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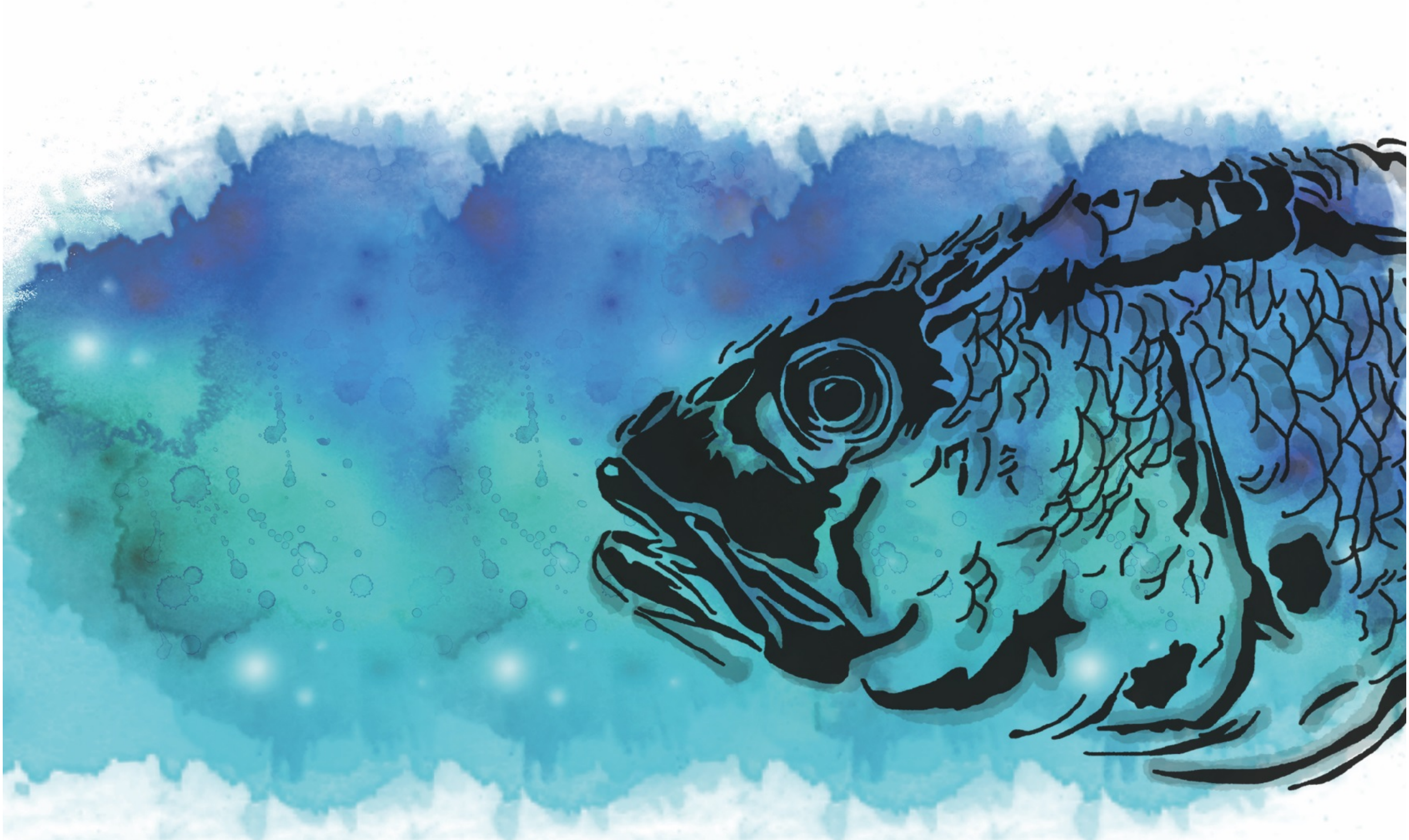
Acoustic repertoire of the meagre *Argyrosomus regius* (Asso 1801): The influence of development, gender and context in temporal and spectral variation of calls



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FACULDADE DE CIÊNCIAS E TECNOLOGIA

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Acoustic repertoire of the meagre *Argyrosomus regius* (Asso 1801): The influence of development, gender and context in temporal and spectral variation of calls

Mestrado em Biologia Marinha

Trabalho efetuado sob a orientação de:
Professora Doutora Clara Amorim
Professora Doutora Teresa Modesto

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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.

Beatriz Palinhos Pereira

(Beatriz Palinhos Pereira)

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Abstract

Sciaenids are among the largest families of vocal sonic fishes. The meagre (*Argyrosomus regius*, Asso 1801) was shown to emit sounds during the spawning season, but there is a lack of information on its wider vocal repertoire.

The aims of this study were (1) to compare spontaneous sounds and distress calls recorded in captivity from juveniles and adults, and from female and male adults, (2) to describe seasonal variation in the sound production of *A.regius* (3) to characterize the acoustic activity and the sounds produced during social contexts in relation with spawning events (monitored by the collection of eggs from the tanks), and (4) to compare sounds recorded in captivity with sounds recorded in nature to validate ongoing passive acoustic monitoring carried out in Tagus estuary.

Sound recordings were obtained from a group of adult breeders and juvenile meagre, *A. regius*, hosted at the aquaculture facilities of Instituto Português do Mar e da Atmosfera – Estação Piloto de Piscicultura de Olhão (IPMA – EPPO), Portugal.

The present dataset demonstrates for the first time that in *A. regius* dominant frequency has an inverse relationship with fish size, that females emit sounds that differ from those of males and that sounds vary according to context and throughout ontogeny. Sounds produced by this species during the breading season showed a richer repertoire than previously observed by Lagardère and Mariani (2006), with meagre producing not two, but six call types. Additionally, besides the dense choruses of grunts already known for this species, meagre emitted successive series of isolated pulses, audible as 'knocks', as an acoustic signal related to spawning events. A seasonal trend was noted in knocking sounds but not in drumming due to the short study period. Captive meagre produced sounds which vary in their characteristics as reproduction proceeded, with acoustic parameters of sounds being significantly different among pre-spawning, spawning and post-spawning periods. Finally, sounds produced by meagre in captivity and in the Tagus estuary showed significant differences, probably associated with disparities between the environment and between individuals (i.e. temperature fluctuations in the field; larger size of wild individuals).

The study of distress and social calls produced in captivity by *A. regius* will contribute to monitor natural populations of meagre including identify the presence and location of juveniles and breeding adults in nature.

Key words: Fish sounds, distress calls; ontogeny of sound production; sexual dimorphism; spawning; Sciaenidae

Resumo

Mais de 800 espécies pertencentes a 109 familías de peixes teleósteos são atualmente reconhecidas como vocais. No entanto, este número representa apenas as espécies vocais descritas na literatura, estando certamente muito aquém do real número de espécies capazes de produzir sons.

Os peixes ósseos possuem uma audição bem desenvolvida e apresentam diversos mecanismos de produção de som que lhes permitem gerar uma vasta diversidade de vocalizações.

O repertório acústico de espécies de peixes vocais pode incluir diversos tipos de sons (pelo menos até cinco em algumas espécies), sendo que estes não são tão variáveis como os produzidos por outros taxa. No entanto, espécies próximas apresentam diferenças consistentes nos seus sons e, em algumas espécies, parece até mesmo existir individualidade nas vocalizações. Diferenças nos sons produzidos durante a corte por espécies simpátricas, que podem promover isolamento reprodutivo, são de particular interesse. Diferenças interindivíduais podem, por sua vez, ter um papel na escolha sexual, por competição entre machos e escolha de parceiro por parte das fêmeas. A variabilidade dos sons pode ainda estar associada com motivação e status social, estação do ano, hora do dia, alterações ontogenéticas e dimorfismo sexual. A variabilidade nos sons de peixes é principalmente observada nos padrões temporais dos sons, no número e forma de onda dos pulsos que compõem cada som e em variações da frequência do som (por vezes apresentam modulação de frequência). Esta variabilidade parece ter um papel fundamental na vida social dos peixes.

Entre os grupos de peixes que comunicam acusticamente, os cienídeos apresentam uma elevada diversidade de sons, alguns descritos como “drumming, croaking, knocking, clucking, or purring”. Os mecanismos, no entanto, seguem aproximadamente o mesmo modelo: a bexiga natatória é rodeada dorsalmente por músculos sónicos extrínsecos e simétricos bilateralmente, com origem na musculatura hipaxial, e que se inserem indiretamente na bexiga através de um tendão central. A rápida contração cíclica dos músculos sónicos (espasmos) provoca oscilações transientes da parede da bexiga natatória, que nos peixes desta família resulta em sucessões de pulsos. Os sons dos cienídeos apresentam uma frequência fundamental entre 100 e 200 Hz e frequências dominantes até 1000 Hz. No entanto os parâmetros das vocalizações podem variar de acordo com o sexo, com desenvolvimento das gónadas, com o tamanho do peixe, a temperatura da água e a estação do ano. Na maior parte das espécies de cienídeos, a capacidade de produção sonora é restrita aos machos. No entanto, em algumas espécies como a corvina preta (*Pogonias cromis*), a corvina branca (*Genyonemus lineatus*) e a corvina legítima

(*Argyrosomos regius*), tanto os machos como as fêmeas possuem músculos sónicos extrínsecos comuns à família.

A corvina (*Argyrosomus regius*, Asso 1801) é um teleósteo marinho que pode atingir grandes dimensões, comummente encontrada ao longo do Mar Mediterrâneo e do Mar Negro, bem como por toda a costa Atlântica da Europa e África. No início do período de desova, durante a Primavera, grupos de corvinas entram em estuários onde permanecem até ao final do Verão, quando a época de reprodução termina. Áreas como os estuários dos rios Tejo (Portugal), Guadiana (Sul de Espanha e Portugal) e Gironde (França) ainda são de grande importância para a desova desta espécie. A capacidade de *A. regius* para produzir sons é conhecida há seculos. De facto, os sons que estes peixes produzem durante a desova são de tal amplitude que dominam o ambiente acústico em que estão inseridos, sendo esta informação utilizada por pescadores em diversos locais. As vocalizações produzidas por agregações desta espécie durante a desova já foram caracterizadas. Está em falta, no entanto, informação mais completa sobre o seu repertório vocal, incluindo se as fêmeas produzem sons e se a variabilidade dos sons estará associada com o contexto social (e.g. agonístico, reprodutivo) ou fase ontogenética (repertório vocal de juvenis *vs.* adultos e variação com o tamanho).

Os objetivos deste estudo foram (1) comparar sons espontâneos e vocalizações de alarme, gravados em cativeiro, de juvenis e adultos, e de fêmeas e machos adultos, (2) descrever as variações circadianas e sazonais na produção de sons de *A. regius*, (3) caracterizar a atividade acústica e as características dos sons produzidos durante contextos sociais em relação com a desova (monitorizada através da colheita de ovos dos tanques), e (4) comparar sons gravados em cativeiro com sons gravados na Natureza, para validar a monitorização acústica passiva realizada no estuário do Tejo até ao momento.

As gravações sonoras de um grupo de corvinas juvenis e adultas foram realizadas nas instalações do Instituto Português do Mar e da Atmosfera – Estação Piloto de Piscicultura de Olhão (IPMA – EPPO), Portugal. Para a gravação de vocalizações de alarme, tanto de juvenis como de adultos, os peixes foram transferidos para recipientes de plástico, nos quais foram mantidos dentro de uma manga de rede e ocasionalmente estimulados (por exemplo por manipulação do pedúnculo caudal). As vocalizações foram gravadas durante 3 minutos por peixe, usando uma cadeia de registo portátil, com o hidrofone colocado a aproximadamente 10 cm do abdómen do peixe. No caso dos adultos, uma pequena concentração de anestésico foi adicionada à água do tanque para minimizar o stress e evitar que o peixe saltasse e se ferisse aquando da captura ou manuseamento. Foi dado aos peixes tempo para recuperar e as gravações foram realizadas quando estes não mostravam sinais de estarem sob efeito do anestésico.

Vocalizações voluntárias foram gravadas usando uma cadeia de registo portátil no caso dos juvenis e uma cadeia de registo fixa no caso dos adultos, com o hidrofone colocado verticalmente no centro do tanque, a aproximadamente 30 cm do fundo.

Os dados recolhidos mostram, pela primeira vez, que em *A. regius* a frequência dominante tem uma relação inversa com o tamanho do peixe, que as fêmeas emitem sons que diferem dos produzidos por machos e que os sons variam consoante o contexto e durante a ontogenia. Sons produzidos por esta espécie durante a época de desova mostraram um repertório mais rico do que o previamente observado por Lagardère and Mariani (2006), com a corvina a produzir não dois, mas seis tipos de vocalizações. Adicionalmente, além dos densos coros de grunhos já conhecidos para esta espécie, as corvinas emitiram sucessões de pulsos isolados que soaram como ‘knocks’, um sinal acústico possivelmente associado a desova. Um padrão sazonal foi observado nos sons de ‘knock’ mas não nos de ‘drumming’, devido ao curto tempo de estudo. Finalmente, as corvinas em cativeiro produziram sons que variam nas características à medida que a desova prossegue, com os parâmetros dos sons a mostrarem diferenças significativas entre os períodos de pré-desova, desova e pós-desova. Finalmente, sons produzidos por *A. regius* em cativeiro mostraram diferenças significativas dos e no estuário do Tejo, provavelmente associadas a disparidades entre o ambiente e entre os indivíduos estudados (por exemplo, flutuações de temperatura no campo; maior tamanho de indivíduos selvagens).

É esperado que o estudo de vocalizações sociais e de alarme produzidos em cativeiro por *A. regius* possa contribuir para monitorizar a população natural de corvina, incluindo para a delimitação espacial de juvenis e adultos dentro e fora do estuário do Tejo.

Palavras chave: Peixes, sons de alarme, ontogenia da produção de sons; dimorfismo sexual; reprodução; Sciaenidae

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General introduction

1. Bioacoustics: Historical Background

Among vertebrates, fish have developed the largest diversity of sound-generating mechanisms. Yet, investigation on this subject has proven challenging and controversial for hundreds of years. In the fourth century B.C., Aristotle mentioned sound-producing mechanisms in fish for the first time (Gohlke, 1957), but investigations devoted to this topic are only known from the 19th century (e.g. Müller, 1857; Dufossé, 1874; Bridge and Haddon, 1889, 1892; Sörensen 1895). It was only in the middle of the 20th century that systematic studies of sounds and sound production began. During World War II, the widespread use of engineering hydroacoustics for the disclosure of enemy submarines and communications, led to the awareness that "silent" waters were loaded with various biological sounds interfering greatly with the acoustic apparatus. The intensities and spectral characteristics of these sounds were considerably different in various seas and oceans. Furthermore, they varied between the time of the day, season, climate conditions, etc. From this point on, there was an increasing need for the recognizable proof of underwater sounds, as well as for the understanding of their origin in connection with different biotic and abiotic factors. In addition, there was a growing demand for the discovery of new methods and settings that ensured the normal operation of hydroacoustic. These requirements prompted the appearance of a new science direction at the border between acoustics and biology – underwater bioacoustics (Mann et al., 2008; Kasumyan, 2008).

In recent years, the great number of publications, reviews, and sections in books dedicated to fish sensory systems and behavior reflect the increasing attention that has been given to the study of acoustic communication in these animals (Fendall and Sewell, 2009; Von der Emde et al., 2012; Ladich and Fine, 2006). With passive acoustic monitoring (PAM), marine biologists can take advantage of these sounds to study many aspects of fish biology relevant to stocks management and conservation (e.g. location/timing of spawning, populations abundance and distribution, habitat/environmental health, behavioral responses to biological and anthropogenic stimuli) (Hawkins and Amorim, 2000; Gannon, 2008). The basic technique involves using one or more hydrophones to convert natural vocalizations made by fishes into a voltage that can be recorded by a data acquisition system, and can potentially be analysed in real time (Luczkovich et al., 2008; Mann et al., 2008). Studies of vocalizations through PAM are non-invasive, can be conducted at low cost, and can cover a large study area at high spatial and temporal resolution. However, the analysis of such large datasets can also be an overwhelming task. Since fish sounds are usually accompanied by signals of other sensory modalities, such as visual displays, direct observation of the target species are crucial to establish the role of acoustic signals (Amorim et al., 2015). In that respect, field studies are also challenging because of the frequent visibility restrictions found in underwater environments that hinder fish behavior (Zelick et al., 1999). Analysis of sounds registered in laboratory conditions (small aquariums and tanks) also present technical difficulties. The complex acoustic background noise and distortions of sounds observed in such conditions may hinder the correct recording of fish sounds and confinement may limit natural fish behavior (Kasumyan, 2008).

2. Acoustic Communication in Fish

Fish represent the largest group of sound producers among vertebrates. However, due to aforementioned technical difficulties to study acoustic communication in this taxon (in comparison with terrestrial taxa), the knowledge of the function of fish acoustic signals is incomparably inferior when compared to other animals, such as amphibians, birds and mammals (Ladich, 2004).

Over more than 800 fish species from 109 families belonging to teleost fish have now been reported as vocal. However, this number essentially reflects the scientific expenditure instead of the actual distribution of sound-producing families (Rountree et al., 2003). In some taxa (e.g. Cichlidae, Doradidae, Batrachoididae, Gobiidae, Gadidae, Sciaenidae, and Carapidae), the majority of species have the ability to communicate acoustically, though mute species exist (Fine and Waybright, 2015). On the other hand, large taxa such as Cyprinids are mostly mute with a few species producing socially relevant sounds (Johnston and Johnson, 2000; Holt and Johnston, 2014). Considering the aquatic medium characteristics, many studies suggest benefits and even a need for fishes to produce sounds. However, it is still not clear why sounds are produced only in some taxa (Parmentier and Fine, 2016).

In comparison with air, water is characterised by high density and low acoustic elasticity, making sound propagation, and hence acoustic communication, more efficient (Kasumyan, 2008). Underwater, sounds are characterised by slow attenuation and rapid propagation (Kasumyan, 2008), with a speed close to 5 times faster than in air (*ca* 1,500m/s) (Fine and Parmentier, 2015). These and some other features represent clear advantages for species that rely on sound as a source of environmental cues, especially considering the low- light conditions (due to water depth, turbidity, early and late diurnal periods) of aquatic habitats that limit visual communication (Myrberg and Lugli, 2006).

Water is also characterised by some restrictions for acoustic communication, which are most pronounced in shallow water bodies and coastal zones of seas and oceans, due to the strong acoustic noise from overlapping of sound waves. Because fish mostly use low frequency signals (< 1 kHz), in shallow water their communication active space is reduced to few meters (Kasumyan, 2008).

The most important communicative feature of sounds generated by fishes is considered to be the variation of temporal patterns (e.g. number of pulses, pulse rate in a sound and frequency components of the sounds) related with differences between and within species, and context (Winn, 1964; Amorim, 2015). This variability in acoustic communication may play a role in social life of fishes and promote reproductive isolation, stimulation and facilitation of courtship activities, female mate choice (vocalization as a secondary sex characteristic to increase fitness), evaluation of opponents during contests, or recognizing the identity of competitors (e.g. Myrberg et al., 1986; Ladich et al., 1992; Amorim and Neves, 2008; Simões et al., 2008).

3. Sounds produced by Fishes

Sounds produced by soniferous fish consist of elementary units – low frequency pulses (typically less than 1000 Hz) - that vary in amplitude, duration, number and acoustic frequency (Winn, 1964; Myrberg et al., 1978). However, their ability to modulate sound frequency is poorer than those of other taxa and, therefore, they demonstrate a restricted acoustic repertoire (Lugli et al., 1997; Kaatz, 1999).

Fishes generate both simple and complex sounds (Kasumyan, 2008). Simple sounds (Figure 1a, b, c), produced by most fish species, consist of alternating pulses, usually represented by a series of several pulses that can be characterised by: the duration of the sound, the duration of the series, the number of pulses in the sound, the frequency of the pulses, the amplitude of the pulses, the pulse period (the time between the maximum amplitudes of adjacent pulses), dominant frequency (corresponding to the band with the greatest energy), the maximum and minimum frequencies of the sound. When simple sounds have a narrow diapason of frequencies they are called tonal sounds, as they are relatively long and include harmonic components in which the frequencies are the multiple of the basic frequency in the tonal sound (Kasumyan, 2008). In most species sounds are broadband in frequency. In several species, simple sounds are considered to be highly stereotyped (Mann et al., 1997). However, despite their apparent simple structure and usual short durations, simple sounds can be subject to large temporal and spectral variation (i.e. number of pulses, pulse rate in a sound and frequency components of the sounds) (Lugli et al.,1996; Finstad and Nordeide, 2004).

Complex (Figure 1d), produced by a smaller number of species, are a sequence of several simple sounds followed by a tonal element, that results from the continuous series of high muscle contractions. In this case, contraction rate is so fast that sound pulses fuse together and become almost like a pure tone, in such a way that is no longer possible to distinguish them aurally (Pruzsinszky and Ladich, 1998).

