Sound production in Sciaenidae spp.: the relationship between acoustic activity and spawning in an aquaculture perspective

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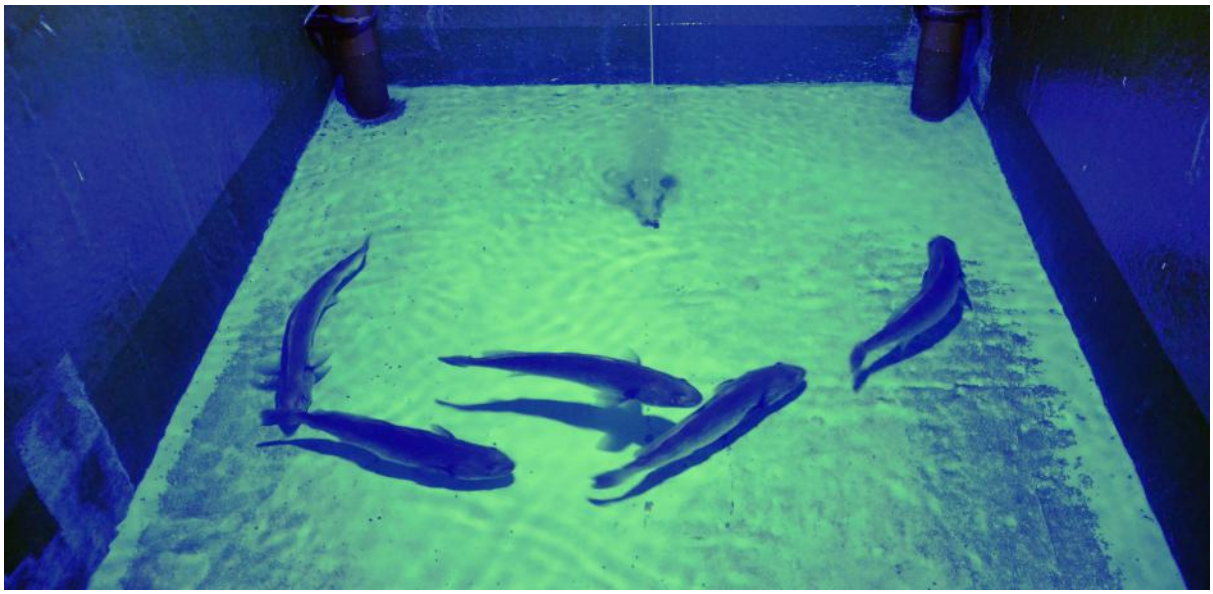
Sound production in Sciaenidae spp. : the relationship between acoustic activity and spawning in an aquaculture perspective.



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Abstract

During this study, sound production of three Sciaenidae species (*Argyrosomus regius*, *Umbrina cirrosa* and *Sciaenops ocellatus*) was investigated before (t0 and t1) and during spawning season (t2) in rearing facilities. The study showed a clear increase in calling activity during the spawning season and changes in the acoustic parameters in all three species. A shift in the diel pattern of sound production before and during reproduction was observed in all three species. The calling activity out of reproductive season was mainly emitted during daytime hours whereas calls emitted during the reproduction season occurred mainly during nighttime hours. Fine acoustic parameters like number of pulses and pulse period seemed indicative of spawning as more quickly repeated pulses were found during the reproductive season. This implies that the sonic muscles contract faster during reproduction which was supported by the increase in sarcoplasmic reticulum on analyzed cross-sections.

Résumé

Durant cette étude, la production sonore de trois espèces de Sciaenidae (*Argyrosomus regius*, *Umbrina cirrosa* et *Sciaenops ocellatus)* a été investiguée dans une aquaculture avant (t0 et t1) et durant la période de reproduction (t2). L'étude a pu démontrer une augmentation significative de la production sonore pendant la période de reproduction ainsi que des changements dans les paramètres fins pour les trois espèces étudiées. Un changement dans la production sonore journalière a été observé chez les trois espèces entre la période non reproductive et la saison de reproduction. En dehors de la période de reproduction, la production sonore était principalement émise durant les heures diurnes alors que les sons émis durant la saison d'accouplement survenaient principalement durant les heures nocturnes. Les paramètres fins tels que le nombre de pulsations et la période de pulsation semblaient indicatifs de ponte sachant que des sons avec plus de pulsations et plus souvent répétées ont été obtenus lors de la saison de reproduction ce qui implique que les muscles soniques se contractent plus rapidement durant la saison de ponte. Ceci est appuyé par l'augmentation du réticulum sarcoplasmique en coupe transversale.

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Introduction

A. Acoustic communication in aquatic environments

As other taxa, teleost fish depend on communication during crucial activities such as reproduction or agonistic interactions (Amorim et al. 2015). Acoustic communication is the intentional exchange of information, beneficial at least for the sender, between an emitter and a receiver thanks to an acoustic signal, i.e. sound (Bradbury & Vehrencamp 1998).

When compared to visual or chemical communication, acoustic communication shall be one of the most successful ways for aquatic organisms to exchange information (Tavolga 1971; Southall & Nowacek 2009). Visual comunication can be attenuated by turbidity, darkness, distance or obstacles (Fine & Parmentier 2015). Moreover, more than 87.5% of the sunlight is absorbed in the euphotic zone (first 200 meters) of the ocean (Chiang et al. 2011). Below 200 meters visual communication is restricted or absent, or must resort to light produced by chemical reactions or cellular secretion in the cells of living organisms or in the cells of symbiotic organisms, which live together with them, i.e. bioluminescence (Altun et al. 2008; Popper & Ketten 2008; Chiang et al. 2011; Fine & Parmentier 2015). Chemical signals do not propagate in all directions but are limited by water dynamics such as currents to close by areas (Fine & Parmentier 2015). On the contrary, sound propagates in all directions and five times faster in water than in the air (Tavolga 1971; Popper & Ketten 2008). However, the speed of sound can change with temperature, pressure and salinity of the medium, i.e. water (Brewer et al. 2015). Also, sound travels for a longer distance in water because of the compressive characteristics of the medium (Fine & Parmentier 2015).

Aquatic organisms have first evolved in an environment where the soundscape, i.e. the acoustic environment, was filled only by geophonical and biophonical sound sources (Krause 2012). The geophony includes all sounds produced by natural physical sources (e.g. wind, currents, meterological events, tectonic movements etc.), while biophony refers to the sound made by living organisms with exception of humans (Pijanowski et al. 2011). Since the mid of the last century, an additional source of noise, anthropophony or man made noise, has regularly increased in the soundscape (Frisk 2012).

1. Sound production and morphology in fishes

Contrary to terrestrial vertebrates, teleost fishes do not use a laryngeal or syringeal structure for sound production (Popper et al. 2003) but possess many different sound producing mechanisms that have evolved independently several times. It results that fish possess the widest diversity of soniferous mechanisms among vertebrates (Parmentier et al. 2017). These different mechanisms are however based on common principles and each teleost group has to deal with similar constraints. It means different mechanism can be found but they are all based on the same principle. Although it is quite simplistic, it can be fairly divided into three main groups: stridulatory, hydrodynamic and swimbladder mechanisms (Popper et al. 2003).

Stridulatory mechanism

The stridulatory mechanism involves rubbing of bones or other hard elements like teeth or vertebrae between each other's (Ladich & Bass 2003; Fine & Parmentier 2015). These sounds can be used for communicative purpose or can be made involuntary during activities such as feeding (Parmentier et al. 2014; Fine & Parmentier 2015; Parmentier et al. 2017). An example of stridulatory mechanism is the rubbing of the pectoral fin against the shoulder girdle in catfishes (Ladich & Bass 2003; Fine & Parmentier 2015; Parmentier et al. 2017).

Hydrodynamic sounds

Hydrodynamic sounds are produced when a fish displaces water by swimming. They are more important during acceleration and spontaneous changes in direction (Tavolga 1971).

Swimbladder mechanism

Mechanisms that imply the swimbladder can be broadly divided into two groups on the basis of the contraction speed of specialized muscles, i.e. the sonic muscles, which are directly or indirectly attached to the swimbladder. Extrinsic muscles typically originate on the skull and insert on the swimbladder or on a bone or tendon connected to the swimbladder. Intrinsic muscles attach exclusively to the bladder wall (Fine & Parmentier 2015).

Additional mechanisms

Some sound production mechanism could not be categorized in the three groups mentioned above like the sound production created due to tendon vibrations or the forced flow through an orifice (Parmentier et al. 2017).

The slow mechanism encountered in some carapids is driven by slowly contracting muscles going from the orbital part of the skull to the swimbladder by attaching to it directly or indirectly (Fine & Parmentier 2015). As the sonic muscles contract, the swimbladder fenestra

stretches. When the pressure is released and the fenestra pops back to its natural position, sound is emitted and the swimbladder vibrates (Fine & Parmentier 2015).

The fast mechanism appears in different species or families like Mochokidae, Bagridae, Serrasalmidae or Sciaenidae (Fine & Parmentier 2015). The mechanism consists on a muscle contraction stretching and releasing the swimbladder, i.e. a muscle twitch. This movement is responsible for the sound production (Fine & Parmentier 2015).

Fast sonic muscles are extremely rapid contracting organs. In fact, their contraction can lead to frequency of more than 100 Hz, whereas locomotory muscles do usually not exceed 15 Hz (Rome & Lindstedt 1998). Histology on *Opsanus tau* (Linnaeus 1766) revealed that the amount of lipids, glycogen and mitochondria surrounded by myofibrils and endoplasmic reticulum may play a role in this unique functioning (Devincenti et al. 2012). This has also been investigated by Fine & Parmentier (2015). They explain that sonic muscles need to be able to contract faster than other muscles in order to produce audible fish sounds. For this reason, the muscle undergoes several adaptations. Mitochondria, myofibrils and sarcoplasmic reticulum are the three major components of the muscle fibers. Sonic fibers and myofibrils are both smaller compared to those in other muscles; this affects the contraction force of the muscle because smaller more numerous muscle units can generate more force. Mitochondria are more abundant in sonic muscles than in other muscles; this affects the fatigue resistance as mitochondria provide energy to the cells. Sarcoplasmic reticulum is found in higher volume than in other muscles; this affects the contraction rate because more sarcoplasmic reticulum ensures more available calcium which leads to more contraction and relaxation cycles of the muscle. In addition, an important vascularization of the fibers provides sufficient oxygen. (Ramcharitar et al. 2006; Fine & Parmentier 2015; Parmentier et al. 2017).

Fish sounds variability due to the behavioral context

Teleosts emit sounds to convey different behavioral (Lobel 2001; Ladich & Myrberg 2006; Amorim et al. 2015), sexual, ontogenetic (Amorim 2006) and physiological (Amorim et al 2010; Amorim et al. 2015) information (Zelick et al. 1999). Acoustic signals can also be influenced by environmental conditions, such as water temperature (Kéver et al. 2015; Ladich 2018).

Advertisement sounds are made by males while being at a long distance from females to attract them to their area during the reproductive season (Ladich & Popper 2004; Amorim et al. 2015). Advertisement calls are species-specific and can convey information on the males'

identity (species, sexes) and their location (Amorim et al. 2015). Choruses (sounds produced by an aggregation of individuals vocalizing together at the same place) of advertisement calls are easily detected by females and are an effective way to indicate a spawning area (Amorim et al. 2015). Courtship calls are made when female and male are already close (Ladich & Popper 2004; Amorim et al. 2015). They play an important role to indicate the motivational state and quality of the male and can be mate choice cues (Amorim et al. 2015). Territoriality and agonistic behaviors can be exhibited by multiple signals as visual or acoustic cues (Ladich & Myrberg 2006) to prevent attacks that could injure the opponents or even be lethal. Sounds can be made to repel a predator or rival that comes too close to the nest or to the territorial space. Agonistic calls are also made while competing or foraging for food (Ladich & Myrberg 2006) as it is the case in sea robins (Hawkins & Amorim 2000).

Distress sounds are also widespread among soniferous fishes and are made when fish feel disturbed or are handled (Ladich & Myrberg 2006). A supposed function for these signals would be to alert conspecifics of a potential or apparent danger (Ladich & Myrberg 2006).

Due to darkness or turbidity, the visibility can sometimes be restricted in the water column (Fine & Parmentier 2015). Group cohesion sounds (also called contact sounds) are made to ensure the unity of an aggregation. These sounds aim to communicate the location of the group and avoid that individuals deviate from the way (Lagardère & Parmentier 2014).

Fish sounds variability due to the physical characteristics

Sounds produced by one species can vary depending on different factors like age, body size, lipid content or liver mass.

During the growth of juveniles into adults, morphological changes happen in the body (Koumoundouros et al. 2005) which explain ontogenetic modification in sound features (Amorim 2006). Nevertheless, the relation between the body size and the dominant frequency depends strongly on the species (Parmentier & Fine 2016). In fact, species producing short repeated pulses like sciaenids or croaking gouramis see their dominant frequency decrease with the body size (Amorim 2006) as larger sonic muscle lead to longer sounds with lower dominant frequency (Connaughton et al. 2000). As an example, the short pulsed sounds of the croaking gourami *Trichopsis vittata* (Cuvier 1831) decrease in dominant frequency with age but increase other features like call duration, the number of pulses, the pulse period and the SPL (Amorim 2006).

Body size, lipid content or liver mass are features linked to the physiological state and can modulate the temporal and/or spectral parameters of the sound (Amorim et al. 2010).

Also, temperature can play a role in these poikilotherms (Kéver et al. 2015; Ladich 2018). It affects the physiology of the neural system and musculature, which are both involved in the sound production (Ladich 2018). Another study, Monczak et al. (2017), was able to find a positive relationship between positive temperature anomalies and the increase in calling activity in some of the studied species (*Bairdiella chrysoura* (Lacepède, 1802), *Cynoscion nebulosus* (Cuvier, 1830), *Opsanus tau, Pogonias cromis* (Linnaeus, 1766).

2. Sound production and morphology in Sciaenidae

The members of the Sciaenidae are commonly called croaker or drummer (Lagardère & Mariani 2006; Ramcharitar et al. 2006; Parmentier et al. 2014) due to their ability to produce drumming-like sounds. The Sciaenidae counts for around 270 species (Ramcharitar et al. 2006; Tellechea et al. 2010; Tellechea et al. 2011) among which 14 species (**Appendix 1**) have been recorded. They are multiple-batch spawner with group-synchronous oocytes (Barbaro et al. 2002; Falguière 2001; Mylonas et al. 2004) which means that Sciaenidae spawn several times a year in numerous aggregations (Picciulin et al. 2016).

In all studied species, sound-producing mechanisms imply contraction of sonic muscles surrounding the swimbladder (Sprague 2000) with pulsed sounds reflecting the contraction rate of the sonic muscles.

Among the 270 Sciaenidae species, six different swimbladder shapes were found. They are classified into type I to VI according to their shape. Both, extrinsic and intrinsic sonic muscles are found within the Sciaenidae family (Ramcharitar et al. 2006), although extrinsic sonic muscles are more common. In fact, the only known sciaenid with an intrinsic mechanism is the black drum *Pogonias cromis* (Ramcharitar et al. 2006).

While the fish ages the muscles grow bigger, this might change the emitting frequency and amplitude in relation with body size (Fine & Parmentier 2015). In some species the sonic muscles are only active when hypertrophied during the spawning season (Amorim et al. 2015). Highly vascularized, sonic muscles can be recognized by their dark red coloration and are consisting in thin and uniform fibers (Ramcharitar et al. 2006).

Chorus emitted by Sciaenidae suggests to help creating breeding aggregation (zone of lek) to facilitate mating (Amorim et al. 2015) and the spawning success by using spawning

synchronization (simultaneous release of gametes) (Aalbers & Drawbridge 2008). The white seabass or white weakfish *Atractoscion nobilis* (Ayres, 1860) creates spawning aggregations from spring to summer near California and Mexico and emits five different sounds depending on its behavioral state (Aalbers & Drawbridge 2008). A group formed by one to nine male individuals surrounds the female during spawning in order to fertilize the pelagic eggs. The Atlantic croaker *Micropogonias undulatus* (Linnaeus, 1766) is found in aggregations in proximity to estuaries in North Carolina and spawns between September and February; in this species both sexes possess sonic muscles and sounds are characterized by an unusual spectral content (wideband up to 5 kHz and dominant frequency > 1 kHz) (Gannon 2007).

Micropogonias furnieri (Desmarest, 1823) possess an extrinsic mechanism in both sexes (Devincenti et al. 2012). Two kinds of sound are produced: the advertisement sounds produced by males and the disturbance sounds made by both sexes (Tellechea et al. 2010). In *Pogonias cromis*, both sexes produce sounds thanks to their well-developed intrinsic sonic muscles. This species is present in temperate waters from the Southern to the Northern hemisphere. Its segregation into two populations hypothesizes that the sound production in both sub-species may vary (Tellechea et al. 2011). In fact, sounds made by individuals inhabiting the waters of Florida are three times longer than the ones made by the Southern population. This divergence in sound features suggests a genetical divergence because of the isolation of these two populations (Tellechea et al. 2011).

Passive Acoustic Monitoring (PAM) is an acoustic detection system technique consisting in the use of hydrophones for monitoring the underwater soundscape, including fish vocalization (Nedwell & Parvin 2007). The technique has been proved successful in mapping the spawning location of several Sciaenidae (Picciulin et al. 2016). This could lead to the conception of protected areas (Tellechea et al. 2010) to preserve vulnerable species like the shi drum *Umbrina cirrosa* (Linnaeus, 1758), *Sciaena umbra* (Linnaeus, 1758) and etc. (Picciulin et al. 2016).

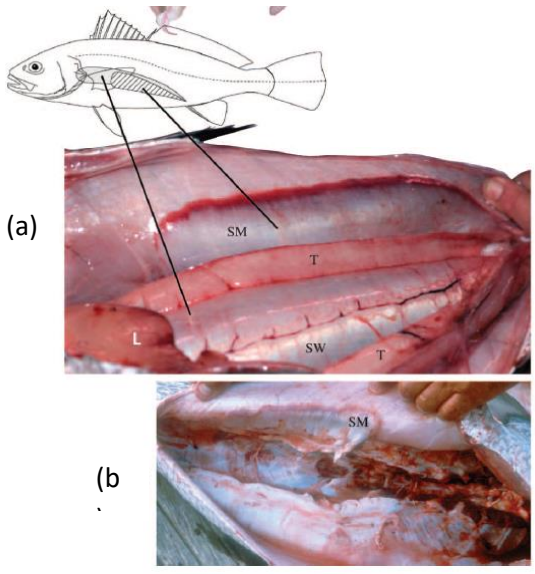
This thesis focuses on 3 Sciaenidae species (**Appendix 2**): *Argyrosomus regius* (Asso, 1801), *Umbrina cirrosa* and the red drum *Sciaenops ocellatus* (Linnaeus, 1766).

2.1. Sound production and morphology in Argyrosomus regius

Argyrosomus regius, commonly called meagre, is a sciaenid found in the Mediterranean Sea (Picciulin et al. 2016) and the Atlantic Coast, from Congo to the North Sea (Cabral & Ohmert

2011). The reproductive season of *A. regius* occurres in late spring and summer months in the Gulf of Cádiz and the Gironde estuary (Cabral & Ohmert 2001; González-Quirós et al. 2011; Lagardère & Mariani 2006). The sound production mechanism involves fast sonic muscles attached to the swimbladder. The genus *Argyrosomus* possesses a type IV bladder characterized by paired diverticula found laterally of the structure (Ramcharitar et al. 2006).

The meagre is one of five species where both sexes possess sonic muscles. The other species are *Nibea albiflora* (Richardson 1846), *Argyrosomus argentatus* (Houttuyn 1782), *Micropogonias undulatus* and *Pogonias cromis.* Male and female *Argyrosomus regius* both have been recorded vocalizing at slightly different frequencies (Lagardère & Mariani 2006). Male vocalize with a higher dominant frequency than females (Lagardère & Mariani 2006). These findings were also shown in closely related species *Argyrosomus argentatus*, *Nibea albiflora* (Takemura et al. 1978) and *Argyrosomus japonicus* (Temminck & Schlegel, 1843) (Ueng et al. 2007). Male and female *A. japonicus* were from the same age and size. Males *A. japonicus* vocalized with a frequency of 686 ± 203 Hz (N=210) whereas females reached a dominant frequency of 587 ± 190 Hz (N=164) (Ueng et al. 2007). Also, females produced longer sounds with more pulses and a shorter pulse period (Ueng et al. 2007). Normally, shorter pulse periods should lead to a higher frequency. Therefore, the lower dominant frequency of females found by Ueng et al. 2007 could be related to the weaker tension occurring in the thinner sonic muscles of females compared to males (Sprague 2000; Ueng et al. 2007).



Some morphological differences have also been detected between the two sexes. Indeed, the sonic muscles of a female appear in a more pink-like coloration and seem less hypertrophied than the ones belonging to a male (Lagardère & Mariani 2006; Takemura et al. 1978). Fishermen observed that when fish were taken out of the water, it was possible to see that the males' sides were still moving, i.e. contracting the swimbladder while females were motionless (Lagardère & Mariani 2006).

Figure 1: Picture showing in (a) the left sonic muscle (SM), the swimbladder (SW) and the testicles (T) of a male *Argyrosomus regius* and in (b) the right sonic muscle of a female of the same species. A difference in coloration can clearly be seen between the sonic muscles of the two sexes (Lagardère & Mariani 2006).

Lagardère & Mariani 2006 reported two different sound types emitted by *Argyrosomus regius* in the Gironde estuary between May and July (i.e. the spawning period). The most abundant sounds where named long grunts, which were defined as series of 30 to 112 closely repeated pulses with dominant frequency around 336- 444 Hz. These grunts were suggested to function as a mean to gather all individuals in one place. Short grunts were recorded less frequently than long grunts and were characterized by 4 to 6 closely repeated pulses with a dominant frequency of 383 Hz. Short grunts were emitted while regular emission of long grunts ceased. Several authors (Connaughton & Taylor, 1996; Connaughton et al., 2002) indicated that drumming ceased in *C. regalis* during the moment of gamete release. Also in the red drum, no sounds were heard when males were actively nudging the female or during gamete release (Guest & Lasswell, 1978). Because meagre short calls correlated with the cessation of drumming, they were probably emitted by a female meeting a male to involve a courtship with it, or by a male starting a pursuit of a female (Lagardère & Mariani 2006).

2.2. Sound production and morphology in Umbrina cirrosa

The shi drum *Umbrina cirrosa* is a species of Sciaenidae living in the Mediterranean Sea (Francescon & Barbaro 1999; Picciulin et al. 2016) and in the Black Sea (Ayala et al. 2013; Francescon & Barbaro 1999). It inhabits different bottom substrates in slightly to highly salty waters (Koumoundouros et al. 2005) which makes *Umbrina cirrosa* an euryhaline species (Barbaro et al. 2002).

