ACOUSTIC COMMUNICATION IN TRIGLIDS AND OTHER FISHES

by

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"There was something soothing about the murmur of the water at my ear (while lying on the bottom of a boat), and at once I noticed a peculiar sound, a musical clicking, not at all like the swash of water on the ripple of waves, a clear distinct musical note which came from all about and gradually disappeared."

Fish stories - Holder and Jordan's (1909 in Bass 1990)

Excerpt providing an appealing account of sounds made by the plainfin midshipman fish, *Porichthys notatus.*

DECLARATION

The work presented in this thesis is the result of my own investigations. The thesis has not been submitted for any other degree, and all sources of information have been properly acknowledged in the text.

Work from Chapter 6 on "Sound production in the blue-green damselfish, *Chromis viridis* (Cuvier, 1830) (Pomacentridae)" was published in *Bioacoustics*, **6**: 265-272 (1996).

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Sound production is widespread among teleosts and is usually observed in social contexts. Many fish, such as triglids, produce sounds by contracting a pair of specialised striated muscles attached to both sides of the swimbladder wall, yielding low frequency sounds made up of repeated brief pulses. Temporal features of acoustic emissions can be controlled by the rate of muscle contraction, thus providing a rich means of conveying information.

In this study the sound features of the grey (*Eutrigla gurnardus*), the streaked (*Trigloporus lastoviza*), the red (*Aspitrigla cuculus*) and the tub (*Trigla lucerna*) gurnards were compared. The grey and the red gurnards emitted 3 different sound types, knocks, grunts and growls, whereas the streaked and the tub gurnards only emitted one sound type, growls and grunts respectively. Interspecific differences of calls were marked and based on the temporal patterning and on the grouping of the pulses. In the grey gurnard, ontogenetic changes in sound production were found. The sound production rate, the proportion of emitted sound type and the physical features of sounds varied with fish size. A study of diel and seasonal variations of sound production in the grey gurnard showed that more sounds were uttered during the day than at night and that grunts were more important and intense during the Spring-Summer period.

The sonic apparatus was examined in the species mentioned above and also in the large-scaled gurnard (*Lepidotrigla cavillone*) and the piper (*Trigla lyra*). The swimbladder and the sonic muscles grew throughout life in all species. Variations in the sonic apparatus with fish gender or time of the year were not detected. This suggests that the ability to vocalise is similar in both male and female gurnards, probably even during courtship. The shape of the swimbladder was species-specific. All species possessed a pair of intrinsic sonic muscles except for the piper whose sonic muscles were extrinsic. The pair of intrinsic sonic muscles of the grey gurnard contracted synchronously and each contraction generated a pulse of sound.

The biological significance of sound production was studied in the grey, the streaked and the tub gurnards. Few other studies have made a comparison of the behavioural context of sound production in different species of fish of the same family. Competitive feeding interactions were examined and compared between species. The different sound types emitted were also correlated with different behavioural categories. Typical sequences of behaviour were found in each species. Disputes over food items were settled by either reaching food first or by being aggressive, but never involved fighting escalation. The knocks of the grey gurnard and the growls of the streaked gurnard were associated with feeding and low levels of aggressive behaviour, and the grunts of grey and tub gurnards were associated with agonistic behaviour, such as approach, chase and frontal displays. The grey gurnard was the most vocal and aggressive species during competitive feeding. Smaller grey gurnards interacted more frequently than did larger fish, and a larger proportion of their interactions were accompanied by grunt calls.

The metabolic costs of sound production in fish were calculated theoretically for different types of acoustic activity. If the assumptions made are valid then it seems that producing acoustic signals in fish is cheap. The biological implications of these low energetic costs were discussed.

Finally, the acoustic repertoire of several non-triglid species of fish was examined. Sounds from 7 species were described for the first time. These species uttered sounds during territorial defence and courtship; contexts quite different from those studied for triglids.

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CONTENTS

CHAPTER 1

INTRODUCTION

1 - INTRODUCTION

It has long been known that fish produce sounds (Moulton 1963), but, it is only in the past few decades that we have become aware of how widespread sound production is amongst fish (Myrberg 1981). During the early phases of investigations on sound production, Griffin (1950 in Tavolga 1958b) stated: "The discovery that a wide variety of underwater sounds are produced by fish and other marine animals has raised many unsolved problems concerning the biological significance of these sounds. Are they purely accidental by-products of other activities, are they used for communication from one animal to another, or do they serve in any way for orientation?" In the past 50 years a considerable amount of work has been carried out to answer these questions. However, much is yet to be learnt in relation to the biological significance of fish sounds. The recent advances in sound detection, recording and analysis, together with improvements in our knowledge of fish behaviour and the methodology for unravelling animal behaviour, provide an excellent opportunity to answer such questions.

1.1 - UNDERWATER ACOUSTICS

1.1.1 - The nature of underwater sounds

Sound is a mechanical disturbance in any material medium, generated by the movement or vibration of an object. When sound is generated, kinetic energy is imparted to the medium and is passed on as a travelling wave, within which the component particles of the medium display a to-and-fro motion. The sound wave propagates away from the source at a particular velocity (v, measured in ms^{-1}), which depends on the density and the elasticity of the medium (Hawkins & Myrberg 1983, Hawkins 1993). In any medium, a propagating sound wave is characterised by its pressure (P, measured in Pascals), a scalar quantity, and the vector properties, acceleration, particle displacement and velocity (Schwarz 1985). Sound pressure is the oscillatory change in pressure above and below the hydrostatic pressure, that accompanies the particle displacement. In a free sound field, where there are no obstacles to sound propagation, sound behaves approximately as a plane-wave and particle velocity is directly proportional to sound pressure; i.e. $v = P/\rho c$, where c is the propagation velocity and ρ is the density of the medium (kgm^{-3}). A measure of the acoustic properties of the medium is its acoustic impedance or the specific acoustic resistance of the fluid, which is the product ρc (Urick 1983). As water is much denser than air (about 1000 times), the velocity of sound underwater is much higher (approximately 5 fold: 1500 ms^{-1} (water) vs 330 ms^{-1} (air)). Since $\lambda = c/f$ (where λ is the wavelength and f the frequency of the sound), for a given frequency the wavelength of sound in water is about 5 times longer than in air (Tavolga 1971). Also, since water is much denser than air, a much larger input of energy may be required to initiate the propagation of a sound in water. However, especially low frequency sounds, like those uttered by fish, travel extremely rapidly through the sea and may travel great distances (Tavolga 1971). Furthermore, in the sea, in contrast with air, sounds are relatively unaffected by absorption and scattering. The limitations for sound propagation in the sea are the sea surface and the sea bed, which behave as primary reflecting surfaces, and discontinuities in the medium caused by variations of salinity, temperature and pressure (Hawkins & Myrberg 1983, Urick 1983).

As sound is propagated, the ratio of P to v changes with distance from the source. Close to the source, in the so-called near-field, v predominates, whereas far away from the source, in the far-field, P predominates. For a particular frequency, the boundary between near-field and far-field is defined as the point at which P and v reach simultaneously their maximum values, i.e. where they are in phase (Schwarz 1985). Within the near-field, v declines with the inverse square of distance (Harris 1964), whereas within the far-field sound behaves as a plane-wave (see above) (Schwarz 1985). The effects of the near-field and the far-field, in terms of their influence upon a sound detector have been reviewed, for example by Harris (1964). Because water is more incompressible and dense than air, the near-field extends further from the sound source. Generally, terrestrial animals are considered to be always in the far-field, whereas aquatic animals are often in the near-field, especially with sounds at frequencies lower than 1000 Hz (Schwarz 1985).

Sound intensity (the amount of energy transmitted as sound propagates, per second and per unit area) is usually expressed as a logarithmic measure, the decibel (dB), relative to a reference quantity. As intensity (I) is related to pressure ($I = P^2/\rho c$), sound pressure can also be expressed in decibels with the following equation:

$$\text{sound pressure level (SPL)} = 20\log_{10}P/P_{ref}\ \text{dB}$$

where P is the measured sound pressure and P_{ref} is a reference pressure, usually 1μPa for water (Au 1993). SPL is expressed in dB re 1 μPa.

1.1.2 - Detection of underwater sounds

Underwater sounds are detected with hydrophones (underwater microphones) which are commonly piezoelectric transducers that convert sound pressure into proportional electrical voltages (Au 1993). The detected signals are usually amplified and filtered with low- and high-pass bands to eliminate noise at extremely low and high frequencies, and then recorded. Monitoring of sounds can be achieved with the use of earphones, or visually with an oscilloscope or with real-time spectrographic analysis (Pavan 1992).

1.1.3 - Analysis of sounds

Sounds may be analysed by looking at their temporal and frequency structures, most commonly with specifically designed software such as that used in this work (see section 2.2). Temporal measurements can be taken from a sound pressure wave (or oscillogram) of the sound. Frequency analysis can be made by passing the signal through a number of parallel narrow-band filters and displaying the spectrum depicting the relative energy at different frequencies (Hawkins 1993). A more elaborate and common analysis involves looking at the variations of the sound frequency structures with time, with a spectrograph display (Pye 1982). Spectrograms provide a representation of frequency against time and are based on the fast Fourier transform (FFT). The function of the FFT is equivalent to a bank of bandpass filters, each centred at a slightly different analysis frequency. The output of each filter is proportional to the signal's amplitude in a discrete frequency band, centred on the analysis frequency of the filter. The spectrogram results from overlapping the outputs of each bandpass filter at varying time, thus giving information on the amplitude of different frequency bands and how those vary with time. The bandwidth of a spectrogram is the range of input frequencies around the central analysis frequencies that are passed by each filter. Narrower filter bandwidths have better frequency and poorer time resolution, and wider filter bandwidths have worse frequency and better time resolution. Indeed, there is a time-frequency uncertainty principle that has been discussed by Beecher (1988), for example. The relation between time and frequency resolution are optimised depending on the study in question. As fish are thought to favour temporal rather than frequency information in acoustic communication (e.g. Myrberg *et al.* 1978, Fay 1985), wider filter bandwidths are preferred for the study of fish sounds.

If sounds are pure tones (or frequencies), such as the ones arising from a tuning-fork, the waveform is sinusoidal and a single frequency band is shown in the spectrogram. However, most sounds are complex and the spectrogram may depict several frequency bands. When these are evenly spaced, the sound is described as harmonic. The lowest band is then the fundamental frequency while the other frequency bands are called the higher harmonics. Generally, such sounds have a "pulsed" structure, looked at in the time domain. Where sounds are made up of pulses, the spacing between the frequency bands or harmonics changes with the pulse repetition rate.

1.2 - SOUND PRODUCTION IN FISH

1.2.1 - Historical background

The knowledge that fish produce sounds dates from the time of the ancient Greeks. Aristotle compared the sound of fish to those of other animals and named them according to the sounds they produced (Moulton 1963). Listening to fish sounds with the aid of pipes, oars and other objects that transmit underwater sounds to the ear has been used in fisheries for centuries, probably since prehistoric times (Moulton 1963). More recently, reports by Dufossé (1874), Sörensen (1894-1895), Tower (1908) and Dijkgraaf (1932), among others, have produced major contributions to the description of fish sounds and the mechanisms of sound production. But it was not until World War II that the refinement of suitable listening equipment - hydrophones, amplifiers, and recorders - made possible a quantitative description of fish sounds (Tavolga 1971). Following the war, the pioneer studies of Fish *et al.* (1952) and Fish (1954) and the reviews of Moulton (1963), Protasov (1965) and more recently Tavolga (1971) and Myrberg (1981), have provided descriptions of sounds produced by a wide variety of species belonging to many families, and have shown that sound production in fish is much more widespread than previously thought.

1.2.2 - Peripheral mechanisms of sound production

Fish sounds can be classified into 3 different categories depending on the mechanisms used to produce them (see reviews by Marshall 1962 and Tavolga 1971). Stridulation sounds are sounds resulting from the friction between hard parts of the body. They resemble rasps and creaks and contain a series of very rapidly produced and irregular transient pulses (Hawkins 1993). Typically these sounds have fundamental frequencies and harmonics between 1 - 4 KHz (Bass & Baker 1991).

Hydrodynamic sounds are fast pressure pulses generated when fish make rapid swimming movements (Gray & Denton 1991). With both stridulatory and hydrodynamic sounds the swimbladder may have a complementary role and impart a hollow resonant quality to the sound (Hawkins 1993).

In the most highly-developed mechanism of sound production, sounds result from the contraction of specialised intrinsic or extrinsic muscles (called drumming or sonic muscles) upon the swimbladder wall, which may then act as a resonator (Marshall 1962, Tavolga 1971, Demski *et al.* 1973, Hawkins 1993). Swimbladder sounds are often highly stereotyped and are made up of single or a series of separate low-frequency pulses, usually with fundamental frequencies below 1KHz. These low-frequency pulses generally result from highly synchronized contractions of both sonic muscles; each synchronous muscle contraction generates a pulse of sound (Fish 1954, Tavolga 1971, Hawkins & Myrberg 1983, Hawkins 1993). When the muscle contraction rate is very fast the pulses may merge together. Longer sounds arise from a greater number of rapidly repeated contractions. The pulsed sounds produced by swimbladder mechanisms usually show a harmonic structure, as discussed earlier.

The sonic muscles of fish are among the fastest-acting of all known vertebrate muscles (Tavolga 1964, Fine *et al.* 1993). They can complete a twitch in a little over 10 msec (Skoglund 1961) and can make unfused contractions up to about 300 pulses/sec (Tavolga 1964). Sonic muscles have numerous adaptations for fast aerobic function (see Fine 1989). The fibres of these muscles tend to be shorter and of smaller diameter than those of non-sonic striated muscles. They may show innervation by a large number of afferent nerve fibres, with many nerve terminals along the length of each muscle fibre. Moreover, the sonic muscles are highly vascularised and rich in myoglobin, which is an oxygen reservoir, and have a well-developed sarcoplasmic reticulum, consistent with the ability of these muscles to maintain a high contraction rate over long periods (Demski *et al.* 1973).

The peak frequency of a sound (frequency at the sound's highest intensity) is determined by the resonance characteristics of the swimbladder, which is usually similar to that of a free gas bubble of identical size in water (Protasov 1965). Larger swimbladders resonate at lower frequencies than smaller ones for a given depth.

1.2.3 - Central control of sound production

Neuromuscular system

Sonic muscles are innervated by branches of the occipital nerves that arise just in front of the first true set of spinal nerves issuing from the spinal cord (Fig. 1.1a) (Tavolga, 1962, 1964, 1971, Hawkins 1968, Bass 1990). An exception is found in sciaenids in which the sonic muscle are derived from the lateral body wall and are innervated by true spinal nerves (Tavolga 1971). Each sonic muscle fibre shows polyaxonal innervation along its length. Indeed, up to 4 or more axons may innervate each muscle fibre in both the oyster toadfish (*Opsanus tau*) and the squirrelfish (*Holocentrus rufus*) (Gainer & Klancher 1965, Gainer *et al.* 1965). This special neuromuscular organisation provides a means of evoking simultaneous and distributed action potentials throughout the whole muscle (Gainer & Klancher 1965, Gainer *et al.* 1965).

Sonic motor systems

The axons of the occipital nerves originate from sonic motoneurons that form a nucleus (a specific collection of cells in the central nervous system) (Bass 1990). In batrachoidids, such as in the plainfin midshipman fish (*Porichthys notatus*), the sonic motor nucleus is an unpaired midline structure that originates at the caudal end of the brain and extends into the rostral spinal cord (Fig. 1.1a). Each half of the nucleus sends its axons to innervate the sonic muscle on the same side of the body axis (Fine *et al.* 1984, Bass 1985). Sonic motoneurons fire synchronously (Bass & Baker 1991) and each nerve volley causes one synchronous contraction of the sonic muscles (Skoglund 1961). In contrast, the sea robin (*Prionotus carolinus*) (Triglidae) has a pair of sonic motor nuclei with each nucleus lying along the ventrolateral margin of the brain and spinal cord (Fig. 1.1b); each nucleus sends again its axons to the sonic muscle on the same side of the body axis (Bass 1985). The firing of the paired motor nuclei in the sea robin is out of phase, resulting in asynchronous contractions of the sonic muscles (Bass & Baker 1991).

Sonic motoneurons are electrically coupled by pacemaker neurons which determine the firing frequency of motoneurons (Weiser *et al.* 1986) by

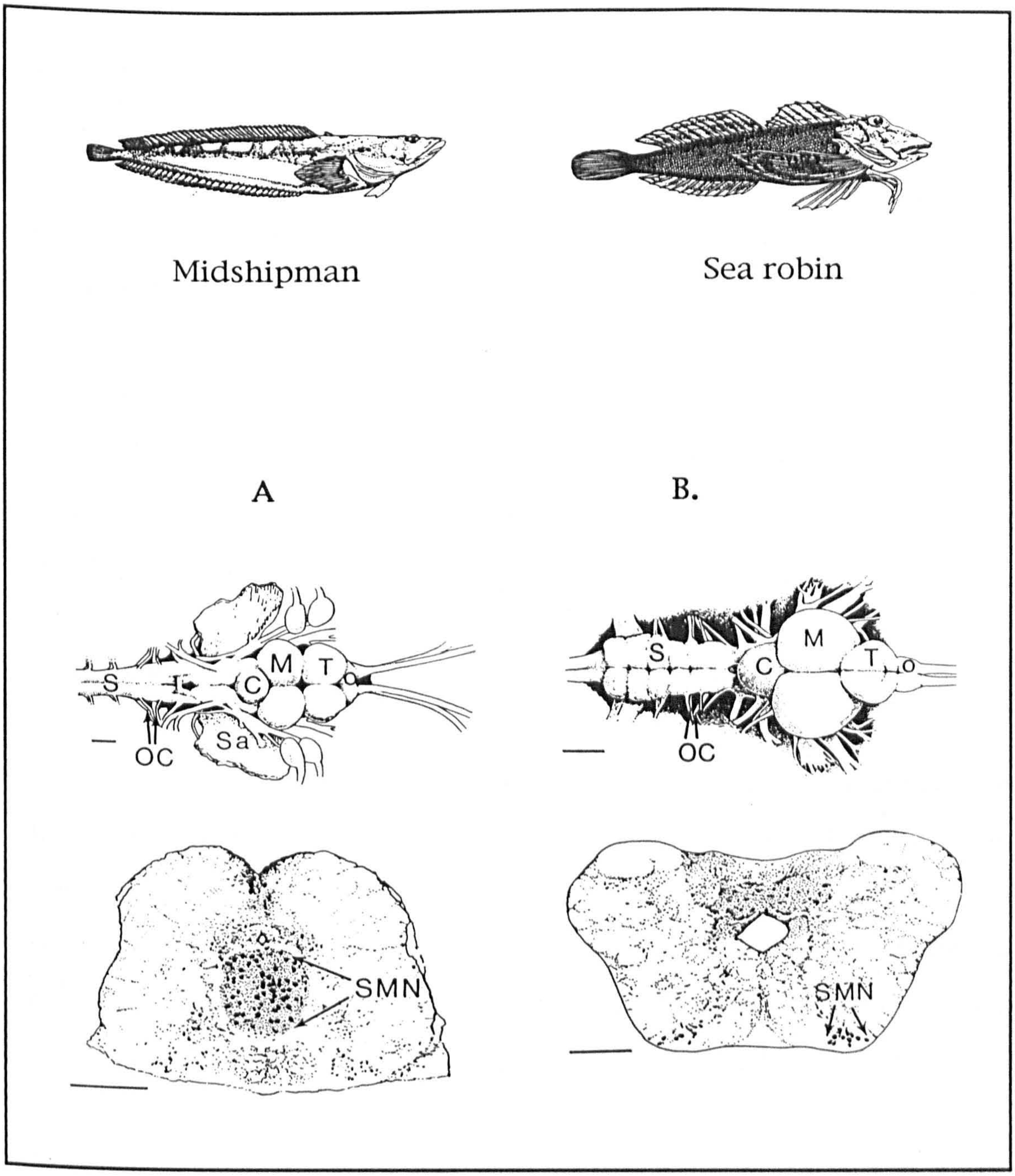


Figure 1.1 - Sonic motor system of the plainfin midshipman, *Porichthys notatus* (A) and of the sea robin, *Prionotus carolinus* (B). On top (of both A and B) is the surface view of the brain with the paired sonic occipital nerve roots (OC). The transverse section (bottom) shows in A the unpaired midline sonic motor nucleus (SMN) and in B the bilateral SMN. The small bar equals 0.5 mm and the larger bar equals 1mm. C - cerebellum; M - midbrain; S - spinal cord; T - telencephalon; Sa - sagitta (otolith of sacculus); O - olfactory bulb. After Bass & Baker (1991).

having both excitory and inhibitory inputs to the sonic motor nucleus (Bass & Marchaterre 1989, Marchaterre *et al.* 1989).

There is only limited information on brain control of sound production in fish (Fine & Perini 1994). Neural pathways controlling sound production descend from higher centres in the forebrain in amphibians, birds and mammals, contrasting with fish in which only the lower centres (brainstem and spinal centres) have been implicated in sound production (Bass 1989). However, the work of Fine & Perini (1994) with the oyster toadfish suggested that the forebrain is possibly involved in the control of sound production in fish.

1.2.4 - Inter- and intraspecific variations of fish sounds

Fish calls are usually found to be species-specific. Indeed, several authors have reported that sounds produced by species of fish belonging to the same family differ mainly in the temporal organization and the grouping of the pulses in their sounds; frequency differences are mainly related to differences in sizes of their swimbladders (Gerald 1971, Hawkins & Rasmussen 1978, Myrberg *et al.* 1978, Spanier 1979, Ladich *et al.* 1992a).

