Instituto Superior de Psicologia Aplicada Ψ

Mestrado em Etologia

SOUND PRODUCTION IN THE NORTH-EASTERN ATLANTIC TOADFISH *HALOBATRACHUS DIDACTYLUS* (PISCES: BATRACHOIDIDAE)

by

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ABSTRACT

Batrachoidids usually show a well developed acoustic repertoire, with at least two main types of calls related to different social contexts: mate attraction, and aggression. *Halobatrachus didactylus* (Schneider, 1801) is an eastern Atlantic toadfish, usually found in sandy or muddy bottoms, under rocks or tiles. The breeding ecology of this species is still poorly studied. In this study the basic acoustic repertoire of *H. didactylus* was recorded and analyzed.

The present work shows that this species has acoustic emissions similar to other toadfishes. It produces a rhythmically long tonal sound, similar to the boatwhistle and hum signals of *Opsanus tau*, *Opsanus beta* and *Porichthys notatus*, and a complex of sounds which include the grunt-like signals. Other types of sounds were identified in *H. didactylus*: croaks, mixed grunt/croak calls and double croaks. A discriminant analysis of the sounds showed that the boatwhistle is highly differentiated from the other types of calls, and that signal organisation and differentiation is mainly based on the temporal arrangement and repetition of pulses, rather than differences in frequency structure.

No correlation was found between peak frequency and body size in the *H. didactylus* sounds, recorded in a tank. It was also demonstrated that this sound feature is not significantly different between males and females. However male sounds were significantly longer than female fish. The meaning of this sound duration difference between the sexes remains unclear.

Only double grunt calls showed significant differences between night and day periods. The actual meaning of this difference was not clear.

In this study the boatwhistle call latency distributions of two neighbouring toadfish were compared. This analysis showed that two individuals never overlapped their calls, and that the alternation pattern may not be produced only by chance. One of the individuals appears to be calling independently while the other is coupling its call. However more tests are needed to further support this hypothesis.

This work represents the first attempt to study the acoustic behaviour of *Halobatrachus didactylus*, and it should be viewed as a starting point for future investigations.

RESUMO

A familia Batrachoididae é conhecida por posssuir espécies com um reportório acústico bastante desenvolvido. Normalmente existem pelo menos dois tipos de emissões acústicas produzidas em diferentes contextos comportamentais: atracção de femeas e interacções agonísticas. *Halobatrachus didactylus* (Schneider, 1801) é um batrachoidideo do Atlântico Leste, que habita fundos arenosos ou lodosos, onde pode ser encontrado debaixo de pedras ou rochas. Pouco se sabe sobre a ecologia e o comportamento reprodutor desta espécie. Neste estudo analisou-se o repertório acústico básico de *H. didactylus*.

Este trabalho demonstrou que *H. didactylus* possui emissões acústicas semelhantes às outras espécies de batrachoidideos. Produz ritmicamente um som com uma estrutura semelhante ao 'boatwhistle' de Opsanus tau, Opsanus beta e ao 'hum' de Porichtys notatus, e um conjunto de sons nos quais estão incluidos os 'grunts' agonísticos. Os outros tipos de sons identificados, foram: os 'croaks', sons mistos com uma componente 'grunts' e outra de 'croaks', e 'croaks' duplos. O resultado da aplicação de uma análise discriminante usando os vários tipos de sons, mostrou que o 'boatwhistle' era um sinal bastante diferenciado dos restantes sinais, e que a differenciação e organização da estrutura dos vários tipos de sinais, é baseada principalmente no arranjo temporal e repetição dos impulsos, e não na variação da frequência.

Segundo a análise de sons gravados em laboratório, verificou-se existir uma ausência de correlação entre o tamanho dos indivíduos e a frequência dominante (peak frequency) do sinal, e que não existem diferenças significativas dos valores desta frequência entre machos e fêmeas. Contudo foi possível verificar uma diferença significativa na duração do sinal. O significado desta diferença não nos parece claro.

Diferenças na produção de sons entre a noite e o dia, foram apenas dectadas nos duplos croaks. Estes sinais são produzidos em maior quantidade durante o dia.

Realizou-se ainda a análise da relação entre vocalizações de dois machos vizinhos num coro de 'boatwhistles'. Os dois indivíduos apresentavam um padrão de vocalizações alternado, sem a sobreposição de sinais. Esta interacção pode não ser produzida ao acaso, dado que um dos machos aparenta estar a seguir o ritmo do vizinho, tentando ao mesmo tempo manter sempre a sua vocalização a uma distância mais ou menos igual à vocalização do outro indivíduo.

Este trabalho representa uma primeira abordagem ao estudo do comportamento acústico de *Halobatrachus didactylus*, e deverá ser encarado apenas como uma base para a elaboração futuros trabalhos.

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1. INTRODUCTION

1.1 Underwater acoustics

Sound can be defined essentially as any local mechanical disturbance in any material medium, produced by the vibration or movement of an object (Hawkins 1993). This mechanical disturbance produces a longitudinal elastic wave, within which the particles of the medium are forced together and apart in an oscillatory movement. These movements are also accompanied by an oscillatory change in pressure above and below the dominant hydrostatic pressure of the medium.

Measurements in underwater acoustics may be made in terms of units of pressure, in Pascal ($1\mu Pa = 1\times10^{-6}\ Nm^{-2} = 1\times10^{-5}\ \mu bar$), and particle motion (usually particle velocity, cms^{-1}, but also particle displacement and acceleration, m and ms^{-2}, respectively). Sound pressure is a scalar quantity that acts in all directions in the medium, while particle velocity is a vector quantity with its direction determined by the axis of propagation. These two quantities are related to one another, with their relationship depending primarily upon the acoustic impedance of the medium, that is by its density and elasticity ($v = p/\rho c$; where v - sound velocity; p - pressure; c - propagation velocity and ρ - density of the medium; the product $c\rho$ is the acoustic impedance).

Comparative to air, water is a more dense (about 850 times) and less elastic medium, presenting consequent overall differences in the properties of sound emission and propagation. For example the input energy required to initiate sound emission is higher in water than in air, but after the sound is initiated it's transmission is faster than in air, the value of sound velocity is almost 1500 ms^{-1} for water and 330 ms^{-1} for air. This means that underwater sounds have wavelengths of almost five times the length produced by the same frequency in air ($\lambda = c/f$; c - sound velocity, ms^{-1}; λ - wavelength, m; f - frequency, Hz). Larger wavelengths may travel greater distances within the medium, because less acoustic energy is scattered by reflection and absorption phenomena (Tavolga 1971).

Because of water's high resistance to compression, the propagation of a sound wave involves not only compression but also particle displacement. This effect is stronger at close range to the sound source where the wave front has an spherical form and particle velocity is much higher for a given pressure value, This phenomenon is called near-field effect. Away from the sound source the advancing wavefront has an almost planar surface, and particle velocity and sound pressure are directly proportional to one another, giving origin to the far-field effect (Hawkins 1993). In most field or tank work conditions both of the effects take place simultaneously, and are very difficult to discriminate with the standard equipment (Tavolga 1971).

Sounds can also be affected by physical boundaries of different densities, such as the sea bottom, water surface and water interfaces of different temperature or salinity. Near these boundaries phenomenon like wave reflection or absorption can occur, giving origin to complex acoustic conditions (Tavolga 1971, Hawkins 1993). When one tries to interpretate sound measurements, produced in aquaria or tanks, one has to bare in mind the acoustic field generated by wave reflection (Parvulescu 1967). Small tanks compared to the wavelength of the signal present serious problems of sound analysis because most of the acoustic energy has its origin on the reflection phenomenon and produce unpredictable frequency-dependent nodes of pressure and particle motion (Popper & Fay 1993).

Underwater recordings usually present a high level of background noise, even when one is measuring the sound level in ideal conditions (when there are no boats and sound producing animals near by and the sea is dead calm) one can still detect a high level of ambient noise, with pressure of about 15 dB below 1μPa (Tavolga 1971). The ambient noise at sea can be originated by rain, seismic activities, wave motion on the surface, friction of water currents with the substrate and against each other, biological sources, and human activities (boats, dredging, driling). All this sources may potentially mask the signal one want's to measure (Hawkins 1993).

Another problem that one faces when analysing underwater sound recordings, is the difficulty to differentiate between biological signals produced by a specialised mechanism and simple motion activity. Particle movements and compression exerted by the fins of a moving fish and even the water currents are essentially an acoustic phenomena, and the distinction between these phenomena and the production of sound by fish (for example with a swimming bladder mechanism) is sometimes difficult to evaluate (Tavolga 1971, Hawkins & Myrberg 1983).

1.2 Sound recording and representation

For many years, sound recording equipment have been used and developed by naval military forces of several countries, in order to detect and identify enemy vessels (Hawkins 1993). Some of the investigation involved identifying possible noise sources from biological organisms (Fish et. al. 1952). Gradually this equipment was made available to civil research institutes and the public in general.

The hydrophone is an underwater transducer that captures mechanical signals transforming them in to electric signals. Most of the hydrophones are sensitive to sound pressure, and the pressure-sensitive element is constituted by an impermeable transducer made of barium titanate or lead zirconate, connected to a high-input impedance preamplifier (Hawkins 1993). The sound is recorded in a magnetic tape or in a digital audiotape.

The first attempt to describe sounds was by using a verbal description of the signal. This method has been showed to be rather inadequate and often lead to confusion. With the development of new technologies and techniques some of these problems were solved.

One of the techniques that became important in sound representations was the sound spectrogram or sonogram. Almost all current fish sound publications use this representative technique. In brief, this method takes the recorded signal and transforms it mathematically (Fast Fourier transform) representing it in a frequency *versus* time graph. However, one must be aware of the limitations in the analysis of a sonogram; for example, the display of apparent harmonics can result from repetition of pulses, and not due to resonance phenomena (Tavolga 1971).

Another way to represent sound is plotting a pressure intensity versus time graph, usually known as waveform representation or oscillogram. Time related measures are normally done on this type of graph.

In our days computers can be used for sound digitalisation, representation and analysis. Sound analysis software and hardware are now more available, becoming also more user friendly.

1.3 Sound production in fishes

Sound production is a widespread phenomenon in fishes. In the last 5 decades, more than 50 families have been reported to have at least one specie that produce sound (Hawkins & Myrberg 1983, Hawkins 1993), and every year more are being added to the list (example: *Cottus gobius*, Ladich 1989; *Gaidropsarus mediterraneus*, Almada *et. al.* 1996; *Chromis viridis*, Amorim 1996a). The context of sound production is not always clear, for example some fish produce sounds incidentally when eating or moving near the substrate (Fish et. al. 1952, Hawkins & Myrberg 1983, Amorim 1996b). Though these signals can be intercepted by other fish that can use them to extract information (for example: predators may use these caustic emissions to detect potential preys), it does not involve the production of a specially designed signal or call. Still several species have a well defined acoustic repertoire, with different types of signals, used in specific social contexts, and elicits modifications in other individuals behaviour (Hawkins 1993). The diversity of signals and sound production mechanisms suggest that acoustic communication has evolved independently in several groups (Hawkins & Myrberg 1983).