The shi drum enters in sexual maturity after approximatively three years of age and has a natural spawning period from June to August (Picciulin et al. 2016). It is a good rearing species (Koumoundouros et al. 2005) because of its high commercial value. It can also be weaned early to artificial nutriments and does not need nauplius larvae (Barbaro et al. 2002; Mylonas et al. 2004).

In comparison with two other vocal Mediterranean sciaenids *Argyrosomus regius* or *Sciaena umbra*, less is known about the sound production in this species (Picciulin et al 2016). Lagardère & Parmentier 2014 illustrates eleven sounds taken thanks to the recordings of six specimens found in the aquarium of Biarritz. They could highlight the contact sound made by *Umbrina cirrosa* recorded in the morning (8:00 to 09:00) with one to three pulses ranging from 129 to 155 dB re 1µPa at a frequency around 200 Hz. They found two categories of sounds, one lasting from 60-90 milliseconds (ms) and the other ranging from 140 to 160 ms.

In another study (Picciulin et al. 2016), 30 mature specimen of *Umbrina cirrosa* were recorded during spawning season in an outdoor aquaculture tank belonging to a rearing facility center in Italy. The sex ratio was unknown. The recorded sounds where characterized by one to eleven pulses, although the majority of sounds were made of two pulses. Their peak frequency was on average 400 Hz. In this study, sound duration had a wider range (i.e. from 150 to 1400 ms). This can be explained by the difference in the number of pulses per call found in the two studies. On average, the pulse period was 180 ms and pulse duration 40 ms (Picciulin et al. 2016).

2.3. Sound production and morphology in Sciaenops ocellatus

Native from the Atlantic coast up to the Gulf of Mexico, *Sciaenops ocellatus*, also called red drum or red fish, has been introduced in several overseas departments as a good candidate for aquaculture (Falguière2011). Like the Latin name already suggests, *Sciaenops ocellatus* can be recognized by the fact that it has at least one black spot on the caudal fin mimetizing an eye to create a diversion preventing predators from attacking the real eyes (Falguière 2011).

In the red drum, only the male is capable of emitting sounds thanks to his extrinsic sonic muscles based mechanism. As shown in **figure 2**, the sonic muscles follow laterally the swimbladder and merge on the dorsal part to attach to a flat tendon called aponeurosis (Parmentier et al. 2014). *Scieanops ocellatus* possesses a species-specific type V bladder which is characterized by diverticula, an expanded anterior part or both features (Ramcharitar et al. 2006).

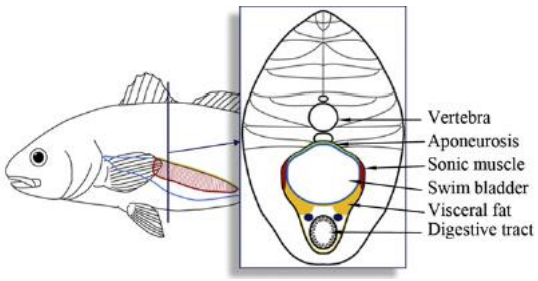


Figure 2: Drawing showing a synthesized view of the morphology inside the body cavity of a male *Sciaenops ocellatus* (Parmentier et al. 2014).

The red drum creates spawning aggregation in estuaries from July to November with a spawning peak between August and October (Falguière 2011), which is supported by histological examination of gonadal development (Holt 2008). In contrast to previous references, Holt (2008) suggests that red drums located around the coast of Mexico do not really spawn in aggregation but are more widespread during spawning (Holt 2008).

Maturation occurs on average around three years of age, even if males are often mature already after one year and a half and females around three to six years (Falguière 2011). In captive reproduction, hormonal induction (releasing hormone LHRH) used in conjunction with the playback of callings of males allows for the spontaneous expulsion of eggs into the water column and avoid the problems linked to the stripping of this species (Falguière 2011).

Guest & Lasswell 1978 noticed male *Sciaenops ocellatus* nudging on the urogenital papilla of females before gamete release during which sound production ceased (Guest & Lasswell 1978; Lagardère & Mariani 2006; Holt 2008). The same behavior was observed in the white seabass (Aalbers & Drawbridge 2008), in the weakfish (Connaughton & Taylor 1996) and in *Umbrina cirrosa* (Francescon & Barbaro 1999). Also, similar to *Atractoscion nobilis* (Aalbers & Drawbridge 2008), *Sciaenops ocellatus* was observed spawning in groups of one female and three males (Guest and Lasswell 1978).

Holt 2008 recorded sounds made by *Sciaenops ocellatus*. The general call rate was mostly less than 16 calls/min. It was possible to distinguish two types of sounds: one was emitted by a group of fishes while the other was made by one specimen alone or in a really small cluster of fish. (Holt 2008). Sound production was characterized by a diel pattern of emission, i.e. sounds were more numerous in the evening, from one hour before to three hours after the sunset during the spawning season (Holt 2008). In a captive setting, Parmentier et al. 2014 observed sound production at the end of the spawning period. The calling activity peaked during morning hours. Also, sounds were produced in significantly higher numbers in tanks with natural photoperiod than during delayed photoperiod (i.e. fish out of reproductive season) (Parmentier et al. 2014).

Guest & Lasswell (1978) and Holt (2008) found an important difference in the fundamental frequency (**Table 1**). This variation may be explained by the size of the fish. Moreover, knowing that over 20 years have passed between the studies, improvements made on recording devices could potentially be an impacting factor in explaining this difference.

Table 1 : Comparison of the fundamental frequency found in three different studies.

	Guest & Lasswell 1978	**Holt2008**
Fundamental frequency (Hz)	240 - 1000	140 - 160

Montie et al. 2016 continued the research in this area by transferring groups of wild *Sciaenops ocellatus* (three females and two males) into four fiberglass rearing tanks where different parameters could be adjusted in order to reproduce a complete reproductive period. The aim of the study was to investigate if a relationship can be found between the amount of collected eggs or other spawning features and the calling rate or other fine parameters of the produced sounds.

Montie et al. 2016 found that the temperature and photoperiod had an effect on the sound production. In fact, callings peaked at 25 °C with a photoperiod ranging from 12.5 to 13.0 hours of light, which corresponds to the natural parameters found in autumn during the spawning season. Daily recordings showed that the majority of the sound was found between half an hour before to one hour after sunset. Also, if no calling happened, no spawning occurred. In addition, days with more, longer and louder calls where associated with more productive spawnings. They concluded that a positive correlation between spawning success and sound production exists and that calls longer than 0.8s and containing more than seven pulses are most likely to have an implication in spawning (Montie et al. 2016).

B. Objectives

The aims of this thesis were to further investigate the relationship between call rate and fine acoustic features with reproductive stage and spawning success in three Sciaenidae species, *Argyrosomus regius*, *Umbrina cirrosa* and *Sciaenops ocellatus* hosted in rearing facilities.

Therefore, sounds were recorded in different experimental phases for each species. Call rate, duration of the sound, number of pulses, pulse period and frequency were inspected and related to the number of eggs collected and the fertilization success obtained. Also, sonic and epaxial muscle samples of *Argyrosomus regius* and *Sciaenops ocellatus* were taken, fixed and stained. They were visualized under an optical microscope and the fiber sizes were compared between phases.

Material and methods

1. *Argyrosomus regius* and *Umbrina cirrosa*; acoustic recordings at Hellenic Centre for Marine Research – Crete (Greece)

The meagre (*Argyrosomus regius)* and shi drum (*Umbrina cirrosa)* specimens observed during this study were breeding stocks hosted at the Research facility of the Institute of Marine Biology, Biotechnology and Aquaculture at the Hellenic Center for Marine Research (HCMR Crete, Greece). There, 17 sexually mature, 5-years-old *Argyrosomus regius*, originating from HCMR Souda, and 5 sexually mature, 15-years-old *Umbrina cirrosa*, originating from the Department of Fisheries and Marine Research (TATHE) in Cyprus were kept in two 15 m^3 cement tanks filled with seawater (meagre tank: 1.95m x 3.32m x 2.45m; shi drum tank: 1.96 m x 3.30 m x 2.4m). Sex ratio was 11 females and 6 males for the meagre and 3 females and 2 males for the shi drum.

The acoustic activity of both species was monitored by using underwater acoustic dataloggers (SNAP, hydrophone sensitivity; −170 dB re 1 V/µPa, Loggerhead Instruments, FL, USA) deployed in the center of each tank. The SNAP records .wav files at 44100 Hz, 16 bits. The two SNAPs were set for recording 300 seconds every 900, meaning that 3 recordings lasting 5 minutes each were taken per hour (total of 3765 min of recordings for *A. regius* and 3730 for *U. cirrosa*). The SNAPs recorded with this schedule for a total of 11 days over a 24-hour cycle. Acoustic recordings were interrupted only shortly in the morning (less than 1 hour) in order to retrieve the dataloggers for storing the data. As Sciaenidae are known to call around sunset hours (Montie 2016), airlifts, pumps and filtration were shut off one hour before to three hours after sunset for further reducing of the signal to noise ratio (SNR).

In both species, sound production was studied during three different phases (t0, t1, t2), which vary with temperature, photoperiod and hormonal conditions (**Table 2**). A first recording session was carried out from March 5 to 9 2018 (t0), when water temperature was below the one characterizing the reproductive season, the photoperiod was that of March, i.e. less hours of light than during the reproductive period and fish were not in spawning conditions. A second recording session was carried out from May 3 to 7 2018 (t1), when water temperature and photoperiod were the one of the reproductive season, a few days before the hormonal induction which occurred on May 7. Meagre were not able to spawn spontaneously whereas shi drum naturally released very few unfertilized eggs, i.e. less than 1000, before the hormonal treatment. Recordings collected from May 7 to 10 (t2) were considered as part of

t2. During this phase, following hormonal induction (GnRHa), the meagre spawned three times and the shi drum twice.

Table 2 : Water temperature and hormonal condition in both species during the different phases (t0, t1, t2).

	T0		T1		T2	
	T°	**hormones**	**T°**	**Experimental state**	**T°**	**hormones**
Argyrosomus regius	16.2 C°	Out of reproduction season	20 C°	Few days before hormonal induction	20C°	After hormonal induction
Umbrina cirrosa	19.3 C°	Out of reproduction season	22C°	Few days before hormonal induction	22C°	After hormonal induction

The photoperiod was adjusted to natural photoperiod.

Hormonal induction protocol

On Monday the 7th May 2018, water level was lowered in both thanks until reaching a water depth of 50 cm. Once the water level was reached, 0.01% of clove oil was added per liter of seawater. The purpose of the clove oil was to mildly anesthetize the fish. Afterwards, a member of the research facility went inside the tank and started collecting the fish one at the time. The fish was then immersed in a smaller tank with a higher concentration of clove oil (0.03%) in order for it to be completely anesthetized.

As *Umbrina cirrosa* had already been weighted on March 1st, it was not done again for this species while *Argyrosomus regius* were weighted one fish at the time. A biopsy was realized on the females of both species to check for the oocyte's stage of development. Semen samples of the males were collected to evaluate the spermation condition. By putting pressure on the side of the fish, the maturation of the male was evaluated. The stages went from s0 to s3, where s0 indicated a non-mature individual. The s3 stage was the most advanced stage of maturity and could be detected when sperm I was ejected as soon as the side of the fish was gently pressed. Depending on the sex of the fish, an implant or injection was used for the hormonal induction. Males were provided with an implant containing 350 µg of GnRHa while females got an injection. The dose for the injection was calculated in function of the weight of the fish by injecting 15 µg of GnRHa for each kg of weight (**Table 3**).

After the hormonal induction, fish were placed back in the tanks, which in the meantime had been emptied from the clove oil water, washed and fully refilled with fresh seawater.

For *Argyrosomus regius*, one treated male and female were put in a separate tank and were euthanized on 11/05/2018 in order to obtain sonic muscle at the end of t2. Another three individuals had already been euthanized before for collecting sonic muscle samples before spawning induction (t1). All three individuals were females. The samples were placed in tubes with a fixation solution containing 4% formaldehyde + 1% glutaraldehyde.

Table 3 : Table showing the sex, weight, date of the weight, spermation stage, oocyte diameter, the injected or implanted dosis of GnRHa and the histology samples.

Species	Individual	Sex	Weight (g)	Date of weight	Spermiation stage	Oocyte diameter (µm)	Treatment (µg)	Histology
Umbrina cirrosa	1	M	4400	01/03/2018	S2		imp 350	
	2	M	4300	01/03/2018	S3		imp 350	
	3	F	4700	01/03/2018		550	inj 380	
	4	F	4500	01/03/2018		550	inj 320	
	5	F	4000	01/03/2018		500	inj 350	
Argyrosomus regius	1	F	4800	07/05/2018		600	inj 300	
	2	F	5000	07/05/2018			inj 300	
	3	F	11300	07/05/2018		450	inj 600	
	4	F	4736	07/05/2018				Killed t1 (sample1)
	5	F	4575	07/05/2018				Killed t1 (sample2)
	6	M	5450	07/05/2018	S3		imp 350	
	7	F	5700	07/05/2018				
	8	F	6000	07/05/2018		550	inj 300	
	9	F	6000	07/05/2018		500	inj 300	
	10	F	8000	07/05/2018		500	inj 450	
	11	F	6400	07/05/2018		500	inj 300	
	12	M	3800	07/05/2018			imp 350	Killed t2 (sample4)
	13	F	5400	07/05/2018		500	inj 300	Killed t2 (sample5)
	14	M	6700	07/05/2018	S2		imp 350	
	15	M	5750	07/05/2018	S3		imp 350	
	16	M	6400	07/05/2018	S2		imp 350	

	17	M	5900	07/05/2018	S2		imp 350	

In addition, older *Argyrosomus regius* (13 years old) hosted in a different tank, (**Table 4**) were hormonally induced on 09/05/2018 during t2 following the same protocol. Their loud sound production (which could clearly be detected while walking in the facility at night) was recorded by using an omnidirectional hydrophone Aquarian H2a (sensitivity −180 dB re 1 V/Pa; frequency response 10 Hz–100 kHz) connected to a ZoomH1 Handy recorder (sampling rate 44.1 kHz, 24-bit) operating on batteries and recording .wav files. Recordings were collected from 21:48 to 23:59 on 10/05/2018 and from 00:00 to 08:15 on 11/05/2018 by lowering the hydrophone at mid water from one tank wall. The call rate and fine parameters were analyzed and compared with those obtained in t2 for the younger meagre individuals.

The older individuals spawned during the night of the 10/05/2018. The next morning 2 907 000 eggs with a 95 % of fertilization success were found in the egg collector.

Table 4 : Table showing the sex, weight, spermation stage, oocyte diameter and the injected or implanted dosis of GnRHa.

Species	Individual	Sex	Weight (g)	Spermiation stage	Oocyte diameter (µm)	Treatment (µg)
Argyrosomus regius	1	M	16200	S3		imp 900
	2	F	14800		550	inj 500
	3	M	13900	S3		imp 900
	4	F	15800		570	inj 550
	5	F	12800		550	inj 450
	6	M	/	/		imp 900

Spawning data

After completing the spawning induction in both tanks, the egg collectors were verified each morning for potential spawns and ca. every hour during spawning nights. For the meagre, spawning events occurred in the evenings from the 08/05/2018 until the 10/05/2018. Eggs were collected in the morning and were counted under the microscope in order to estimate the number of fertilized eggs for each spawning event. The night of the 08/05/2018 two spawning events occurred. One was estimated around 22:00 to 23:00 and the other one around 00:30 to 01:00 thanks to the development stages of the eggs. The next morning a sum of 62 000 eggs were counted with a fertilization success of 48%. The eggs released between 22:00 and 23:00

during the spawning event of the 09/05/2018 were counted the next morning: 149 000 eggs with a 61% fertilization success.

As for the shi drum, the fish spawned for the first time after the treatment on the 09/05/2018 in the evening around 18:00 to 19:00. The next morning 1.531 million eggs were collected. The fertilization rate was 22%. Another spawning event occurred the following night (10/05/2018). This spawning event counted 197.000 eggs with a fertilization rate of 48 %.

Data analysis

The .wav files (total 3765 min of recordings for meagre and total 3730 min of recordings for the shi drum) were analyzed by visual and aural inspection by using Raven 1.5 for Windows (Bioacoustic Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA).

Spectrograms were visualized by using a Hanning window, 2933 FFT. The number of calls was manually counted in each minute of recording; call rate was defined as the number of sounds per minute divided by the number of males. However, the call rate obtained should be taken as an approximation that does not reflect individual call rate (as it might vary significantly between speciemens), and does not account for the possibility that also females produce sounds. Nevertheless, it permitted to reduce the bias given by the different number of *A. regius* individuals recorded in each phase (due to the removal of specimens at the end of t1 for histological samples). Call rate has been calculated for both species for every hour of recording. For each species, and for each day of recording, during the hour of vocal activity peak, a sample of at least 26 sounds was taken in order to characterize the acoustic features of 26 calls. When possible, the sample taken was even bigger to ensure a better confidence level (total number of sounds analyzed: 995 sounds for younger meagre, 30 sounds for older meagre and 91 sounds for the shi drum).

The following sound features were measured: sound duration (DUR; ms), number of pulses (NP), pulse period (PP; ms) and dominant frequency PF; (Hz). The duration of the sound was the time from the onset of the first pulse to the offset of the last pulse. The number of pulses was obtained manually by counting the number of pulses in each sound. Also, the number of pulses was confirmed by adding one to the number of pulse periods within each sound. Pulse period was the time interval between the peaks of two consecutive pulses in a sound, and was measured for all the pulses succession within a sound. The dominant frequency was measured from the power spectrum as the frequency with the highest energy.

Statistical analysis

The statistical analysis was obtained thanks to the following two programs: Microsoft Excel and PAST statistics 3. The Shapiro-Wilk test was used to determine if the data was parametric. Furthermore, tests like Kruskal-Wallis and Mann-Whitney were made to compare the data of each parameter (call rate, DUR, NP, PP, PF) in the different phases (t0, t1, t2). The experimental phase t2 was subdivided in $t2_{(1)}$ and $t2_{(2)}$ in correspondence to the two spawning events. The different statistical tests were visualized with boxplots.

To examine the relationship between sound production and reproduction, the number of eggs collected and the fertilization success were plotted to the call rate or the fine parameters measured. The aim was to see if some relationship between sound production (increase in call rate, change in duration, number of pulses, pulse period and peak frequency) and spawning success could be determined.

Histology

The histological samples of *Argyrosomus regius* taken after t1 (3 females samples) and t2 (2 females and 1 male sample) were fixed with a 4% formaldehyde and 1% glutaraldehyde solution. The paraffin samples were stained with hematoxylin-eosin coloration. The sections were visualized on an optic microscope (Leica) combined to a Leica MC 170 HD camera connected to a computer. Pictures of sonic muscle and epaxial muscle fibers were taken with the program LAS EZ. The fibers' diameters and area were determined and compared on the imageJ program.

2. IFREMER facility – Martinique

Sciaenops ocellatus was recorded in two tanks with different conditions with a digital spectrogram long-term acoustic recorder (DSG; Loggerhead Instruments, Sarasota, FL, USA; -186 dB re 1 V/µPa). The first communitarian tank was $15m^3$, made of cement and contained 20 sexually mature (11 females and 9 males), 5 to 7 years old *Sciaenops ocellatus* out of reproduction season (t0). The second communitarian tank was $12.5m^3$, made of fiberglass and counted also 20 sexually mature (8 females and 12 males), 4 to 5 years old *Sciaenops ocellatus*. The temperature and photoperiod of the second tank was artificially modulated to reproduce reproductive season conditions and activate gonad maturation and spawning (t2) (**Table 5**). Unlike for the meagre and the shi drum, the red drum sounds recorded in t0 and t2 were not emitted by the same individuals, and spawning conditions were naturally induced without hormonal treatment.

Recordings of the first communitarian tank were made from 03/07/2015 to 10/08/2015, while the second tank was recorded from the 06/07/2015 to the 08/09/2015. Out of this data set, 4 and 3 consecutive days were randomly subsampled in order to measure the call rate and fine parameters in t0 and t2. The recordings of tank 1 were analyzed from 21 to 25 July 2015. For the reproduction season t2, the analysis was made from 6 to 9 July. The data analysis and statistics (led on 129 sounds) used on these data are the same as those applied for *Argyrosomus regius* and *Umbrina cirrosa*.

Spawning data

Sciaenops ocellatus spawned the 09/07/2015 around 23:00. The next morning 198 000 eggs were collected with 88.9% fertilization success.

Table 5 : Water temperature and hormonal condition in *Sciaenops ocellatus* during the different phases t0 an t2.

	T0		T2	
	T°	**Experimental state**	**T°**	**Experiental state**
Sciaenops ocellatus	28.6 C°	Out of reproduction season	30 C°	Photoperiod and temperature induced reproduction season

Histology

The histological samples of *Sciaenops ocellatus* taken at different ontogenic stages were fixed with a glutaraldehyde solution and placed in a cacodylate buffer. The sections were stained with toluidine blue coloration. They were visualized on an optic microscope (Leica) combined to a Leica MC 170 HD camera connected to a computer. Pictures of sonic muscle and epaxial muscle fibers were taken with the program LAS EZ. The fibers' diameters and area were determined and compared on the imageJ program.

Results

1. Sound production of *Argyrosomus regius*

Argyrosomus regius emitted pulsed sounds (i.e. acoustic energy peaks emitted in close repetition, where each peak corresponds to a muscle contraction) for all the duration of this study and in all observed phases (t0, t1, t2). Lagardère et al. (2006) reported the presence of two sound types in *A. regius*: short and long grunts. Short grunts are pulsed sounds ranging from 4 to 6 pulses while long grunts are characterized by series of at least 30 pulses. However, this study highlights the range of emitted pulses is higher than first expected. Three sound types could be identified at a qualitative level. They included the short and long grunts described by Lagardère et al. (2006) (**Appendix 3, a, b and c respectively**) and a third sound type recorded only during t2, which we called "weak grunt" (**Appendix 4**).

However, the overlapping in the features was very difficult to discriminate between these sound types because the division "short" and "long" drums proposed by Lagardère et al. (2006) excludes all sounds ranging from 7 to 29 pulses, which were very abundant in our study. This suggests that the sound types chosen by Lagardère et al. (2006) shall be revised. Considering that our experimental hypothesis was to investigate if sound features change depending on the experimental phase (t0, t1 and t2) and that in Sciaenidae species the temporal parameters are indicative of muscle contraction dynamics, in all the statistical analysis carried out hereafter (i.e. call rate and sound features) all sound types emitted by *A. regius* were pooled together.

1.1. Call rate of *Argyrosomus regius*

Call rate has been calculated for each hour of each day of recording for t0, t1 and t2 (**Appendix 5, 6, 7**). The average call rate diel pattern of each phase (t0, t1 and t2) can respectively be visualized separately in **Appendix 8, 9 and 10**.