Intraspecific differences in fish calls may also be found. For example, different sizes of fish may produce different calls. Typically, smaller fish produce higher frequency sounds than their larger conspecifics, as found, for example, for some Sciaenidae (Dobrin 1947), the sea robin (Fish 1954), the perch-pike (*Stizostedion lucioperca*) and the haddock (*Melanogrammus aeglefinus*) (Protasov 1965).

Within a species, sound production may vary according to seasonal and diel rhythms. For example, Tavolga (1960), Fish (1964) and Breder (1968) have reported the existence of seasonal spawning choruses of fish of the Ariidae and Sciaenidae families. Seasonal variations of fish calls related to breeding have also been mentioned, for example, in batrachoidids (Fine 1978, Bass & Andersen 1991), in gobies (Tavolga 1956, Torricelli *et al.* 1986) and in sciaenids (Guest 1978, Connaughton & Taylor 1995b). Many fish species also show considerable diel variations in their acoustic activity, and peaks of sound production may be diurnal, nocturnal or crepuscular. For example, the catfish (*Ariis felis*) and the squirrelfish show dawn and dusk peaks of sonic activity, possibly correlated with feeding or territorial behaviour (Winn *et al.* 1964, Breder 1968).

Sexual dimorphism in sound production is likewise a cause for intraspecific differences in fish sounds. Sexually dimorphic sound production is usually associated with differences in the sound-producing apparatus which may be related to different hormonal levels in males and females (Templeman & Hodder 1958, Fine & Pennypacker 1986). Androgens may cause an increase of sonic muscle mass, hypertrophy of the sonic muscle fibres as well as an increase in the fibre number (e.g. Fine & Pennypacker 1986, Brantley *et al.* 1993, Fine *et al.* 1993). In many species, only the males produce sounds during courtship and spawning and they often show territorial behaviour (e.g. gobies, Tavolga 1958a, Torricelli & Romani 1986), or are more active sound producers than females (e.g. gadoids, Hawkins & Rasmussen 1978). Interestingly, in the plainfin midshipman fish, intraspecific differences in sound production are polymorphic, with two different reproductive morphotype males. Differences in vocal behaviour of females and type I and II males are parallelled by differences in sonic muscle size (Brantley & Bass 1994).

1.3 - ACOUSTIC COMMUNICATION IN FISH

1.3.1 - Some thoughts on communication

Most textbooks state that communication occurs when the signals emitted by the sender alter the behaviour of the receiver. However, various refinements are introduced to this basic definition to distinguish communication from other sorts of effects that the behaviour of one animal might have on another, such as the interception of signals by predators (Dawkins 1986). It is these refinements that give such a confusing picture to the definitions of "communication" and also of "signal" and "information transfer" (Dawkins 1986). Whether signals have been shaped by natural selection or are only by-products of other behaviours, whether signals are conspicuous or subtle, and whether signals are costly or cheap, are examples of divergent of opinions found in the literature (e.g. Zahavi 1977, 1993, Dawkins 1986, Guilford & Dawkins 1991, 1992, Blumberg & Alberts 1992).

The definitions of communication given by Myrberg (1981) and Krebs & Davies (1987) are two examples of those found in the literature. Myrberg (1981) has carefully sought a definition of communication. He characterised it as the transfer of information between individuals whose functional aim or intent rests solely in obtaining adaptive advantage for the sender. Myrberg's definition has the pitfall of including animals' intentions in his definition of communication since these are quite problematic to assess. His definition does not rule out the dissemination of deceptive information by the sender, i.e. it does not imply benefits for the receiver. Although Myrberg (1981) accepts that receivers may also gain benefits and possess specialised receiving mechanisms, he does not consider them relevant for defining communication. However, increasing attention is being focused on the selection pressures affecting animals as receivers of signals (McGregor 1991, Guilford & Dawkins 1991, 1992). Krebs & Davies (1987) characterized communication as the process in which actors use specially designed signals or displays to modify the behaviour of reactors. In contrast to Myrberg their definition of communication does not exclude the possibility of cooperative signalling, i.e. the case when both actor and reactor benefit from sending and receiving the signal. In cooperative signalling the reactor is thought to have evolved high sensitivity to the signal (Krebs & Davies 1987). Both definitions stress the fact that signals have been shaped by natural selection, while Blumberg & Alberts (1992) disagree on the importance given to natural selection in shaping signals.

In the present work, communication is used in accordance with the definition of Krebs & Davies (1987), and signals are defined as the means by which communication is achieved.

1.3.2 - Advantages and disadvantages of acoustic signalling underwater

Acoustic signalling has advantages as a means of communication underwater. For instance, sound propagates very rapidly and it can convey information through variations in both frequency structure and temporal patterning. Additionally, it propagates in all directions and is effective even if visibility is poor. Low frequency sounds, in particular, may propagate around solid objects or through dense cover without being absorbed (Hawkins 1993). Acoustic signalling also has disadvantages, such as interception by predators or perhaps a high energetic cost of production. Further, it can convey only limited information compared, for example, with visual communication, which is more information-rich.

However, other means of communication show greater constraints underwater. Tactile communication demands close proximity of both receiver and sender, vision is very often restricted by low visibility, especially at greater depths or at night, and chemical communication is slow and non-directional. Although signalling with sound has certain constraints it is not surprising that acoustic communication is widely used by aquatic animals (Myrberg 1981, Gerdhardt 1983, Hawkins & Myrberg 1983) such as teleosts (Myrberg 1981, Bass & Baker 1991). Indeed, field studies suggest that the highly stereotyped and species-specific vocalization of fish (Bass & Baker 1991) contribute to both survival and reproductive success (reviewed by Bass 1990).

1.3.3 - The behavioural context of sound production

Acoustic behaviour is known to play an important role in social contexts, involving interactions between individuals. Several studies have dealt with various aspects of sound production, particularly concerning the importance of conspecific sounds in agonistic and reproductive interactions.

Sounds are commonly produced by fish when they are in the presence of a predator or other noxious stimuli. These sounds are intense and have a sudden onset, characteristic of animal alarm calls, and may be accompanied by other behavioural acts (e.g. visual displays) (Hawkins 1968).

Aggressive fish are often vocal and sound may play a role in spacing individuals out. Indeed, aggressive calls are often emitted by territorial fish (Fine *et al.* 1977a). On the other hand, it has also been suggested that sound production may play an important role in the social aggregation of schooling fish (Moulton 1960), although the sounds produced may be overheard by predators and the advantages of schooling lost (Hawkins 1993). Sound production by aggressive fish is also often related to competitive feeding (Fish 1954, Protasov 1965).

Perhaps the most common occurrence of sound production is during the reproductive period. In many species calls are uttered by mating male fish that often hold territories. In these cases, the distinction between aggressive and mating calls becomes difficult. During reproductive activities, sounds usually accompany complex visual displays and have an important role in the successful accomplishment of courtship, culminating in spawning (e.g. Fine *et al.* 1977a, b, Myrberg 1981, Hawkins & Myrberg 1983, Hawkins 1993).

1.3.4 - Hearing in fish

A large volume of literature exists on the hearing sensitivity and discriminative capacities of fish. For reviews see: Popper & Fay (1973), Coombs & Popper (1979), Fay & Popper (1980), Schuijf & Buwalda (1980), Blaxter (1981), Hawkins (1981), Popper & Coombs (1982), Hawkins & Myrberg (1983), Fay 1985 and Hawkins (1993).

The fish auditory system consists of a pair of inner ears, embedded in the cranium on either side of the head, close to the midbrain (Fig. 1.2a). Each inner ear includes a series of canals and sacs filled with endolymph (Fig. 1.2b). The 3 canals of each ear are arranged orthogonally, and each incorporates a bulbous expansion across which mechanoreceptive hair cells are mounted. The 3 otolith organs, the saccule, the lagena and the utricule, each consists of a heavy calcareous mass, lying over a sensory membrane or macula (Fig. 1.2b) (Fay & Popper 1980, Fay 1985, Hawkins 1993). The utricular macula lies mainly in the horizontal plane, while the

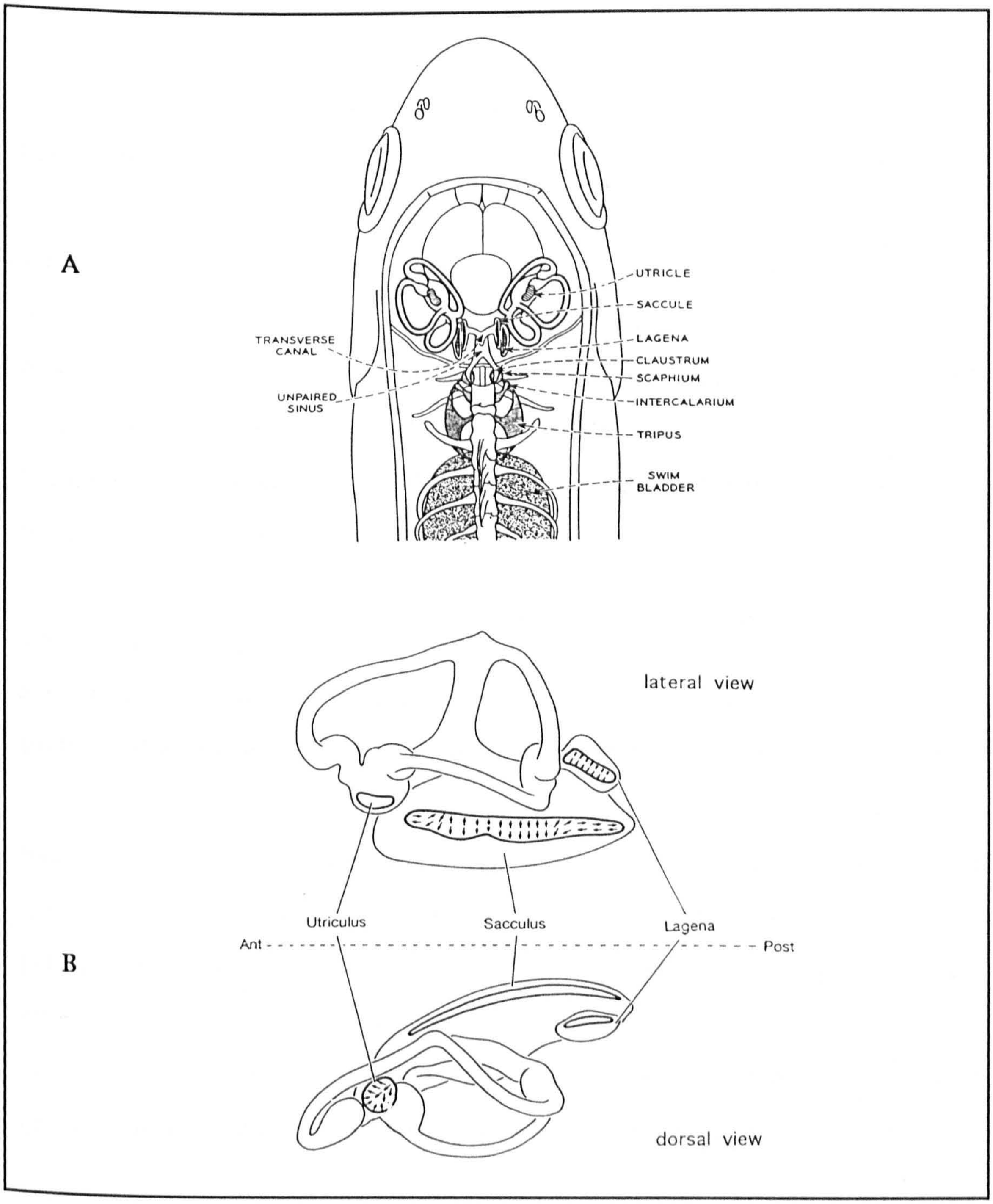


Figure 1.2 - **A**: Dorsal view of the auditory system of an ostariophysan fish (*Cyprinus idus*), showing the swimbladder, the Weberian ossicles (tripus, intercalarium, scaphium and claustrum), and the inner ear with the utricule, the saccule and the lagena otoliths. After von Frisch (1938), taken from Fay (1978). **B**: Lateral and dorsal view of the left inner ear of the cod (*Gadus morhua*), showing the three orthogonal semicircular canals and the 3 otoliths organs. The sensory membranes or maculae of the utriculus, sacculus and lagena are depicted, with the orientation of the hair cells shown by the arrows. After Hawkins (1993).

saccular and the lagenar maculae and their respective otolith lie mainly in the vertical plane (Hawkins & Myrberg 1983). Each macula contains many hair cells, which are organised into two or more groups with opposing directional orientation (Fig. 1.2b) (Fay & Popper 1980, Fay 1985, Hawkins 1993). The hair cells are extremely sensitive to mechanical deflection and are directional in their response (Hawkins & Myrberg 1983). Acceleration of the body causes relative motion between the otoliths and the endolymph in the canals (both acting as inertial masses), and the hair cells, exciting the latter (Fay 1985). The otolith organ is sensitive to particle motion, either to particle acceleration or to particle velocity (Hawkins 1993).

Some fish are hearing-specialists and show an acute sensitivity to sounds at an extended frequency range. They include many ostariophysan species, such as the cypriniformes, and some holocentrids (Tavolga & Wodinsky 1963, Coombs & Popper 1979, Fay & Popper 1980, Fay 1984). Cypriniform fish have the saccules efficiently coupled with the swimbladder via a set of specialised bones, the Weberian ossicles. The swimbladder serves as a primary transducer and vibrates in response to the incident sound pressure waves; the vibration is transmitted to the Weberian ossicles and then to the inner ear (Tavolga 1971). Such hearing-specialists are sensitive to sound pressure, at least at high frequencies (Fay & Popper 1975).

The clupeiform fish are also hearing-specialists, though their auditory system is quite different from that of the cypriniform fish (see comprehensive description in Blaxter *et al.* 1981). In Clupeoids, the swimbladder, the inner ear and the lateral line system are all linked. Each bulla of the auditory bulla system (mechanically linked with lateral line neuromasts) is divided into a gas-filled and a liquid-filled parts by a membrane, which is elastic and under tension (Blaxter *et al.* 1981). The liquid-filled part (perilymph) is connected to the inner ear via a fenestra in the upper wall of the bulla and the gas-filled part of the bulla is connected to the swimbladder by very fine pre-coelomic gas ducts (Blaxter *et al.* 1979, 1981). The swimbladder acts as a gas reservoir to maintain the gas-filled bulla system constant at different depths (Denton & Blaxter 1976, Blaxter *et al.* 1979). Pressure changes acting on the fish cause displacements on the elastic bulla membrane, which lead to liquid displacements in the perilymph, which in turn are trasmitted to the inner ear and the rest of the lateral system (Blaxter *et al.* 1981).

Species with poor sensitivity and narrow frequency range lack the swimbladder altogether and are only sensitive to particle displacement. Examples of these are various species of flatfish, the goby (*Gobius niger*), the toadfish and the salmon (*Salmo salar*) (Dijkgraaf 1952, Fish & Offutt 1972, Chapman & Sand 1974, Hawkins & Johnstone 1978).

Other fish that have intermediate hearing capacities, such as the cod (*Gadus morhua*), damselfish and other coral reef fish, have the swimbladders close to the inner ears and are sensitive to sound pressure at high frequencies and particle motion at low frequencies (Myrberg & Spires 1972, Chapman & Hawkins 1973, Fay & Popper 1980).

On the whole, the frequency range of fish is much less than that of most terrestrial vertebrates, especially birds and mammals. Even specialist hearing fish are not sensitive to frequencies above 2 to 3 KHz, whereas Man can detect sounds above 15 KHz and Cetaceans above 100 KHz (Dijkgraaf 1960). However, fish hear extremely well within their range of frequencies. Fish can discriminate signals from the background noise with the use of a frequency-selective auditory filter (Hawkins & Chapman 1975). Fish are also capable of distinguishing pure tone frequencies that differ by as little as 3 - 5% (Fay 1985). This ability is poorer than that of Man, but comparable to that of birds and mammals. Furthermore, fish are able to distinguish sounds with intensities as different as 1.3 dB (50 Hz tone), and resolve short-duration pulses better than Man. Finally, fish are able to detect sound direction, even in the vertical plane, an ability only poorly developed in Man (Hawkins & Sand 1977, Fay 1980, 1985, Schuijf & Hawkins 1983).

Fish seem to be particularly well adapted to the analysis of sounds in the time domain but not in the frequency domain (Fay 1985), consistent with the fact that temporal cues are important for sound discrimination by fish (Myrberg *et al.* 1978).

1.4 - THE TRIGLIDS AS SOUND PRODUCERS

Triglids (also called gurnards in Europe or sea robins in America) are common species in British waters and live in a range of habitats from inshore to the deep sea. Both off the British Isles and in the Mediterranean, they can generally be found from 20 - 50 m down to 100 - 200 m, except for the piper, *Trigla lyra* Linnaeus, 1758, that is typically found at depths of 300 - 700 m (Wheeler 1969, Papaconstantinou 1983). Gurnards are benthic gregarious fish and tend to form small (Protasov 1965) and occasionally, extremely large shoals (Heesen & Daan 1994). The 3 lowest rays of the pectoral fins are free and are used as tactile organs to search for food on the bottom (Wheeler 1969). Triglids usually migrate inshore in the summer, probably to spawn, and into deeper waters in the autumn (Meek 1915).

The sound-producing ability of triglid fish was already known to the fishermen of Aristotle's time (Moulton 1963). Together with the Sciaenidae, Siluroidae and Pristipomatidae, this is one of the families that contain the greatest number of sound-producing species (Sörensen 1894-1895). The Triglidae produce typical swimbladder sounds and according to various authors, they seem to be responsible for grunt-like calls wherever they occur in Europe, America and Australia (e.g. Moulton 1964).

It is during their reproductive period, in summertime, that their acoustic activity is especially evident (Protasov 1965). One Scots term for the grey gurnard, *Eutrigla gurnardus*, is "crooner", reflecting their conspicuous acoustic activity (Watt 1989).

Triglids also show a daily rhythm of sound emission. More sounds are produced by the grey gurnard during the day (Freytag 1964). In contrast, Shikhova (1963 in Protasov 1965) stated that the drumming sounds of the gurnards of the Black Sea (not identified) are more frequent during dusk and at night.

Outside the breeding period, sound production in gurnards seems to be mainly related to aggressive behaviour, and is easily observed during competitive feeding. For example sound production in the sea robins, *P. carolinus* and *P. evolans*, become louder and more frequent when foraging in groups, or even when feeding alone (Fish 1954). The tub gurnard produces sound associated with aggressive behaviour when feeding (Hawkins 1968). Threatening sounds while feeding are also known to occur in other fish. For example, the perch (*Perca fluviatilis*) usually displays aggressive behaviour towards other members of the group when being fed: the fish chases an individual that has taken some food while raising the dorsal fins and uttering threatening sounds (Protasov 1965). Another example is the female haddock, that in spite of being silent during courtship, will readily produce sounds during competitive feeding, outside the breeding season (Hawkins 1993).

Sounds made by gurnards have been mentioned in the literature many times, although few studies gave detailed descriptions of sounds or related them to behaviour. A review of the sounds produced by members of the Triglidae family will be presented in Chapter 2 (see Table 2.1).

1.5 - THE AIMS OF THE STUDY

The aim of this work was to examine more closely the biological significance of sound production in fish. The main questions asked were: What sounds do fish produce? How do they produce sounds? In what context do they produce them? And finally, what is the significance of their sounds?

Triglids were chosen as the subject species because they are active sound producers and survive well under laboratory conditions. As opportunities arose, other species of fish that became available were also studied.

Since previous descriptions of sounds uttered by gurnards were limited or non-existent, the first step was to characterise the acoustic repertoire of the following fish: the grey gurnard, the streaked gurnard (*Trigloporus lastoviza*), the red gurnard and the tub gurnard (see Table 1.1 for synonyms of scientific names and for common names). In order for fish sounds to have a communicatory value they should be stereotyped. That is, if different sound types convey different messages the variation within sound types should be smaller then the variation between them. Likewise, if an acoustical signal is relevant for intra- or interspecific communication, the variability within individuals or species should be smaller than the variability between them. To examine whether the sounds produced by these species of gurnards possess potential cues for communication the

Table 1.1 - Scientific names, common synonyms and common names of the triglid species studied in the present work (Wheeler 1969, Hureau 1986, Almada pers. com.). The last two species listed were only studied in Chapter 3.

Scientific name	**Common synonym**	**Common names**
Eutrigla gurnardus (Linnaeus, 1758)	*Trigla gurnardus* Linnaeus, 1758 *Trigla milvus* Lacepéde, 1801	Grey gurnard (En) Grondin gris (Fr) Grauer Knurrhahn (Ge) Cabra-morena (Pt)
Trigloporus lastoviza (Brünnich, 1768)	*Trigla lineata* Gmelin, 1789	Streaked gurnard (En) Grondin camard (Fr) Cabra-riscada (Pt) Rubio (Sp)
Aspitrigla cuculus (Linnaeus, 1758)	*Chelidonichthys cuculus* (Linnaeus, 1758) *Trigla cuculus* Linnaeus, 1758	Red gurnard (En) Grondin rouge (Fr) Cabra-vermelha (Pt) Arete (Sp)
Trigla lucerna Linnaeus, 1758	*Trigla hirundo* Bloch, 1785 *Trigla corvus* Risso, 1810 *Trigla corax* Bonaparte, 1834	Tub gurnard (En) Grondin perlon (Fr) Roter Knurrhahn (Ge) Cabra-cabaço (Pt) Bejel (Sp)
Lepidotrigla cavillone (Lacepéde, 1801)	*Trigla aspera* Viviani, 1805	Large-scaled gurnard (En) Cavillone (Fr) Ruivo (Pt) Cabete (Sp)
Trigla lyra Linnaeus, 1758	none	Piper (En) Grondin lyre (Fr) Cabra-lira (Pt) Garneo (Sp)

features of their sounds were compared across and within species. To examine how sound production may change within species, the diel, the seasonal and the ontogenetic changes of sound production were studied in the grey gurnard (see Chapter 2).

