <i>Padogobius nigricans</i> | March-May | Italy | Lugli et al. 1997 |
| | March-June | Venice lagoon | Malavasi et al. 2008 |
| <i>Pomatoschistus canestrinii</i> | March-June | Venice lagoon | Malavasi et al. 2008 |
| <i>Pomatoschistus marmoratus</i> | March-June | Venice lagoon | Malavasi et al. 2008 |
| <i>Pomatoschistus minutus</i> | May-August | Baltic Sea | Lindström and Lugli 2008 |
| | March-June | Venice lagoon | Malavasi et al. 2008 |
| <i>Pomatoschistus pictus</i> | January-May | Portugal | Amorim et al. 2013 |
| <i>Zosterisessor ophiocephalus</i> | March-June | Venice lagoon | Malavasi et al. 2008 |

nesting sites (Magnhagen and Kvarnemo, 1989). Larger nesting sites have a greater surface area and as such a greater number of eggs are deposited (Lugli et al., 1992). However, larger males are not necessarily preferred by females (Magnhagen and Kvarnemo, 1989). Likewise, females are not more likely to choose the winner of a fight (Forsgren, 1997). In the sand goby, preferred males receive more eggs and provide greater parental investment (Forsgren et al., 1996). These factors are somewhat confounding however, as males that provide more care to larger clutches may not inherently be better fathers (Forsgren, 1997). The importance of individual features of interest to females choosing mates is difficult to discern and may differ between species and across habitats.

Sound Producing Mechanisms

Sound production has been documented in at least 23 species in the Gobiidae (see Lugli et al., 1997 and Parmentier et al., 2013) and is likely displayed in other species as well. Several hypotheses exist on the mechanism behind sound production in this family and, due to the diversity of the group, more than one mechanism seems likely. Most recently, Parmentier et al. (2013) proposed that the levator pectoralis muscles are key to sound production, especially in species lacking swim bladders, though the exact mechanism remains unknown. Other possible mechanisms are by rubbing the pharyngeal plates together, coupled with an amplification through the swim bladder in species that possess one (Takemura, 1984; Lugli 1995) as well as squirting water through the opercular openings (Tavolga, 1958) though this latter method is debated (Stadler, 2002; Parmentier et al., 2013). Calls produced by *Odontobutis obscura* are amplified by the muscular connection between the pharyngeal teeth to the 2-4 vertebrae, which in turn are

in direct connection with the thin-walled swim bladder (Takemura, 1984). At least one vocal species lacks necessary structures for producing stridulatory calls (Stadler, 2002). However, the absence of pharyngeal teeth seems unlikely as other members of the *Bathygobius* have pharyngeal teeth (Miller and Smith, 1989). In vocal species of gobies, the large pectoral fins are often erected when fish are calling, potentially using the membrane as an amplifier for the sound (Parmentier et al., 2013) though this behaviour is usually attributed to being part of visual or olfactory displays (Meunier et al., 2013).

Gobies may use their nest cavities as a way of amplifying their calls, as nests tend to only have one opening, which alters the properties of the sound being emitted (Lugli, 2012). The amplificatory effect depends on the material of the nest as well as the frequency of the call. Nesting structures that have a density similar to water do not seem to have the capacity to act as resonance chambers (Lugli, 2012). Similarly, some structures may not interfere with the amplitude of calls, as toadfish boatwhistles were unaltered by terra cotta nesting structures (Barimo and Fine, 1998) due to the resonance frequency of the calls (Lugli, 2012). The form of these nesting structures is another factor to consider, as the opening to a cavity can often affect the radiation of the sound from its source (Fletcher, 2004). In ground crickets, the burrow from which the animal calls significantly increases the loudness of the calls (Bailey et al., 2001). The effectiveness of the nest cavity acting as a resonance chamber also depends on the coupling between the nest cavity and the substrate, as any discontinuity causes a large drop in the shelter's ability to act as an amplifier (Lugli, 2012). Sand gobies are known to move sand around the site of their nest, such that a sand pile forms over top of the nest and the entrance can become quite small (Lugli, 2013). This would prevent the goby's calls from escaping

from any openings other than the entrance, ensuring that the nest cavity could best act as a single-opening resonance chamber.

Bioacoustics as Sexually Selected Signals

In the Gobiidae, calls are produced both for aggressive purposes and in reproductive contexts (Lugli et al., 1995; Kasurak et al., 2012). In some species, sound is only produced in the presence of a gravid female, usually in combination with other courtship displays (Lindström and Lugli, 2000). In the freshwater goby complex calls are produced in combination with tail beats to encourage the female to enter the nest and sound production slowly decreases as spawning proceeds (Lugli et al., 1995). Sound may be produced as a means of encouraging females to approach or enter the nest (Malavasi et al., 2009) or once the female has entered as part of a multisensory display to show paternal reliability (Amorim et al., 2013b). The panzarolo goby (*Knipowitschia punctatissima*) calls only when a female is present inside the nest and calling ceases upon the female's departure (Lugli et al., 1995). Female round goby (*Neogobius melanostomus*) are known to be able to differentiate between calls of several species and show a preference for conspecific calls (Rollo and Higgs, 2008). This species is able to localize to sound sources (Rollo et al., 2007) and males may use calls to attract mates from a distance. Actively displaying males are more attractive mates ensuring proper parental care (Forsgren, 1997).

Recommendations and Conclusions

There is still much to discover about sound production and its uses in fish. In many cases, bioacoustics has only been explored in a few species within a family, leaving

gaps in knowledge about how these sounds are used. Since sounds produced by fish are species specific, a wide range of possibilities exist for human use. Bioacoustics has the capacity to help researchers better understand species interactions and aid in conservation and management without the often destructive consequences of capturing fish. However, in order to get to the point where bioacoustics could replace more traditional techniques, more research on a greater diversity of fish must be done. Some recommendations that could increase the success of future studies include 1) Researchers should share calls of known species through online sound libraries; 2) For management purposes, acoustically active fish should be characterized, enabling the use of passive acoustic monitoring of population health and invasion fronts; 3) The phylogenetic backgrounds of calling species should be analyzed in greater detail; and 4) The linkages between male quality and sound production should be further examined.

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Chapter II

VARIATION IN THE CALL STRUCTURE OF MALE ROUND GOBIES (*NEOGOBIUS MELANOSTOMUS*) IN RELATION TO BODY MORPHOMETRICS

Introduction

The sounds produced by animals can often be used by listeners and eavesdroppers as a means of determining information about the call producer (reviewed in Mock et al., 2011; Kaplan, 2014). Many terrestrial animals perform acoustic displays to attract potential mates (Peters and Peters, 2010), act as a mode of species recognition (Rivero *et al.* 2000), and advertise their quality (Vannoni and McElligott, 2007; Voituron *et al.*, 2012). Inherent differences in sound production mechanisms can create signals that are variable, both within and between individuals (Ryan and Guerra, 2014) and a great deal of the variability in an acoustic display of an individual is based on the individual's "condition". For example, in chacma baboons (*Papio hamadryas ursinus*), a calling bout will contain signals of varying intensity, where intensity decreases as the individuals exhaust themselves (Kitchen et al., 2003). In cases where these signals are costly to produce, it is expected that females can use the display as a measure of the potential mate's condition (Zahavi, 1975). Honest signals should be those that accurately advertise a desirable trait, whether it is male size and resource holding capabilities (Wells, 1977), lipid reserves to protect against filial cannibalism (Manica, 2004) or possession of desired heritable genes (Bentsen et al., 2006). Acoustic honest signalling has been found in many terrestrial species across a wide range of taxa (birds: Spencer et al., 2003; amphibians: Gingras *et al.*, 2013; mammals: Vannoni and McElligott, 2008, Kitchen *et al.*, 2013) and

is suspected to be in many others (arachnids: Rivero et al., 2000; tortoises: Galeotti *et al.*, 2005).

