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Universidade do Algarve
Faculdade de Ciências e Tecnologias
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Mestrado em Biologia Marinha

Trabalho efetuado sob a orientação de:
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Declaração de autoria de trabalho:

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída

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Resumo

O góbio pintado, *Pomatoschistus pictus*, é uma espécie de peixe marinho bentónico que habita em ambientes costeiros em substrato arenoso ou rochoso. A época de reprodução ocorre de Janeiro a Março quando os machos cortejam as fêmeas ao realizarem várias exibições visuais e vocais. Depois da desova, as fêmeas abandonam o ninho, ficando responsável pela descendência. Machos que apresente um maior conteúdo lipídico aparentam investir mais na corte acústica do que na corte visual, que sugere que a atividade acústica é um bom indicador das reservas lipídicas do macho. Como tal, machos com uma taxa de canto elevada podem indicar uma melhor competência no cuidado parental, visto que esta depende da sua condição. Foram testados os efeitos de diferentes taxas sonoras na escolha de parceiro pela fêmea através de emissões de playback de modo a determinar se os machos com taxas de canto mais elevadas são, de facto, preferidos pelas fêmeas. Durante cada experiência a fêmea podia escolher entre machos de tamanhos similares que estavam associados a diferentes taxas de canto, sendo estas elevadas ou reduzidas. Consistentemente, a fêmea mostrou preferência pelos machos associados à taxa de canto mais elevada, tendo em conta que estas tinham acesso aos machos. Porém, quando as fêmeas não tivessem contacto visual com os machos, não era demonstrada nenhuma preferência. A preferência da fêmea aparenta ser somente determinada pela taxa de canto pois a corte visual total assim como a condição do macho (fator K) eram similares entre machos associados a taxas de canto elevadas e reduzidas. Estes resultados sugerem que a preferência das fêmeas é determinada por uma intensidade de corte acústica mais elevada pois esta pode ser um indicador de qualidade parental.

Palavras chave: *Pomatoschistus pictus;* escolha de parceiros, comunicação acústica; emissões de playback; atividade de chamamento

Abstract

The painted goby *Pomatoschistus pictus* is a small marine benthic fish species that inhabits coastal sandy, rocky or gravel substrates. Breeding occurs from January to May, when nesting males court nearby females by performing displays with both visual and vocal components. After spawning females abandon the nest while the males take care of the brood. Males with a higher lipid content appear to invest more in acoustic (drum signals) rather than in visual courtship, suggesting that drumming activity is a good indicator of male fat reserves. Thus, males with higher acoustic activity could be signalling better male quality and likely better parental care abilities, as the latter is typically condition-dependent. Here we tested the effect of different calling rates on female mate choice with playback experiments to assess if the males with highest acoustic signalling rates are the most successful in mating. During trials females could choose between matched size males associated with either high or low calling rate. Females consistently showed preference for males associated to high drum playback rates, provided they had access to the males. However, if females did not have visual contact with males, no preference was shown. The females preference appeared to be solely determined by the calling rate since the total visual courtship and male condition (K factor) were similar between males associated with high and low calling rates. Taken together these results suggest that females select males based on higher acoustic courtship intensity because this may be an indicator of parental quality.

Key words: *Pomatoschistus pictus*; mate choice; acoustic communication; playback experiments; calling activity

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Chapter I – General Introduction

1.1 Introduction

Fishes are known to rely on their sensory systems to successfully extract important information from the environment (e.g. Amorim *et al*., 2015). Such exchange of information may occur via acoustic, chemical, vibrational, olfactory, electrical and visual cues/signals (Sargent, Rush, Wisenden, & Yan, 1998) and includes conspecific communication. Wiley (1994) defined acoustic communication as an alteration in the behaviour of the receiver as a consequence of a signal's reception in the form of sound. Acoustic communication is highly convenient in the aquatic environment allowing for large distance signalling in a medium where visibility is usually low (Amorim *et al*., 2015; Ladich & Fine, 2006; Popper & Schilt, 2005). Despite the increasing number of fish species known to vocalise and detect acoustic signals, the role of acoustic communication remains poorly documented in fishes (Amorim & Neves, 2007; Amorim, *et al*., 2015; Maruska, *et al*., 2012). This is in part due to the limited behavioural responses exhibited by fish in lab conditions while field research is challenged by ever-changing weather conditions, poor visibility and limited access to underwater environments. In addition, there is a lack of commercial underwater loudspeakers that can accurately reproduce low frequency fish pulsed sounds, thus further limiting playback experiments (Zelick et al., 1999; Fonseca, & Alves, 2013).

Nevertheless, understanding the role of different communication channels in fish behaviour, namely the acoustic one, is fundamental to understand the evolution of communication in vertebrates. Here we will focus on the role of acoustic communication in mate choice decisions in fish.

1.1.1 Sexual selection

Individuals of the same sex and species often exhibit different traits or characteristics that are modelled by sexual selection (Endler & Basolo, 1998; Lindström & Mary, 2006). Sexual selection is a process that occurs when there is competition over potential sexual partners leading to a higher or lower reproductive success of the individuals that present the most advantageous traits (Forsgren, 1997; Parker & Partridge, 1998). According to Miller (2001) sexual selection can also be defined as a selective process that plays an important role in the evolution of a species since it can affect several biological phenomena, such as variations in phenotypic and genotypic traits.

Darwin distinguished two main components of sexual selection: intra-sexual and inter-sexual selection. Intra-sexual selection manifests in the form of competition between individuals of the same sex (usually males), like male-male competition or territorial defence (Endler & Basolo, 1998; Sargent et al., 1998;). This type of selection usually leads to the development of traits that will benefit the male increasing its mating success, like increased body size or weaponry (Forsgren, 1997; Sargent et al., 1998). In inter-sexual selection females choose potential mates with the most advantageous morphological and behavioural characteristics that reflect male condition, such as body size, parental care, courtship, coloration and territory defence from predators and/or intruders, as a way to enhance the viability and survival of the offspring (Amorim *et al.*, 2015; Andersson, 1994; Knapp, *et al.*, 1991; Lehtonen, *et al.*, 2007). Furthermore, female preference may not be based on only one characteristic but a combination of them. For example, Lehtonen *et al*. (2007), observed that, in the case of *Pomatochistus minutus*, mate choice did not depend on male body size or nest size alone since female preference relied on the combination of these two cues, as larger males with larger nests were preferred over larger males with small nests.

According to Darwin, mating decisions should benefit the fitness of both individuals. Both females and males equally contribute to the genetic pool of the offspring. However energetic effort is much higher for females than it is for males. This is due to the fact that females spend a great deal of energy producing eggs, making them the limiting sex (Andersson, 1994). This means that their reproductive success is mainly limited by the number of eggs they can produce, as opposed to male's reproductive success, which is mainly limited by the number of females they mate with (Williams, 1975). Because females spend more energy in the reproductive process, they must make sure to choose males with high quality/condition that will ensure the offspring's survival and enhance the females' own individual fitness (Andersson, 1994).

1.1.2 Agonistic interactions

Resources like food, shelter, nesting sites, or availability of mates are often scarce. Therefore, in order to survive and reproduce, individuals (usually males) will compete over these resources (Pereira et al., 2009). The outcome of such interactions is strongly determined by body size, weaponry, motivation, experience or prior residence of the contestants (Hsu & Wolf, 1999; Parker, 1974). Sound production plays an important role in territorial defence, as agonistic sounds act like a warning signal towards possible intruders (Pereira et al., 2009). They are produced by fish in the early stages of conflict when males try to figure out each other's fighting capabilities and motivation. This suggests that sound may convey important information regarding the contestant's fighting abilities. Studies have shown that sound emission can greatly influence the outcome of territorial defence contests (e.g. Valinsky & Rigley, 1981; Vasconcelos, *et al*., 2010). For example, in the case of the damselfish *Stegastes partitus* (Pomacentridae) emission of sounds from artificial nests greatly discouraged territorial invasion (Riggio, 1981).

Such vocalisations are often complemented by visual displays, like colour changes or body size enhancement e.g. by expanding fins or opening opercula. For example in the painted goby, *Pomatoschistus pictus*, fin erection, frontal and/or lateral displays and quivering are common aggressive behavioural displays (Pereira *et al*., 2009).

1.1.3 Mate choice

Mate choice decisions often involve the exchange of information depending on the sensory adaptations of the individuals involved in the process and on the environment (Forsgren, 1997). Females can select males based on several traits, like visual cues (body size, coloration, courtship intensity or ornaments), chemical cues (e.g. pheromones), electric cues (e.g. electric pulses) or acoustical cues (e.g. advertisement calls) (Amorim & Neves, 2007; Amorim et al., 2015; Endler & Basolo, 1998; Sargent et al., 1998; Vasconcelos et al., 2010, 2011). Furthermore mate choice decisions may also be based on benefits that the female obtains, like nuptial feeding, territory quality or parental care (Anderson, 1994). Moreover, some studies showed that mate choice can also be influenced by other factors, such as availability/density of males, timing and levels of oxygen in the water (Jones & Reynolds, 1999; jo *et al.,* 1995; Kvarnemo & Forsgreen, 2000).

Several studies have tried to figure out why mate choice exists and what particular traits are being chosen over others. Five main tactics for mate choice have been identified (Real, 1990; Janetos, 1980): 1) sequential comparison tactic; 2) random mating; 3) threshold-criterion tactic; 4) pool comparison tactic and 5) one step decision tactic. In sequential comparison tactic females compare two recently encountered males and chose one according to some rule. In random mating females will mate with any male with equal probability. In threshold criterion tactic, the chosen males are the ones who present some specific characteristic. In pool comparison tactic, females chose the preferred male among a number of potential mates. In one step decision tactic females decide whether to accept or reject the potential male at each encounter, in this tactic females begin by being very selective and choosy, becoming less and less as time runs out.

Most studies on mate choice decision focus on visual cues (Lehtonen et al., 2007). Visual communication may be useful when assessing courtship, territory quality, body size, coloration and nest characteristics (Bolgan et al., 2012; Pedroso et al., 2013). Visual courtship plays an important role in mate choice, since it can increase the receptivity of the female (Oliver & Lobel, 2013). For example, in *Oreochromis niloticus* spawning will occur even though there is no physical or chemical contact between males and females (Castro et al., 2009). Chemical cues also have an important role in mate assessment and reproductive behaviour (Sargent et al., 1998). However, most of these studies focus on females detection by males (e.g. Dulka et al., 1987; Sorensen, 1992). In some fish species, females may use chemical cues to assess the reproductive state of the male and respond accordingly. Some species of fish, for example *Oreohromis mossambicus* males release urine when in the presence of females because it may contain pheromones that reflect the reproductive condition of the male and may influence the receptivity of the female (Stacey, 2006; Almeida et al., 2005). Moreover, during courtship male threespine sticklebacks (*Gasterosteus aculeatus*) release sex pheromones that attract prespawning females and repel other males (Waas & Colgan, 1992). Electric communication as also been linked to reproductive behaviour in several studies (Moller, 1995). Electric pulses emitted by males may contribute to mating success since bigger and more intense electric discharges attract females because they reflect more developed electric organs (Tobias et al., 2011). Electric signalling may also serve a purpose in communication, orientation, courtship and territorial interactions of fishes (Maruska et al., 2012; Tobias et al., 2011).