The call rate diel pattern of *Argyrosomus regius* (**Figure 3, Table 6**) has been realized for all three phases t0 (N=96), t1 (N=96) and t2 (N=72).

The average call rate during t0 (four days of recordings) was 0.091 ± 0.161 sounds per minute (N=96) (**Figure 3)**. A progressive increase of the number of calls was observed in the afternoon until reaching a peak of 0.250 ± 0.285 sounds per minute at 17:00. Afterwards, the number of calls decreased and became very low from 22:00 to 05:00. Then, the call rate increased again and reached a peak of 0.375 ± 0.398 at 08:00 in the morning. This morning

peak was more intense than the afternoon peak. As the calling activity averages four days of recordings, an important standard deviation can be noticed due to a big variability between days (**Appendix 8, 9 and 10**). The coefficient of variation for t0 was measured in order to quantify the dispersion of the data (CV_{t0} = 177.66 %)**.**

As in t0, an important gap in the calling activity can be noticed during t1 from 22:00 to 05:00 (**Figure 3**). During the rest of the day calling activity was more homogenous than for t0, where a clear call peak could not be found. Nevertheless, the maximum sound production for this phase occurred between 13:00 (0.358 ± 0.237 sounds per minute) and 14:00 (13:00 and 0.358 ± 0.298 sounds per minute). The average number of calls for t1 was 0.170 ± 0.213 sounds per minute (N=96). The coefficient of variation CV t1 = 125.42%.

During t2 (**Figure 3)**, the diel call activity showed remarkable differences as compared to the previous phases (t0 or t1). In fact, the sound production in the afternoon and evening (12:00 to 22:00) was less important and a remarkable increase occurred during the night up to the morning (23:00 to 11:00). So, while out of reproduction (t0 and t1) the calling activity occurred during daytime, sound production shifted to nighttime hours during spawning. The call rate for the whole phase was 0.545 ± 0.644 (N=72). The coefficient of variation CV $_{t2}$ = 118.07%.

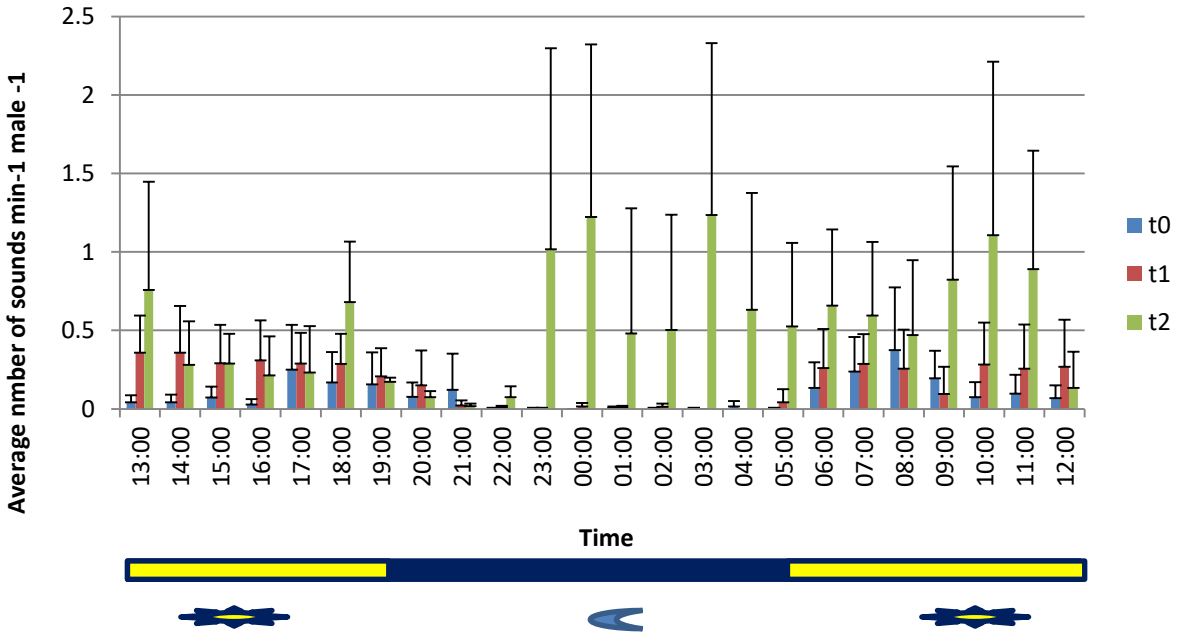


Figure 3: Average call rate per minute per male in *Argyrosomus regius* in different phases (t0, t1, t2).

In order to compare the call rate within t0, t1 t2 and the variation between phases, the average number of calls is shown in **Table 6**.

Table 6: The average call rate ± SD of *Argyrosomus regius* for each phase (t0, t1, t2) and the interphase. The coefficient of variation was calculated for each phase and expressed in percentage.

	Average call rate (min^{-1} $male^{-1}$)	SD (min^{-1} $male^{-1}$)	CV (%)
T0 (N=96)	0.091	0.161	177.66
T1 (N=96)	0.170	0.213	125.42
T2 (N=72)	0.545	0.644	118.07

The coefficient of variation was compared between phases (t0, t1 and t2) to determine the evolution of the call rate. The variability of the call rate observed outside in contrast to during the reproduction season is more widespread than the variability of the call rate within each phase (i.e. variability occurring due to differences between individuals). These results support the hypothesis that the change in variability can be attributed to the change in the experimental phase (reproductive phase) and in physiological characteristics of the sonic muscles (increase in fiber size and sarcoplasmic reticulum). This suggests an increase in contraction force and contraction rate of the sonic muscles during the reproductive season.

The normality test showed a non-parametric distribution of the call rate in all phases (t0, t1, t2). The Kruskal-Wallis test (N = 264 and H = 41.0128) was significant ($p<0.05$) and demonstrated that call rate (CR) changed significantly between experimental phases (**Figure 4a**). A Mann-Whithney test was added to compare the call rate of the three phases, pair by pair. The call rate of t1 was significantly higher than the call rate of t0 (Mann-Whithney, N = 96 ; 96, U = 3576, $p<0.05$). The call rate of t2 was significantly higher than the call rate of t1 (Mann-Whithney, N = 96 ; 72, U = 2125, $p<0.05$) and t0 (Mann-Whithney, N = 96 ; 72, U = 1532, $p<0.05$).

The call rate during the two spawning nights during t2 (**Figure 4b**) did not show significant difference (Mann-Whithney, N = 24 ; 24, U = 266.5, $p>0.05$) while the number of eggs were 62 000 against 149 000.

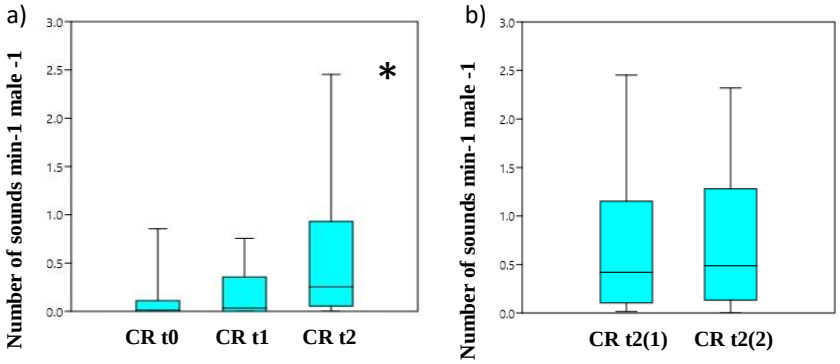


Figure 4: Boxplot showing the distribution around the mean of the call rate of Argyrosomus regius a) in each phase (t0, t1, t2) b) in t2(1) and t2(2).

1.2. Fine acoustic features variation of *Argyrosomus regius* pulsed sounds.

Comparison of each acoustic feature (sound DUR, NP, PP and Fr) shows that *A. regius* sounds differed between phases in all recorded parameters (Kruskal- Wallis, $p < 0.05$) (**Figure 5**).

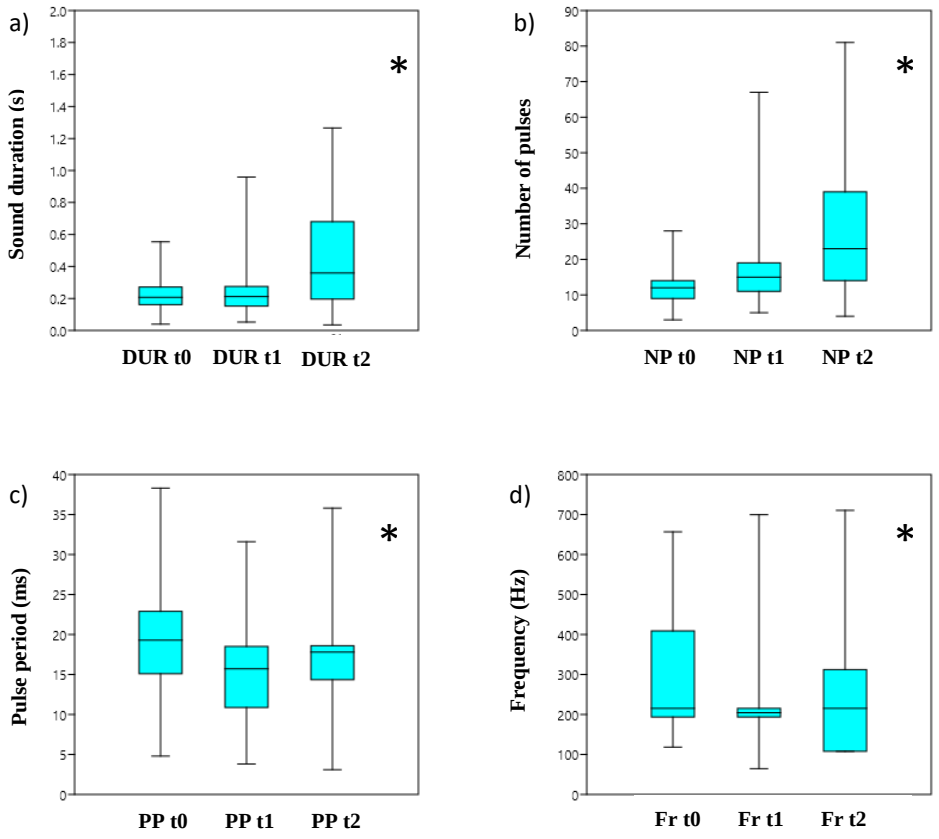


Figure 5: Boxplot showing the distribution around the mean of the fine parameters a) duration, b) number of pulses, c) pulse period and d) Frequency. Kruskal-Wallis tests were performed for each parameter. a) DUR : N = 704, 1091, 510, H = 224.455. b) NP : N = 670, 905, 511, H = 407.095. c) PP : N = 1182, 1182, 1182, H = 400.376. d) Fr : N = 149, 202, 161, H = 20.564.

The sound features of *A. regius* during t0 and t1 were compared (**Appendix 11**), when the only difference in recording conditions was water temperature (T_{t0} = 16.2°C and T_{t1} = 20°C).

The duration of sounds in t1 was not significantly different than the duration of sounds in t0 (Mann-Whithney, N = 704 ; 1091, U = 3.83E05, p >0.05), number of pulses was shorter in ???(Mann-Whithney, N = 670 ; 905, U =1.9836E05, p <0.05), pulse period was significantly shorter in t1 (Mann-Whithney, N = 1482 ; 1182, U = 4.8612E05, p <0.05) and the dominant frequency was lower in t1 (Mann-Whithney, N = 149 ; 202, U = 11 050, p <0.05).

Comparison of sound features between t1 and t2 (**Appendix 12**) reflects differences in the hormonal treatment (t2= spawning induction).

Sounds recorded in t2 (*i.e.* during spawning) were significantly longer than sounds recorded during t1 (Mann-Whithney, N = 1091 ; 510, U = 1.58E05, p <0.05), and were characterized by a significantly higher number of pulses (Mann-Whithney, N = 905 ; 511, U =1.323E05, p <0.05). The pulse period of sounds recorded in t2 was significantly longer than the one of sounds recorded in t1 (Mann-Whithney, N = 1182 ; 4369, U = 2.09E06, p <0.05). The peak frequency was not found to change between phases (Mann-Whithney, N = 202 ; 161, U = 15 246, p >0.05).

Comparison of sound features between t0 and t2 (**Appendix 13**) reflects differences in temperature and hormonal treatment (t2= spawning induction).

The reproductive period t2 was separated into two: $t2_{(1)}$ and $t2_{(2)}$. These two groups correspond to spawning day 1 and spawning day 2 of t2. Below, the data of $t2_{(1)}$ to the data of $t2_{(2)}$ are compared (**Figure 6**).

Sounds emitted during $t2_{(2)}$ were significantly shorter (Mann-Whithney, N = 200 ; 99, U = 7 792.5, p <0.05), showed a higher number of pulses (Mann-Whithney, N = 201 ; 310, U = 27 517, p <0.05) and shorter pulses period (Mann-Whithney, N = 2498 ; 1871, U = 2.0902E06, p <0.05) than sound emitted during $t2_{(1)}$. Sound peak frequency did not change significantly between phases (Mann-Whithney, N = 132 ; 97, U = 5 863, p >0.05).

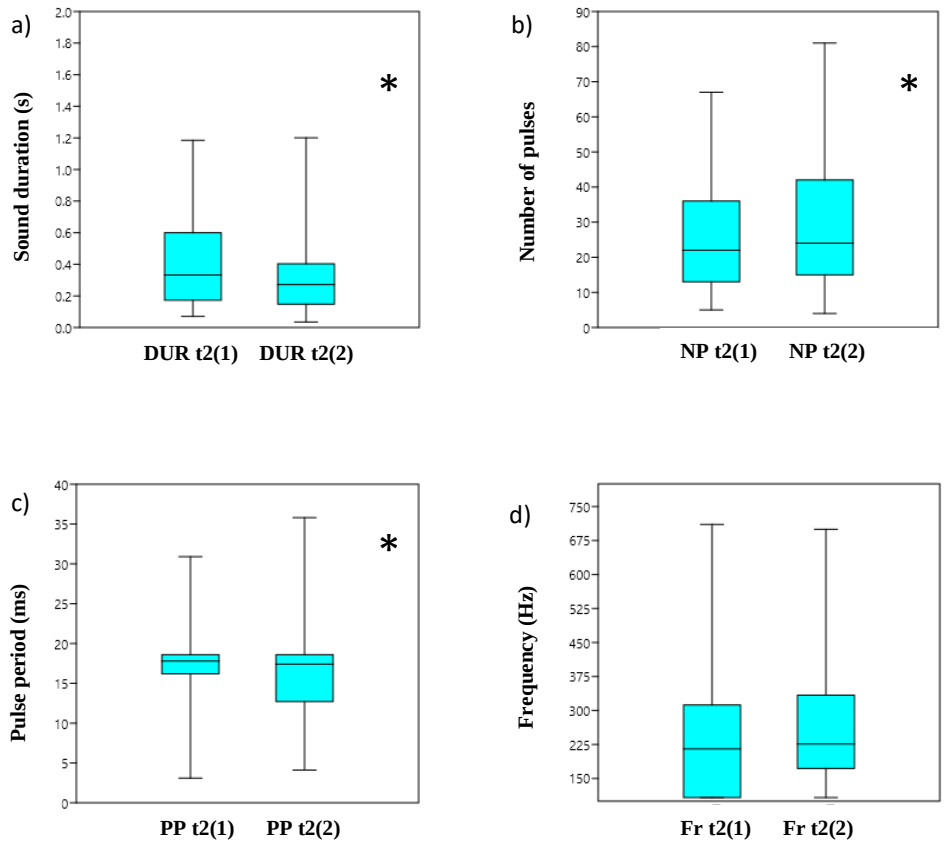


Figure 6: Boxplot showing the distribution around the mean of the fine parameters a) duration, b) number of pulses, c) pulse period and d) frequency between t2(1) and t2(2). Mann-Whithney tests were performed for each parameter. a) DUR : N = 200 ; 99, U = 7 792. b) NP : N = 201 ; 310, U = 27 517. c) PP : N = 2498 ; 1871, U = 2.0902E06. d) Fr : N = 132 ; 97, U = 5 863.

1.3. Sound production in older *A. regius*; call rate and fine acoustic feature variations.

Thirteen years old *Argyrosomus regius* (N= 6) emitted one sound type (**Appendix 14**). The calls were characterized by an average of 48 ± 25 pulses (N=30), repeated every 20 ± 2 ms (N=30) and with an average peak frequency of 183 ± 57 Hz (N=30).

The call rate between 21:00 to 08:00 of the group of thirteen-years-old specimen is shown in **Appendix 15**. The average call rate during t2 was 1.602 ± 1.353 sounds per minute (N=12) (**Figure 7**). The sound production peaked at 03:00 in the morning with 3.757 ± 0.049 sounds per minute. Afterwards, calling activity decreased drastically between 04:00 and 07:00 before reaching 0 sound per minute at 08:00. The coefficient of variation for t2 was measured in order to quantify the dispersion of the data (CV_{t2} = 84.45 %).

The call rate of five years and thirteen years old *Argyrosomus regius* during the experimental phase t2 were compared (**Table 7)**.

Table 7: The average call rate ± SD of *Argyrosomus regius* for different ages (5 and 13) during t2. The coefficient of variation has been calculated for each group of fish and expressed in percentage.

	Mean call rate (min^{-1} $male^{-1}$)	SD (min^{-1} $male^{-1}$)	CV (%)
T2 (5) (N=12)	0.619	0.384	62.08
T2 (13) (N=12)	1.602	1.353	84.45

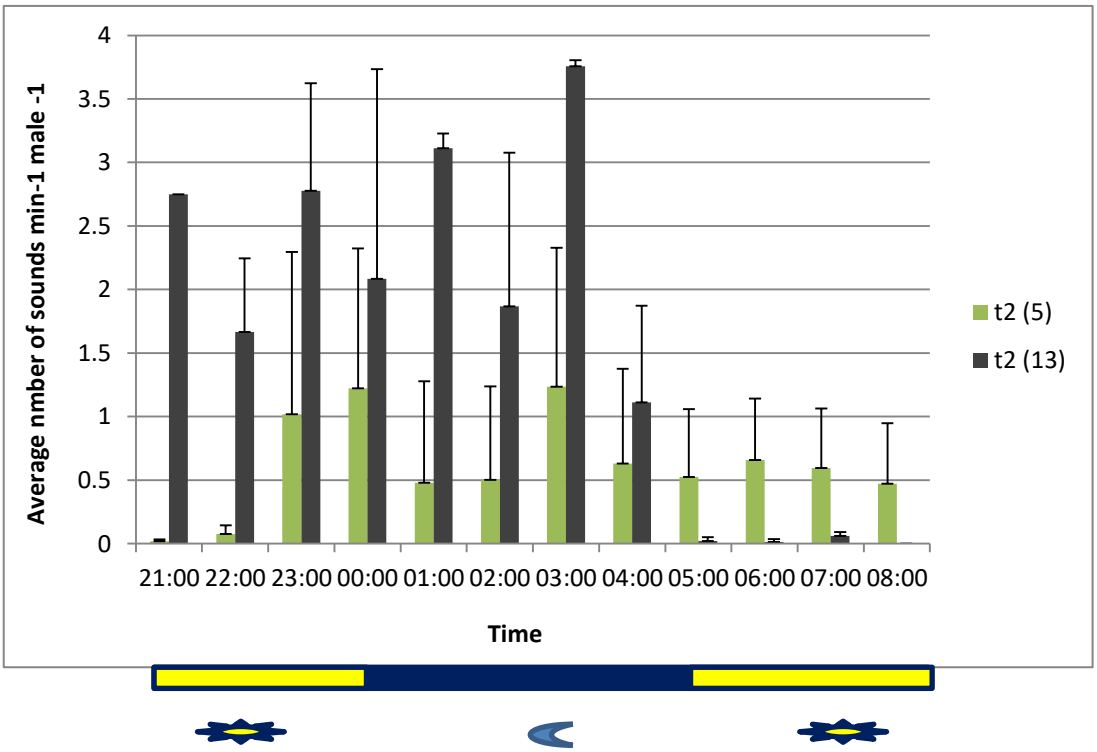


Figure 7: Average call rate per minute per male for the 5-years-old and 13-years-old *Argyrosomus regius* fish in t2 named respectively t2 (5) and t2 (13).

The normality test showed a non-parametric distribution of the call rate for both age groups. The call rate (**Figure 8**) of the older specimens was significantly higher than the call rate of the younger ones (Mann-Whithney, N = 72 ; 12, U = 263.5, $p<0.05$).

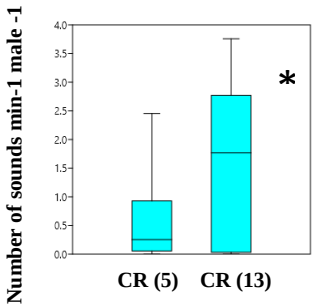


Figure 8: Boxplot showing the distribution around the mean of the call rate of Argyrosomus regius in t2 depending on the age of the fish (5 and 13 years).

A. regius sound features differed with age in three (DUR, NP, PP) recorded parameters (Mann-Whithney, p <0.05) (**Figure 9**).

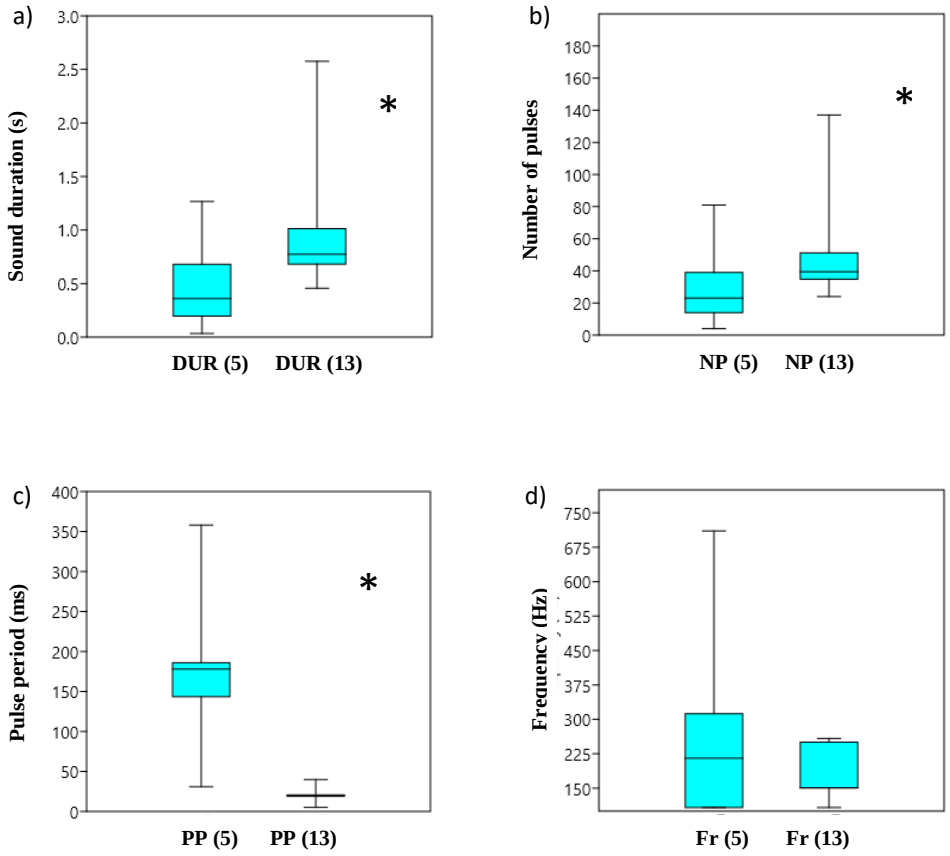


Figure 9: Boxplot showing the distribution around the mean of the fine parameters a) duration, b) number of pulses, c) pulse period and d) frequency. Mann-Whithney test were performed for each parameter. a) DUR : N = 510 ; 30, U = 2394.5. b) NP : N = 510 ; 30, U = 3269.5. c) PP : N = 4369 ; 1404, U = 3.5. d) Fr : N = 161 ; 30, U = 1950.5.