In fish, the role of acoustic signalling as an honest signal is much less clear. It is often difficult to break down acoustic cues to determine the information being carried with them and what may be true for some groups does not necessarily apply to others. Dominant frequency, an acoustic feature that is often negatively correlated with male size in some species (e.g. Myrberg et al. 1986; McKibben and Bass, 1998; Amorim et al. 2003; De Jong *et al.*, 2007), appears to have no relationship to male size in others (Boyle and Tricas, 2011). In the painted goby (*Pomatoschistus pictus*), acoustic activity is related to lipid stores, with fatter males displaying more and with a greater sound pressure level (Amorim *et al.*, 2013). Female midshipman (*Porichthys notatus*) prefer calling bouts that are longer and uninterrupted (McKibben and Bass, 1998) and both sound amplitude and calling activity are indicators of condition in sand goby, *Pomatoschistus minutus* (Pedroso et al. 2013). There is also limited evidence that acoustic parameters influence mate choice in some fish species (Myrberg et al. 1986; Rowe & Hutchings 2008), suggesting that sound can be a true honest signal in fish, but the data remain sparse and more investigation is needed.

The purpose of the current study was to determine if the sounds produced by male round goby (*Neogobius melanostomus*) have the potential to act as size indicators. Previous work shows that round goby males vocalize and females are able to localize to calling males (Rollo et al., 2007), but whether or not these calls also function as honest signals is not yet known. I examined the correlations between male body characteristics and call variability in several call components of the round goby as a means of determining the potential use of calls as a measure of male condition. I test the prediction

that acoustic signals will correlate to at least one measure of body size, thus creating a link to body condition. Determining a linkage between calls and body condition is the first step in determining if acoustic honest signalling has the potential to exist in this species and to explain observed variability in call characteristics.

Methods

Model Organism

The round goby is a benthic fish, originally from the Ponto-Caspian region that has established populations throughout the Great Lakes (Jude et al., 1992). This fish is a brood guarder (Meunier et al. 2009), with the males keeping and defending nests.

Territory holding males, hereafter referred to as parental males, are very readily identified by their nuptial characteristics of black colour, swollen cheeks, a slimy coating and an enlarged urogenital papilla (Marentette et al., 2010). Parental males are known to call to attract females (Rollo et al. 2007; Rollo & Higgs 2008) much like other species of gobies (Lugli et al. 1996). The acoustic component of courtship is accompanied by visual and olfactory cues, whereby the male will fan with his pectoral fins (Meunier et al., 2013). It has been hypothesized (Marentette et al., 2009) that these parental males must defend their nests from sneaker males, reproductive males that do not display nuptial characteristics.

Sampling Methods

Round gobies were caught by angling at the Erieau Marina (N 42.2572945 W 81.9084025), on the north shore of Lake Erie and in the Detroit River, Ontario, in the summers of 2013 and 2014. Fish were then transported to the Animal Care Facility at the

University of Windsor where they were kept on a 12L:12D light cycle at $18\pm 1^{\circ}\text{C}$ and fed daily (Hikari Cichlid Gold; Kyorin Corporation, Japan). Males were visually and acoustically isolated from each other in a Z-Hab System (Aquatic Habitats Incorporated; Apopka, Florida, USA) and kept for no longer than one month. A length of PVC tube was supplied in each tank to act as a shelter. Females were kept in communal tanks of up to ten females.

Experimental Procedure

Only reproductive round gobies were used in this study. Male reproductivity was assessed by the enlargement of the urogenital papilla (Marentette et al., 2010). Both parental and “sneaker” males may have been used as parental males lost their nuptial colouration after transport and could not be differentiated by size. Reproductive females were identified by the enlargement and colour of the urogenital papilla; the papilla appears round and swollen in reproductive females, with the colour shifting from white to yellow-orange (Marentette et al., 2010).

Fish were tested at night as round gobies are more vocal between the hours of dawn and dusk than during daylight hours (Higgs and Humphrey, 2014). During testing all filters, lights and air pumps were turned off to reduce abiotic noise and a plastic, grid barrier was placed into the tank with the male. The PVC shelter was removed to remove any chance of noise being produced by banging against the shelter and a hydrophone (RESON TC4013-4, $-210.9\text{ dB re } 1\mu\text{Pa/V at } 1\text{m}$) was inserted into the male’s side of the tank. Recordings were made using a solid state recorder (Marantz PMD670) after running through a preamplifier (RESON VP2000 Voltage Preamplifier EC6081) with a high pass setting of 1 Hz and a low pass setting of 15 kHz. The hydrophone remained in close

proximity to the male throughout the trial. After five minutes of acclimation, the recording was started for a 30 minute control recording and then a reproductive female was added to the unoccupied side of the tank. The male could see and smell the female, but could not access her. After each half hour trial, the female was placed back into the communal female tank to be used for other trials.

After the experiment, males were euthanized using clove oil (>20 ppm after anaesthesia with 10 ppm) and measured for head width above the eyes (HW), total length (TL), total wet weight (TW) and gonad weight (GW). Total weight and gonad weight were used to calculate gonadosomatic index (GSI) using the equation

$$GSI = \left(\frac{\text{gonad mass}}{\text{total body mass}} \right) \times 100\%.$$

Call Analysis

Male calls were analyzed using Adobe Audition® 3.0 software (Adobe Systems Incorporated, USA). Sound files were cleaned of background noise by using the software's Noise Reduction feature and each call was measured for the dominant frequency (DF, characterized as the frequency with the greatest energy determined by the power spectra, FFT size 32768, Blackmann-Harris window), number of pulses, total duration of the call and the interpulse interval (IPI). Interpulse interval was defined as the time in between two peaks of consecutive pulses when viewed using the waveform setting of Adobe Audition® 3.0.

Statistical Analysis

Data was Log_{10} transformed to obtain a normal distribution. Call and body characteristics were associated against each other using principal components analysis (PCA) on JMP 10 software (SAS Institute Incorporated, North Carolina, USA) to determine general correlative relationships between body quality and call characteristics. Redundant and non-correlative variables identified by the PCA were removed from all subsequent tests. Generalized Linear Models (GLMs) were performed using call characteristics of IPI, DF and duration as dependent factors with body morphometrics of total length, head width and total weight acting as explanatory variables. For these analyses, only calls of more than one pulse were included. In cases where a three-way or two-way interaction between these body characteristics was non-significant, the interaction term was removed and the model run again. Effects of interaction terms on call characteristics were examined using procedures provided by Dr. Jeremy Dawson (<http://www.jeremydawson.co.uk/slopes.htm>). The residuals of all of the GLMs were normally distributed.

The variation of the calls' dominant frequency component was analyzed by examining the coefficients of variation between and within male calls. Coefficient of Variation (CV) is defined with the function $= \frac{SD}{\bar{x}} \times 100\%$. For this purpose, only the first pulse of each call was examined and any males with at least 3 calls were included in the within male variability (CV_w) analysis. As in Bee et al. (2001), between-male variability (CV_b) uses the mean of all of the first and single pulses across all males recorded. Variation is considered greater between males than among males when the ratio of $CV_b/CV_w > 1$.

Results

A total of 36 males were tested, of which 24 produced calls, one of which only called during the control trial. Sixteen actively calling males produced at least one call of more than one grunt, with two of these males producing more than a single call of one pulse. The total number of calls was 37 with 20 of these being calls of more than one pulse. Only 4 males produced at least three calls during a single recording, these calls were analysed for CV_w , with 3 of the four having much lower variation in frequency within than between males (Table 2.1).

The principal components analysis identified three main components (Table 2.2), of which, the first principal component accounted for nearly 41% of the variability in the model. Three body characteristics, total length, head width and total weight were strongly associated with scores of PC1, as was a single call characteristic, interpulse interval. The second component accounted for 26% of the variability. Call characteristics of frequency, duration and number of pulses were all strongly associated with this component. Total width and total size were weakly positively associated with this component. Gonadosomatic index is most strongly associated with scores of PC4 and is not tightly correlated with any feature of the call or other body measure so GSI was discounted from further analyses.