While the role of acoustic signalling regarding territoriality (e.g Parker et al., 1998; Pereira et al., 2009) and courtship (e.g. Amorim, et al., 2013a, b; Maruska et al., 2012) has been studied by many authors, studies on the function of acoustic communication in mate choice are scarce. In the few studies where mate choice has been addressed females seemed do have preferred vocal features associated to male condition and other quality aspects. For example, females of the painted goby, *Pomatocistus pictus*, show preference for males with longer and more intense bursts that reflect the male's condition (Amorim et al., 2013b). However, female preference for calling activity has never been experimentally ascertained.

1.1.4 Sound production mechanisms

Fish bioacoustics is a research field in expansion and studies on sound production mechanisms in fish are increasing continuously (Ladich & Fine, 2006; A. N. Popper & Schilt, 2005). Since teleost fishes have a great diversity of sound producing mechanisms they serve as good models for understanding the evolution process of vocal communication in vertebrates (Ladich & Fine, 2006; Popper & Schilt, 2005).

Sound production can occur through diverse mechanisms, depending on the species (Popper & Schilt, 2005). Unlike birds and mammals that have homologous sonic mechanisms, fish exhibit a wide range or sound producing mechanisms that appear to have evolved independently (Fine & Parmentier, 2015). However, a simple classification of these mechanisms has not yet been defined. According to Ladich and Fine (2006) sound production occurs through either the swimbladder or stridulation with bones involvement. The most commonly known swimbladers' based sound producing mechanism is the rapid contraction of drumming/sonic muscles. Several types of muscles can be involved, which based on their origin and insertion are classified as intrinsic drumming muscles, that are attached to the swimbladder, and extrinsic drumming muscles, that originate on several other structures like vertebrae, skull and ribs (Ladich & Fine, 2006). Besides the contraction of drumming muscles, rubbing the pectoral fins against the body can also excite the swimblader (Moulton, 1960).

In addition to swimbladers, fish have evolved other mechanisms for sound production. These are located in the neurocranium and include pectoral adaptations that affect the pectoral fin rays, the pectoral girdle and fin tendons (fig. 1.1). These pectoral sound generating mechanisms include pectoral girdle vibrations, pectoral tending plucking and pectoral spine rubbing.
Sound production organs are usually more specialized in males and vary intrasexually according to age, size and species (Ladich & Fine, 2006).

Few research has been done on sound production in Gobies (Parmentier et al, 2013), but the mechanism(s) is (are) not yet fully understood and some of the assumptions advanced were not demonstrated. According to Lugli et al., (1996) sound production in *Padogobius nigricans* results from specialized muscles on the pectoral girdles (fig.1.1). However, the same author suggested that in the case of *Padogobius martensii* sound could be due to the contraction of muscles on the swimbladder (Lugli et al., 1995), but such muscles were not identified.

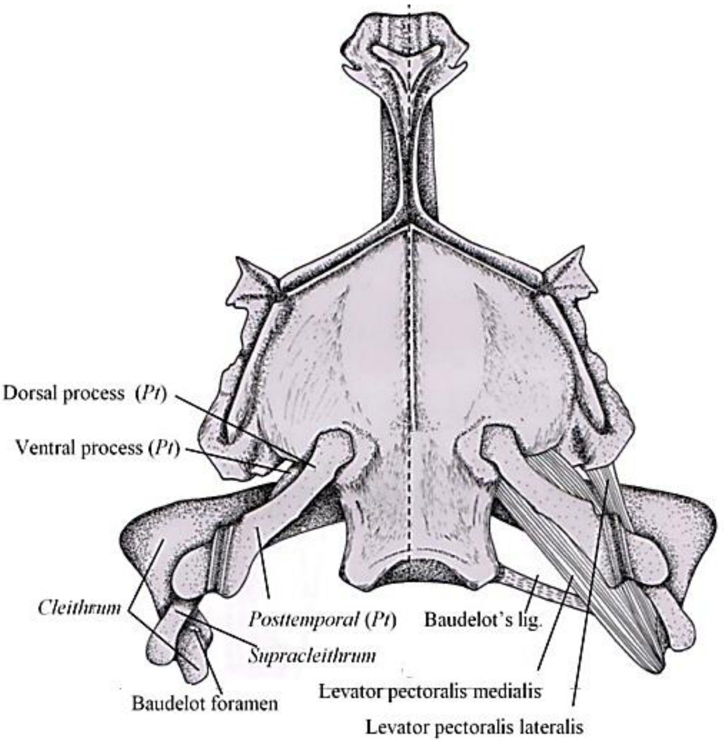


Figura 3.1 Dorsal view of the neurocranium of *Gobius paganellus* depicting the sonic muscles on the right and the pectoral girdle on the left. Adapted from Parmentier et al., 2013.

1.1.5 Sound detection mechanisms

Sound detection is accomplished through receptors localized in the internal ear, since fishes do not possess an external or middle ear (e.g. Ladich & Fine, 2006; Popper *et al.*, 2003; Popper & Schilt, 2005). The internal ear of bony fishes is comprised of three otolithic end organs (utricle, saccule, and lagena) and the semi-circular canals (Popper & Schilt, 2005). The otolithic end organs are responsible for determining the head position in relation to gravity (like in terrestrial vertebrates) and most are likely involved in sound detection (Popper, *et al.*, 2003; Popper & Schilt, 2005). Each otolith end organ contains a calcified structure (the otolith) that overlies the macula, which is a sensory epithelium that contains hair cells (Ladich & Fine, 2006). The movements of the otolith relative to the macula, determined by the different acoustic impedance, cause the hair cells' cilia to deform, thus stimulating neural transmission that is processed by the brain and interpreted as sound (Moyle & Cech, 2004). Moreover, these hair cells present specific orientation patterns that may allow the fish to determine the direction of incoming sound (Popper *et al.*, 2003; Popper & Schilt, 2005). Nevertheless, the precise role of each end organ is yet unknown and the way they contribute to the detection of sound varies among species, since each have different shaped otoliths (Bradbury & Vehrencamp, 2009; Popper *et al*., 2003; Popper & Schilt, 2005). For example, regarding the otophysan fishes the saccule may be the primary sound detector, due to the connection of this organ to the swim bladder (Popper & Schilt, 2005), while in clupeiform fishes, sound detection role may be attributed to the utricle (at least for higher frequencies) (Higgs *et al*., 2004; Mann, 2012; Popper *et al*., 2007). Bolgan et al., (2012) showed that hearing sensitivity of *P. pictus* is higher for lower frequencies (between 15 and 400 Hz). Furthermore, the author suggested that the improved hearing sensitivity for lower frequencies could be the result of an evolutionary adaptation, helping with the detection of predators, prey and mates (Bolgan et al., 2012).

1.2 The Gobiidae family

The gobiidae family is the largest marine fish family, being mostly comprised of benthic species (e.g. Miller, 1986). It has reportedly 230 genera and 1875 species (Hoese, 1998). Most species inhabit marine coastal waters, while others are adapted to brackish or even freshwater environments (Miller, 1986). They are commonly found in tropical and subtropical regions. However their distribution is worldwide, thus suggesting that this family has great adaptation abilities, since they successfully settled in a variety of different ecosystems (Miller, 1986). Most species in the gobiidae family are small sized fishes, no longer than 10 cm.

The Gobiidae family comprises many genus, one of them being *Pomatochistus*, which comprises a total of 12 species: *P. montenegrensis, P. norvegicus, P. quagga, P. tortonesi, P. bathi, P. canestrinii, P. knerii, P. lanzanoi, P. marmoratus, P.microps, P. minutos and P. pictus*; the latter being the one used for this study.

1.2.1 *Pomatochistus pictus* (the painted goby)

Pomatochistus pictus is a teleost fish usually found in the Eastern Atlantic Ocean and Mediterranean Sea (fig. 1.2). The painted goby is a benthic species that inhabits coastal sandy, rocky or gravel bottoms up to 55 meters deep. It lives up to 2 years, attains a maximum of 57 mm in length, reproducing from February to July, depending on the region (Miller, 1986). Small crustaceans, like amphipods or copepods, are their main source of food (Bouchereau et a., 2003). *Pomatochistus pictus* body is usually short and rounded, with rounded caudal fin and dorsal eyes (fig. 1.3) (Patzner, *et al*., 2012). The two dorsal fins, one with soft rays and the other with spinous rays, have dark spots displayed in a row with pink and blue bands (Patzner, *et al*., 2012). Its colouration its yellowish making it very difficult to distinguish from sand, suggesting this may be an adaptation acquired to avoid predation or hunt for food without being detected by the prey (Patzner, *et al.*, 2012; Bouchereau *et al.*, 2003). They usually look for shelter under empty shell valves or rock crevices, where they build their nests by covering them with sediment (Bouchereau *et al*., 2003).

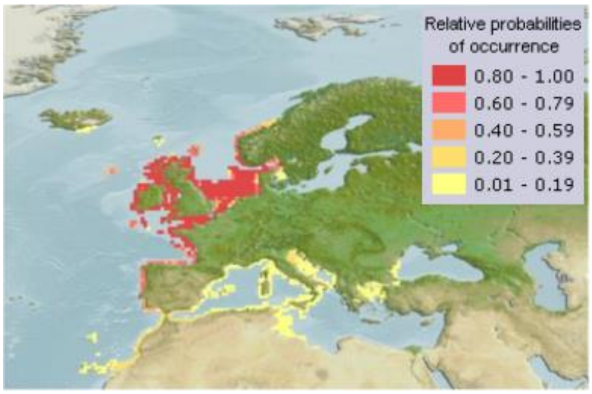


Figura 1.2 Distribution map of *Pomatochistus pictus*. Retrieved from: http://www.aquamaps.org/receive.php?type_of_map=regular

Figura 1.3 Painted Goby *Pomatoschistus pictus*. Retrieved from http://www.jonolavsakvarium.com/blog/200708/sand_goby.jpg

1.2.2 Acoustic signalling in Gobies

Gobies are highly vocal fishes; they emit low pitched, short sounds, with low dominant frequencies (bellow 1 kHz) (Lugli, *et al.*, 1997). Gobies generate sounds during agonistic interactions or in the reproductive season, during courtship, both in pre-spawning or spawning events (Lugli et al., 1997).

Few studies have focused on sound production mechanisms in gobies (Parmentier et al., 2013). Experimental manipulations suggest that the swimbladder is not involved in sound production, mostly because many species do not have a swimbladder, and the contraction of the levator pectoralis muscles and the vibration of the large radials were recognised as involved in sound production in *G. paganellus* (Parmentier *et al.*, 2013). According to Lugli *et al.*, (1997) most of the sounds emitted by gobies appear to be non-harmonic, i.e., composed of unrelated frequencies. In other fishes, non-harmonic sounds are originated by stridulation or by scraping the teeth of the lower and upper jaws (Tavolga, 1964). According to Takemura (1984) this could be how the male goby *Odontobutis obscura* produces acoustic signals. In addition, Lugli *et al*. (1996) suggested that muscle contraction on the pectoral girdles could be responsible for sound production in *Padogobius nigricans.* Moreover, Myrberg and Stadler (2002) proposed that in the case of *Bathygobius curacao*, sound production was made by ejection of water through the gill openings.