Fish sounds are usually composed of low frequencies, not higher than 4Khz (often less than 1Khz), and its temporal and frequency structure varies with the type of mechanism used .

1.3.1 Mechanisms of sound production

The morphology of sound producing mechanisms appears to be independent, or weakly correlated to the phylogenetic position in fishes (Tavolga 1971).

One can find three general types of sonic mechanisms: stridulatory sounds (rubbing of hard parts of the body together), air bladder mechanism (drumming of specialised muscles) and hydrodynamic sounds (production of sound during swimming activity).

Stridulation is the sound produced by rubbing two hard materials together. Fish have developed several mechanisms which they use to produce such sounds. Some species use the pharyngeal denticles as stridulatory elements. For example *Balistes carolinensis* (Balistidae) makes a kind of metallic scratching sound with it's pharyngeal teeth. In this specie the air bladder acts as a resonator structure (Fish et. al. 1952). However one must differentiate these sounds from the ones produced during feeding activities like the grasping of teeth on rocks or against each other. Another way to produce stridulatory sounds is by the use of specially modified fin rays and spines. The croaking gourami (*Trichopsis vittatus*: Anabatidae, Belontiidae) produces low click sounds with a sonic apparatus modified from its pectoral fin (Ladich & Fine 1992). Finally fish can also use the friction of skeletal elements (adjacent bones) to produce sounds, such as the river bullhead, *Cottus gobius* (Cottidae), which produces a single or a train of knocks by moving the pectoral girdle and skull against each other (Ladich 1989).

When displayed in a sonogram the stridulatory sounds usually show a nonharmonic structure, and can be divided in two general categories: a) high frequency signals with the dominant frequency ranging from 1000Hz to 4000Hz, and a variable time duration; b) and low frequency signals that have dominant frequencies under 1000Hz. These latter sounds are signals where the air bladder plays some part (Tavolga 1971).

As we saw in the previous paragraph the air bladder can be associated with other sound producing mechanisms (example: stridulatory mechanisms), functioning as the resonant element of the apparatus. There are also several ways by which the air bladder can act as a sound producing mechanism by itself.

Air expulsion from the air bladder is one of the possible mechanisms of acoustic emission, for example the American common eal (*Anguilla rostrata*; Anguillidae) produces a bubbling ("put-put") sound by letting the gas escape from the air bladder, and through the pneumatic duct and branchial aperture (Fish et. al. 1952).

A very common sound production mechanism is the use of the air bladder as a drumming element. Usually this mechanism consists of a pair of muscles derived from the body wall or skeletal elements, that vibrate against the wall of the air bladder. The size and position of these muscles can be very variable even within the same family (Tavolga 1971). Artificial stimulation of the nerves that connect with the air bladder show that these muscles are capable of very rapid contractions and recovery cycles, and that they are also highly resistant to tetanization (Schneider 1967). The sonic muscles show a well developed sarcoplasmatic reticulum and have a high degree of vascularization which contributes to its fast acting properties (Tavolga 1971). Usually the fundamental frequency of the sound is a direct translation of the contraction frequency of the muscles (Schneider 1967). These contractions produce changes in the volume and pressure of the air bladder, making its entire surface pulsate. These pulses are transmitted through the body of the fish with little loss to the medium (Tavolga 1971).

These muscles can be devided in two types: extrinsic or intrinsic muscles. The first are contiguous to the lateral walls of the air bladder, but still attached to the walls of the body. An example of these types of muscles can be found in the family Sciaenidae (example, *Leiostomus xanthurus*). The typical sound producing mechanism of the members of this family is composed of two extrinsic muscles located on each side of the air bladder, these muscles originate on the central tendon overlying the dorsal surface of the air bladder and insert via connective tissue to the lateral wall of the body cavity (Hill et al 1987). The intrinsic muscles are completely attached to the air bladder, for example, the sea-robin (*Prionotus carolinus*, Triglidae) has a large bi-lobed air bladder with two muscles attached (one on each lobe) that extend from the anterior end of the lobe to the posterior end. The muscles adhere strongly to the underlying coat of the air bladder and are red in colour (Tower 1908, Schneider 1967).

The air bladder mechanism sounds usually have an harmonic structure and a tonal quality. In most of the fish species they consist of short pulses (20-100 ms) that can be emitted singly or in a train, with a fundamental frequency that can range from 75-100 Hz (Tavolga 1971).

However in some cases where sounds have been reported to have an air bladder quality, no specialised muscles where found. Some authors suggest that these sounds might be produced by contraction of non-specialised body wall muscles (see Tavolga 1971, Almada et al 1996).

Virtually any movement in water will produce particle displacement and compression waves. These types of waves are sub-sonic vibrations, predominantly generated by near field-effects, that can be intercepted by fish. Tavolga (1971) recognised three possible ways in which the locomotion of fish could produce pressure and displacement effects: the more and less rhythmic effects of ondulatory movement; turbulence generated by flow noise; and internally generated motor sounds produced by skeletal and muscular movement in the body of the animal.

The hydrodynamic sounds have a nonharmonic structure, with dominant frequencies below 100 Hz, with the standard pressure sensitive recording equipment one can only detect these sounds near the sound source or if the signal is generated at high intensity (Tavolga 1971).

1.3.2 Hearing in fish

In the last 30 years there has been a great deal of research on the hearing capabilities and hearing mechanisms in fish. Most of the studies where conducted on bony fish (class Osteichtyes) (for revisions see: Popper & Fay 1973, Hawkins & Myrberg 1983 and Popper and Fay 1993).

Fish hearing sensitivity can be graphically depicted in an audiogram. This type of graph consists on a curve that represents the thresholds or minimum sound levels (in dB *re* μbar) to which the fish responds for a given frequency value. Depending on the ability of the sound detecting mechanism the sound level can be expressed in sound pressure or in particle motion amplitude (Hawkins 1993). Most of the audiograms data, showed that fish can be highly sensitive to sound intensity (as low as 55 dB *re* 1 μPa (Popper & Fay 1973)), although this sensitivity is restricted to a limited frequency range. Even the best hearing fish are quite insensitive to sounds produced over 2 or 3 kHz (Hawkins 1993).

Some species appear to decode the sound spectrum using auditory filters, which may allow them to discriminate between pure tones of different frequencies. These filters may also be important in detecting a given signal from background noise, because the fish has the possibility of selectively tuning to a specific frequency (Popper and Fay 1993).

Another important feature of hearing in fish (at least in some species) is its apparent adaptation to decode and process acoustic information in the time domain. Some authors (see Popper and Fay 1993, Hawkins 1993) refer that temporal patterning recognition may play an important role in the detection, identification and classification of sound sources, and to decode the information contained in some features of fish vocalisations (example: individual recognition) (Popper and Fay 1993).

The anatomical, physiological and behavioural features of fishes are highly variable between and within the different groups. This is also true for the hearing system anatomy. This variation can suggest that different species may detect and process sounds in different ways, depending on their anatomy and acoustic conditions of their habitat (Hawkins & Myrberg 1983, Popper & Fay 1993).

The main sound receptors of fish are two paired structure located in the cranium called the inner ears. In general each structure consists of three semicircular canals and three otholit organs. These canals are arranged in an orthogonal geometry and are filled with a fluid called the endolymph. Each canal incorporates a bulbous expansion, called ampulla, which is occluded by a diaphragm (the cupula). Across the ampulla exists a saddle-shaped wall covered with mechanoreceptive hair cells (the crista) (Hawkins & Myrberg 1983). Each of the otholit organs (the saccule, utricle and lagena) consist of an expanded sac linked with one another and to the semicircular canals. Inside each sac there is a dense calcareous structure named otholit that lays on top of a mechanosensory epithelium, the macula (Hawkins & Myrberg 1983). This epithelium contains sensory hair cells very similar to those found in the lateral line of fish and the ears of other vertebrates. These cells form different groups in the macula depending on its hair orientation and each individual cell is directionally sensitive to motion stimuli (Popper & Fay 1993). In the utricle the macula lies usually on the horizontal plane, while in the saccule and lagena it lies on the vertical plane (Hawkins 1993). As the otholits are set in motion on top of the macula, different orientated sensory hair cells from the three otholit organs of both ears are stimulated, providing the central nervous system with information about direction or pathway of motion (Popper & Fay 1993).

Regarding to the type of hearing mechanisms, fishes can be classified as specialists and nonspecialist or generalists. This classification considers the degree of structure modification and specialisation of the hearing apparatus. The Otophysans (superorder Otophysi) are one of the best well studied groups of hearing specialists. These fish posses a well defined structure linking the inner ears to the air bladder, called the Weberian ossicles. The expansion or contraction of the air bladder induces motion on the ossicles, this motion by its turn causes the perilymph of a small sinus to flow. This structure is connected to the semicircular canals and transmits the motion in to the endolymph (Hawkins & Myrberg 1983).

Another degree of specialisation is present in the cupleiform fish, where the air bladder is coupled to the inner ear by a pair of pre-optic bullae. Each bullae is divided in a gas and liquid chamber, the first is connected with the air bladder, while the other is filled with perilymph and connects with the labyrinth. The liquid chamber is also linked to the lateral line canals. When the air bladder suffers a change volume, the gas filled chamber of the bullae also changes its volume and sets the perilymph in motion. This motion is then transmitted to the endolymph of the inner ear (Hawkins & Myrberg 1983).

There are also other species that do not have any connection between the air bladder and the ear, these are called nonspecialists. In these fish sound detection is made by the analysis of particle motion and acceleration (the otholits are sensible only to particle motion), while in the specialist species where the ear is connected to the air bladder, fish can detect also sound pressure change (Hawkins & Myrberg 1983).

1.3.3 Underwater communication

Acoustic signalling has evolved in several groups of animals, and in different environments. Hawkins and Myrberg (1983) suggest that sound communication has several advantages: acoustic signals are flexible, a variation in the frequency structure and temporal pattern can produce a large number of signals working with only a few basic elements; it propagates in all directions; its transmission is very fast and may travel great distance. Acoustic signals may be advantageous also when the animal lives in an environment where light is scarce and visibility is low (Hawkins 1993). However there may also exist some drawbacks in acoustic signalling, for example, sounds are easy to detect and the signaller might be exposed to signal interception by predators or by rival individuals; the calls may also suffer a great degree of interference from noise or obstacles in the environment (see chapter 2.1.2).

In almost every study of sound production in fish, sounds were recorded when a fish interacted with other individuals (of the same or different species) and it was assumed that these sounds had a communication value (Hawkins 1993). These authors have been following an operational definition of communication, which states that: a communication process occurs when a specially designed modification in the environment (signall) caused by one individual (emitter) elicits a modification on other individual's (receiver) behaviour. This signalling behaviour results in a higher fitness for the individual that emits the signal (Hawkins & Myrberg 1983, Hawkins 1993, Krebs & Davies 1993).

As it was briefly discussed in the beginning of this chapter, fish produce acoustic emissions during different social contexts. These emissions often peak during the reproductive season when interactions between individuals are more frequent (Winn 1964).