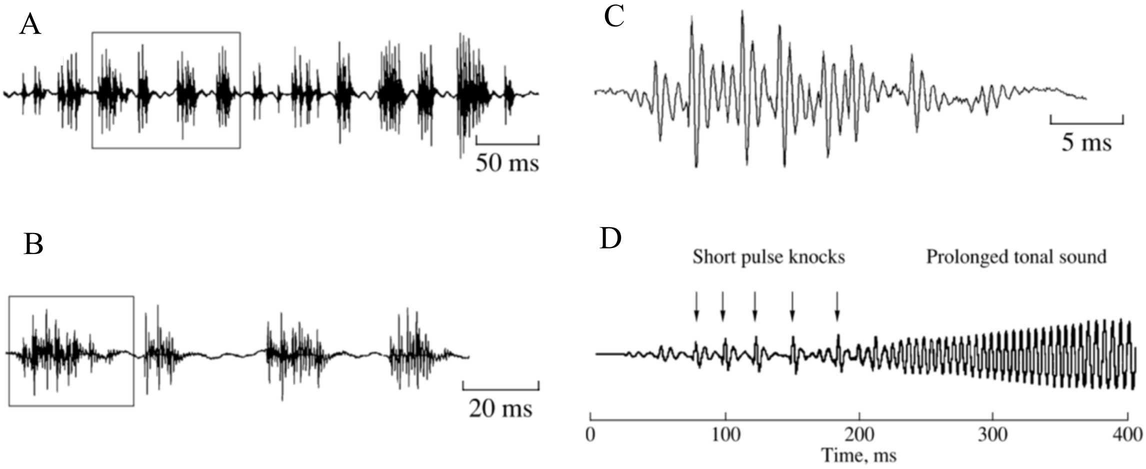

Figure 1: (A) Oscillogram of a series of simple sounds (B) a fragment of the oscillogram with four consecutive simple sounds, and (C) an oscillogram of a simple sound from the fragment, (D) oscillogram of a complex sound. The rectangular selections show the corresponding parts of the oscillogram. Adapted from Kasumyan (2008).

There are three main tools for depicting fish sounds: oscillograms (amplitude against time) (Fig. 2a); sonograms (a representation of frequency against time) (Fig. 2b); and frequency spectra (amplitude against frequency and indicate dominant frequencies within a sound) (Fig. 2c). These outputs are obtainable on various software programs.

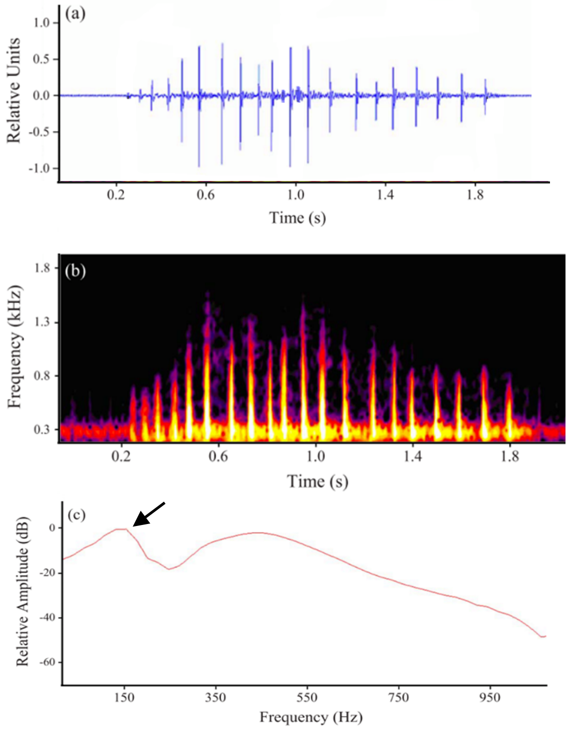

Figure 2: (a) Oscillogram, (b) sonogram, and (c) power spectrum of a *Pseudotropheus zebra* male courtship sound. Arrow indicates the peak frequency of the sound. Adapted from Simões et al., (2008).

4. Sound-generating mechanisms in Fishes

Sound production can occur through diverse mechanisms, depending on the species (Kasumyan, 2008). Unlike birds and mammals that have homologous sonic mechanisms, fish exhibit a wide range of sound producing mechanisms that appear to have evolved independently (Fine and Parmentier, 2015). Due to this great diversity, a simple classification of these mechanisms has not yet been defined.

According to Ladich and Fine (2006) sound production occurs predominantly through either the swimbladder or stridulation of bony elements. Most commonly, mechanism based on the swimbladder produce drumming (or sonic) sounds as a result of fast-contracting sonic muscles that cause the swimbladder to suffer quick volume changes – pulsations (Kasumyan, 2008). Besides the contraction of sonic muscles, the swimbladder can also be excited by rubbing the pectoral fins against the body wall that covers it. In this case, the swimbladder acts as resonator that amplifies sounds produced by stridulation mechanisms (Moulton, 1960). Depending on the origin and insertion of drumming muscles, various types are distinguished. Intrinsic drumming muscles, likely a more-derived condition (Fine and Parmentier, 2015), are attached directly to the bladder wall (e.g. in Batrachoididae: toadfishes; Ladich, 2014; Fig. 3A). If extrinsic, muscles originate on structures such as the skull, vertebral processes or body wall musculature (Ladich and Fine, 2006; Kasumyan, 2008; Ladich, 2014) and insert either directly (in pimelodid catfishes; Ladich 2001; Fig. 3B) or indirectly on the swimbladder, either via bony plates (elastic springs in doradid, mochokids and ariid catfishes) or broad tendons (piranhas, drums; Ladich and Bass 2005). Some species with extrinsic muscles may have both directly and indirectly vibrating mechanisms (Bass and Ladich, 2008).The force these muscles exert and the rate at which they activate, contract and relax, affect the spectral characteristics of the call and are dependent on the size, position and contractile properties of the muscles (Connaughton et al., 2000; McCauley, 2001; Rome, 2005), which can, in turn, be related to the size, condition, and sex of the fish (Parsons and McCauley, 2017).

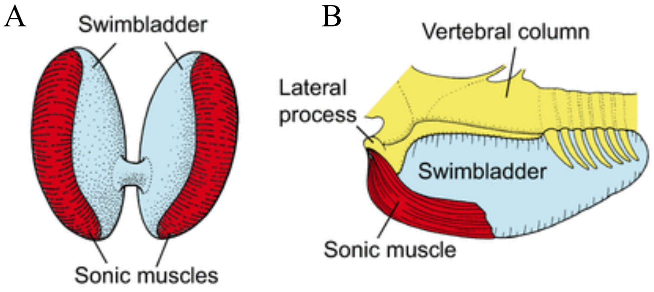

Figure 3: Ilustrations of swimbladder sound-generating mechanisms in teleost fishes. (A) Intrinsic sonic muscle attached directly to the swimbladder wall (Lusitanian toadfish, *Halobatrachus didactylus*), (B) Extrinsic drumming muscles originating at the lateral process of the vertebral column and inserting directly on the ventral part of the swimbladder (pimelodid catfish, *Pimelodus sp*,). Adapted from Ladich and Winkler (2017).

5. Sound detection mechanisms in Fishes

All fishes detect sounds and likely use them to learn about their environment (i.e. detecting prey or predators). However, only some species have actually adapted hearing capabilities for intra-specific communication (Popper, et al., 2003).

Teleost fishes, unlike terrestrial vertebrates, have no external or middle ear structures. Nonetheless, they do possess a pair of inner ears on each side of the head, near the midbrain, where receptor organs of acoustic stimuli are located. The fishes' internal ear is comprised of three semicircular canals and three otolithic end organs (utricle, saccule, and lagena), each containing a sensory epithelium (maculae) covered by an otolithic membrane, and a calcareous otolith (Ladich and Popper, 2004; Ladich and Schulz-Mirbach, 2016). Most fishes utilize the saccule for sound perception. As fishes generally lack a sound-pressure-detecting mechanism, they rely on a sensor that responds to small particles movements: sensory hair cells that cover the maculae in close contact with the otolith. This way, when the otolith, which is denser than the surrounding tissue, vibrates, the sensory cells are excited, stimulating neural transmission that is processed by the brain and interpreted as sound (Hawkins, 1993; Moyle, 2004; Ladich and Winkler, 2017) . Moreover, these hair cells present specific orientation patterns that may allow the fish to determine the direction of incoming sound (Popper, et al., 2003). Nevertheless, the precise role of each end organ is yet unknown but the way they contribute to the detection of sound varies among species, since each have different shaped otoliths (Popper et al., 2003; Ladich and Schulz-Mirbach, 2016).

Because the majority of fishes detect particle motion rather than sound pressure (Ladich and Winkler, 2017), most species of fish respond to a relatively narrow range of frequencies, generally between 50Hz (or below) to 1000 or 1500 Hz (Webb et al., 2008). However, approximately one-third of fish species (e.g. otophysine fish like minnows and carps, catfishes, tetras and piranhas) have morphological specializations (gas-filled vesicles or bony adaptations attached to the anterior part of the gas bladder) that may be close to or in direct contact with the inner ear, improving their hearing capacity to lower sound levels and higher frequencies (above 3000 kHz) (Popper and Fay, 1999; Ladich and Popper, 2004; Webb et al., 2008). This increase in auditory performance is owed to the transmission of the pressure fluctuations induced by the acoustic stimulus in the gas bladder, to the inner ear (Fig. 4).

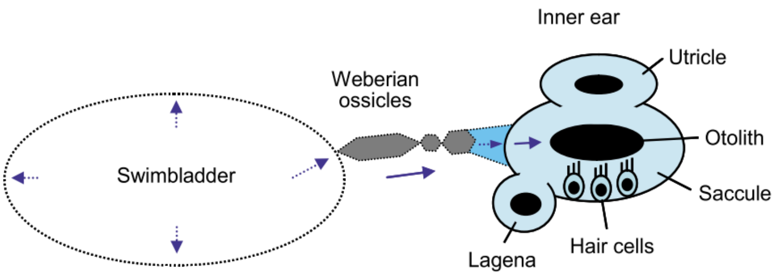

Figure 4: Schematic illustration of fishes' ears. Fishes detect sound with otolithic end organs of the inner ear. The swimbladder is shown as an accessory structure that impoves the hearing capacity of otophysine fish. Oscillations of the swimbladder wall (arrows in swimbladder) result in the transmission of the pressure fluctuations to the endolymph of the inner ear, via a chain of tiny ossicles (Weberian ossicles). Adapted from Ladich and Winkler (2017).

6. Diversity of sounds in fish

The study of the sound production mechanisms of fishes can aid the understanding of the sounds produced and their variation (Parsons and McCauley, 2017). However, to understand the scope for acoustic communication, the extents of call variations must be associated with differences between species; between individual callers of the same species (e.g., large versus small individuals) and between call types issued by the same individual under different behavioral contexts (spawning, courtship displays, feeding, territory defence, convey distress, etc.) (Myrberg 1997; Zelick et al., 1999; Amorim and Hawkins 2000; Ladich 2004); and between the same call type of the same individual (e.g., under different temperature regimes) (Connaughton et al., 2000). This variability is mainly based on temporal patterning of sounds or pulses within a sound and on frequency variation (sometimes modulation) (Winn, 1964).

6.1. Interspecific Diversity

Species-specific differences in vocalization are commonly related to species recognition and reproductive isolation. This may be explained by the fact that vocal fish from closely related species often live in sympatry (Amorim at al., 2004; Amorim et al., 2008; Colleye et al., 2011) and have similar morphologic characteristics making it difficult to recognize conspecifics. In these situations, the diversity of sounds found within the same family of vocal fishes is a valuable asset, allowing the female to find a partner of the right species to mate with while males emit sounds to attract them to the spawning site (Amorim, 2006). For example, males of *Pyxicephalus adspersus* and *Pollimyrus isidori*, two related species, show differences in their courtship sounds during the spawning season. While *P. adspersus* emit a steady sequence of alternating grunts and moans, *P. isidori* produce a single grunt followed by a train of short duration moans (Crawford et al., 1986; Crawford et al., 1997a; b).

6.2. Intraspecific Diversity

Advertisement calls

Some species are also known to show individuality in male courtship sounds. Taking in consideration the aquatic medium, acoustical modality is essential for fish to accomplish these events (Myrberg and Lugli, 2006). This allows females (that invest a lot more than males in gamete production) to extract information about the several prospective mates, not only based on their sex and size but also on their spawning readiness and specific location (advertisement calls), so that successful reproduction occurs (Alcock, 2001; Amorim, 2006; Amorim et al., 2015). Thus, choosy females can increase their fitness either through direct benefits (e.g. higher territory quality, better parental care) or through indirect benefits (e.g. receiving favourable genes) (Andersson et al., 2006). Advertisement calls are typically loud, have low frequencies, long duration and high repetition rate (i.e. a high duty cycle) so that they can be transmitted over long distances to attract females. Furthermore, these signals must contain features that are highly stereotyped within and between individuals to transmit unambiguous information on species identity (Laidre, 2012). On the other hand, courtship and spawning signals, exchanged during close proximity interactions that lead to coupling, are generally softer, high-frequency sounds to avoid the attraction of nearby competitive mates and predators, having an increased duty cycle to synchronize spawning events (Amorim et al., 2015). Since fish size strongly correlates with male reproductive success, in some species small males have been reported to have alternative reproductive strategies (Myrberg and Lugli, 2006). For example, small males grass gobies (*Zosterisessor ophiocephalus*) silently sneak into the larger, sound emitting goby's burrow to steal fertilizations while a female is spawning. Nonetheless, in the absence of a larger dominant male, small fish of this species produce courtship sounds and can occupy a burrow (Malavasi et al., 2003). Furthermore, sounds produced by males during reproductive activities often vary depending on the situation of the prospective partners. Not only reproductive competitors acoustically communicate among themselves but often interrupt spawning activities, inciting aggression between the participants (Myrberg and Lugli, 2006). Males of spawning haddock have been reported to increase the duration of courtship sounds when competition for the female increases by the introduction of another male (Bremner et al., 2002).

Agonistic and distress calls

Vocalization during agonistic behavior is widespread in many fishes and has been described in representatives of more than 30 families, though this is likely to be a great underestimate (Ladich and Myrberg, 2006). Sounds are produced in several agonistic contexts, including in distress or disturbance situations. A great diversity of fish sounds was described when fish were caught or hand held (Fish and Mowbray, 1970; Myrberg, 1981). However, distress sounds are not only related to an anthropogenic context, but towards potential predators as well (Ladich and Myrberg, 2006). In example, Atlantic cods (*Gadus morhua*) were observed to produce grunts toward a conger eel and click sounds in the presence of seals or human divers (Brawn, 1961; Vester et al., 2004). The functional importance of the latter could represent an altruistic behavior or be related to the alarming of conspecifics, enhancing their odds of survival and thus increasing the general fitness of the transmitter (if protected conspecifics are closely related to the sender). Another hypothesis could be that distress calls are used to attract other predators and by that disrupt the predation event allowing the prey an opportunity to escape. Moreover, numerous fish produce sounds during aggressive, intraspecific competitive encounters (Amorim, 2006). Confronts related to competitive feeding usually occur at a low level of aggressions as disputes end quickly after a few behavioral acts (Ladich and Myrberg, 2006). Foraging fish, such as gurnards, regularly circle the feeding area, frequently with aggressive displays towards competitors while grasping food and producing knocks, grunts and growls (Amorim and Hawkins, 2000). Higher levels of aggression are exhibited when fishes are competing for mates (as referred above) or while establishing and maintaining territories. Aggressive interactions towards conspecifics associated with competition for space begin, in most cases, after an invader comes into the territory owner's scope of vision (Ladich and Myrberg, 2006). However, these are not always related with the domination of territories; they can also function to maintain distances between individuals within an aggregation, as this may diminish the chances of more than one individual being caught by predators; or to space out individuals as they advertise territorial ownership (e.g. In Batrachoididae; Salmon, 1967). In these circumstances, contests usually begin with visual displays in which individuals try to increase their body size/contour by erecting fins, opercular covers and gill membranes, while emitting complex acoustic signals (low cost) (Ladich and Myrberg, 2006). Animals try to gain information about fighting abilities through assessing signals and solving conflicts without escalated fighting which may end up in injury or death (high cost) (Krebs and Davies, 1993). Acoustic displays can comprise one kind of sound or several types, demonstrating diverse levels of hostility or different meanings (Ladich and Myrberg, 2006). Usually, advertisement or courtship sounds are longer and with a faster pulse repetition rate than agonistic sounds (Amorim, 2006), which are brief and harsh (Ladich, 1997a). For example, gouramis produce one type of sound – croaks. However, intervals between croaks produced during fights were shown to be significantly longer than during courtship (Marshall, 1967).

Fish size and ontogeny

Acoustic differences between individuals of the same species can vary between social context, mostly conspecifics agonistic interactions and mate choice, as well as with ontogeny and size. Dominant frequency is the most common intraspecific variation in fish sounds. Several studies on species that produce sounds with repeated broadband pulses, such as the weakfish (Connaughton et al., 2012), have demonstrated an inverse relation between dominant frequency and fish size. Typically, this relationship is expected to be a mechanism related to the size and acoustical properties of the swimbladder – larger fish have larger swimbladders which are associated with lower frequencies. However, Connaughton et al., (2012) argues against resonance interpretation, stating that the decrease of dominant frequency in larger fish may be explained by the scaling of the sonic muscles. This variability between frequency of calls and size may be a valuable asset for females to discriminate males on the basis of their size, as larger fish are usually dominant, occupy better territories or spawning sites, and have greater reproductive success. Additionally, it is useful to discriminate the opponent's size in agonistic displays (Amorim, 2006). However, the relationship between these variables and size is not invariant and exceptions do exist. Examples include species that either do not possess a swimbladder, e.g. some gobies (Mok, 1981; Lugli et al., 1996), or that produce tonal sounds, in which, as previously mentioned, high rates of muscle contraction determines the fundamental frequency (Skoglund, 1961; Fine et al., 2001), such as in "boat-whistles" of the toadfish *O. tau* (Fine and Lenhardt, 1983).

In what concerns ontogeny, it is known that sound generation is widespread among juvenile fishes since the competition for food and territory may occur regardless of reproductive stage (Bass and Ladich, 2008). According to Wysocki and Ladich (2001), the characteristics of sounds produced by the croaking gourami varies widely during ontogeny, which is probably related to the development of sound-producing structures and to a larger body size (as described before). This species has been shown to decrease dominant frequency and to increase croak duration, number of pulses within a croak, pulse period and sound pressure level, from juveniles to adults. Furthermore, the frequency and intensity of agonistic behavior associated with competitive feeding may also change with ontogeny in fish (Ryer and Olla, 1991). Small juvenile grey gurnards were reported to change their sound production features throughout ontogeny during competitive feeding, with smaller fish being more aggressive (producing more grunts) than larger ones (that mainly emitted knocks), possibly because a larger body size gives an advantage in tracing, capturing and handling prey (Amorim and Hawkins, 2005).

Sexual and seasonal variations

Intraspecific differences in sound characteristics can also vary between gender. Sound-generating mechanisms can be exclusive to males, as in many sciaenid species (Chao, 1978) or sexually dimorphic, such as in gadoids, gobies, some sciaenids and batrachoidids (Amorim, 2006), with the sound-producing apparatus usually being better developed in males (Ladich and Fine, 2006). For example, in toadfishes several studies have uncovered differences in size of swimbladders, intrinsic swimbladder muscles and in fine structure of muscle fibers. This dimorphism arises during development, as juveniles present similar sonic structures in both sexes which after puberty start to grow at different rates (Brantley et al., 1993; Fine et al., 1997). Sexual dimorphism is also found in fishes with pectoral mechanisms. In callichthyid catfishes pectoral fins standardized to body length are longer in males than in females and differences in sound characteristics were detected (Pruzsinszky and Ladich, 1998). Although sound production is commonly associated with male courtship, most fish females with smaller sonic muscles vocalize, not only during spawning events but especially during agonistic interactions (Ladich, 1997b). According to Myrberg and Lugli (2006) the reason that could explain the low number of reported sounds produced by females during courtship display is that for a large number of species (mainly benthic), eggs are placed in the males' nest to where ripe females must be attracted to, thus there is no need in being acoustically active.

The condition and size of sonic muscles don't just vary between gender. In fact, this sound-producing apparatus have been reported to undergo a yearly hypertrophy-atrophy cycle in line with seasonal dynamics, in many fishes. For example, in the males of haddock and weakfish, seasonal changes of photoperiod and temperature have been reported to induce the production of high levels of androgens, which results in the hypertrophy of the sonic muscles. In both of these species, muscles increased in size by more than two times up to the beginning of the spawning season. Together with their size, the coloration of the muscles changes to dark pink. During the peak of the mating season, sound amplitude, like the sonic muscles, is maximal. After the spawning, the muscles gradually atrophy and colour becomes lighter (Templeman and Hodder, 1958; Hawkins et al., 1967; Connaughton and Taylor, 1996; Lagardère and Mariani, 2006).

Other Variabilities

Another noteworthy point is that, depending on the time of day, species can be more or less acoustically active, with some emitting more sounds during the night, others during the day, and some showing peaks at dawn or dusk. This may be correlated with different events, such as formation of breeding choruses, feeding and territorial behavior (Amorim, 2006). Furthermore, sound parameters associated with muscle contraction have been observed to change with temperature. Generally, rising temperatures cause muscles to take less time to complete a twitch and contract with a faster velocity (Connaughton et al., 2000). Hence, rate of sound emissions, sound pressure level (SPL), sound duration, pulse repetition rate and fundamental or dominant frequency tend to increase, and pulse duration tends to decrease (Fine, 1978a,b; Brantley and Bass, 1994; Connaughton et al., 2000, 2012). Additionally, geographical differences in acoustic signals have been studied for the oyster toadfish *Opsanus tau* (Fine, 1978a; 1979) and the damselfish, *Dascyllus albisella* (Mann and Lobel, 1998). Boatwhistle duration and fundamental frequency emitted by male oyster toadfish vary along the Atlantic coast of the USA (Fine 1978a,b).