Generalized Linear Models of call characteristics against body morphometrics identify several significant interactions between body features affecting the call. Frequency is affected by two two-way interactions of HWxTW and HWxTL. The HWxTW cross showed heavier males having lower frequency calls than their smaller counterparts (X^2 ; $df=1$, $N = 20$ $p = 0.0293$; Figure 2.1a). On the other hand, the HWxTL cross showed longer males had higher frequencies than smaller males (X^2 ; $df=1$, $N = 20$

Table 2.1

Table 2.1. Inter- and intra-male variability of call frequency. The coefficients of variation in dominant frequency for four male round goby that each called at least 3 times compared to the variation in the calls of all recorded males. Only the dominant frequency of the first pulse of each call was used in the analysis. CV_w within male variability, CV_b between male variability.

| Male | CV_w | CV_b | CV_b / CV_w |
|------|--------|--------|---------------|
| 5 | 13.6 | 36.0 | 2.7 |
| 31 | 17.3 | | 2.1 |
| 32 | 7.0 | | 5.1 |
| 36 | 50.0 | | 0.7 |

Table 2.2

Table 2.2. Loadings of call and body measures. Principal components loadings

showing interpulse interval, total length, head width and total weight were most highly associated with the first component, frequency, duration and number of pulses grouped in the second component and GSI most strongly in the fourth component by itself.

| Characteristic | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 |
|--------------------------|--------------|---------------|--------|--------------|--------|--------|--------|--------|
| Log#pulses | 0.191 | 0.783 | 0.524 | 0.152 | -0.091 | -0.158 | 0.131 | 0.001 |
| LogDuration | 0.471 | 0.772 | 0.280 | 0.224 | 0.019 | 0.170 | -0.151 | -0.002 |
| LogFreq | -0.180 | -0.572 | 0.506 | 0.554 | 0.275 | -0.005 | 0.007 | 0.001 |
| LogTW | 0.916 | -0.378 | 0.082 | 0.045 | -0.058 | 0.023 | 0.054 | -0.020 |
| logGSI | -0.450 | 0.102 | -0.498 | 0.702 | -0.198 | 0.056 | 0.044 | -0.001 |
| logTL | 0.820 | -0.464 | 0.214 | 0.020 | -0.209 | 0.137 | 0.049 | 0.014 |
| logHW | 0.877 | -0.125 | -0.297 | 0.235 | -0.016 | -0.238 | -0.117 | 0.004 |
| logIPI | 0.700 | 0.380 | -0.508 | 0.022 | 0.296 | 0.070 | 0.113 | 0.004 |
| Total variability | 40.839 | 25.847 | 15.738 | 11.660 | 3.243 | 1.733 | 0.932 | 0.009 |

Figure 2.1

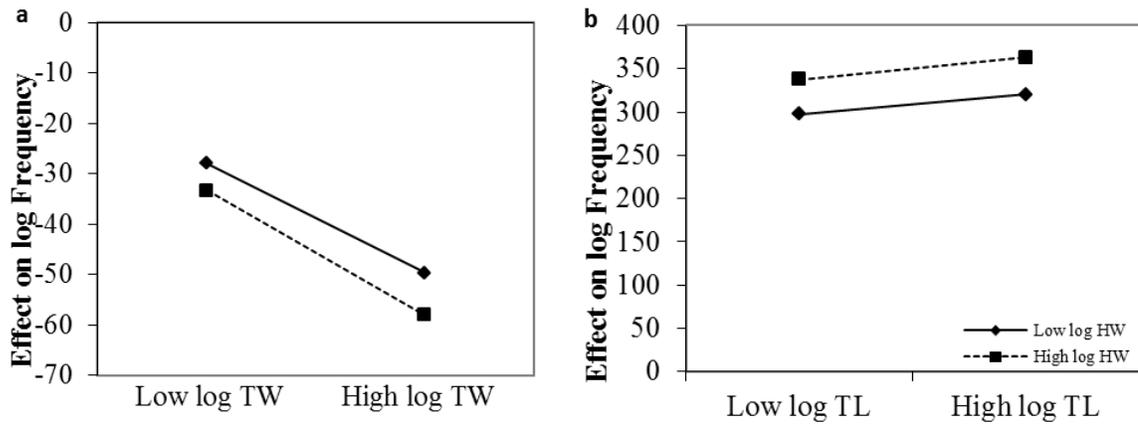


Figure 2.1. The relationship between HWxTW, HWxTL and DF. a.) The negative relationship between the log₁₀ transformed dominant frequency of calls and the two-way interaction effect of head width and total weight. This interaction suggests larger males have lower dominant frequencies of their calls. b.) The positive relationship between the log₁₀ transformed dominant frequency of calls and the two-way interaction effect of head width and total length. This interaction suggests larger males have higher dominant frequencies of their calls.

$p = 0.0213$; Figure 2.1b. These interactions work in opposite directions with large headed, heavier males having lower frequency calls, but large headed, long males having higher frequency calls.

The IPI is also affected by two two-way interactions of HWxTW and HWxTL. The HWxTW cross showed heavier, large-headed males having longer IPIs compared to thinner, small-headed males (X^2 ; $df=1$, $N = 20$ $p = 0.045$; Figure 2.2a). This interaction is contradicted by the HWxTL cross, which shows shorter, small-headed males having longer IPIs (X^2 ; $df=1$, $N = 20$ $p = 0.0383$; Figure 2.2b).

The GLM indicated that duration was significantly influenced by a three way interaction of HWxTLxTW X^2 ($df=1$, $N = 20$ $p = 0.0005$; Figure 2.3). This interaction suggests that long duration calls are produced by short, heavy males and short duration calls are produced by thin, long males. In this case, males with a greater TW did not change duration as considerably as males with a lesser TW. Males producing the shortest duration calls were those with long, thin bodies and a large head. Long, thin males with a small head produced calls with durations comparable to short, thin males with large heads.

Discussion

Honest signalling requires that a signal accurately and consistently displays the trait of interest (Zahavi, 1975; Johnstone, 1995). In the current study, the accuracy of call features predictive ability for body measures is quite high, suggesting that these acoustic signals could be used by conspecifics to determine the “quality” of the caller. There is also a greater inter-male variability in calls than intra-male variability,

Figure 2.2

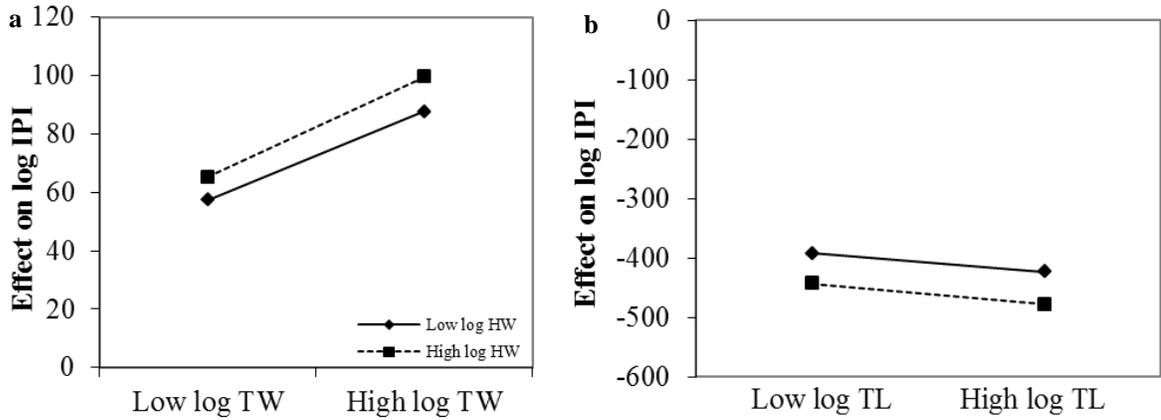


Figure 2.2. The relationship between HWxTW, HWxTL and IPI. a.) The positive relationship between the log₁₀ transformed interpulse interval of calls and the two-way interaction effect of head width and total weight. This interaction suggests larger males have greater interpulse intervals in their calls. b.) The negative relationship between the log₁₀ transformed interpulse interval of calls and the two-way interaction effect of head width and total length. This interaction suggests larger males have shorter interpulse intervals in their calls.