The production of sound during a stressful situation is very common in fishes, for example, the oyster toadfish (*Opsanus tau*, Batrachoididae) produces a bout of grunt like signals when handled out of the water or when a strange object is introduced in the nest (Gray & Winn 1961). Whether these signals are of alarm or defence against other individuals or predators is still not well understood (Tavolga 1981).

Sounds are also produced in association with reproductive behaviour, the value of these signals in this context and its relation to visual displays is still not well known. In the majority of the studied species sounds related to reproduction are only produced by males (Winn 1964, Hawkins & Rasmussen 1978, Tavolga 1981, Hawkins & Myrberg 1983, Hawkins 1993, Brantley and Bass 1994). Ladich (1989) suggests that sounds may act as an advertisement feature of male individual quality or resource ownership, used in female choice. As it was mentioned before, sound production has associated costs to the emitting individual: the calls may be detected by predators or rival males, and it involves energetic costs that otherwise could be used in other activities. This author also proposes that in some cases the male may use sound production just before spawning, as a mechanism to ensure egg and sperm synchrony release with its spawning mate. Hawkins (1978) found that in the male Haddock (*Melanogrammus aeglefinus*, Gadidae) courtship display, there was a close association between the rate at which knock sounds were produced and the closeness of the behaviour to the spawning climax.

Torriceli *et al* (1986) suggested that the coupling of visual and acoustic displays may provide a more conspicuous display, acting as an element of redundancy and reduce the chance of behaviour misinterpretation.

Threat displays and other types of aggressive behaviours may also be accompanied with the production of sounds. Most of these sounds and behaviours are associated with territorial behaviour (Hawkins 1993). There is evidence that acoustic signals may be used as reliable signals for assessing motivation and/or fighting abilities of opponents. For example, in many species calling frequency is highly correlated with body size, success of agonistic encounters and territory size (Ladich 1990). Myrberg et al (1993) showed that in the bicolour damselfish (*Pomacentrus partitus*, Pomacentridae), the peak frequency of the chirp sound that is emitted in aggressive encounters, can be used as a predictor of body size, with larger males having a lower peak frequency value. This author also proposes that the fish may use this as a mechanism of individual recognition that can be used in future agonistic encounters. The recognition process within members of the colony would be promoted by the exposure to the neighbours acoustic emissions and correlated actions.

It is common that one species has different types of signals that are used in different behavioural contexts. For example, the haddock (*Melanogrammus aeglefinus*, Gadidae) produces at least two acoustic emissions, one associated with aggression and the other with courtship behaviour. These sounds have also distinct sound duration and repetition rates (Hawkins & Rasmussen 1978).

In some species the male has a better developed sonic apparatus than females, which may not produce sounds at all. In the scaenid, *Cynoscion regalis* (weakfish, Sciaenidae) drumming muscles are only present in the males, while in *Micropogonias undulatus* (another sound producing sciaenid) females posses sonic muscles, but they are less developed than in males (Hill *et al* 1987). Hill et al (1987) observed that the differential development of the sonic muscles in the two sexes is correlated with gonadal maturation and hormone differential production. Other studies have related the differential development of the sonic apparatus with hormone production (Fine & Pennypacker 1986).

Some fish present a circadian cycle in sound production activity, with peaks generally at dusk, night or dawn (Winn 1964, Schneider 1967). Schneider (1967) showed that the drumming sound of the triggerfish (*Therapin jarbua*) were more frequent when light intensity corresponded to advanced twilight. Evolutionary pressures related to predation or ambient noise may determine patterns of sound production during the day to night periods (Endler 1993).

Fish are ectothermic animals, which means that a variation in the ambient water temperature also causes a modification of the body temperature of the animal. This change of body temperature may influence the contraction speed of the muscles involved in sound production, for example, Torriceli *et al* (1990) observed that sound duration decreased significantly with the increase of ambient water temperature.

1.4 Sound production in Toadfishes (Family Batrachoididae)

The batrachoidids are coastal benthic fishes of temperate and warm seas. This family has nineteen genera spread along the coasts of the Atlantic and Pacific oceans (Roux 1971). Toadfish can usually be found in shallow waters under rocks or boulders, which they use as shelters and nesting sites. Parental care is present in all of the studied species. The males occupy a

shelter in the beginning of the reproductive season, in which the females lay their eggs. Males will then guard the eggs until the young leave the nest (Gray & Winn 1961, Fine 1978, Brantley & Bass 1994).
In *Opsanus tau*, individuals migrate to shallow waters in the beginning of the breeding season (in winter time they stay in deeper waters). The eggs are laid in shelters usually attached to the ceiling of the shelter. The males are polygamous and may guard clutches of various females in the nest (Gray & Winn 1961). In another species from the Pacific coast, *Porichtis notatus*, this breeding system may be modified by the existence of two different types of males (Brantley & Bass 1994). Some of the males are large in body size, they occupy a shelter during the breeding period and take care of the eggs an young (type I male), while other males are small in size, do not hold a shelter during the spawning season and try to sneak in the type I males nest to fertilise the eggs.

The family Batrachoididae has long been known to include sound producing species. Tower (1908) was one of the first to describe the acoustic emissions and sound apparatus of the oyster toadfish *Opsanus tau*, an eastern coast batrachoidid of North America.
Two different types of sounds associated with distinct behavioural contexts, have been identified in the batrachoidid species: a long duration tonal sound, which is thought to be analogous in function to insect and frog mating calls and to bird song (Fine 1989); and an agonistic short sound usually produced during aggression or alarm contexts - grunt sounds.
In *Opsanus tau* and *Opsanus beta* the long duration tonal sound is called 'boatwhistle' while in *Porichtis notatus* the call is denominated as a 'Hum'. In this last species, the animal may sustain the call for at least thirteen minutes. These calls are usually produced only by males at the nest (Gray & Winn 1961, Brantley & Bass 1994), although in some species female boatwhistles could be evoked by electrically stimulating parts of the central nervous system (Fine 1979). Type II males of the specie *P. notatus* do not produce the hum sounds (Fluet & Bass 1990).

Male toadfish tend to form calling chorouses and in *Opsanus tau* individual males were found to respond to playback calls from other fish with an increase of calling rate (Winn 1967). The functional role of this call and its chorousing behaviour is still not completely understood. As noted above in this chapter one of the possible functions might be related to mate attraction, and because this kind of sound has low shallow-water propagation (Fine & Lenhardt 1983), males would group in a chorus to produce a higher global sound intensity so that females could localise them more effectively. Another hypothesis may be that this call results from male to male space competition and acts as a spacing assesment signal. This last hypothesis is reenforced by the observation that in *Opsanus tau* the fish space their nest in a precise matter (Winn 1967).

A circadian calling cycle is present in some species, *Porichtis notatus* has higher call production during the night period, with the males initiating call production at dusk (Brantley & Bass 1994). Fine et al (1977) found no calling cycle in *Opsanus tau*, although the authores agree that there still may exist maximal and minimal periods of sound production.

Seasonal variation of this type of call is also present in the males, with the fish starting to call in the beginning of the breeding period and decreasing at the end until it ceases a short period after. Fine (1978) also reported that in *Opsanus tau* some of the sound charecteristics may show some degree of variation during the breeding season; the fundamental frequency of the boatwhistle increases in the beginning of the breeding period and decrease at the end. The author suggests that temperature alone can not explain this pattern and sugests that other factors like periods of hormone production could be involved (Fine 1978).

The grunt sounds are thought to be agonistic or defensive signals. Gray & Winn (1961) observed that when a male or a strange object apeared in front of a male *Opsanus tau* nest, the fish would emitt a series of grunt call bouts. This reaction is more frequent when the male has eggs or young in its nest. These types of signals are produced by both sexes, but some degree of dimorphism (or even polymorphism) is found in some species. For example, the single grunts produced by female *P. notatus* have a longer sound duration than type II male grunts (females are larger in body size) (Brantley & Bass 1994).

The general sound producing mechanism of Batrachoidid fish consists of the presence of two intrinsic muscles attached to the wall of the air bladder. Toadfish have a large bilobed air bladder (which may occupy two thirds of the visceral body cavity), with each lobe connecting to each other in the mid-line. Internaly the two parts are united by an opening giving origin to only one large chamber (Fish & Mobray 1959).
On each side of the air bladder the lateral wall is covered by a thick muscle mass, that meet but not fuse posteriorly (Fish & Mobray 1959). Sounds are generated by simultaneously contracting both muscles at high frequency rate (Fine & Pennypacker 1988). These muscles are highly adapted to rapid contraction, they show a well developed sarcoplasmatic reticulum, ambundant parvalbumin and calcium, and specialized myosins (Fine 1989). Growth and enzyme activity of sonic muscles are androgen sensitive (Fine & Penneypacker 1986, Walsh et al 1989), and the males have a higher growing muscle rate than females (Fine 1989). The sonic muscles are innervated by a large cluster of neurons in the caudal medulla and occipital spinal cord, the sonic motor nucleus (Fine 1979, Fine and Mosca 1995). Sounds have been evoked by stimulating a variety of sensory and motor sites in the toadfish brain (midbrain and medulla), both grunt sounds and boatwhistles seam to be produced from the stimulation of the same sites (Fine 1979). The motoneurons in the spinal cord are usually larger in males than in females (Fine & Pennypacker 1986), the degree of sexual dimorphism varies among toadfish species (Fine & Mosca 1995).
The toadfish air bladder also shows a dimorphic pattern (being larger in males) and its growth and dimensions are correlated with sonic muscle size (Fine & Penneypacker 1986). It appears that the primary acoustic function of the air bladder is to act as a device for impedance, matching the muscle contraction to water, rather than a ressonant amplifier used in hearing (Fine

1983). The absence of a connection between the air bladder and the internal ear suggests that hearing in toadfish is dominantly particle displacement sensitive (Fay & Popper 1993).

The hearing threshold of the toadfish was determined to range between 150 to 500 Hz, with the best hearing frenquency at 75 to 100 Hz (Fish & Offut 1971, Fine 1981). Unlike most other animals, toadfish present a mismatch between the matting call production and its hearing. The fundamental frequency of grunts (90 - 100 Hz) match the fish's most sensitive auditory region, while the boatwhistle frequency does not directly correspond to the tuning of its most sensitive neurons. Instead they posess a secondary tunning around 200 Hz, that can aid boatwhistle detection.

The biologically useful harmonics whithin a call may vary a lot depending on the conditions when the call is emitted, if the toadfish showed a frequency domain detection of the sounds, it had to be capable to respond to that variation. A more simpler system is the detection of sounds based on its more reliable temporal cues, this seams to be what the toadfish does when detecting the boatwhistle call (Fine & Lenhardt 1983).

Halobatrachus didactylus (Schneider, 1801) is an eastern Atlantic toadfish, with a distribution ranging from the Gulf of Vizcaya to the Coast of Ghana, including the Madeira and Açores Islands, Western Mediterranean and Adriatic Sea (Roux 1977, Roux 1986). This species is also benthic and individuals are usually found in sandy or muddy bottoms, under rocks or tiles. In Portugal (Lopes da Cunha 1994) and Spain (Gonzalez De Canales et al 1991) the breeding season ranges from May to June. The eggs are demersal and they are attached to the roof of the shelter. Male *Halobatrachus didactylus* care for the eggs and young. Both sexes have an assymetrical bilobed air bladder with intrinsic sonic like muscles. There is some degree of sexual dimorphism in the size of the body and air bladder (Teresa Modesto, personal communication).