Given that the mechanisms of sound production determine the characteristics of the resulting sounds, the sound-producing mechanism of gurnards was studied. In Chapter 3, the ontogenetic, sexual and seasonal changes of the sound-producing apparatus of different gurnard species were investigated. Also, the neuromuscular mechanism of sound production in the grey gurnard was examined.

The biological significance of the sounds produced by gurnards during competitive feeding interactions was investigated by associating different sound types with different behavioural categories in each species. Playback experiments were also carried out with the grey gurnard since they provide a valuable means of investigating animal communication (see Chapter 4).

The metabolic costs of sound production have not been studied in fish and are important to the understanding of the behavioural strategies used by a species. Chapter 5 contains theoretical calculations on the costs of sound production in fish. These theoretical costs were compared with those reported for other groups of animals. Further, the implications of the costs of sound production in the life of gurnards and other fish were discussed.

Finally, potential sound-producing species not previously studied were examined. A description of the acoustic repertoire of the successful candidates and, when possible, the sound-producing mechanism is given in Chapter 6.

CHAPTER 2

SOUND PRODUCTION IN GURNARDS

2 - SOUND PRODUCTION IN GURNARDS

2.1 - INTRODUCTION

Sound production in gurnards has been mentioned by a number of researchers including Dufossé (1874) (*Trigla* sp. and *Peristidion cataphractum*), Burkenroad (1931) (*Prionotus tribulus* and *P. puntactus*), Moulton (1958a) (*P. evolans*), Dobrin (1947) (*P. carolinus*), Freytag (1961, 1964) (*T. lucerna*). Few studies, however, have given detailed information on the calls made by members of the triglid family (see Table 2.1). The sounds produced by the Triglidae range from 40 Hz up to 4000 Hz with very variable fundamental frequencies, though usually higher than 150Hz. The gurnard species found in American and Japanese waters appear to produce a much wider frequency range than the European species (Table 2.1). The European gurnards, *E. gurnardus, T. lucerna, A. cuculus* and *T. lastoviza*, produce two different kinds of calls, knocks and grunts, which are composed of pulses repeated at different rates (see Table 2.1). It is not known whether the "gu" sounds produced by the Japanese gurnards are composed of pulses (Bayoumi 1970). As for the American species, only Moulton (1956) reports that the staccato calls uttered by the common sea robin (*P. carolinus*) consist of fast repeated paired pulses, and no further information is given on the calls described by other authors as to their composition. Furthermore, Moulton (1956) suggests that these staccato calls (quite different from those of other gurnards) result from an asynchronous contraction of the intrinsic sonic muscles, whereas Evans (1973) proposes that, instead, the swimbladder's extrinsic muscles are responsible for these calls. Evans (1973) adds that the grunts are the sounds that result from the intrinsic muscles' action. According to Bass & Baker (1991), the wide range frequency sounds produced by this American species can be partially explained by the asynchronous vibration of the sonic muscles.

The behaviour associated with sound production is outlined in Table 2.2, for species, fish size and number of fish studied. The conditions of sound recording - *i.e.* whether it is recorded in the field or under laboratory conditions, and whether sounds are produced naturally or artificially stimulated, is also given. It is important to note that the sound characteristics measured in an aquarium may depend on the environment or be affected by local resonances.

Sounds related to courtship behaviour have never been studied in triglids, except for the possible reproduction-related staccato calls uttered by the sea robins. This is probably because field work is greatly limited by the depth at which triglids live. There is no report of these fish breeding in captivity. Aggressive and alarm signals, however, have received more attention, but the acoustic repertoire has not been studied in much detail for any species and no consistent comparison between different species' calls has been made.

In this Chapter, extensive recordings of sounds produced during competitive feeding by four different species of European triglids - *E. gurnardus*, *T. lastoviza*, *A. cuculus* and *T. lucerna*, (see Appendix 1) were made for the first time, and the structural features of their calls compared. For one species, the grey gurnard, diel rhythms, seasonal variation and ontogenetic changes of sound production were also studied for the first time.

Table 2.1 - Sounds produced by triglids. SPL = sound pressure level.

Species	Author	Description of sound	Pulse duration	Call duration	Frequency range	Main energy concentration	SPL
Prionotus carolinus (common sea robin)	Fish (1954)	single clucks and more often a series of squawks consisting of 10-15 signals	--- ---	cluck: 2.5-3 s squawk: 1-15 s	40-2400 Hz* "	150 -750 Hz (300, 150, 450, 600 p. e. c.)*	46.5 dB re 1 µPa at 1 m *
"	Moulton (1956)	grunt and staccato call (composed of pulses produced in pairs)	--- 22 pulses/s	grunt: 0.1 s staccato: 2.5-3 s	44-1700 Hz 500-4000Hz	--- 700-2500 Hz	--- ---
"	Fish *et al.* (1952)	single squawks and series of rapid clucks	--- ---	--- ---	40-1400Hz* "	300, 150, 450, 600 Hz (p. e. c.)*	--- ---
P. evolans (southern striped sea robin)	Fish (1954)	grunt	---	---	40-800 Hz	200, 100 and 300 Hz (p. e. c.)	51.88 dB re 1 µPa at 1 m
"	Fish *et al.* (1952)	single grunts or a burst of croaks (less staccato than *P. carolinus*)	--- ---	--- ---	40-800 Hz* "	" * "	--- ---
Chelidonichthys kumu (Japanese gurnard)	Bayoumi (1970)	"gu" sounds and grunts consisting of pulses repeated rapidly	--- grunt pulses: 0.04 s	"gu": 0.2 s ---	50-4000 Hz* "	250-300 Hz* "	--- ---

* it is not specified for any of the two call types. p. e. c. : principal energy components (listed in order of magnitude).

Table 2.1 - *Continued.*

Species	Author	Description of sound	Pulse duration	Call duration	Frequency range	Main energy concentration	SPL
Black Sea gurnard (species not identified)	Protasov & Romanenko (1961 in Protasov 1965)	clucking sounds and double drumbeat sounds	- - - - - -	0.2-0.3 s * "	- - - - - -	400, 1200, 1700 Hz (p. e. c.)	
Eutrigla gurnardus (grey gurnard)	Hawkins (1968)	single and repetitive knocks (1 knock = 1 pulse)	knocks: 7-42 ms/ interval between knocks (pulses): 26 ms-4.3 s	up to 15 s	100-630 Hz	variable	variable
		growls composed of pulses (resembling a single knock) repeated at a very fast rate	- - -	less than 400 ms	"	"	"
"	" (a)	growls	6.5-8.0 ms/ interval between pulses: 6 ms	- - -	315-630 Hz	- - -	122.86 dB re 1 μPa at 1 m (105-120 dB higher than in tanks)
"	Freytag (1964)	growls	- - -	- - -	200-630 Hz	315 Hz	- - -

* it is not specified for any of the two call types. (a) - sounds recorded in anechoic cages in the sea.

Table 2.1 - Continued.

Species	Author	Description of sound	Pulse duration	Call duration	Frequency range	Main energy concentration	SPL
Trigla lucerna (tub gurnard)	Hawkins (1968)	repeated knocks (pulses)	10-32 ms/ interval between pulses < 400 ms	0.19-12.2 s (2-31 pulses respectively)	100-500 Hz	---	---
		growls, consisting of many pulses repeated at a faster rate than knocks, tending to overlap each other	---	up to 200 ms	" (*?)	---	---
"	Freytag (1961, 1964)	deep purring sound	---	---	---	---	---
Aspitrigla cuculus (red gurnard)	Hawkins (1968)	repetitive knocking sounds. Growls (similar to the ones uttered by the grey and the tub gurnards)	---	---	---	---	---
Trigloporus lastoviza (streaked gurnard)	"	"	---	---	---	---	---

* it is not specified for any of the two call types.

Table 2.2 - *Context of sound production in triglids.*

Species	Author	Behavioural context of sound production	Sizes of fish studied	Conditions of recording	Comments
P. carolinus (common sea robin)	Fish (1954)	presence of a new fish in a tank (of the same or of a different species); crowding, prodding or direct attack; competitive feeding	no sizes specified; higher frequencies were produced by smaller fish	tanks; electric stimulation	possible pharyngeal noises
"	Moulton (1956)	grunt accompanied by fin erection when fish was handled - probably produced as a general alarm reaction; staccato call probably associated with breeding behaviour	not specified	not specified	--
P. evolans (southern striped sea robin)	Fish (1954)	feeding alone or competitively; handling; alarm; presence of a new fish in the tank	"	tanks; electric stimulation	possible pharyngeal noises
C. kumu (Japanese gurnard)	Bayoumi (1970)	not specified	the character and pattern of sounds did not change with fish size; frequency range of large (45-52 cm) and medium (38-41 cm) specimens: 200-600 Hz, small fish (27-29 cm): 200-800 Hz.	life cage kept in a natural pond, 5 m deep, with sandy bottom	sound characteristics did not change regardless of group size: 1-9 individuals
Black Sea gurnard (species not identified)	Protasov & Romanenko (1961 in Protasov 1965)	sounds used as danger signals	not specified	not specified	sounds produced at dusk or night time; expected swimbladder diameter:1.3-1.5 cm (Protasov 1965)

Table 2.2 - Continued.

Species	Author	Behavioural context of sound production	Sizes of fish studied	Conditions of recording	Comments
E. gurnardus (grey gurnard)	Hawkins (1968)	sounds produced during defensive and aggressive displays: spreading of the pectorals and erection of dorsal fins	not specified ----------> 7 gurnards ranging ----> from 26-29 cm length	tanks anechoic cages in the sea	
"	Freytag (1964)	aggressive behaviour produced during lateral displays to conspecifics or members of another species	3 specimen of 26-29 cm	not specified	the amount of sounds produced depends quantitatively on the time of day
T. lucerna (tub gurnard)	Hawkins (1968)	sounds accompanied by pectoral fins extension and first dorsal fin erection. Aggressive or defensive behaviours with 2 intensities: low - pectoral fins extension with no sound production; severe - growl, fleeing with spread pectorals accompanied by knocking sounds production. Growling sounds seem to be associated with a more intensive reaction than the knocking sounds	groups of small individuals (< 30 cm) and large fish (individuals or in pairs). No more specifications	tanks	the sounds described were not descriminated for different sized fish
"	Freytag (1961,1964)	approach of another fish of the same or different species. Sound production was accompanied by the erection of the dorsal fin and the extension of the brightly coloured pectorals	not specified	not specified	1 specimen; sound production induced by mechanical stimulation

Table 2.2 - Continued.

Species	Author	Behavioural context of sound production	Sizes of fish studied	Conditions of recording	Comments
A. cuculus (red gurnard)	Hawkins (1968)	aggressive and defensive behaviour: sounds were accompanied by visual displays - spreading out the pectorals and dorsal fin erection	not specified	tanks	only individual specimens studied
T. lastoviza (streaked gurnard)	"	aggressive and defensive behaviour: sounds were accompanied by visual displays - spreading out the pectorals and dorsal fin erection. Knocking sounds accompanying persistent pectoral flicking	not specified	tanks	only individual specimens studied

2.2 - METHODS

2.2.1 - Fish collection and maintenance

Grey, streaked and red gurnards used in this study were captured with the use of trawls. Trawls were kept short to minimise fish skin and fin damage. Fish were captured from as shallow waters as possible to lessen the swelling (or even bursting) of the swimbladders, that fish may experience when pulled to the surface due to the rapid pressure changes. Only fish in apparent good condition were taken to the laboratory.

The grey gurnard - *Eutrigla gurnardus*

The grey gurnards used for investigating sound production were trawled at depths of 15 - 40 m, in the North Sea. After capture, fish were kept in containers with renewed or aerated sea water to keep them in good condition until they were landed and transported to the aquarium (at the Fish Behaviour Unit of the Marine Laboratory, Aberdeen). There, the fish were kept in fibreglass tanks either outdoors under natural light conditions (stock fish), or indoors under a computer-controlled day-night schedule, set to simulate natural light/dark conditions (experimental fish). Thus, the length of daylight hours varied according to the time of the year. The outdoor stock tanks were provided with raw sea water in open circulation, with temperatures similar to the sea: 5.5°C (Winter) to 13°C (Summer). The experimental indoor tanks were provided with treated recirculated sea water (filtered and sterilised), with temperatures ranging from 7°C (Winter) to 12°C (Summer). For experiments, smaller specimens were kept indoors in 1.5 and 3 m diameter fibreglass tanks (respectively the small and the medium-sized fish, see section 2.2.2), and larger specimens in a swimming-pool (3.5 m (width) × 7.0 m (length) × 1.5 m (depth)) (large and extra-large fish, see section 2.2.2), with sand bottoms and aeration. The fish were fed three times a week, with fish or shrimps.

The streaked gurnard - *Trigloporus lastoviza*

Fish were trawled in the Mediterranean off the Bay of Iraklion, Crete (Greece), from depths of 10 to 15 m, and kept in a tank on board the fishing vessel with continuously inflowing sea water, until transfer to the experimental tanks at the Institute of Marine Biology of Crete, Iraklion. The streaked gurnards were kept in 1.5 m fibreglass tanks, provided with aeration and recirculated sea water (filtered and sterilised) running in both closed and open circuits. The water temperature was approximately the same as the sea temperature, ranging from approximately 17° to 24°C in the months of May to July (1994). A natural photoperiod was maintained, with natural light supplied by large windows in the tank room. The gurnards were fed with chopped fish 3 times a week.

The red gurnard - *Aspitrigla cuculus*

The red gurnards were also trawled in the Mediterranean, off the Bay of Iraklion, at depths of 200 - 300 m. Live specimens were kept in a tank on board the fishing vessel, with renewed sea water. None of the specimens survived for more than 30 - 45 min due to swimbladder decompression problems. Since it was impracticable to decompress the fish, remedy was attempted by piercing the swimbladders with a syringe needle to release the gas. This proved unsuccessful. Sounds from red gurnards could therefore only be obtained during the period while the fish remained alive, on board the research vessel.

The tub gurnard - *Trigla lucerna*

The tub gurnards used for recordings were residents in 2 exhibition tanks at the Vasco da Gama Aquarium Exhibition Centre in Lisbon (Portugal). The fish were originally caught in the Tagus estuary, near Lisbon, and had been kept in the aquarium for 1 month to 2 years. The 2 tanks were made of concrete with glass fronts: 0.82 m (width) × 1.50 m (length) × 0.60 m (water depth), and 1.75 m (width) × 3.40 m (length) × 1.20 m (water depth). Both tanks were provided with aeration and recirculated sea water (in a closed circuit). The smaller tank had a sandy bottom, and the larger tank a gravel bottom with several big rocks, starfish and anemones. Because these were exhibition tanks, the tub gurnards were kept with other fish species. In the smaller tank there were also 2 lesser weavers (*Trachinus vipera*, Trachinidae) and 1 boar-fish (*Capros aper*, Caproidea). In the larger tank the tub gurnards were kept with 1 rainbow wrasse (*Coris julis*, Labridae). Fish were subject to a natural photoperiod by virtue of windows in the roof above the tanks and artificial lights which were switched on and off with a timer; the artificial lights were only used in the winter when the level of natural light level was low. The water temperature ranged from 15.5° - 17.5°C during the months of study: December - January.

2.2.2 - Fish length and group size classes

The grey gurnard - *Eutrigla gurnardus*

Five arbitrary size classes of fish were studied for sound production: individuals ranging from approximately 10 to 15 cm (small), 15 to 20 cm (medium), 25 to 30 cm (large), and 30 - 40 cm (extra-large) in total length. Only the medium-size class of fish were used to study the complete sound repertoire of this species over a year, since they were the only ones available all year round. Sounds recorded from the other size classes were used to study ontogenetic changes of sound production in this species. The group sizes used during recordings varied over the study period between: 3 - 5 small (S), 2 - 5 medium (M), 3 - 4 large (L), and 8 extra-large (XL) individuals.

The streaked gurnard - *Trigloporus lastoviza*

Fish used in recordings ranged from 10 - 15 cm total length and were divided into two separate groups (one group in each tank), that varied over the study period from 3 to 5 individuals in one group and from 9 to 10 in the other.

The red gurnard - *Aspitrigla cuculus*

The fish size range of the individuals recorded on board the vessel (see section 2.2.1) was from 10 - 25 cm total length.

The tub gurnard - *Trigla lucerna*

Fish in groups of 4 and 6 were studied, measuring respectively approximately 20 - 30 cm and approximately 40 cm in total length (which were respectively in the smaller and the larger tanks; see section 2.2.1). Only the smaller-sized fish were heard to emit sounds during the period of study.

2.2.3 - Diel rhythms of activity and sound production

Diel rhythms of activity and sound production were measured during the Spring period and for the grey gurnard only. Five medium-sized individuals were observed in a 3 m diameter fibreglass tank, using a low-light sensitivity CCD video camera equipped with a 6 mm lens, set overhead at the centre of the tank, approximately 1.5 m above the water surface. Fish activity was recorded using a SVHS video recorder (Panasonic, model AG-6720) on a time lapse basis (one frame every 0.48 s) over a period of 96 hours. The maximum number of fish engaged in swimming during each 15 min intervals of the 96 hours period was scored. The proportion of active fish expressed as % was then compared between the different times of the day.

The diel rhythms of sound production were studied in a group of 7 small to medium fish, kept in a 1.5 m diameter fibreglass tank. Sound production was monitored for 10 min every hour with a professional digital audio tape recorder (DAT) (Casio, model DA-2). The complete equipment and settings used for recording the sounds uttered by the fish are described in section 2.2.4. As the water had to be switched off during the periods of recording to decrease the background noise and the fish density was high, recordings lasted only 12 hours. Water was allowed to run for a minimum of 12 hours between recording periods. A total of 144 hours were sampled for sound emissions, over a total of 6 days and 6 nights. Fish were never fed during or close to the 10 min recording periods since the increased acoustic activity associated with feeding could bias the results.

2.2.4 - Recording of fish calls

For the purpose of sound analysis acoustic emissions from all species of gurnards were obtained with hydrophones (MS.83 Sound Range Hydrophone from Plessey Company Ltd.) with a sensitivity of -100 dB re 1V/μbar and with a flat frequency response up to 40 kHz, placed approximately in the middle of the experimental tanks. The sounds were amplified and filtered with a low-noise amplifier (Brookdeal, model 450), calibrated for 30 to 50 dB gain and set for frequency limits between 10 Hz and 10 KHz, and recorded with a digital audiotape recorder (DAT) (Casio, model DA-2 and DA-1). Recorded sounds were monitored with RS dynamic headphones connected to the Brookdeal amplifier. The settings of both the DAT recorder and the amplifier were chosen in order to achieve the highest signal/noise possible without sound distortion (Fig. 2.1). Because sound production increased significantly during feeding bouts in all gurnard species studied (except for the red gurnard; see section 2.2.1), sounds were always recorded during these periods.

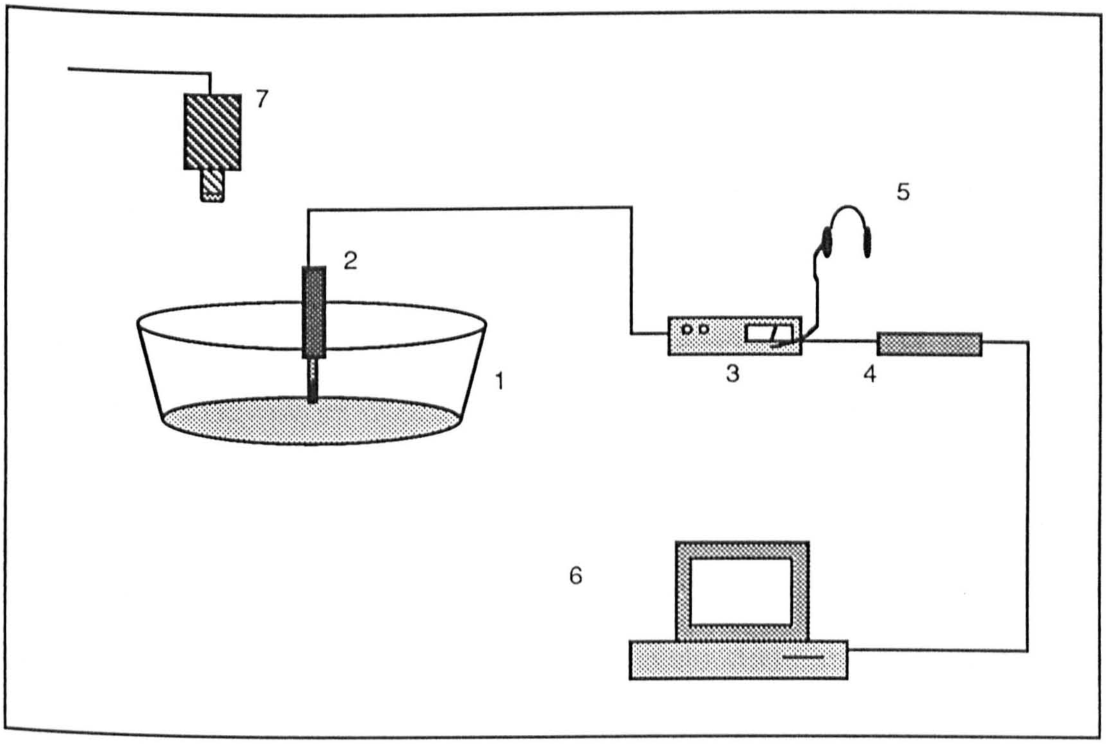


Figure 2.1 - Schematic representation of the equipment used to maintain fish, record and analyse fish sounds and behaviour. 1- Fibreglass tank filled with flowing sea water, 3 m in diameter; 2- Plessey hydrophone; 3- Brookdeal low-noise amplifier with frequency filter; 4- Professional digital audio tape recorder (DAT); 5- RS dynamic headphones; 6- IBM compatible PC provided with Loughborough Sound Images Workstation for fish sound analysis; 7- Video camera to monitor fish behaviour.