Figure 2.3

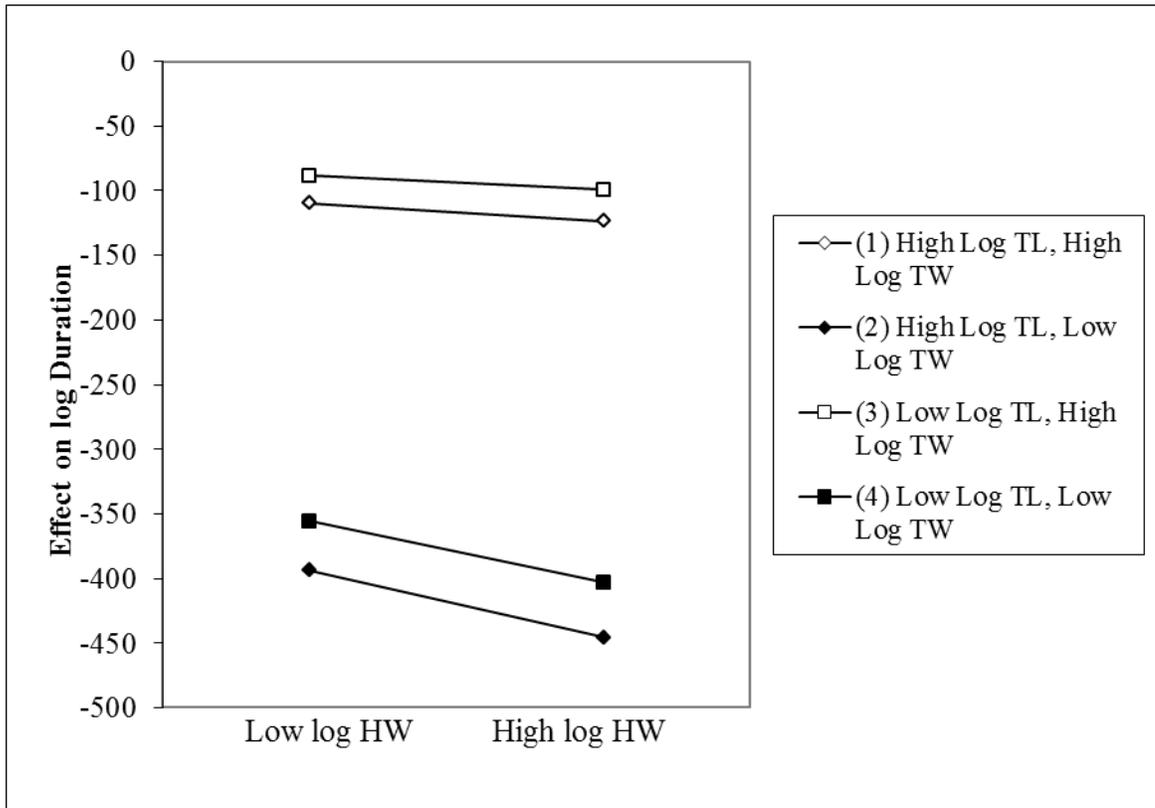


Figure 2.3. The relationship between HWxTLxTW and duration. The negative relationship between the log₁₀ transformed duration of calls and the three-way interaction effect of head width, total length and total weight. Males with a greater weight show a considerably longer call duration regardless of head size and length. The shortest call durations are produced by long, thin males with large heads. Call produced by short, thin, big-headed males and long, thin, small-headed males are of a comparable length.

another factor necessary for honest signalling to exist. The calls produced by individual males were consistent over the short time frame of this study, showing a high reliability in signalling body condition. While the mechanism for sound production is not known in this species, calling may not be overtly costly (Amorim et al 2013) as honest signals are usually thought to be (Gintis et al., 2001; Számadó, 2011), however, the physical mechanism of sound production is likely limited by the body traits measured. In species, such as the weakfish (*Cynoscion regalis*), which use sonic muscles, larger fish produce sounds of lower frequencies due to the increased time it takes for the longer muscle fibres to contract and relax. Similarly, these larger fish produce sounds of greater intensity because they have greater muscle mass (Connaughton et al., 2002). A size-restricted sound production mechanism can be assumed to be the case where head size and body length of the fish affect characteristics of the call, as was seen in the current study. In other species of gobies where the mechanism of sound production has been examined, the call invariably originates from the head region of the fish. Some suspected modes of call production in gobies include projection of water through the operculum (Tavolga, 1958), grinding pharyngeal teeth together (Takemura, 1984; Lugli, 1995) and vibration of the levator pectoralis muscle which originates on the skull and inserts on the pectoral girdle (Parmentier et al., 2013). Should one of these modes of call production prove to be true for the round goby, the physical properties of the fish's head would alter the call, consistent with my results.

In the current study, long males with big heads produced short, high frequency calls with short IPI, while heavy, big headed males produce calls with lower frequencies and longer IPI. Depending on female preference for male traits, the female may be able to tease apart the various levels of a call to determine which nests to visit. In other species of

gobies, there is very little evidence supporting female preference for male size (Magnhagen and Kvarnemo, 1989; Forsgren, 1997; Amorim et al., 2013). Conversely, larger males are more capable fighters and tend to win larger territories, for which females do display a preference (Magnhagen and Kvarnemo, 1989). This contradiction may in part reflect the effort put into courtship. In Pacific blue-eye fish (*Pseudomogil signifier*) females do not mate more frequently with larger, more competitive males, instead choosing males that put greater effort into courtship (Wong, 2004). Bicolor damselfish (*Stegastes partitus*) females are less likely to mate with males that are not actively courting, perhaps because these males have lower energy reserves (Knapp and Kovach, 1991). Female gobiids are thought to prefer males with greater lipid stores (Amorim et al., 2013) and in the current study total weight was a significant predictor of IPI, DF and call duration. Choosing males with greater lipid stores would be adaptive because fatter males may be less likely to cannibalize their eggs (Neff, 2003), larger testes allowing for greater sperm production (Taborsky, 1998), and larger males may have greater brood survival rates (Sabat, 1994).

Call duration provides some counterintuitive results, wherein the longest call durations were performed by short, heavy males with small heads. There is not as great a difference between the groups of heavy males with HW and TL varying as there is between the groups of light males. This could suggest that males must have attained a minimum weight to be able to call for any substantial length of time, perhaps resorting to alternate, less costly modes of communication. While frequency and IPI might be related to morphological restrictions of the sound production mechanism, duration of the call may be more heavily influenced by the male's condition. Plainfin midshipman males that are in better condition and have greater mass, also have a greater number of young at the

end of the season (Sisneros et al., 2009). These males likely use their greater energy reserves for increased courtship effort, to produce calls of longer duration, to which females are more attracted (McKibben and Bass, 1998). Greater fat stores would allow the male to increase courtship, gain more mating opportunities and receive more eggs. Male gobies that care for more eggs exhibit lower incidence of filial cannibalism and more fanning behaviour than those that care for fewer eggs (Forsgren et al., 1996).

For both IPI and dominant frequency I saw opposite relationships for total length or total weight. Longer fish had a shorter IPI and higher frequency and heavier fish had a longer IPI and lower frequency than their counterparts. This disparity may be explained by the different information portrayed to the female by these two metrics of size. Total body weight is a more immediate measure of condition in fish (Cargnelli and Gross, 1997), with immediacy due to ability to thrive under current conditions while total length may be a sum total of growth capability over longer time frames (Nate and Bremigan, 2005). A heavier fish will be more capable of putting in the reproductive effort in the current season rather than waiting until it is larger, but possibly in worse condition. While I do not know which metric is more preferred by female round gobies, it is possible that the two metrics convey different information, or conversely that the call characteristics are influenced more by weight than length. This possibility seems unlikely however, as these two characteristics are very tightly correlated in the reproductive males used in this study (Pearson's $r = 0.959$). Until we can ascertain how calls are produced in round goby however I cannot assess what is driving these differences.