The different types of sounds studied in gobies can be gathered into 4 major groups:

- Tonal sounds, which consist of fast pulse repetition rates resulting in a tone with sinusoidal form; the repetition rate of tonal sounds decreases throughout the emission (Lugli et al., 2009) (fig. 1.4D)
- Pulsed sounds or drums characterized by slow rate of pulsed trains that can vary in amplitude (Amorim & Neves, 2007) (fig 1.4A)
- Complex sounds, which show a combination of the previous two (Lugli et al., 2009) (fig. 1.4C)
- Thumps, which are non-pulsed low frequency sounds (under 100 Hz) (M. C. Amorim & Neves, 2007) (fig. 1.4B)

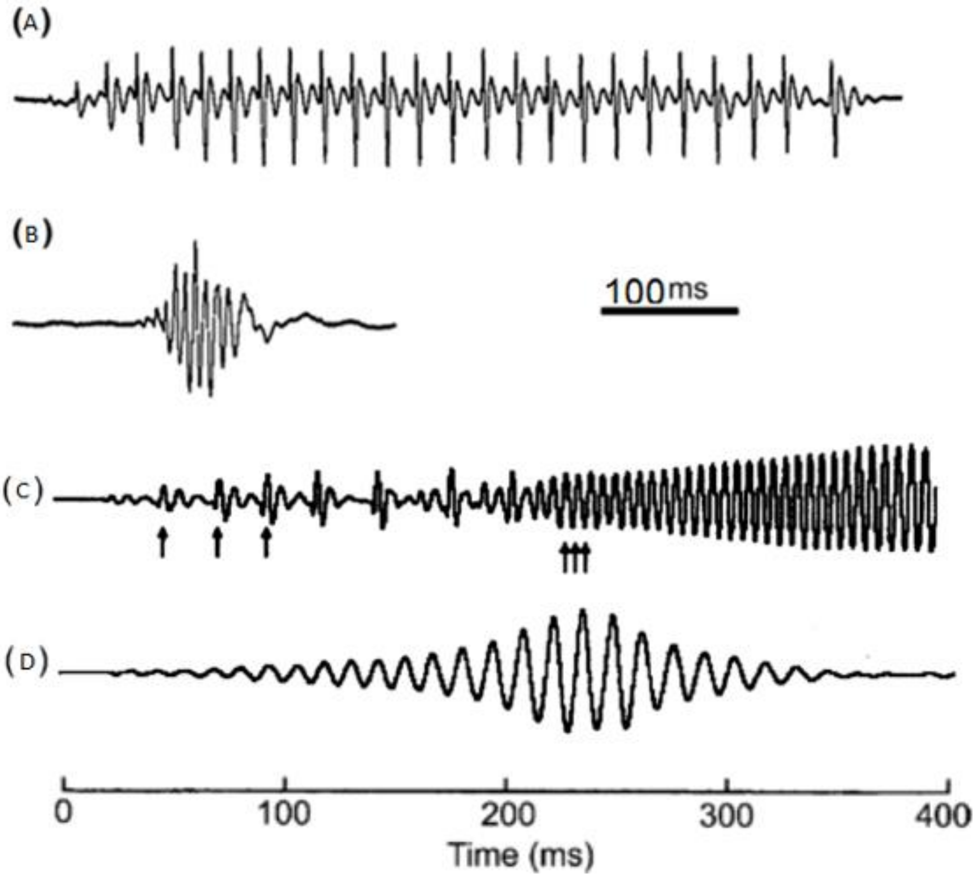


Figure 1.4. Oscillogram of acoustic signals of four species of gobies. Drumming sounds emitted by the *Pomatoschistus pictus* (A); Thump sounds of *Pomatoschistus pictus* (B); Complex sounds emitted by Padogobius martensii (C) and tonal sounds of *Padogobius nigricans* (D). The arrows depicted indicate the beginning of three pulses. (Adapted from Lugli et al., 2009; Amorim et al., 2013a).

Amorim *et al*. (2013a) distinguished between two major sound types produced by *P. pictus*. One was a thump-like sound, made up by a long sound wave that lacked a pulsed structure; the other one, a drumming sound, more common than the previous, was composed of repeated short, low frequency pulses (fig. 2, appendix I) (Amorim *et al*., 2013a). Males produce thumps and drums in order to attract females and drums only when in agonistic situations (Amorim & Neves, 2007; 2008).

1.2.3 Courtship behaviour of gobies

As mentioned previously, gobies are known for vocalising in several occasions. During courtship, some goby species modulate sound production, as they emit different acoustic signals according to the courtship phase or the female's position in the male territory (Lugli et al., 2009).

Male acoustic signalling can either occur inside or outside the nest. When vocalising outside the nest it is common for males to also engage in visual courtship (e.g. Pedroso et al., 2013). For example the male sand goby, *Pomatoschistus minutus*, will engage in a series of quiver displays inside the nest with its head out while emitting drum sounds (Lindstrom & Lugli, 2000). These signals are considered pre-spawning calls as they are used to attract females into the nest (Amorim & Neves, 2007).

In another goby species, *Knipowitschia punctatissima*, drum sounds are only produced when the females are inside the nest (Lugli et al., 2009). Furthermore, *Padagobius martensii* produce different sounds depending on the behavioural context as they produce drumming sounds when the female is inside the nest and tonal sounds during courtship (Lugli et al., 1996).

Generation of drumming sounds during pre-spawning and spawning events suggest that these vocal signals could have a role in mate choice because males usually only emit them inside the nest and females may enter a number of nests before deciding where to spawn (Lindström & Mary, 2006; Pedroso, *et al*., 2013).

In addition, drumming activity by males during courtship show inter-male variability in terms pulse repetition rate and sound duration, which could convey information on male quality and motivation (Amorim & Neves, 2008; Bolgan et al., 2013; Bouchereau et al., 2003; Lindström & Mary, 2006).

1.2.4 Mate choice and the role of acoustic signals in *P. pictus*

Goby females chose their mate based on several cues like courtship intensity, nest condition, nuptial coloration, acoustic signalling, etc. (Forsgren, 1997; 1992; Takahashi & Kohda, 2004). Lindstrom and Lugli (2000) suggested that acoustic signals produced within the nest by goby males during courtship may influence the female's choice, since they often enter more than one nest before deciding where to spawn. Moreover, in species where males provide exclusive parental care, parental qualities may be more important than phenotypic cues usually associated with mate choice (Amorim & Neves, 2007).

In the case of P. *pictus*, the intensity of calling activity is positively correlated with body lipid reserves, which suggests that lower body lipid reserves hinder an intense and sustained calling activity (Amorim et al., 2013a, b; Amorim & Neves, 2008). Males with a higher lipid condition invest more in acoustic signalling (drums) than in visual courtship thus suggesting that drumming activity is a good indicator of male fat reserves (Amorim *et al.*, 2013a). Thus, larger males with higher acoustic courtship rate and longer sound bursts could signal better parental care abilities and male quality (Amorim *et al.*, 2013a, b). Consistently, males with a higher drumming rate were more successful in mating, suggesting that visual courtship and body size are less important for mating success. Moreover, in Amorim *et al*. (2013a) females showed preference for males with higher acoustic activity but did not show preference for male body size. In another study, using playback experiments, Amorim *et al.*, (2013b), showed that female preference was greater for the male associated with courtship sound than the one associated with white noise or silence, suggesting that males that vocalise more often have a higher reproductive success.

1.3 Aims of the study

The aim for this study is to assess the role of acoustic communication in mate choice in *Pomatochistus pictus*, particularly the preference for different male calling rates, in order to assess if the males with higher acoustic signalling rates are the most successful in mating.

Chapter II – Materials and Methods

2.1 Study site and sample collection

The individuals were collected between January and April 2017 at Praia da Parede, Cascais (38° 41’N, 9° 21’W), (fig.2.1), during full/new moon low tides, approximately every 15 days, with the help of hand nets and dive torches during night time. Sample collection also occurred via SCUBA-diving in shallow waters at Arrábida, (38°28′ N, 8°58′ W) (fig.2.2).

After capture, the individuals were placed in a recipient with aerated seawater (Nirox X-18 air compressor) to prevent anoxic conditions until arriving at the laboratory. Once there, the individuals were sorted by gender and placed in stock aquaria, with no more than 8 fish per each aquarium. Gender differentiation was based on the observation of swollen/large bellies typical of pregnant females, and a black stripe near the genital papilla and/or red stripe near the dorsal fins characteristic of male nuptial colours (Bouchereau *et al*., 2003).

The individuals were fed daily a mixture of chopped shrimp, clam and mussel crumb. The light conditions followed a natural regime (12 hour light and 12 hour dark).

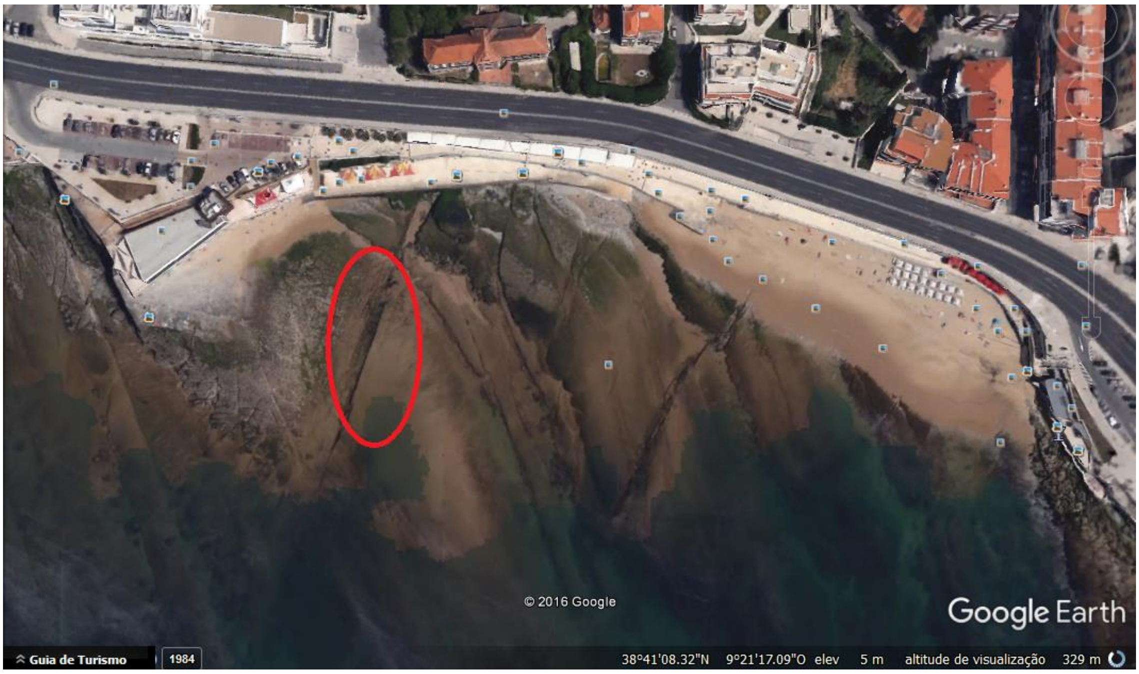


Figura 4.1 Sample collection location, Praia da Parede, Cascais, Portugal (38° 41’N, 9° 21’W). Red circle depicting the canal where the individuals were captured.

Figura 2.2 Sample collection location, Serra da Arrábida, Portugal (38°28′ N, 8°58′ W). Red circle depicting the canal where the individuals were captured by SCUBA diving (38º25’54.82’’N 9º04’37-75’’).

2.2 Experimental design and set-up

The experimental work took place at FCUL laboratory facilities, in Lisbon, where 17 stock aquaria (c. 18L, 24 x 24 x 32 cm) and 2 experimental aquaria (20 x 30 x 50 cm, c.35L) were used. Aquaria were filled with artificial seawater which was partially changed weekly. The stock aquaria were provided with a closed system responsible for the flow of artificial sea water that was be kept at approximately 16ºC by refrigerators (Hailea 1000A) (one per aquaria) and salinity at 31-33 g/L. The water system common to all stock aquaria included biological filters (bio balls), a skimmer (Tunze 9006) to remove excess protein and an ultraviolet filter (Vecton 600 Tropical marine centre UK).