7. Sciaenidae family

The teleost family Sciaenidae, collectively known as croakers and drums, is among the largest families of vocal sonic fishes comprising roughly 270 species within 70 genera worldwide. Though some species are found exclusively in freshwater habitats, sciaenids occur mostly on the continental shelves of coastal marine waters of the tropical and temperate regions of the Atlantic, Indian and Pacific oceans (Chao, 1986; Nelson 1984). The members of this family are typically demersal, inhabiting sandy or muddy substrates, where they feed on mollusks, crustaceans, polychaetes, and fish (Murdy et al., 1997). Estuaries are commonly used as nursery areas by the larvae and juveniles of many of the marine sciaenid species (Chao, 1986; Flores-Coto and Warlen, 1993; Hettler and Barker, 1993). Usually these fishes are solitary or live in small groups, with some species forming large aggregations during the spawning season (Saucier and Baltz 1993; Murdy et al., 1997).

Among other groups of fishes that also communicate acoustically, the sciaenids are unique in the diversity of sounds produced, variously described as drumming, croaking, knocking, clucking, or purring (Ramcharitar et al., 2006). Mechanisms, however, follow roughly the same design: the swimbladder is dorsally surrounded by bilaterally symmetrical and extrinsic sonic muscles that originate from the hypaxial musculature and insert indirectly on the swimbladder via a central tendon (Hill et al., 1987; Ono and Poss, 1982; Tower 1908; Connaughton et al., 1997). The fast contraction of sonic muscles (twitches) stimulates the swimbladder in a transient response, which in this fish family results in trains of repeated pulses of sound, with each pulse decaying before the next one begins (Fish and Mowbray, 1970; Demski et al., 1973; Guest, et al., 1978; Tower, 1908; Lagardère and Mariani, 2006). Sciaenid fish producing sound in this manner generate fundamental frequencies between 100 and 200 Hz and dominant frequencies up to 1000 Hz (Ramcharitar et al., 2006). However, within this family the parameters of vocalizations have been showed to vary, depending on gender (Ueng, et al., 2007), gonads development, fish size, water temperature, season and geography (Hill et al., 1987; Connaughton and Taylor, 1994; Connaughton et al., 1997, 2000, 2002 2012; Gannon, 2007; Tellechea et al., 2010; 2011). As such, although no experiments have been conducted to test whether sciaenids can discriminate calls based on temporal characteristics, data from other teleost groups suggest that the information contained in their calls (e.g., species identity or behavioral state) is probably encoded in the temporal domain (Winn, 1964; 1972).

Sciaenids produce at least two different types of calls: reproductive calls and disturbance calls. Most commonly, sciaenids were reported to produce drumming sounds during the spawning season (Fish and Cummings, 1972; Takemura et al., 1978; Saucier and Baltz, 1993; Connaughton and Taylor, 1995), suggesting that it might play an important role in reproduction, including formation of spawning aggregates and courtship behavior. Sciaenids have been reported to have highly marked circadian rhythms producing more sounds at twilight and early evening (Holt et al., 1985; Connaughton and Taylor, 1995; Lagardère and Mariani, 2006; Parsons et al., 2009; Tellechea et al., 2010; Borie et al., 2014; Montie et al., 2016) . Usually, the ability to produce species-specific sounds during the spawning period is attributed to male courtship (Fish and Mowbray, 1970; Connaughton and Taylor, 1995, 1996; Luczkovich et al., 1999). In most sciaenid species (e.g., weakfish, red drum, and silver perch) only males possess sonic muscles (Tower, 1908; Fish and Mowbray, 1970; Hill et al., 1987) indicating that sound production is restricted to males. There are, however, species (e.g., Atlantic croaker, whitemoth croaker, meagre, and mullway) in which both sexes present sonic muscles (Hill et al., 1987; Lagardère and Mariani, 2006; Ueng et al., 2007).

Because sound production in sciaenids is associated with spawning, passive acoustics has been used to record temporal and spatial patterns of fish reproduction. Despite the vast literature on sciaenid sound production, there is a lack of understanding about the functional significance of their calls, which leaves plenty of room for further investigations (Ramcharitar et al., 2006).

Disturbance calls indicating distress, pain, aggression, or a similar state have been studied for several sciaenid species (Dijkgraaf, 1947; Schneider and Hasler, 1960; Connaughton et al., 2002; Fine et al., 2004; Tellechea et al., 2010, 2017). Physical disturbance or distress calls usually consist of a long series of rapid pulses. In weakfish (Connaughton et al., 2002), Atlantic croaker (Fine et al., 2004) and whitemouth croaker (Tellechea et al., 2010) the similarities between the waveform of advertisement and disturbance calls suggest that both sounds are produced by the same mechanism, though the number of pulses varies more widely in disturbance calls then in advertisement calls, as the latter are characterised by longer inter-pulse intervals. As advertisement and disturbance calls present similarities, recordings and characterization of disturbance calls may be valuable to understand intraspecific variability in a species of interest and thus use this in studies using PAM, such as detecting juveniles *vs.* adults in nature.

8. Meagre, *Argyrosomus regius* (Asso 1801)

The meagre (*Argyrosomus regius*, Asso 1801) is one of the world's largest marine teleosts commonly found throughout the Mediterranean and Black Sea, as well as along the Atlantic coasts of Europe and Africa (Poli et al., 2003). This sciaenid can be found in estuaries, coastal lakes and in inshore and shelf waters, close to the bottom or near the surface, within a depth range of 15-200 m (Griffiths and Heemstra, 1995). In the beginning of the spawning period, during Spring, meagre enter the mouths of estuaries in groups where they remain until late summer, when the spawning season ends (Cárdenas, 2010). Areas that remain important for spawning are the estuary of the river Tagus (Portugal), river Guadiana, (Southern Spain and Portugal) (González-Quirós et al., 2011) and river Gironde (France) (Quéméner et al., 2002). In the winter, adult meagre return to deep waters to feed (Quéméner et al., 2002) while juveniles leave estuaries to spend 2-3 years in coastal waters before migrating to offshore feeding areas. The most important factor determining meagre migrations and reproduction appears to be water temperature (FAO, 2005–2010b).

Meagre have a significant commercial value for recreational and small-scale commercial fisheries and aquaculture. They present high fertility and growth rates (ca. 1kg/year), attaining 180 cm in total length and 50kg in the wild, costing up to 200€/specimen (FAO, 2005-2010b). Furthermore, being euryhaline species, these fish can adapt to a variety of environments, allowing their cultivation in land based brackish water media and in tanks, as they withstand captivity very well (Pastor et al., 2002) and tolerate a wide range of temperatures and salinities (Duncan et al., 2013).

It is known for centuries that *A. regius* generates sounds. In fact, the sound these fish produce during spawning season is so loud that they dominate the acoustic environment where they occur, facilitating fishing in certain sites. While advertisement calls produced during spawning aggregations have been characterised in this species (Lagardère and Mariani 2006) we lack information on their wider vocal repertoire, including whether females make sounds, and the variability of sound production associated with social context (agonistic *vs.* reproductive) or ontogenetic phase (vocal repertoire of juvenile *vs.* adult, and variation with size). However, studies with a congeneric species *Argyrosomus japonicas* point to the existence of acoustic variability associated with gender and social context, that will allow passive acoustic monitoring to be a valuable tool to study natural populations of *A. regius*.

8.1.Advertisement calls in Meagre (*Argyrosomus regius*)

Advertisement calls produced during spawning aggregations have been characterised in this species solely by Lagardère and Mariani (2006). In this study, recordings of vocalizations produced by meagre during the spawning season were carried out in the Gironde estuary (with water temperature 16-21ºC and high turbidity) in order to describe spawning sound types produced by the meagre. Pre-spawning female and sperm and non-sperm producing males were caught and dissected for analysis of the sound-generating apparatus (Fig. 5). The results showed that both sexes possess sonic muscles. In males, the swimbladder was large, covering the entire length of the abdominal cavity, and was surrounded by bilaterally symmetrical sonic muscles forming two broad bands (Fig. 5A,B). These extrinsic muscles were inserted on a connective tissue sheet (aponeurosis) sitting on the top of the swimbladder (Fig. 5A,B). Differences were found between male individuals. In the sperm- producing male, muscles were enlarged (Fig. 5A,B) in contrast with the non-producing one (Fig. 5C). In pregnant females of the same size, sonic muscles were thinner (Fig. 5D).

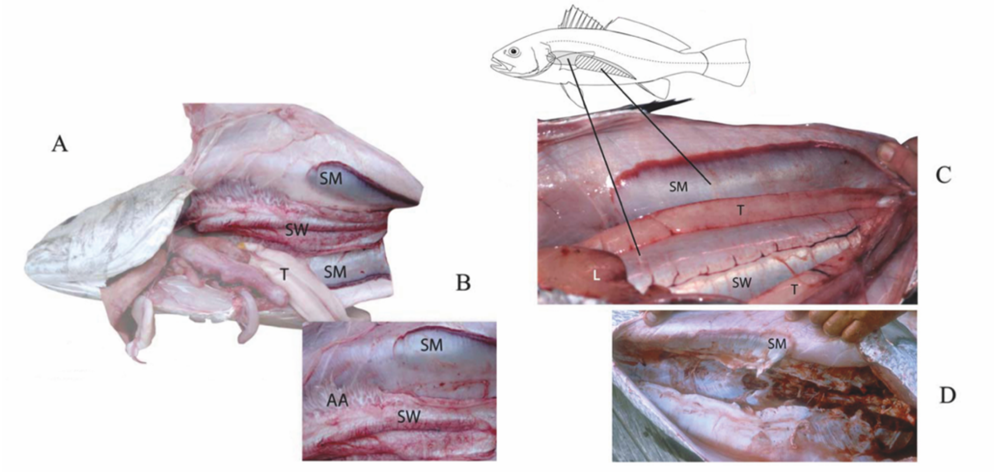

Figure 5: Sexual dimorphism of sonic muscles in the meagre *Argyrosomus regius* : (A) body cavity and body wall of a sperm producing male (B) amplification of the same picture exhibiting anterior part of swimbladder (SW), with its arborescent appendages (AA) and the left sonic muscle (SM); (C) body cavity of a non-sperm producing male sonic muscle and its extent on the left body wall, T = testicle, L = liver; (D) right body wall of a pre-spawning female showing the less developed sonic muscle. Adapted from Lagardère and Mariani, (2006).

Meagre sounds were produced in dense choruses (Fig. 6A), thus individual (Fig. 6B) sound emissions were not possible to differentiate. Furthermore, with both sexes having sonic muscles, in field conditions sounds could not be associated to gender, hence it is still unknown weather females produce sound or not. Meagre sounds were of two types: long and short grunts. Long grunts consisted of trains of 30–112 pulses (Fig. 6C,D), with the pulse period (i.e. sonic muscle contraction rate) being very regular. Short grunts consisted of trains of 4-6 pulses and resulted in the cessation of long grunts (Fig. 6E,F).

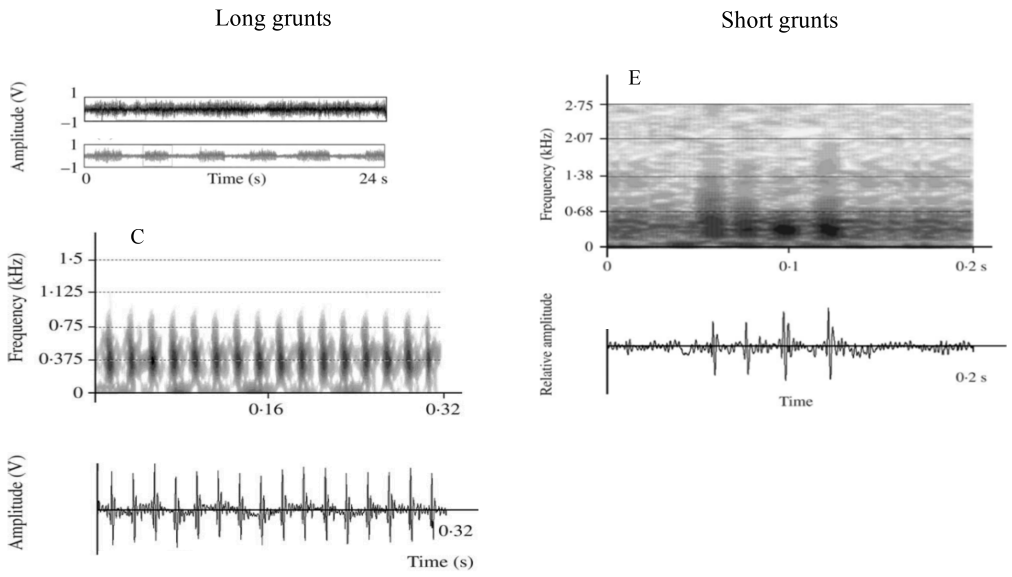

Figure 6: Oscillograms of (A) chorus and (B) individual long grunts produced by meagre during the spawning period in the Gironde estuary. (C) Sonogram and (D) oscillogram of a typical meagre long grunt consisting of a series of individual sound pulses. (E) Sonogram and (F) oscillogram of one meagre short grunt (heard much less often than long grunts) recorded during spawning period under field conditions. Adapted from Lagardère and Mariani (2006).

9. Objectives

The goals of this project are to characterize the sounds emitted in alarm and social contexts of the meagre in different phases of their life cycle, namely in juveniles and adults. Specifically, it aims to:

- Compare voluntary sounds and distress calls recorded in captivity (in aquaculture facilities of IPMA, Instituto Português do Mar e da Atmosfera, in Olhão) from juveniles and adults, and from adult female and male.
- Describe seasonal variation in sound production of *A. regius.*
- Characterize the acoustic activity and characteristics of sounds produced during social contexts in relation to spawning events (monitored by the collection of eggs from the tanks).
- Contrast sounds recorded in captivity with sounds recorded in nature to validate ongoing passive acoustic monitoring carried out in Tagus estuary.

We expect that the study of distress and social calls produced in captivity will contribute to monitor natural population of meagre including delimiting spatial use of juveniles and adults inside and outside the Tagus estuary.

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Sound production in the Meagre, *Argyrosomus regius* (Asso 1801): Intraspecific variability associated with size, gender and context

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Abstract Advertisement calls produced during spawning aggregations have been documented for *Argyrosomus regius*, but there is lack of information on the variability of the acoustic features of these calls and on their wider vocal repertoire. The goals of this work were to characterize the sounds emitted in alarm and social contexts by meagre in different phases of their life cycle, namely in juveniles and adults. Spontaneous sounds and distress calls were recorded in captivity (IPMA, Algarve, Portugal). Sounds produced by juveniles and adults recorded during handling (distress) were related to size, and in the case of adults, also gender. The present dataset demonstrates for the first time that in this species dominant frequency is inversely related to fish size, that females emit sounds that differ from those of males and that sounds vary according to context and throughout ontogeny. Sounds produced in captivity were comparable to sounds emitted in the field. We expect that this study will contribute to monitor natural populations of meagre including delimiting spatial use by juveniles and adults inside and outside the Tagus estuary.

Key words: fish acoustic communication; distress calls; advertisement calls; ontogeny of sound production; sexual dimorphism; Sciaenidae

Introduction

More than 800 species from 109 teleost fish families emit sounds which in some cases, play an important role in social communication (Rountree et al., 2003). Fishes have evolved several sound production mechanisms, including the vibration of the swimbladder through the contraction of sonic mussels (Tavolga, 1964, 1971; Fish and Mowbray, 1970; Fine and Parmentier, 2015). The frequency spectrum and the pulse repetition rate of a swimbladderdriven call can be affected by the biological attributes of the structures involved in the mechanism (Connaughton et al., 2000; Sprague, 2000; Ramcharitar et al., 2006). Consequently, fish sounds can be species specific (Hawkins, 2002), and can vary with size (Myrberg et al., 1993; Connaughton et al., 2000). Additionally, sound production and variability is frequently related to behavioral functions, such as courtship, spawning, agonistic behavior, competitive feeding, and disturbance (Ladich and Myrberg, 2006).

Sciaenidae, collectively known as the croakers and drummers, is one of the largest families of vocal fishes (Chao, 1986). Representatives of this family produce swimbladder-related sounds (reviewed in Fine and Parmentier 2015). In most sciaenid species, sound-producing ability is restricted to males (Tower, 1908; Fish and Mowbray, 1970; Hill et al., 1987; Connaughton and Taylor, 1995). However, in some species, such as the black drum (*Pogonias cromis*), white croaker (*Genyonemus lineatus*), and meagre (*Argyrosomus regius*), both males and females possess extrinsic sonic muscles common to the family (Fish and Mowbray, 1970; Takemura et al., 1978; Lagardère and Mariani, 2006).

Passive acoustics monitoring of sciaenids has been used to assess spatial and temporal patterns of fish reproduction by using sounds related with spawning (Fish and Cummings, 1972; Takemura et al., 1978; Saucier and Baltz, 1993; Lagardère and Mariani, 2006; Ueng et al., 2007; Parsons et al., 2009; Montie et al., 2016). However, although several studies on breeding vocalizations have been published for a number of sciaenid species, detailed descriptions of sound parameters and their size-dependent variations are scarce and often restricted to distress calls (Connaughton et al., 2000; Colleye et al., 2009; Tellechea et al., 2010). For example, Connaughton et al., (2000), found an inverse relation between dominant frequency of distress calls and fish size. These authors showed that for weakfish, *Cynoscion regalis*, throughout a range of 11cm of total length (25–36cm) the dominant frequency decreased by 91Hz from 560 to 479Hz. Gender-related differences of vocalizations are also rarely reported since for the majority of the studied sciaenid species, sonic muscles are absent in females. Ueng et al., (2007) observed on Japanese croaker that advertisement calls of the male and female differ; females generated significantly more pulses per call, and their calls had a longer call duration, a shorter pulse period, and a lower dominant frequency than those of males. Nevertheless, similarities between social (e.g. advertisement) and disturbance calls, namely in dominant frequency, were observed in Sciaenid species (Connaughton et al., 2002; Fine et al., 2004; Tellechea et al., 2010) suggesting that the intraspecific variability of disturbance calls may bear a parallel with that of advertisement calls.

The meagre, *Argyrosomus regius* (Asso, 1801), is a vocal sciaenid widely distributed along the Atlantic coast of Europe and Africa, and in the Mediterranean (Chao, 1986). It is a species with a high commercial value, being farmed in several countries since the 1990s, including Portugal (Monfort 2010; FAO 2011). While advertisement calls produced during spawning aggregations and the sexual dimorphism of sonic muscles have been characterised in this species (Lagardère and Mariani 2006), there is a lack of information on their wider vocal repertoire, including whether females make sounds, and the variability of sound production associated with social context (agonistic *vs.* reproductive) or ontogenetic phase (vocal repertoire of juvenile *vs.* adult, and variation with size). Such knowledge could be useful to interpret field-recorded sounds, e.g. by allowing to recognize reproduction areas or young fish shoals.

The present study aimed to describe disturbance and social sounds produced by juveniles and adults to examine intraspecific variability of the acoustic signals with context, size and, in the case of adults, also gender. To validate this variability with field recordings advertisement sounds registered in the aquaculture setup were also compared with sounds registered in the Tagus river during the breeding season.

Methods and Materials

Captive fish

Sound recordings were obtained from a group of adult breeders and juvenile meagre, *A. regius,* hosted at the aquaculture facilities of Instituto Português do Mar e da Atmosfera – Estação Piloto de Piscicultura de Olhão (IPMA – EPPO), Portugal (37◦02′ N, 7◦49′ W). All fish were reared in aquaculture tanks under a natural photoperiod, natural temperature ranging between 14 and 23ºC, continuous water supply, controlled pH (8± 0.4), salinity (35± 0.2 psu) and oxygen level close to saturation (80±7.6%.). Meagre were fed dry food 4 times a day. Adult meagre breeders (n=10) with 6 and 9 years old, were housed in an indoor unisex concrete parallelepipedic tank (3 m^2 area, 1.2 m deep), exhibited an average total length (TL) of 87cm ranging 69-102 cm, and a 4:6 (M:F) sex ratio (Fig. 1A,B). Subject juveniles were reared in two interior 1500L fiberglass circular tanks (3 m diameter, 1.6 m deep) (Fig. 1 C,D). One tank housed 1 and 2 years old juveniles (n=208) ranging in size from 31-51cm. The other tank reared 2,5 months old juveniles (n=4000), with an average TL of 9 cm.

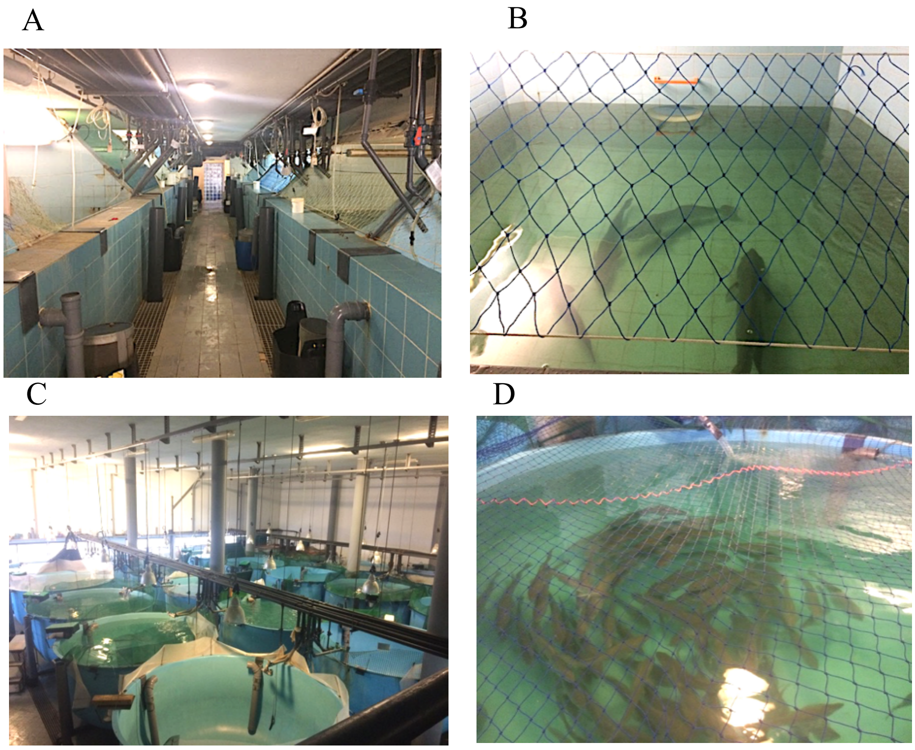


Figure 1: Parallelepipedic concrete interior tanks used to rear adult meagre breeders in IPMA facilities (A, B); (C) and (D) depict circular fiberglass interior tanks rearing meagre juveniles.