The breeding ecology of this species has still not been studied in detail, and although it was known that this specie might produce sound, no sound production description has ever been made.

The objectives of this study are: to determine if *Halobatrachus didactylus* produce sounds and describe its acoustic repertoir; comparison of call features between males and females; Comparison of the calls frequencies between day and night period; and analisys of the call interaction, in the context of chorus behaviour, between two individuals.

2. METHODS

2.1 Sound recordings

2.1.1 Field recordings

The study areas were located at Ria Formosa and the Guadiana estuary (37º 10' N, 7º 24' W), on the southern cost of Portugal, where the north-western Atlantic toadfish, *Halobatrachus didactylus*, is an abundant species. At Ria Formosa, a sea water lagoon complex, two sites were chosen: one near the docks of Olhão (37º 01' N, 7º 49' W) and the other near the islet of Faro (37º 00' N, 1º 59' W). Substrate at all three sites was mud and fine sand, with loose rock barriers. The tosdfish find shelters in these rock aggregations.

The recordings were made between 17 and 24 July 1996. Each site was sampled twice, in sessions of 15 to 60 minutes duration; once in daytime, and once at night, from the same shore-based location. Six TDK DA-R60EB tapes were used during these field sessions, recording a total of 219 minutes of signals.

Two sets of equipment were used: a Brüel & Kjær 8101 hydrophone (piezoelectrical ceramics) with a Sony DAT recorder TCD-D10 Pro, and a Sea Systems 200/K hydrophone (polymer-based) with a Sony TCD-D7. Both Systems had a flat frequency response (± 1 dB) between at least 50 Hz and 22 kHz.

In order to verify that the toadfish was the source of the sounds recorded, a dive was made simultaneously with a recording session.

2.1.2 Laboratory recordings

Some further recordings were made at the University of the Algarve, in tanks with isolated toadfish of known sex and length, captured at Ria Formosa. Nineteen males and nineteen females were studied. The hydrophones were placed inside the 1 m^3 fibber-glass tanks, with a sand bottom and half flower pots as shelters. Each fish was carefully held and softly massaged in the ventral area to stimulate sound emissions. Aeration was stopped at the beginning of each recording session.

The equipment used for these recordings was the Sea Systems 200/K hydrophone with a Sony TCD-D7 DAT tape recorder.

2.2 Analysis

2.2.1 Sound classification and description

All six tapes were first aurally indexed according to the different types of sounds that the listener could distinguish, and the background noise level of the recordings was noted. From each of the tapes only the signals with higher signal-to-noise ratio sound intensity and with no overlapping of other acoustic emissions (e.g. boats or a different type of call) were selected for analysis. The sounds were analysed using the Canary 1.2 software (Cornell University) on a PowerMacintosh 7100 computer, using a sampling frequency of 11.1 kHz (Nyquist frequency, 5550 Hz).

In order to perform the measurements the signals were first graphically depicted in time (waveform) and frequency (spectrogram) domain representations (Filter bandwidth 1411.78 and 176.47 Hz; Grid resolution 10.87 and 43.47 Hz). The sounds were classified in 5 types or groups: Boatwhistle, Grunt, Croak, Mixed grunt/croak and Double croak.

To describe each type of acoustic emissions the following features were measured:

Boatwhistle parameters:

Sound duration (ms)
Number of pulses
Pulse rate (Number of pulses per second)
Pulse duration (ms)
Pulse peak-to-peak interval (ms)
Peak frequency (Hz)

The last three parameters were measured in three different stages of the call: third pulse from the beginning of the sound emission; middle of the signal (first complete pulse from the middle point of the call, Sound duration × ½); and third pulse counting from the end of the call. Differences were tested with a non-parametric Friedman-Anova test (Siegel & Castellan 1988).

Grunt parameters:

- Bout duration (ms)
- Number of Grunts
- Grunt duration (ms)
- Interval between Grunts (ms)
- Number of pulses
- Pulse duration (ms)
- Pulse peak-to-peak interval (ms)
- Peak frequency (Hz)

Pulse duration and peak frequency were measured on the 1st pulse of the signal. Pulse peak to peak interval was measured between the 1st and 2nd pulse.

Croak parameters:

- Sound duration (ms)
- Number of pulses
- Pulse duration (ms)
- Pulse peak-to-peak interval (ms)
- Peak frequency (Hz)

Pulse duration and peak frequency were measured on the 2nd pulse of the signal. Pulse peak to peak interval was measured between the 2nd and 3rd pulse.

Mixed grunt/croak parameters:

Sound duration (ms)
Grunt-croak interval (ms)

Croak portion

For each croak of the Mixed grunt/croak sound the features measured were the same as in the Croak sound with the adition of number of croaks.

Grunt portion

For the grunt part of the Mixed grunt/croak sound the features measured were the same as in the Grunt sound.

Double croak parameters:

Sound duration (ms)
Interval between the 1st and 2nd croak (ms)

For each croak from the Double croak sound the parameters measured were the same as in the Croak sound.

In all types of sound the fundamental frequency was measured, in the third pulse of the signal.

For all of the 5 types of sound a descriptive statistics table presenting the average, maximum, minimum and standard deviation values, was constructed.

In order to confirm the classification of the above mentioned sound types a discriminant analysis (Manly 1994) was run using the following variables: sound duration, number of pulses, pulse duration, pulse peak-to-peak interval and peak frequency. The grunt and croak signals of Mixed grunt/croak calls, were introduced in the analysis as different signals in order to see if they would be classified as the same signals as the grunts and croaks from grunt and croak calls. After the discriminant analysis it was performed a Kruskal-Wallis test followed by a post-hoc Dunn test, to test which groups each variable differentiates (Glantz 1996).

To determine if the 3 stages of the boatwhistle were different a Friedman ANOVA test (Siegel & Castellan 1988) was applied to each of the three following variables: Pulse duration, Pulse peak-to-peak interval and Peak frequency.

2.2.2 Differences between the sexes

During fish manipulation in the laboratory, only grunt like sounds were recorded. Ten of these signals were randomly chosen from each individual's call bout and the following features were measured: signal duration (ms) and Peak frequency (Hz). signal duration measurements should not be taken as precise values due to possible reverberation in the tank. It also was not possible to measure other sound parameters (for example pulse duration or even number of pulses) because of the unclear signal structure generated by the acoustic field during sound production in a small tank (Parvulesco 1967). In spite of these constraints one can use this data to compare between the two groups if it is assumed that both of them suffered the same sound interference, and that differences will prevail.

A descriptive statistics was applied to each individual in order to determine the median value of the parameters. The median values were then grouped by sex, and a Mann-Whitney U test (Siegel & Castellan 1988) was used to test if the measured features were different between the sexes.

To investigate if the size of the fish influenced the measured parameters, a Pearson correlation (Glantz 1996) was computed between the different features and the total length of the fish.

2.2.3 Differences in sound production between day and night periods

From each study site and day/night time field recordings, a 10 minute periods of sound recordings was chosen. Following the previous classification, these selections were aurally and visually analysed (waveform representation), in order to count the number of grunts, croaks, double croaks and mixed grunt/croak signals produced during the different periods.

Differences between day/night-time for each study site, were statistically analysed with a χ^2 goodness-of-fit test (Siegel & Castellan 1988).

2.2.4 Call-timing between two individuals in a Toadfish chorus

The objective of this analysis is to investigate if an individual adjusts the timing of its own calls in relation to those of neighbours. For example, one can test whether the timing of one individual's call is non-random relative to the timing of the neighbours call (Klump & Gerhardt 1993).

A straightforward way to do this analysis is to measure the time delay (A-B and B-A, ms) between an individual own call and the preceding calls of the neighbour, and demonstrate that the data follows a non-random distribution, with overlapping or non-overlapping calls (Klump & Gerhardt 1993).

This analysis was run using a 6 minute period from Olhão daytime tape. On these recordings one could identify two individuals by observing the waveform of the signal.

The following features were measured: inter-call interval (A-A and B-B; ms), and between individuals call latency (A-B and B-A; ms).

In order to perform this type of analysis the following assumptions were made:

a) The boatwhistle sound is an advertisement call produced by nesting males.

b) Both of the selected individuals had the same sound pressure intensity therefore it was assumed that they were the nearest neighbours.
c) The constant time delay between the neighbour's call and the individual's own call is not the result of a random but constant phase difference between the two uncoupled periodic-call oscillators.

All statistical tests were applied using the computer software Statistica 5.0 for Windows, StatSoft, Inc. 1995. The Kruskal-Wallis and Dunn tests were applied using the statistics program Primer of Biostatistics software (v. 4; Glantz 1996).

3. RESULTS

3.1 Sound classification and description

The recorded sounds were aurally and visually classified into 5 categories or groups: boatwhistle, grunt group, croak, double croak and mixed grunt/croak call. The results from the discriminant analysis confirm this classification (see Table 3.1 and figures 3.1.a and 3.1.b).

	Classification Functions Coefficients			
Variables	Grunt	Croak	Double Croak	Boatwhistle
Duration	-0.0903	-0.1182	0.0994	0.4480
Number of pulses	1.3425	3.0445	1.0124	1.8080
Pulse duration	0.5568	-0.0971	1.7736	3.9020
Pulse peak-peak interval	22.2751	21.5937	19.1993	46.2830
Peak frequency	0.0234	0.0288	0.0289	0.0234
Constant	-86.2242	-98.1651	-94.5431	-609.5060

Table 3.1 - Discriminant analysis results.

The analysis correctly classified 98.2 % of the cases (Wilk's $\lambda = 0.00094$, F (15,132) = 101.82, $p < 0.001$). The criteria that best accomplished sound discrimination were sound duration, pulse peak-to-peak interval and number of pulses.

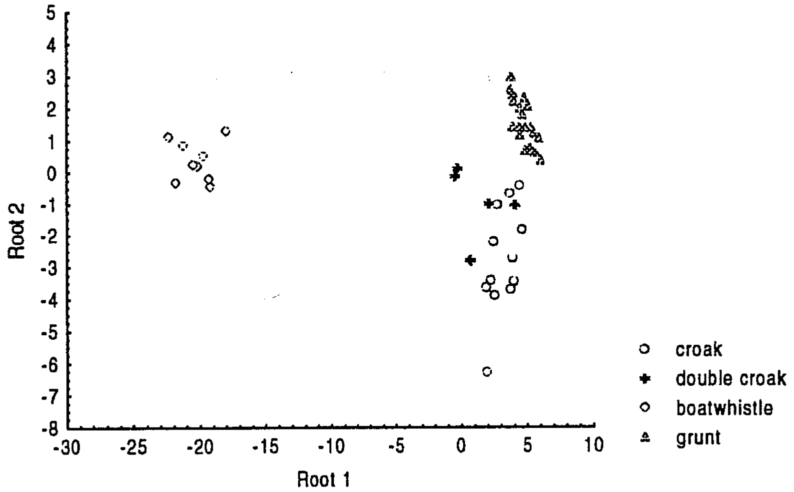

Figure 3.1.a - Plot of four acoustic signals against their values for two canonical discriminant functions: Root 1 vs. Root 2.