The experimental aquaria were placed on top of two marble layers interspaced with rubber foam shock absorbers, which significantly reduce vibrations and floor borne noise to the aquaria, thus improving the quality of fish sound recording (Amorim *et al.*, 2013a).

Each experimental aquarium was provided with sand substrate (previously washed with freshwater) and two artificial PVC shelters (5.5 x 3 x 7cm, fig.2.3) placed in opposite ends of the aquarium. The inner side of the nests was covered with a plastic sheet that was removed after spawning occurrences, for egg observation and counting purposes. Each aquarium was divided into 3 sections by 2 removable opaque acrylic partitions, to prevent visual contact between the individuals (fig. 4, appendix I). A third removable partition, of smaller length, was placed in the middle of the aquarium to prevent visual interaction between males. The aquaria were lined with light green plastic to prevent visual contact between fish in adjacent aquaria.

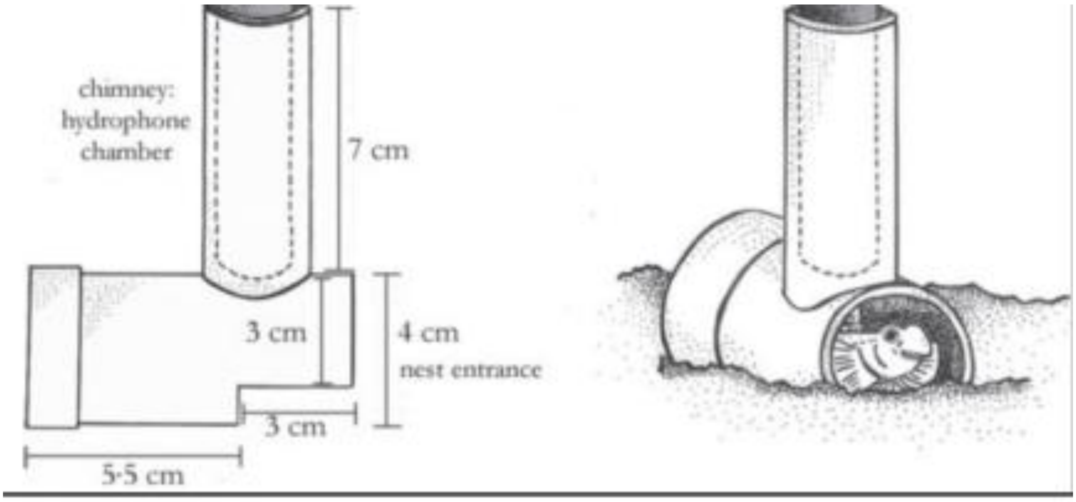


Figure 2.3: Male's artificial PVC nest depicting the hydrophone housed in the chimney. Adapted from Amorim *et al.*, 2013b.

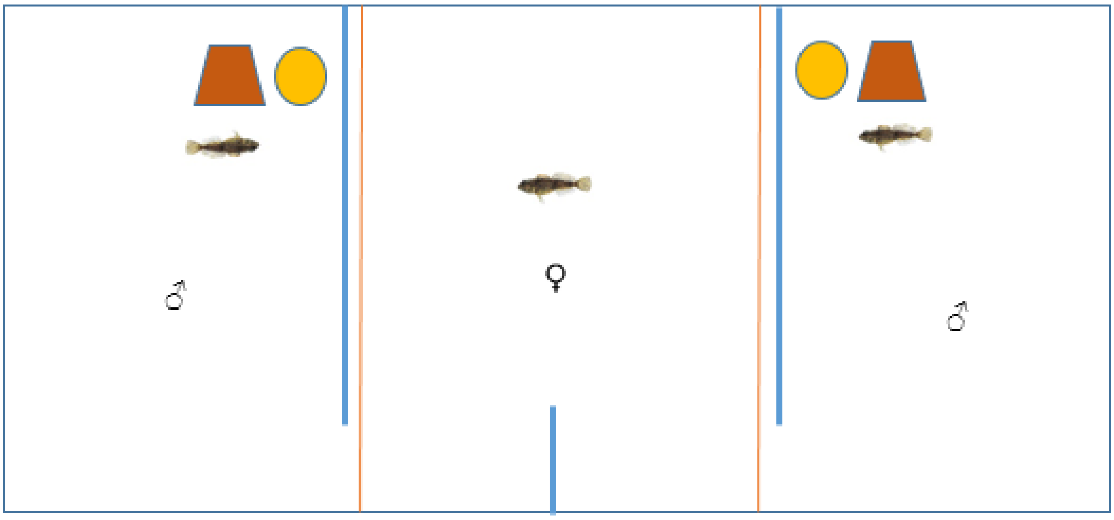


Figure 2.4: Experimental aquaria set up depicting the opaque acrylic partitions separating the aquaria, represented in blue; removable opaque partitions represented in red; the artificial PVC nests in brown; the playback speakers in yellow; the males (in opposite partitions) and the female in the middle partition.

2.3 Behavioural observations

A total of 39 experimental trials were conducted from January until April 2017. The test subjects, males and females, were occasionally used more than once due to the lack of gobies in the stock aquaria. Before each trial the individuals were measured and weight.

For each experimental trial two males matched in size were selected and taken from the stock to the experimental aquarium. Each male was placed in opposite compartments of the aquarium and left for 2 days to acclimatize until they became territorial (acknowledged by the male covering the nest with sand, nest occupation and male colouration). A pregnant female was then collected from the stock aquaria and placed in the middle compartment to stimulate males’ territorial and courtship behaviour. Nest sand coverage was made similar to each other before trials, to rule out female preference based on nest building abilities, which may be an indicator of male sexual motivation (e.g. Briffa & Sneddon, 2007; Pereira *et al*., 2009), since the aim was to test female preference regarding calling rates.

Before starting the trials, aeration and cooling systems of both experimental and stock aquaria, as well as the remaining aquaria in the lab, was turned off in order to reduce background noise during sound recordings. Each trial lasted one hour and comprised three periods: Pre-playback (PRE) (5 min duration), with sound stimuli playback but no visual contact between males and females, followed by a period (playback, PBK) (20 min duration) when the complete opaque partitions were removed and the female was allowed to freely interact with both males, keeping the sound playback. Finally, the post playback period (POST) consisted of a 40 min period with contact between individuals but no sound stimuli. This latter step was included to allow more time for females to spawn.

2.3.1 Sound recording, playback and analysis

Two hydrophones were placed next to each nest about 1 cm off the substrate to monitor sound playback and possible sound production by the males. The hydrophones Brüel & Kjær 8104 (Brüel & Kjær, Naerum, Denmark; frequency response from 0.1 Hz to 180 kHz, sensitivity -205 dB re1V/µPa) conditioned by a Brüel & Kjær Mediator type 2238 (Brüel & Kjær, Naerum, Denmark), and High Tech 94 SSQ (High Tech Inc., Gulfport, MS, USA; sensitivity 165 dB re. 1 V/µPa, frequency response within ± 1dB from 30 Hz to 6 kHz) were connected to an A/D converter device Edirol UA25 EX (Roland, Japan;16 bit, 8 kHz) controlled by Adobe Audition 3.0 software (Adobe Systems Inc., Mountain View, CA, USA).

Two underwater speakers were placed next to each nest, as close to the partition as possible (fig. 2.4) and emitted different playback stimuli. The playback treatment consisted of previously recorded sequences of drums emitted by four different gobies (see details in Amorim et al. 2013b). Each file contained sounds from a single male. Playback treatment simulated the males calling at high (8 drums/min) and low (2 drums/min) rates (see Amorim et al. 2013b). In the beginning of each trial the playback files were randomly selected, but the high and low rate were from the same male. The side of the aquarium in which the high or low rate was played was randomized to avoid aquarium side effects. Playback amplitude was similar in each nest, and similar to the average amplitude of drums emitted by the males in the nest (Amorim et al. 2013a). Natural courtship patterns were followed regarding the intervals between the playback calls. The speakers were connected to a home-made amplifier (Fonseca and Maia-Alves, 2012) fed through a D/A converter device (Edirol UA-25 EX) controlled by Adobe Audition 3.0 (Adobe Systems Inc., Mountain View, CA, USA).

During trials sound playbacks were real time inspected and recorded using Cool Edit Pro 2.1 for Windows. After each trial the acoustic file was saved for further analysis using Raven 1.2.1 for windows (Bioacoustic Research Program, Cornell Laboratory of, Ithaca, NY, USA).

2.3.2 Video recording and analysis

All 39 trials were video recorded (Sony handycam DCR-HC39). The camera was placed approximately 50 cm in front of the experimental aquarium in order to frame the part in which the encounters took place and zoom in for better observation of courtship behaviour. The camera output was digitalized to a laptop using Pinnacle Dazzle DVD Recorder Plus and Windows Movie Player. Video recordings were analysed using EthoLog (v2.2). Each recording was analysed three times; one for the assessment of female preference and the other two for quantification of courtship and agonistic behaviour of the two males.

For female preference assessment, each trial was separated into two phases: first phase, the pre playback period (PRE), when the female did not have access to the males, and second phase the playback and post-playback periods (PBK and POST), when the partitions were removed and the female had free access to the males. During the first phase virtual preference areas were created (fig 2.5). Female preference was evaluated by observing how long it spent in each preference area and in the middle of the central compartment. For the second phase six categories were created and accounted for: 1) time spent in each male compartment during playback and post playback period, after the partitions were removed; 2) nest entrances, i.e. the number of times the female entered each nest and for how long it stayed inside; 3) rolling, i.e. how many times the female rolled over once inside the nest; 4) spawning, accounting for if the female spawned and how many eggs it laid; 5) first compartment visited by the female after the partitions were removed.

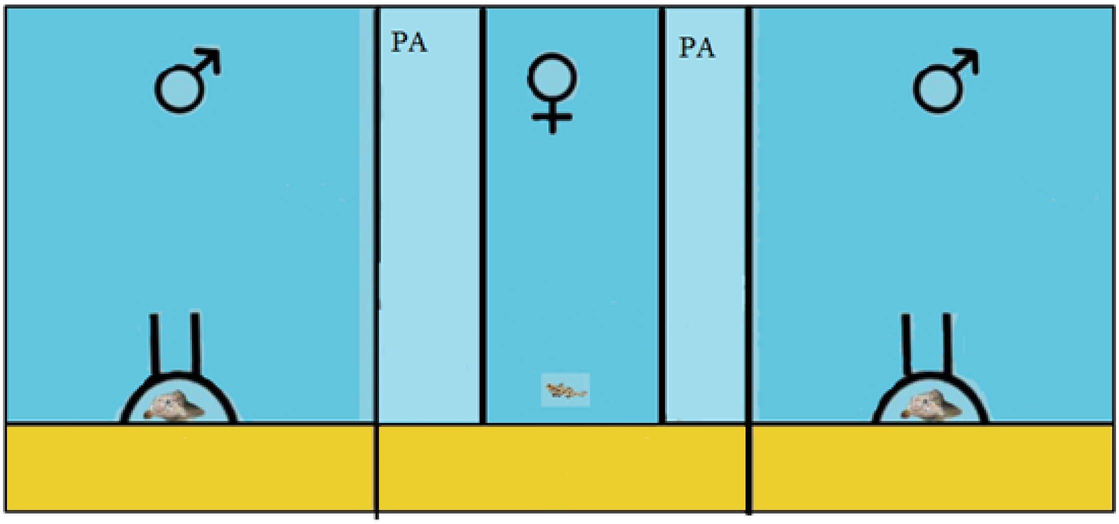


Figure 2.5 Experimental aquaria set up. Side compartments are occupied by size-matched territorial males while the female is in the middle compartment. Lighter blue areas "PA" indicate the preference areas of the female that consist in the areas next to the male partitions. The PA width was 5 cm which is approximately one body length.