The medium-size class of grey gurnards were recorded throughout the year (from March 1993 to February 1994) as previously mentioned, but all other size classes were only studied when fish were available: that is, for small fish - June to July (1993); for large fish - March to April (1994); for extra-large fish - October to November (1995). These recordings enabled the creation of a complete catalogue of the sounds produced by this species as well as the study of ontogenetic and seasonal variations of sound production.

Streaked and red gurnards were recorded over the months of May to July (1994), and tub gurnards during December (1994) and January (1995).

Fish sex was not discriminated during recordings since these species do not present any external sexual dimorphism. However, when opportunity arose (e.g. death of experimental animals), gonad development and sex was noted.

2.2.5 - Sound analysis

The sounds produced by gurnards were analysed using a Loughborough Sound Images Workstation (version 2.0; 1986-Metagraphics Software Corporation©) with IBM compatible hardware (Elonex PC-333) (Fig. 2.1).

In order to objectively describe and compare the acoustic emissions produced by the different species of gurnards studied, the following criteria were used: a call is here defined as the entire sound produced by a fish in a given event. A phrase is considered to be a subdivision of a call; thus a call may be composed of several repeated phrases. A phrase can be further divided into smaller elements, the pulses. Often pulses within a phrase are clustered into groups of 2 or more pulses. Different pulses within a phrase cannot always be discriminated by the human ear, but different phrases within a call can. Gurnards' sounds were classified into three phrase types: knocks, grunts, and growls, all consisting of repetitive pulses. Knocks are audible to the human hear as a single pulse, whereas grunts and growls are heard as longer, continuous sounds, composed of several pulses.

The following features were measured in order to analyse the various sounds recorded from different gurnard species:

Call

- duration of the call
- number of phrases within a call
- interval between phrases

Knocks

- duration of the phrase
- duration of the first pulse
- number of pulses
- interval between the 1st and the 2nd pulses
- mean pulse peak to peak interval (i.e. mean of the time elapsed between the peak amplitude of two pulses; equivalent to the pulse repetition rate).
- peak frequency (frequency measured where the phrase presents its highest intensity)

Grunts and growls

- duration of the phrase
- mean duration of pulses
- number of pulses
- if pulses in a phrase are arranged in groups: number of groups of pulses and number of pulses in each grouping.

- mean pulse peak to peak interval
- group peak to peak interval, if the pulses in a phrase are further rearranged in smaller groups (i.e. mean of the time elapsed between the peak amplitude of the first pulses of consecutive groups of pulses).
- peak frequency (frequency measured where the phrase presents its highest intensity)

The acoustic emissions used for interspecific comparisons were all produced by sexually inactive individuals.

2.3 - RESULTS

2.3.1 - The grey gurnard - *Eutrigla gurnardus*

It is likely that both males and females of the studied species have similar abilities to produce sounds since both males and females were found during the examination of the mortalities that occurred during the course of experiments. Females were, however, predominant. Further, the different individuals recorded did not show any apparent difference in their ability to vocalise.

2.3.1.1 - Diel rhythms of activity and sound production

The 5 medium grey gurnards studied showed an increased activity during the hours of light including dawn and dusk periods (Fig. 2.2). A smoother was fitted to the data of each of the four days of video recording (using Cricket graph, version 1.3.2 ©), in order to reveal the overall trend.

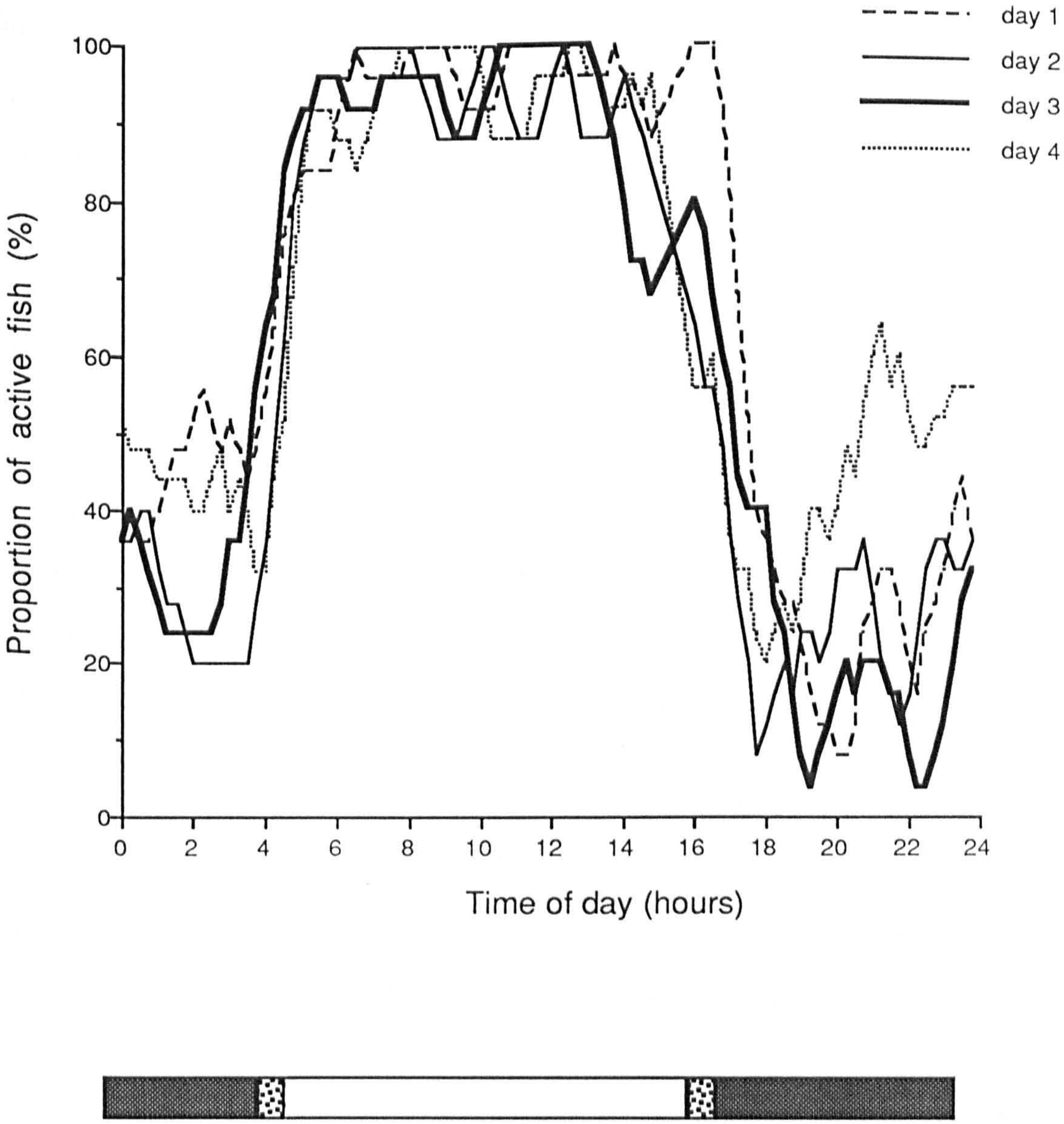


Figure 2.2 - Rhythm of activity of 5 grey gurnards (15≤TL<20 cm) recorded over a period of 4 consecutive days. The fish were subject to a computer-controlled day-night schedule. The graphic depicts the proportion of active fish sampled during each 15 minutes interval of every hour of the day. A smoother was fitted to the data of each of the 4 days.
□ - Day time; ▨ - Dawn and dusk; ■ - Night time.

Calls occurred infrequently. The typical number of calls registered per 10 min of recordings during the different periods of the day is shown in Figure 2.3. Each call was made up of on average 7.42 phrases (s.d. = 10.65; min = 1 and max = 52). The existence of diel rhythms of sound production was checked by generalized linear models (GLM). The response variable used was the number of calls produced per 10 min of recording (see section 2.2.3). The explanatory variables were time of the day (day; night; dawn and dusk) and date (the different days when the recordings took place). Both variables were used as factors with 3 and 12 levels, respectively. The models were fitted with a Poisson distribution and a log link function. This distribution assumption was selected because the response variable consisted of count data (many zero observations and few with large values) (Crawley 1993). The number of calls was preferred to the number of phrases as the response variable to reduce potential problems with overdispersion. The above model was tested against the null model and was found to be highly significant (Table 2.3). It was then compared with models including only time of day or date to test for the relative importance of date and time of day effects. These comparisons suggest that date is only marginally significant ($P = 0.04$) whereas time of day is highly significant (Table 2.3). In addition, a model fitted with only the time of day as explanatory variable was tested against the null model confirming the previous results (Table 2.3). Furthermore, the fitted effects of date indicated that date was marginally significant only because on one experimental day no calls were registered. Observations of the fitted effects indicated that call emission was significantly higher during day time and during dawn and dusk periods than at night time. Significant differences between day time and dawn and dusk could not be found.

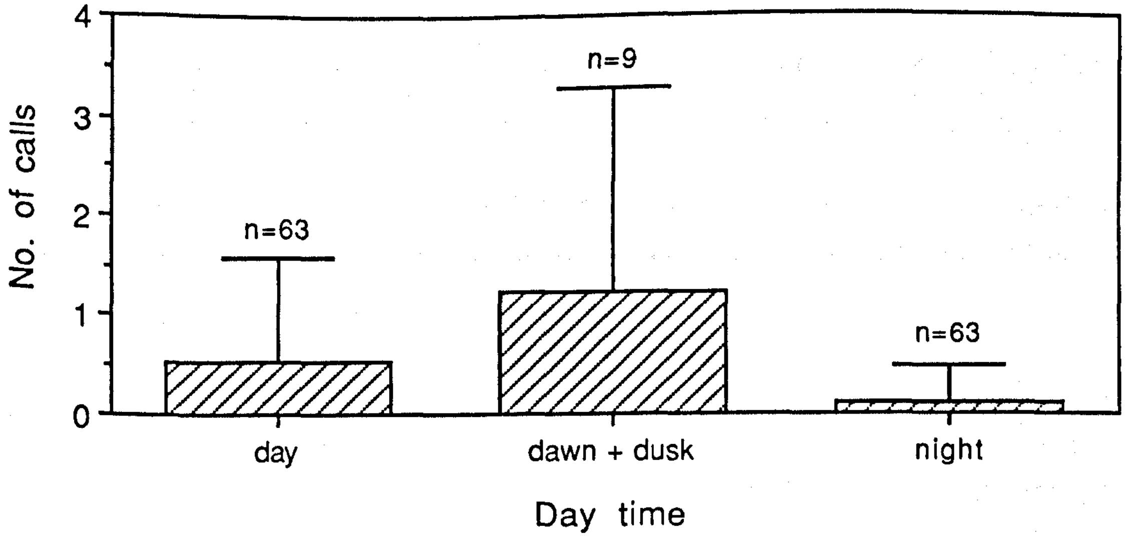


Figure 2.3 - Mean and standard deviation of the number of calls registered per 10 min of recording over a 144 h period during, day, dawn and dusk, and night time. Sound production was studied outside feeding bouts.

Table 2.3 - Results of χ^2 tests between models comprising time of day (T) and date (D) effects. 1 is the null model. * = P<0.05; *** = P<0.001.

Model comparisons	Testing:	Probability χ^2
T + D vs 1	T + D effect	***
T + D vs T	D effect	*
T + D vs D	T effect	***
T vs 1	T effect (with no D effect)	***

2.3.1.2 - The sound repertoire

The calls produced by grey gurnards were categorised on the basis of common acoustic characteristics and subjective aural impression into three kinds of phrases: knocks, grunts, and growls.

Figure 2.4 shows sound duration against pulse peak to peak interval to assess whether the classification of sounds presented here is appropriate. Preliminary analysis showed that these 2 sound parameters were the most appropriate for categorising the grey gurnards' sounds into the different types (uncorrelated variables most significant in a classification tree for sound type). Sounds from all 4 fish size classes studied were used (n = 1081). Figure 2.4 also explores whether the different sound types formed continuous or discrete categories of acoustic emissions. It is important to note that none of the knocks consisting of single pulses are shown in the plot. The sounds fall into 3 distinct clusters with few overlapping points. Some of the sounds classified as knocks lie between typical knocks and grunts and are probably a second subtype of either phrase. Figure 2.5 shows the distribution of the number of pulses for each phrase type.

Since the identification of sound type achieved during the video analysis relies on the aural capability of distinguishing sounds, the original sound classification will be the one used in this Chapter. Verification of the biological validity of these categories will be achieved by correlating the different sound types with different behaviours or sound contexts in Chapter 4.

Sonograms and oscillograms of knocks, grunts and growls produced by grey gurnards are presented in Figures 2.6 - 2.8. A quantitative description of the different phrases is presented in Tables 2.4 (for all fish sizes). Descriptive statistics for the interval between calls, call duration, number of phrases in a call and interval between phrases are given in Table 2.5. Although, the mean values for intervals between knocks are greater than the mean values for the intervals between grunts (Table 2.5), 61% of the intervals between grunts are smaller than 200 ms, whereas 60% of the intervals between knocks are either smaller than 100 ms or greater than 500 ms (Fig. 2.9). This means that grunts are repeated in fairly typical intervals, smaller

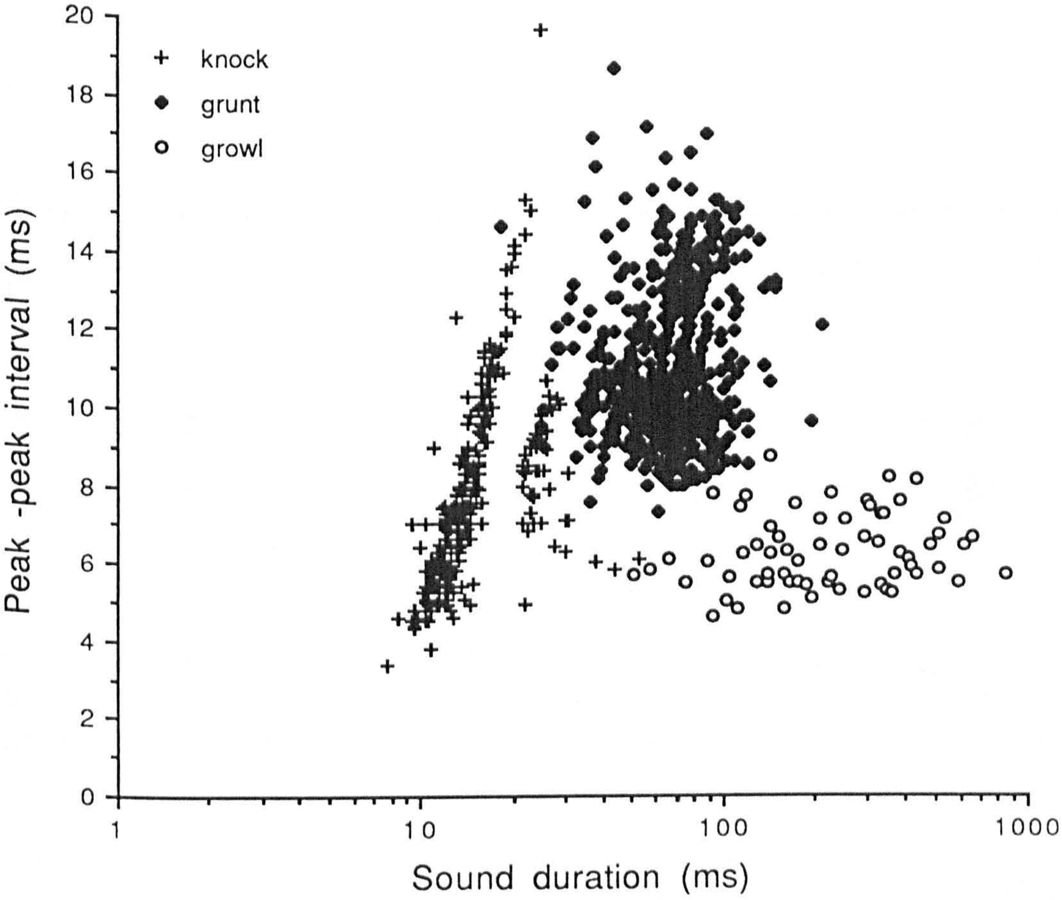


Figure 2.4 - Sound duration (ms) against pulse peak to peak interval (ms) of knocks, grunts and growls for all sizes of grey gurnards. N = 1081.

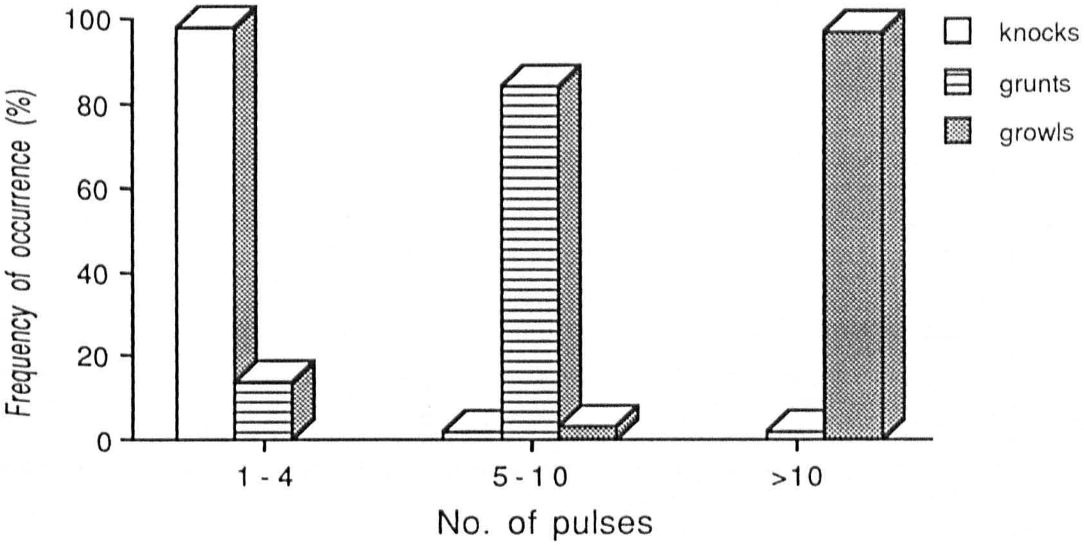


Figure 2.5 - Distribution of the number of pulses in knock, grunt and growl phrases.

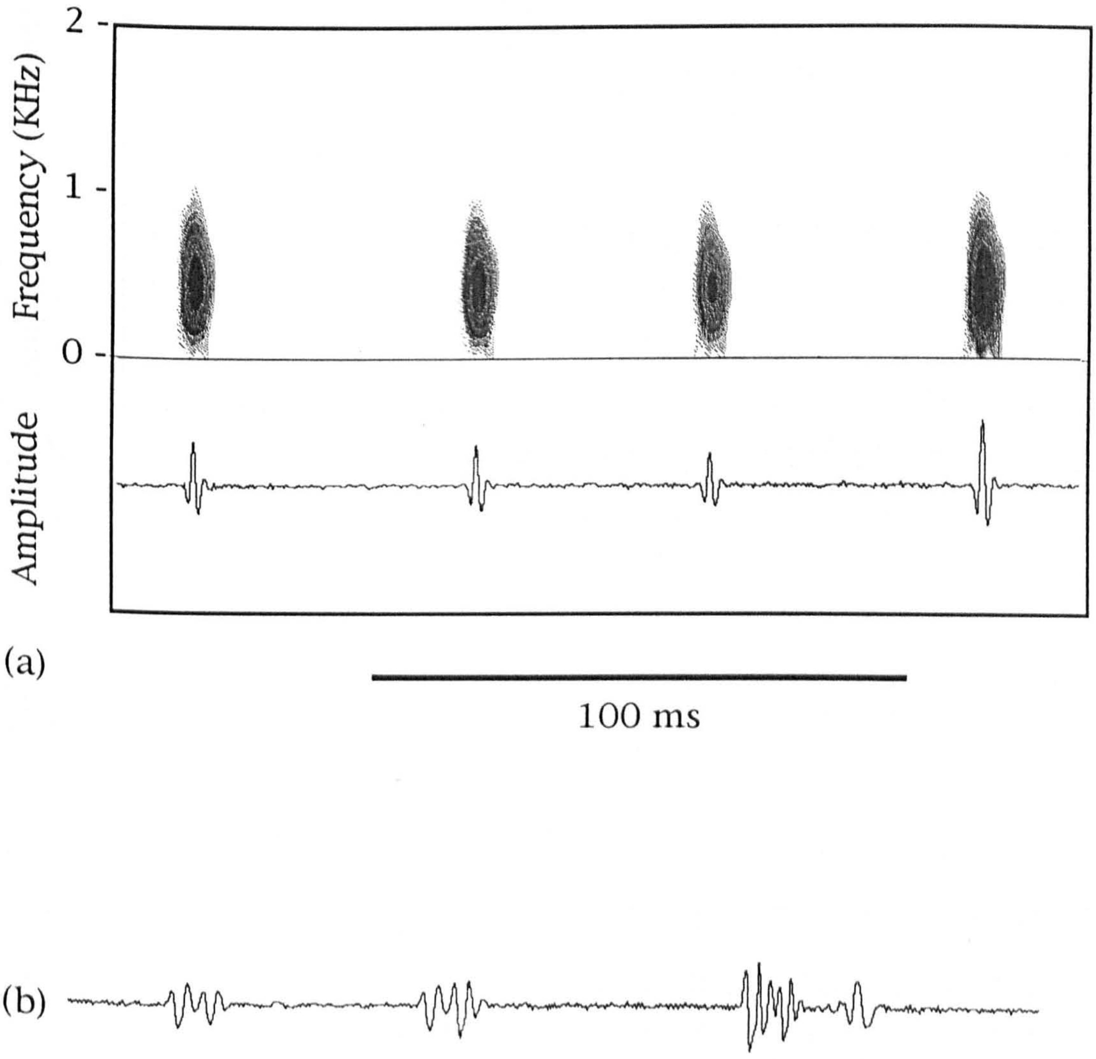


Figure 2.6 - (a) Sonogram and oscillogram of a series of knocks made up of 1 pulse, produced by medium grey gurnards (filter bandwidth = 125Hz). (b) Oscillogram of a train of knocks made up of 2 and 3 pulses emitted by extra-large grey gurnards. The time scale is the same for (a) and (b).