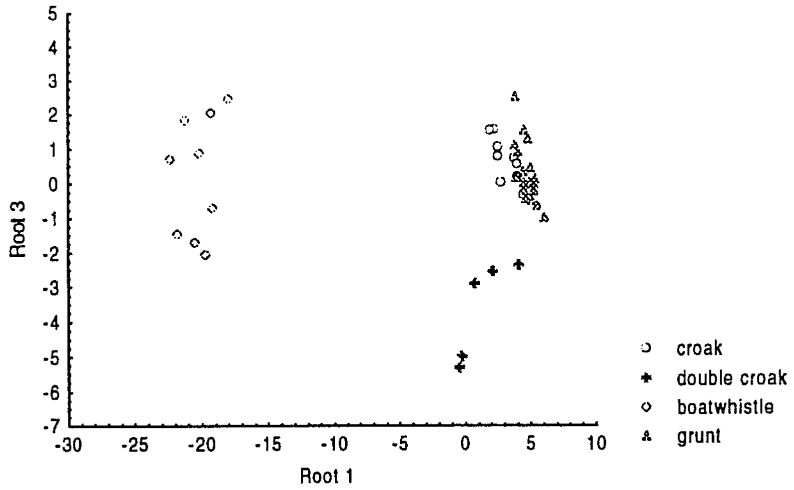

Figure 3.1.b - Plot of four acoustic signals against their values for two canonical discriminant functions: Root 1 vs. Root 3.

Sound spectrograms and descriptive statistics tables of the signal parameters are presented in figures 3.2 - 3.7 and tables 3.2 - 3.6.

The boatwhistle call (figure 3.2) is a relatively long sound emission, ranging from 434 to 790 ms (see table 3.2), with a pulsed structure and low frequency tone (peak frequency: $\bar{x} = 184$ Hz, N = 21).

Boatwhistle - parameters	N	mean	s.d.	min.	max.
Sound duration (ms)	21	647,6	113,9	433,9	789,8
No. of pulses	21	44,7	6,9	31	54
Pulse rate (no. pulses per second)	21	68,4	5,5	54,4	78,5
Pulse duration (ms)	33	11,2	2,4	6,6	16,2
Pulse peak-peak interval (ms)	33	13,6	2,8	8,3	18
Peak frequency (Hz)	52	183,9	87,9	74	460
1st Stage					
Pulse duration (ms)	11	12,7	2	9,6	16,2
Pulse peak-peak interval (ms)	11	16,6	0,8	15,5	18
Peak frequency (Hz)	16	157,7	52,3	87	240
2nd Stage					
Pulse duration (ms)	11	12	1,7	9,7	14,3
Pulse peak-peak interval (ms)	11	13,8	0,8	12,7	14,9
Peak frequency (Hz)	17	218	98,3	87	370
3rd Stage					
Pulse duration (ms)	11	8,9	1,6	6,6	11,8
Pulse peak-peak interval (ms)	11	10,2	1,2	8,3	12,2
Peak frequency (Hz)	18	180,4	97,1	74	460

Table 3.2 - Descriptive statistics of the measured features used to describe the boatwhistle call.

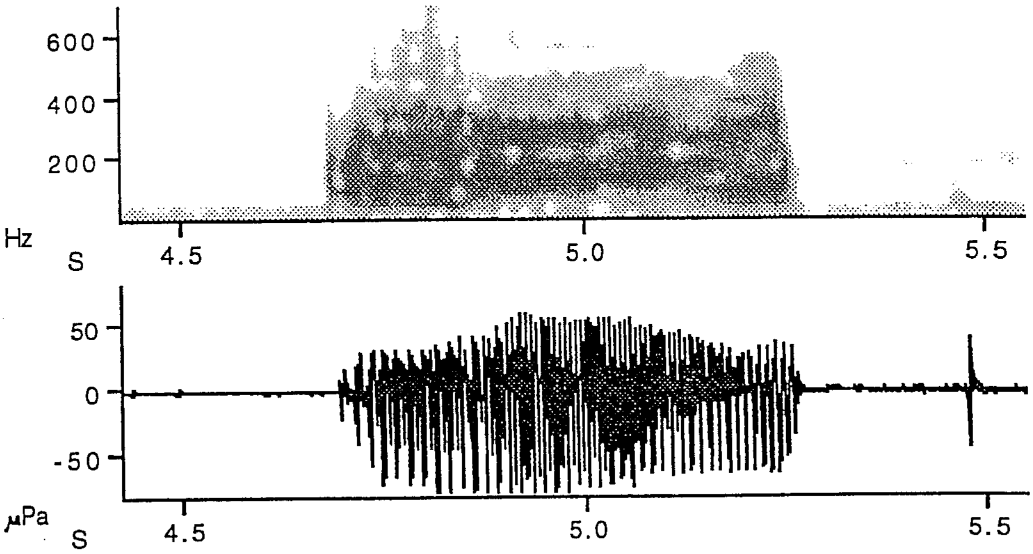

Figure 3.2 - A representative sample of a boatwhistle call. The sound spectogram was made with Canary 1.2. recording parameters: 11.1 Khz sampling frequency, 8 - bit sampling. Analysis parameters: FFT, hamming analysis window; frequency resolution: 43.47 Hz / 256 points filter bandwith; grid resolution: 11.5 ms, 50% overlap, 176.47 Hz / 256 points.

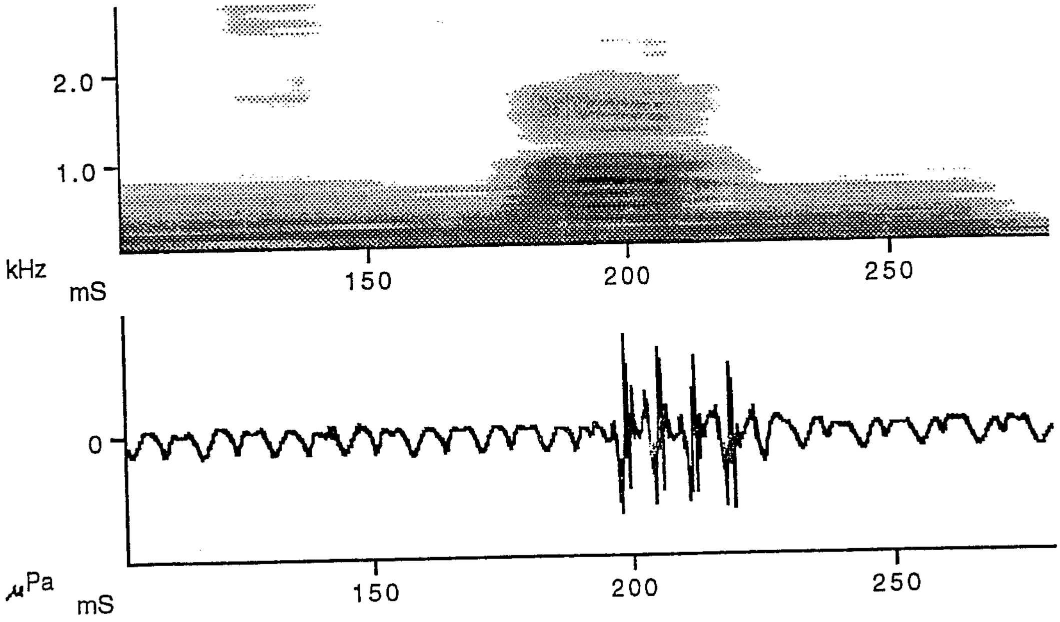

Figure 3.3 - A representative sample of a single grunt call. The sound spectogram was made with Canary 1.2. recording parameters: 11.1 Khz sampling frequency, 8 - bit sampling. Analysis parameters: FFT, hamming analysis window; frequency resolution: 43.47 Hz / 256 points filter bandwith; grid resolution: 11.5 ms, 50% overlap, 176.47 Hz / 256 points.

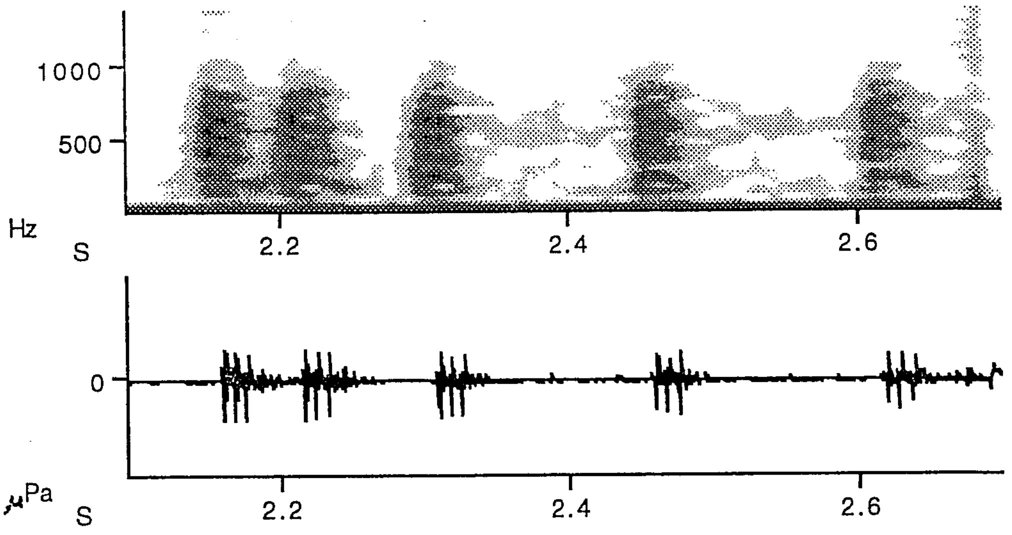

Figure 3.4 - A representative sample of a grunt bout call. The sound spectogram was made with Canary 1.2. recording parameters: 11.1 Khz sampling frequency, 8 - bit sampling. Analysis parameters: FFT, hamming analysis window; frequency resolution: 43.47 Hz / 256 points filter bandwith; grid resolution: 11.5 ms, 50% overlap, 176.47 Hz / 256 points.