Females that did not leave their compartment, did not approach a male's nest or did not show any reproductive behaviour or interest in any male were considered non responsive and discarded back to the stock aquaria.

Male behavioural analysis took place in the second phase of the experiment when the partitions were removed and the males had access to the females. Both courtship and agonistic displays were accounted for. Male K factor was calculated for all males.

Male K factor=$\frac{male\ weight}{male\ lenght^3}$

After all trials were concluded, all individuals were released back to their natural habitat.

2.4 Statistical analysis

Statistical analysis were conducted using the software Statistica (version 10, Statsoft, Tulsa, OK, USA).

To test for preferences for drum rate during the PRE period differences between time spent in preference areas by each female were compared using a non-parametric Wilcoxon matched pairs test. Similarly, the time spent in each male compartment during the PBK and POST periods, i.e. the number of times the female entered and how long it stayed inside the each male's nest, were compared with non-parametric Wilcoxon matched pairs tests.

To ascertain whether male behaviour did not influence female preference, total male courtship and total visual courtship were compared between the high rate and low rate treatments with analyses of covariance (ANCOVA), using playback treatment as a factor and the time the female spent in the male compartment as a covariate. Male behaviour data was log-transformed to follow the homogeneity of variances assumption. To verify for homogeneity of variances a Levene's test was carried out. To check for normality of data Normal p-p plots were performed. Finally, a Mann-Whitney test was conducted to check if male condition factor differed between high and low rate males.

Chapter III – Results

3.1 Female preference assessment

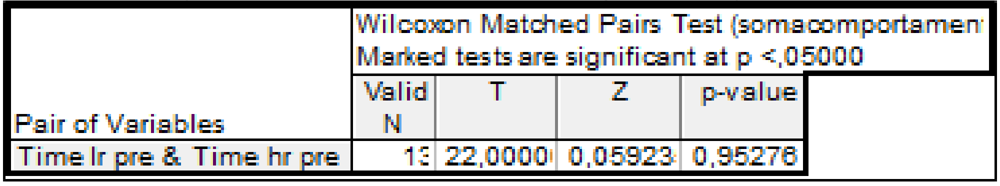
During the 13 trials, the same female was used twice, therefore the final sample is composed of 12 females that showed preference for one of the males. Each female had an ID that corresponded to the date of the trial, number of aquarium the trial took place, the playback treatment in each side of the aquarium and the sound stimuli used in each experiment (Table 3.1, Appendix I).

Five major female behavioural patterns were observed during trials: 1) escape, when male approaches the female, the female quickly swims away in the opposite direction; 2) not interested, when the female swims around but not showing any interest in the males' courtship; 3) interested, when the male approaches and starts courtship, the female turns its head towards the male; 4) follow, the female follows the male into the nest after approach and courtship, sometimes the female stops at the entrance of the nest while the male courts from inside; 5) enters the nest, after the male enters the nest the female often follows immediately or sometimes it waits before entering.

First phase of the experiment: (Pre-playback period with no visual stimuli)

Here we compared the time spent by the female in each preference area, high and low rates, during the 5 minutes of the pre-playback period to assess the female preference for low or high rate playback without visual or physical contact with the males. There were no differences between time spent in the high or low rate preference area (N=13, Z=0.06, P>0.05; Table 3.2, fig. 3.1), therefore showing no particular preference for low or high rate calls in the absence of visual and physical stimuli.

Table 3.2 Wilcoxon matched pairs test for the total time spent by the female between the low rate playback preference area and the high rate playback preference area



Wilcoxon Matched Pairs Test (somacomportamen
Marked tests are significant at p <,05000

Pair of Variables	Valid N	T	Z	p-value
Time lr pre & Time hr pre	1	22,0000	0,05923	0,95276

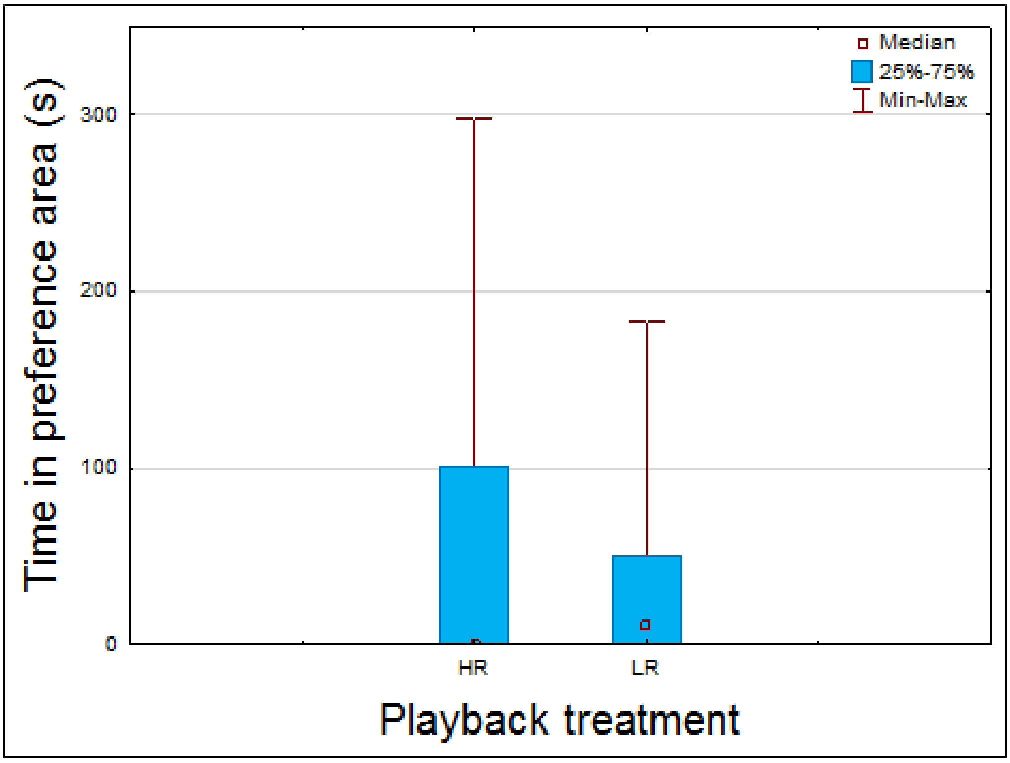
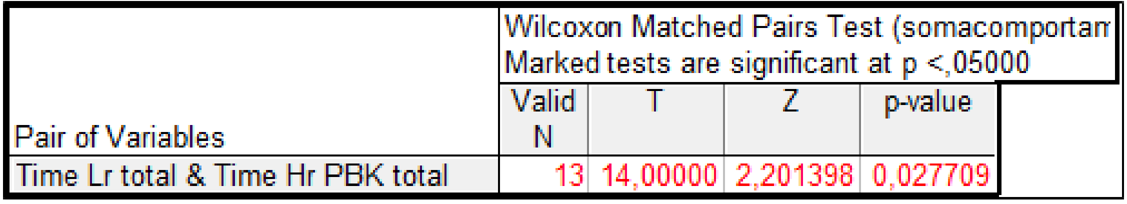


Figure 3.1 Box and Whisker plot comparing the playback treatments (high and low rate playbacks) with the time spent in each preference are by the female depicting the median, quartiles and maximum and minimum sample values for each treatment. High rate – HR; Low rate - LR

Second phase of the experiment: (Playback period with acoustic and visual stimuli)

Here we compared the total amount of time the female spent in each compartment during the whole 60 minutes (PBK + POST) to assess the preference for high or low playback calling rates with visual and physical contact with the males. The mean time the female spent in the high rate compartment was of 2085.46 seconds and in the low rate compartment of 545.17 seconds. A non-parametric Wilcoxon matched pairs test indicates that there was a preference for the high rate male (N=13, Z= 2.2, P<0.05, table 3.3). A box and whisker plot comparing the time spent in each preference area with the playback treatment shows a clear preference by the female for the high rate playback (fig 3.2).

Table 3.3 Wilcoxon matched pairs test for the total time spent by the female between the low rate playback compartment and the high rate playback compartment.



	Wilcoxon Matched Pairs Test (somacomportam Marked tests are significant at p <,05000			
Pair of Variables	Valid N	T	Z	p-value
Time Lr total & Time Hr PBK total	13	14,00000	2,201398	0,027709

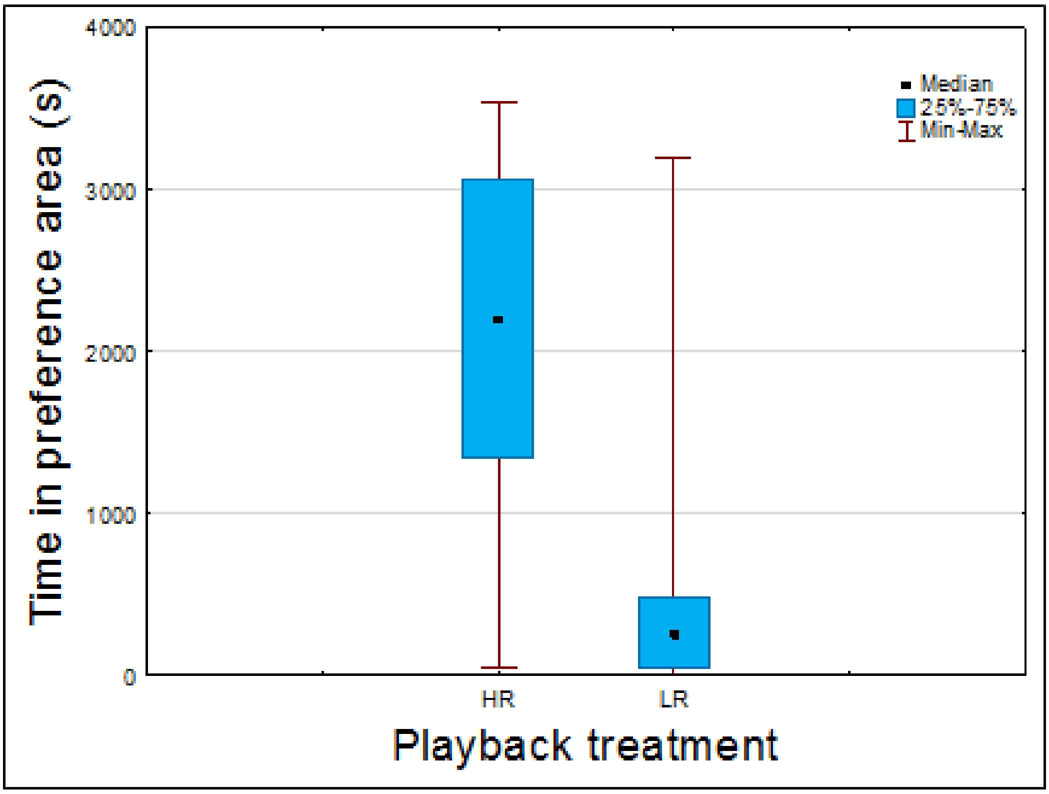
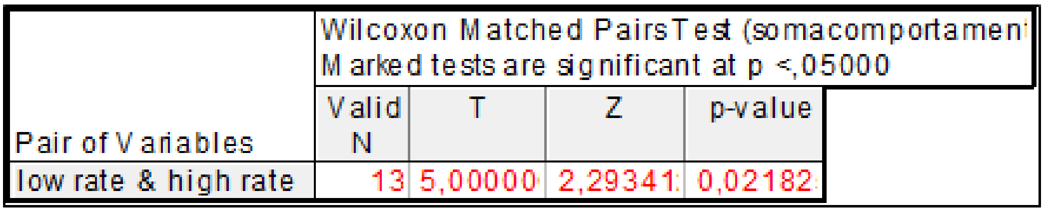


Figure 3.2 Box and Whisker plot comparing the playback treatments (high and low rate playbacks) with the time spent in each preference are by the female. Median, quartiles and minimum and maximum sample values for each treatment are depicted. High rate – HR; Low rate - LR

The same test was conducted for analysing the time spent inside each nest. This indicated that there are significant differences between time spent inside each nest. There is a clear preference for the nest in the high rate side of the aquarium (N=13, Z=2.3, P<0.05; table 3.4). In addition, the average time the female spent inside the high rate nest was 693 seconds and only 8 seconds for the low rate nest (fig 3.3).