The sounds made by 13-year-old fish were significantly longer (Mann-Whithney, N = 510 ; 30, U = 2394.5, p <0.05), with a higher number of pulses (Mann-Whithney, N = 510 ; 30, U = 3269.5, p <0.05), where pulses were more quickly repeated than in younger males (Mann-Whithney, N = 4369 ; 1404, U = 3.5, p <0.05). There was no significant difference between the frequency of sound made by younger or older specimens (Mann-Whithney, N = 161 ; 30, U = 1950.5, p >0.05).

1.4. Spawning data of *Argyrosomus regius*

After the hormonal induction of reproduction, several spawning events occurred (**Table 8)**.

Table 8: Summary of the information regarding the spawning data of *Argyrosomus regius*.

Spawning name	Date	Hour of spawning	Number of eggs	Fertilization success
Spawning 1	08/05/2018	22:00 – 23:00	62 000	48 %
Spawning 2	09/05/2018	00:30 – 01:00		
Spawning 3	09/05/2018	22:00 – 23:00	149 000	61 %

During the reproductive season, *A. regius* sound production is significantly more important than out of reproduction. Nevertheless, in the reproductive period, by comparing the approximate hours of spawning with the calling activity, the sound production seemed to decrease during spawning hours which might suggest a decrease or even ceasing of sound production during the gamete release.

During the spawning hours, sounds of the spawning 3 (t2(2)) had quicker more repeated pulses than sounds of the spawning 1+2 (t2(1)) which suggests that sounds with quicker more repeated pulses might be related to spawning with more eggs with higher fertilization success.

1.5. Histology

Histology cross sections of epaxial and sonic muscles (**Figure 10**) were done in three females before reproduction (t1) and in one female and one male during reproduction (t2). Area and diameter of the fibers were compared (**Table 9**) to observe if histological changes occurred between phases.

Table 9: Table showing the area and the related diameter of epaxial and sonic muscle fibers (N=20) of Argyrosomus regius non-reproductive female, reproductive female and reproductive male.

	Female t1 epaxial		Female t2 epaxial		Male t2 epaxial	
(N=20)	Area (µm²)	Diameter (µm)	Area (µm²)	Diameter (µm)	Area (µm²)	Diameter (µm)
Mean	4883	79	8316	103	7473	98
SD	1736	47	2995	62	2506	56
Min	2658	58	2655	58	3937	71
Max	8822	106	13909	133	11391	120
(N=20)	Female t1 sonic		Female t2 sonic		Male t2 sonic	
Mean	451	14	800	32	938	35
SD	201	16	136	13	129	13
Min	172	15	579	27	721	30
Max	1084	37	1014	36	1190	39

2. Sound production in *Umbrina cirrosa*

Umbrina cirrosa emitted pulsed sounds for all the duration of this study and in all observed phases (t0, t1, t2). Lagardère & Parmentier (2014) reported the presence of 1 to 3 pulses per sound. The recorded sounds were divided into two categories: one type had a duration of 60 to 90 ms, the other type lasted from 140 to 160 ms. Picciulin et al. (2016) reported only one sound type with 1 to 11 pulses.

During this study, one sound type could be identified and included 1 to 7 pulses (**Appendix 16**). Shi drum calls were characterized by an average of 2.31 ± 1.10 pulses (N=91), repeated every 304 ± 58 ms (N=91) and with an average peak frequency of 96 ± 24 Hz (N=74).

The relative occurrence of sounds with more or less pulses changed in relation with the experimental phase (t0, t1 and t2). In order to visualize in more details the ratio with which the different number of pulses occurred, three aerograms were created showing the percentage of each number of sound pulses applying to each analyzed phase (**Figure 11**).

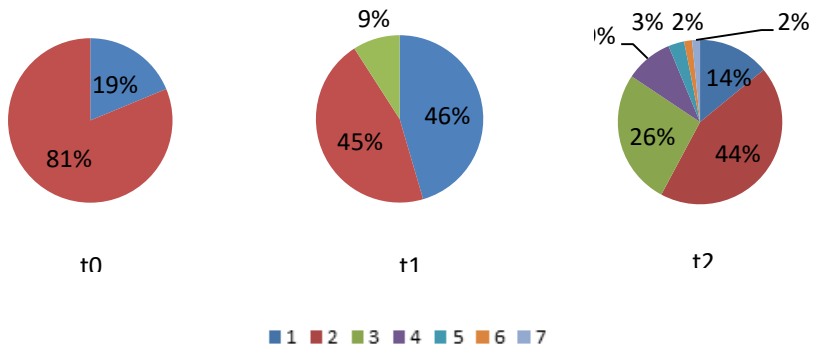


Figure 11: Distribution of the number of pulses found in Umbrina cirrosa sounds in t0 (N=13), t1 (N=11) and t2 (N=64).

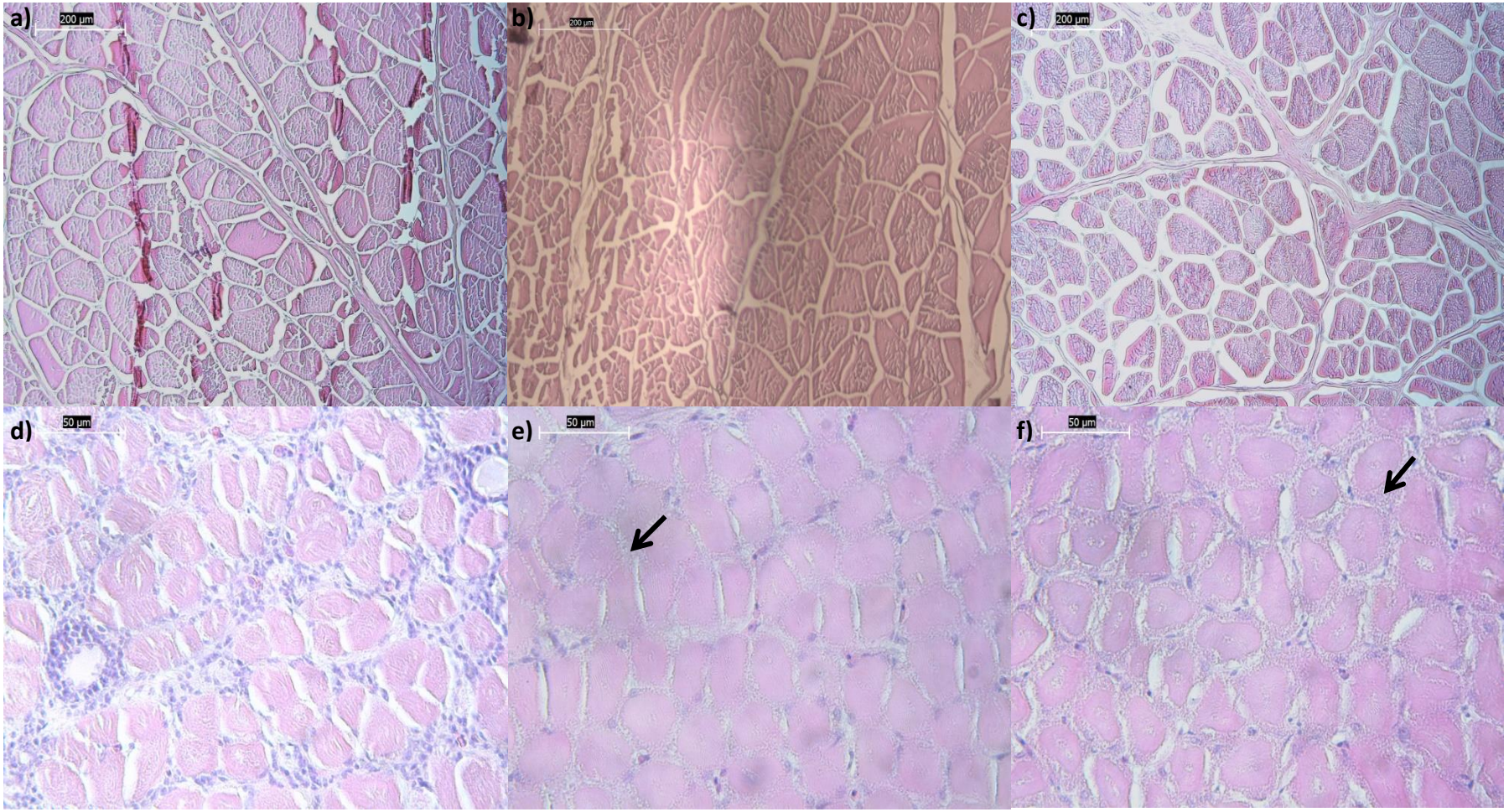


Figure 10: Semi-thin cross-sections of the epaxial (a, b and c) and sonic (d, e and f) muscles of *Argyrosomus regius*. a) and d) were respectively epaxial and sonic muscle fibers of a 4.7 kg female out of reproduction season (t1). b) and e) were respectively epaxial and sonic muscle fibers of a 5.4 kg female during reproduction season (t2). c) and f) were respectively epaxial and sonic muscle fibers of a 3.8 kg male during reproduction season (t2). The comparison between d), e) and f) sonic muscle fibers suggest an increase in sarcoplasmic reticulum volume (arrows). Scale bar : 200µm for a), b) and c) and 50µm for d), e) and f).

2.1. Call rate of *Umbrina cirrosa*

The average call rate diel pattern of each single phase (t0, t1 and t2) can respectively be visualized in **Appendix 17, 18 and 19** respectively. The call rate of *Umbrina cirrosa* for all three phases t0 (N=96), t1 (N=96) and t2 (N=72) can be found below (**Figure 12**).

The average call rate during t0 was 0.006 ± 0.025 sounds per minute (N=96); the call rate diel pattern is shown in **Figure 12**. Almost no calling activity was found during the afternoon and night. Indeed, the majority of the sound production occurred between 06:00 and 09:00. A progressive increase of the number of calls could be observed in the morning until reaching a peak of 0.050 ± 0.100 sounds per minute at 07:00. Afterwards, the number of calls decreased and became very low, reaching 0 sounds per minute at 10:00 and 11:00. Then, the call rate increased again at 12:00. As the sound production averages four days of recordings, an important standard deviation can be noticed due to a big variability in between the days. The coefficient of variation for t0 was measured in order to quantify the dispersion of the data (CV_{t0} = 449.56 %)

For t1 (**Figure 12**) the calling activity is more dispersed through the day than for t0, as in t0, no sound production occurred in the afternoon. Nevertheless, the fish vocalized during the night (22:00, 23:00 and 02:00). Afterwards, calling activity ceased until 07:00 in the morning. Like in t0, the call rate peaked at 07:00 and reached 0.042 ± 0.083 sounds per min. Vocalization decreased at 08:00 and reached 0 sound per min until increasing again but only slightly at 11:00. The average number of calls for t1 was 0.004 ± 0.021 sounds per minute (N=96). The coefficient of variation CV_{t1} = 495.88%.

During t2 (**Figure 12)**, the diel call activity showed remarkable differences as compared to the previous phases (t0 or t1). In fact, an important increase in vocalization occurred revealing two peaks could be detected. The highest peak was found at 10:00 in the morning with 0.311 ± 0.539 sounds per minute. This finding differed with the results of the call rate of t0 and t1 where the highest peak occurred at 07:00. In t2 a second peak appeared at 15:00 reaching 0.122 ± 0.212 sounds per minute. The remaining calling activity was much lower and distributed homogeneously over the rest of the day. When comparing t2 to the other two phases t0 and t1, less hours with no sound activity at all were found in t2. The average number of calls per minute and per male for the whole phase was 0.029 ± 0.119 (N=72). The coefficient of variation CV_{t2} = 406.87%.

To visualize the call rate between t0, t1 and t2, the average number of calls is shown in **Table 10**.

Table 10: The average call rate ± SD of *Umbrina cirrosa* for each phase (t0, t1, t2). The coefficient of variation was calculated for each phase and expressed in percentage.

	Mean call rate (min^{-1} $male^{-1}$)	SD (min^{-1} $male^{-1}$)	CV (%)
T0 (N=96)	0.006	0.025	449.56
T1 (N=96)	0.004	0.021	495.88
T2 (N=72)	0.029	0.119	406.87

The coefficient of variation was compared between phases (t0, t1 and t2) to determine the evolution of the call rate. For all three phases the variability within phases was lower than the variability between phases which means that there is a more important variability in sound production between the experimental phases than between individuals. These results support the hypothesis that the change in variability can be attributed to the change in the experimental phase (reproductive phase).

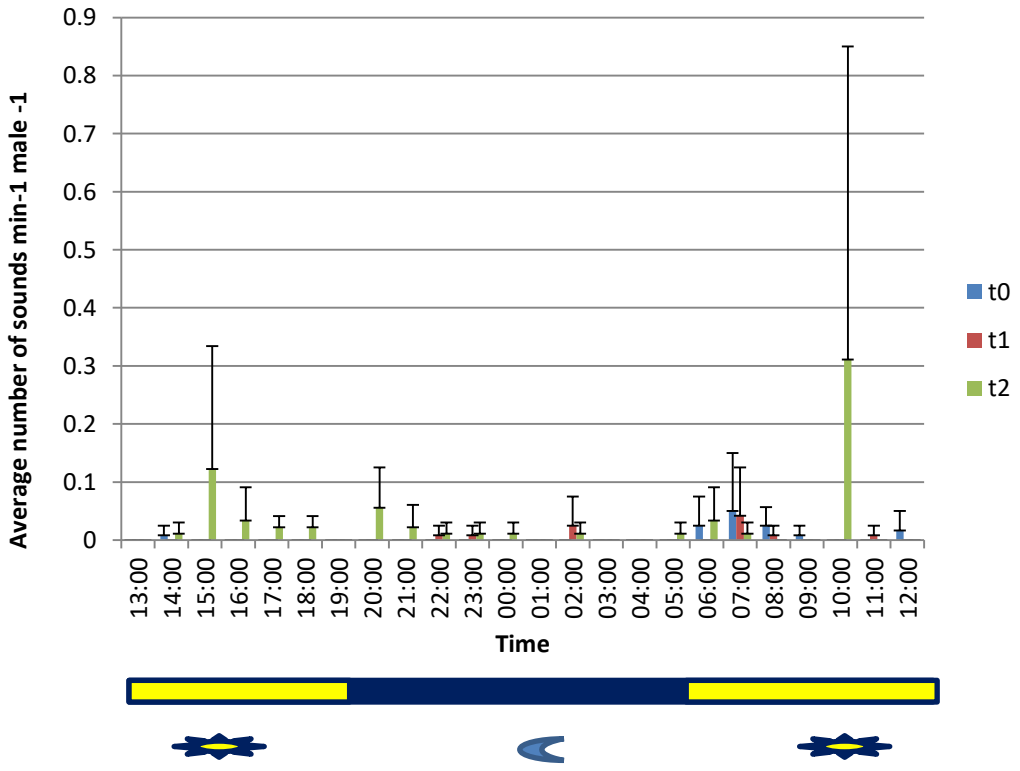


Figure 12: Average call rate per minute per male in different phases (t0, t1, t2) in *Umbrina cirrosa*.

These findings were confirmed by statistical analysis. The normality test showed a non-parametric distribution of the call rate in all phases (t0, t1, t2). The Kruskal-Wallis test (N = 96, 96, 72 and H = 5.008) was not significant ($p>0.05$). Nevertheless, a Mann-Whithney test was added to compare the call rate of the three phases, pair by pair. The call rate of t1 was not significantly higher than the call rate of t0 (Mann-Whithney, N = 96 ; 96, U = 4556.5, $p>0.05$). The call rate of t2 was significantly higher than the call rate of t1 (Mann-Whithney, N = 96 ; 72, U = 2784, $p<0.05$). The call rate of t2 was significantly higher than the call rate of t0 (Mann-Whithney, N = 96 ; 72, U = 2837, $p<0.05$).

2.2. Fine acoustic features variation of *Umbrina cirrosa* pulsed sounds.

Kruskal-Wallis test was performed to compare each acoustic feature measured as part of this study (DUR, NP, PP and Fr) during the three experimental phases. Results show that *U. cirrosa* sounds differed significantly between phases in two (NP and PP) recorded parameters (Kruskal-Wallis $p<0.05$) (**Figure 13**). Sounds in t2 had significantly more quickly repeated pulses than t1 and t0. A Mann-Whithney test was added to compare the fine parameters of the three phases, pair by pair. For the other two parameters (DUR and Fr), the Kruskal-Wallis test was not significant ($p > 0.05$) (**Figure 13**).

The duration, number of pulses, pulse period and frequency were plotted separately to better visualize the pair-to-pair differences. **S**ound features of *U. cirrosa* were compared during t0 and t1 (**Appendix 20**), the only difference in recording conditions was water temperature (T_{t0} = 19.3 °C and T_{t1} = 22 °C).

The sound duration in t1 was significantly shorter than the sound duration in t0 (Mann-Whithney, N = 13; 6, U = 13, $p<0.05$). As for the number of pulses, there was no significative difference between the number of pulses in t1 and t0 (Mann-Whithney, N = 16 ; 11, U = 71, $p>0.05$). The pulse period of sounds recorded in t1 was significantly shorter than the one of t0 (Mann-Whithney, N = 13 ; 7, U = 20, $p<0.05$). The frequency of the sounds recorded in t1 was not significantly different than the one of t0 (Mann-Whithney, N = 14 ; 6, U = 30, $p>0.05$).

The data of t1 was compared to the data of t2 (**Appendix 21**). The difference between these two phases resulted exclusively from hormonal treatment (t2 = spawning induction).

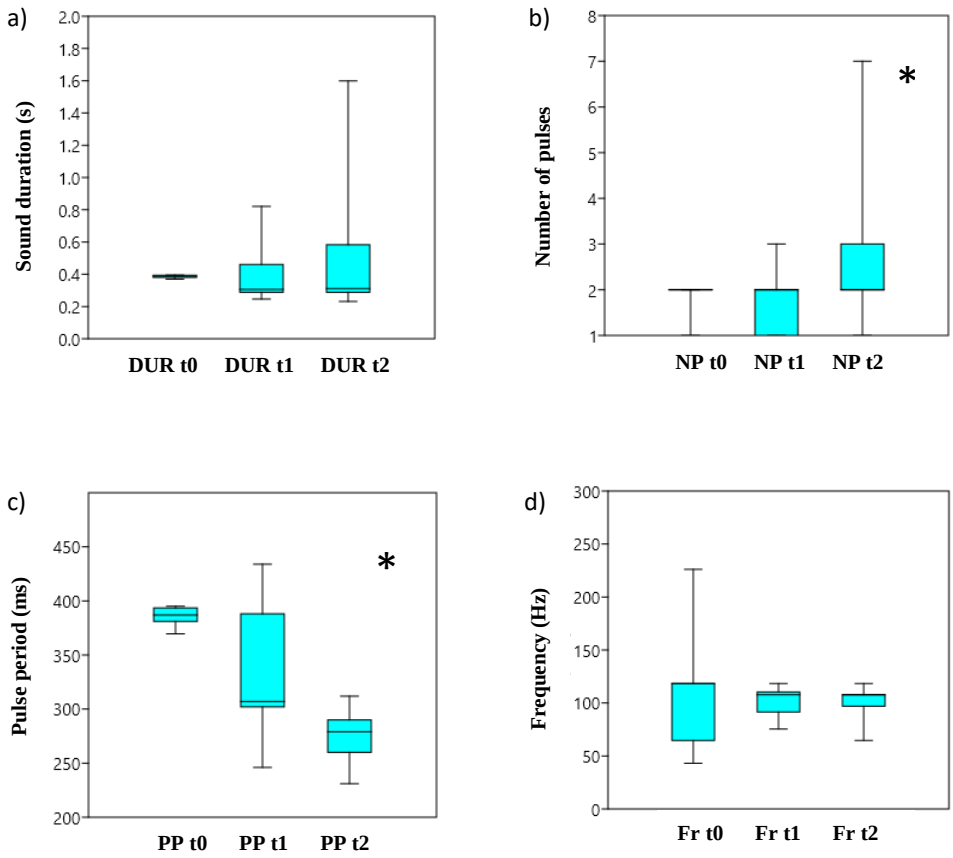


Figure 13: Boxplot showing the distribution around the mean of the fine parameters a) duration, b) number of pulses, c) pulse period and d) frequency. Kruskal-Wallis tests were performed for each parameter. a) DUR : N = 13, 6, 55, H = 0.7112. b) NP : N = 16, 11, 64, H = 10.533. c) PP : N = 13, 7, 99, H = 41.2807. d) Fr : N = 14, 6, 55, H = 5.2616.

Sounds recorded in t2 (i.e. during spawning) were not significantly longer than sounds recorded during t1 (Mann-Whithney, N = 6 ; 55, U = 152.5, p >0.05), and were characterized by a significantly higher number of pulses (Mann-Whithney, N = 11 ; 64, U = 183, p <0.05). The pulse period of sounds recorded in t2 was significantly shorter than the one of sounds recorded in t1 (Mann-Whithney, N = 7 ; 99, U = 103.5, p <0.05). The peak frequency was not found to change between phases (Mann-Whithney, N = 6 ; 55, U = 136.5, p >0.05).

Acoustic features of sounds emitted in t0 were compared to those emitted in t2 (**Appendix 22**). These two periods differed in temperature and the hormonal condition.

The duration of sounds in t2 was not significantly different than the duration of t0 (Mann-Whithney, N = 13 ; 55, U = 351, p >0.05). The number of pulses of t2 were significantly more than in t0 (Mann-Whithney, N = 16 ; 64, U = 312.5, p <0.05). The pulse period of t2 was significantly shorter than the one of t0 (Mann-Whithney, N = 13 ; 99, U = 0, p <0.05). The

frequency of t2 was significantly lower than the one of t0 (Mann-Whithney, N = 14 ; 55, U = 235, p <0.05).