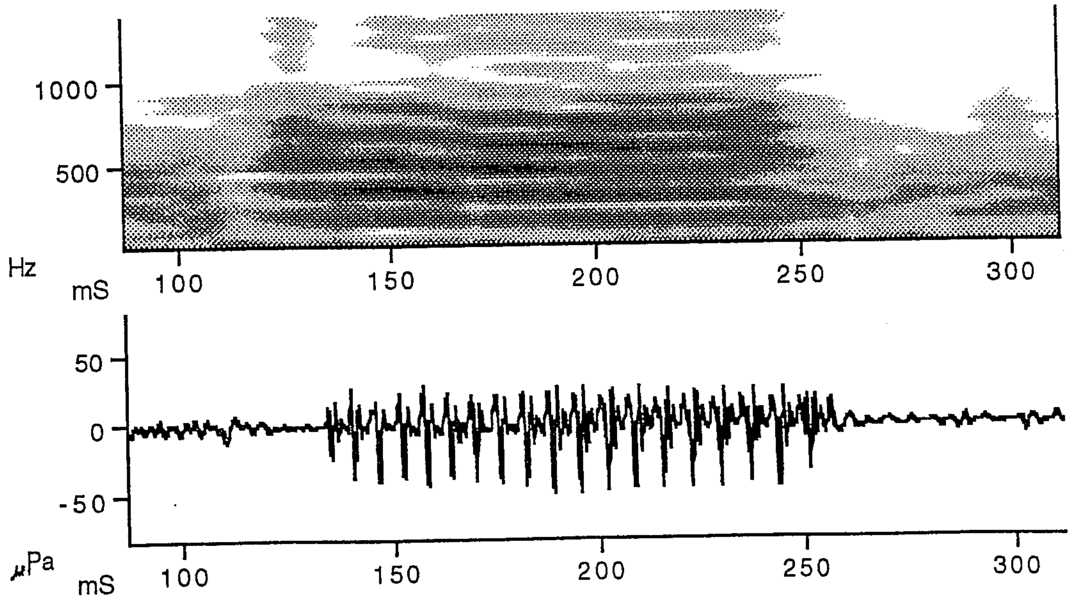

Figure 3.5 - A representative sample of a croak call. The sound spectogram was made with Canary 1.2. recording parameters: 11.1 Khz sampling frequency, 8 - bit sampling. Analysis parameters: FFT, hamming analysis window; frequency resolution: 43.47 Hz / 256 points filter bandwith; grid resolution: 11.5 ms, 50% overlap, 176.47 Hz / 256 points.

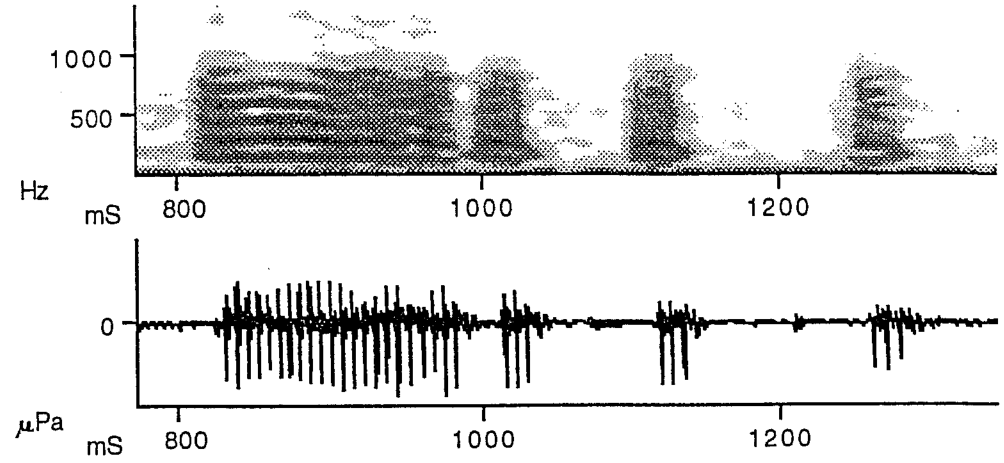

Figure 3.6 - A representative sample of a mixed grunt/croak call. The sound spectogram was made with Canary 1.2. recording parameters: 11.1 Khz sampling frequency, 8 - bit sampling. Analysis parameters: FFT, hamming analysis window; frequency resolution: 43.47 Hz / 256 points filter bandwith; grid resolution: 11.5 ms, 50% overlap, 176.47 Hz / 256 points.

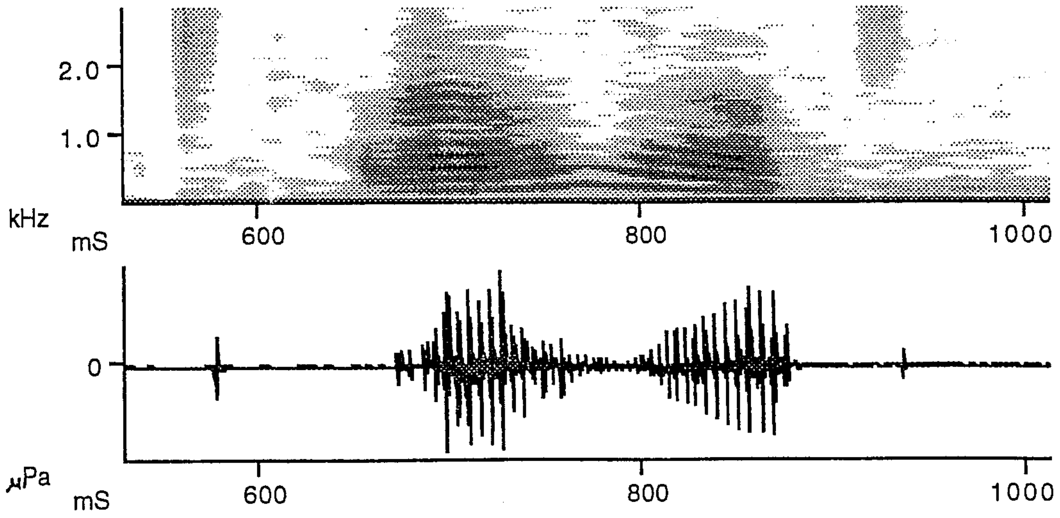

Figure 3.7 - A representative sample of a double croak call. The sound spectogram was made with Canary 1.2. recording parameters: 11.1 Khz sampling frequency, 8 - bit sampling. Analysis parameters: FFT, hamming analysis window; frequency resolution: 43.47 Hz / 256 points filter bandwith; grid resolution: 11.5 ms, 50% overlap, 176.47 Hz / 256 points.

This signal can be emitted by the same individual in a rather regular rhythm, that can range from 4 to 11 calls per minute (in htree identified individuals). In a more detailed analysis of this type of call, three different stages can be detected: beginning, middle and end of the signal.

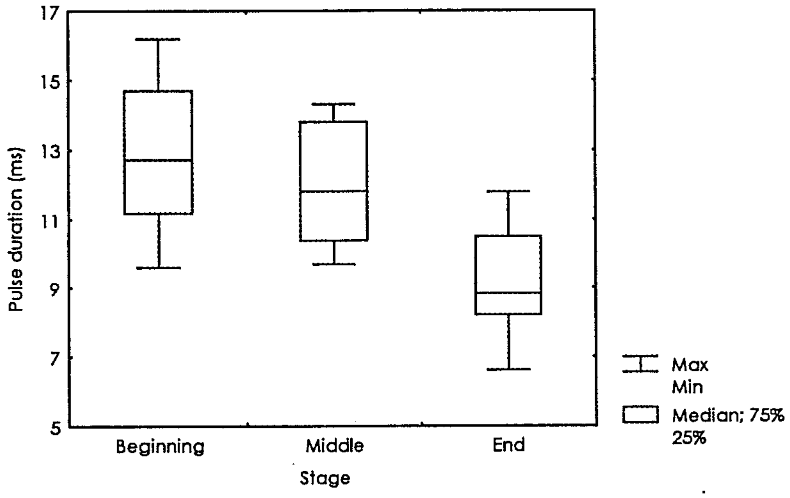

Figure 3.8 - Pulse duration of three different stages of the boatwhistle call.

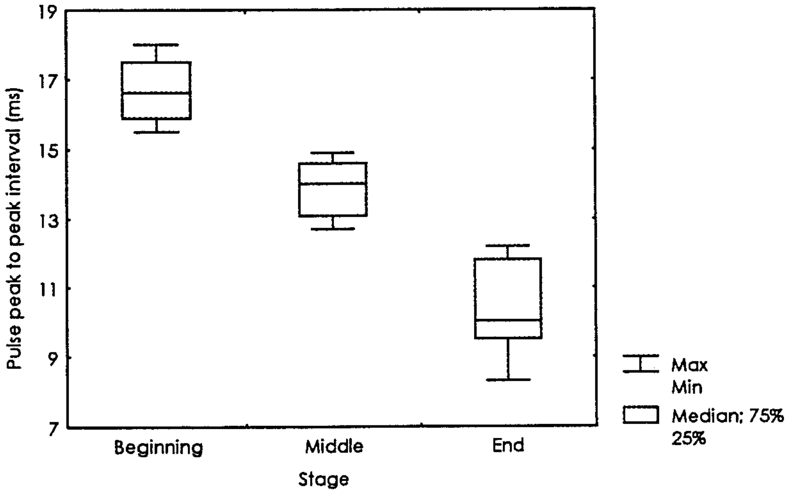

Figure 3.9 - Comparison of the pulse peak to peak interval values measured on three different stages of the boatwhistle call.

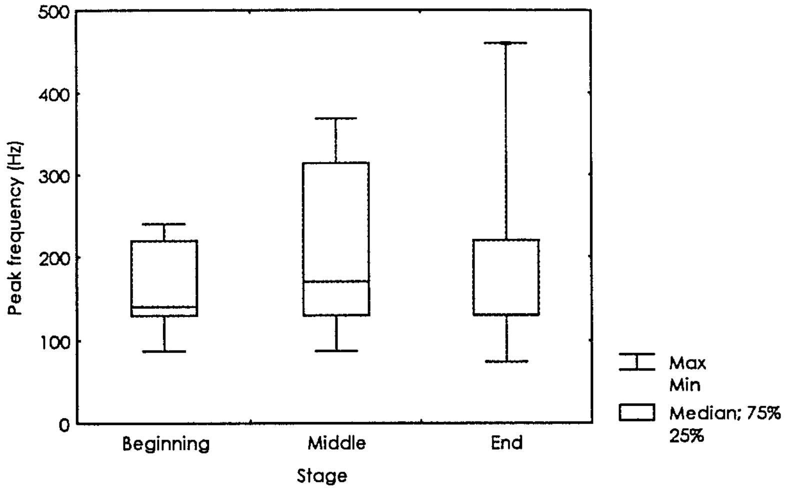

Figure 3.10 - Comparison of the peak frequency values measured on three different stages of the boatwhistle call.

Each one of these stages is characterised by differences in pulse duration, pulse peak to peak interval and peak frequency (Friedman-Anova N = 11, d.f. = 2, $p < 0.01$, $p < 0.001$, $p < 0.05$, respectively, figures 3.8 - 3.10).

This type of call was observed during a diving session, with the fish at the entrance of a shelter.

The grunt group consist of short duration signals (single grunt $\overline{x} = 25.1$ ms, N = 11; Grunts from grunt bouts, x = 22.5 ms, N = 211) that can be either emitted singly (figure 3.3) or in bouts of 3 to 21 signals (figure 3.4). Grunt bouts have an average duration of 1600 ms (see table 3.4). The signal peak frequency is higher than the boatwhistle sound ($\overline{x} = 376.4$ ms, N = 167).

This type of call can be triggered by massaging the ventral area of the fish.