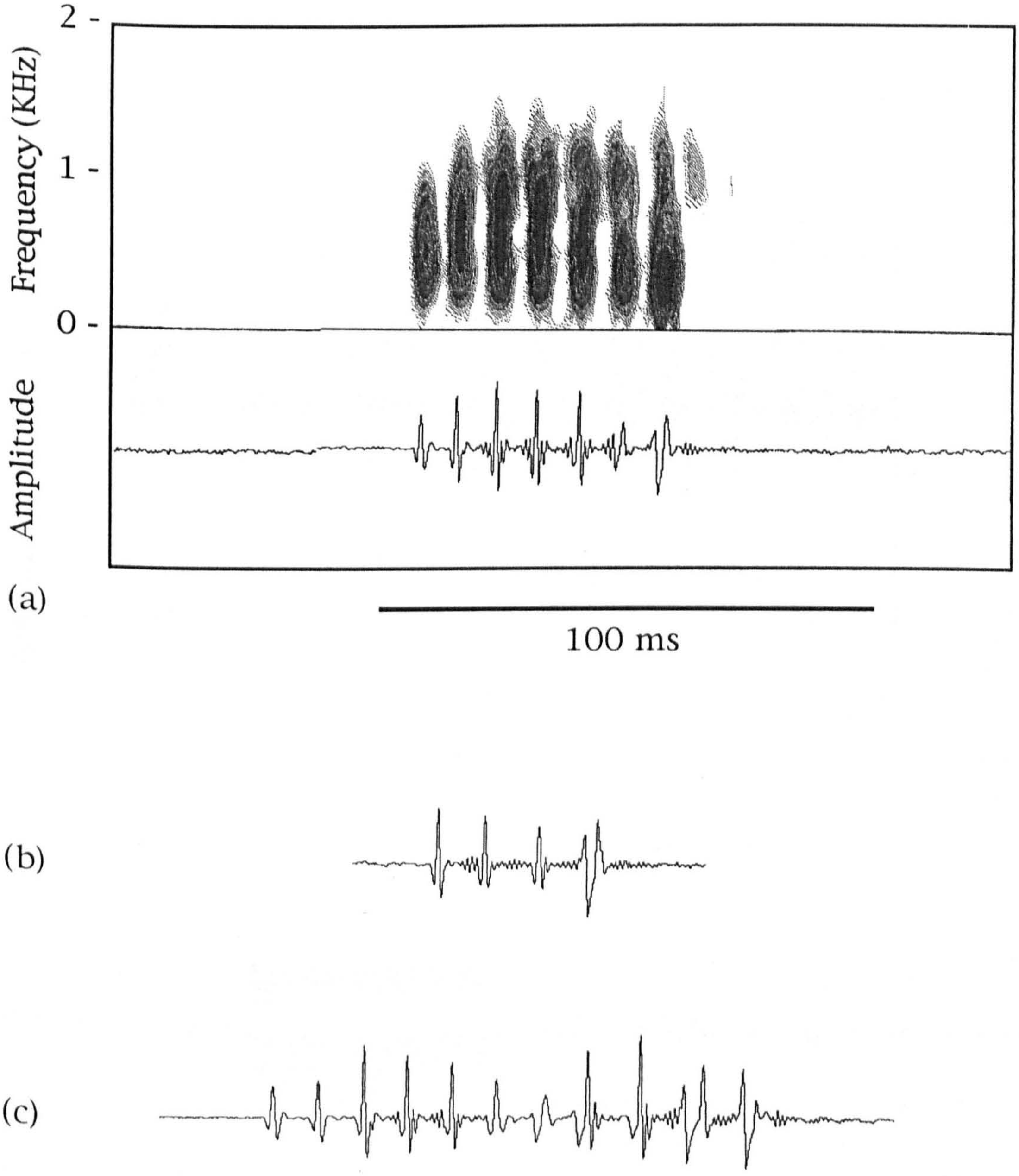


Figure 2.7 - (a) Sonogram and oscillogram of a grunt with 7 pulses produced by medium grey gurnards (filter bandwidth = 125Hz). (b) and (c) are the oscillograms of two grunts produced by medium grey gurnards with respectively 4 and 11 pulses. The time scale is the same for all grunts.

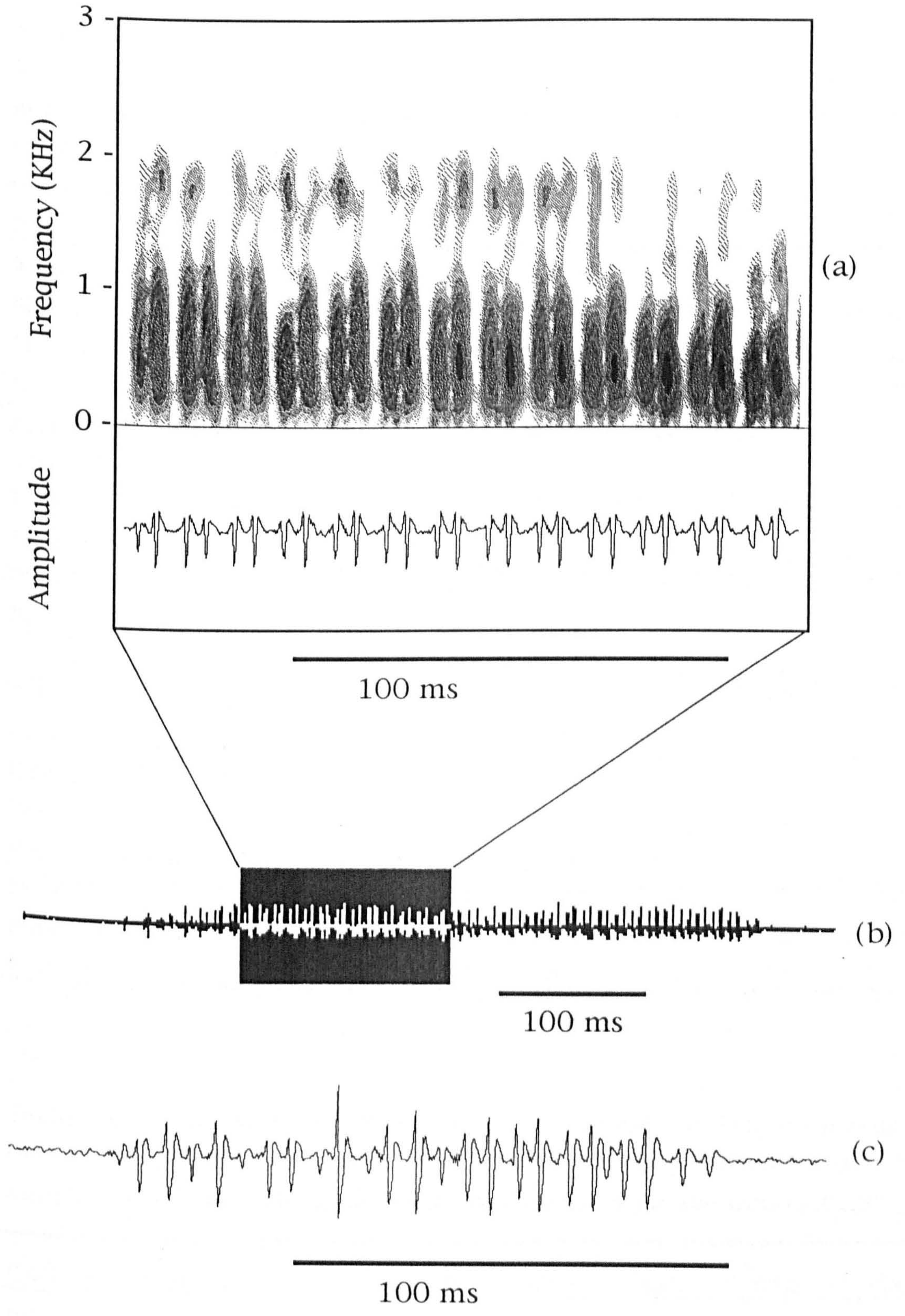


Figure 2.8 - Example of 2 growls produced by small grey gurnards. (a) Sonogram and oscillogram of a section of growl 1 (filter bandwidth = 125Hz). (b) Envelope of the complete growl 1 (78 pulses). (c) Oscillogram of growl 2 (24 pulses).

Table 2.4 - Mean, standard deviation (s.d.), minimum (min) and maximum (max) of the parameters measured to characterise knocks, grunts and growls produced by all sizes of grey gurnards. N = sample size. For an explanations of the parameters listed see section 2.2.5.

Phrase parameters	N	mean	s.d.	min	max
Knocks					
Phrase duration (ms)	985	12.19	7.51	2.6	53.0
Pulse duration (ms)	985	5.65	0.94	2.6	9.8
No. of pulses	985	1.84	0.90	1	7
Pulse peak-peak interval (ms)	333	7.76	2.28	3.4	19.6
1st-2nd pulse interval (ms)	589	2.02	1.85	0	8.7
Peak frequency (Hz)	985	515.51	165.52	246	1064
Grunts					
Phrase duration (ms)	693	70.14	24.26	18.3	297.5
Pulse duration (ms)	692	5.55	0.84	3.6	8.4
No. of pulses	693	6.15	2.30	2	37
Pulse peak-peak interval (ms)	693	11.03	1.94	5.7	18.6
Peak frequency (Hz)	691	478.31	201.82	229	1170
Growls					
Phrase duration (ms)	64	263.55	167.31	50.8	846.5
Pulse duration (ms)	64	4.61	0.40	3.9	5.5
No. of pulses	64	41.13	26.51	9	140
Pulse peak-peak interval (ms)	64	6.24	0.94	4.6	8.7
Peak frequency (Hz)	64	498.80	159.08	304	1018

Table 2.5 - Mean, standard deviation (s.d.), minimum (min) and maximum (max) of the parameters measured to characterise grey gurnards' calls. N = sample size. For an explanations of the parameters listed see section 2.2.5.

Call parameters	N	mean	s.d.	min	max
Interval between calls (s)	182	10.91	15.75	0.12	116.00
Knock call duration (s)	130	1.40	2.11	0.006	9.192
No. of knocks in a call	130	5.30	5.05	1	30
Interval between knocks (ms)	873	324.02	368.13	5.6	2172.0
Grunt call duration (s)	85	1.14	2.01	0.025	12.666
No. of grunts in a call	85	4.60	6.48	1	52
Interval between grunts (ms)	354	252.99	287.73	6.4	1858.0

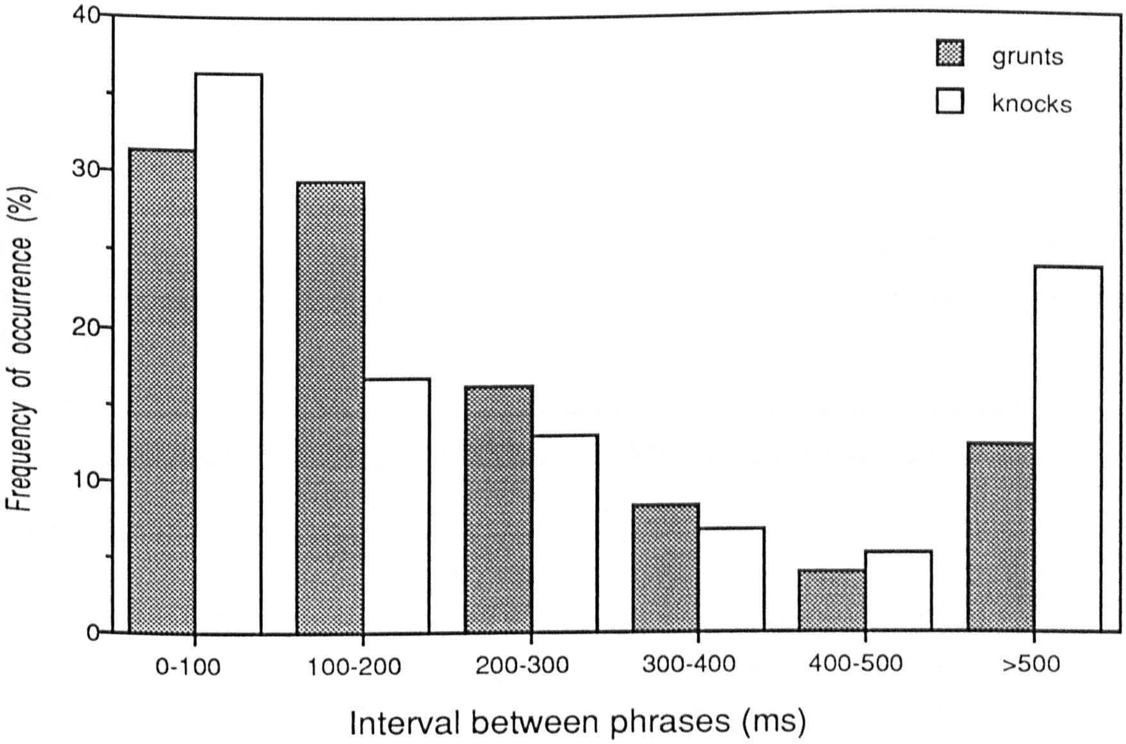


Figure 2.9 - Distribution of the intervals between phrases. N (knocks) = 873; N (grunts) = 354.

than 200 ms. Knocks, on the other hand, are either repeated very rapidly or slowly.

Knocks were the most frequent phrases produced, followed by grunts (Table 2.6). Growls, on the other hand, were heard only very rarely and were usually produced within or at the end of a sequence of grunts. Only 8 growls were recorded for the medium grey gurnards during one year, and only 52 and 4 growls were registered respectively for the small and the extra-large grey gurnads over 2 months of study (Table 2.6). A growl seems to be very intense sort of grunt, with a longer phrase duration, higher number of pulses and shorter peak to peak intervals than a grunt.

Table 2.6 - Descriptive statistics for the number of knocks, grunts, growls, and total number of phrases produced per minute, during feeding bouts (recording periods of 5 min). Data are presented separately for each fish size class.

Fish size		Knock	Grunt	Growl	Total
Small	n	11	11	11	11
	mean	19.51	3.36	1.67	24.55
	s.d.	9.67	2.63	0.84	10.46
	min	5.80	0.60	0.80	11.20
	max	32.80	8.00	3.80	42.60
Medium	n	32	32	32	32
	mean	9.10	5.31	0.04	14.46
	s.d.	7.78	5.79	0.11	9.92
	min	0.40	0.00	0.00	1.40
	max	31.20	21.60	0.40	38.00
Large	n	10	10	10	10
	mean	6.86	4.00	0.00	10.86
	s.d.	5.09	2.47	0.00	6.00
	min	0.00	0.00	0.00	3.00
	max	15.20	8.20	0.00	20.60
X-large	n	11	11	11	11
	mean	4.53	4.27	0.05	8.85
	s.d.	4.06	3.60	0.09	5.11
	min	0.00	0.40	0.00	1.40
	max	10.80	9.80	0.20	18.20

2.3.1.3 - Seasonal variations

The sound production rate (no. of phrases / min) observed for medium-sized grey gurnards throughout a year, is shown in Figure 2.10. Seasonal variations in the acoustic parameters of knocks and grunts (see section 2.2.5) were investigated with a Kruskal-Wallis non-parametric test. The data were pooled for each two consecutive months (January + February; March + April, etc) to allow an adequate sample size. When the p-value of the Kruskal-Wallis was similar to the p-value of the parametric ANOVA, it was inferred that the ANOVA's assumptions were not seriously violated. In such cases, and when the effect of the explanatory variable was significant, 95% confidence intervals were used as an *a posteriori* test.

Variations of water temperature and hours of light (photoperiod) during the study period are depicted in Fig. 2.11. Both these parameters are known to affect sound emissions (e.g. Ladich 1989, Torricelli *et al.* 1990b).

Seasonal variation in the features of growls could not be studied since only 8 were recorded over a one-year sampling period for the medium grey gurnards. Nevertheless, 5 out of the 8 growls recorded were produced in the months of June and July.

Knocks

Although the period of the year had significant effects on the knocks' physical features (Table 2.7), the observed variations showed no seasonal patterns except for the interval between the first and the second pulse which decreased significantly from March to October (Fig. 2.12).

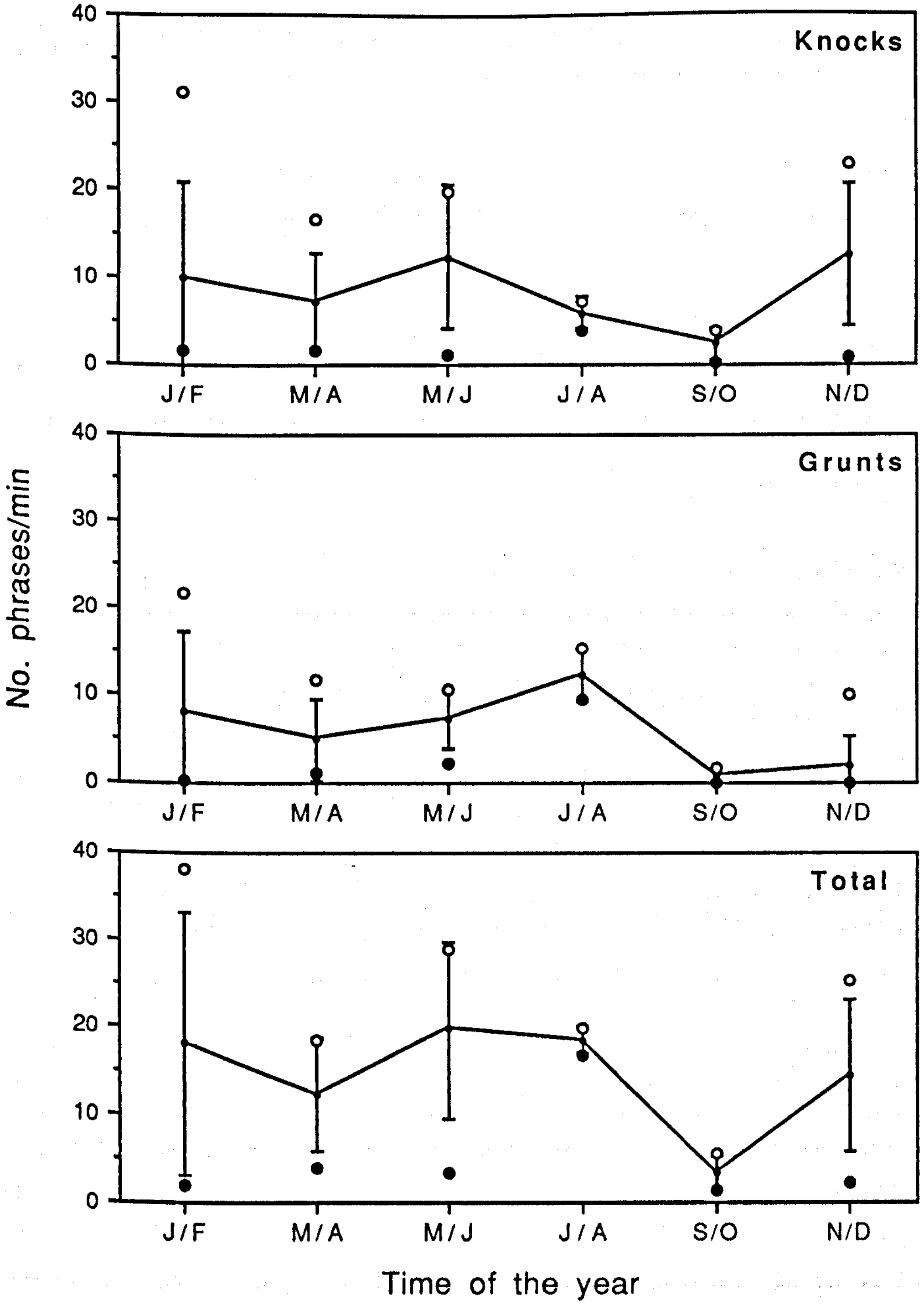


Figure 2.10 - Bimonthly means of sound production rate (no. of phrases per minute) of knocks, grunts and total number of phrases (including growls) during feeding bouts. Bars indicate standard deviations; closed and open circles indicate minimum and maximum values, respectively. Data concern medium-sized grey gurnards.