2.3. Spawning data of *Umbrina cirrosa*

After the hormonal induction of reproduction, several spawning events occurred (**Table 11)**.

Table 11: Summary of the information regarding the spawning data of *Umbrina cirrosa*.

Spawning name	Date	Hour of spawning	Number of eggs	Fertilization success
Natural spawning	08/05/2018	17:00 ?	1000	0%
Spawning 1	09/05/2018	18:00	1 531 000	22 %
Spawning 2	10/05/2018	20:00	197 000	48 %

During the reproductive season, *Umbrina cirrosa* sound production is significantly more important than out of reproduction. Nevertheless, in the reproductive period, by comparing the approximate hours of spawning with the calling activity, the sound production seemed to decrease during spawning hours which might suggest a decrease or even ceasing of sound production during the gamete release.

During the spawning hours, sounds of the spawning 1 and 2 differed in number of eggs and fertilization success which might suggest changes in sound features between the two spawnings.

3. Sound production in *Sciaenops ocellatus*

Sciaenops ocellatus emitted pulsed sounds for all the duration of this study and in all observed phases (t0 and t2). Montie et al. (2016) reported the presence of 1 to 29 pulses per sound. Parmentier et al. (2014) reported calls characterized by 1 to 7 pulses in reproduction season and only 3 to 4 pulses when out of the season.

During this study, one sound type could be identified and included 2 to 19 pulses (**Appendix 23**). Red drum calls were 0.592 ± 0.198 s (N=129) long and characterized by an average of 8.74 ± 3.66 pulses (N=129), repeated every 91 ± 37 ms (N=129) and with an average peak frequency of 428 ± 286 Hz (N=129).

The relative occurrence of sounds with more or less pulses changed in relation to the experimental phase (t0 and t2). In order to visualize in more details the ratio with which the different number of pulses occurred, two aerograms were created showing the percentage of each number of sound pulses for each phase analyzed (**Figure 14**).

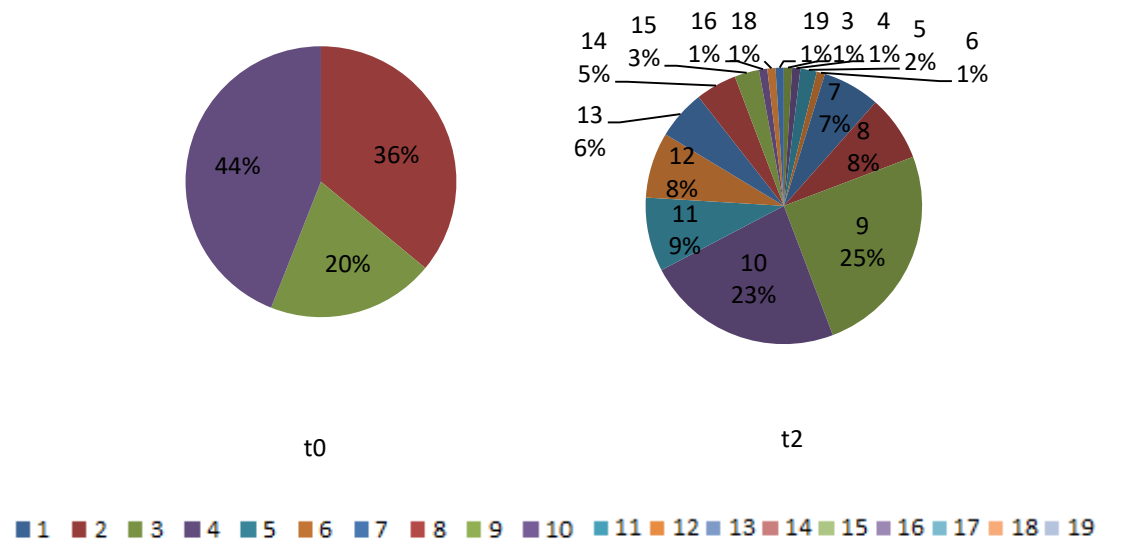


Figure 14: Distribution of the number of pulses found in Sciaenops ocellatus sounds in t0 (N=25) and t2 (N=104).

3.1. Call rate of *Sciaenops ocellatus*

The call rate diel pattern of *Sciaenops ocellatus* for the two phases t0 (N=96) and t2 (N=96) can respectively be found in **Appendix 24 and 25**.

The average call rate during t0 was 0.012 ± 0.063 sounds per minute (N=96) (**Figure 15)**. *Sciaenops ocellatus* did not vocalize from 19:00 to 05:00 in the morning. A mild sound production was detected between 15:00 and 18:00 with a peak at 16:00 characterized by 0.037 ± 0.074 sounds per minute. Another peak of 0.194 ± 0.263 sounds per minute appeared at 11:00. This morning peak was much higher than the one in the afternoon. As the calling activity averages four days of recordings, an important standard deviation can be noticed due to a big variability between days. The coefficient of variation for t0 was measured in order to quantify the dispersion of the data (CV_{t0} = 528.79 %).

During t2 (**Figure 15)**, the diel call activity showed remarkable differences as compared to the previous phase (t0). In fact, an important increase in vocalization occurred. The calling activity peak in t2 is almost 10 times the calling activity peak found during t0. Sound activity was mild from 13:00 to 21:00. Then it increased significantly and reached a major peak at 23:00 with 3.896 ± 0.765 sounds per minute. This finding differed from the results of the call rate of t0 where the major peak was at 11:00. After this the sound production decreased a bit at 00:00. Finally, no vocalizing could be detected from 01:00 to 08:00 in the morning. If compared to t0, less hours with no sound activity at all were found in t2. The average number of calls per minute and per male for the whole phase was 0.372 ± 0.964 sounds per min

(N=72). As for *Argyrosomus regius*, in *Sciaenops ocellatus* an increase in the amount of calls happened during the reproductive season. Also, the sound production occurred during daytime hours out of reproduction season and shifted to nighttime hours during the spawning season.The coefficient of variation CV_{t2} = 259.58%.

In order to compare the call rate between t0 and t2, the average number of calls is shown in **Table 12**.

Table 12: The average call rate ± SD of *Sciaenops ocellatus* for each phase (t0 and t2). The coefficient of variation has been calculated for each phase and expressed in percentage.

	Mean call rate (min^{-1} $male^{-1}$)	SD (min^{-1} $male^{-1}$)	CV (%)
T0 (N=96)	0.012	0.063	528.79
T2 (N=72)	0.372	0.964	259.58

The coefficient of variation was compared between phases (t0 and t2) to determine the evolution of the call rate. The variability of the call rate observed outside in contrast to during the reproduction season is more widespread than the variability of the call rate within each phase (i.e. variability occurring due to differences between individuals). These results support the hypothesis that the change in variability can be attributed to the change in the experimental phase (reproductive phase) and in physiological characteristics of the sonic muscles (increase in fiber size and sarcoplasmic reticulum). This suggests an increase in contraction force and contraction rate of the sonic muscles during the reproductive season.

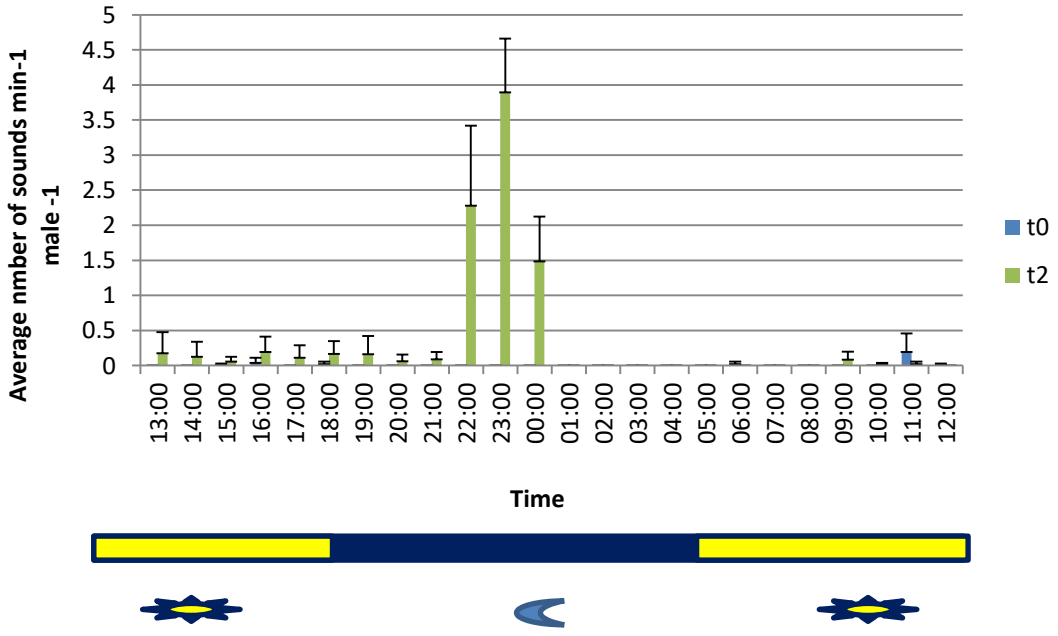


Figure 15: Average call rate per minute per male in different phases (t0, t2) in *Sciaenops ocellatus*.

These findings were confirmed by statistical analysis. The normality test showed a non-parametric distribution of the call rate in all phases (t0, t2). A Mann - Whithney test was run (**Figure 16**). The call rate of t2 was significantly higher than the call rate of t0 (Mann-Whithney, N = 96 ; 96, U = 3306, p<0.05).

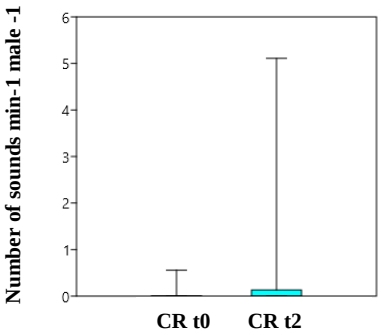


Figure 16: Boxplot showing the distribution around the mean of the call rate of *Sciaenops ocellatus* in each phase (t0, t2).

3.2. Fine acoustic features variation of *Sciaenops ocellatus* pulsed sounds.

Mann-Whithney test was performed to compare each acoustic feature measured as part of this study (DUR, NP, PP and Fr) along the two experimental phases. Results show that *S. ocellatus* sounds differed between phases in all recorded parameters (Mann-Whithney, p <0.05) (**Figure 17**). This means that there is a significant difference between the duration of the sound between the two phases. The same conclusion can be made for the number of pulses, the pulse period and the frequency.

The fine acoustic features shown below compare the data of t0 to the data of t2 (**Figure 17**). The difference between these two periods was the temperature and the photoperiod. Latter parameters induced the reproductive season in *Sciaenops ocellatus*.

The duration of sounds in t2 was significantly longer than the duration of t0 (Mann-Whithney, N = 25 ; 104, U = 69, p <0.05). The number of pulses of t2 were significantly more than in t0 (Mann-Whithney, N = 25 ; 104, U = 19, p <0.05). The pulse period of t2 was significantly shorter than the one of t0 (Mann-Whithney, N = 52 ; 947, U = 232, p <0.05). The frequency of t2 was significantly higher than the one of t0 (Mann-Whithney, N = 25 ; 104, U = 653.5, p <0.05).

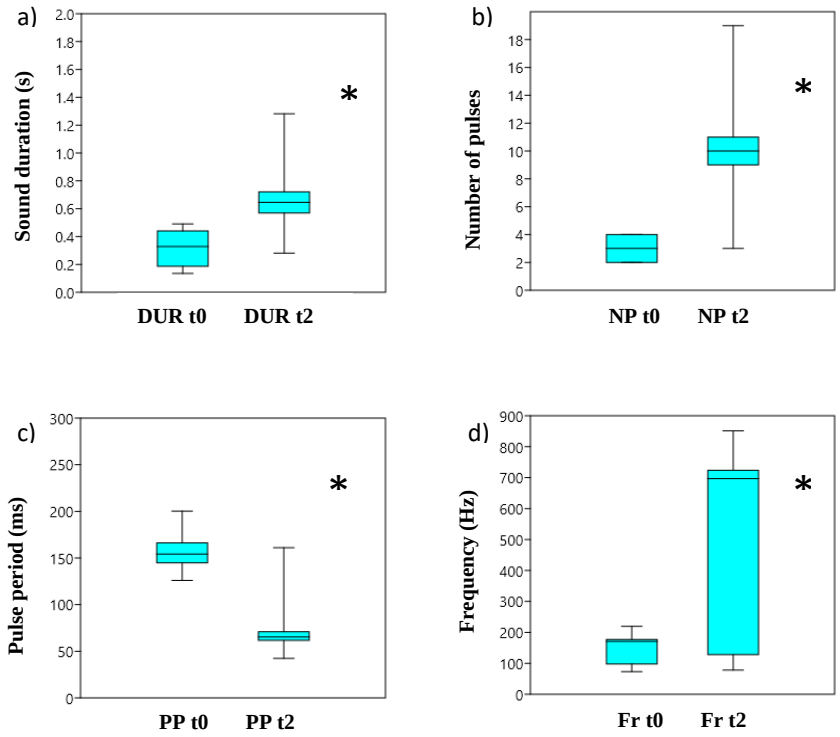


Figure 17: Boxplot showing the distribution around the mean of the fine parameters a) duration, b) number of pulses, c) pulse period and d) frequency between t0 and t2. Mann-Whithney tests were performed for each parameter. a) DUR : N = 25 ; 104, U = 69. b) NP : N = 25 ; 104, U = 19. c) PP : N = 52 ; 947, U = 232. d) Fr : N = 25 ; 104, U = 653.5.

3.3. Spawning data of *Sciaenops ocellatus*

After induction of the reproductive season by temperature increase and change in photoperiod, several spawning events occurred (**Table 13**).

Table 13: Summary of the information regarding the spawning data of *Sciaenops ocellatus*.

Spawning name	Date	Hour of spawning	Number of eggs	Fertilization success
Spawning 1	09/07/2015	23:00	198 000	88.9%

During the reproductive season, *Sciaenops ocellatus* sound production is significantly more important than out of reproduction. Nevertheless, in the reproductive period, by comparing the approximate hours of spawning with the calling activity, the sound production seemed to decrease during spawning hours which might suggest a decrease or even ceasing of sound production during the gamete release. The sound production might also be indicative of egg production with a high fertilization success.

3.4. Histology

As for *Argyrosomus regius*, the same procedure was used to analyze the histological sample of a male *Sciaenops ocellatus* during reproduction season. The area and diameter of the epaxial and sonic muscle fibers measured can be found below (**Table 14**).

Table 14: Table showing the area and the related diameter of epaxial and sonic muscle fibers (N=20) in reproductive *Sciaenops ocellatus*.

	Male t2 epaxial		Male t2 sonic	
(N=20)	Area (µm^2)	Diameter (µm)	Area (µm^2)	Diameter (µm)
Mean	5301	82	247	18
SD	2509	57	52	8
Min	876	33	165	14
Max	10544	116	396	22

Discussion

This study investigates the relationship between sound production in three species of Sciaenidae (*Argyrosomus regius*, *Umbrina cirrosa* and *Sciaenops ocellatus*) before (t0 and t1) and during spawning season (t2) in captivity conditions.

All three species vocalized before and during the spawning season which is in contrast with previous findings (Connaughton & Taylor 1996; Mok & Gilmore 1983; Ueng et al. 2007) suggesting that calling activity in Sciaenidae is limited to reproduction. Nevertheless, calling activity and fine acoustic characteristics were found to change significantly in accordance to the reproductive state of the individuals where the temperature and hormonal treatment acted as inductors for reproduction. These differences in acoustic activity are supported in part by the histological sections.

In *Sciaenops ocellatus*, only the male possesses sonic muscles (Parmentier et al. 2014). Both sexes have sonic muscles in *Argyrosomus regius* but do not have similar appearances as the one belonging to males are more hypertrophied and more red colored (Lagardère & Mariani 2016). The situation is still not known in *Umbrina cirrosa*, as it was not possible to dissect this fish during this study.

In *Argyrosomus regius*, sonic muscle fibers area in a reproductive female (weight = 5.4 kg) was two times bigger than for the non-reproductive one (weight = 4.7 kg) which means that in the female an increase in the sonic muscle mass occurred during the reproduction season.

However, it should be taken as an approximation due to the weight difference between the individuals. The sonic muscle fibers area of the reproductive male (weight = 3.8 kg) was similar to the one of the reproductive female which suggest that at same weight, males should have more hypertrophied sonic muscles than females. These findings might be confirmed by Lagardère & Mariani (2006) that, as already mentioned above, observed bigger and darker colored sonic muscles in males *Argyrosomus regius*. Also, the histological cross-sections of the present study indicate an increase in volume of the sarcoplasmic reticulum in the sonic fibers during reproduction which suggests a higher contraction rate of sonic muscles during spawning season.

These changes of sonic muscle fiber characteristics might influence the sound production found during the present study, as bigger sonic muscles with a higher sarcoplasmic volume might contract for a longer time with a higher contraction rate (i.e. sounds with shorter repeated pulses) which also leads to a higher number of pulses emitted per sound.

Sonic muscle fibers diameter were more or less 4 times smaller than epaxial one, so for the same volume, sonic muscles have smaller more numerous fibers which means that sonic muscles have a higher contraction force than epaxial ones.

For *Sciaenops ocellatus* samples were only taken in reproductive males as female do not possess the structure. As in *Argyrosomus regius*, epaxial muscle fiber area was bigger than sonic muscle fiber area (by a factor 21) as already found by Parmentier et al. (2014), which means that sonic muscles have a higher contraction force than epaxial muscles.

1. *Argyrosomus regius*

The diel pattern of sound production in *Argyrosomus regius* differed from the study of Lagardère & Mariani (2016). However, these authors did not record on a 24 hour cycle and the peak of sonic activity is based on fishermen knowledge that pointed it in the late afternoon (Lagardère & Mariani 2016). Sciaenids are known to vocalize mainly around sunset (Holt 2008; Lagardère & Mariani 2006; Mok & Gilmore 1983; Montie et al. 2016; Picciulin et al. 2012). Mok & Gilmore (1983) found a temporal shift in Sciaenidae sound production (*Bairdiella chrysoura*) with the onset of the reproductive season. Out of reproduction, wild *B. chrysoura* vocalizes around one hour after sunset while during reproduction the peak shifted to three hours after sunset (Mok & Gilmore 1983). The present study also showed a shift in the diel pattern of calling activity before and during reproduction. The calling activity of *Argyrosomus regius* out of reproductive season was mainly emitted during daytime hours

whereas calls emitted during the reproduction season occurred in a significantly higher number and at night. These results can be compared to the "regular calls" of *Sciaena umbra* emitted in the wild during spawning season (Picciulin et al. 2012) during the nocturnal sound production peak (presence of one peak around sunset and one around sunrise).

The comparison between different age groups (5 and 13 years old) of *Argyrosomus regius* during this study showed that older males produced longer sounds with faster repeated pulses. Lagardère & Mariani (2006) recorded wild *A. regius* with longer sounds and more pulses than in the present study suggesting that they recorded a wider size range, between which older individuals were present.The pulse period (18 ± 3 ms and 20 ± 2 ms) of five- and thirteen-year-old *A. regius* sounds recorded as part of this study was similar to the pulse period (20.1 ± 0.9 ms) found in Lagardère & Mariani (2006) which indicates a similar contraction rate of the fish sonic muscles in this two studies carried out at similar temperatures. Also, the pulse periods of calls emitted by meagre are particularly short, compared to other Sciaenidae, which suggests a much faster contraction rate of the sonic muscles compared to other species (Lagardère & Mariani 2006) which indicates an important volume of sarcoplasmic reticulum as found on the histological samples. The frequency of the studied sounds of five-year-old fish ranged from 64 to 710 Hz with a dominant frequency of 305 ± 142 Hz. For the older specimen, the range of frequency was from 107 to 258 Hz with a dominant frequency of 183 ± 57 Hz. The results differed from the dominant frequency of 336 to 444 Hz (Lagardère & Mariani 2006). The wider range of frequency found in our study might be influenced by the length of the fish as the range of frequency becomes narrower in species like *Cynoscion regalis*, *Micropogonias furnieri* and *Pogonius cromis* with increasing fish size (Connaughton & Taylor 1966; Tellechea et al. 2010; Tellechea et al. 2011). This hypothesis was also reinforced by the comparison between five- and thirteen-year-old *Argyrosomus regius* where the frequency decreased with fish size. In fact, bigger muscles have a slower contraction rate which determines a lower frequency.

As the aim of the thesis was to describe the relationship between sound production and reproduction, the calling activity (call rate) was compared between phases (t0, t1 and t2). Fish calling activity increased progressively from t0 to t2, where the call rate of t2 was significantly higher than the call rate of t1 and t0 and the call rate of t1 was significantly higher than the one of t0. So, the increase in temperature and the action of a hormonal treatment influenced positively the calling activity (Aalbers & Drawbridge 2008; Connaughton & Taylor 1996; Luczkovich et al. 1999; Mok & Gilmore 1983; Montie et al.

2016). The acoustic features (DUR, NP, PP and Fr) differed significantly between phases. Sounds recorded during the reproductive season were characterized by a higher number of pulses, a shorter pulse period and a similar dominant frequency. This means that on a morphological point of view, mature and hypertrophied sonic muscles contracted faster and more often during the reproductive period.

Interestingly, a significant difference was also found when comparing the fine parameters of the two spawning nights $t2_{(1)}$ and $t2_{(2)}$. Calls emitted during the second spawning night were shorter, had a higher number of pulses with shorter pulse periods and no difference in dominant frequency. From a physiological point of view, it indicates that sonic muscles contracted even faster during t2(2) and but were able to sustain this contraction speed for a shorter period of time. The results suggest that fine features could change significantly within a really restricted time (less than 3 hours) and influence close by spawning events.

As the sound duration in t2(2) was longer, with more pulses, shorter pulse periods, and similar dominant frequency than in t2(1) and the number of eggs and fertilization success were more important in t2(2), this could indicate that there might be a relationship between some fineacoustic features (sound duration, number of pulses and pulse period) and spawning success.

2. *Umbrina cirrosa*

The calling activity out of reproductive season in *Umbrina cirrosa* peaked around 07:00 in the morning whereas it is around 10:00 during the spawning season.

Table 15: Table comparing the fine acoustic parameters of *Umbrina cirrosa* obtained in the present study and two other researches.