Although acoustic recordings are greatly affected by small tank dynamics (Akamatsu et al. 2002), the recordings in the current study were taken with the hydrophone very close to the fish and all experiments were done in the same conditions

so individual comparisons would not be affected. The removal of the male's shelter for the duration of the recording may also have negatively impacted call production. This does not seem to be the case however, as male call rate is similar to that found in nature (unpublished data), as are the call characteristics (Rollo et al., 2007). There also remains the possibility that male mate choice affected the results of this study. However, females were size matched and always checked for status of the urogenital papilla before being placed in the experimental setup. Regardless, male courtship effort may have been altered by the use of several females across trials. The production of calls by males thought to have been "sneakers" may suggest that this reproductive stage could be an alternate behavioural tactic when more dominant males are present.

In conclusion, male round goby have call parameters that are strongly linked to several body characteristics. These acoustic traits may act as an honest signal to females during courtship of the male's condition or parental capabilities. Male courtship calls may carry a large amount of information not only of male lipid stores, but also of overall size. These traits, if the female is able to detect them, would be beneficial for determining risks of filial cannibalism, effort put into rearing, as well as fighting capabilities. Future studies should aim to determine the relative importance of acoustic cues in relation to other male courtship signalling modalities and test the response of females to these courtship signals as a true test of honest signalling in this species.

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Chapter III

EXAMINING THE POTENTIAL FOR ACOUSTIC HONEST SIGNALLING IN THE ROUND GOBY (*NEOGOBIUS MELANOSTOMUS*) THROUGH FEMALE CHOICE

Introduction

Acoustically-mediated honest signalling is dependent upon two factors: the first being that the acoustic signal correlates with male condition and the second being that females can discern this relationship through the sound. Both of these aspects are well studied in terrestrial systems. Male condition is usually examined as a function of male size (Schulte-Hostedde et al., 2005), though some studies are beginning to look into condition as a factor of social ranking (Kitchen et al., 2003, Vannoni and McElligott, 2008). Larger males are often more capable combatants, winning themselves a greater social rank (Le Boeuf , 1974; Bowyer, 1986). From the aspect of size, sound production should be a reliable signal as sound tends to scale linearly with the size of the larynx or syrinx and diameter of the mouth, or in the case of insects, the size of the vibrating organ producing the sound (Fletcher, 2004). When presented with acoustic signals produced by conspecific males, females are often able to judge male size accurately, often by the frequency of the call (Ryan et al, 1990). Female crickets (*Teleogryllus commodus*) prefer males that present greater calling effort, a trait that is condition dependent and suspected of being heritable (Bentson et al., 2006). Having offspring that in turn produce more attractive calls fits into the “sexy son” hypothesis, increasing overall fitness as the female would ultimately have more descendants (Weatherhead and Robertson, 1979). Males in better condition put greater effort into raising young in species that do so (Iwasa and Pomiankowski, 1999).

In fish, evidence for female choice for male acoustic signals is limited. While male acoustic signals often correlate strongly with male body size (Amorim et al., 2010; Chapter 2), few studies examine honest signalling from the female choice perspective. In cases where female preference for male calls is examined, females tend to prefer calls where the temporal aspects of the call correlate with male body characteristics. In the plainfin midshipman (*Porichthys notatus*), male calls that are uninterrupted and longer in duration are preferred by females (McKibben and Bass, 1998). Greater size and muscle mass may be attributed to the longer call duration (Connaughton et al., 2002) and if larger males are preferred, duration may act as an honest signal of size. Greater courtship effort is also linked to greater male energy reserves, reducing the chances that males will cannibalize their brood (Knapp and Kovach, 1991), thus increasing female fitness.

The current study focuses on the potential for honest signalling in the round goby (*Neogobius melanostomus*). Previous work has shown that acoustic characteristics do correlate strongly with male body conditions (Chapter 2). Where male calls function as indicators of body size, the potential exists for females to exploit this trait. The preference for larger males and the ability to tell a male's "condition" from his call would make the acoustic signal a true honest signal. The objective of this chapter is to determine if female round goby show some level of preference for male call characteristics, providing evidence for true honest signalling of male size in this species.

Methods

Sampling Methods

Round gobies were caught by angling at the Eriean Marina (N 42.2572945 W 81.9084025), on the north shore of Lake Erie and in the Detroit River, Ontario in the

summers of 2013 and 2014. Fish were then transported to the Animal Care Facility at the University of Windsor where they were kept on a 12L:12D light cycle at $18\pm 1^{\circ}\text{C}$ and fed daily (Tetramin Fish Flakes; Tetramin Inc, Blacksburg, VA, USA). Fish were housed in communal tanks of up to 10 females. Lengths of PVC tubes and clay pots were provided as artificial shelters.

Effects of Hormone Injection

Once recordings of reproductive males had concluded, out-of-season female round gobies were tested for male call preference. Initial tests were run at night during the months of October and November, 2013. Previously reproductive females were given an intraperitoneal injection of $0.5\ \mu\text{L/g}$ fish body mass of Ovaprim (Syndel Laboratories Ltd., Vancouver, B.C.) 5 days before testing, and then again after testing. The second test was done 48 hours after the second injection. This timing was decided as the fish were no longer in reproductive condition, thus the longer timeline for injection with a booster was chosen. This method has been used previously for inducing maturation in juvenile fish (Hansen and Routledge). Two trials were done in order to see if female choice was consistent across time within the same female. A total of 10 fish were used for this test. Hormone injection was decided upon in order to enhance female response rates, since the fish used in this time frame were no longer in reproductive condition. The experimental procedure for the hormone injection study was the same procedure used in subsequent tests. After the second trial females were euthanized with clove oil and measured for total length (mm), total weight (g) and gonad weight (g). When females were in reproductive condition during the summer of 2014, Ovaprim injection did not occur, and an additional 34 females were tested for call preference.

Male Call Playback

I had recordings from several reproductive male round gobies from previous work (Chapter 2) that I used in female call preference experiments. Both parental and “sneaker” males were used in this portion of the study, as parental male nuptial colouration returned to normal colouration and the two morphs were not distinguishable by size. These calls were recorded in individual tanks in a Z-Hab System (Aquatic Habitats Incorporated; Apopka, Florida, USA), at night during the summer months of 2013 and 2014 while in the presence of a reproductive female. All calls used were produced by males while in the presence of a reproductive female. The longest call train from each male was sectioned out of a 30 minute recording using Adobe Audition 3.0 software (Adobe Systems Incorporated, USA) so that the file used for playback was of a single, clear fish call. Calls were saved with a 15 decibel gain to allow for easy playback.

A 1020-L rectangular ($243.8 \times 91.4 \times 91.4$ cm) fibreglass tank with a holding area in the center and speakers (UW-30, Lubell Labs, Columbus, OH, USA) at either end was used for all female choice trials (Figure 3.1). The barriers had four 10 cm wide exits located approximately 20 cm apart so that fish must navigate through the barrier to gain access to the speaker of choice. Speakers were on acoustic foam to reduce vibration and were surrounded by a nylon mesh barrier so that fish could not use the speaker as a shelter. A GoPro Hero 2 camera (Woodman Labs Inc., USA) was situated above the tank and recorded 960p definition video of each thirty minute trial. The tank was filled with dechlorinated tap water to a depth of 30 cm and was held at 13 ± 1 °C. To start a trial, a reproductive female was placed in the centre of the holding area using a net. In order to reduce the chances that a female would seek shelter and refuse to move when calls began,