Females entered the nests associated with high playback drumming rate significantly more often that they did the low-rate nests (N=13, Z=2.20, P<0.05, table 3.5 and figure 3.4).

Table 3.4 Wilcoxon test to compare for the time spent inside the high rate and low rate nests. Depicting the number of the sample and the p-value.



Wilcoxon Matched Pairs Test (somacomportament
Marked tests are significant at p <,05000

Pair of Variables	Valid N	T	Z	p-value
low rate & high rate	13	5,00000	2,29341	0,02182

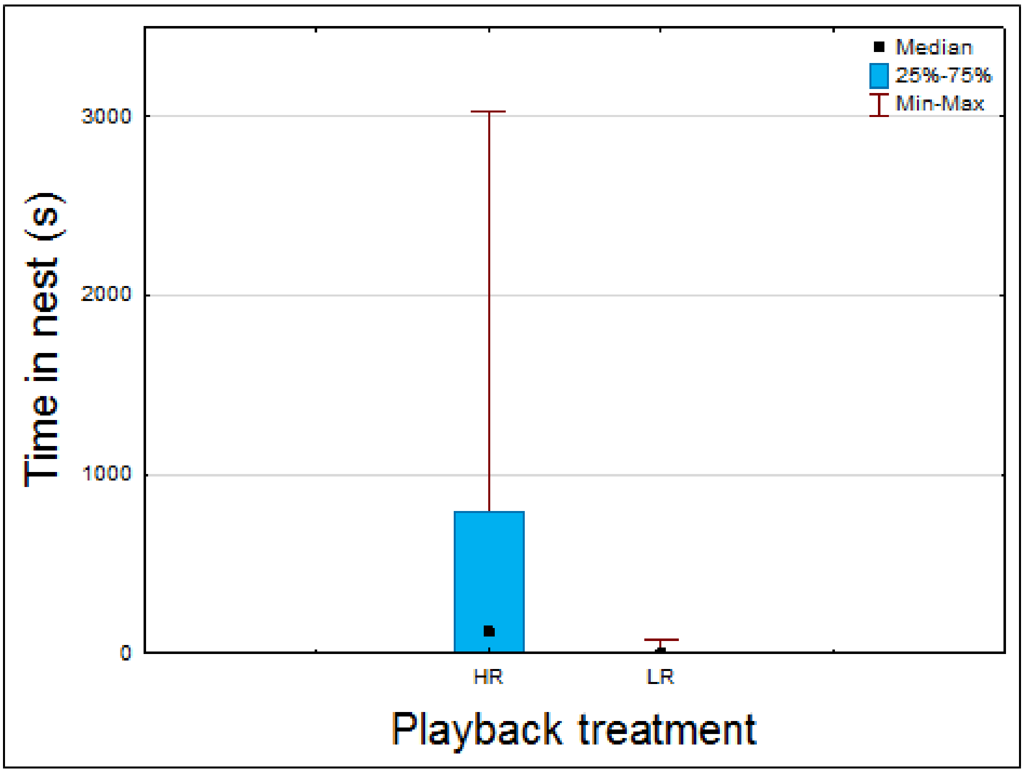
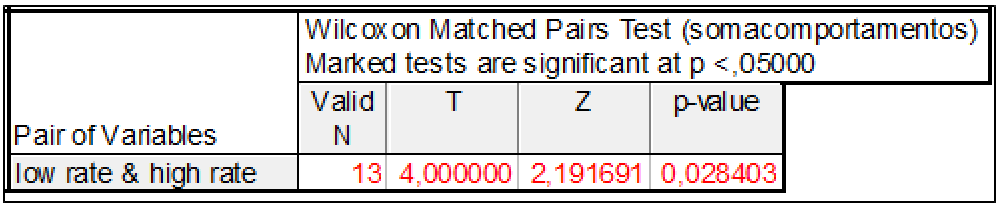


Figure 3.3 Box and whisker plot to compare between time inside the high rate and low rate nest. Median, quartiles and minimum and maximum sample values for each treatment are depicted. High rate – HR; Low rate - LR

Table 3.5 Wilcoxon test to compare for the number of times the female entered the high rate and low rate nests. Depicting the number of the sample and the p-value.



	Wilcoxon Matched Pairs Test (somacomportamentos) Marked tests are significant at p <,05000			
Pair of Variables	Valid N	T	Z	p-value
low rate & high rate	13	4,000000	2,191691	0,028403

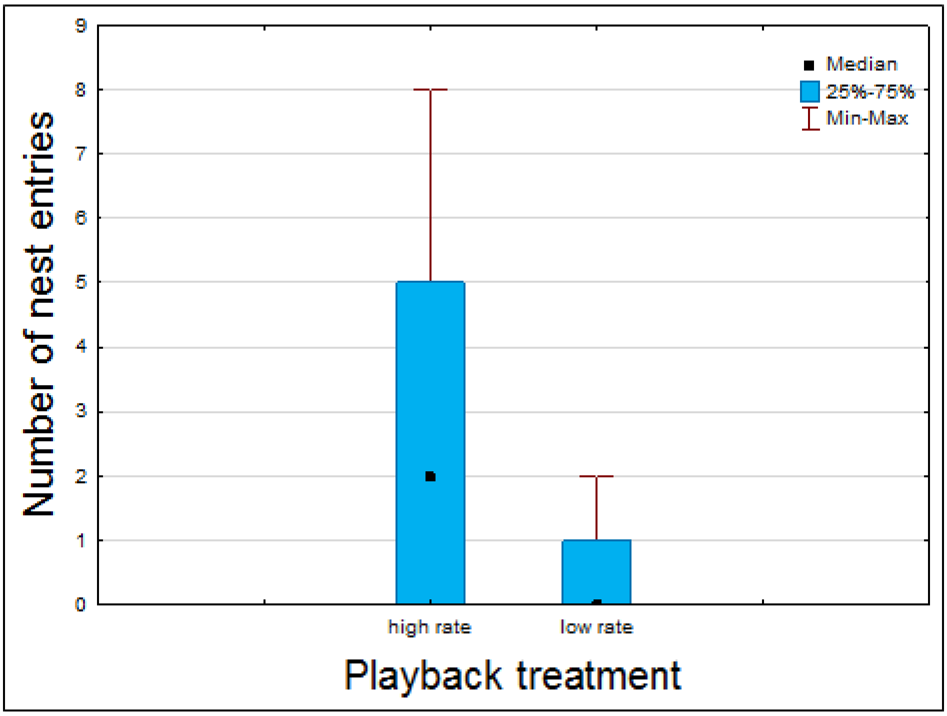


Figure 3.4 Box and whisker plot to compare between number of entries for the high rate and low rate nests. Median, quartiles and minimum and maximum sample values for each treatment are depicted. High rate – HR; Low rate - LR

These results are supported by visual observations during the trials. From the 13 responsive females, only 3 spawned, however all the spawning events occurred in the high rate nest. In addition, the first compartment to be visited was the high rate, 9 out of thirteen times.

3.2 Male behavioural analysis

A total of twenty four males were tested. Due to the scarce number of individual males, the same two were used twice. Each male had an ID that corresponded to the date of the trial, same as the females.

Six courtship visual displays were observed during trials: 1) *approach*, the male starts moving towards the female, undulating his body and fins, darkening its head and erecting its fins (dorsal, pectoral and pelvic), the *approach* is usually followed by *lead*; 2) *lead*, male swims around the female waving the caudal and dorsal fins in an attempt to lead her back into the nest; 3) *nudge*, the male swims towards the female, nudging against her flank; 4) *eight display*, male swims in front of the female performing an eight-like pattern; 5) *nest rubbing*, the male rubs its belly on the ceiling of the nest, belly rubbing may release pheromones used to attract females and synchronize spawning activities and also allows for mucus trails to get attached to the ceiling which may contain active sperm, thus facilitating the fertilization process; 6) *nest display*, inside the nest with the head out, the male proceeds to a series of movements with its head and fins.

We started by analysing the males' total visual courtship, which included the sum of the displays described above. These analysis only took place in the second phase of the experiment, when the males had access to the females. According to visual observations during the trials, males often exhibited courtship displays when the female was near. Male-male competition was also frequent whenever one of the males entered the other male territory. In these situations there always seemed to be a "winner" which was usually the territorial male, regardless of the treatment. Agonistic displays also occurred when one of the males was in proximity of the other one whether in or out its territory.

The factorial ANOVA used to compare the high rate male and the low rate male visual courtship showed that the time the female spent with each male had a significant effect on the visual courtship intensity of the males ($P<0.05$, table 3.6), since the high rate males courtship is significantly higher than the low rate (fig. 3.5). However, if we control the time the female spent with the males, then the courtship intensities do not differ from high to low rate males (fig 3.6), indicating that the male visual courtship intensity did not influence female preference.