	Present study	Picciulin et al. (2016)	Lagardère & Parmentier (2014)
DUR(ms)	231 - 1599	150 - 1400	370 - 874
NP	1 - 7	1 - 11	1 – 3
PP(ms)	275	180	/
Fr (Hz)	64 - 226	400	150 - 250

The duration of the sounds (231 to 1599 ms) is in the same range than data (150 to 1400 ms) from Picciulin et al. (2016). The shorter duration (370 to 874 ms) in the study of (Lagardère & Parmentier (2014) can be explained by the lower number of pulses (1 to 3) compared to this study (1 to 7) and Picciulin et al. (2016) data (1 to 11).This variation of duration and number of pulses could be explained by the season in which the recordings were taken. In fact, while

this study and Picciulin et al. (2016) recorded sounds during May (which corresponded to the reproductive season), Largardère & Parmentier (2014) recorded *Umbrina cirrosa* during September to November which was already out of the reproductive season. In fact, the findings of this study in t0 and t1 are similar to those found in Lagardère & Parmentier (2014) whereas the findings of t2 are comparable to the results found in Picciulin et al. (2016) for the duration of the sound and number of pulses which indicates that the duration of the sounds is longer and with more pulse during reproduction compared to sounds out of the reproductive season. However the pulse period is much lower in Picciulin et al. (2016) than in this study (180 ms vs 275 ms in t2). On a morphological point of view this changes mean that the fish recorded by Picciulin et al. (2016) had a much higher contraction rate than the fish in this study. The dominant frequency range going from 64 to 226 Hz, was comparable to the 150 to 250 Hz found in Largardère & Parmentier (2014) but really different than the 400 Hz found by Picciulin et al. (2016). This could be attributed to the recording conditions. This study (T° = 19.3°C and 22°C) and the study of Lagardère & Parmentier (2014) were both recorded in tanks (T° not specified) and could have been affected by the "small tank effect" described by Akamatsu et al. (2002) whereas Picciulin et al. (2016) recorded in semi-natural conditions (T° = 18 to 22°C). Also, the fish age (which can be related to the fish size (Abou Shabana et al. 2012)) could have influenced the frequency. In fact, the specimens of this study were 15-year-old specimens and might have vocalized at a lower frequency than younger 3-year-old specimen in Picciulin et al. (2016).

The call rate of t2 was significantly higher than the call rate of t1 and t0 but the call rate of t1 was not significantly different than the one of t0. So, the temperature and hormonal treatment seemed to influence positively the calling activity of *Umbrina cirrosa*. The acoustic features (DUR, NP, PP and Fr) differed significantly between phases. Sounds recorded during the reproductive season were more abundant and longer than sounds out of spawning season. They were characterized by a higher number of pulses, a shorter pulse period and a similar dominant frequency. Like for *Argyrosomus regius*, mature and hypertrophied sonic muscles of *Umbrina cirrosa* contracted faster and more often during the reproductive period.

As the number of eggs and fertilization success were very reduced during the natural spawning compared to the hormonal induced spawning and that sound activity increased during the reproduction phase t2, this might indicate a relationship between sound production and spawning success.

3. *Sciaenops ocellatus*

Sciaenops ocellatus calling activity during spawning season could be compared to the sound production described by Montie et al. (2016). The present study showed that calling activity out of reproductive season peaked at 11:00 in the morning. For the spawning season, the calling activity of *Scieanops ocellatus* peaked in the evening at 23:00 which is close to the peak occurring one hour after darkness in Montie et al. (2016), the peak sound production between 18:00 and 22:00 found in Holt (2002) and the peak sound production between 19:00 and 21:45 of Guest & Lasswell (1978). Nevertheless, it differed with the results (peak from 06:00 to 09:00) found in Parmentier et al. (2014). These results were actually more similar to the calling activity found in t0, before the reproductive season, which confirms that the spawning period was already over.

The duration of the sounds found in this study (136 to 1282 ms) was shorter than the duration of the sounds recorded by Montie et al. (2016) which was from 190 to 3530 ms. In fact, Montie et al. (2016) counted more pulses, 2 to 29 pulses instead of 1 to 19 pulses. Sound duration of this study (656 ± 153 ms) (N=104) was longer than the sound duration of 320 ± 28 ms (N=100) found in Parmentier et al. (2014). These results can be explained by the lower number in pulses found in Parmentier et al. (2014): 1 to 3 pulses whereas in this study the range was 1 to 19 pulses. Lowerre-Barbieri et al. (2008) found evidence supporting that sounds with up to 4 pulses did not lead to reproduction whereas sounds counting 8 or more pulses could be related to spawning events. Results of this study are similar as in the 104 sounds analyzed during t2 only 12 sounds had less than 8 pulses and the sound production was followed by a spawning event of 198 000 eggs with a fertilization success of 88.9%.

Concerning the pulse period, during the reproductive season the average pulse period (contraction rate of the sonic muscles) was 72 ± 19 ms (N=104) whereas Parmentier et al. (2014) found a much higher result: 144 ± 12 ms (N=100). As the pulse period was found to be higher out of reproduction season, it reinforced the hypothesis of Parmentier et al. (2014) suggesting that the spawning season might already have been over. The dominant frequency of the calls of this study was of 428 ± 286 Hz (N=104) and similar to the dominant frequency found in Parmentier et al. (2014) with an average of 500 ± 237 Hz (N=100) but different to the frequency found in other studies: 145 to 155 Hz in Holt (2002), 50 to 300 Hz in Montie et al. (2016).

The call rate of t2 was significantly higher than the call rate of t0. So, an increase in temperature and a natural photoperiod influenced positively the calling activity. Also, overlap of sound production occurred occasionally but to simplify the analysis these sounds engaged in chorus activity were not used for fine parameters. Acoustic features (DUR, NP, PP and Fr) differed significantly between phases. Sounds recorded during the reproductive season were more abundant and longer than sounds out of spawning season. They were characterized by a higher number of pulses, a shorter pulse period and a higher dominant frequency. This means that on a morphological point of view, mature and hypertrophied sonic muscles contracted faster and more often during the reproductive period.

Conclusion

In conclusion, the findings obtained during this research indicated that, for all three species, sound production was more important during the reproductive period and that spawning events occurred. Also, the number of pulses and the pulse period of sounds emitted seemed indicative of spawning as more quickly repeated pulses were found during the reproductive season. This implies that the sonic muscles contract faster during reproduction. This is supported by histology made on *Argyrosomus regius* and *Sciaenops ocellatus* as an increase of sarcoplasmic reticulum (storage for calcium), was observed on the sonic muscle fiber cross-sections.

A potential application that could emerge concerns the maximization of rearing production thanks to acoustic playbacks. Playbacks would consist in the use of underwater loudspeakers reproducing optimized sounds (playback of sounds with fine parameters that induce a better spawning production and success) in the proximity of mature individuals in order to improve spawning in rearing conditions (e.g. better synchronization of gamete release, influence on gonadal maturation etc.). Another potential application would involve the use of PAM for finely localize breeding areas in space and time; this could improve the resolution of fisheries management survey and could provide critical information for the creation and the maintenance of protected areas for spawning.

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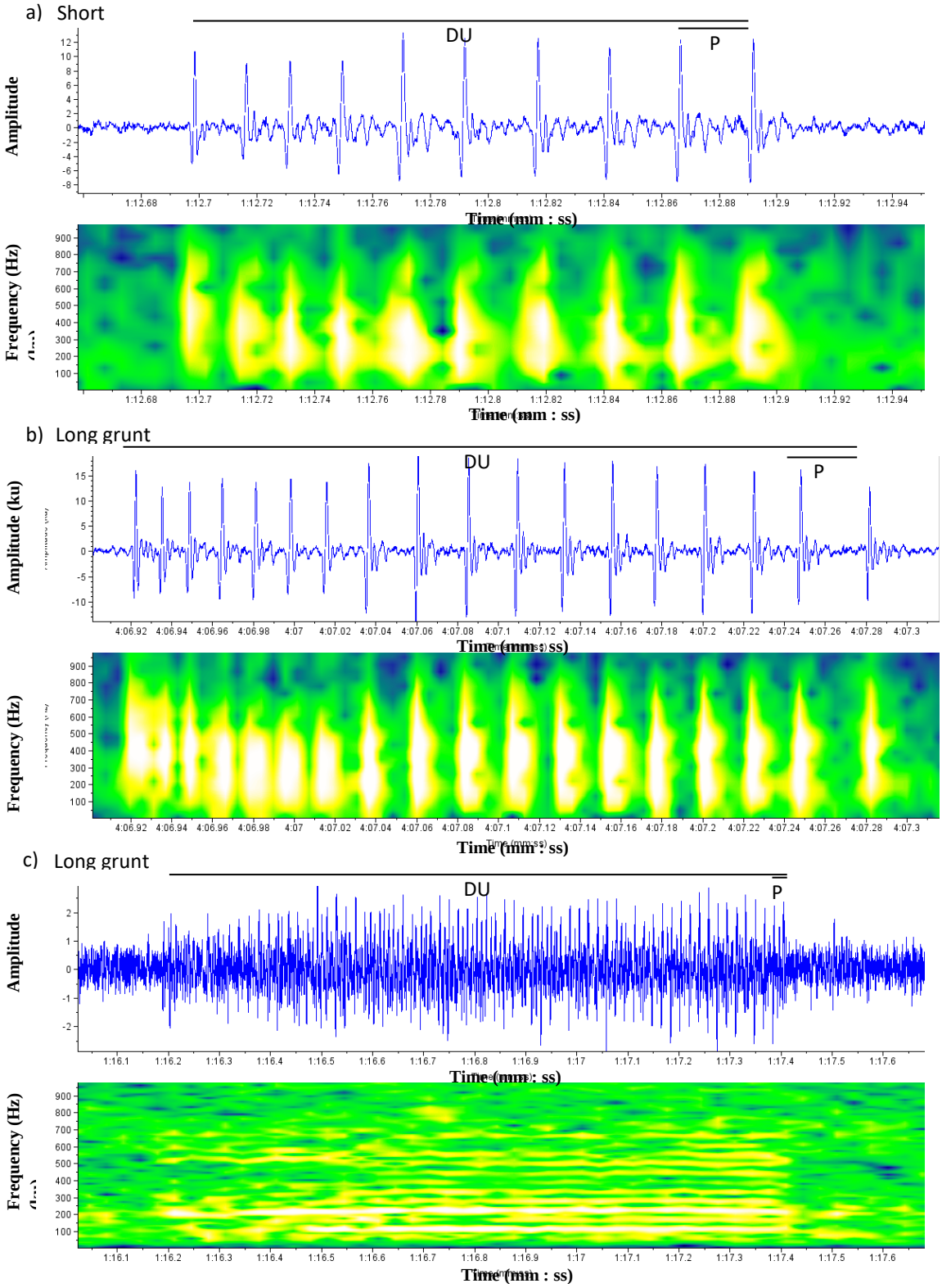
Appendix

Appendix 1: Table showing some information concerning 14 Sciaenidae species reported vocalizing.

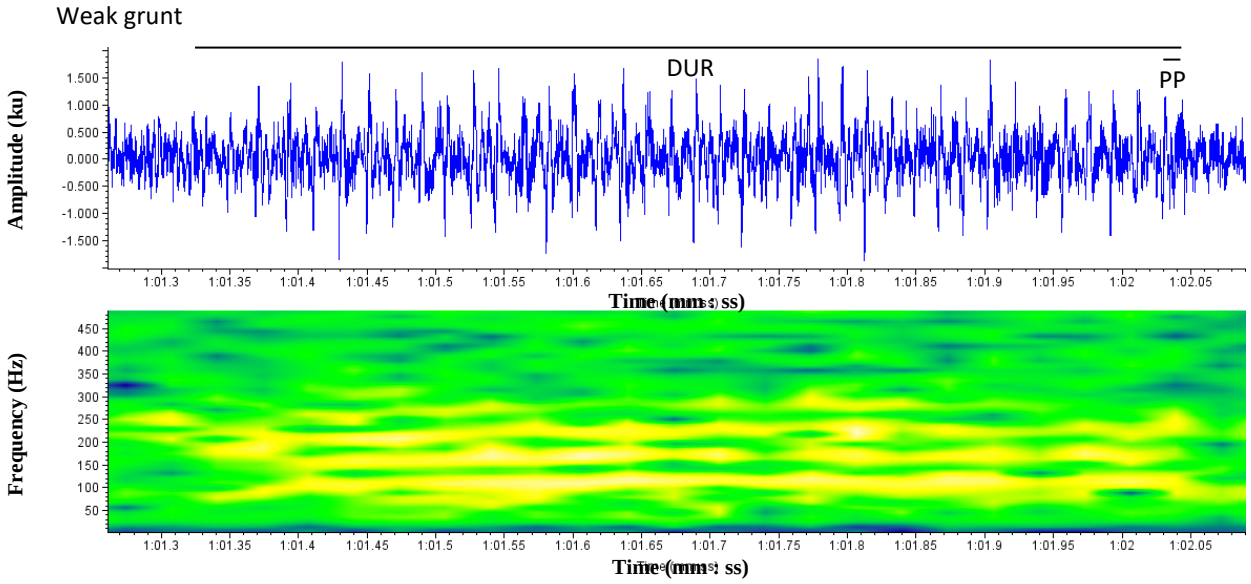
Scientifical name	**Common name**	**Reference**
Atractoscion nobilis (Ayres, 1860)	white seabass	Aalbers & Drawbridge 2008
Pennahia argentata (Houttuyn, 1782)	silver croaker	Borie et al 2014
Micropogonias undulatus (Linnaeus 1766)	Atlantic croaker	Gannon 2007
Umbrina canosai (Berg, 1895)	Argentine croaker	Tellechea et al 2017
Cynoscion nebulosus (Cuvier, 1830)	spotted seatrout	Luczkovich 2008; Montie et al 2017
Cynoscion regalis (Bloch & Schneider, 1801)	weakfish	Connaughton & Taylor 1996; Sprague 2000; Perkins 2001; Luczkovich et al 2008; Tellechea et al 2012
Bairdiella chrysoura (Lacepède, 1802)	silver perch	Luczkovich et al 2008
Argyrosomus japonicus (Temminck & Schlegel, 1843)	mulloway	Parsons et al 2006; Parsons et al 2013; Parsons & McCauley 2017
Sciaena umbra (Linnaeus, 1758)	brown meagre	Picciulin et al 2012; Picciulin et al 2013
Micropogonias furnieri (Desmarest 1823)	whitemouth croaker	Tellechea et al 2010
Pogonias cromis (Linnaeus 1766)	black drum	Tellechea et al 2011
Sciaenops ocellatus (Linnaeus 1766)	red drum	Holt 2002; Holt 2008; Lowerre-Barbieri et al 2008; Luczkovich et al 2008; Parmentier et al 2014; Montie et al 2016
Argyrosomus regius (Asso, 1801)	meagre	Lagardère & Mariani 2006
Umbrina cirrosa (Linnaeus, 1758)	shi drum	Lagardère & Parmentier 2014; Picciulin et al 2016

Appendix 2: Table showing some general information, acoustic features, behavioral context and morphology for each of the three studied species.

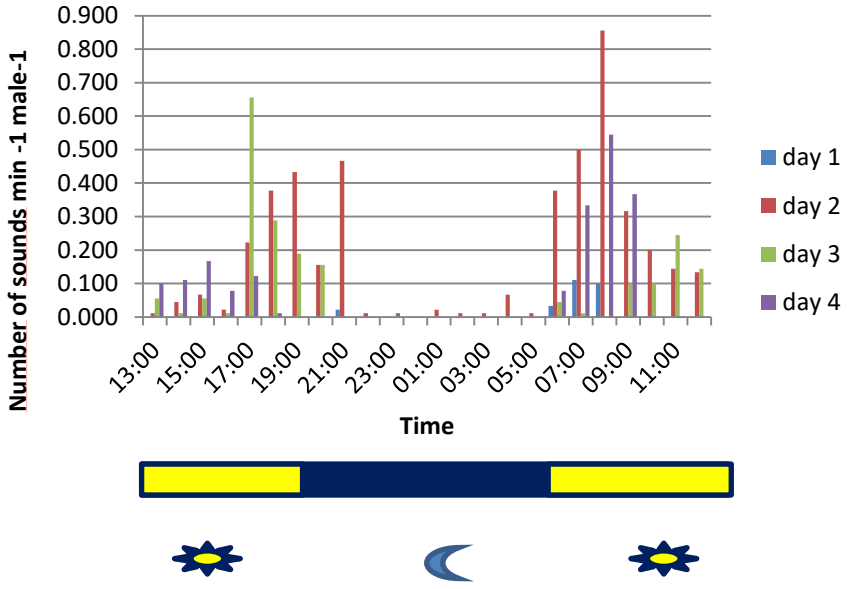
Species			General information			Acoustic features							Behavioral context			Morphology			Reference
scientifical name	named by	common name	research period	number of individuals	sexe ratio F : M	sound type	call rate (calls/min)	duration (ms)	number of pulses/call	pulse period (ms)	pulse duration (ms)	dominant frequency (Hz)	context	wild/captivity	temperature (°C)	intrinsic	extrinsic	male/female	
Sciaenops ocellatus	Linnaeus, 1776	Red drum	5 Febr - 25 March	20	10:10	drumming sound	/	0.392 ± 0.036	3	171 ± 18	0.043 ± 0.018	500 ± 237	out of spawning	captivity	26 -29	/	/	only male	Parmentier et al 2014
Sciaenops ocellatus	Linnaeus, 1776	Red drum	23 July - 19 Dec 2012	5	03:02	drumming sound	/	0.19 to 3.53	2 to 29	/	/	50-300	spawning	captivity	25	/	/	only male	Montie et al 2016
Argyrosomus regius	Asso 1802	Meagre	June - July 2003 and 2004	/	/	long grunt	12 to 13	598 to 2496	30 to 112	20.1 ± 0.9	7.4 to 14.4	336 to 444	spawning	wild	17 -21	/	x	both	Lagardère & Mariani 2006
Argyrosomus regius	Asso 1803	Meagre	June - July 2003 and 2004	/	/	short grunt	/	69.9 to 99.2	4 to 6	594 to 5281	4.5 to11.7	/	spawning	wild	18 -21	/	x	both	Lagardère & Mariani 2006
Umbrina cirrosa	Linnaeus, 1758	Shi drum	Sept - Nov 2008	6	/	"son de contact"	/	0.370 to 0.874	1 to 3	/	/	150 to 250	contact sound	captivity	/	/	/	/	Lagardère & Parmentier 2014
Umbrina cirrosa	Linnaeus, 1759	Shi drum	14 May 2011	30	/	call	/	0.15 to 1.400	1 to 11	180	40	400	/	captivity	18 - 22	/	/	/	Picciulin et al 2016



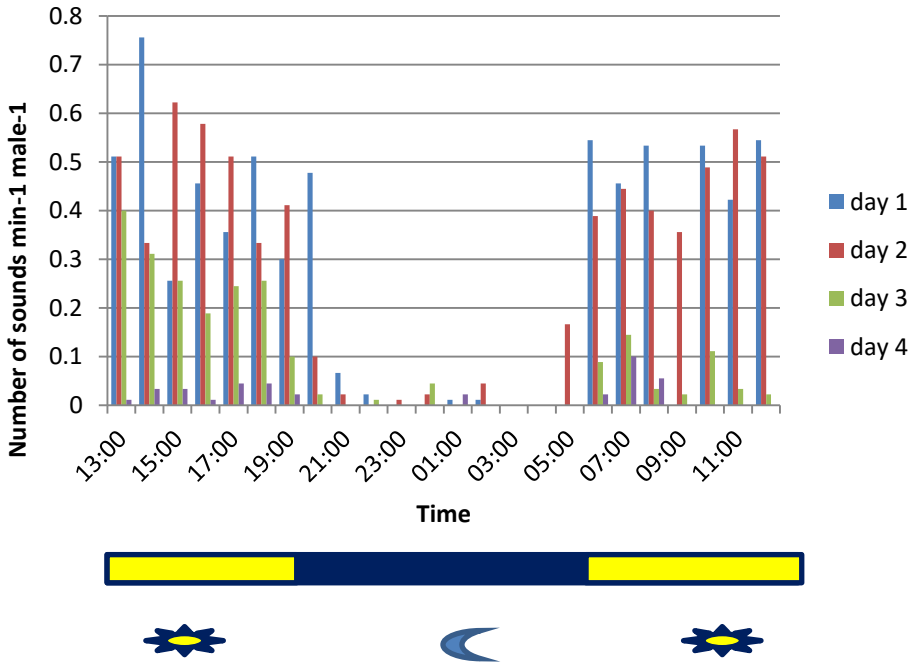
Appendix 3: Waveform and spectrograms (Hanning window, FFT= 516, 516 and 2933 respectively) of respectively a) short grunt, b) long grunt recorded in t0 and c) long grunt recorded in t2 (75 pulses long) of *Argyrosomus regius*. The three sounds respectively show a dominant frequency of 323 Hz (FFT 516), 376.8 Hz (FFT 516) and 215.5 Hz (FFT 2933).



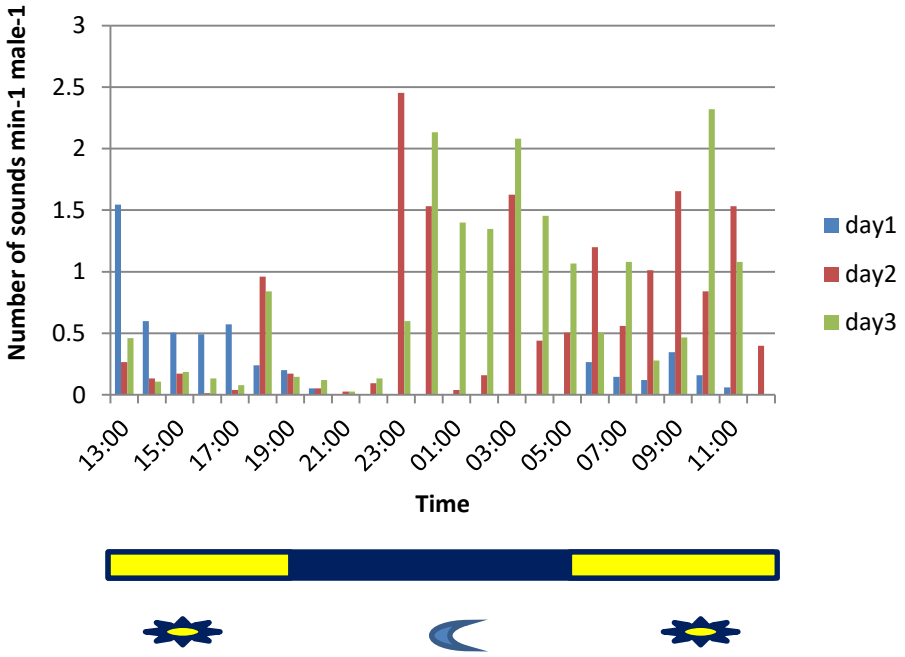
Appendix 4: Waveform and spectrogram (Hanning window, FFT 2933), of a weak grunt (t2) of *Argyrosomus regius*. The dominant frequency is of 107.7 Hz (FFT 2933).