Single Grunt - parameters	N	mean	s.d.	min.	max.
Sound duration (ms)	13	25,1	4,7	18,4	34,2
No. of pulses	13	3,8	0,7	3	5
Pulse duration (ms)	13	4,3	0,9	2,7	5,8
Pulse peak-peak interval (ms)	12	6,7	0,5	5,7	7,8
Peak frequency (Hz)	13	376,9	198,8	130	740

Table 3.3 - Descriptive statistics of the measured features used to describe the single grunt.

Grunts - parameters	N	mean	s.d.	min.	max.
Bout duration (ms)	19	1607,3	1167,4	233,7	3932
No. of Grunts	19	12,6	8,3	3	29
Grunt duration (ms)	211	22,5	7,7	3,2	45,7
Grunt interval (ms)	193	116,4	80,7	5,4	659
No. of pulses	107	2,4	0,9	1	4
Pulse duration (ms)	104	4,2	1,2	1,8	7,9
Pulse peak-peak interval (ms)	87	7,5	0,7	4,2	8,8
Peak frequency (Hz)	167	376,4	230,2	87	1300

Table 3.4 - Descriptive statistics of the measured features used to describe the grunt calls.

The croak sounds (figure 3.5) are single signals, with a rapid pulse-train structure. This call type has an intermediate duration, relative to the last two call types $\bar{x}$ = 110.1 ms, N = 8). Its pulse structure is similar to the single grunt calls (see tables 3.3 and 3.5), although differences in pulse peak-to-peak interval can be detected (Dunn's Test: Q = 3.75 $p < 0.05$).

Croak - parameters	N	mean	s.d.	min.	max.
Sound duration (ms)	8	110,1	39,7	72,1	196,9
No. of pulses	8	17,5	5,9	11	30
Pulse duration (ms)	8	3,8	0,7	2,7	4,6
Pulse peak-peak interval (ms)	8	6	0,4	5,6	6,9
Peak frequency (Hz)	8	647	448,4	170	1350

Table 3.5 - Descriptive statistics of the measured features used to describe the croak calls.

The grunt/croak mixed call (figure 3.6) is an arrangement of two different types of signals: a bout of grunt signals preceded or followed by one or two croak signals. Each of the different type of signals in this call share the same pulse structure properties (see table 3.6), with similar pulse duration and pulse peak to peak frequency.

The double croak sound (figure 3.7) is a tonal acoustic emission with two distinct croak phases. These two signals have the same pulse structure and similar duration (see table 3.7). Although there is a difference in the peak frequency value between the two phases, (which is aurally perceived), no statistical significance was encountered (Mann-Whitney U test: N = 6 , z = -1.94, p = 0.053). Each of the croak phases have similar properties with the croak sound.

Mix - parameters	N	mean	s.d.	min.	max.
Sound duration (ms)	6	652,9	199,4	464,8	969
Croak/Grunt interval (ms)	7	31,9	22,6	7,9	80
Croak fase					
No. of Croaks	6	1,2	0,4	1	2
Croak duration (ms)	7	125,7	31,9	63,5	155,9
No. of pulses	7	18,3	5,2	9	23
Pulse duration (ms)	7	4,8	0,7	3,7	5,7
Pulse peak-peak interval (ms)	7	6,7	0,3	6,3	6,9
Peak frequency (Hz)	7	534,3	177,3	250	770
Grunt fase					
No. of Grunts	6	6,2	3	3	11
Grunt duration (ms)	37	24,5	8,9	2,3	43,8
Grunt interval (ms)	30	65,8	48,4	9,1	179,3
No. of pulses	37	3,4	0,9	1	5
Pulse duration (ms)	37	4,3	1,1	2,3	7
Pulse peak-peak interval (ms)	35	7	0,6	5,7	8,4
Peak frequency (Hz)	28	559,9	258,4	217	258,4

Table 3.6 - Descriptive statistics of the measured features used to describe the grunt/croak mixed calls.

Double croak - parameters	N	mean	s.d.	min.	max.
Sound duration (ms)	6	203,8	56,9	112,6	258,9
Total No. of pulses	5	27,4	5,7	20	34
1st/2nd fase interval (ms)	5	30,9	22,9	5,3	64,4
1st fase					
Duration (ms)	5	86,9	21,5	60,5	117,2
No. of pulses	5	14,8	11	21	3,8
Pulse duration (ms)	6	5,1	0,5	4,4	5,7
Pulse peak-peak interval (ms)	6	6,3	0,5	5,6	6,6
Peak frequency (Hz)	6	733,8	228,6	570	1170
2nd fase					
Duration (ms)	5	76,3	19,1	50,1	103
No. of pulses	5	12,6	3,4	9	18
Pulse duration (ms)	6	4,6	1,1	2,9	6,4
Pulse peak-peak interval (ms)	6	6,2	5,4	6,7	0,5
Peak frequency (Hz)	6	505	167,7	300	780

Table 3.7 - Descriptive statistics of the measured features used to describe the double croak calls.

All signals presented similar fundamental frequency values ranging from 76 Hz minimum (measured in a boatwhistle) and 240 Hz maximum (measured in a croak signal). Average values of the signals ranged from 133 Hz (boatwhistle) minimum and 175 maximum (croak).

3.2 Differences between the sexes

There is an overall significant difference of sound duration between grunts produced by females and males (Mann-Whitney U test: female N = 19, male N = 19, z = - 3.28, p < 0.01), with the male signals biased towards a longer duration (male and female median sound duration - 154.4 and 118.5 ms respectively, figure 3.11).

The two groups showed no significant differences in the call's peak frequency (Mann-Whitney U test: female N = 18, male N = 19, z = - 1.94, p = 0.052).

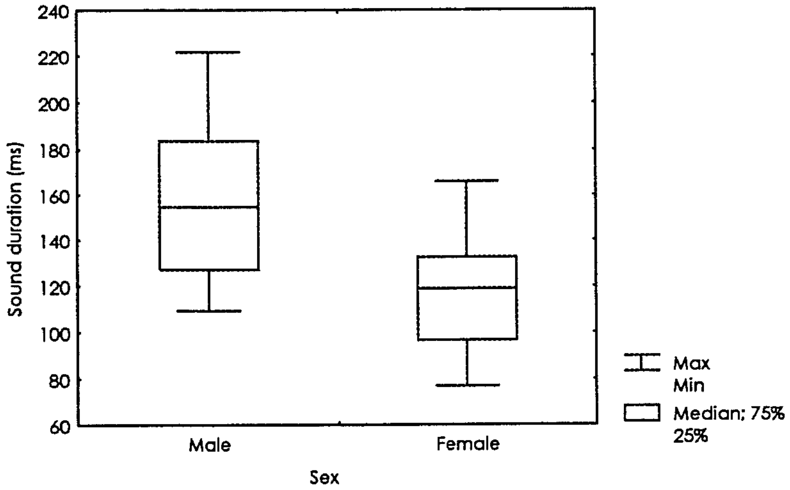

Figure 3.11 - Comparison of sound duration of male and female grunt signals recorded in a tank. Note that sound duration is larger than the grunts recorded in the field, this may happen because of tank wall reflection of the sound waves.

Although one could detect significant body length differences between the two sexes (Mann-Whitney U test: female N = 19, male N = 19, z = - 2.07, $p < 0.05$), no trend was found between any of the measured sound features and the body length of the fish (Pearson correlation: peak frequency, r = - 0.16, $p < 0.36$; and sound duration, r = 0.24, $p < 0.15$).

3.3 Difference of sound production between day and night periods

During this analysis and because of the aural difficulty in separating the croak signal from the grunt/croak mixed call, these two classes were merged together.

The double croak signals are emitted more frequently at night, although in the study site near the docks of Olhão no significant differences were found (see figure 3.12), probably because of the small sample size (N = 8). The other types of calls show no significant differences between both periods, with the exception of the grunt calls in the islet of Faro (Chi-square Goodness fit test: $X^2 = 7.25$, d.f. = 1, $p < 0.01$), where they were heard more frequently during daytime.

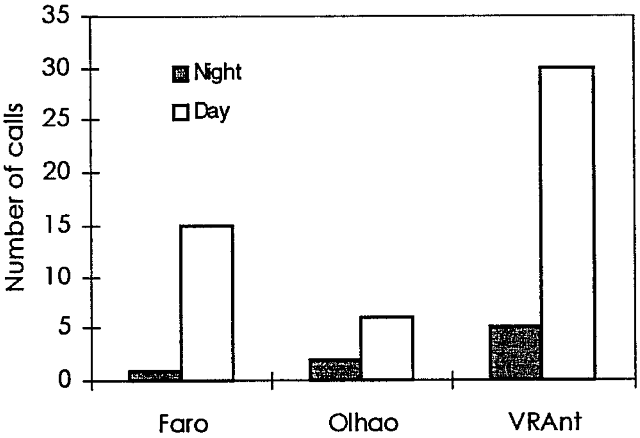

Figure 3.12 - Differences of the frequency emission of double croak calls between day and night periods, recorded at three different sites. Significant differences were observed in Faro (Chi-square Goodness fit test: $X^2 = 12.25$, d.f. = 1, $p < 0.001$) and VRAnt (Chi-square Goodness fit test: $X^2 = 17.9$, d.f. = 1, $p < 0.001$).

3.4 Call-timing between two individuals in a Toadfish chorus

Figures 3.13 and 3.14 show the call latency distributions for two neighbouring individuals. One can observe that no calls are given in the first 1100 ms after the onset of the neighbours call (call average duration 647.6 ms, N = 21).

The individual from figure 3.13 (individual B) presents a higher frequency of calls in the first 1100 to 2200 ms with a gradual decrease to longer latency periods. On the other hand, the response latencies of individual A (figure 3.14) show no particular high frequency class, with the calls ranging in similar frequencies from 1100 to 6600 ms.

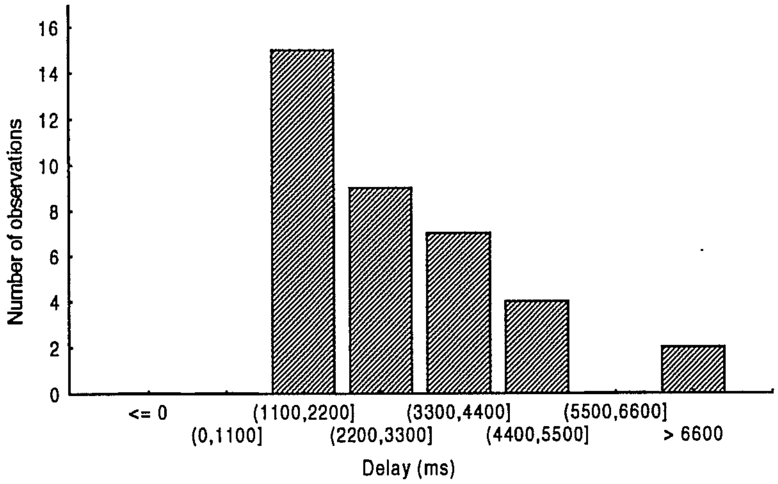

Figure 3.13 - The distribution of time intervals between the beginning of male A call and the beginning of male B call (time delay of male's B call).