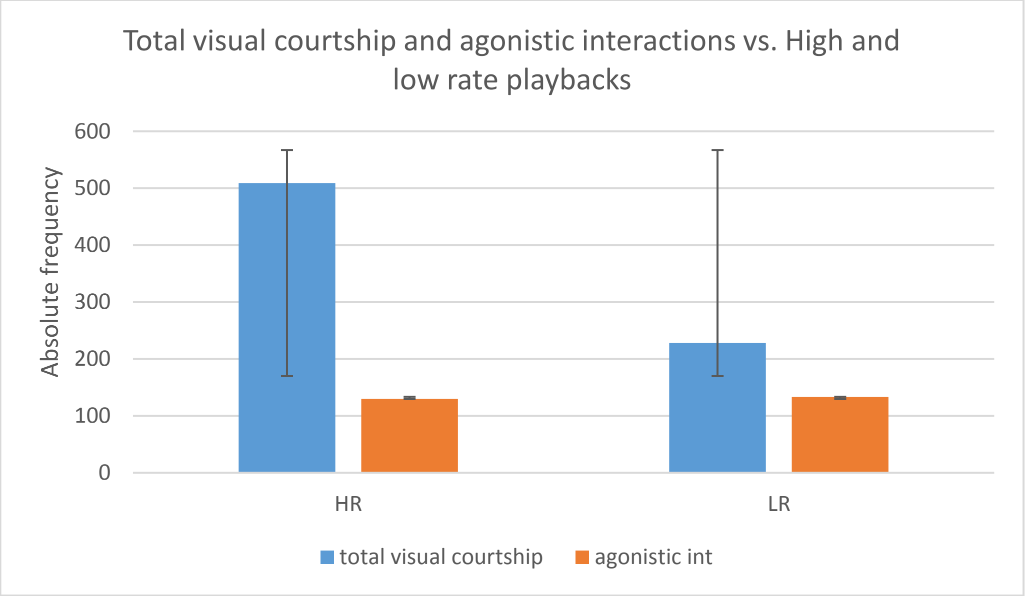
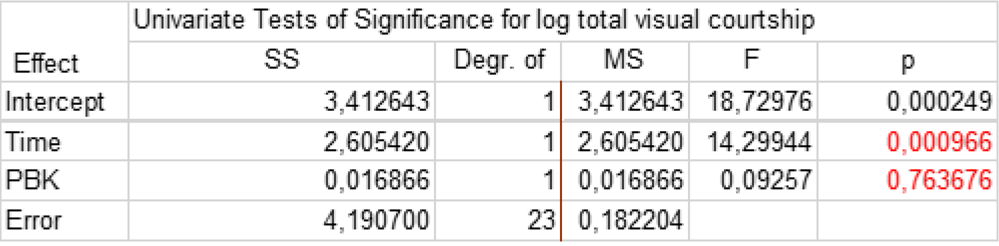


Figure 3.5 Comparison of the total males' visual courtship and agonistic interactions between high and low rate playbacks. Note that values are not controlled for the time the female is in the male compartment (the time females spent with males is considered here). Vertical black lines represent the standard deviation. High rate – HR; Low rate - LR

Table 3.6 Results of an ANCOVA comparing the total visual courtship between males assigned to both treatment levels (factor: PBK). The time spent by the females was included as a covariate (Time).



	Univariate Tests of Significance for log total visual courtship				
Effect	SS	Degr. of	MS	F	p
Intercept	3,412643	1	3,412643	18,72976	0,000249
Time	2,605420	1	2,605420	14,29944	0,000966
PBK	0,016866	1	0,016866	0,09257	0,763676
Error	4,190700	23	0,182204		

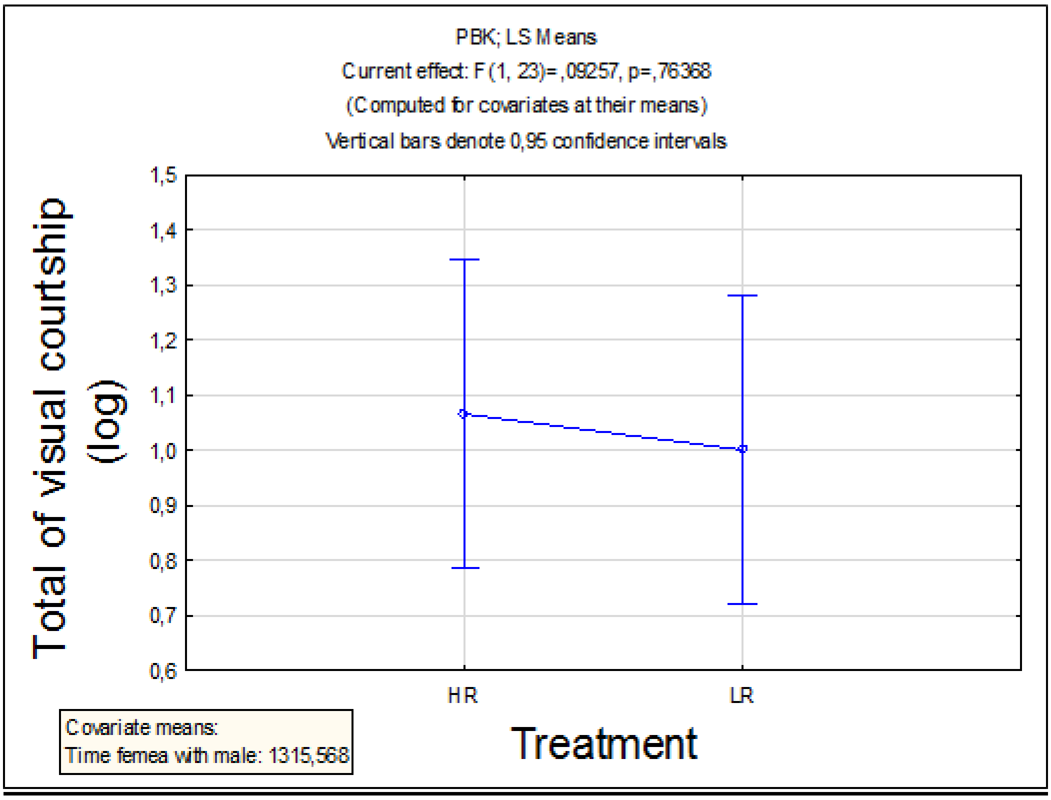
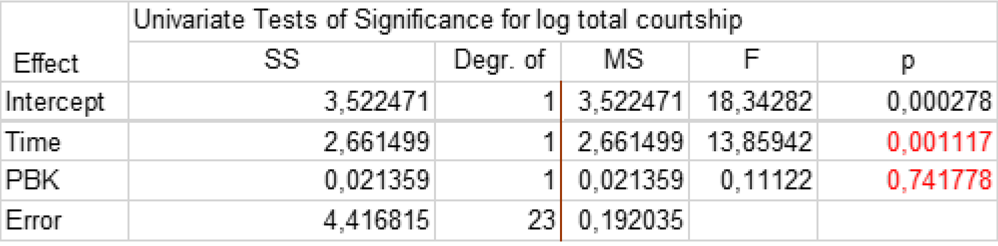


Figure 3.6 Comparison between the total visual courtship of males and the playback treatments. The vertical bars denote 95% confidence intervals and the circles represent the mean. High rate – HR; Low rate - LR

Total courtship intensity, which included the visual displays and the sounds produced by the males was further compared between high and low rate males. From a total of twenty four males only nine produced sounds. From these nine males, seven belonged to the high rate playbacks. Sounds produced by males were all drums. The time females spent with males similarly affected total male courtship intensity (p-value=0.001, table 3.7, figure 3.7), but the playback treatment had no significant effect on male courtship intensity (p-value=0.74, table 3.7).

Table 3.7 Results of an ANCOVA comparing the total courtship between males assigned to both treatment levels (factor: PBK). The time spent by the females was included as a covariate (Time)..



Effect	Univariate Tests of Significance for log total courtship				
	SS	Degr. of	MS	F	p
Intercept	3,522471	1	3,522471	18,34282	0,000278
Time	2,661499	1	2,661499	13,85942	0,001117
PBK	0,021359	1	0,021359	0,11122	0,741778
Error	4,416815	23	0,192035		

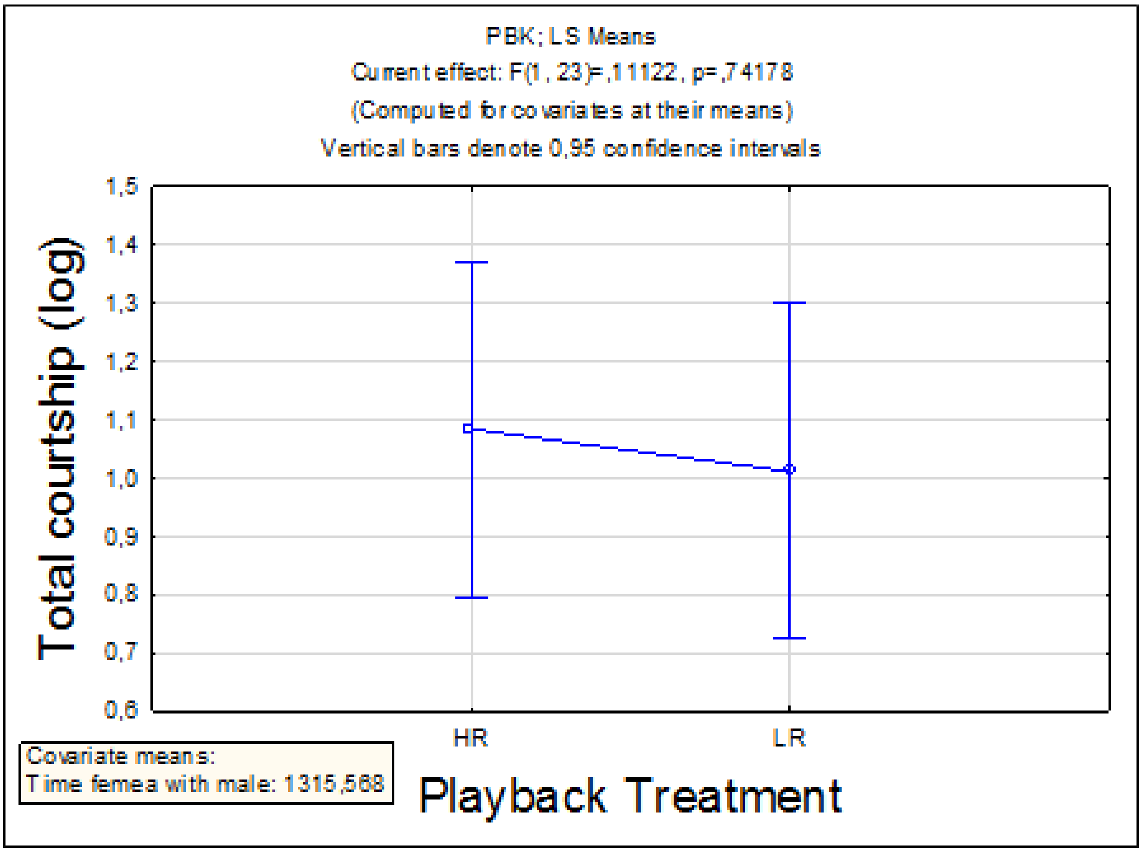
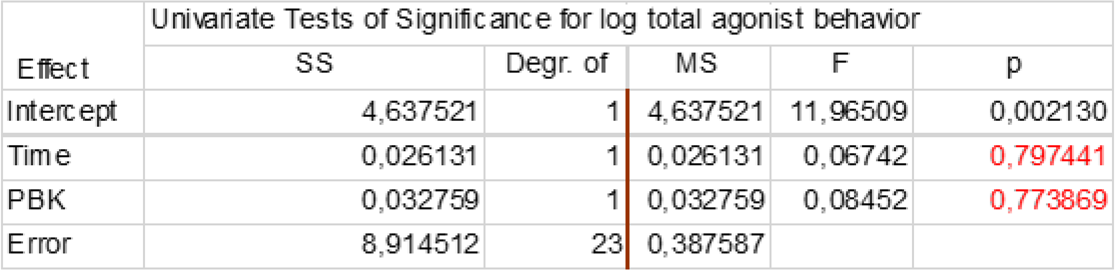


Figure 3.7 Comparison between the total courtship (including sounds) of males and the playback treatments. The vertical bars denote 95% confidence intervals and the circles represent the mean. High rate – HR; Low rate - LR

Moreover, agonistic behaviour between high and low rate males was also analysed but there were no differences in male aggressiveness. In addition, the playback treatments and the time spent with the female did not have a significant effect on the agonistic repertoire of males (P>0.05, table 3.8). Therefore, the time females spent with the males and the playback treatments did not seem to influence the agonistic behaviour of the individuals.