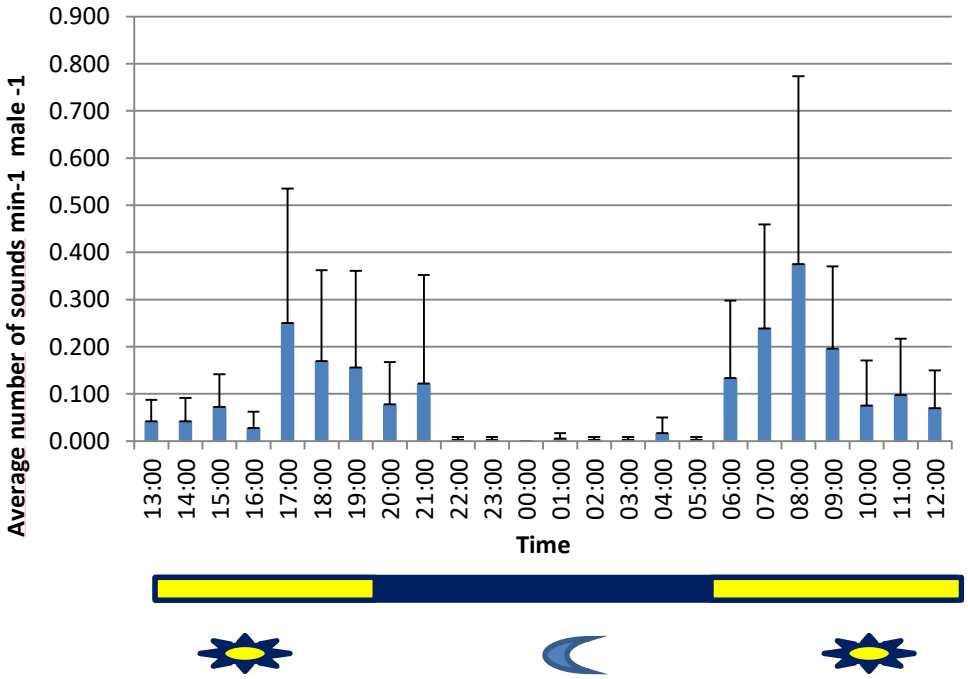
Appendix 5: Diel call rate standardized per minute per male in t0 for each day in *Argyrosomus regius*. Temperature = 16.2 C°.



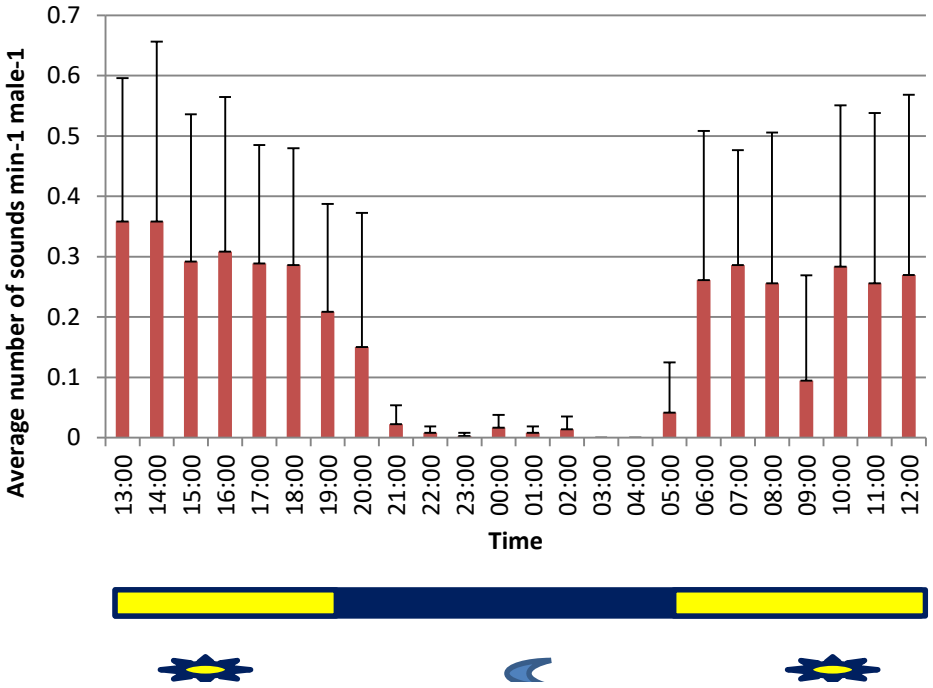
Appendix 6: Diel call rate standardized per minute per male in t1 for each day in *Argyrosomus regius*. Temperature = 20 C°.



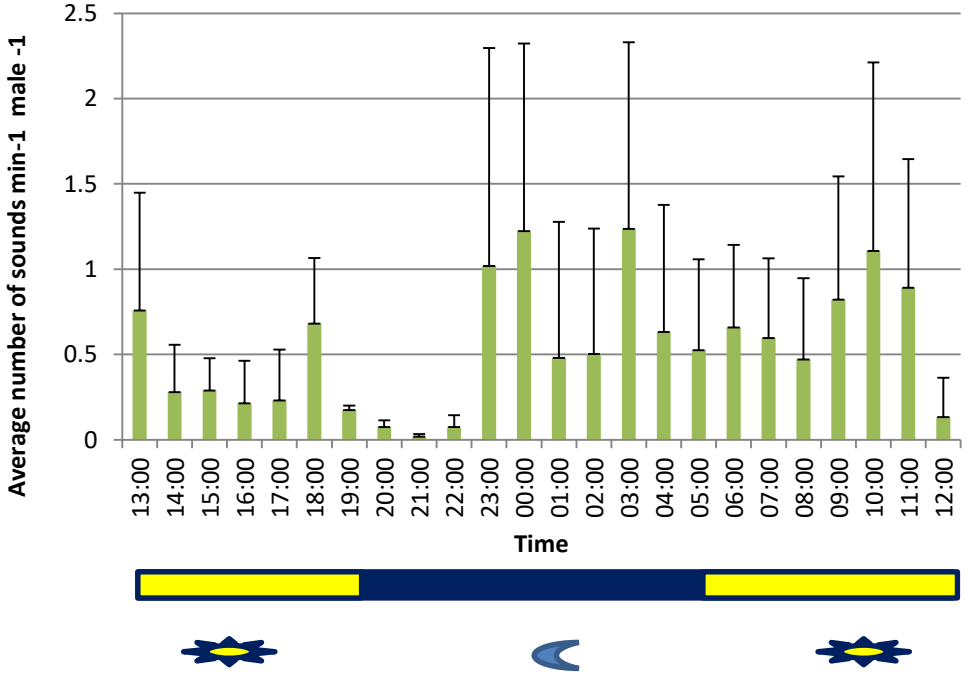
Appendix 7: Diel call rate per minute per male in t2 for each day in *Argyrosomus regius*. Temperature = 20°C. The fish were hormonally induced with GnRHa to obtain reproduction.



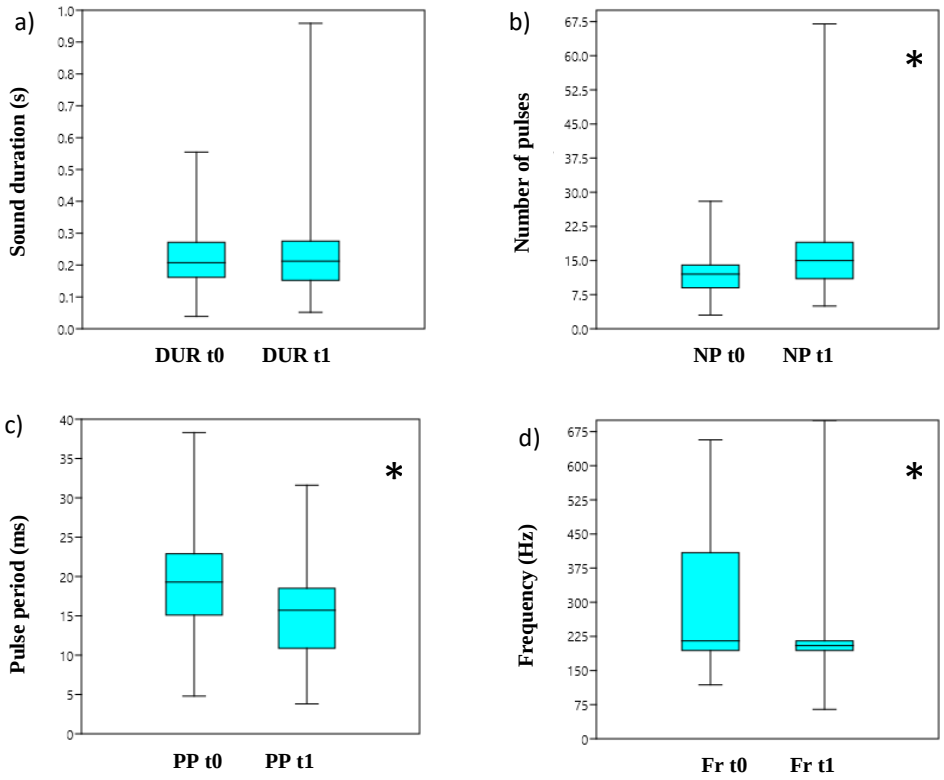
Appendix 8: Diel call rate per minute per male in t0 (average of the four days) in *Argyrosomus regius*. Temperature = 16.2 C°.



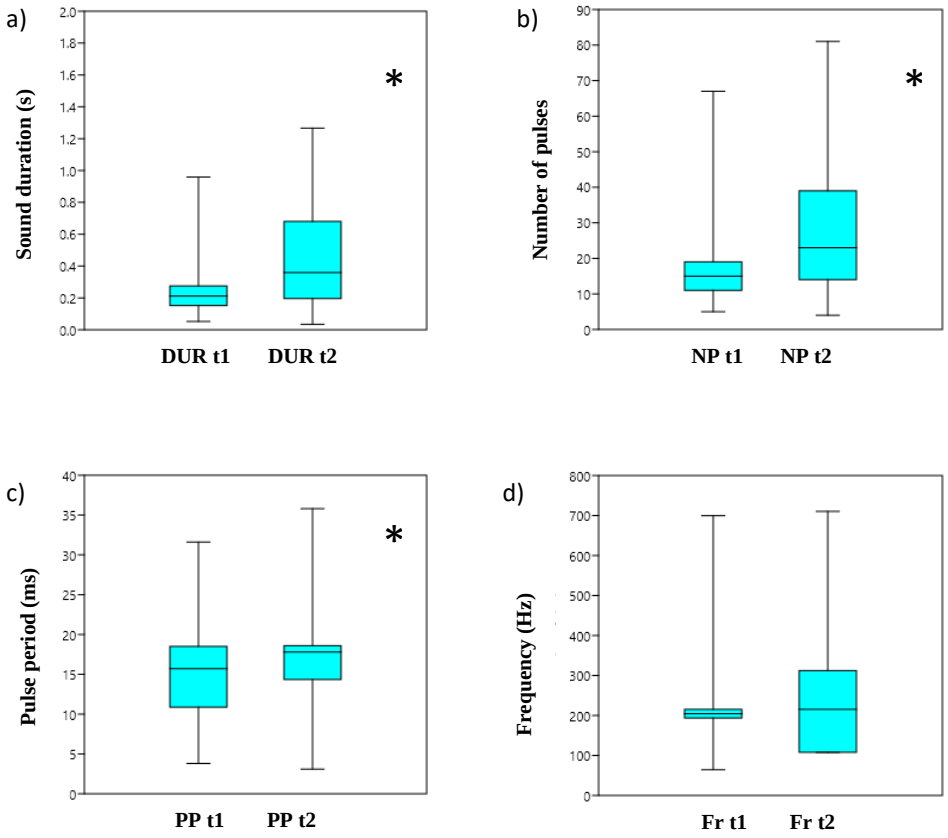
Appendix 9: Diel call rate per minute per male in t1 (average of the four days) in *Argyrosomus regius*. Temperature = 20 C°.



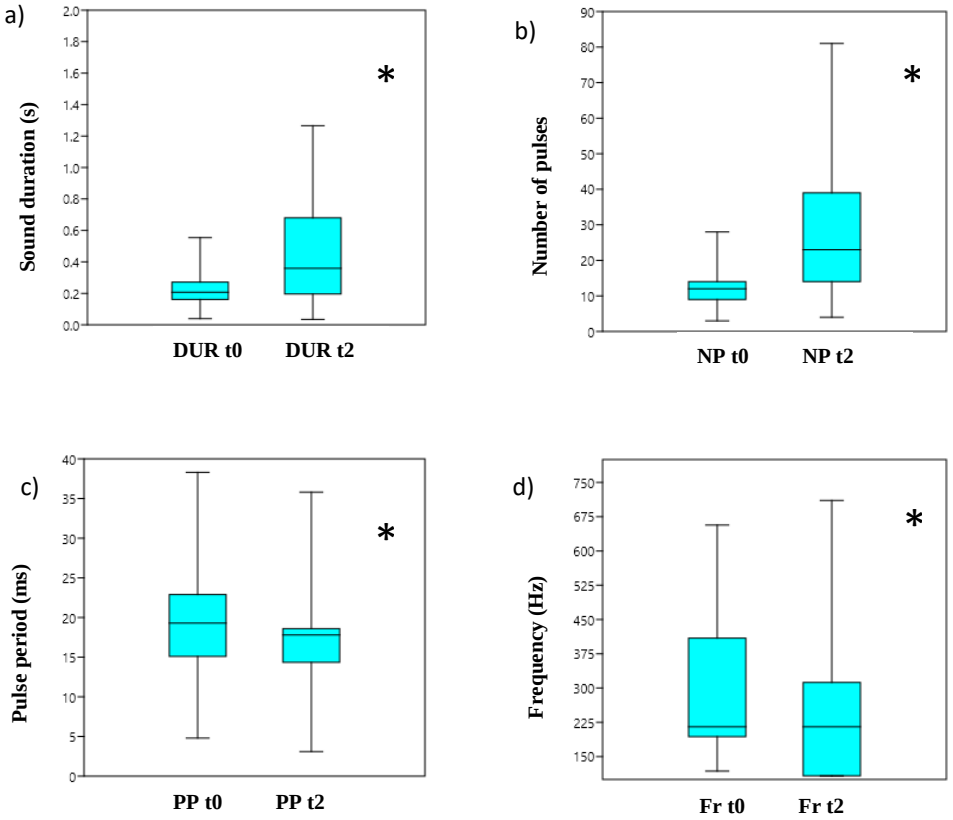
Appendix 10: Diel call rate per minute per male (average of three days) in t2 in *Argyrosomus regius*. Temperature = 20°C. The fish were hormonally induced with GnRHa to obtain reproduction.



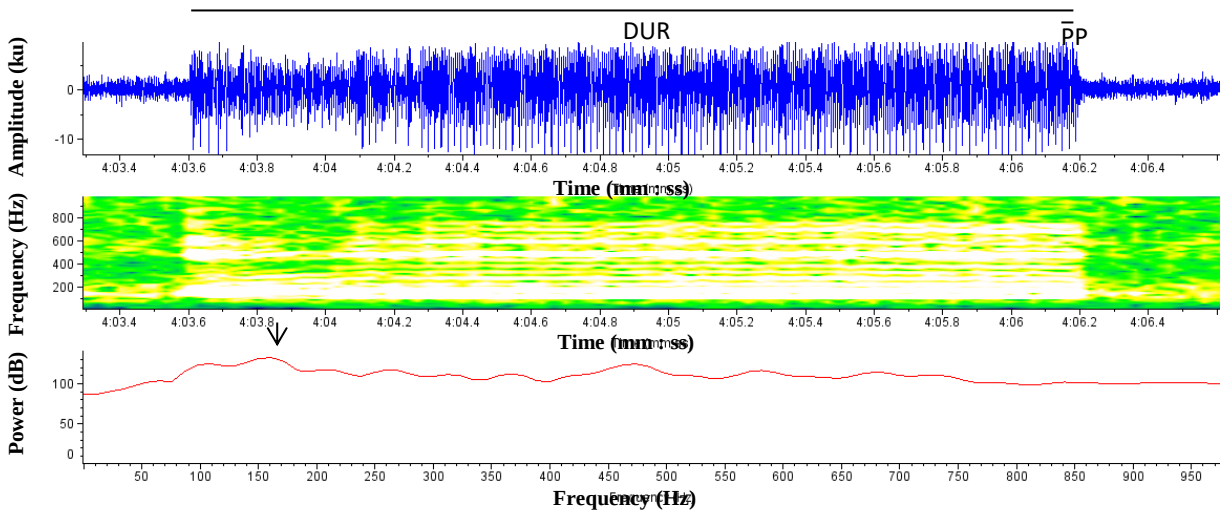
Appendix 11: Boxplot showing the distribution around the mean of the fine parameters a) duration, b) number of pulses, c) pulse period and d) frequency between t0 and t1. Mann-Whithney tests were performed for each parameter. a) DUR : N = 704, 1091, U = 3.8299E05. b) NP : N = 670, 905, U = 1.9836E05. c) PP : N = 1482, 1182, U = 4.8612E05. d) Fr : N = 149, 202, U = 11 050.



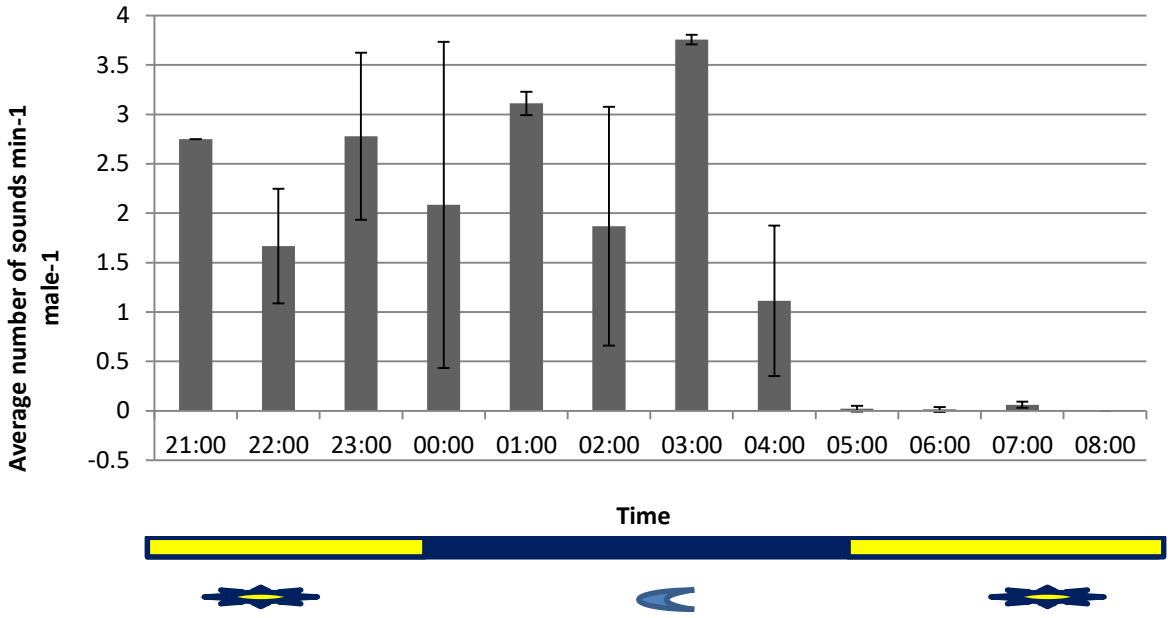
Appendix 12: Boxplot showing the distribution around the mean of the fine parameters a) duration, b) number of pulses, c) pulse period and d) frequency between t1 and t2. Mann-Whithney tests were performed for each parameter. a) DUR : N = 1091 ; 510, U = 1.5803E05. b) NP : N = 905 ; 511, U =1.3229E05. c) PP : N = 1182 ; 4369, U = 2.0901E06. d) Fr : N = 202 ; 161, U = 15 246.



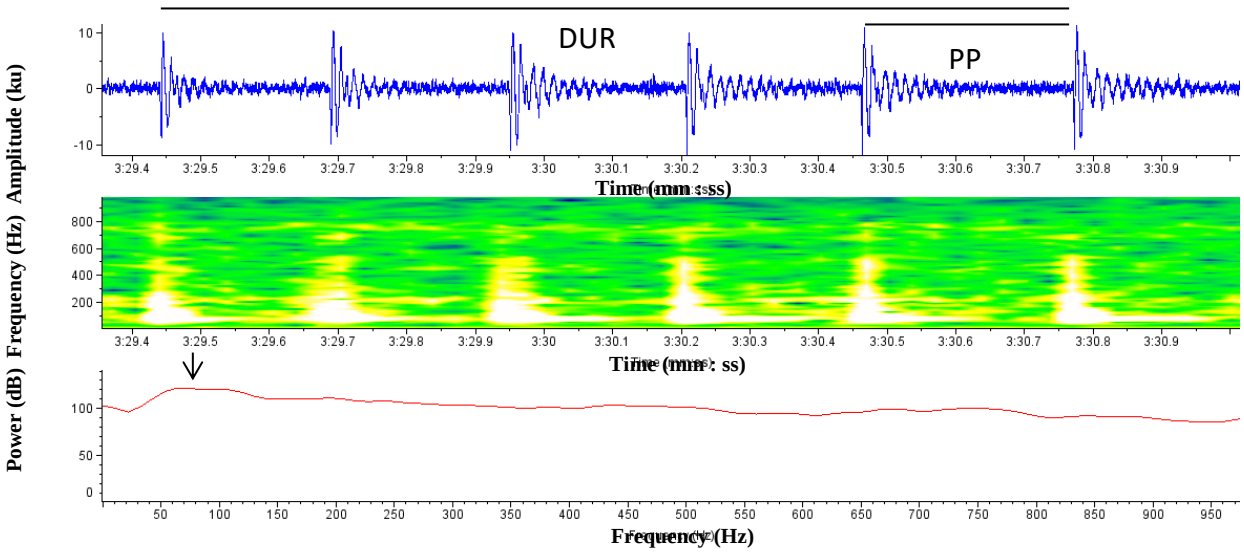
Appendix 13: Boxplot showing the distribution around the mean of the fine parameters a) duration, b) number of pulses, c) pulse period and d) frequency between t0 and t2. Mann-Whithney tests were performed for each parameter. a) DUR : N = 704 ; 510, U = 1.0099E05. b) NP : N = 670 ; 511, U =65 791. c) PP : N = 1482 ; 4369, U = 2.2831E06. d) Fr : N = 149 ; 161, U = 9 234.