Recording of vocalizations in captive fish

Ontogenetic and gender variation of distress calls

Vocalizations of juveniles with different size were individually recorded at approx. 20ºC water temperature in May 2018. Two 200L plastic circular containers (Fig. 2A) were filled with water from the rearing tanks under continuous aeration. For each size class, a group of 10 individuals was transferred with a hand-net to one of these 200L containers. Then a single fish was transferred with a sleeve to the second 200 L container where recordings were carried out. This fish was kept inside the sleeve and sometimes stimulated by pressing the caudal peduncle while distress calls were recorded for 3 min with a High Tech 94 SSQ hydrophone (sensitivity of – 165 dB re 1 V/µPa, flat frequency response up to 6 kHz ± 1 dB), placed at approximately 10 cm from the fish's abdomen, and connected to a Tascam DR-40 Portable Digital Recorder (44.1 kHz sampling rate, 16 bit). Prior to each recording, the total length and weight of each fish were rapidly measured using an ichtyometer and a Kern 60K1DL scale (± 2g), respectively (Fig 2B).

To check for sound production in smaller fish, similar recordings of distress calls were conducted with a group of 10 randomly selected juveniles with approx. 9 cm TL. The recording protocol was similar to the one above, except that fish were transferred to a 10L glass beaker with water from the original tank also at approximately 20º C. As before, distress calls were recorded individually. Furthermore, one individual with 10 cm was transported to the laboratory facilities at University of Algarve, to be dissected to inspect sound-producing muscles. For this purpose, the fish was immersed in a buffered solution of MS222 (tricaine methanesulfonate buffered with sodium bicarbonate to a neutral pH) at a concentration of 250mg/l during approximately 10 min and then euthanized by decapitation.

A similar experimental design was carried out in July 2018 for the adult breeders reared in the concrete tanks. Prior to sampling, the water level in the indoor tank was lowered by 1 m and all fish (fasted for 24h) were anaesthetized with 40 ppm of 2-phenoxyethanol (2-PE) to reduce stress and prevent fish from jumping and harming themselves while being captured. Following the juveniles' recordings protocol, two 200L containers filled with aerated seawater from the rearing tanks and without anaesthetic, were used. Water temperature was the same as in juveniles (~20ºC) to rule out differences in the sounds due to temperature-dependent effects. After 20 min, anaesthetized individuals were safely captured with a plastic sleeve bag and transferred in groups of 3 to the first container, where fish were allowed to recover for approximately 15 min after which they showed no signs of anaesthesia. Posteriorly, each breeder was individually identified by chip reading and transferred with a sleeve to the second 200L container where recordings were carried out. Barata et al., (2016) tested the efficiency of 2-phenoxyethanol (2-PE) in juveniles of this species at IPMA, based on the stages of anaesthesia described by Iwama et al., (1989): total loss of equilibrium (Stage AI), loss of gross body movements (Stage AII), and cessation or imperceptible opercular movements (Stage AIII). According to the study, after being exposed for 10 min to 100 mg/L of 2-PE, meagre juveniles lost equilibrium (AI) but continued to exhibit locomotion and opercular movements and took approximately 4 min do recover. Biometric samplings were conducted a few days later, with breeders fasted for 48h being submitted to a higher dose of anesthetic (200ppm). Additional to total length and weight measurements, gender determination of individuals was attempted. Since external sexual dimorphism is not present in meagre, sex identification was carried out overwater by carefully turning the fish upside down and gently pressuring the abdomen to check if sperm was released through the genital papilla. In the case of sperm release, individuals were considered males. When individuals were not fluid, a flexible polystyrene catheter was inserted in the genital papilla in an attempt to extract gonadal tissue and identify the fish as female. However, with the exception of two female individuals, gonadal tissue extraction was not succeeded, and female gender was attributed to individuals that, according to the chip's information, in various samples over the last 3 years were never fluid.

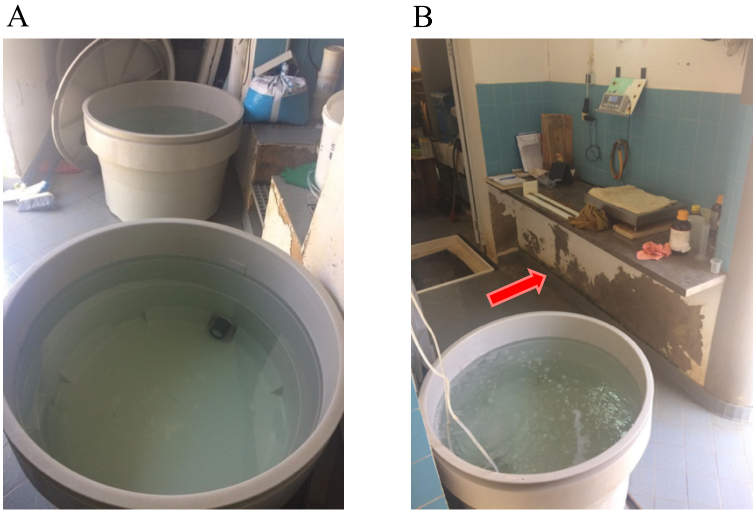


Figure 2: Experimental set up for the distress calls recordings depicting (A) the two 200L containers used for individual recordings and (B) sampling station for biometric measurements (indicated by the red arrow).

Ontogenetic variation of sounds made during social contexts

On May 2018, recordings of juvenile individuals (n=208) with total lengths ranging from approximately 30-50 cm, were conducted for 2 hours using a Tascam DR-40 Portable Digital Recorder (44.1 kHz sampling rate, 16 bit) connected to a High Tech 94 SSQ hydrophone (sensitivity – 165 dB re 1 V/µPa, flat frequency response up to 6kHz ±1dB) vertically positioned at the centre of the tank at approximately 30 cm from the bottom. Water temperature in the tank was 19,8ºC.

Sounds of adult individuals (n=10) with an average length of 87cm (69-102 cm) were recorded during the spawning season on July 2018 with a High Tech 94 SSQ hydrophone (sensitivity – 165 dB re 1 V/µPa, flat frequency response up to 6kHz ±1dB) positioned vertically at the centre of the tank at approximately 30 cm from the bottom and connected to a stand-alone 16 channel datalogger (LGR – 5325, Measurement Computing Corp, Norton Ma USA; 12 kHz sampling rate 16 bit, ±1 V range). Water temperature in the tank was 21,3ºC.

Vocalizations recordings in the field

The data set used in this study consisted of ca. 5 day round-the-clock recordings of sounds obtained from 14 to 18 of June 2018, in the Tagus estuary (Air Force base 6, Montijo, Portugal; 38º42'N, 8º58'W). For this purpose, High Tech 94 SSQ hydrophone (sensitivity – 165 dB re 1 V/μPa, flat frequency response up to 6kHz ±1dB) was anchored at about 20 cm from the bottom to a stainless-steel holder projecting from a concrete base where the cable was attached to minimise current-induced hydrodynamic noise. The signal from the hydrophone was recorded by a 16 channel stand-alone data logger (LGR – 5325, Measurement Computing Corp, Norton Ma USA; 4 kHz sampling rate 16 bit, ±1 V range). Diurnal temperature variation was highly influenced by the tide, with a mean of 20.2 ºC and a range between 18.3-23.2ºC. Water depth varied approximately between 3-6 m, depending on tide.

Sound Analysis

Calls were edited with Cool Edit Pro Software and analysed with Raven 1.2.1. Only the sounds with a good signal-to-noise ratio were used in the analyses. The following temporal and spectral parameters were measured from distress and social sounds (Fig. 3): sound duration (ms), as the time from the onset of the first pulse to the offset of the last pulse; number of pulses; pulse period (ms) (the average time interval between the peaks of two consecutive pulses in a sound), obtained by dividing the duration of the sound by the number of pulses minus 1 (Mann et al., 1997); and dominant frequency (Hz), the frequency presenting the highest energy level in the sound. Temporal parameters were measured from oscillograms while dominant frequency was measured from power spectra (6 kHz, Fourier transform (FFT) size 1024 points, Hamming window, 50% time overlap).

To examine ontogenetic and gender variation of distress calls a total of 600 calls were randomly selected and manually analysed (20 sounds per individual for each of the 20 juveniles and 10 adults). To study ontogenetic variation of sounds made during social context in captivity a total of 100 sounds were analysed; 50 for juveniles and 50 for adults. To compare voluntary calls produced in captivity with those produced in the field, 30 sounds emitted during the spawning season in the Tagus estuary were analysed. Meagre sounds were produced in dense choruses and individual calls could not be distinguished in field recordings. Accordingly, individual sound emissions that occurred before and after the main chorus were analysed.

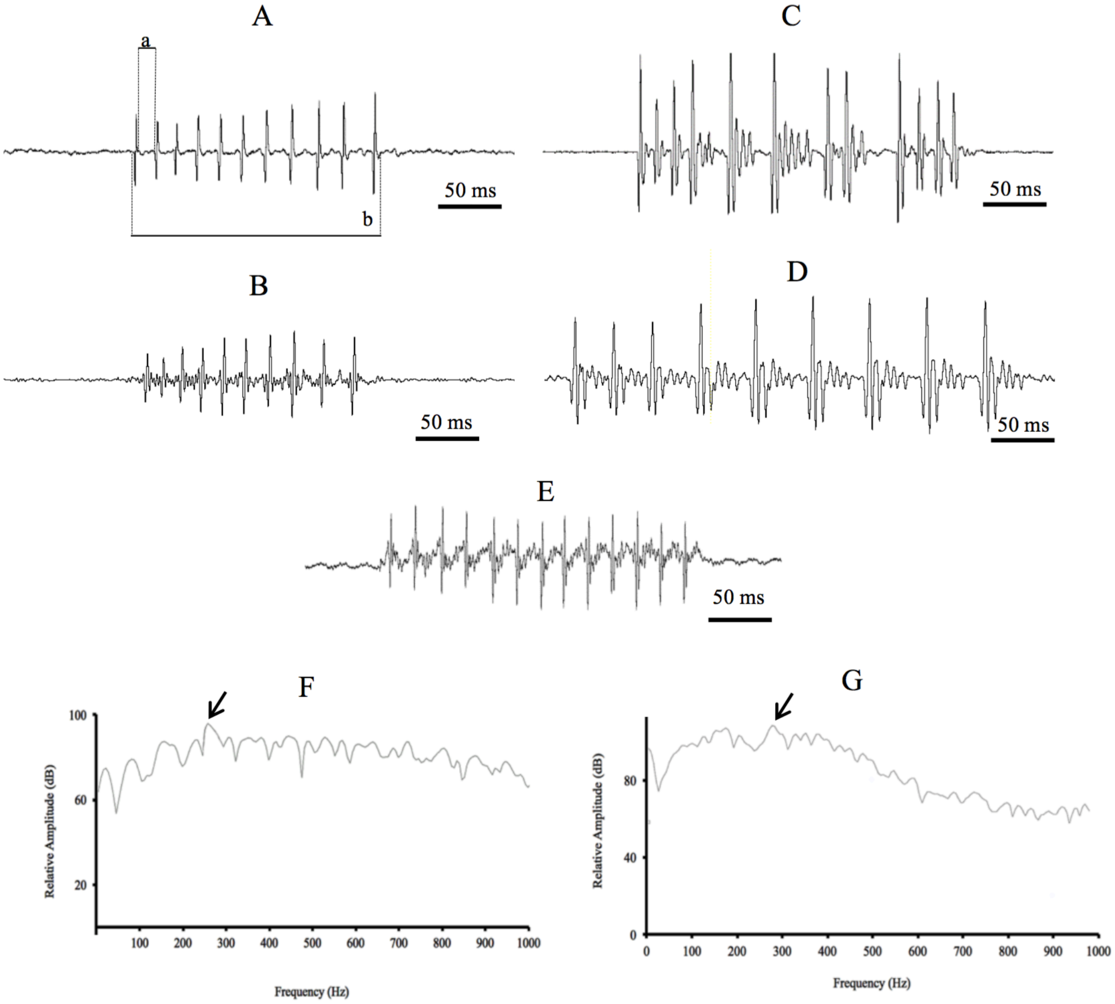


Figure 3: Oscillograms (A-D) of sounds produced by captive *A. regius* associated with different contexts and ontogeny; oscillogram of sound produced by meagre in the field (E); and power spectrum (F, G). (A) juvenile distress call representing some of the acoustic parameters measured ((a) pulse period) and (b) sound duration), (B) juvenile social call, (C) adult distress call, (E) spontaneous call produced by a wild individual, (F) power spectrum of a juvenile distress call and (G) power spectrum of an adult social call. Arrows depicting the peak frequency. Sampling frequency 6 kHz, 1024 point FFT, Hamming window, and 50% overlap.

Statistical Analysis

Statistical analysis was conducted using the software Statistica (version 10, Statsoft, Tulsa, OK, USA). A 0.05 significance level was used for all analyses (Zar, 1984). To assess the influence of fish total length (TL) on all acoustic variables of disturbance calls, correlation matrices were used to examine changes in all acoustic variables across total length. The data used examine changes in mean values of all acoustic variables (20 sounds per fish, n=30). For the analyses of the variation of sounds produced in different context and ontogenetic groups, Mann-Whitney U Tests were conducted considering 50 sounds per context for each ontogenetic group. In these analyses, data concerning distress sounds were restricted to 50 randomly selected sounds from the whole data set to avoid large imbalances between factor levels sample sizes. To examine gender-related differences of agonistic calls characteristics, an analysis of covariance with gender as a factor and TL as the covariate was performed. As the covariate had a non-significant effect, it was removed from the analysis. Since variables did not violate assumptions of normality, male and female sound characteristics were then compared with Student's t-tests. The data used in this analysis were mean values of 20 sounds for each of the 4 males and 6 females (n=10). 50 voluntary calls recoded in captivity and 30 spontaneous calls recorded in the field, were compared with Student's t-tests.

Results

Ontogenetic variation of distress calls

All acoustic features of distress calls were significantly related with total length (Table 1, Fig. 4). Sound duration and number of pulses increased with fish size (Fig. 4A,B) while pulse period and dominant frequency decreased (Fig. 4C,D). Across a range of 71 cm in total length (31–102 cm), sound duration increased 8ms from 126 to 135ms, number of pulses increased by 3 pulses, pulse period decreased by 2 ms from 11 ms to 9 ms and dominant frequency decreased by 79 Hz from 340 Hz to 261 Hz.

Table 1: Relation between total length (cm) and sound features (Pearson correlation).

Variables (*x versus y*)	*r*	*P*
Total length versus sound duration	0.44	0.014
Total length versus nº of pulses	0.64	< 0.001
Total length versus pulse period	-0,64	< 0.001
Total length versus peak frequency	-0,56	0.001

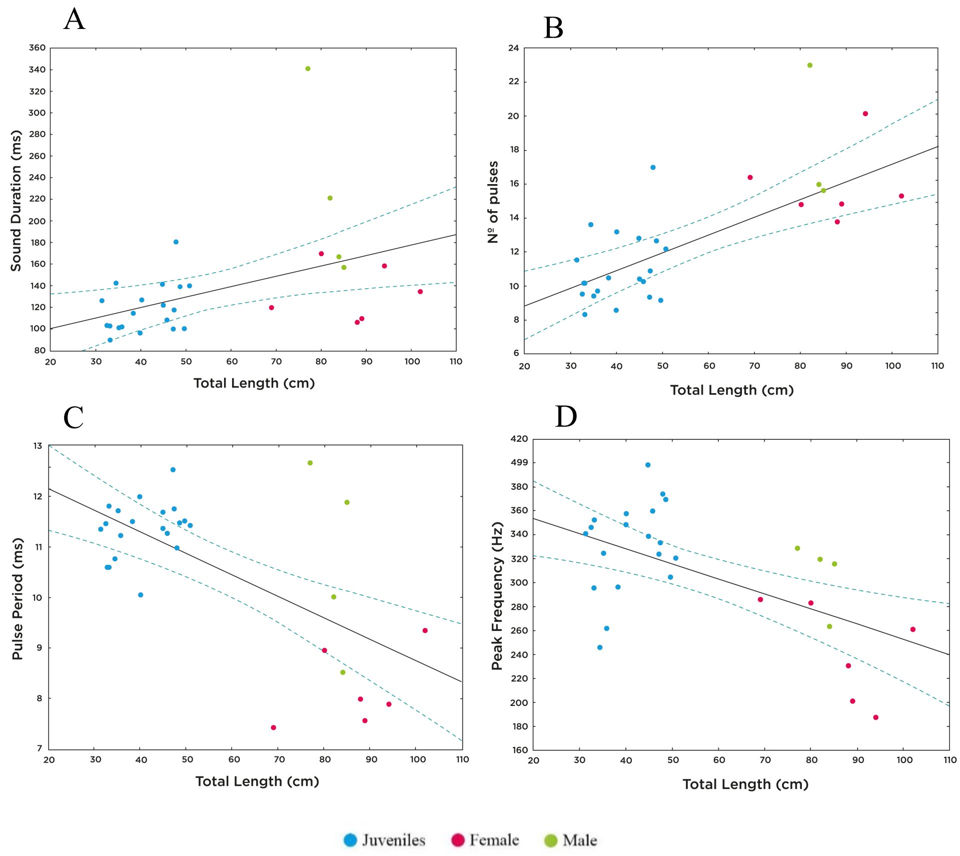


Figure 4: Influence of fish size on disturbance calls of *Argyrosomus regius*. Relation between total length (cm) and (A) sound duration (ms), (B) number of pulses, (C) pulse period (ms), (D) peak frequency (Hz). Gender is depicted by different colors. Fish ranged from 31 to 102cm in total length (n=30) and all recordings were made at 20°C. Regression lines and 95% confidence interval are shown.

No sounds were registered from the small juveniles of c. 9 cm. Dissection of one individual showed that it was a male (testicles were visible) but sonic muscles were absent (Fig. 5). Fig. 5 depicts the swimbladder, consisting of a single chamber, located between the viscera and the vertebral column.

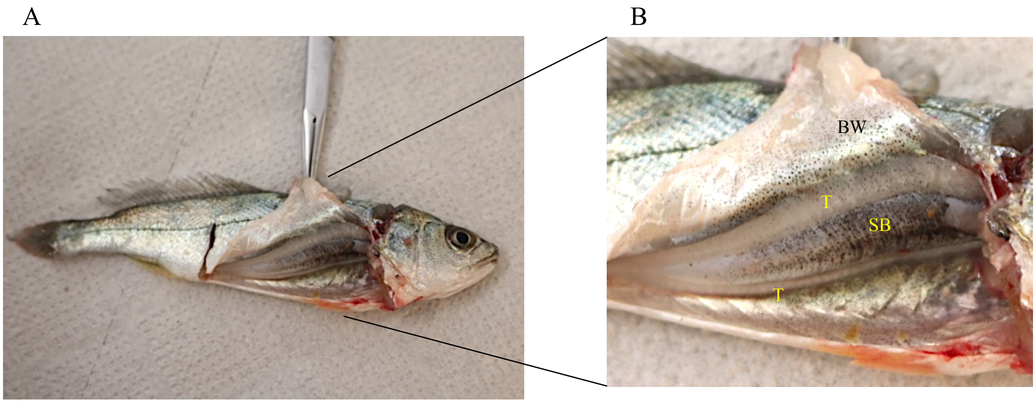


Fig. 5: Photography of dissected Argyrosomus regius juvenile (TL= 9 cm) (A) and enlargement of the same picture (B) showing body cavity, raised body wall (BW), anterior part of the swimbladder (SB) and testicles (T).

Distress calls produced by adults and juveniles when manipulated presented significant differences in all temporal and spectral parameters of sounds (Table 2, Fig. 6). Adults emitted longer calls (Fig. 6A) with a higher number of pulses (Fig. 6B) and a lower pulse period (Fig. 6C). The frequency of juveniles' sounds was higher than that of adults (Fig. 6D).

Table 2: Sound duration, number of pulses, pulse period and peak frequency of sounds produced by *A. regius* adults (n=10) and juveniles (n=20) during distress context. 50 sounds were analysed per ontogenetic group and per context. *p*-values from Mann-Whitney U Tests are shown.