Figure 3.1

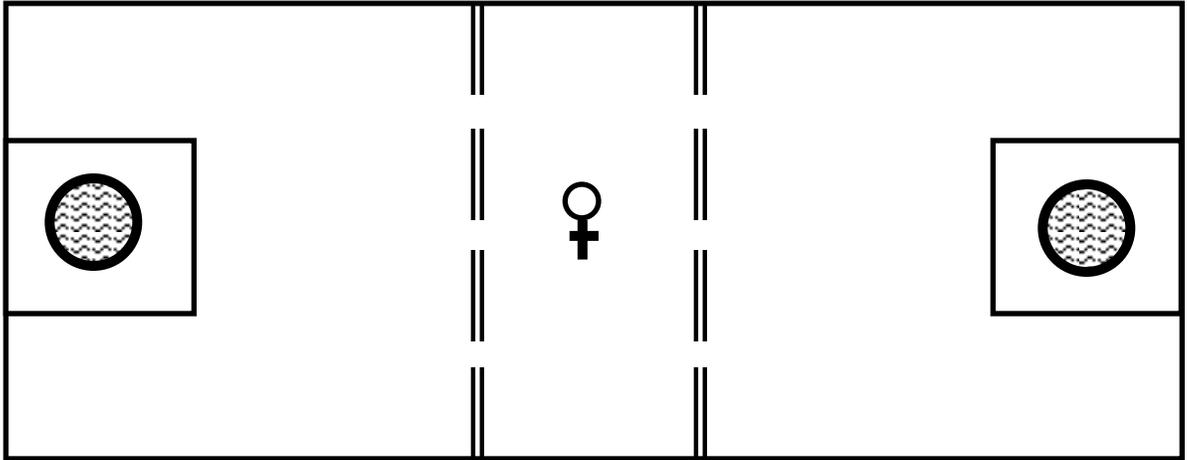


Figure 3.1. Diagram of the female choice experimental set-up. The tank in which female call preference was tested consisted of three “zones”, the two outer zones in which a speaker played a call from and the centre or starting zone. Dashed lines represent the barrier from which females had to navigate out of to reach a speaker. Speakers are represented by circles and the nylon mesh cage around the speakers are represented by the boxes.

there was no acclimation period. Two calls were chosen at random from a total of 12 male calls, and the speakers never played the same call during the same trial. A call was then played from each speaker simultaneously and each at an intensity of 140 dB re 1 μ Pa as measured before each trial by a precalibrated hydrophone (Interocean Inc., San Diego, CA, U.S.A.). Calls were left playing on a continuous loop for the duration of the 30 minute trial. The fish was left without any other outside stimulus for the duration of the trial. After the trial, females were euthanized with clove oil and measured for total length (mm), total weight (g) and gonad weight (g).

Female behavioural responses to male playback were quantified from video to determine speaker of choice for male preference, latency of response before approach began, and time spent near speaker. A responsive female was any female that oriented suddenly to a call and navigated through the barriers to approach one of the speaker zones. Females that oriented towards a call but stopped at the barriers were counted as non-responders as the motivation for moving from the starting zone could not be judged. A female was considered to have chosen a speaker when she was within 10 cm of the cage in which the speaker was placed. "Time spent near a speaker" was determined by recording the period of time during which a female was situated within 10 cm of a speaker cage.

Statistical Analysis

All statistical analyses were performed using JMP 10 statistical software (SAS Institute Incorporated, North Carolina, USA). A matched pairs t-test was performed to compare the mean amount of time spent within each speaker region between trials of hormone injected females that responded in both trials. The responses of injected and

non-injected fish were tested and found not to be significantly different. Therefore, all females were analyzed as a single group.

Data from trial 1 of each responsive fish was \log_{10} transformed to obtain a normal distribution. A Generalized Linear Model (GLM) was run using latency to respond as the dependent variable and the dominant frequency (DF) and interpulse interval (IPI) of the first male chosen as explanatory variables. A separate GLM was run with time spent in the speaker area as the explanatory variable and the dominant frequency and interpulse interval as explanatory variables. The GLM of time spent at the speaker of choice was then repeated with the initially chosen speaker's DF and IPI.

To determine if females exhibited a preference for particular male call characteristics, males' calls were ranked as attractive or unattractive based on the amount of time a female spent in their respective speaker arena. An attractive male was ranked a 1 if that male had a lower frequency than the unattractive male and a 2 if that male had a higher frequency than the unattractive male. For IPI, attractive males were given a rank of 1 if that male had a longer IPI than the unattractive male and a 2 if that male had a shorter IPI than the unattractive male. The binomial probability of each outcome was calculated using the equation $P(X) = \frac{n!}{(n-x)!x!} \cdot (p)^x \cdot (q)^{(n-x)}$ with a 50% chance of occurrence in either direction.

Results

Of the total 44 female fish used, 24 responded to the male call stimulus. Of these 24 fish, 7 were from the hormone injection trials. Of the 7 responsive Ovaprim-injected females, 3 females were only responsive during one of the trials. From the matched pairs

t-test, females consistently spent the same amount of time in each speaker region across both trials ($t(df=7) = -0.47, p = 0.651$; Figure 3.2). The 4 females that responded in both trials spent more time with the same male across each of their trials. Injected and non-injected fish were not significantly different in their responses ($F\text{-Ratio}(df=1,38) = 3.3907, p = 0.0738$).

In trial 1, 21 females responded to male call stimulus. Winning calls were those where the female spent more time in the speaker's arena. One of the males (male 31) was consistently rejected by females in all 5 of the trials where females were responsive; this male was atypical in sound, with a call that was "tinny" in quality and probabilities were calculated both including and excluding data where this male was an option. Females did spend time at the speaker playing this call and would occasionally choose this speaker first; however, females consistently spent less time at this speaker. Of the responding females, 1 had the option of choosing between males of the same frequency and was discounted from the probability calculation. The remaining 20 females showed an even distribution of choice between high and low frequency males ($P(x=10) = 0.176$). When data from male 31 was removed, the total number of trials dropped to 17 and choosing the low frequency male had a probability of random occurrence of ($P(x=10) = 0.148$) (Figure 3.3a).

Examination of the IPI chosen by responsive females shows that females had a strong preference for male calls with a longer average IPI. When including data from male 31, females chose the male with the longer interpulse interval in 15 of the 21 trials ($P(x=15) = 0.0259$). Without the data using male 31, the number of trials was 17 and the probability of random occurrence of 14 "wins" dropped to ($P(x=14) = 0.00519$) (Figure 3.3b).