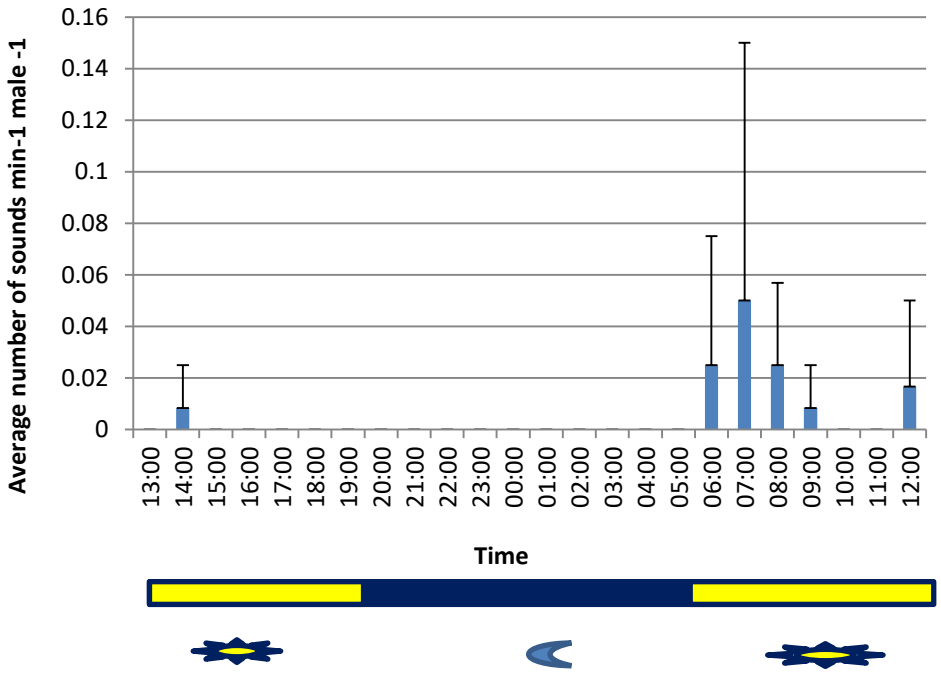
Appendix 14: Waveform, spectrogram (Hanning window, FFT=2933), and power spectrum of a 137 pulsed long grunt (t2) of 13-years-old *Argyrosomus regius*. The power spectrum indicates a frequency of 161.5 Hz.



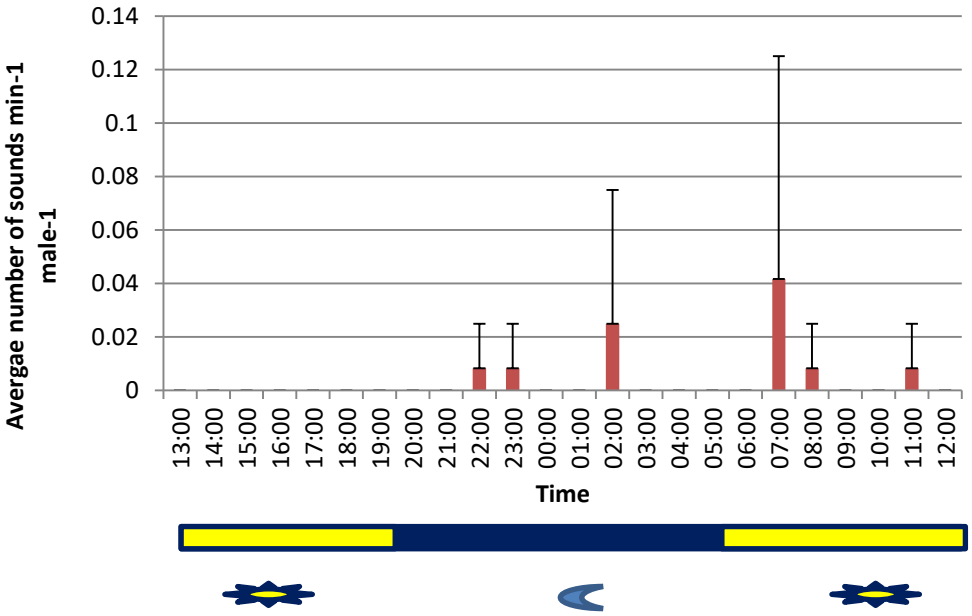
Appendix 15: Call rate per minute per male (between 21:00 and 08:00) in t2 for older *Argyrosomus regius*. The fish were hormonally induced with GnRHa to obtain reproduction.



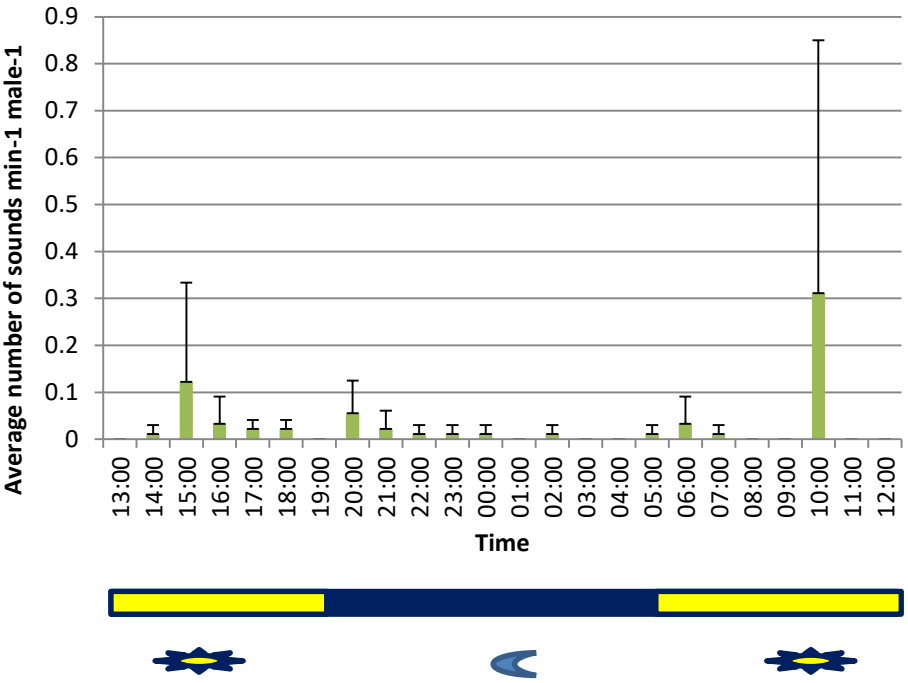
Appendix 16: Waveform, spectrogram (Hanning window, FFT = 2933) and power spectrum of a 6 pulsed *Umbrina cirrosa* sound in t2. The power spectrum shows a dominant frequency of 64.6 Hz.



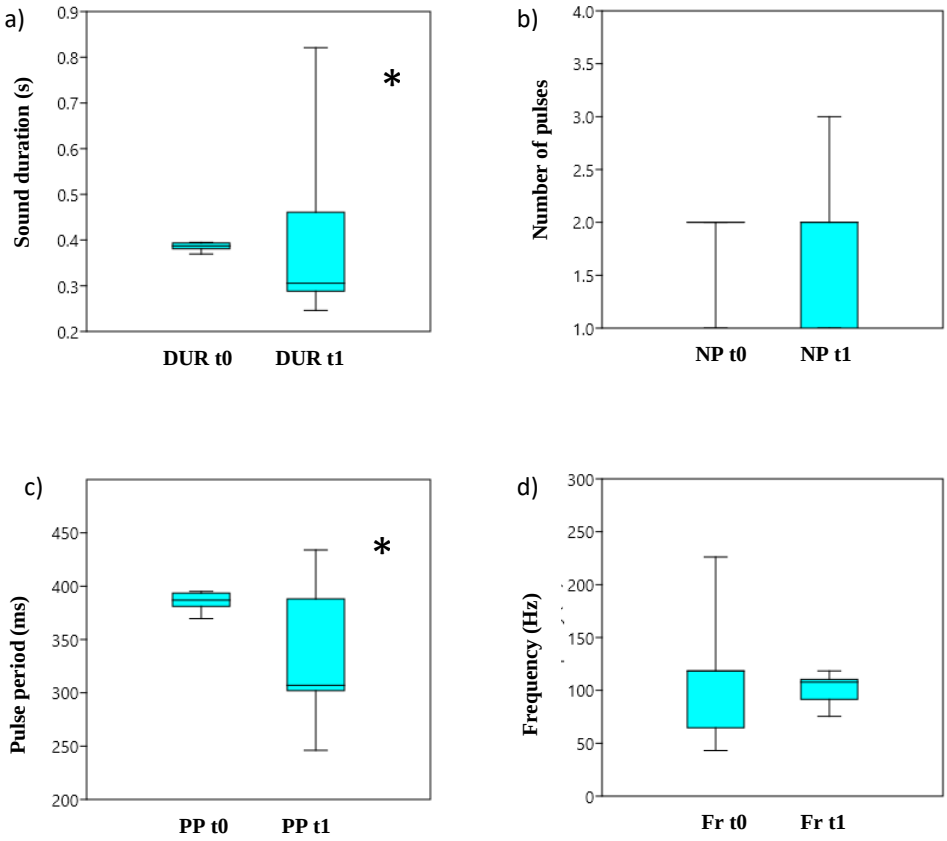
Appendix 17: Diel call rate per minute per male in t0 (average of the four days) for *Umbrina cirrosa*. Temperature = 19.3°C.



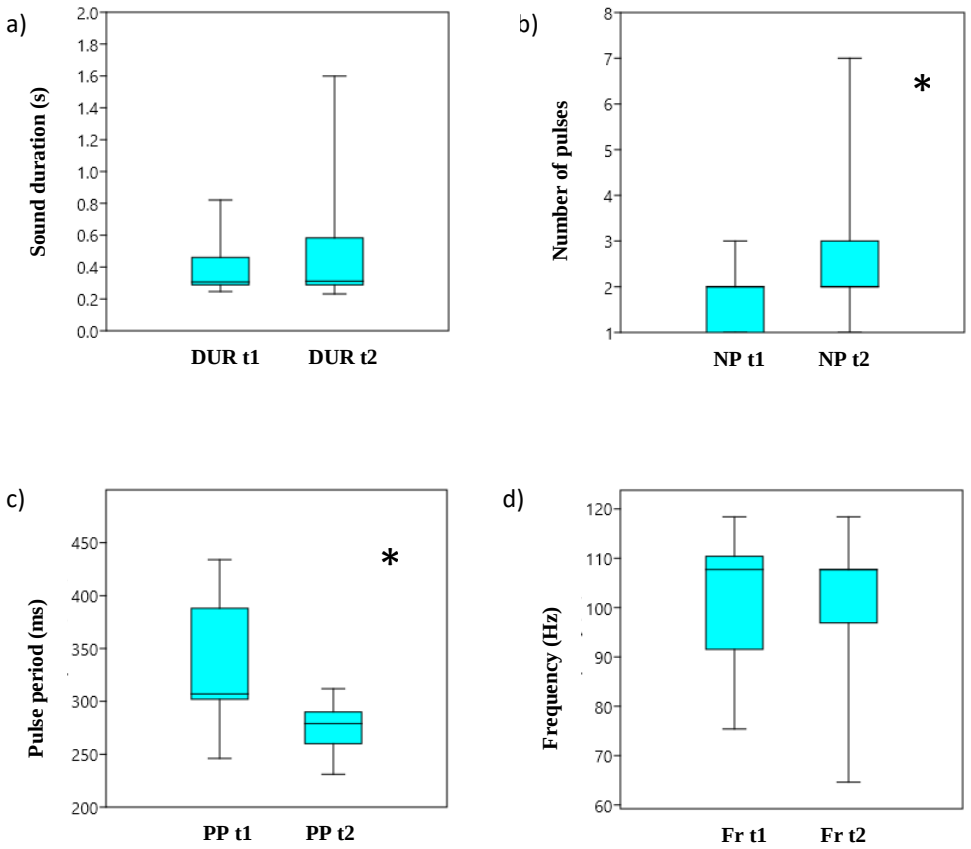
Appendix 18: Diel call rate per minute per male in t1 (average of the four days) for *Umbrina cirrosa*. Temperature = 22°C.



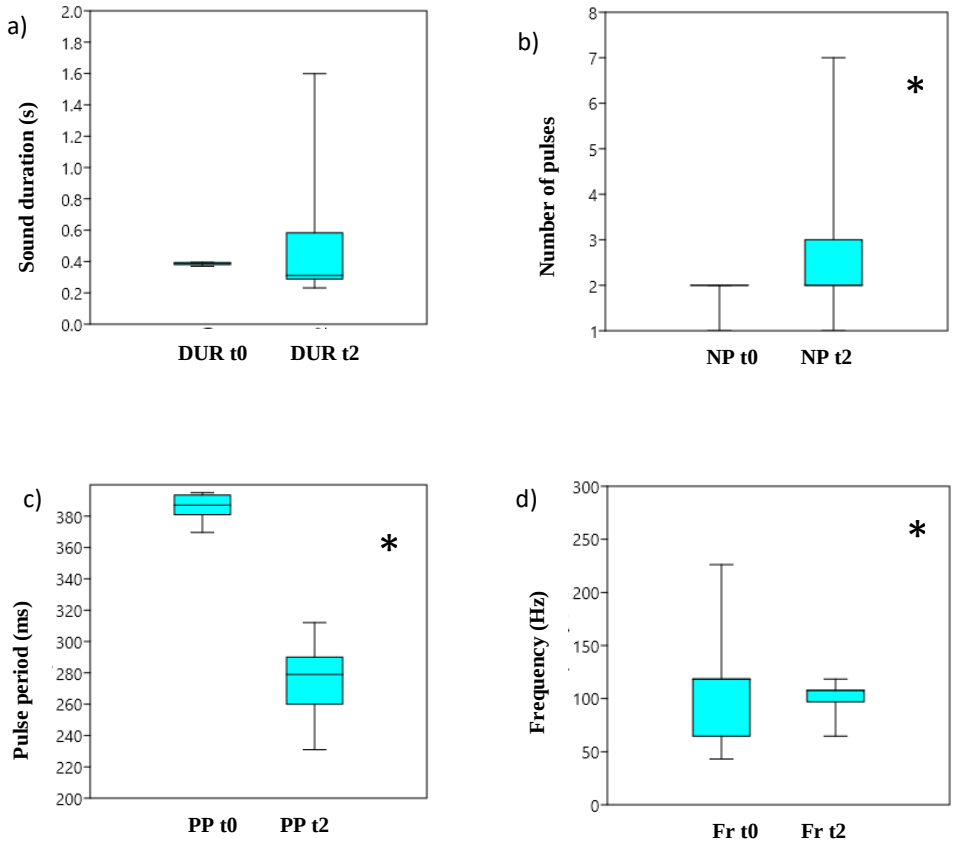
Appendix 19: Diel call rate per minute per male in t2 (average of three days) for *Umbrina cirrosa*. Temperature = 22°C. The fish were hormonally induced with GnRHa to obtain reproduction.



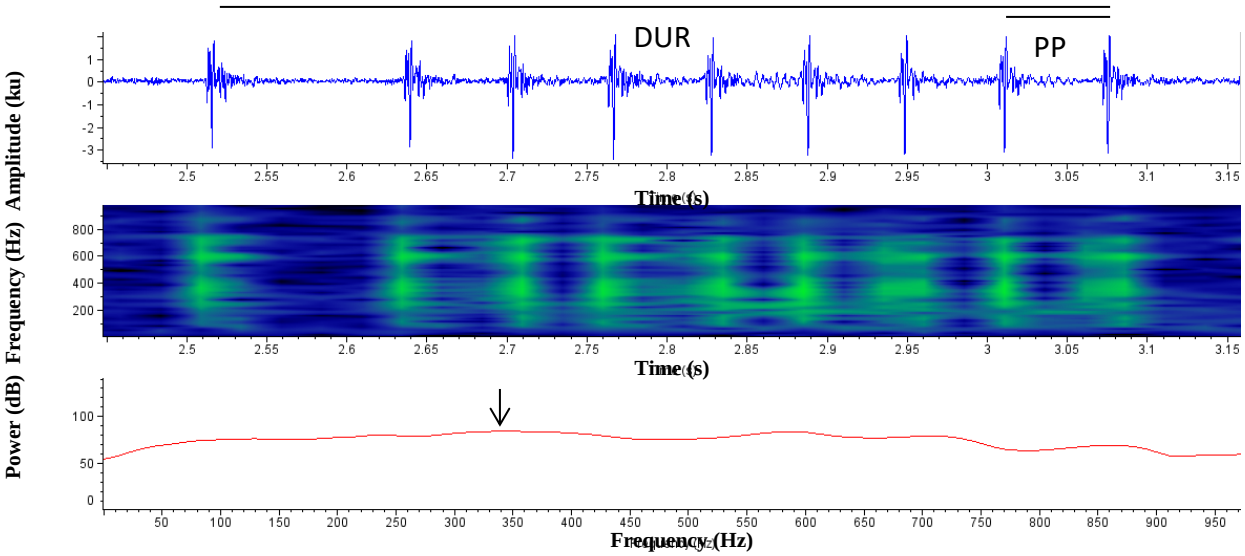
Appendix 20: Boxplot showing the distribution around the mean of the fine parameters a) duration, b) number of pulses, c) pulse period and d) frequency between t0 and t1. Mann-Whithney tests were performed for each parameter. a) DUR : N = 13; 6, U = 13. b) NP : N = 16 ; 11, U = 71. c) PP : N = 13 ; 7, U = 20. d) Fr : N = 14 ; 6, U = 30.



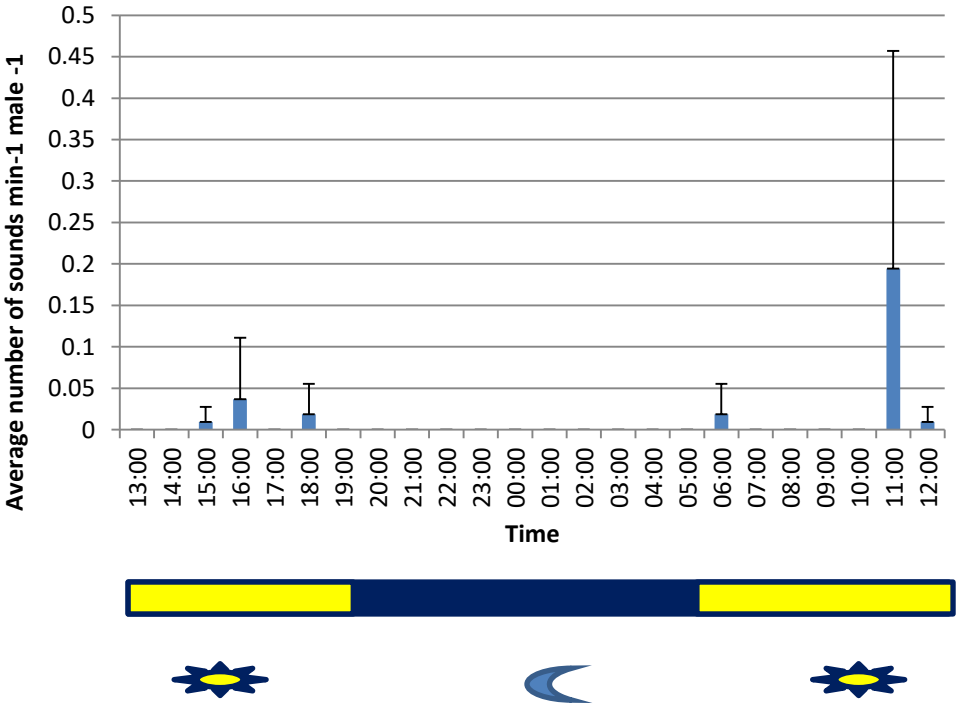
Appendix 21: Boxplot showing the distribution around the mean of the fine parameters a) duration, b) number of pulses, c) pulse period and d) frequency between t1 and t2. Mann-Whithney tests were performed for each parameter. a) DUR : N = 6 ; 55, U = 152.5. b) NP : N = 11 ; 64, U = 183. c) PP : N = 7 ; 99, U = 103.5. d) Fr : N = 6 ; 55, U = 136.5.



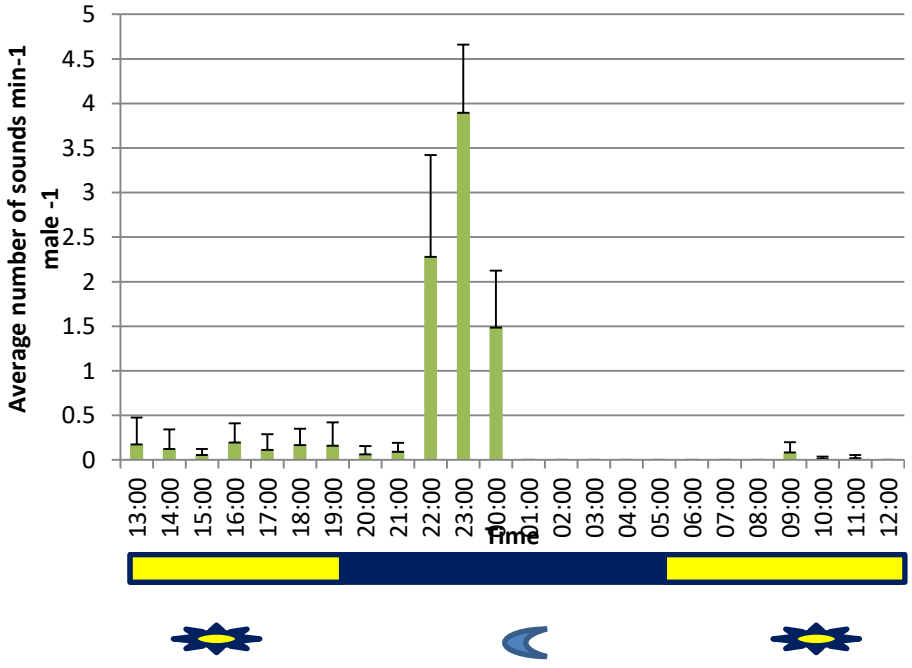
Appendix 22: Boxplot showing the distribution around the mean of the fine parameters a) duration, b) number of pulses, c) pulse period and d) frequency between t0 and t1. Mann-Whithney tests were performed for each parameter. a) DUR : N = 704, 1091, U = 3.8299E05. b) NP : N = 670, 905, U = 1.9836E05. c) PP : N = 1482, 1182, U = 4.8612E05. d) N = 149, 202, U = 11 050.



Appendix 23: Waveform, spectrogram (Hanning window, FFT = 275) and power spectrum of a 9 pulsed *Sciaenops ocellatus* sound in t2. The power spectrum shows a dominant frequency of 343.8 Hz.



Appendix 24: Diel call rate per minute per male in t0 (average of the four days) for *Sciaenops ocellatus*. Temperature = 28.6°C.



Appendix 25: Diel call rate per minute per male in t2 (average of the four days) for *Sciaenops ocellatus*. Temperature = 30°C. The fish were in reproductive season induced by an increase in temperature and the reproduction of the natural photoperiod found during spawning season.