Sound Parameters	Mean ± SD		Range		Significance
	Adults	Juveniles	Adults	Juveniles	
Sound Duration (ms)	192 ± 202	114 ± 48	24 - 1298	37 - 322	0.004
Nº of pulses	17 ± 9	11 ± 4	3 – 42	2 - 28	< 0.001
Pulse Period (ms)	10 ± 3	11 ± 2	7 - 25	7 - 21	< 0.001
Peak Frequency (Hz)	273 ± 73	321 ± 69	106 - 469	188 - 457	< 0.001

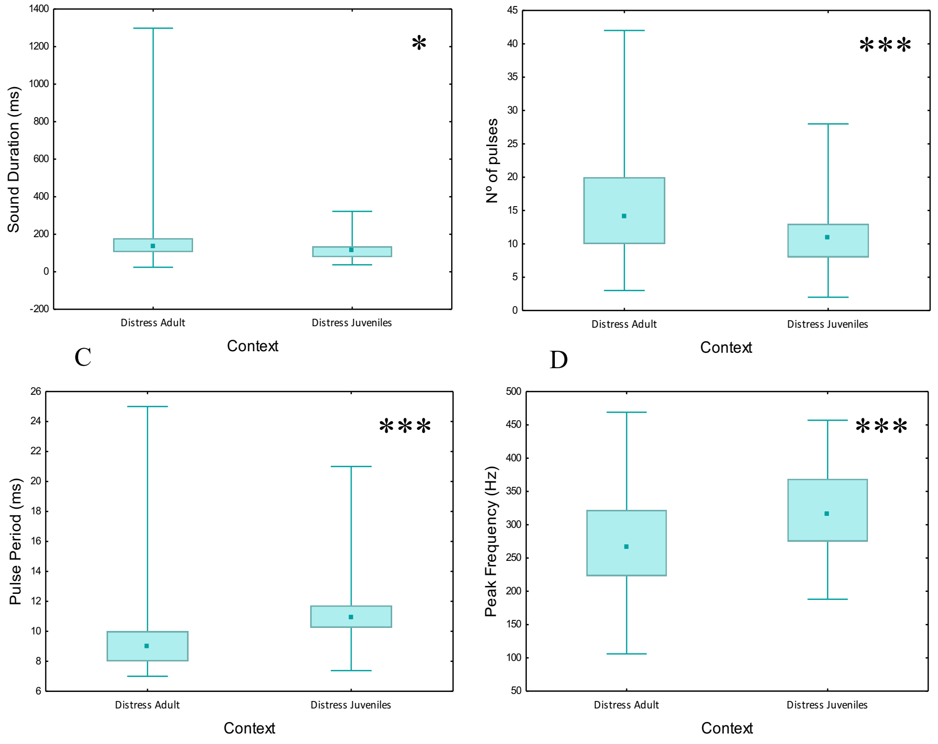


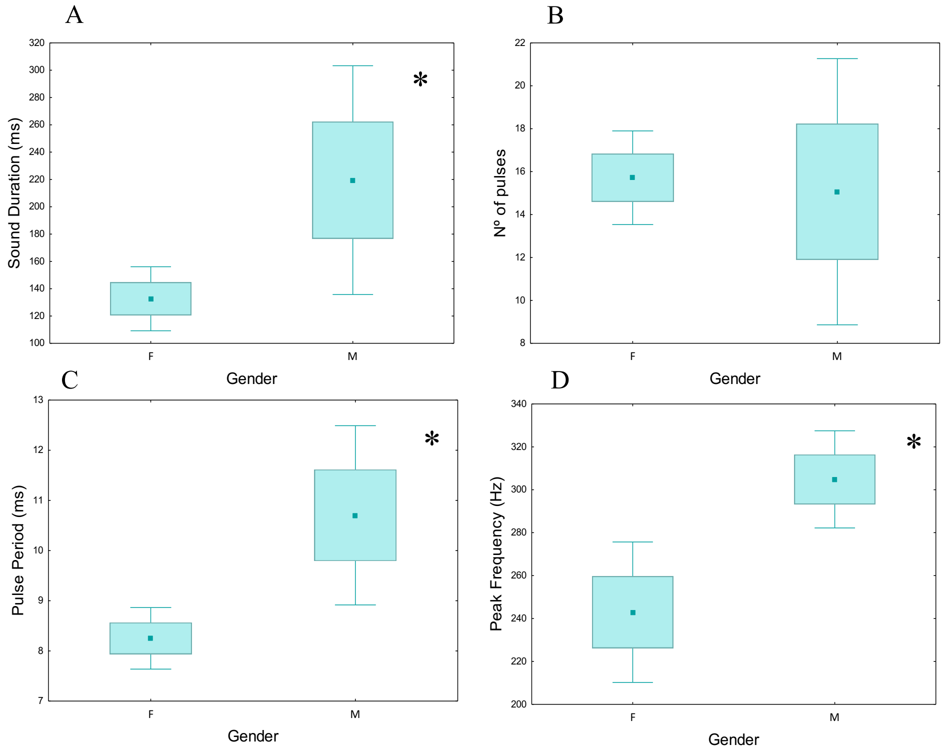
Figure 6: Box and Whisker plots comparing acoustic parameters of sounds emitted by adults and juveniles A. regius in distress context. (A) Sound duration (ms), (B) number of pulses, (C) pulse period (ms) and (D) peak frequency (Hz). (Mann-Whitney U Test; 50 sounds per ontogenetic group, per context). Parameters that are significantly different are indicated by (*) for $p < 0.05$ and by (***) for $p < 0.001$.

Gender variation of distress calls

Gender-related differences of distress calls characteristics were analysed for 80 male and 120 female sounds (Table 3, Fig. 7). Distress sounds produced by males and females differed significantly in all acoustic parameters, with the exception of the number of pulses ($t = 0.23$; $p = 0.826$; Fig. 7B). Male sounds were longer than those emitted by females ($t = -2.35$; $p < 0.047$; Fig. 7A), had a longer pulse period ($t = -2.99$; $p < 0.017$; Fig. 7C) and a higher peak frequency ($t = -2.72$; $p < 0.026$; Fig. 7D).

Sound Parameters	Mean ± SD		Range		Significance
	Female	Male	Female	Male	
Sound Duration (ms)	133 ± 29	220 ± 85	97- 170	156 - 341	0.046
Nº of pulses	16 ± 3	15 ± 6	12 - 21	7 - 22	0.826
Pulse Period (ms)	8 ± 1	11 ± 2	7 - 9	8 - 13	0.017
Peak Frequency (Hz)	243 ± 41	305 ± 23	192 - 286	276 - 329	0.026

Table 3: Sound duration, number of pulses, pulse period and peak frequency of distress calls produced by 4 male and 6 female *Argyrosomus regius* during handling. Sounds were recorded individually; fish of different sexes did not vary significantly in body length. Data correspond to the mean values of 20 sounds per individual. *p*-values from t-tests are shown.



Figure

7: Box and whisker plot comparing gender-related differences in distress calls acoustic parameters of *A. regius* (A) sound duration (ms), (B) number of pulses, (C) pulse period (ms) and (D) peak frequency. (Student's t-test; 50 sounds per context). Parameters that are significantly different are indicated by (*) for $p < 0.05$. Female – F, Male – M.

Ontogenetic variation of social calls

Social calls produced by adults and juveniles presented significant differences in the sound duration and number of pulses (Table 4, Fig. 8). Adult calls were longer (Fig. 8A) and included a higher number of pulses (Fig. 8B). Pulse period (Fig. 8C) and peak frequency (Fig. 8D) were similar in both ontogenetic groups.

Table 4: Sound duration, number of pulses, pulse period and peak frequency of sounds produced by *A. regius* adults (n=10) and juveniles (n=20) during social context. 50 sounds were analysed per ontogenetic group and per context. *p*-values from Mann-Whitney U Tests are shown.

Sound Parameters	Mean ± SD		Range		Significance
	Adults	Juveniles	Adults	Juveniles	
Sound Duration (ms)	586 ± 355	231 ± 101	59 - 1332	83 - 554	< 0.001
Nº of pulses	39 ± 23	16 ± 6	7 – 88	7 - 35	< 0.001
Pulse Period (ms)	15 ± 2	15 ± 1	6 - 22	13 - 16	0.678
Peak Frequency (Hz)	439 ± 110	424 ± 68	129 - 598	223 - 510	0.172

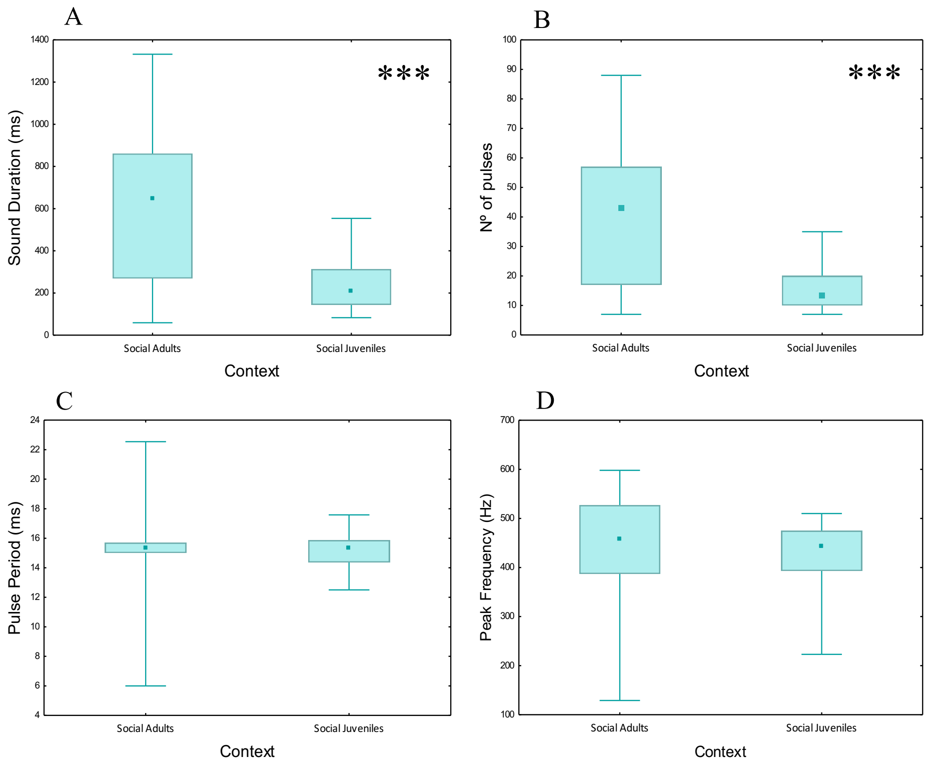


Figure 8: Box and whisker plots comparing acoustic parameters of sounds emitted by *A. regius* adults and juveniles in a social context. (A) Sound duration (ms), (B) number of pulses, (C) pulse period (ms) and (D) peak

frequency (Hz). (Mann-Whitney U Test; 50 sounds per context). Parameters that are significantly different are indicated by (***) for $p < 0.001$.

Variation with context

Distress *vs.* Social

Sounds produced by juveniles differed significantly between contexts ($p < 0.05$) in all temporal and spectral parameters of sound (Table 5, Fig. 9). Social sounds showed a longer call duration (Fig. 9A), a higher pulse number (Fig. 9B), a longer pulse period (Fig. 9C) and a higher peak frequency (Fig. 9D) than those produced in a distress context

Table 5: Sound duration, number of pulses, pulse period and peak frequency of sounds produced by *A. regius* juveniles (n=20) during distress and social contexts. 50 sounds were analysed per context. *p*-values from Mann-Whitney U Tests are shown.

Sound Parameters	Mean ± SD		Range		Significance
	Distress	Social	Distress	Social	
Sound Duration (ms)	114 ± 48	231 ± 101	37 - 322	83 - 554	< 0.001
Nº of pulses	11 ± 4	16 ± 6	2 - 28	7 - 35	< 0.001
Pulse Period (ms)	11 ± 2	15 ± 1	7 - 21	13 - 16	< 0.001
Peak Frequency (Hz)	321 ± 69	424 ± 68	188 - 457	223 - 510	< 0.001

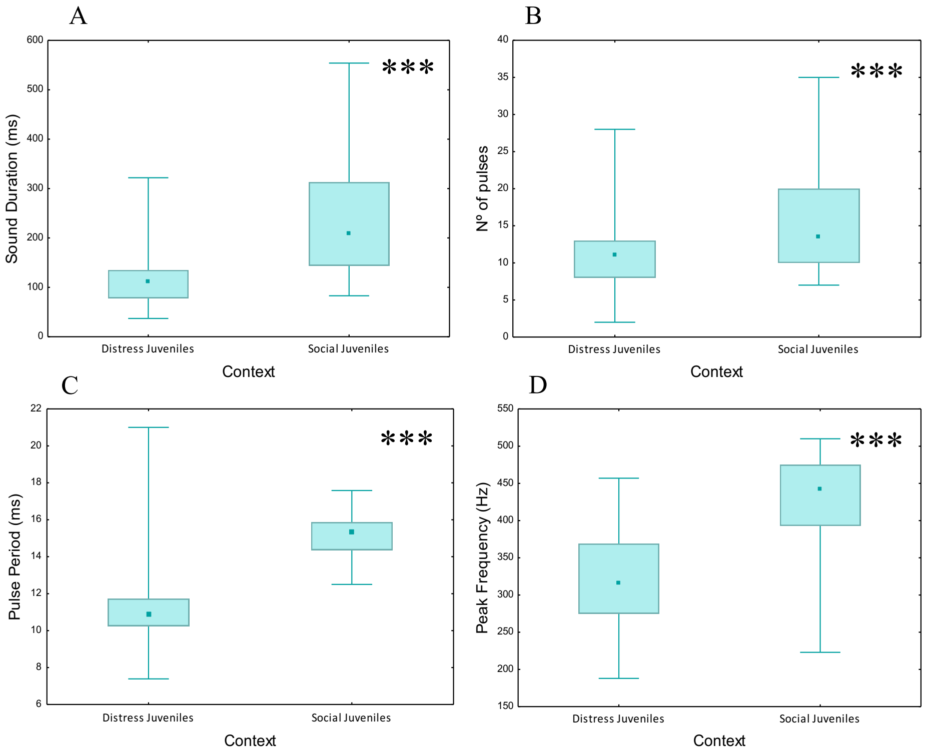


Figure 9: Box and whisker plot of the variation of the acoustic parameters of sounds emitted by *A. regius* juveniles

in distress and social contexts. (A) Sound duration (ms), (B) number of pulses, (C) pulse period (ms) and (D) peak frequency (Hz). (Mann-Whitney U Test; 50 sounds per context). Parameters that are significantly different are indicated by (***) for $p < 0.001$.

Like in juveniles, sounds produced by adult meagre differed significantly between contexts ($p < 0.05$) in all temporal and spectral parameters of sound (Table 6, Fig. 10). Sounds produced in social context were longer (Fig. 10A), included more pulses (Fig. 10B) and had a longer pulse period (Fig. 10C) than distress sounds. The peak frequency was significantly higher in the social context (Fig. 10D).

Table 6: Sound duration, number of pulses, pulse period and peak frequency of sounds produced by *A. regius* adults (n=10) during distress and social contexts. 50 sounds were analysed per context. p-values from Mann-Whitney U Tests are shown.

Sound Parameters	Mean ± SD		Range		Significance
	Distress	Social	Distress	Social	
Sound Duration (ms)	192 ± 202	586 ± 355	105 - 179	59 - 1332	< 0.001
Nº of pulses	17 ± 9	39 ± 23	10 – 20	7 – 88	< 0.001
Pulse Period (ms)	10 ± 3	15 ± 2	8 - 10	6 - 22	< 0.001
Peak Frequency (Hz)	273 ± 73	439 ± 110	223 - 333	129 - 598	< 0.001

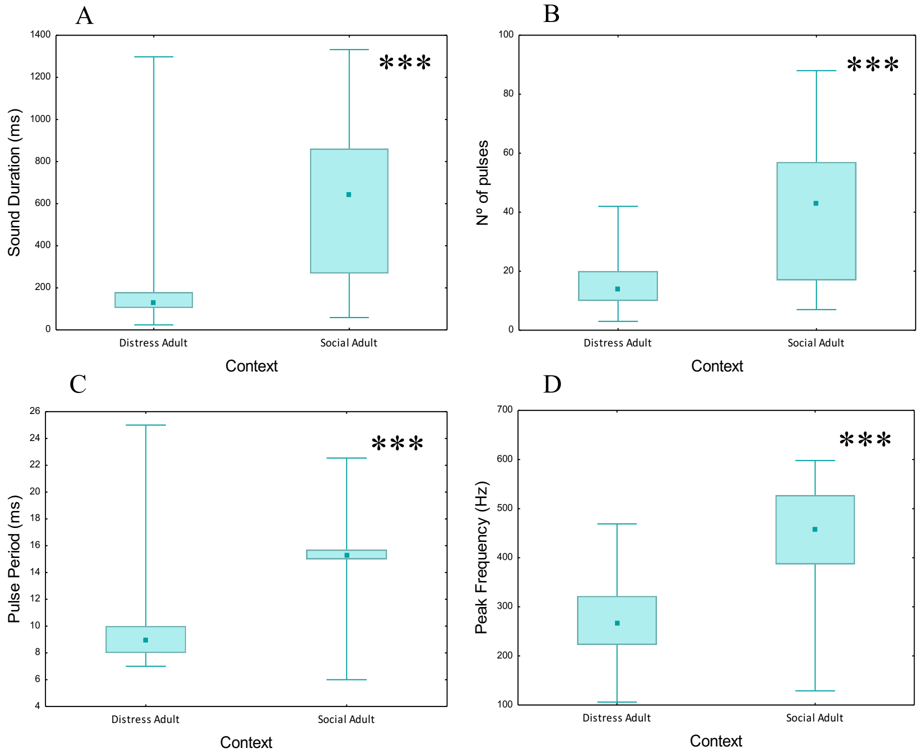


Figure 10: Box and Whisker plot of the variation of the acoustic parameters of sounds emitted by *A. regius* adults in distress and social contexts. (A) Sound duration (ms), (B) number of pulses, (C) pulse period (ms) and (D) peak

frequency (Hz). (Mann-Whitney U Test; 50 sounds per context). Parameters that are significantly different are indicated by (**) for $p < 0.001$.

Plots between the number of pulses and the other acoustic parameters further illustrate the aforementioned differences between distress and social calls observed in juveniles and in adults. Sound duration increased linearly with the number of pulses per sound both in social and distress contexts for both ontogenetic groups, but in social calls the number of pulses increased more steeply than in distress calls (Fig. 11A,B). Pulse period and peak frequency presented higher magnitude in social sounds in both groups (Fig. 11 C-F). In both ontogenetic groups, peak frequencies of distress and social calls varied greatly with increasing number of pulses, showing no clear pattern (Fig. 11E,F).

Other interesting patterns were visible in Fig. 11. For example, calls produced by adults were longer and included a higher number of pulses than the ones produced by juveniles (as shown above). In contrast to juveniles, the range of the number of pulses in adults was clearly wider in social context than in distress. (Fig. 11A,B). Pulse period was quite irregular in distress sounds with less than 5 pulses. Social sounds always presented more than 5 pulses.

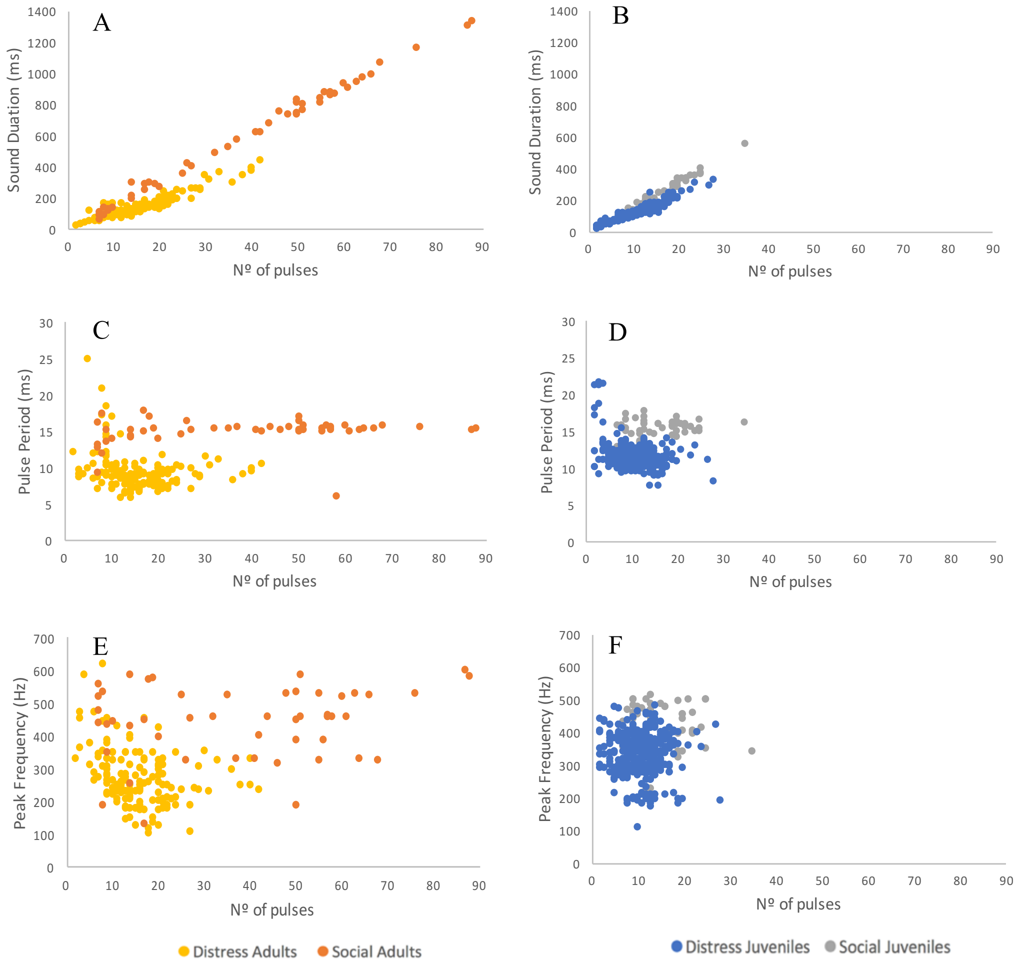


Figure 11: (A-F) Variation in sound duration, pulse period and peak frequency with the number of pulses for distress and social calls, made by juvenile and adult meagre. Scatterplots were performed with 100 social sounds

(50 sounds per ontogenetic group) and 500 distress sounds (200 sounds for 10 adults; 400 sounds for 20 juveniles).