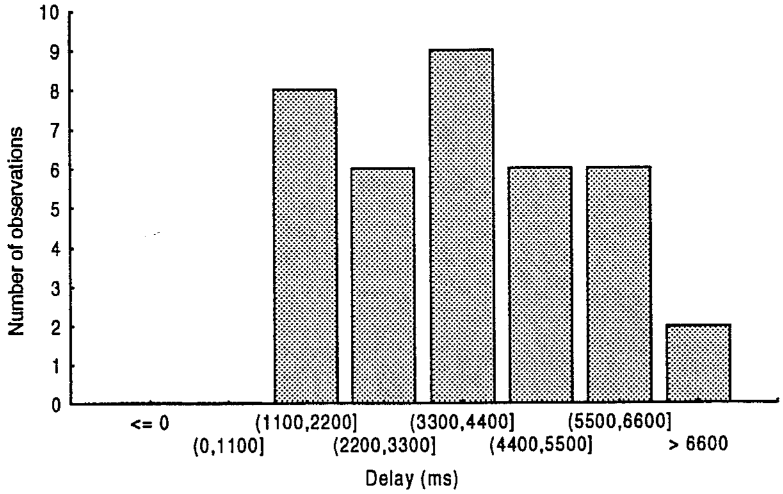

Figure 3.14 - The distribution of time intervals between the beginning of male B call and the beginning of male A call (time delay of male's A call).

4. DISCUSSION

Batrachoidids usually show a well developed acoustic repertoire, with at least two main types of calls related to different social contexts: mate attraction, and aggression (Fish & Mowbray 1959, Gray & Winn 1961, Brantley & Bass 1994). The present study in *Halobatrachus didactylus* shows that this species has acoustic emissions similar to other toadfish. It produces rhythmically a long tonal sound, similar to the boatwhistle and hum signals of *Opsanus tau*, *Opsanus beta* and *Porichthys notatus*, and a complex of sounds which include the grunt-like signals.

The boatwhistle sounds are similar in the various species of toadfishes: it posses a typical sound spectrogram image, with well defined frequency harmonics; and it is produced in the same behavioural context. These signals may have suffered some degree of modification in some species, for example *Porichthys notatus* has a very long sound duration compared to other toadfish mating signals (Bass & Andersen 1991). In *Halobatrachus didactylus* the boatwhistle appears to present three different stages defined by different temporal pulse structure, and frequency. Fine (1982) has also reported that in *O. tau* this sound begins and ends with a grunt like portion. The author also states that the functional significance of this signal structure is not well understood.

The behavioural context of the boatwhistle in *H. didactylus* is not clear, although the observation of an individual emitting this call in front of the shelter suggests behavioural similarities with the other toadfish species, were this call is used by nesting males in mate attraction or male-to-male nest spacing competition (Gray & Winn 1961, Winn 1967, Brantley & Bass 1994). Although in other species this sound is only produced by males, in this study this was not possible to determine, if this kind of sound is sexed.

The grunt signals are present in several species of toadfish, for example type I male of *P. notatus* produces grunts in singly or in regularly spaced sequences when another male approaches the nest (Brantley & Bass 1994). In both *O. tau* and *P. notatus* this sound has been identified as a display used in aggressive interactions. *H. didactylus* also produces single grunts and sequences of different grunt number, with variable inter-grunt interval. Behavioural context was not observed for this type of sound, however its acoustic similarities to the grunts produced by other toadfish may suggest that this might be an aggressive context signal.

In this study other types of sounds were identified: croaks, mixed grunt/croak calls and double croaks. The first type of signal has the same pulse structure as the grunts, but it presents a higher number of pulses and correlated sound duration (usually more than nine, compared with the maximum five pulse grunt). Also, this sound was never observed to be produced in bouts.

As the name suggests, the mixed grunt/croak calls is a complex sound with usually one croak signal followed or preceded by a sequence of grunt signals. The last type of sound is composed by two croak signals emitted very close to each other. This sound is peculiar since it presents distinct frequency upsweep in the first croak, and by an equivalent downsweep on the second signal. However this apparent signal form can also result from differences in pulse repetition, rather than frequency variation (Tavolga 1971). Further analysis is needed to determine which of the phenomena is responsible for its form.

The discriminant analysis of the sounds showed that there are four basic signals in *H. didactylus* acoustic activity. The boatwhistle is highly differentiated from the other types of calls, it possesses typical temporal pulse structure, number of pulses, and signal duration. The pulse of the boatwhistle sound presents a more complex form than the rest of the acoustic emissions. No differences were detected in pulse organisation between the other signals; this may suggest that they are based on the same acoustic unit (pulse). The different signals are formed by varying the number of pulses and their rate. These signals can then be emitted singly or can be arranged in sequences producing the different sounds (see Table 4.1). This pattern of pulse and signal organisation is manly based on the temporal arrangement and repetition of its pulses.

<table>
<tr><th>Pulse</th><th>Signal</th><th>Sound</th></tr>
<tr><td rowspan="6">Type I pulse</td><td rowspan="3">Grunt</td><td>Single grunt</td></tr>
<tr><td>Grunt bout</td></tr>
<tr><td rowspan="2">Mixed grunt/croak call</td></tr>
<tr><td rowspan="2">Croak</td></tr>
<tr><td>Croak</td></tr>
<tr><td>Double croak</td><td>Double croak</td></tr>
<tr><td>Type II pulse</td><td>Boatwhistle</td><td>Boatwhistle</td></tr>
</table>

Table 4.1 - Proposed classification for the recorded sounds of *Halobatrachus didactylus.*

Different authors have stated that in fish acoustic communication, the information is usually encoded in a time scale rather than in frequency variation (Myrberg 1981, Hawkins & Myrberg 1983), particularly in non-hearing specialist species (Popper & Fay 1993). If *H. didactylus* is able to discriminate between the different sounds and to respond differentially to them, these data appear to support the hypothesis that this species also uses time domain signal variation to encode the information.
It was not possible to determine whether this classification has any functional significance, because of the absence of simultaneous observations of behavioural context and sound production.

In toadfish some degree of sexual dimorphism in body size and sound production can be found (Fine 1989, Brantley & Bass 1994). The fish used in this study also showed sex differences in the body size with the males being larger than females. The sounds produced by the captive *Halobatrachus didactylus* were similar to the grunt bouts recorded in the field. Gray & Winn (1961) also observed that when the oyster toadfish (*Opsanus tau*) was handled, it produced grunt-like signals, and they suggested that these sounds could be some kind of alert or defence signals.

In *O. tau* intrinsic muscle contraction rate and not bladder resonance determines the fundamental frequency and harmonic interval of the sound. Fine (1983) stated that the principal frequencies of the grunt signal where not related to fish body size. This study produced similar results, since no correlation were found between peak frequency and body size in the *H. didactylus* laboratory sound, it was also demonstrated that this sound feature is not significantly different between males and females. However male sounds were significantly longer than female sounds. The meaning of this sound duration difference between the sexes it is not clear. One can speculate that this happens because of differences in the sonic muscle development, males having a larger swimbladder and muscles than females (Teresa Modesto, personal communication). It was demonstrated in other species of toadfish that males have a more developed mechanosensory sound apparatus than females which enables them to produce the boatwhistle sound, a long call (Fine 1989).

Daily acoustic activity related to photoperiodism is present in some species of fish (Schneider 1967). In batrachoidids *P. notatus* shows a daily pattern of 'humming' activity, type I males of this specie start to produce this sound shortly after dusk (Brantley & Bass 1994). In this study field boatwhistle choruses of *Halobatrachus didactylus* were recorded in both night and day periods, however it was not possible to determine whether the calling rate varied between these periods. Fine et al (1977) did not find a calling pattern in *Opsanus tau* boatwhistle production, though they do not rule out the possibility of maximum and minimum periods of call production for the toadfish population.

Night versus day period differences were also investigated for the other types of calls. Only double grunt calls showed significant differences between these periods. Differences in general individual activity depending upon photoperiodism are common in teleost fish. Activity may influence the frequency of some behaviours. For example the interaction between two individuals has a higher probability to occur when these fish are more active. Another factor such as the number of individuals present at the recording time may also influence the probability of an interaction to occur. These two factors are often related to each other.

The understanding of the double grunt frequency difference between night and day will be only possible when the behavioural context of this sound is determined.

Signal interactions among chorusing males are very common in insects and anuran species (Greenfield 1994). The males mutually adjust the timing of their sexual advertising calls, generating characteristic temporal patterns.

The boatwhistle signal of batrachoidids is thought to be a male advertising call, used by females to find a mate (Fine 1977). Males usually form nest aggregations, in which each male produces boatwhistles, creating a calling choruses. One of the first approaches to study male calling interactions in batrachoidids, was made by Winn (1967) with playback field experiments in the oyster toadfish (*O. tau*). This author observed that the playback of a boatwhistle set, with a high signal rate production, would increase the calling rate of the target individual. He noticed also that the males never overlapped their calls with the playback calls.

When comparing the boatwhistle call latency distributions of two *H. dydactilus* individuals it was observed that, like in *O. tau*, no call overlap occurred. Furthermore one of the individuals presented a higher frequency (more than 50%) of calls in the first 1100 to 3300 ms after the onset of its neighbour calls. These data might suggest that this individual waits a certain amount of time after its neighbours call to produce the boatwhistle. Winn (1967) has argued that call alternation in *O. tau* is produced by chance. He states that if a regular calling fish stimulates another fish to call regularly then the two rates may present an alternation pattern, only by chance. This would be enhanced if the individuals avoided call overlapping. In *H. didactylus* this alternation pattern may not be produced only by chance. In fact one of the individuals appears to be calling independently while the other is coupling its call. further tests are needed to confirm these hypothesis.

Winn (1967) suggests that because of male call facilitation, boatwhistle chorus may function as a male-to-male stimulation that maintains each male in a sexually reproductive state over a longer period of time (it helps to maintain a higher rate of calling in an individual male). Another possible function is the maintenance of the spacing between individuals, which is achieved by the continuous production of boatwhistles.

FINAL COMMENTS

his work represents the first attempt to study the acoustic behaviour of *Halobatrachus didactylus*. The description of the acoustic repertoire raises mportant questions regarding the relation between sound production and behavioural context. Boatwhistle and grunt calls appear to be conservative n the various species of batrachoidids. Its signal form and behavioural context are similar in all studied toadfish species (Fish & Mowbray 1959, Gray & Winn 1961, Brantley & Bass 1994). The sound classification proposed in this work is only based on signal form rather than biological function, and it is unknown how the fish might perceive the different types of signals.

In captivity only sounds similar to grunts were recorded. This work suggested the existence of sexual dimorphism in sound production, with males possessing a longer call period. It would be interesting to verify if this pattern would be maintained under natural conditions, and with the other types of calls.

In this study it was not possible to determine individual boatwhistle rate production. It should be interesting to follow the variation of the boatwhistle production rate with the photoperiod, and its relation to the general activity.

Very few studies have been done in chorusing behaviour in fish. The results of these studies have suggested that the calling rate of one individual is influenced by the adjacent individual's call. However, this interaction was described as based only on the rate's increase, and not as an adjustment in the synchronisation of both calls. This study suggests that this interaction may be more complex, with some individuals leading the call cycles.

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