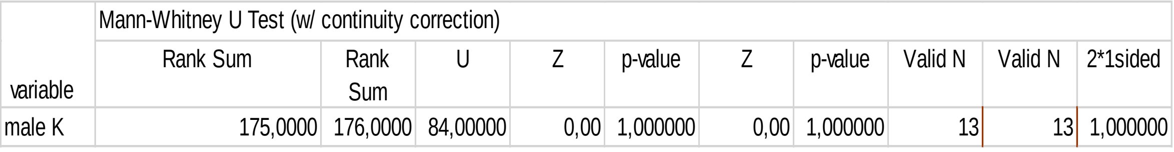
Table 3.8. Results of an ANCOVA comparing the total agonistic displays between males assigned to both treatment levels (factor: PBK). The time spent by the females was included as a covariate (Time).



	Univariate Tests of Significance for log total agonist behavior				
Effect	SS	Degr. of	MS	F	p
Intercept	4,637521	1	4,637521	11,96509	0,002130
Time	0,026131	1	0,026131	0,06742	0,797441
PBK	0,032759	1	0,032759	0,08452	0,773869
Error	8,914512	23	0,387587		

Male condition factor (K) did not differ with treatment (Mann-Whitney test, P>0.05, Table 3.9, Fig. 3.8).

Table 3.9 Comparison of male fitness (K factor) between high and low rate males



	Mann-Whitney U Test (w/ continuity correction)									
variable	Rank Sum	Rank Sum	U	Z	p-value	Z	p-value	Valid N	Valid N	2*1sided
male K	175,0000	176,0000	84,00000	0,00	1,000000	0,00	1,000000	13	13	1,000000

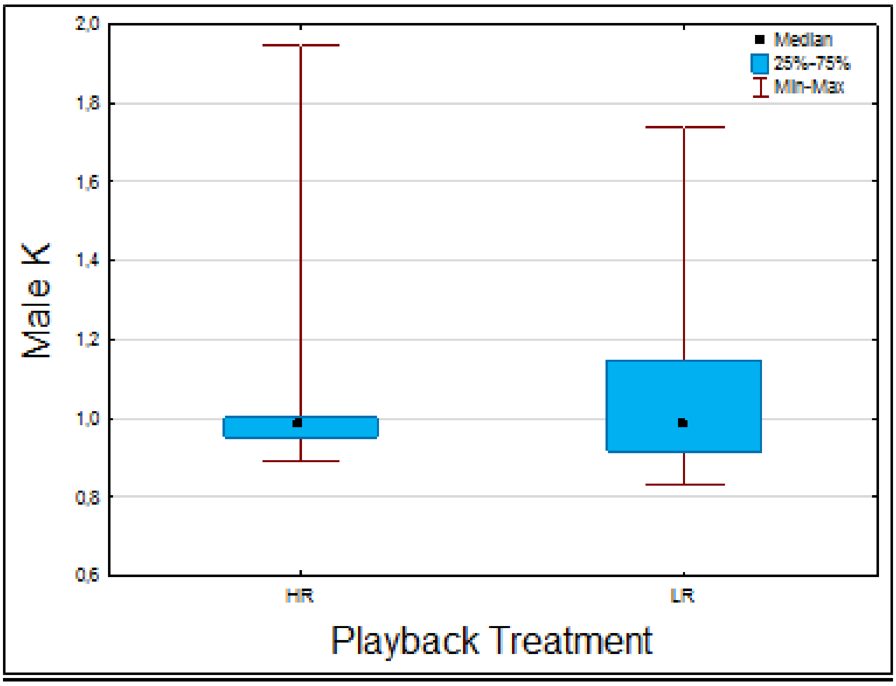


Figure 3.8 Comparison of male condition factor (K) between playback treatments (high rate, HR and low rate, LR). Median, quartiles and minimum and maximum sample values for each treatment are depicted.

Chapter IV - Discussion

This study intended to investigate if calling activity affected mate choice decisions by painted goby, *Pomatoschistus pictus*, females. Females showed a clear preference for males associated to the high rate playbacks but did not show any preference for acoustic cues alone. This suggests that painted goby females show their preferences only when they have the possibility to interact with the males, allowing for visual, acoustical, chemical and tactile (multimodal) communication.

We started by comparing the amount of time females remained in the preference areas associated to low and high rate calling activity, but without visual contact with males. Amorim et al., 2013b observed that females tended to prefer males with higher acoustic activity when exposed to additional visual stimuli. However acoustic courtship stimuli alone did not have an effect on female preference. The same was observed in this study, where no particular preference for high rate calling activity in the absence of additional stimuli was found.

Female preference was also evaluated when not only visual stimulus was allowed but also females had free access to males. Here, female preference was assessed by comparing how long it remained in the high rate or low rate compartment. In contrast to sound alone, a significant difference between the time spent by females in the low and high rate compartments was found. Furthermore, once in the male compartment, the number of times the females entered the nest and the amount of time it remained inside it with the male was significantly higher for the high rate nests. In addition, every spawning event occurred in high rate nests. These results are in line with previous studies. Amorim et al., (2013a) carried out mating experiments with painted gobies where females were allowed to interact freely with the males and they found that males with higher drumming rate enjoyed a higher mating success, but visual courtship did not seem to influence female mating decisions. In another study, Myrberg and Stadler (2002) showed that vocal males of the *Bathygobius curacao* were preferred over the silent ones, since the females were only attracted to the males when additional acoustic stimuli was provided. Furthermore, Gerhardt, et al., (2000) showed that females of the grey tree frog, *Hyla versicolor,* preferred males that emitted longer and higher rate acoustic calls.

In addition, experiments with the african cichlid fish, *Astatotilapia burtoni,* also showed that, when additional visual and acoustic stimuli were provided, females were more attracted to the males associated with courtship sounds rather than the control males (Maruska et al., 2012).

To ascertain if male behaviour could have influenced female mate choice, visual and total courtship (acoustic and visual displays) were compared between males associated with high or low rate. As expected, males associated to the high rate playbacks showed higher courtship activity because courtship displays are highly correlated with the time the female spends with the male. Indeed, if the female spends more time with the high rate male, then this male will have more opportunity and a higher motivation to court the female, as opposed to the low rate males. However, after controlling for the time the female spent with the male, male visual and total courtship did not differ between the high rate and low rate males. This suggests that female choice was driven by male calling activity (manipulated by the playback) and not by other male behaviour. Interestingly male behaviour did not seem to be influenced by playback treatment as it did have an effect on the male's courtship or aggression.

According to the study of Amorim and Neves (2007), significant differences were found in the courtship intensities of the males, both visually and acoustically, since larger males courted females more intensively. Moreover, the painted goby acoustic courtship rate and duration is positively correlated with male condition, with males exhibiting higher lipid levels producing and maintaining acoustic displays at a higher rate (Amorim et al., 2013a). Therefore, males that present better condition are more likely to provide better parental care. In species with parental care, males with higher fitness condition should be preferred by females since they maximize their fitness (Amorim et al., 2013a; 2015). Courtship displays are energetically costly and therefore reflect male condition, since males with better condition are capable to sustain high courtship rates better than others. This may be the reason why males associated to the high courtship rate were preferred over the low rate males. Moreover, Wisenden, (1999) suggested that females tend to prefer males based on several cues, like presence of healthy eggs or nest condition that may hint on male care capabilities. In addition, according to Bartsch et al., (2015), acoustic features of male nightingales may highly influence female preference, since these assess male care qualities based on the acoustic cues of the males.

Male-male competition can occur whether in the presence or absence of females, however, female presence can highly influence these agonistic interactions, as well as their outcome (e.g. Procter et al., 2012; Matos & Mcgregor 2002). However, results obtained from the analysis of the agonistic displays show otherwise, suggesting that female presence did not have any particular effect on these interactions, since male aggressiveness remained similar in the presence and absence of females. Also, male condition revealed to be similar between high and low rate males, and this may be the reason why courtship and agonistic displays were so similar between males.

From a total of 39 trials conducted during this study, only a total of 13 were considered for analysis. The sample was small since the total number of females captured from the natural habitat was low and among these, those sufficiently ripe and ready to mate were very scarce, which hinders the success of the trial.

In conclusion, painted goby females clearly preferred the males that were associated to the high rate acoustic displays, despite these being similar to the males associated to the low rate playback in terms of courtship intensity and male condition. Taken together these results suggest that females select males based on higher acoustic courtship intensity likely because this may be an indicator of good parental quality. Future studies should confirm the role of acoustic signalling in mate choice. In addition, it would be interesting to test if males with higher calling activity are indeed more capable of providing paternal care.

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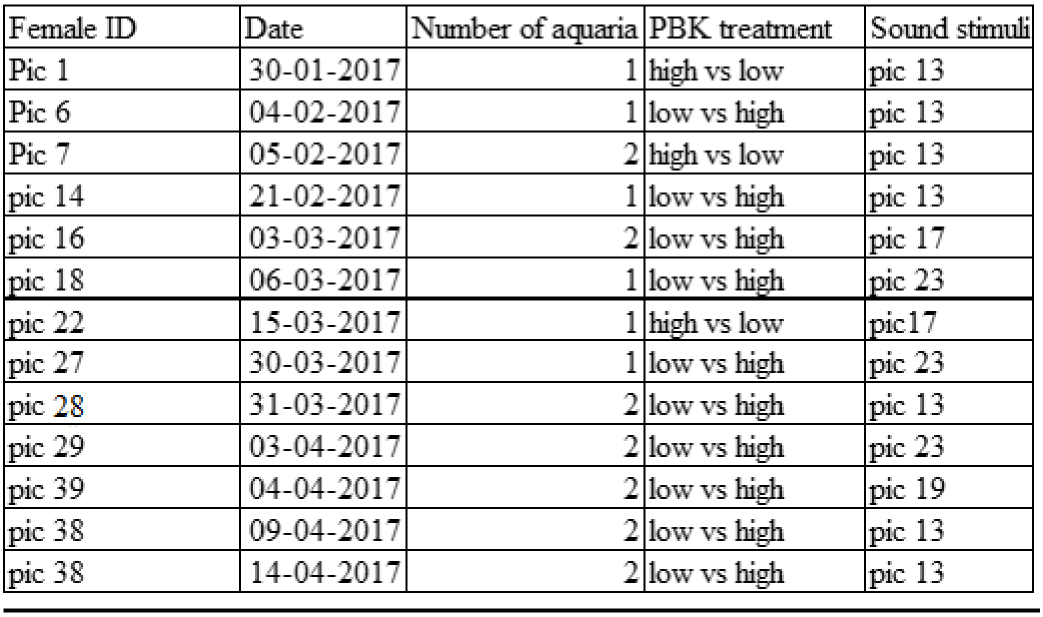
Appendix

Table 3.1 Table depicting the ID of the 13 responsive females, together with the date of each trial, the number of experimental aquaria where the trial took place, the playback treatment used in each male compartment and sound stimuli.



Female ID	Date	Number of aquaria	PBK treatment	Sound stimuli
Pic 1	30-01-2017	1	high vs low	pic 13
Pic 6	04-02-2017	1	low vs high	pic 13
Pic 7	05-02-2017	2	high vs low	pic 13
pic 14	21-02-2017	1	low vs high	pic 13
pic 16	03-03-2017	2	low vs high	pic 17
pic 18	06-03-2017	1	low vs high	pic 23
pic 22	15-03-2017	1	high vs low	pic17
pic 27	30-03-2017	1	low vs high	pic 23
pic 28	31-03-2017	2	low vs high	pic 13
pic 29	03-04-2017	2	low vs high	pic 23
pic 39	04-04-2017	2	low vs high	pic 19
pic 38	09-04-2017	2	low vs high	pic 13
pic 38	14-04-2017	2	low vs high	pic 13