Field *vs.* Captivity

Sounds produced in the field showed considerable differences from those emitted in captivity (Table 7, Fig. 12) in all acoustic parameters of sounds, with the exception of sound duration (Fig. 12A). Animals in captivity produced calls with more pulses (Fig. 12B), a longer pulse period (Fig. 12C) and with higher peak frequencies (Fig. 12D).

Table 7: Sound duration, number of pulses, pulse period and peak frequency of sounds produced by *A. regius* in the field and adults in captivity. 30 sounds were analysed for sounds produced in the field and 50 sounds were analysed for sounds produced in captivity. *p*-values from t-tests are shown.

Sound Parameters	Mean ± SD		Range		Significance
	Field	Captivity	Field	Captivity	
Sound Duration (ms)	548 ± 271	586 ± 355	158 - 1268	59 - 1332	0.586
Nº of Pulses	29 ± 19	39 ± 15	8 - 67	7 - 88	0.02
Pulse Period (ms)	19 ± 0,9	14 ± 1.9	18 - 21	6 - 17	< 0.001
Peak Frequency (Hz)	389 ± 44	439 ± 110	281 - 457	129 - 598	0.005

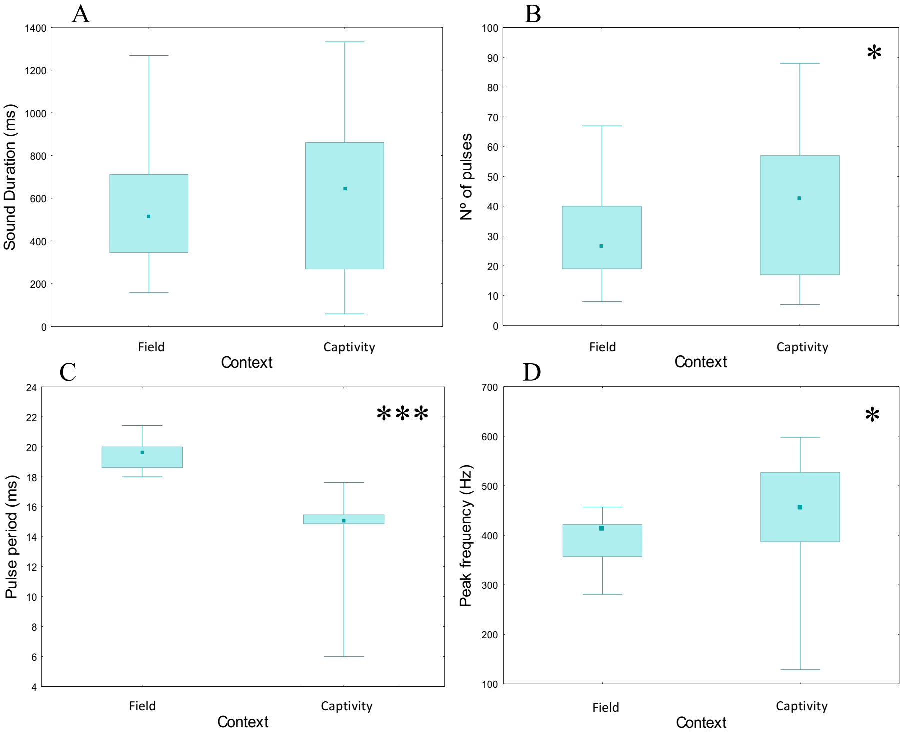


Figure 12: Box and Whisker plot of the differences between acoustic parameters of sounds emitted by *A. regius* in the field and in captivity. (A) Sound duration (ms), (B) number of pulses, (C) pulse period (ms) and (D) peak frequency (Hz). (Kruskal-Wallis Anova; 30 sounds for field and 50 for captivity). Parameters that are significantly different are indicated by (*) for $p < 0.05$ and by (***) for $p < 0.001$.

Discussion

Our knowledge on the detailed acoustic repertoire and variability is lacking in most vocal fish species. The present dataset demonstrates for the first time that *A. regius* calls vary with fish size, that females emit sounds that differ from those of males and that sounds vary according to context and throughout ontogeny. In this study, sound duration increased and peak frequency decreased with increasing fish body size. Sexual dimorphism was significantly present in sound duration, pulse period and peak frequency, with males exhibiting higher values. Sounds produced in social context showed significant differences between juveniles and adults, with adults exhibiting longer calls with a higher number of pulses. Additionally, calls produced by both adults and juveniles showed significant differences between distress and social contexts, with disturbance calls being of shorter duration, including fewer pulses, having a shorter pulse period and a lower peak frequency than social calls. Finally, advertisement calls recorded in Tagus estuary were significantly different from those of captive individuals, probably associated with disparities between the environment and between individuals, such as the existence of higher temperature fluctuations in the field and possibly larger size of wild individuals, as well as lab conditions affecting the measured sound frequency. The variation in call parameters with fish size, gender, context and ontogenetic phase suggests that the study of these calls in captivity may be valuable to fisheries biologists as a non-invasive tool to monitor natural population of meagre.

Ontogenetic variation of distress calls

Argyrosomus regius, like other sciaenids, produced trains of sound pulses when in distress. Though advertisement calls produced during spawning aggregations have been characterised for *A. regius* (Lagardère and Mariani 2006), disturbance calls and their relation with size have never been studied for this species.

In this study, distress calls produced when juveniles (31-51 cm) and adults (69-102cm) were handled varied significantly with fish size. According to Connaughton et al., 2000, sound parameters such as pulse duration and peak frequency are determined by sonic muscle development, rather than the resonant frequency of the swimbladder, which is thought to be strongly correlated with fish size. The increase of sound duration and decrease of peak frequency observed in this study support Connaughton et al., (2000) conclusion. Therefore, longer muscles, presumably with longer fibers, are taking longer to complete a twitch in larger fish resulting in longer pulses (presumably longer sounds) and also in lower sound frequencies.

These results are in line with other studies on species belonging to the family Sciaenidae that demonstrated the same relation between these parameters and body size (Myrberg et al., 1993; Connaughton et al., 2000; Tellechea et al., 2010). This variability between peak frequency and size may be a valuable asset for females to discriminate males on the basis of their size, as larger fish are usually dominant, occupy better territories or spawning sites and have greater reproductive success. Call duration and pulse period may provide similar cues for *A. regius*. Locascio and Mann (2008) suggested that time between pulses could be used for species-specific recognition in sciaenids. Additionally, it could be useful to assess the size and fighting ability of the opponent in agonistic displays and solve conflicts without escalated fighting (Krebs and Davies, 1993; Amorim, 2006).

In what concerns the ontogenetic variation of calls produced in a distress context, significant differences in all acoustic parameters of sounds were present between juveniles and adults, with juveniles emitting shorter calls with fewer number of pulses, longer pulse period and higher peak frequency. It is known that sound generation is widespread among juvenile fishes since the competition for food and territory may occur regardless of reproductive stage (Bass and Ladich, 2008), but only in a few cases has sound production been mentioned in juveniles during agonistic interactions and in distress situations. Development of agonistic behavior and vocalization has been shown in the croaking gourami (*Trichopsis vittata*) (Henglmüller and Ladich, 1999; Wysocki and Ladich, 2001). Similarly to what was observed in the present study, in agonistic sounds produced by the croaking gourami, dominant frequency decreased and croak duration, number of pulses and pulse period increased, from juveniles to adults. Likewise, in the grey gurnard (*Eutrigla gurnardus*), sound production changes from small juveniles to large adults during competitive feeding, with the sound duration, pulse duration and number of pulses increasing while peak frequency decreased with fish size. Moreover, proportion of knocks (less aggressive) was high in larger fish while smaller fish emitted more grunts (more aggressive) (Amorim and Hawkins, 2005). These differences are probably related to the less developed sound-producing structures and to a smaller body size of juveniles (as described before). Additionally, juveniles may benefit from a more aggressive behavior than larger ones, possibly because a larger body size gives an advantage in agonistic encounters (Amorim and Hawkins, 2005).

Gender variation of distress calls

The present dataset demonstrates for the first time that female meagre emit sounds and that their sounds differ from those of males (see below). Both male and female *A. regius* emitted pulse trains (grunts) when handled. In most sciaenid species (e.g., weakfish, red drum, and silver perch) only males possess sonic muscles (Tower, 1908; Fish and Mowbray, 1970; Hill et al., 1987). There are relatively few species in which these muscles are present in both sexes (e.g., Atlantic croaker, Whitemouth croaker, meagre, and Japanese croaker) (Hill et al., 1987; Lagardère and Mariani, 2006; Ueng et al., 2007). Sexual dimorphism of the sound-producing apparatus has been observed in three species of the genus *Argyrosomus,* including the meagre (Lagardère and Mariani, 2006; Ueng et al., 2007; Takemura et al., 1978). However, no information is available on the ability of females of these species to produce sound, and thus, thoughts regarding the selective factors that lead to the occurrence of sonic muscles in females are still hypothetical. Sound production by females could be associated with agonistic interactions as in other genus (e.g. *Cichlasoma centrarchus,* Schleinzer, 1992; *Trichopsis,* Ladich, 2007). Whether females vocally take part in spawning is still unknown. Future experiments, in which advertisement calls produced by *A. regius* are recorded for a single-sex group during the spawning season should provide answers to this question.

In this study, with the exception of the number of pulses, sexual dimorphism was significantly present in all of the studied acoustic parameters of calls (sound duration, pulse period and peak frequency), with males exhibiting higher values. Usually, size and sex are extremely correlated due to the presence of size dimorphism between males and females (Allen, 1972). As such, males, frequently having better developed sonic muscles associated to their larger body size (Ladich and Fine, 2006), are expected to produce sounds with lower frequencies then females (Hill, 1950; Wainwright and Barton, 1995; Connaughton et al., 2002). In addition, sciaenid male sonic muscles suffer hypertrophy during the mating season (Connaughton et al., 2002; Lagardère and Mariani, 2006). Hence, besides carrying information related to the size, dominant frequency and call duration could also be signals passing on information on the gender of the producer (Colleye et al., 2009). Male and female meager used in this study did not differ significantly in total length but sample size was very small. Females had a tendency to be larger (mean TL of 87 cm for females *vs.* 82 cm for males) which could have affected the peak frequency of sounds. A similar result was observed for the closely-related species *Argyrosomus japonicus* (Ueng et al., 2007). One possible explanation for these observations is that the female's thinner sonic muscles create a weaker tension which may be related to the lower frequency of their sounds (Sprague, 2000). To help understand the role of sonic muscle contraction in generating the acoustic waveform, muscle action potentials (an electromyogram or EMG) should be recorded from a representative individual. Nonetheless, as in this experiment gonadal tissue extraction was only succeeded for two individuals, female gender may have been incorrectly attribute to a male. Also sounds from more individuals should be recorded as the sample size was very low in the present study and results should be seen with caution.

Ontogenetic variation of social calls

Sounds produced in social context showed significant differences between juveniles and adults in the duration of calls and number of pulses, but not in pulse period or peak frequency. In adults, sounds recorded in social context probably corresponded to advertisement calls. Though no spawning events occurred, social sounds were produced in the beginning of July with a water temperature of approximately 21ºC, concurrent with the spawning period that characterizes this species (Costa et al., 2008). Additionally, individuals may have been stimulated by spawning individuals in other tanks emitting high energy grunts. If so, it's only natural that in this context, the sounds produce by mature, ready to spawn meagre are longer and include more pulses than the ones produced by immature juveniles. According to Myrberg et al., (1965) sound duration and pulse repetition rate (and hence number of pulses) may vary according to the fish motivation. Additionally, Lagardère and Mariani (2006) observed that the meagre, *A. regius*, rarely produced short grunts during the spawning season, in contrast with long grunts (trains of 30 to 112 pulses) which were abundant. Since in this study, juveniles exhibited a significantly smaller body size, a higher frequency should be expected. However, this was not the case. The similar frequencies of adults' social calls may be a result of the effects of reverberation and resonance of the tanks. In many cases, a sound presented several frequency peaks with very similar energy (see Fig. 3G). Consequently, it is possible that the relative energy in frequency peaks changed and the wrong frequency peak was measured.

Variation with context

Social *vs.* Distress

In this study, sounds emitted in two types of contexts were characterised for juveniles and adults *A. regius*: social and distress. It is well-known that siaenids are vocal species that produce sounds in courtship and agonistic contexts. As mentioned before, in this species (like in other sciaenids) sound production is stimulated by well-developed extrinsic sonic muscles that surround the swimbladder (Tavolga 1964; Ladich and Fine 2006). The waveform of both the distress and social calls of *A. regius* consisted of similar rapidly-damped pulses, suggesting that both sounds are produced by the same mechanism. Plus, contractions of the sonic muscles could be felt when handling the fish. For both ontogenetic groups, significant differences between the two contexts were found for all parameters of sounds, with disturbance calls being of shorter duration, including fewer pulses, having a shorter pulse period and a lower peak frequency than social calls. In line with the observations made in this study, differences have been found between agonistic and social sounds for other species in which agonistic sounds are brief and broadband (harsh) whereas sounds uttered in other contexts (submission, courtship) are frequently of longer duration and occasionally tonal (Crawford et al., 1986; Ladich and Tadler, 1988; Ladich et al., 1992a; McKibben and Bass, 2001). For example, the male midshipman (*Porichthys notatus*) produce tonal hums of long duration to attract pregnant females to the nest and short broadband grunts to defend the nest from potential intruders (Bass and McKibben, 2001). Physiologically, these differences are mainly related with differences in the contraction rate of sonic muscles (Fine et al., 2001). Furthermore, they are probably associated with differences in production rate and function of the two call types. According to Lagardère and Mariani (2006), advertisement calls of the meagre are mainly characterised by long grunts produced at a constant high rate for several tens of minutes. This implies higher costs of advertisement calls, not only physiologically and metabolically (i.e. higher sonic muscle contraction rates) (Fine et al., 2001), but also at an ecological level (i.e. attraction on predators). Consequently, it's likely that longer calls, higher calling rates and higher peak frequency are used as direct signals of male quality for mate choice by females. Furthermore, while for the formation of spawning aggregations, sounds have to travel long distances to attract females to breeding areas, in agonistic interaction fish are relatively close, which may explain the lower peak frequencies of the latter (Vasconcelos et al., 2010). Finally, as mentioned before, Locascio and Mann (2008) proposed that time between pulses could be used for species recognition in sciaenids. Their suggestion agrees with the separation of disturbance and advertisement calls in *A. regius*. However, studies with other sciaenids, such as the weakfish (Connaughton et al., 2000) and the Argentine croaker (Tellechea et al., 2010) showed similar characteristics between disturbance and advertisement calls. These observations indicate that the acoustic repertoire of the meagre may be richer than previously thought, and surprisingly more differentiated than that of other sciaenids such as the weakfish. Nonetheless, the significantly higher frequencies of social calls may be a result of the effects of reverberation and resonance in the tanks. This is probably not the case in the distress calls, as recordings were performed with the hydrophone 10 cm apart from the individuals' abdomen. These observations emphasize the need of additional research to support these results and if correct, clarify the behavioral functions of the sounds that may have played a role in the speciation of *A. regius.*

Field *vs.* Captivity

Advertisement calls recorded in Tagus estuary and in captivity showed significant differences in acoustic parameters of sounds. This may be explained by the diurnal temperature fluctuations observed in the field (ranging from 18 to 23ºC), which are highly influenced by tides. Sound parameters associated with muscle contraction have been showed to change with temperature (Connaughton et al., 2000, 2002). Moreover, water temperature appears to be the most important factor determining meagre migrations and reproduction (FAO, 2005–2011b). It is also worth to note the "stressful" conditions to which captive fish are submitted that may affect vocal performance (e.g., missing certain sound type； Midling et al., 2002). Additionally, significantly higher peak frequencies of sounds emitted by captive individuals could be caused by their smaller body size when compared to wild calling individuals. As mentioned above, the frequency of sounds emitted by *A. regius* is inversely correlated with total length, showing a peak frequency decrease by 35 Hz (321-286 Hz) across a range of only 10 cm between the larger juvenile and the smallest adult (51-69 cm). In the wild this species can reach over 50 kg, with the largest size recorded being 182 cm total length and 103 kg of body weight (Quéro and Vayne, 1987). However, in field conditions, visual census of fish was compromised due to limited water visibility and calling individuals could not be observed. The size of the spawning aggregations in the field, which could also have an effect on acoustic sound characteristics, could not be inferred. Finally, lab conditions may be affecting the measured sound frequency. Nonetheless, the sounds recorded in captivity fall within the variability of field recorded sounds. As such, they are comparable and sounds recorded in captivity may be used to validate sounds produced in the field

Concluding remarks

The variation in sounds documented in the present study indicates that *A. regius* vocalizations have the potential to carry information about species, size, sex, ontogeny, motivation, and other factors that may play a role in both social and distress contexts. From the researcher's point of view these differences could be used to identify meagre in the field and further attempt to ascertain size and different phases of their life cycle (i.e. juveniles vs. adults) (Sprague and Luczkovich 2001). Indeed, specific sound types conjectured to be made by particular sciaenids have been associated with the spawning season and are being widely used to obtain information about the relative size and location of vocalizing aggregations in the field (Connaughton and Taylor 1995; Luczkovich et al., 1999, 2008; Rowell et al., 2017).

This study revealed an acoustic repertoire richer than the described for other sciaenids. Additional research to clarify differences between gender and other sources of variability (e.g. water temperature) are needed.

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Variation of acoustic activity and acoustic features of meagre, *Argyrosomus regius* (Asso 1801) in relation to season and spawning events

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Abstract The goals of this study were to characterize the acoustic activity and characteristics of sounds produced during social contexts in relation with and spawning events (monitored by the collection of eggs from the tanks). Sounds produced by captive meagre during the breading season showed a richer repertoire than previously observed, with meagre producing not two, but six call types: long grunts, intermediate grunts, short grunts, 1 pulse, 2 pulses and 3 pulses. Additionally, besides the dense choruses of grunts already known for this species, meagre emitted successive series of isolated pulses, audible as 'knocks', in association with spawning. A seasonal trend was noted in knocking sounds but not in grunts likely due to the restricted duration of the chosen period for sound analysis. Captive meagre produced sounds which varied significantly in their acoustic parameters as reproduction proceeded, with sounds produced during the spawning period being characterised by a higher number of pulses, larger pulse periods and lower peak frequencies than those emitted in the pre- and post-spawning periods. These results can be used to help identify the presence and location of meagre breeding adults in nature and potentially help detecting spawning events.

Key words: fish sounds; spawning; advertisement calls; seasonality; Sciaenids

Introduction

Many species of fish are soniferous, producing sound in different activities, such as feeding, territorial behavior and during reproductive activities (Winn, 1964; Connaughton and Taylor, 1995). In some species, the vocalizations associated with the latter are often produced in choruses (McCauley, 2001). These sounds can provide information about the caller to the recipient as they are associated with differences between species; between individual callers of the same species and between call types issued by the same individual under different behavioral contexts (Amorim, 2006). The species-specificity of fish calls and intra-specific variation can also be important for the researcher. Passive acoustics monitoring (PAM) has been widely used to record temporal and spatial patterns of fish sounds. Listening and identifying the source of a chorus of fish breeding aggregations in their natural habitat through PAM can greatly improve a biologist's ability to delimit location and timing of spawning without creating behavioral bias. This information is crucial for the management of exploited species (i.e. seasonal fishery closures designed to protect spawning fish) and conservation of essential fish habitat. However, to understand the temporal and spatial extents of spawning behavior, the function of calls produced during the reproductive period need to be characterised. (Hawkins, 2002; Zydlewski et al., 2006; Parsons et al., 2009, 2010).

Sciaenids, known as croakers and drums, are soniferous fishes that produce breeding sounds (drums or grunts) through the contraction of a pair of sonic muscles typically present in males, and occasionally in both sexes, that cause the swimbladder to vibrate (Parsons et al., 2006). Sound production during the reproductive season has been reported in a number of sciaenid species such as the weakfish, *Cynoscion regalis* (Connaughton and Taylor 1996) meagre, *Argyrosomus regius* (Lagardère and Mariani, 2006) Japanese croaker, *Argyrosomus japonicus* (Parsons et al., 2009) and red drum, *Sciaenops ocellatus* (Montie et al., 2016). The profusion of advertisement calls produced by the representatives of this family during the reproductive season suggest that sound production might play an important role in their reproduction. Observations on behavior and sound production of Sciaenids during spawning, suggest that calling may serve a variety of functions including: announcing the male's spawning readiness, attracting individual pregnant females, stimulating courtship activities, facilitating aggregation of spawning groups, mediating male–male competitive interactions, serving as a secondary sex characteristic used in female mate choice, synchronizing release of gametes and reproductive isolation (Connaughton and Taylor, 1995; Lagardère and Mariani, 2006; Luczkovich et al., 2008; Mok et al., 2009; Picciulin et al., 2012).