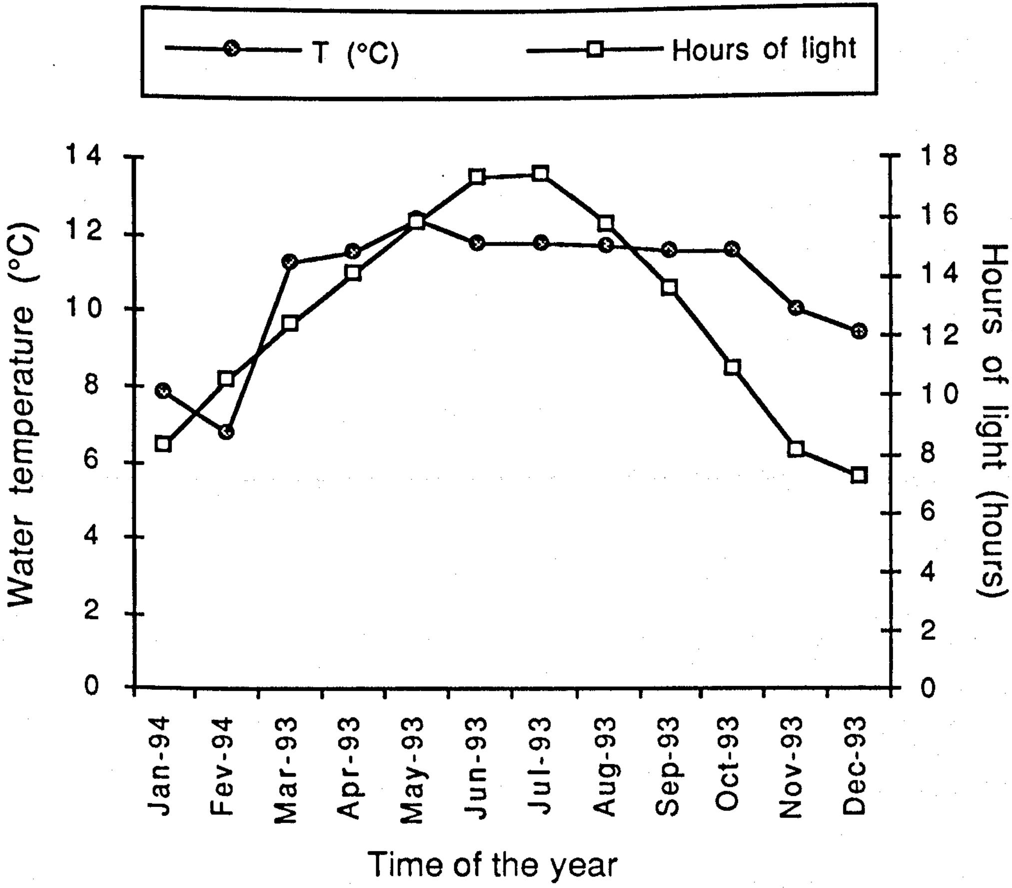


Figure 2.11 - Variation of water temperature and hours of light during the period of March - 93 to February - 94 in the Fish Behaviour Unit, Marine Laboratory, Aberdeen.

Table 2.7 - Results of the Kruskal-Wallis test for seasonal variations of the physical features of knocks produced by medium grey gurnards ($15 \leq TL < 20$ cm) throughout one year. N = total sample size. *** = $P<0.001$.

Knock - parameters	N	d.f.	H	P
Phrase duration (ms)	510	5	59.08	***
1st pulse duration (ms)	"	"	48.59	***
No. of pulses	"	"	71.27	***
Peak frequency (Hz)	"	"	255.26	***
1st-2nd pulse interval (ms)	307	"	80.37	***

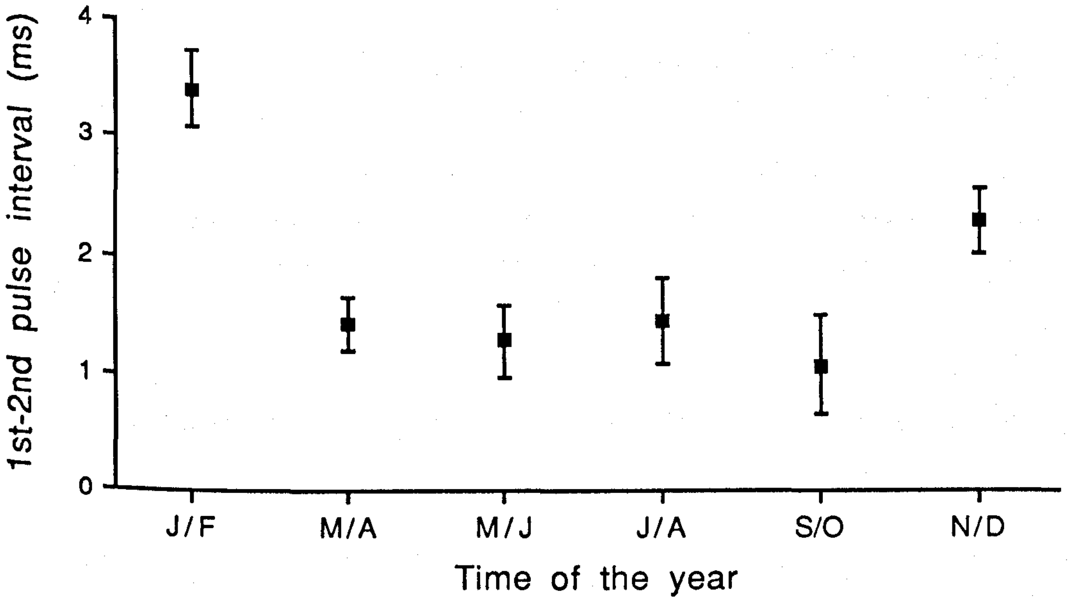


Figure 2.12 - Knock phrases. Bimonthly mean of the interval between the 1st and 2nd pulse throughout one year. Data concern medium grey gurnards. Bars indicate 95% confidence intervals.

Grunts

Seasonal changes in the physical features of grunts were also significant (Table 2.8). The parameters peak to peak interval and peak frequency presented a seasonally-patterned variation. There was a significant decrease in the peak to peak interval (or an increase of the pulse repetition rate) (Fig. 2.13), and a significant increase of the peak frequency (Fig. 2.14) in the Spring-Summer period. Also, the percentage of grunts with a higher number of pulses was greater in the period of May to August (Fig. 2.15). Furthermore, the proportion of grunts produced per 5 minutes of recording increased in the months of July-August (and thus the proportion of knocks decreased in these months) (Fig. 2.16). It seems therefore that in the Spring-Summer period the grunt produced become more intense.

Table 2.8 - Results of the Kruskal-Wallis test for seasonal variations of the physical features of grunts produced by medium grey gurnards throughout one year. N = total sample size. *** = P < 0.001.

Grunt - parameters	N	d.f.	H	P
Phrase duration (ms)	413	5	127.99	***
Pulse duration (ms)	"	"	103.19	***
No. of pulses	"	"	103.25	***
Peak frequency (Hz)	"	"	116.80	***
Peak-peak interval (ms)	"	"	212.94	***

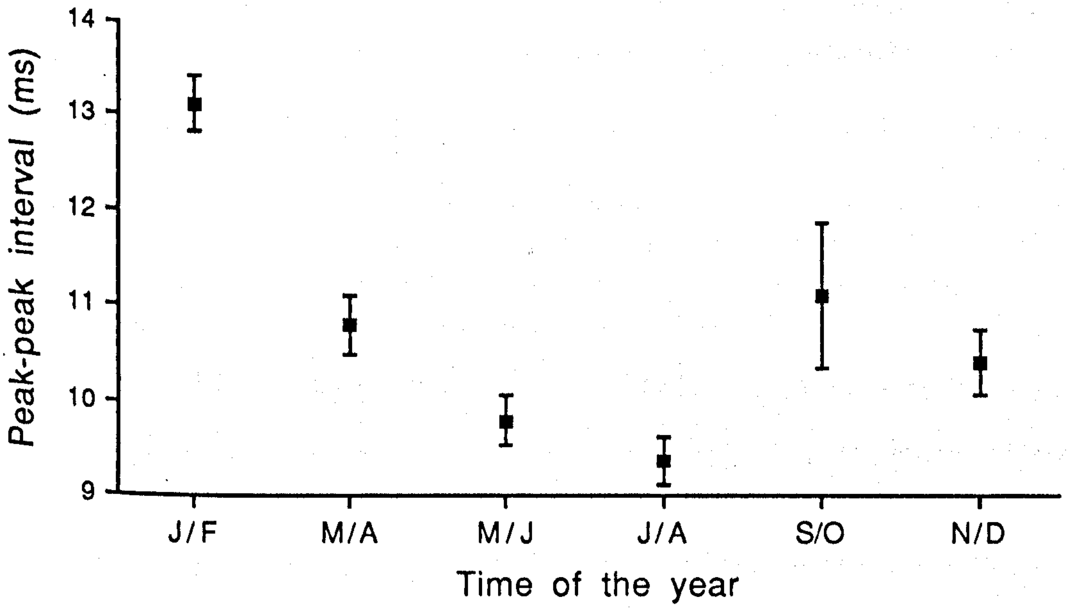


Figure 2.13 - Grunt phrases. Bimonthly mean interval of the pulse peak to peak interval throughout one year. Data concern medium grey gurnards. Bars indicate 95% confidence intervals.

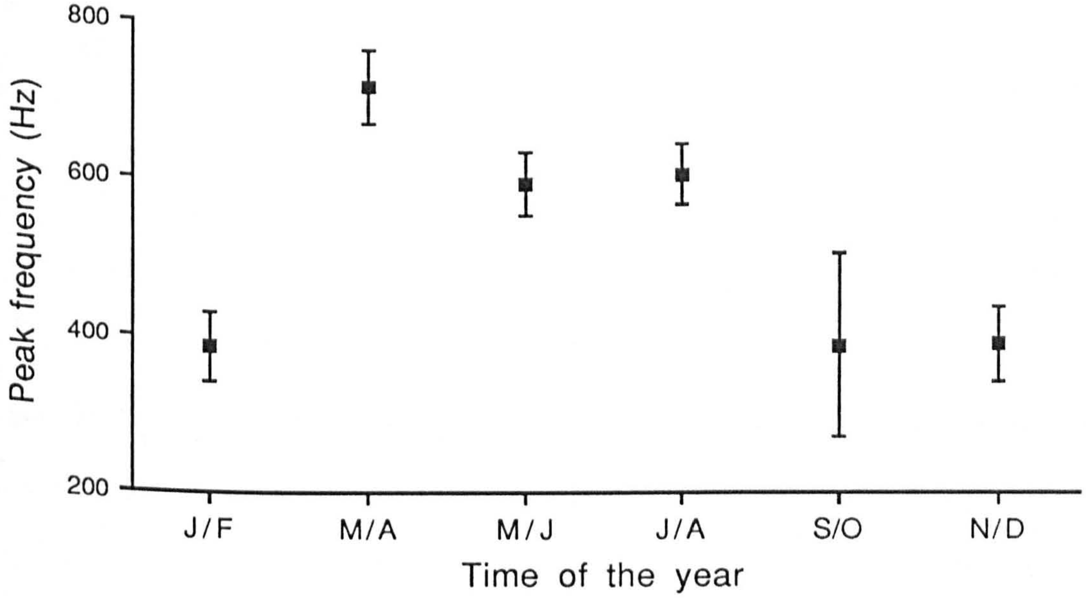


Figure 2.14 - Grunt phrases. Bimonthly mean interval of the peak frequency throughout one year. Data concern medium grey gurnards. Bars indicate 95% confidence intervals.

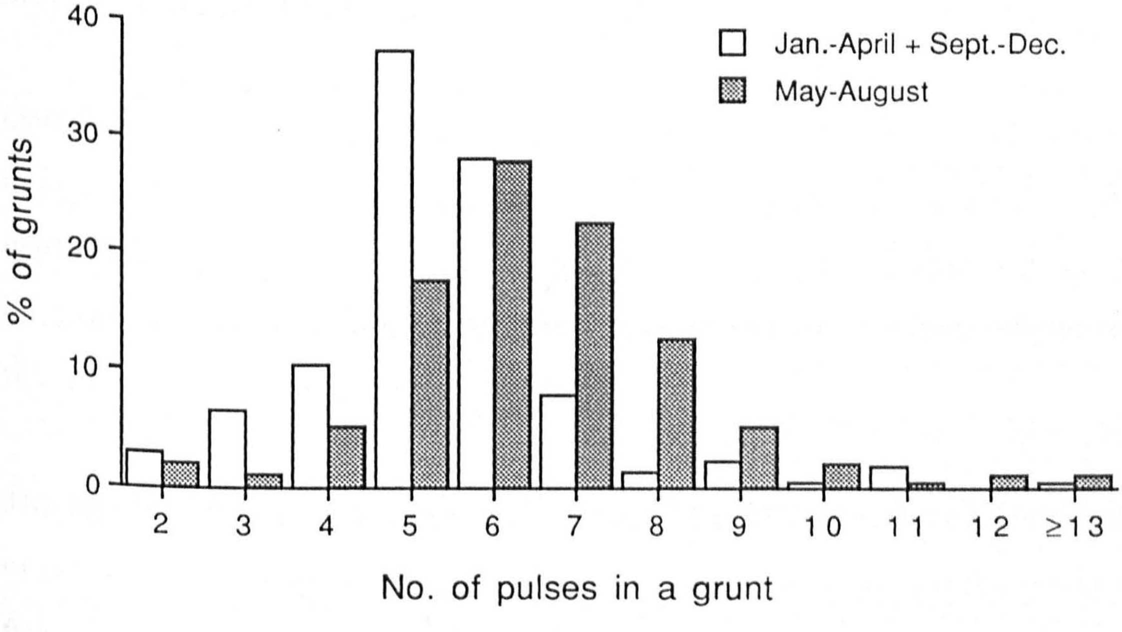


Figure 2.15- Percentage of grunts with different number of pulses produced by medium grey gurnards during the May - August period and during the rest of the year.

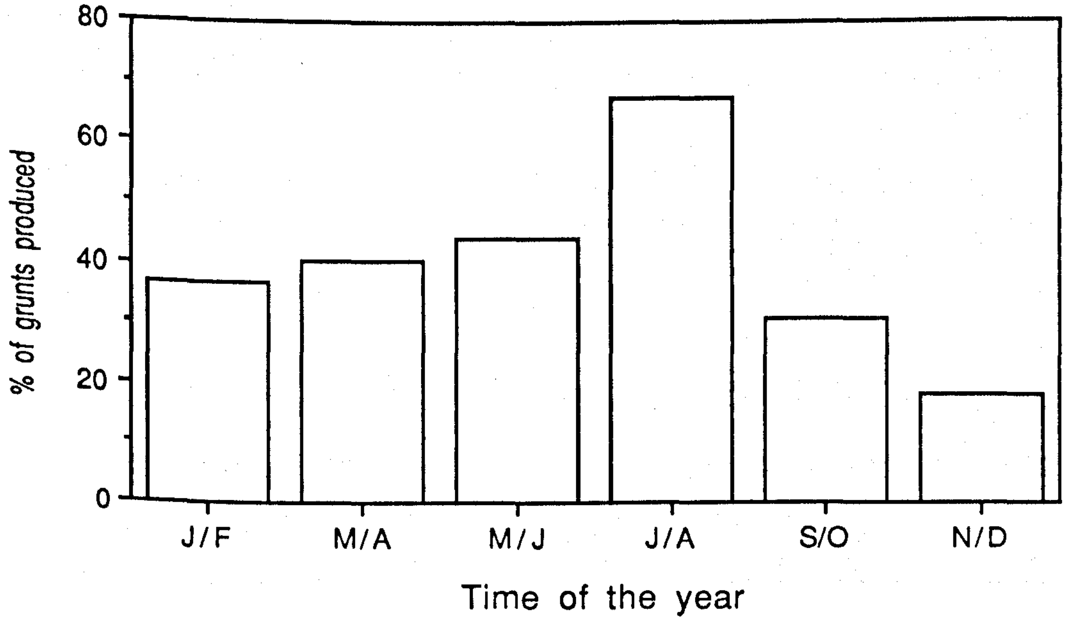


Figure 2.16 - Seasonal variation in the proportion of grunts produced by medium grey gurnards per 5 minutes of recording.

2.3.1.4 - Ontogenetic changes

Ontogenetic changes in sound production were investigated by regression analysis. Median fish total lengths for all size classes were used as the explanatory variable in a simple linear regression with normal errors. In all cases, the examination of residuals against the fitted values suggested that the distribution assumption was correct.

The effect of fish size on the total numbers of phrases produced per 5 min of recording is shown in Table 2.9 and Figure 2.17. In addition, the effect of fish size on the proportion of phrase type emitted per 5 min of recording was also checked for and is likewise presented in Table 2.9 and Figures 2.18. As proportions usually form a binomial rather then a normal distribution the data were transformed by arcsin($\sqrt{x}$) to fulfil the linear regression

Table 2.9 - Results of regression analysis on the effects of fish size on (I) total number of phrases and (II) proportion of each phrase type uttered per 5 min of recording. In II data are transformed by arcsin($\sqrt{x}$). The regression equation is $y = a + bx$. N = sample size; *** = $P < 0.001$; ** = $P < 0.01$. The correlation coefficient r is corrected for the degrees of freedom.

Regression	N	a	b	r	P
I					
Total no. of phrases / 5 min	64	140	-3.23	0.432	***
II					
Proportion of knocks	64	1.29	-0.018	0.306	**
Proportion of grunts	64	0.192	0.021	0.363	**
Proportion of growls	64	0.225	-0.008	0.510	***

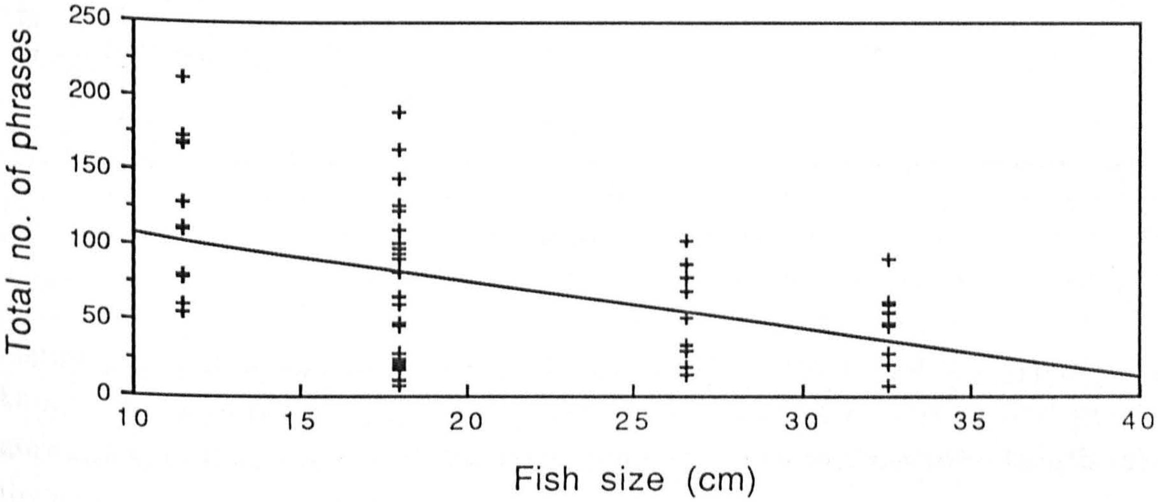


Figure 2.17 - Relationship between the total number of phrases produced per 5 min of recording and grey gurnard size. The median length of the experimental fish was used for each size class.