Figure 3.2

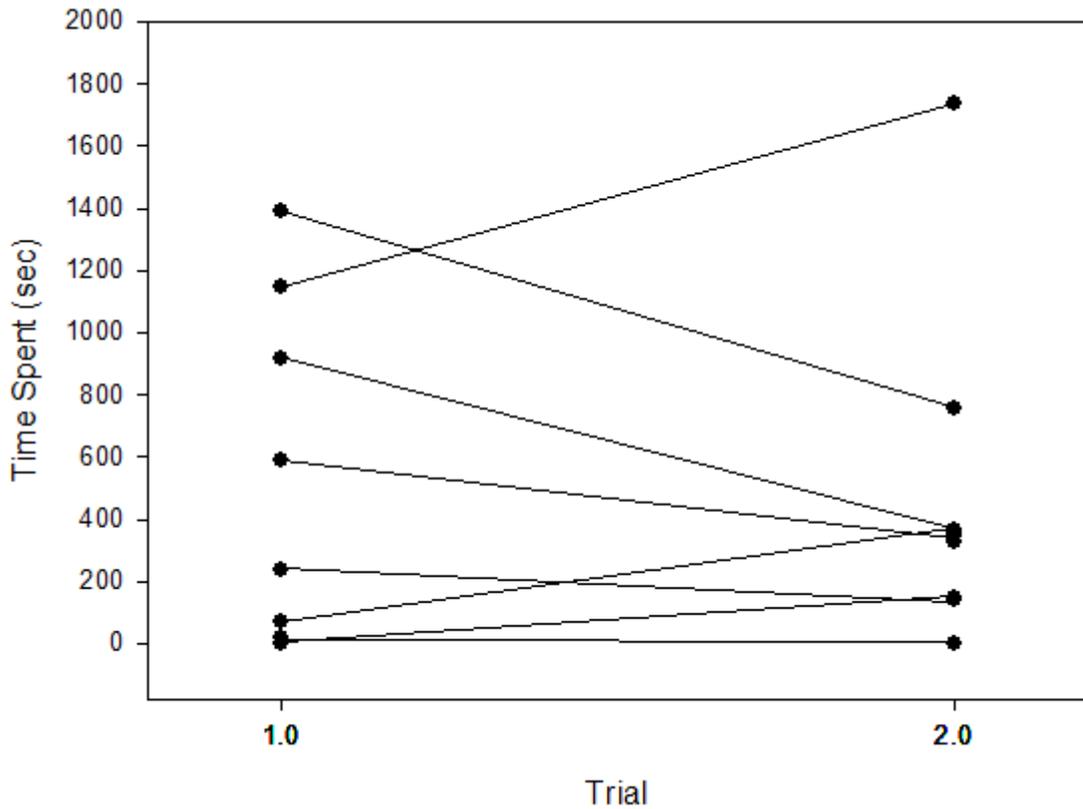


Figure 3.2. Time spent in arena speaker across trials. The 4 hormone injected females that responded in both trials spent similar amounts of time in the same speaker arenas during both trials. These 4 females spent time in both arenas, for a total of 8 choices, during the two trials and time spent in the arena was consistent between trials. Each line represents one chosen speaker, where the time spent at that speaker between trials is not significantly different. The hormone injected females consistently chose the same males across trials, and also spent similar amounts of time in the chosen speaker arena ($t(df=7) = -0.47, p = 0.651$).

Figure 3.3

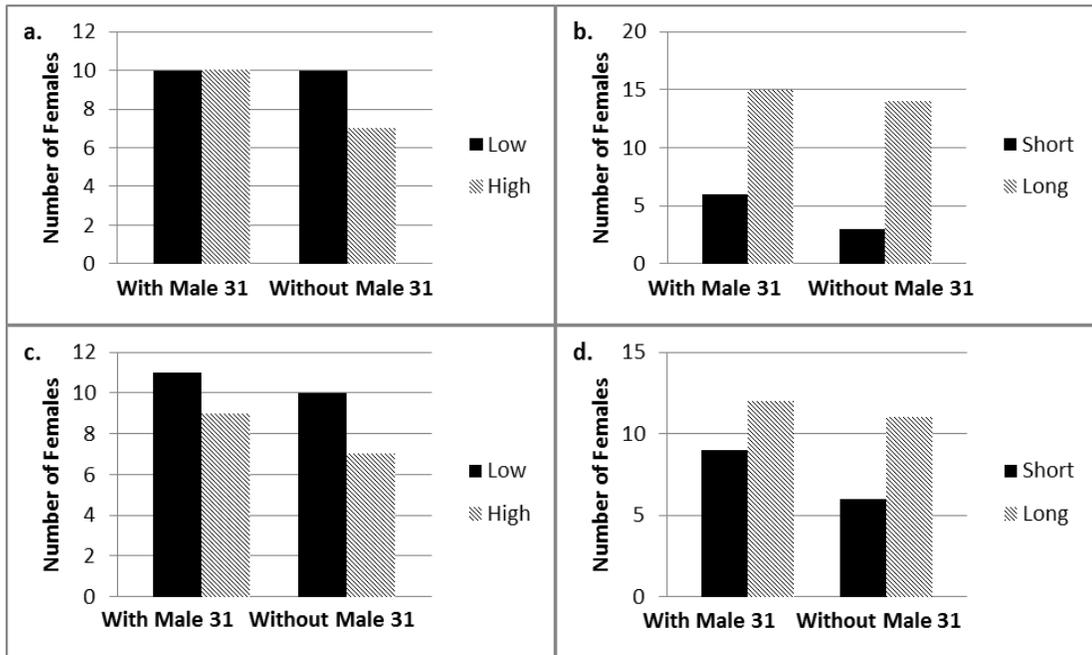


Figure 3.3. Number of females showing call preference. The call with which a female spent the greater amount of time or chose first was classed as the attractive male of the two calls and tallied as the preferred call. a. shows the frequency of the call where attractive males were decided by time spent in the speaker arena by the female; b. shows the interpulse interval of the call where attractive males were decided by time spent in the speaker arena by the female; c. shows the frequency of the call where attractive males were decided by the initially chosen call; and d. shows the interpulse interval of the call where attractive males were decided by the initially chosen call.

Initial call of choice showed a similar pattern, where frequency had a probability of $(P(x=11) = 0.160)$ with male 31 and $(P(x=10) = 0.0944)$ without male 31 (Figure 3.3c). The initial IPI chosen loses some of the distinction, with females only slightly preferring longer IPIs ($(P(x=12) = 0.140)$ with male 31 and $(P(x=11) = 0.0944)$ without male 31) (Figure 3.3d).

None of the GLMs performed showed any clear pattern (Table 3.1). Time spent near speaker was independent of call DF or IPI ($p > 0.05$), nor did it depend on the DF or IPI of the initially chosen call ($p > 0.05$). Removing interaction terms from these models created no change in the results. Latency of the response was also not determined by the initially chosen call's DF or IPI ($p > 0.05$). All residuals were normally distributed.

Discussion

The possibility for female choice is evidenced by the very low probability (2.6% chance of random occurrence) of females choosing males with longer IPI over males with shorter IPI, showing that this was a choice rather than a random occurrence. When choices involving male 31 were removed from the analyses, the probability drops even lower (0.5% chance of random occurrence). The atypical call of male 31 may be due to the hydrophone resting against the wall of the tank, producing a call with a tinny quality. In other fish species, the temporal aspect of the call seems to have a greater impact on female preference (McKibben and Bass, 1998). In the midshipman, females are much more discriminatory over pulse duration than over modulations in frequency, where even short breaks of a few hundred milliseconds in a call would decrease female responsiveness (McKibben and Bass, 2001). Another fish species where temporal

Table 3.1

Table 3.1. Statistical results of female choice GLMs. The results of the Generalized Linear Models with a normal distribution performed on time spent with the chosen speaker and latency of the females' response. No significant results were found.

| | | DF | ChiSquare | Prob>ChiSq |
|------------|-----------------------|------|-----------|------------|
| Time spent | Initial DF chosen | 1,38 | 0.3544 | 0.5516 |
| | Initial IPI chosen | 1,38 | 0.114 | 0.7356 |
| | Initial DFxIPI chosen | 1,38 | 0.0202 | 0.8868 |
| Time spent | DF chosen | 1,38 | 0.1558 | 0.693 |
| | IPI chosen | 1,38 | 0.7538 | 0.3853 |
| | DFxIPI chosen | 1,38 | 0.563 | 0.453 |
| Latency | Initial DF chosen | 1,21 | 0.5266 | 0.468 |
| | Initial IPI chosen | 1,21 | 1.5234 | 0.2171 |
| | Initial DFxIPI chosen | 1,21 | 0.2837 | 0.5943 |

discrimination has been discovered, the bicolour damselfish, *Eupomacentrus partitus*, increases courtship effort when calls with longer pulse intervals are played (Myrberg and Spires, 1972). The enhancement in courtship effort is evident with as little as a 10 msec increase in pulse interval; however, increasing the length of the pulse itself does not affect the courtship effort (Myrberg and Spires, 1972). The fact that female choice was stronger on the basis of time spent rather than speaker of first choice may be suggestive that females will visit multiple male nests before choosing a mate. This seems to be a distinct possibility as round goby will lay their eggs in several nests (Corkum et al., 1998). Acoustic signalling is part of male courtship effort, and so females may assess males on several courtship tactics before mating (Malavasi et al., 2009).

The lack of obvious choice by females based on frequency is not entirely unexpected. Round goby do not have a strong ability to differentiate between low range frequencies (Belanger et al., 2010), however this is condition dependent, as reproductive females are capable of distinguishing differences in frequencies (Zeyl et al., 2013). Even in the midshipman, a species that does display a preference for frequency, the preference is for a call frequency that is expected at a certain temperature and not for a frequency that displays information about the male (McKibben and Bass, 1998). In species that produce sound through vibratory muscles, larger males produce lower frequency calls, as larger muscles take longer to contract (Crawford et al., 1997; Connaughton et al., 2002). Male call frequency may indicate male size accurately, but larger males are not necessarily more capable fathers (Forsgren, 1997). Thus frequency may not be an important factor for females when selecting a mate.