The meagre (*Argyrosomus regius*, Asso 1801) is one of the world's largest marine teleosts and has with a high commercial value for recreational and small-scale commercial fisheries and aquaculture (Quéméner et al., 2002). This sciaenid it's known to be an anadromous species, migrating to estuaries in at the end of May to breed, where they remain until July (Prista, 2013). In the end of the spawning season, fish return to deep waters to feed along the coast during winter (Cárdenas, 2010). Areas that are important for spawning are the estuary of the river Tagus (Portugal), river Guadiana, (Southern Spain and Portugal) (González-Quirós et al., 2011) and river Gironde (France) (Quéméner et al., 2002). Advertisement calls produced during spawning aggregations have been characterised for *A. regius* (Asso 1801) by Lagardère and Mariani (2006). According to these authors, meagre sounds were produced in dense choruses in which two sound types could be identified: long and short grunts. However, there is still a lack of information on the daily and seasonal variation of sounds produced by adult meagre. In addition, it is still not known how acoustic activity and sound features vary in association to spawning events. The latter could be extremely useful to monitor and predict spawning in the meagre both in nature and in aquaculture facilities.

In this work recordings of sounds produced by adult meagre in captivity were made round-the clock (24h/day) during 6 months in aquaculture facilities. Voluntary sounds produced between February and June were characterised to examine seasonal and circadian variations in sound production. Additionally, acoustic activity and characteristics of sounds produced during social contexts were characterised and related with spawning events monitored by the collection of eggs from the tanks. This work will contribute to monitor natural population of meagre and delimit location and timing of spawning, namely inside and outside the Tagus estuary. It will also contribute to monitor and potentially to predict spawning in aquaculture systems.

Material and Methods

Animals in Captivity

Sound recordings were performed from a group of sexually mature meagre, *A. regius* hosted at the aquaculture facilities of Instituto Português do Mar e da Atmosfera – Estação Piloto de Piscicultura de Olhão (IPMA – EPPO), Portugal (37◦02′ N, 7◦49′ W). Fish were reared in an indoor unisex concrete square tank (3 m^2 area, 120 cm deep) (Fig. 1) under natural photoperiod, natural temperature ranging between 14 and 23ºC, continuous water supply, controlled pH (8± 0.4), salinity (35± 0.2 psu) and oxygen level close to saturation (80±7.6%.). Meagre were fed dry food 4 times a day. Individuals housed in the tank (n=8) were 9 and 6 years old and exhibited an average length of 86 cm (71-94cm) and 6:2 (M:F) sex ratio.

Eggs were collected from a skimming port in front of the tank that drained into an egg collection container equipped with a 250 micron mesh net. Collection nets were checked daily (early morning and late afternoon) for the presence of eggs. If eggs were present, they were collected and divided into floating (viable eggs) and non-floating in a graduated beaker, recording the amount in both fractions. Floating eggs were removed, incubated and reared according to protocols developed at IPMA (Pousão-Ferreira et al., 1999). Exact time of spawning is unknown, however, monitoring of the tanks in late afternoon and early morning places egg deposition between 20h and 7h of the following day.

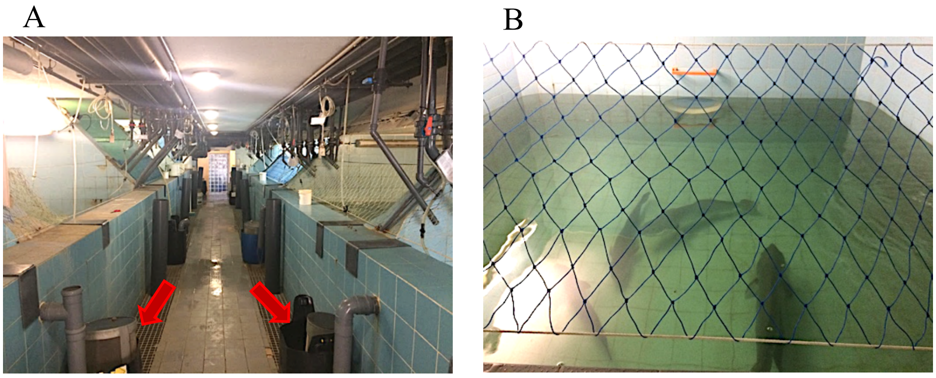

Figure 1: IPMA facilities with (A) breeder's interior tanks with egg collectors (red arrow) and (B) square concrete tank rearing adult meagre

Vocalizations recordings in the captivity

Sounds of adult individuals were recorded round-the clock (24h/day) from February to July 2018, in one tank. A custom-made hydrophone (Fonseca and Maia Alves, 2011) was positioned vertically at the centre of the tank at approximately 30 cm from the bottom (Fig. 2) and connected to a stand-alone 16 channel datalogger (LGR – 5325, Measurement Computing Corp, Norton Ma USA; 12 kHz sampling rate 16 bit, ±1 V range). In an attempt to associate sounds characteristics to the contexts under which they are produced, acoustic recordings were complemented with direct observation. Focal behavioral observations were compromised because water visibility was limited and the total time that fish emitted grunts was low and unpredictable relative to the total acoustic monitoring time. Further, vocalizing meagre were not readily discernible from non-vocalizing meagre, especially when in motion.

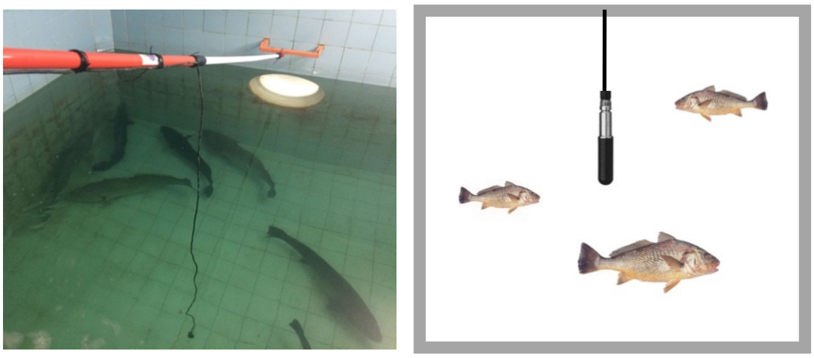

Figure 2: Photo (left) and schematic image (right) of the experimental setup for vocalization recordings in the tank the custom-made hydrophone (Fonseca and Maia Alves, 2011) positioned vertically and centrally at approximately 30 cm from the bottom.

Sound Analysis

Calls were edited with Cool Edit Pro Software and analysed with Raven 1.2.1. Only the sounds with a good signal-to-noise ratio were used in the analyses. The following temporal and spectral parameters of sound were measured: sound duration (ms), as the time from the onset of the first pulse to the offset of the last pulse; number of pulses, obtained manually by counting the number of pulses in each sound; pulse period (ms) (the time interval between the peaks of two consecutive pulses in a sound), obtained by dividing the duration by the number of pulses minus 1 (Mann et al., 1997); and dominant frequency (Hz), the frequency presenting the highest energy level in the sound. Temporal parameters were measured from oscillograms while dominant frequency was measured from power spectra: 6 kHz, fast fourier transform (FFT) size 1024 points, Hamming window, time overlap 50%.

Captive meagre were observed to exhibited diel periodicity, with sound emissions generally beginning at dusk, by 18h, reaching a maximum activity approximately two to three hours later, at 20/21h, and ending at 24h. For this reason, in this study, the sounds analysed were selected in this time frame.

The total number of vocalizations was determined between 18h and 21h, on the 18th and 28th days of each month, from February to June 2018. A total of 7492 sounds were manually counted. Vocalizations were divided into 7 different types based on pulse number: 1 pulse, 2 pulses, 3 pulses, short grunts (4-6 pulses), intermediate grunts (7-29 pulses) and long grunts (more than 30 pulses) and indeterminate in cases in which the number of pulses could not be clearly determined. This classification attempted to include both the short and long grunts reported by Lagardère and Mariani (2006) and the sounds that were registered in the present study. Additionally, temporal features and dominant frequency were measured as above, in 10 to 20 long grunts produced in 1 to 2 consecutive days (depending on the presence/ absence of sounds), every month from March to July.

For the association of sound features and acoustic activity with spawning events, sound production was studied during 4 different periods (T1, T2, T3 and T4) for each of the 3 studied spawning events (which occurred in May, June and July). T1 represented the pre-spawning period corresponding to the 5th day prior to the spawning event; T2 and T3 represented the spawning period, with T2 corresponding to the day that preceded the first egg deposition and T3 to the day(s) when eggs were found in the tank; T4 corresponded to the post-spawning period being the day immediately after spawning (the last egg deposition). Sounds were selected and analysed between 18h and 24h, corresponding to the peak of meagre sound emissions. When present, 10 sounds were randomly selected per day for each of the 3 spawning events studied. A total of 140 sounds were analysed, as for 2 days, only 5 sounds were present.

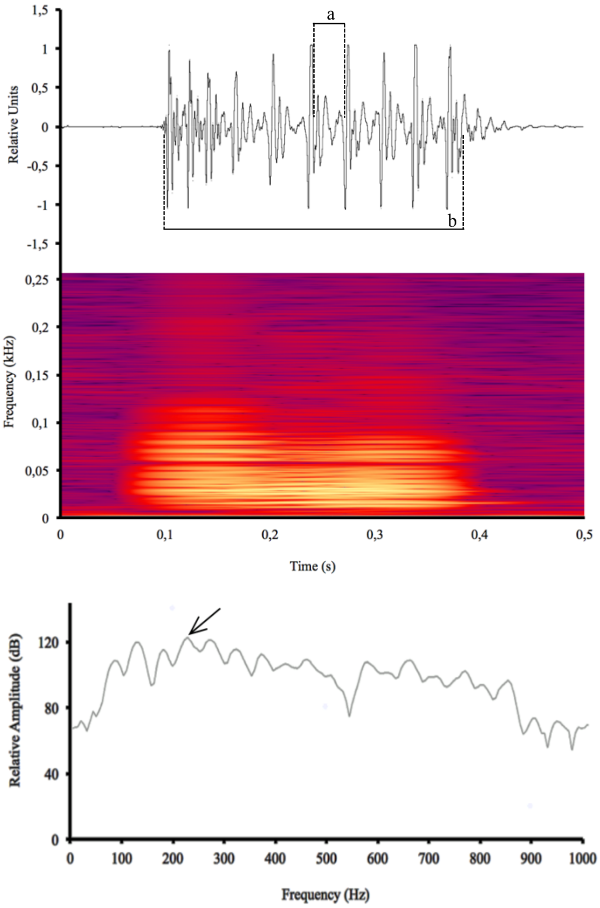

Figure 3: (A) Oscillogram, (B) sonogram, and (C) power spectrum of a *Argyrosomus regius* male breeding sound, representing some of the acoustic parameters measured: pulse period of one pulse (a), and (b) sound duration in (A) and peak frequency (arrow) in (C). Sampling frequency 6 kHz, 1024 point FFT, Hamming window, and 50% overlap.

Statistical Analysis

Statistical analysis was conducted using the software Statistica (version 10, Statsoft, Tulsa, OK, USA). A P-level of 0.05 was used for all analyses (Zar, 1984). Probability plots and Levene test were performed to assess normality of the data and homogeneity of variances, respectively. Nonparametric statistics were used whenever the assumptions for parametric tests were not met after applying the recommended data transformations (Zar, 1984). For the study of seasonal variation of long grunts, one-way ANOVAs were conducted. General linear models (GLM) with temperature as a covariate were fitted to compare the acoustic characteristics of sounds produced during different periods (T1, T2, T3 and T4; factor with 4 levels hereafter named spawning period) correlated with spawning events. As assumptions were not met even after data transformation a Kruskal–Wallis nonparametric test was used for the pulse period using spawning period as a factor.

Results

Acoustic repertoire of A. regius

The sounds emitted by captive *A. regius* were pulsed sounds. Six sound categories were qualitatively recognized in the meagre: long grunts (30-160 pulses, Fig. 4A), intermediate grunts (7-29 pulses, Fig. 4B), short grunts (4-6pulses, Fig. 4C), sounds made up of 3 pulses, 2 pulses and 1 pulse (Fig. 4D).

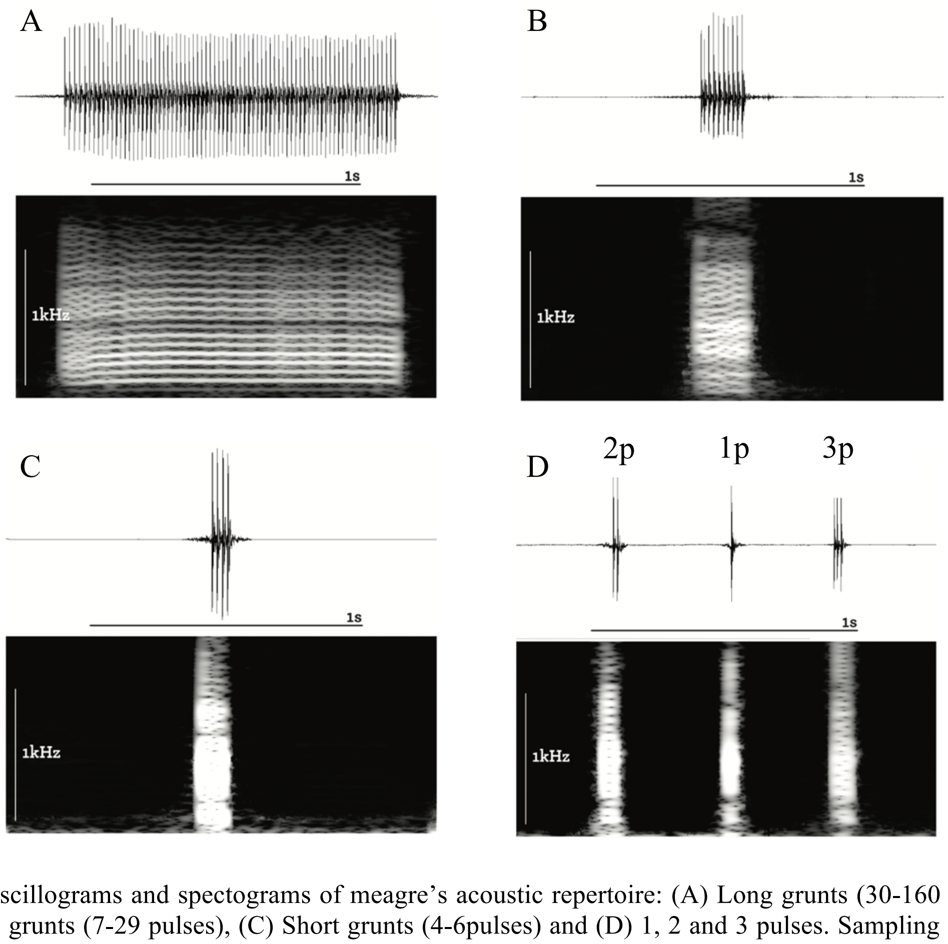

Figure 4: Oscillograms and spectograms of meagre's acoustic repertoire: (A) Long grunts (30-160 pulses), (B) Intermediate grunts (7-29 pulses), (C) Short grunts (4-6pulses) and (D) 1, 2 and 3 pulses. Sampling frequency 6 kHz, 1024 point FFT, Hamming window, and 50% overlap.

In the spawning period, the meagre produced two types of acoustic signals: sequences of isolated pulses (Fig. 5A) and, most commonly, dense choruses (Fig. 5B). Sequences of pulses consisted of a series of 1 (most frequent), 2 and 3 (least frequent) pulse sounds; these sequences could last for 1 hour. Dense choruses comprised long (most common), intermediate and short grunts produced at a constant rate.

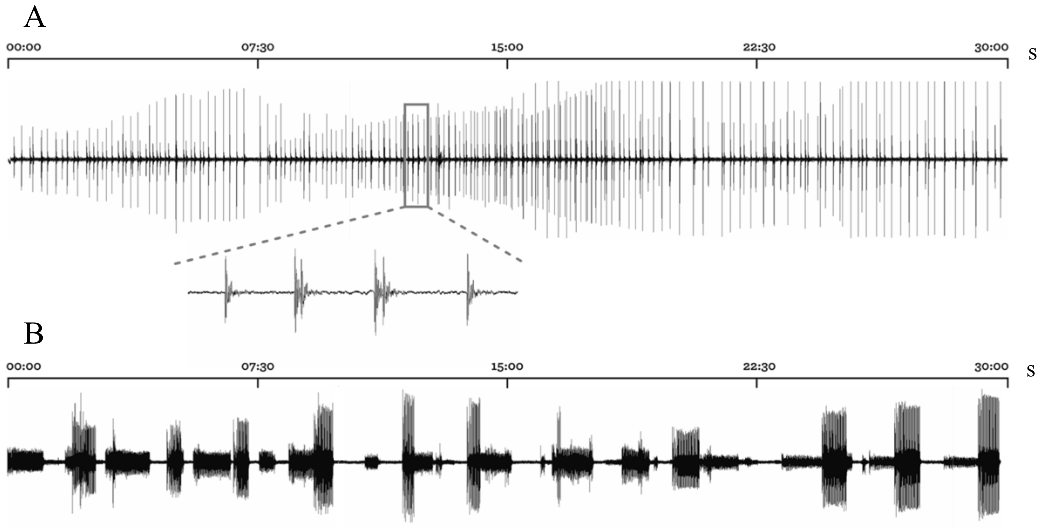

Figure 5: Oscillogram of (A) series of isolated pulses and (B) choruses of long grunts that preceded egg release Sampling frequency 6 kHz, 1024 point FFT, Hamming window, and 50% overlap.

Seasonal changes in sound production

From February to May, the number of sounds produced markedly increased. In February, no sounds were produced during the studied days and hours. 86, 756 and 5678 sounds were counted in March, April and May, respectively. In June, the amount of sounds produced decreased to 416 sounds (Fig. 6B).

In what concerns the type of sound produced (Fig. 6A), from March to June, the percentage of 1 pulsed sounds increased (from 11% to 59%), with a peak in May (82%). Two and three pulse sounds were mainly concurrent with one pulse sounds sequences but represented a much inferior proportion (i.e. in May 2 pulses accounted for 10% of the total number of sounds, and 3 pulses for 2%). April corresponded to the month with the higher percentage of long grunts (30%), with some also being produced in May and June, but their presence was reduced in relation to other call types (1%). The proportion of intermediate grunts decreased from February (17%) to June (1%). The highest percentage of short grunts corresponded to March (34%) and the lowest to May (4%). Mean water temperatures per month increased from February to June (18ºC to 22ºC; Fig. 6C).

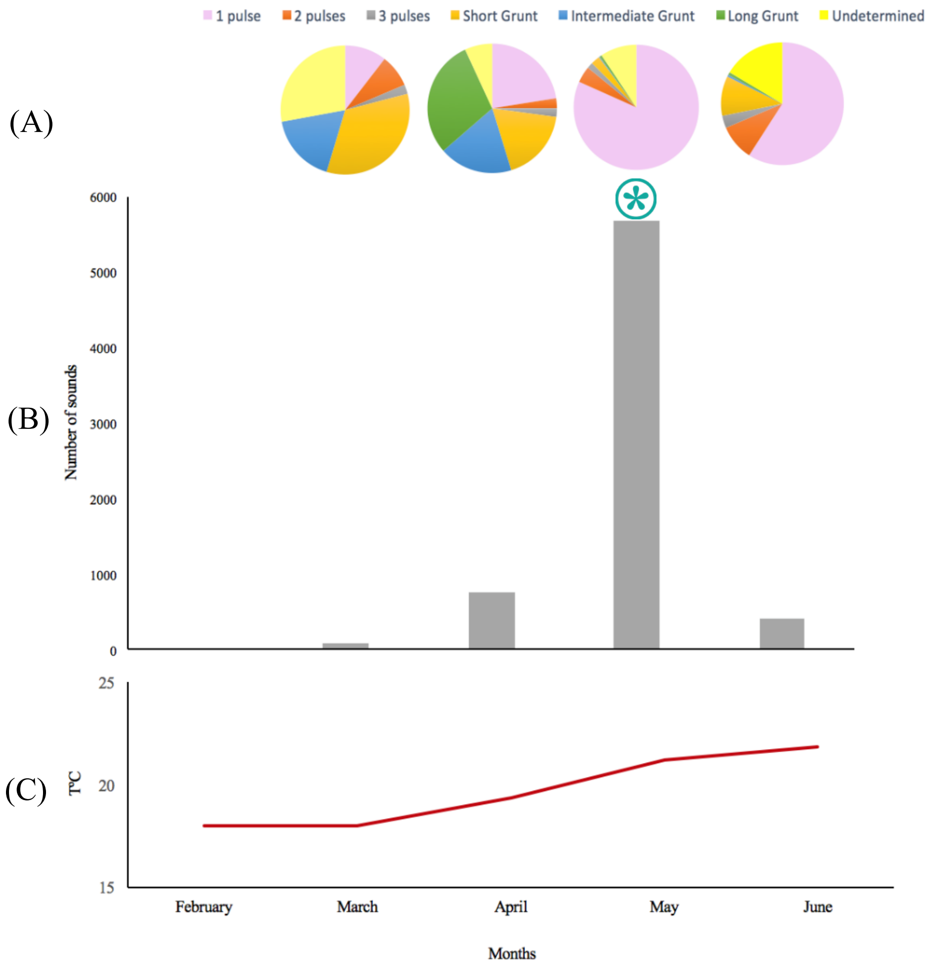

Figure 6: Seasonal variation of (A) the proportion of call types and (B) number of sounds; (C) Water temperature per month obtained by the mean temperature of the two studied days. A total of 7936 sounds were counted and categorized from 5 hours' files, between 18h and 23h, on the 18th and 28th days of each month, from February to June 2018. (*) indicates occurrence of a spawning event.

Acoustic parameters of long grunts varied significantly among months. Sound duration and number of pulses decreased from March to June, increasing in July (Fig. 7A,B). Pulse period and peak frequency decreased from March to May, increasing in June and decreasing again in July (Fig. 7C,D). Water temperatures (Fig.7E) increased exponentially from March to May (18 to 22ºC), decreased in June (from 22 to 20ºC) increasing again in July (21ºC).