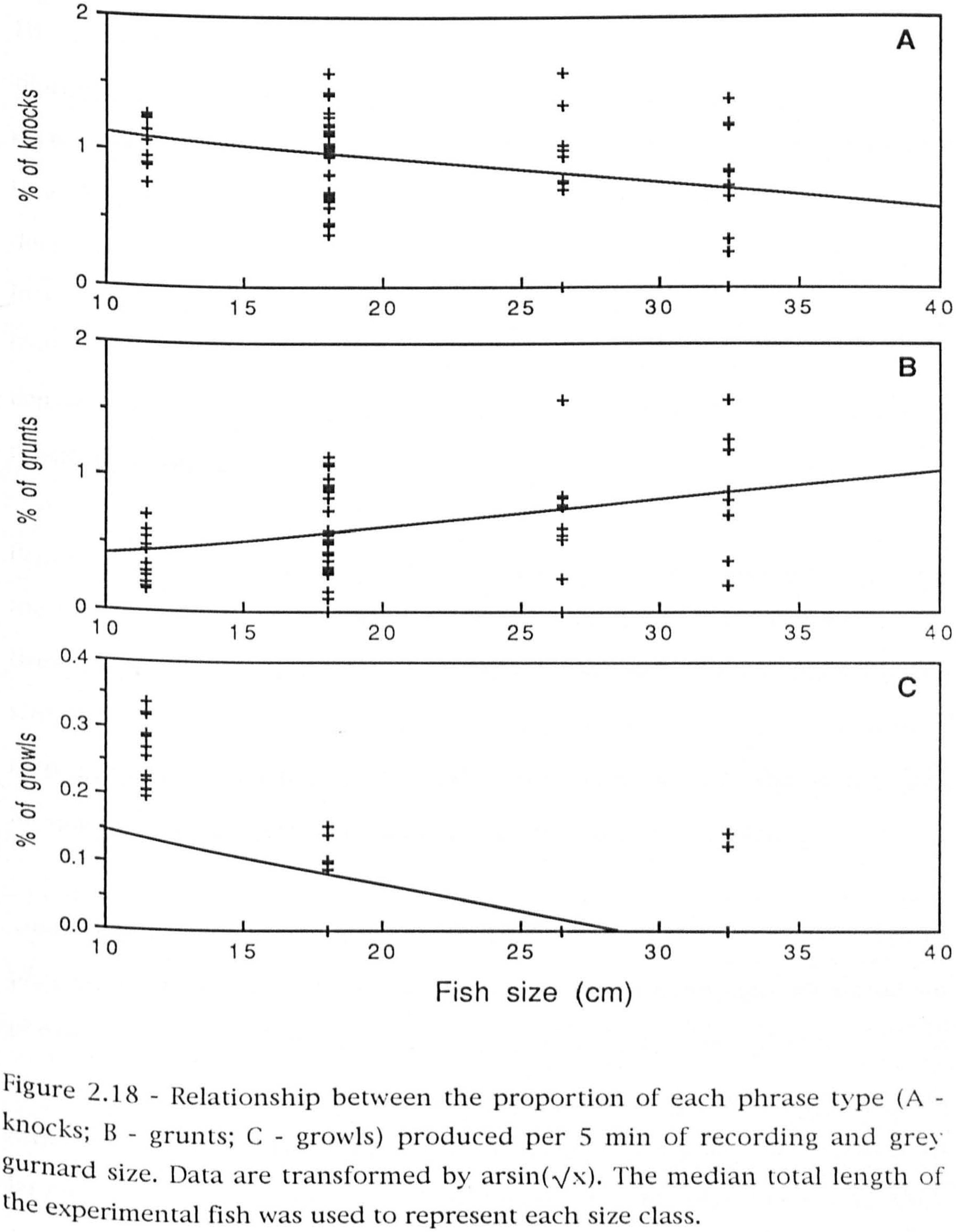


Figure 2.18 - Relationship between the proportion of each phrase type (A - knocks; B - grunts; C - growls) produced per 5 min of recording and grey gurnard size. Data are transformed by arsin($\sqrt{x}$). The median total length of the experimental fish was used to represent each size class.

assumptions (Zar 1984).

The total number of sound emissions over a 5 min period decreased significantly with increasing fish size. Furthermore, the importance of each phrase type in the acoustic repertoire altered significantly with fish size (Table 2.9 and Figure 2.19). The proportion of knocks produced decreased, whereas the proportion of grunts emitted increased with increasing fish size. The most marked change was, however, with growls. Growls constituted on average approximately 7% of the total acoustic emissions of small grey gurnards and almost disappeared from the acoustic repertoire of larger fish.

Regression analysis also showed that fish size had a significant effect on the sound features of the emitted phrases (see Table 2.10 and Fig. 2.20). Growls were not considered in this analysis because of their small sample size. The data used for medium grey gurnards covered only 6 months of recordings (which included 3 seasons of the year) so that the sample size for medium fish was similar to the ones for the other size classes.

There was a general increase in duration of the temporal parameters of phrases with increasing fish size as well as in the number of pulses in phrases (Table 2.10 and Fig. 2.20). The only exception was the decrease in the interval between the 1st and 2nd pulse and in the pulse peak to peak interval in knock phrases. Phrase peak frequency tended to be lower in larger fish, as expected from their larger swimbladder sizes (Protasov 1965).

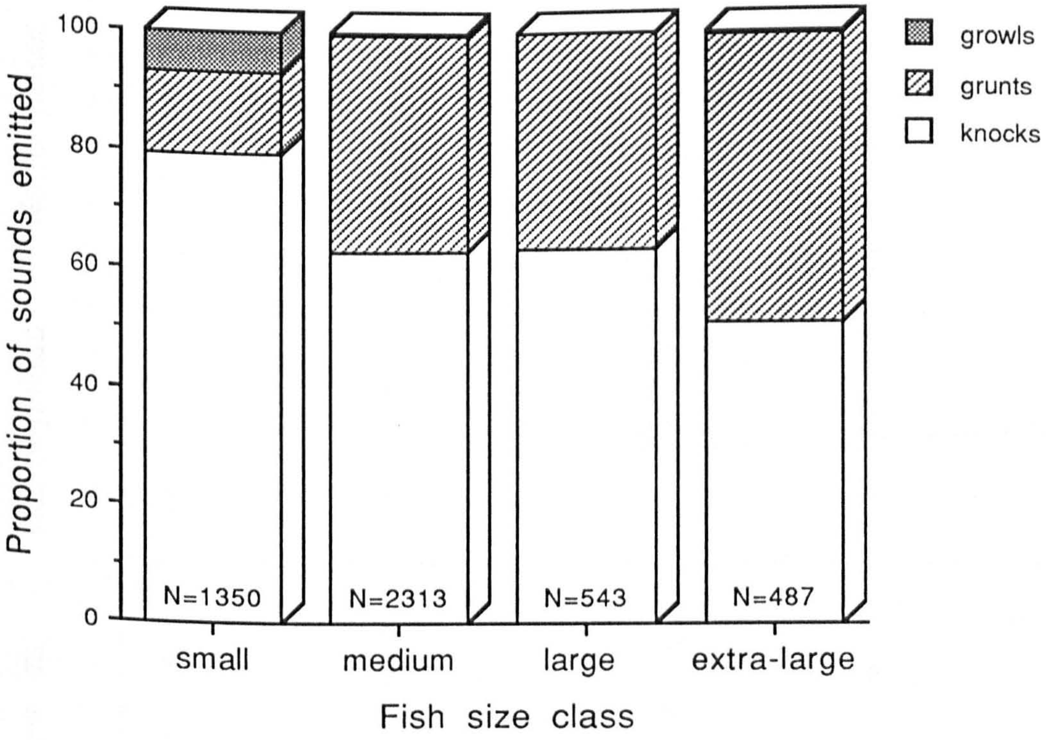


Figure 2.19 - Proportion (%) of each phrase type in the acoustic repertoire of the grey gurnard from all sizes. N is the number of sounds analysed for each size class (counted from 11, 32, 10 and 11 sessions of 5 min recordings for the small, medium, large, and extra-large fish, respectively).

Table 2.10 - Results of regression analysis on the effects of fish size on each of the sound parameters studied. The regression equation is $y = a + bx$. N = sample size; *** = $P < 0.001$; ** = $P < 0.01$. The correlation coefficient r is corrected for the degrees of freedom.

Sound parameters	phrase	N	a	b	r	P
No. of pulses	knock	724	1.11	0.03	0.319	***
"	grunt	440	4.51	0.07	0.342	***
Phrase duration (ms)	knock	724	5.73	0.27	0.321	***
"	grunt	440	46.5	0.96	0.336	***
Pulse duration (ms)	knock	724	4.01	0.08	0.596	***
"	grunt	440	3.82	0.09	0.741	***
1^{st} - 2^{nd} pulse interval (ms)	knock	422	5.51	-0.15	0.550	***
Peak - peak interval (ms)	knock	416	8.76	-0.05	0.148	**
"	grunt	440	9.04	0.09	0.377	***
Peak frequency (Hz)	knock	724	780	-14.10	0.735	***
"	grunt	440	551	-5.57	0.255	***

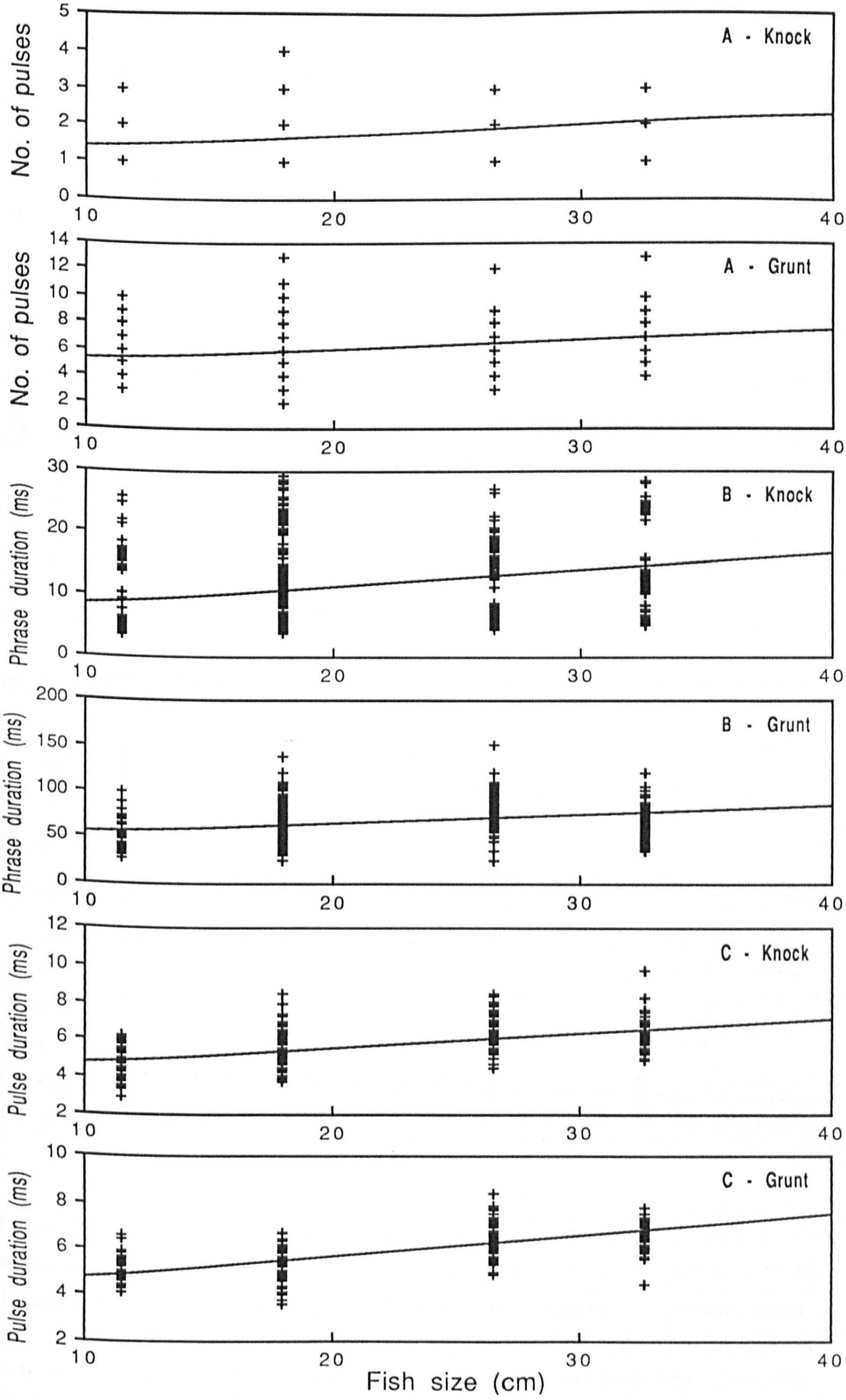

A - Knock
No. of pulses
0
1
2
3
4
5
A - Grunt
No. of pulses
0
2
4
6
8
10
12
14
B - Knock
Phrase duration (ms)
0
10
20
30
B - Grunt
Phrase duration (ms)
0
50
100
150
200
C - Knock
Pulse duration (ms)
2
4
6
8
10
12
C - Grunt
Pulse duration (ms)
2
4
6
8
10
10
20
30
40
Fish size (cm)

Figure 2.20.

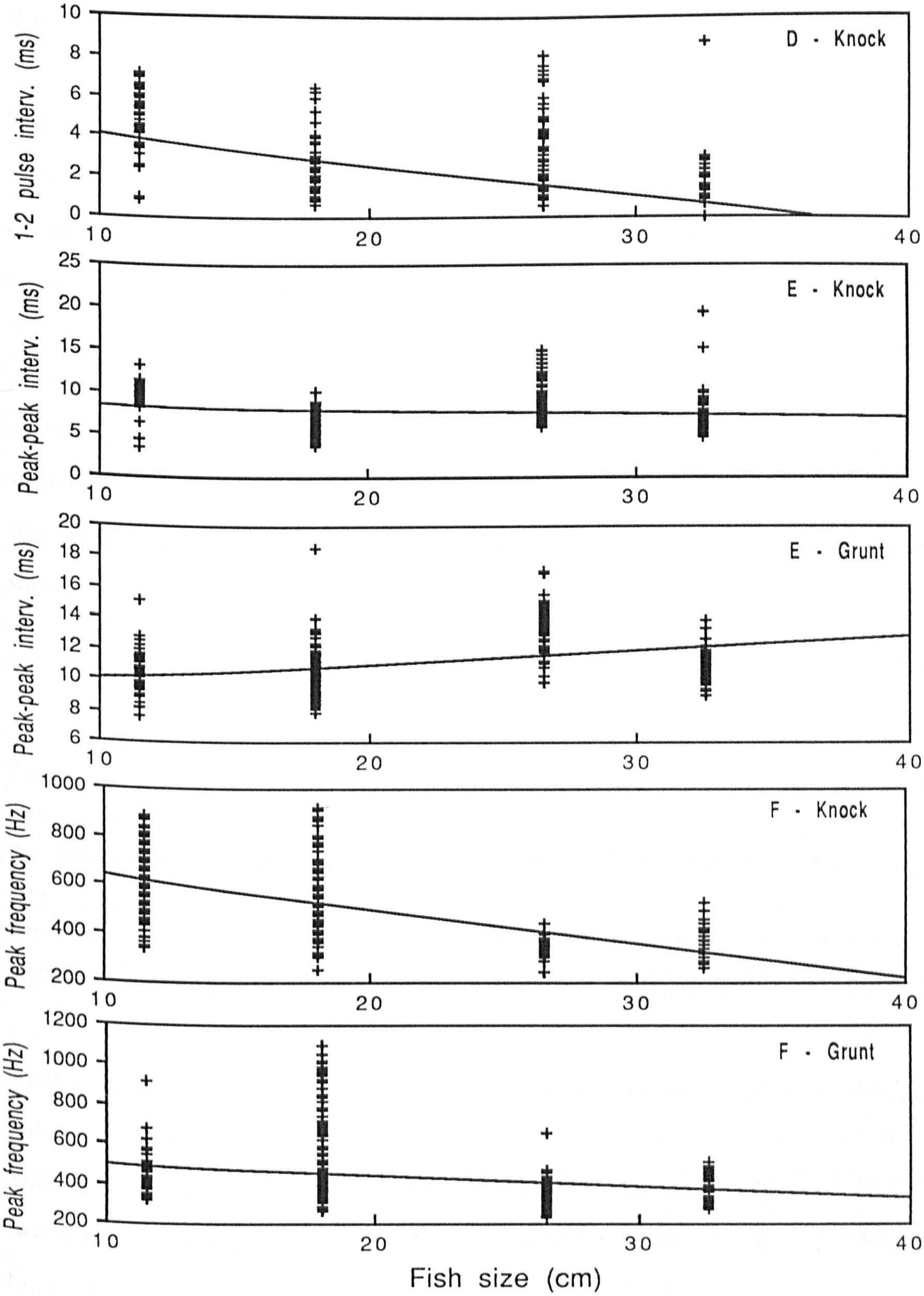


Figure 2.20 - Relationship between the physical features of knocks and grunts and grey gurnard size. A = No. of pulses in a phrase; B = Phrase duration; C = Pulse duration; D = 1^{st} - 2^{nd} pulse interval; E = Pulse peak to peak interval; F = Peak frequency. For explanation of these parameters see section 2.2.5. The median total length of the experimental fish was used to represent each size class.

2.3.2 - The streaked gurnard - *Trigloporus lastoviza*

The acoustic repertoire of the streaked gurnard was recorded during 31 sessions of 5 - 10 min each, made throughout approximately 1.5 months. The acoustic emissions of this species consisted of only one type of phrase, audible to the human ear as long growls, that could last up to approximately 3 seconds. These sound emissions are composed of repeated pulses, but the pulses, unlike in the growls of the grey gurnard, were further organised within a phrase into groups of pulses. The structure of the 42 growls analysed is described in Table 2.11. The sonogram and oscillogram of a section of a typical growl, as well as the envelope of the entire phrase, is shown in Fig. 2.21.

Table 2.11 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features of growl phrases emitted by the streaked gurnard. For an explanation of the parameters listed see section 2.2.5.

Growl - parameters	**N**	**mean**	**s.d.**	**min**	**max**
Phrase duration (ms)	41	1069.01	775.58	52.9	3149.9
Pulse duration (ms)	372	4.69	0.61	2.9	7.0
Total no. of pulses	9	100.67	54.46	30	188
No. of groups of pulses	39	36.45	25.20	3	94
No. of pulses per group	372	2.04	1.28	1	13
Pulse peak-peak interval (ms) (in group)	251	3.81	0.61	2.3	6.3
Group peak-peak interval (ms) (between groups)	358	23.21	10.24	6.8	64.9
Peak frequency (Hz)	372	555.12	125.40	304	1018

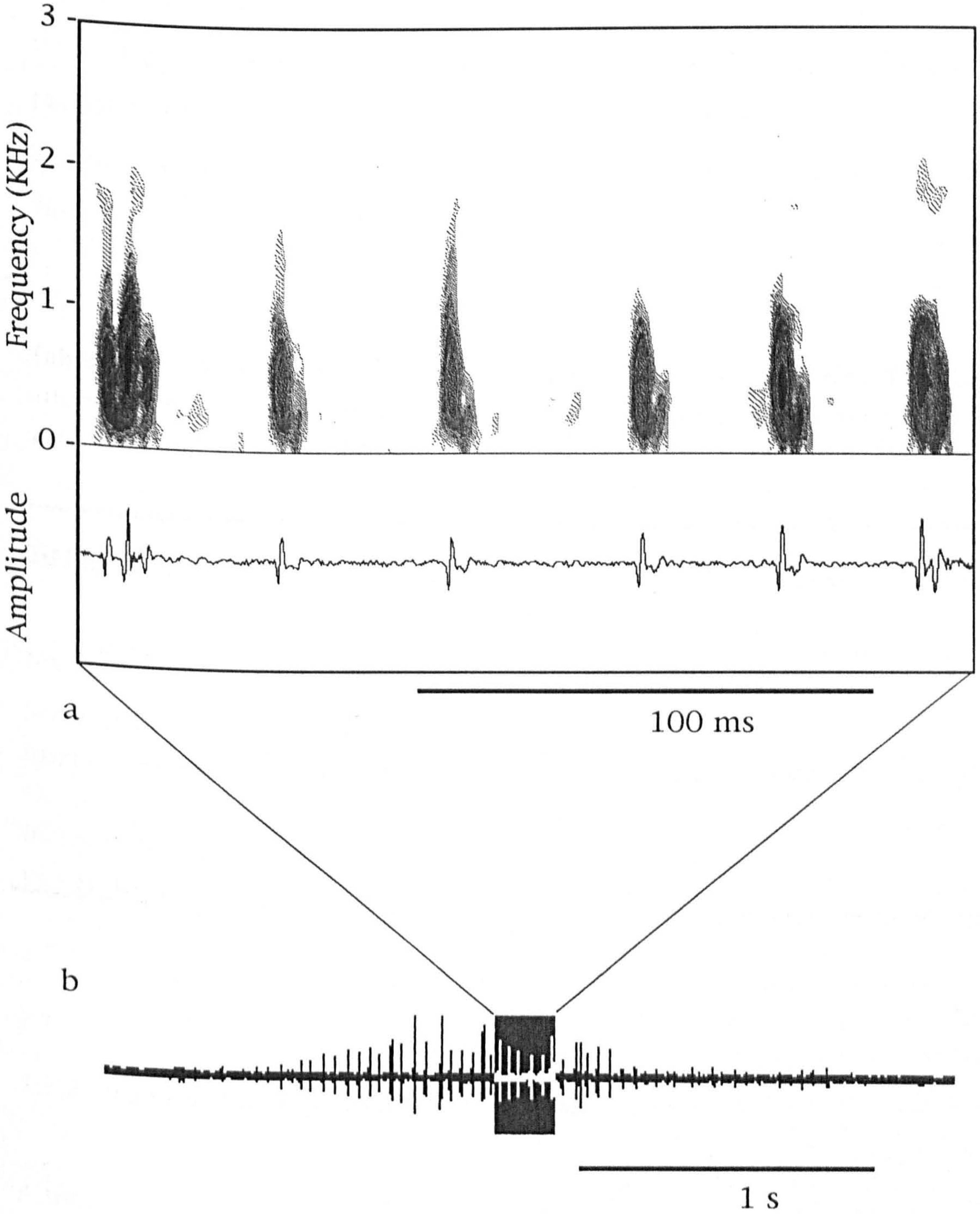


Figure 2.21 - Example of a growl produced by the streaked gurnard. (a) Sonogram and oscillogram of a session of the growl sound (filter bandwidth = 125Hz). (b) Envelope of the whole growl.

The number of growls emitted per 5 minutes of recording was quantified for streaked gurnards in a large group of 9 - 10 fish and a small group of 3 - 5 individuals (Table 2.12). The time interval between growl emissions in a recording session was also measured (Table 2.12). Although calling rate tended to be higher in the large group, significant differences between the 2 group sizes were not found for the 2 parameters measured (Kruskal-Wallis).

Table 2.12 - Number of growls produced per 5 minutes of recordings and interval between growl emission in streaked gurnards, in 2 different group sizes: 3 - 5 and 9 - 10 individuals.