Between trial 1 and trial 2 of the Ovaprim-injected females, all 4 of the hormone treated females that were responsive in both trials were consistent in their responses. The

precision of the amount of time spent between trials at each speaker for the Ovaprim-injected females suggests that female round goby can differentiate between calls and will consistently choose the same male over time (Figure 3.2). Honest signalling likely plays a large part of the reasoning behind choosing the same male consistently. Another possibility may be that females associated certain territories with the call, as each call was played from the same speaker across both trials. Since male gobies have quite high site fidelity (Marentette et al., 2011) and inter-male variability is greater than intra-male variability (Chapter 2), females may have made decisions based on previous experience from trial 1.

The GLMs performed did not show evidence of female choice based on latency of response or time spent in the arena of the speaker of choice. This may be due to the female only having the choice of two males that were randomly selected from the group of 12 male calls. The random playback of two of twelve male calls was deemed necessary so as to avoid pseudoreplication that is so common in sound playback experiments (Slabbekoorn and Bouton, 2008). The limitation of using this method is that comparisons across the entire data set are made. Trends that are clear from individual choices become impossible to discern when examined as a whole. While the individual trials show the female spent more time with the males with longer IPIs, the individual variation of these female choices was great enough to mask patterns present in the overall data set. Females did not spend the most time with the speakers producing the largest IPIs, instead spending roughly equal amounts of time with whichever male of the pair presented to them had the longer IPI. The discrepancy caused by comparing randomly chosen calls could perhaps have been alleviated by having groups of calls from which females could randomly choose, so that a long IPI call would always be compared against a short IPI call. Using

this method would prevent pseudoreplication of only offering the female two choices, while simultaneously presenting two very clear choices that would be easier to analyze.

Certain factors that may be important to female choice were not examined in the current study. The sound pressure level of many species is also a key factor in female choice (Searcy, 1996; Nandi and Balakrishnan, 2013), however all calls were played back at a standard of 140 dB re 1 μ Pa, preventing me from examining this as a potential factor. Likewise, where calls were played continuously to encourage female responsiveness, duration of the call could not be examined as a measure of female choice. Duration is a significant predictor of male size in this species (Chapter 2) as well as in some others (McKibben and Bass, 1998). Future studies delving into female acoustic preference in this species could attempt to modulate these two factors to determine their importance to female decision making.

The current study is one of only a few studies that have examined and found acoustic honest signalling in fish from the female choice perspective. Female choice is critical to the distinction of true honest signals from physiological by-products. This is especially true for acoustic signals, where the characteristics of a call will be highly dependent on the mechanism of production. From an allometric stand point, one would expect call characteristics to scale with the size of the call producing mechanism. While the sound producing mechanism is not known in the round goby, other goby species create sound using the head region (see Chapter 1) and Chapter 2 of this thesis shows that body characteristics interact in complex ways with male head size to alter call characteristics. Future studies examining bioacoustics as honest signals should be sure to incorporate female choice experiments before acoustic signals are deemed honest. Further

research needs to be done to determine what specific benefits female round goby receive by choosing larger males.

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Chapter IV

CONCLUSIONS AND RECOMMENDATIONS

This thesis expands our understanding of the roles of bioacoustics in fish, particularly in terms of honest signals and male call variation. Previous work from our lab showed that the round goby is capable of localizing to a sound source (Rollo et al., 2007), distinguishing conspecifics from heterospecifics (Rollo and Higgs, 2008) and that auditory responses are dependent on the sexual condition of the fish (Zeyl et al., 2013). We have now also added that calls correlate strongly with male body characteristics and that females are capable of detecting these differences.

In Chapter 2, I collected calls from round goby at night in the presence of a reproductive female and analyzed several characteristics of these calls. Principal Components Analysis showed a strong association among Interpulse Interval (IPI), Total Length (TL), Total Weight (TW) and Head Width (HW) as well as links between Dominant Frequency (DF), Call Duration and Number of Pulses. Gonadosomatic Index was not highly related to any call or body features and was discounted from further analyses. Generalized Linear Models used individual call characteristics as dependent variables and incorporated the remaining body characteristics as explanatory variables. Interaction terms in the models were significant for all three call variables. In the case of frequency and IPI, the interactions between HWxTL and HWxTW worked in opposite directions. The three-way interaction of HWxTLxTW that affected call duration showed that a decreased TW greatly decreased call duration. The shortest calls were produced by long, thin males with big heads.

These results suggest that there is a very complex interplay between male body morphometrics and the calls that are produced. The fact that calls do correlate to male

body condition also provides evidence of the potential that these calls function as true honest signals of male body size. The next Chapter focused on validating whether or not females actually could differentiate between male calls and would have a preference for certain call features.

Chapter 3 used 12 of the calls recorded from the male portion of the study to determine if female round goby would display a preference for call characteristics when presented with 2 randomized calls. Calls were played continuously with no breaks for the duration of each trial and female responsiveness in the form of latency to respond, initial speaker of choice and time spent in each speaker zone was quantified. While there was no relationship with latency to respond, there was a pattern observed for time spent in the speaker zone with regards to IPI of the calls being played. The pattern was similar when considering the IPI and the initial speaker of choice, albeit not as strong. The chances of females randomly choosing the longer IPI in 15 out of 21 trials was extremely low ($P(x=15) = 0.0259$), suggesting that female round goby prefer male calls with a longer interpulse interval.

Taken together, the results of both chapters suggest that females prefer males that have large heads and high body mass. Male length affects call parameters in an unexpected way, making the effects of individual body characteristics much harder to discern. While the frequency of a male call was also strongly correlated to an individual's body morphometrics, females did not show any preference for this trait. Male size is known to alter call frequency in several species of fish (Crawford et al., 1997; Connaughton et al., 2002); however, females may be more interested in calling effort typically presented by better condition males than simply the largest males (Amorim et al., 2010). Males that put forth greater effort during courtship may be less likely to

cannibalize their brood, perhaps because of greater energy reserves (Forsgren, 1997). If interpulse interval is directly related to male energy reserves, the female would benefit from increased survival of her young. Further studies into honest signalling in round goby could assess male body lipid content and how this body characteristic affects courtship and paternal care.

Our understanding of the ecology of this invasive species is slowly increasing. Greater knowledge of how the round goby breeds is important to help prevent the spread of this species and other future invaders from this family. Incorporating passive acoustic monitoring into management practices could allow for more specific targeting of the larger more desirable males, and aid in early detection of a moving invasion front. Round gobies at the edges of an established area that disperse to establish new populations are often larger (Brandner et al., 2013) or much more aggressive (Groen et al., 2012). Using acoustic monitoring to determine which invasion fronts are more likely to be successful will more effectively slow the spread of this invasive species. While I realize that the round goby will likely never be successfully eradicated from the regions in which it has now become established, it is still important to prevent the movement of this species into new bodies of water.

This study is also very important in terms of the overall understanding of fish bioacoustics and understanding the diverse uses of underwater acoustic signalling. There are important benefits of determining in which fish species acoustic cues reliably signal mate condition, especially as anthropogenic impacts on aquatic ecosystems increases. Increasing our understanding of fish reproduction could aid us in reducing the detrimental effects of human activities. Where the Gobiidae is such a large family, there is still much to learn about the individual species within this group. It is also very likely that a great

many more species than the ones highlighted in Chapter 1 are soniferous. Ideally, field studies will also be done on this topic as acoustic laboratory studies usually cannot be used to represent the natural environment. The restrictions of the tank affect the sound being produced by the animals, preventing a direct comparison between lab and field-based studies.

True acoustic honest signalling in fish is not widely examined. This thesis highlights the importance of confirming the occurrence of female choice before signals can be labeled as honest indicators of male “quality”. The confirmation of female preference for certain male call variables in the round goby is one of the few studies to take this approach to honest signalling in fish. This study also highlights the abilities for sound detection in a fish with no swim bladder or auditory accessory organs.

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