Table 1: Sound duration, number of pulses, pulse period and peak frequency of long grunts produced by *A. regius* from March to July.

Sound Parameters	27.03.2018		28.04.2018		18.05.18		04.06.2018		05.07.2018	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Sound Duration (ms)	1613 ± 376	1112 - 2230	1208 ± 405	524 - 1835	1034 ± 436	449 - 2176	831 ± 227	517 - 1348	1127 ± 468	642 - 2480
Nº of pulses	77 ± 18	54 - 109	65 ± 21	30 - 95	63 ± 26	30 - 130	48 ± 13	30 - 78	69 ± 30	39 - 160
Pulse Period (ms)	21 ± 1	21 - 23	19 ± 1	18 - 20	16 ± 1	15 - 17	18 ± 0.3	17 - 18	17 ± 0.4	15 - 17
Peak Frequency (Hz)	230 ± 62	146 - 299	200 ± 100	141 - 563	151 ± 64	117 - 305	258 ± 104	117 - 398	124 ± 26	117 - 234

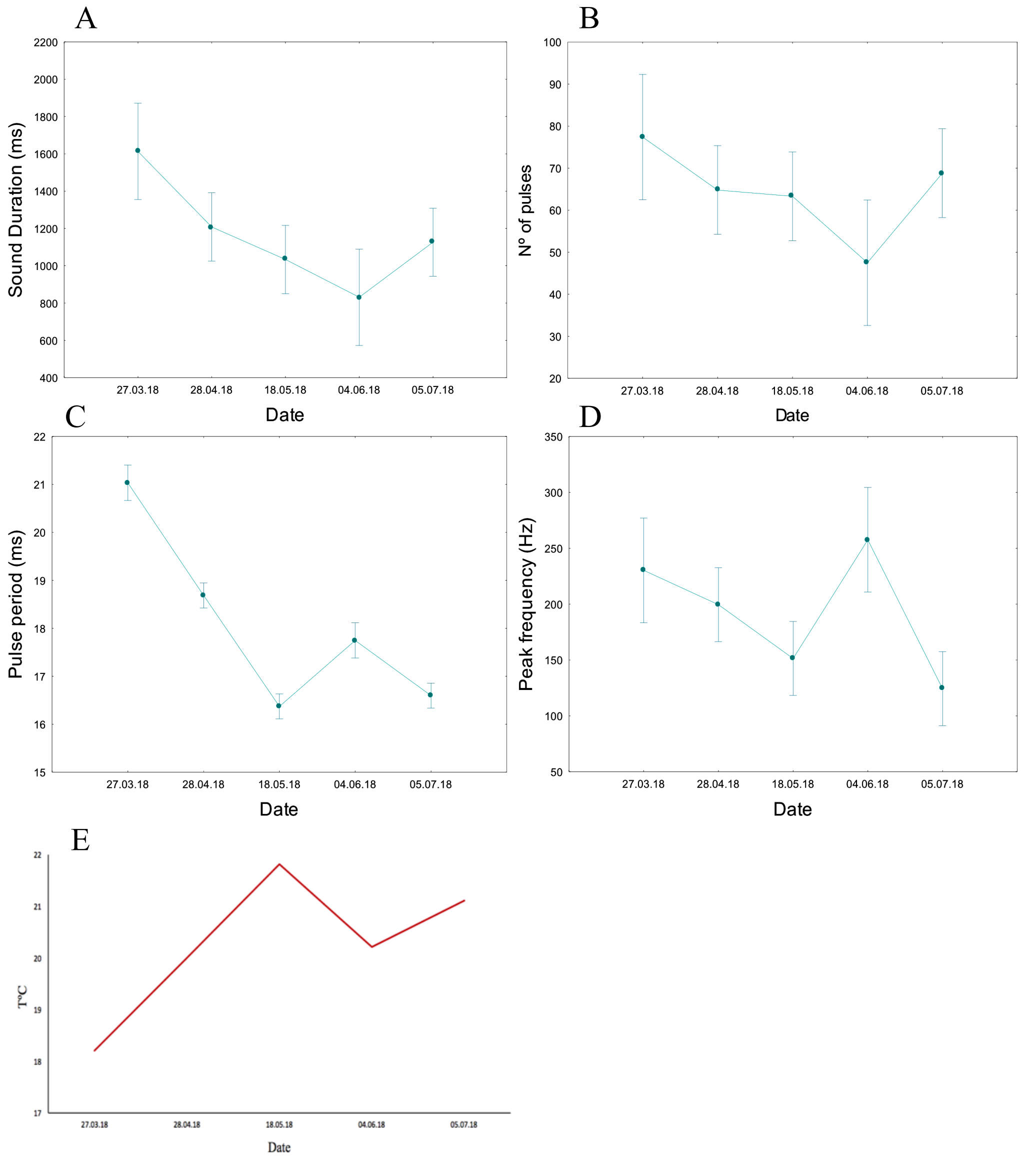

Figure 7: Seasonal variation of (A) sound duration (ms), (B) number of pulses, (C) pulse period (ms) and (D) peak frequency (Hz) of long grunts (One-way Anova; 10 to 20 sounds per month depending on the availability of grunts). 95% confidence intervals are shown. (E) Water temperature variation (ºC).

Sound production associated with spawning

Across the spawning periods, variation in the amount of calling and in the type of sounds produced, was identified at a qualitative level. In T1 (pre-spawning period; Fig. 8A) call activity was erratic and the amount of sounds produced was quite low (sometimes less than 10 calls were produced between 18h and 24h), with long grunts rarely being present. On the other hand, in T2 and T3 (spawning period; Fig. 8B) sounds were produced at a high and constant rate, with 'knocks' preceding dense choruses of long grunts. In this period, short and intermediate grunts were rarely found. T4 (post-spawning period; Fig. 8C) a reduction in the amount of calls produced was perceived, with the number of long grunts decreasing and of short and intermediate grunts increasing.

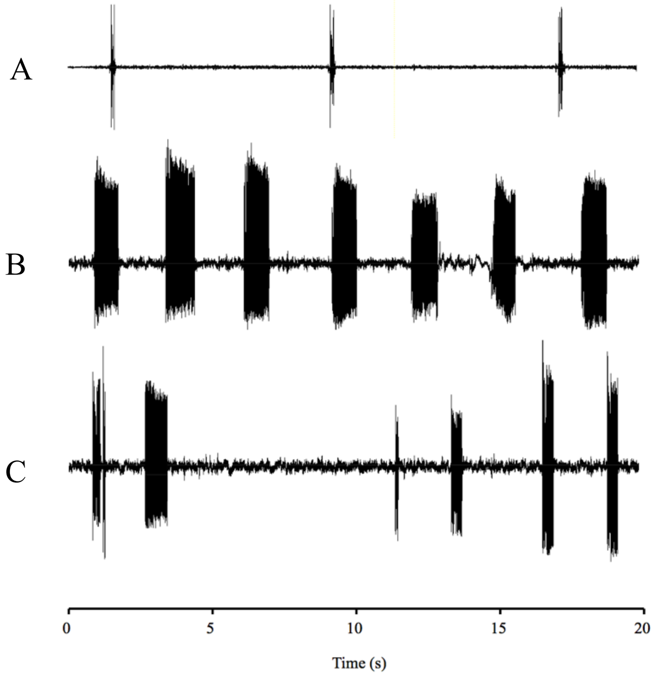

Figure 8: Oscillograms of sounds produced by meagre during (A) pre-spawning period, (B) spawning period and (C) post-spawning period. Sampling frequency 6 kHz, 1024 point FFT, Hamming window, and 50% overlap.

Variability of the acoustic parameters of spawning sounds produced by *A. regius* across the pre-spawning (T1), spawning (T2 and T3) and post-spawning (T4) periods are shown in Figure 9 and Table 1. Sound duration had a positive relation with number of pulses (Table 1), which varied significantly throughout the spawning periods. The number of pulses increased from T1 to T2 and decreased from T2 to T4 (Fig. 9A). The opposite occurred with the peak frequency which decrease with the proximity to the spawning event, from T1 to T3, and increased after egg deposition, from T3 to T4 (Fig. 9B). Regarding the pulse period, it significantly increased from T1 to T2 and significantly decreased from T2 to T3 and from T2 to T4 (Fig 9C).

Table 1: Sound duration, number of pulses, pulse period and peak frequency of sounds produced by *A. regius* during 3 spawning events, across 4 study periods.

Sound Parameters	T1		T2		T3		T4	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Sound Duration (ms)	338 ± 439	38 - 1495	840 ± 568	54 - 2314	743 ± 594	45 - 2420	350 ± 417	46 - 1839
Nº of Pulses	20 ± 24	3 - 84	47 ± 32	4 - 135	44 ± 34	3 - 135	22 ± 26	4 - 116
Pulse Period (ms)	17 ± 3	13 - 23	19 ± 2	16 - 22	17 ± 2	12 - 22	17 ± 2	14 - 24
Peak Frequency (Hz)	394 ± 162	164 - 627	208 ± 112	100 - 580	189 ± 146	106 - 508	306 ± 181	123 - 598

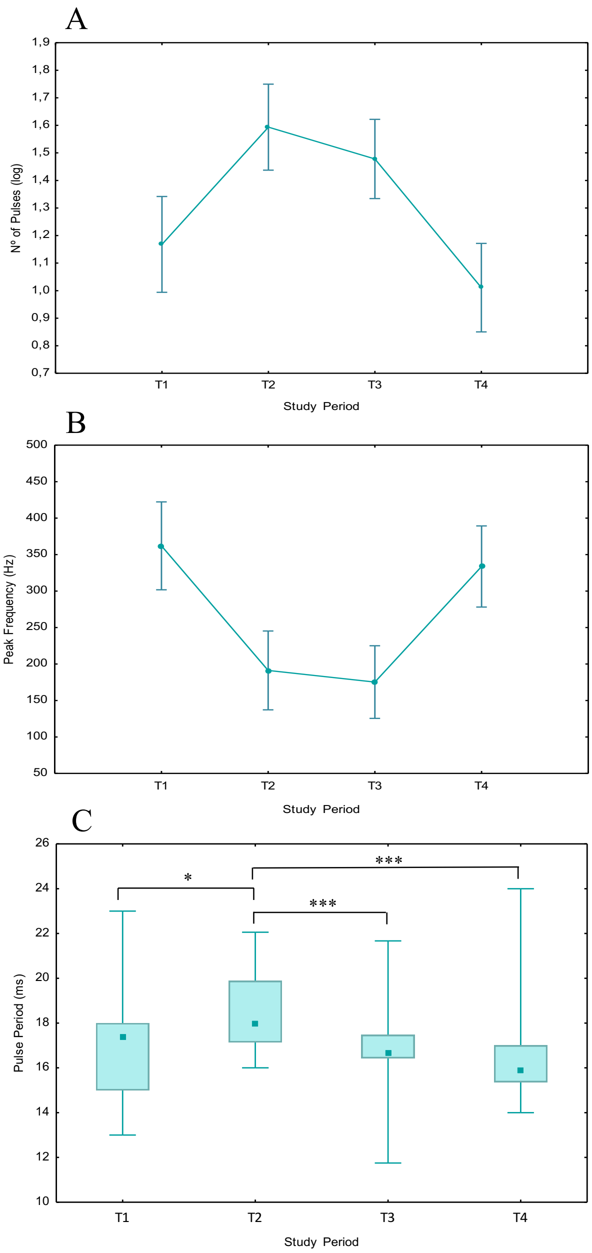

Figure 6: Comparison of the (A) number of pulses, (B) Peak Frequency (Hz) and (C) Pulse Period (ms) of spawning sounds produced by *A. regius* across 4 study periods: T1 (5th day prior to the spawning event), T2 (day before spawning), T3 (day(s) when eggs were found in the tank) and T4 (day immediately the last egg deposition). (A) Number of pulses after log transformation (GLM); (B) Peak frequency (GLM) and (C) Pulse Period (Kruskal–Wallis nonparametric test). In A and B means and 95% confidence intervals are shown. In C Box plots are depicted with significant pairwise differences shown with (*) for $p < 0.05$ and (***) for $p < 0.001$.

Discussion

Recordings made in lab conditions demonstrated that *A. regius* have a greater variety of vocalizations linked to times of spawning than previously thought. According to Lagardère and Mariani (2006), meagre produced two sound types, short grunts (4-6) and long grunts (30-112). However, in this study, 6 types (i.e. short, intermediate and long grunts), could be identified at a qualitative level, all of which were associated with spawning events. The different sounds emitted only showed a quantitative variation, as it is common for the majority of sciaenids (e.g., number of pulses and call duration; Guest and Lasswell, 1978; Luczkovich et al., 1999; Connaughton et al., 2002). Long grunts produced in captivity were similar to the ones found in the Gironde estuary by Lagardère and Mariani, having a short pulse period, with pulses being heard as a unit rather than a train of individual pulses. However, in the same study grunts were observed to have a maximum of 112 pulses, while long duration calls in captivity exhibited a higher number of pulses, with a maximum of 160 pulses. Additionally, personal observations revealed that the initial pulses of these sounds frequently exhibited a lower amplitude than succeeding ones. Hence, it is possible that the sonic muscles take time to attain the tension required to generate amplitudes displayed by later pulses of the long calls (Parsons et al., 2013).

In this study, spawning never happened without a significant increase in sound production. Two types of acoustic signals were associated with successful spawning events: dense choruses, generally of long grunts, and sequences of individual pulses. The former, already described for meagre by Lagardère and Mariani (2006), consisted of sequences of long duration calls heard as 'drums', produced at a constant rate. Sometimes overlapping of sound waves occurred, meaning that they were being produced by different individuals at the same time. In the present study, drumming was so loud that it could clearly be heard without the aid of hydrophones when standing about 3 m away from the tank. Many sciaenids have been reported to produce drumming sounds during the spawning season (Fish and Cummings, 1972; Takemura et al., 1978; Connaughton and Taylor, 1995; Parsons et al., 2013), suggesting that it may play an important role in reproduction, including formation of spawning aggregates and courtship behavior. Interestingly, we also observed long sequences of single pulses audible as 'knocks'. These series of pulses could last for more than one hour and generally preceded the choruses of long grunts. Biological functions for these knocks are unknown. It is possible that meagre are simply triggered by increasing hormonal levels coincident with spawning.

Captive meagre exhibited diel spawning periodicity, with sound emissions generally beginning at dusk, by 18h, reaching a maximum activity approximately two to three hours later, at 20/21h, and ending at 24h. These observations are in accordance with what is known for several other sciaenid species (Mok and Gilmore, 1983; Holt and Holt, 2003). Atlantic croaker (*Micropogonias undulatus*), sand seatrout (*Cynoscion arenarius*), and red drum (*Sciaenops ocellatus*) show similar calling patterns with sound production increasing at laboratory-simulated dusk (Holt et al., 1985; Montie et al., 2015). Saucier and Baltz (1993) showed that spotted seatrout sound production occurred from 17h to 01h and that 92% of the drumming occurred between 19h and 23h. Maintaining daily patterns of calling may be an advantage to broadcast spawners like sciaenids by assuring that a large number of fish would be in spawning condition at the same time, thereby maximizing fertilization of the high number of eggs released into the water column. Dawn or daylight spawning occurs in several species that have visual courtship displays (Middaugh 1981, Doherty 1983) but sciaenids primarily use sound for courtship displays, so spawning is probably not dependent on light (Hobson and Chess, 1978). Mass spawning at dusk in sciaenids is most likely an adaptation to limit the predation on eggs (Holt et al., 1985), since planktivores are inactive during this time (Lobel, 1978; Ferraro, 1980; Robertson, 1983).

The increase in knocking activity (characterised by series of isolated pulses, as mentioned above) from March to May is most likely linked to spawning as it was observed often in association with spawning events. The lower percentage of knocks in March and April may be related with the timing of sampling and spawning events, as the files analysed for acoustic activity did not coincide with spawning activity, in contrast with May, where the analysed sound files concerned hours before the spawning event. This type of sound was also found in June, though no spawning event occurred adjacent to the study period. However, these fish may have been stimulated by meagre from another tank in which a spawning event took place on the 17th. These results call for a more detailed study to ascertain which sounds are made just before spawning as in the present study we only know that spawning occurred sometime in the previous night. The presence of long grunts in April and absence in the previous months indicates seasonality. The low number of long grunts in May suggests that the duration of the observations was insufficient and that it should be extended to midnight, as long grunts were observed after the chosen sampling period. The decrease in the proportion of short and intermediate grunts from March to June indicates seasonality, maybe related with temperature and circulating androgen levels, which could have caused a change in sound type. In addition, as in long grunts it is possible that the circadian vocal rhythms may have shifted to later hours and we may have missed the occurrence of these sounds. Future work should consider the full 24h in the sampling days. However, as this is an extremely time-demanding task an automatic system able to recognize the occurrence of the different sound types should ideally be used.

The study of long grunts across several months revealed, once again, that these sounds are primarily related with spawning events, since this type of call wasn’t found in February and the ones found for the remaining months were always concurrent with reproduction. Variability found in acoustic parameters of long grunts analysed from March to July revealed seasonality, with temperature probably being the major contributor. Because most fishes are ectotherms, the capability and speed of metabolic and physiological processes are influenced by surrounding water temperature. Sound characteristics are expected to change with temperature since it influences muscle contraction properties (Feher et al., 1998). In this study, all parameters were inversely related with temperature. Generally, rising temperatures speed up the velocity of motion and shorten muscle twitches, resulting in an increased rate of sound emissions, sound duration, dominant frequency and decrease in pulse duration (Fine, 1978; Torricelli et al., 1990; Brantley and Bass, 1994; Connaughton et al., 2000). In the present work, the parameter that seemed to be more affected by temperature was the pulse period. It decreased abruptly with rising water temperatures from March to May (18 to 22ºC), significantly increasing when, in June, temperatures declined (to 20ºC) and increasing again with temperature (21ºC) in July. These findings indicate that changes in water temperature as low as 1◦C affect considerably sound characteristics. The decrease in sound duration is intimately related with the decrease in pulse period. Interestingly, the number of pulses and peak frequency did not increase as it would be expected. Instead these parameters decreased with rising temperatures, due to some unknown factor, maybe related with the concurrent spawning events.

In this work, captive meagre produced sounds which vary in their characteristics as reproduction proceeded, with acoustic parameters of sounds (number of pulses, pulse period and peak frequency) being significantly different between pre-spawning (T1), spawning (T2 and T3) and post-spawning (T4) periods. Sounds recorded during the spawning period (T2 and T3) were characterised by a higher number of pulses, larger pulse period and lower peak frequencies than those recorded in the pre- and post-spawning periods (T1 and T4). The variability of acoustical parameters between these periods could be associated with different levels of motivation paralleled by increasing (and decreasing) levels of the male arousal towards (and after) spawning and with increased (and decreased) male competition. Furthermore, the sounds produced with higher proximity to spawning (T2 and T3) probably play a more active role in the formation of aggregations and attraction of females, which explains the lower frequencies and the higher number of pulses (and longer call duration) essential for the transmission of the acoustic signals over long distances. Other species have been showed to produce sounds that vary in characteristics with proximity to spawning. For example, the haddock was observed to emit a series of knocks with increasing repetition rate as courtship proceeded, from short slow knocks to short fast knocks, long slow knocks, long fast knocks, and ending up by fusing together and heard as hums (Hawkins and Amorim, 2000).

Concluding remarks

This study revealed that the meagre has a richer acoustic repertoire than previously thought. Since all the call types found were associated with spawning events, they can be used to identify the presence of breeding adults in nature. Our findings on the diel, seasonal cycles of sound production in *A. regius* can be used to study the movements of meagre on the spawning grounds of Tagus estuary. The variability of sound characteristics found with proximity to spawning (i.e. increase in the number of pulses and decrease in peak frequency) can potentially delimit timing of egg deposition in the field and in aquaculture systems. As in the present study we did not know the exact time of egg deposition, future experiments should focus on a more detailed analysis considering a finer temporal scale of the acoustic activity and sound features in association to the spawning events. In addition, the effects of temperature on *A. regius* sound characteristics should be studied in more detail.

Acknowledgements
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Final considerations

Sounds produced by Sciaenidae fish species have been frequently associated with the spawning season and are being widely used to monitor spawning populations in the field. Advertisement calls of *A. regius* were characterized solely by Lagardère & Mariani (2006) in the Gironde estuary. However, a detailed study on the wider vocal repertoire of the meagre, likely to be encountered during an acoustic census, was not published so far. This lack of knowledge may potentially cause identification uncertainty at sites where more than one species might be emitting sounds.

The variation in sounds we have documented indicates that *A. regius* vocalizations have the potential to carry information about sex, size, motivation, and other parameters that may play a role a role in both social (i.e. courtship and spawning) and distress (i.e. aggressive interactions) contexts. Sounds produced by captive meagre during the breading season showed a richer repertoire than previously observed. A seasonal trend was noted, probably associated with water temperature. Sound production by meagre seemed to be primarily related with spawning events, changing with proximity to spawning, during and after spawning.

The findings of this study help guarantee identification of sounds produced by meagre in the field and are advantageous to study many aspects of this species biology relevant to stocks management and conservation: locate individuals and identify species distribution, delineate crucial habitats for meagre, location/timing of spawning, populations distribution, and monitor behavioural responses to biological and anthropogenic. In addition, being able to monitor sounds that may be indicative of the proximity of a spawning event may also be important for aquaculture.

Research to clarify differences between gender and other sources of variability (e.g. water temperature) are needed. Additionally, a more detailed analysis considering a finer temporal scale of the acoustic activity and sound features in association to the spawning events should be studied for *A. regius.*