Streaked gurnard	N	mean	s.d.	min	max
No. growls/5min (3 - 5 fish)	12	3.75	2.83	1	11
No. growls/5min (9 - 10 fish)	6	7.33	6.02	0	18
Interval between growls (s) (3 - 5 fish)	42	38.45	57.01	1	222
Interval between growls (s) (9 - 10 fish)	45	23.96	30.60	1	126

2.3.3 - The red gurnard - *Aspitrigla cuculus*

A total of 183 phases emitted by red gurnards were recorded and analysed. This species produced 3 classes of phrases: knocks, grunts and growls. As with the streaked gurnard, all phrases were made up of pulses that were further arranged into groups. Knocks were the most frequent phrases produced (66%), followed by grunts (30.5%), and then by growls that were only 2.25% of the total number of phrases recorded. Knocks were typically repeated at intervals ranging from 36.1 to 981.8 ms (n = 84; mean = 173.02 ms; s.d. = 168.37) and were made up of 1 to 3 pulses (see Table 2.13). Grunts were longer phrases made up of several groups of pulses similar to the knock phrases (with 1 to 3 pulses) and were repeated at intervals ranging from 113.3 to 947.1 ms (n = 30; mean = 268.4 ms; s.d. = 198.32). Growls were audibly different from grunts and presented, in total, a much higher number of pulses. In addition, all growls analysed had a smaller or larger section of the phrase similar to the grunt phrase. However, the rest of the growl phrase was clearly different to the grunt and consisted of 1 or 2 groups of 4 to 15 pulses (see Table 2.16). The structure of knock, grunt and growl phrases are described in Tables 2.13 - 2.15. Sonograms and oscillograms of knocks, grunts and growls are also depicted in Figs. 2.22 - 2.24. Because the growls were made up of two distinctive parts (a grunt-like section and a growl-like section), Table 2.16 shows the parameters measured specifically for the typical growl section of the growl phrase, whereas Table 2.15 refers to the complete growl phrase (including the grunt-like section). Apart from these 9 phrases which were analysed as growls, 5 other phrases were recorded that were neither clear grunts or growls (1.25% of the total sounds recorded). These 5 phrases had both grunt-like and growl-like sections but the latter constituted a smaller proportion of the whole phrase, sounding to the human ear as stronger or louder grunts. These phrases were not considered in the analysis of either grunts or growls, but a quantitative description of their growl-like section was included in Table 2.16.

Table 2.13 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features of knocks emitted by the red gurnard. For an explanation of the parameters listed see section 2.2.5.

Knock - parameters	N	**mean**	**s.d.**	**min**	**max**
Phrase duration (ms)	104	9.81	2.33	5.8	21.1
Pulse duration (ms)	104	6.91	1.64	5.2	11.8
No. of pulses	104	1.51	0.52	1	3
Pulse peak-peak interval (ms)	52	3.56	0.51	2.9	6.2
Peak frequency (Hz)	104	394.62	31.92	339	491

Table 2.14 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features of grunts emitted by the red gurnard. For an explanation of the parameters listed see section 2.2.5.

Grunt - parameters	N	**mean**	**s.d.**	**min**	**max**
Phrase duration (ms)	63	109.63	40.38	27.6	227.1
Pulse duration (ms)	250	6.40	1.29	4.4	12.3
Total no. of pulses	63	7.46	1.66	3	12
No. of groups of pulses	63	3.97	0.86	2	6
No. of pulses per group	250	1.88	0.34	1	3
Pulse peak-peak interval (ms) (in group)	218	3.99	0.60	2.6	7.5
Group peak-peak interval (ms) (between groups)	187	32.63	12.16	13.6	80.4
Peak frequency (Hz)	250	421.94	43.89	327	585

Table 2.15 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features of growls emitted by the red gurnard. For an explanation of the parameters listed see section 2.2.5.

Growl - parameters	N	mean	s.d.	min	max
Phrase duration (ms)	9	162.32	37.80	1.26.0	223.0
Pulse duration (ms)	40	6.61	0.97	4.8	10.3
Total no. of pulses	9	18.22	2.73	14	22
No. of groups of pulses	9	4.44	1.24	3	7
No. of pulses per group	40	4.10	4.24	1	15
Pulse peak-peak interval (ms) (in group)	36	5.61	1.09	3.9	8.1
Group peak-peak interval (ms) (between groups)	30	24.82	9.53	16.9	55.9
Peak frequency (Hz)	40	370.95	38.72	304	441

Table 2.16 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the parameters of the typical growl section of the growl phrases emitted by red gurnards.

Growl - parameters	N	mean	s.d.	min	max
Growl section duration (ms)	16	61.07	24.96	29.2	97.8
Pulse duration (ms)	16	6.51	0.20	5.0	7.5
No. of pulses	16	9.19	3.64	4	15
Pulse peak-peak interval (ms)	16	6.08	0.48	4.9	6.6
Peak frequency (Hz)	16	395.4	48.84	316	498

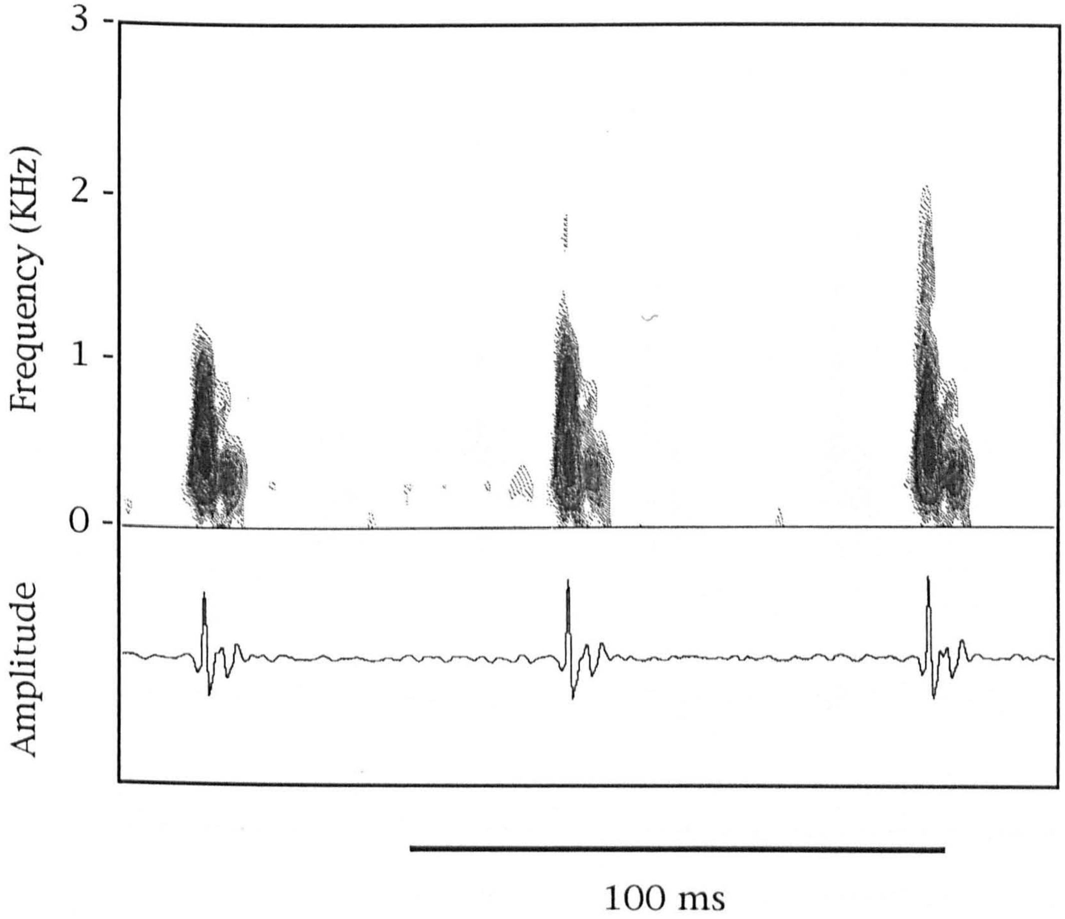


Figure 2.22 - Sonogram and oscillogram of a train of knocks produced by the red gurnard (filter bandwidth = 125Hz).

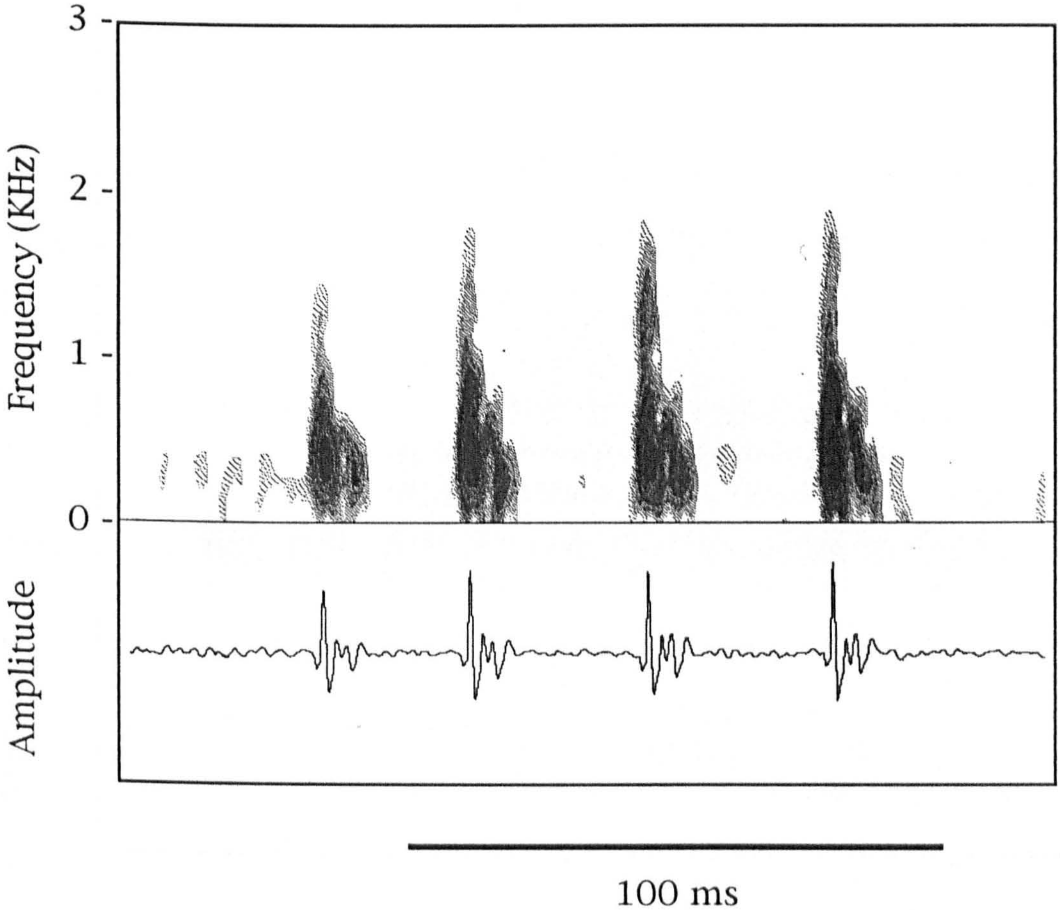


Figure 2.23 - Sonogram and oscillogram of a grunt produced by the red gurnard (filter bandwidth = 125Hz).

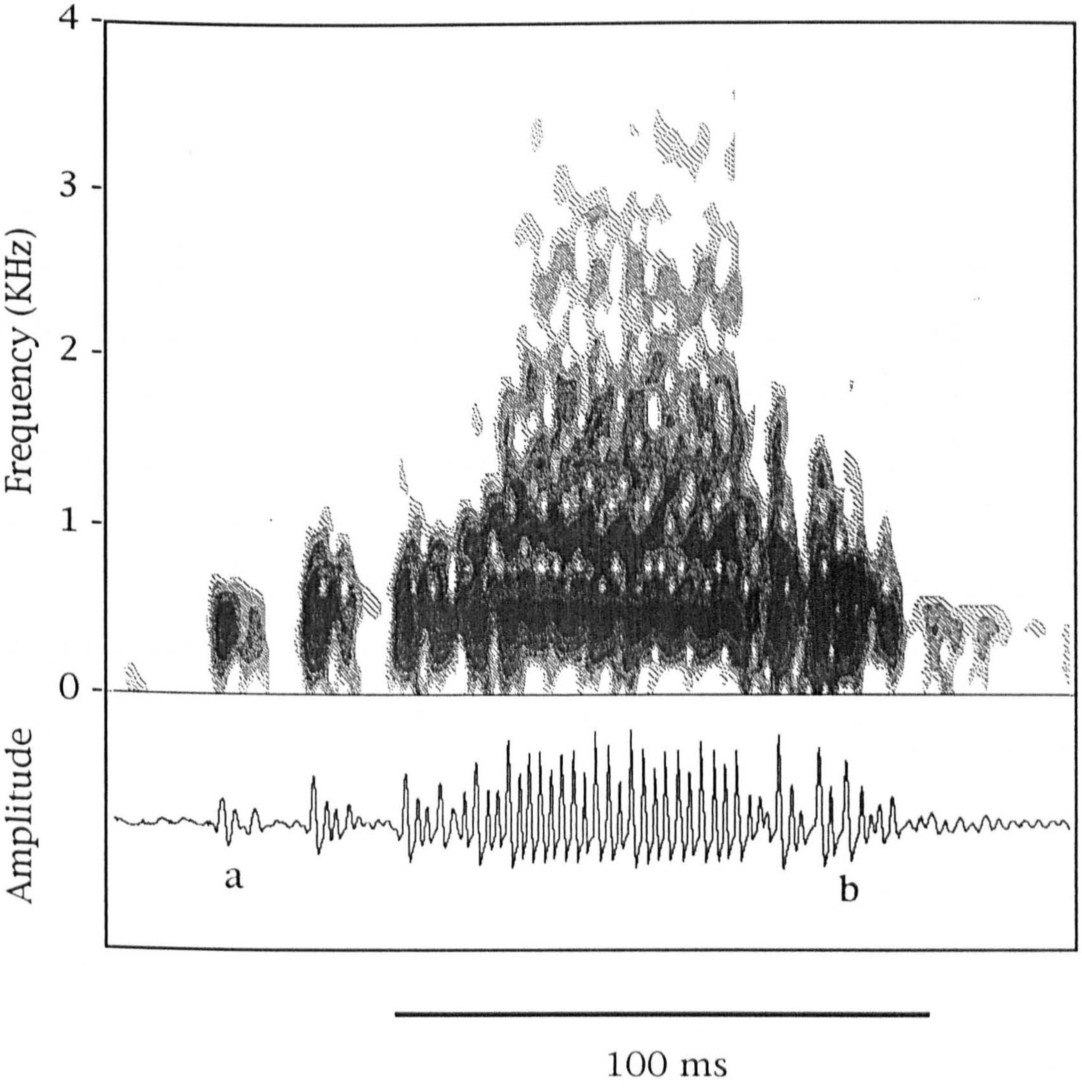


Figure 2.24 - Sonogram and oscillogram of a growl produced by the red gurnard (filter bandwidth = 125Hz). (a) and (b) represent the grunt-like and the growl-like sections of the growl phrase respectively.

2.3.4 - The tub gurnard - *Trigla lucerna*

Sound production was only registered for the smaller-sized tub gurnards in this study. A similar amount of effort to record the acoustic repertoire of this species was made for both medium (20 - 30 cm TL) and large (± 40 cm TL) tub gurnards: approximately 25 sessions of recording of 10 to 15 min each made throughout approximately 2 months. Sounds were never heard from the larger tub gurnards, and only in 6 of the 25 sessions did the smaller tub gurnards emit grunt-like sounds. Of all the grunts recorded, only 13 were analysed since all others were distorted and thus not suitable for analysis. Distortion was probably caused by the bad acoustic conditions inherent to the tank where the study took place. A quantitative description of these 13 phrases is presented in Table 2.17. Figure 2.25 shows the sonogram and the oscillogram of one grunt. The grunts uttered by this species are, as in all other gurnard species, made up of pulses. The phrase's unit was a single pulse such as found for the grey gurnard.

Table 2.17 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features of grunts emitted by the tub gurnard. For an explanation of the parameters listed see section 2.2.5.

Grunt - parameters	**N**	**mean**	**s.d.**	**min**	**max**
Phrase duration (ms)	13	26.36	3.75	20.9	31.1
Pulse duration (ms)	13	6.72	0.36	6	7.5
No. of pulses	13	3.46	0.52	3	4
Pulse peak-peak interval (ms)	13	7.98	0.41	6.9	8.6
Peak frequency (Hz)	13	311.31	121.05	175	561

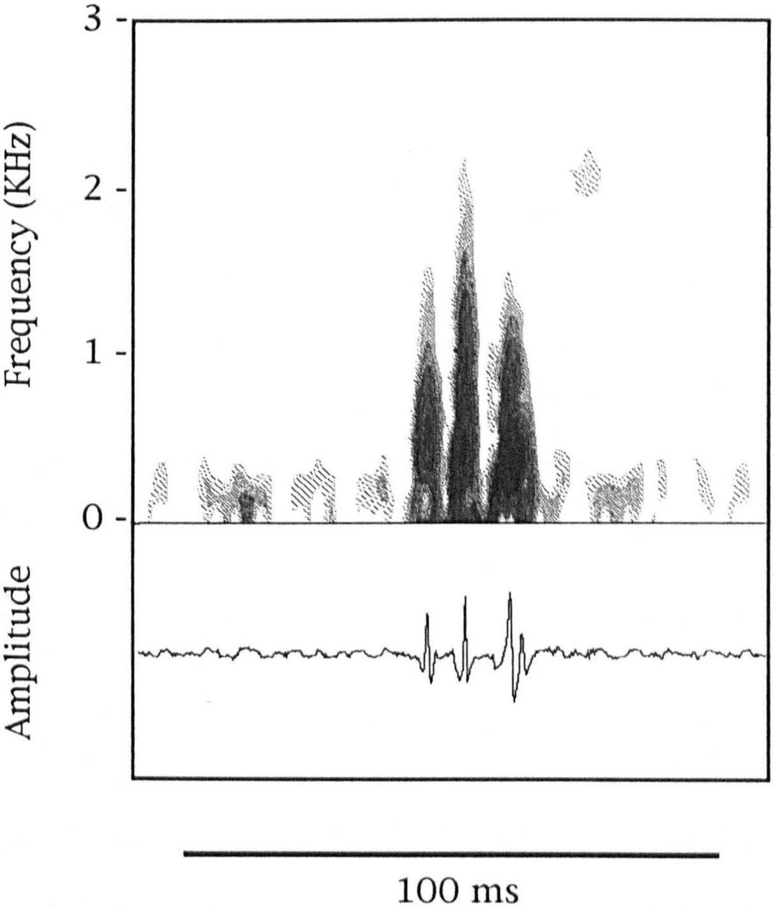


Figure 2.25 - Sonogram and oscillogram of a grunt produced by the tub gurnard (filter bandwidth = 125Hz).

2.3.5 - Differences in call structure of different species

In this Chapter, the differences in call structure of the grey, the streaked, the red and the tub gurnards are studied. The size of the acoustic repertoire varied between species. The grey and the red gurnards emitted three types of phrases whereas only one type of phrase was heard from the streaked and the tub gurnards.

Another striking difference between the acoustic emissions of these 4 species of gurnards was the way in which the pulses were grouped in a phrase. The unit of the phrases produced by the grey and the tub gurnards was a single pulse whereas the pulses in the phrases of the streaked and the red gurnards are further arranged into groups of several pulses (see Tables 2.11, 2.13 - 2.15, for the latter species).

Comparisons between species for each sound parameter and each phrase type were made with a Kruskal-Wallis non-parametric test (Table 2.18). As in section 2.3.1.3, it was inferred that when the p-value of the Kruskal-Wallis was similar to the p-value of the parametric ANOVA, the ANOVA's assumptions were not violated, and therefore parametric 95% confidence intervals could be used as an *a posteriori* test. Results of 95% confidence intervals are shown in Figure 2.26.

There were no significant differences found for knock duration of grey and red gurnards. However, the number of pulses and the peak-peak interval were significantly higher for the grey gurnard (Fig. 2.26a).

Grunts of the grey and red gurnards had a significantly larger number of pulses and a significantly longer phrase duration than the grunts of tub gurnard. The red gurnards' grunts were also longer than those of the

Table 2.18 - Results of the Kruskal-Wallis test for differences in phrase structure between the grey, the streaked, the red and the tub gurnards. (a) - Knock phrases; (b) - Grunt phrases; (c) - Growl phrases; (d) - Peak-peak interval between pulses in the same group; (e) - Pulse duration for the grey and red gurnards include data for all phrase types. For an explanation of the parameters listed see section 2.2.5. *** = P<0.001; ** = P<0.01; * = P<0.05; n.s. = P>0.05.

Kruskal-Wallis	N	d.f.	H	P
(a) **Knock** (grey and red)				
No. of pulses	254	1	5.85	*
Phrase duration (ms)	254	1	0.66	n.s.
Peak-peak interval (ms)	202	1	110.01	***
Peak frequency (Hz)	254	1	32.20	***
(b) **Grunt** (grey, red and tub)				
No. of pulses	176	2	43.22	***
Phrase duration (ms)	176	2	63.61	***
Group peak-peak interval (ms)	213	2	166.74	***
Peak frequency (Hz)	213	2	9.64	**
(c) **Growl** (grey, streaked and red)				
No. of pulses	82	2	22.22	***
Phrase duration (ms)	113	2	39.23	***
Group peak-peak interval (ms)	221	2	134.12	***
Peak frequency (Hz)	230	2	80.28	***
(d) Peak-peak interval within group (ms) (streaked and red)	107	1	51.25	***
(e) Pulse duration (ms) (grey, streaked, red and tub)	697	3	264.24	***

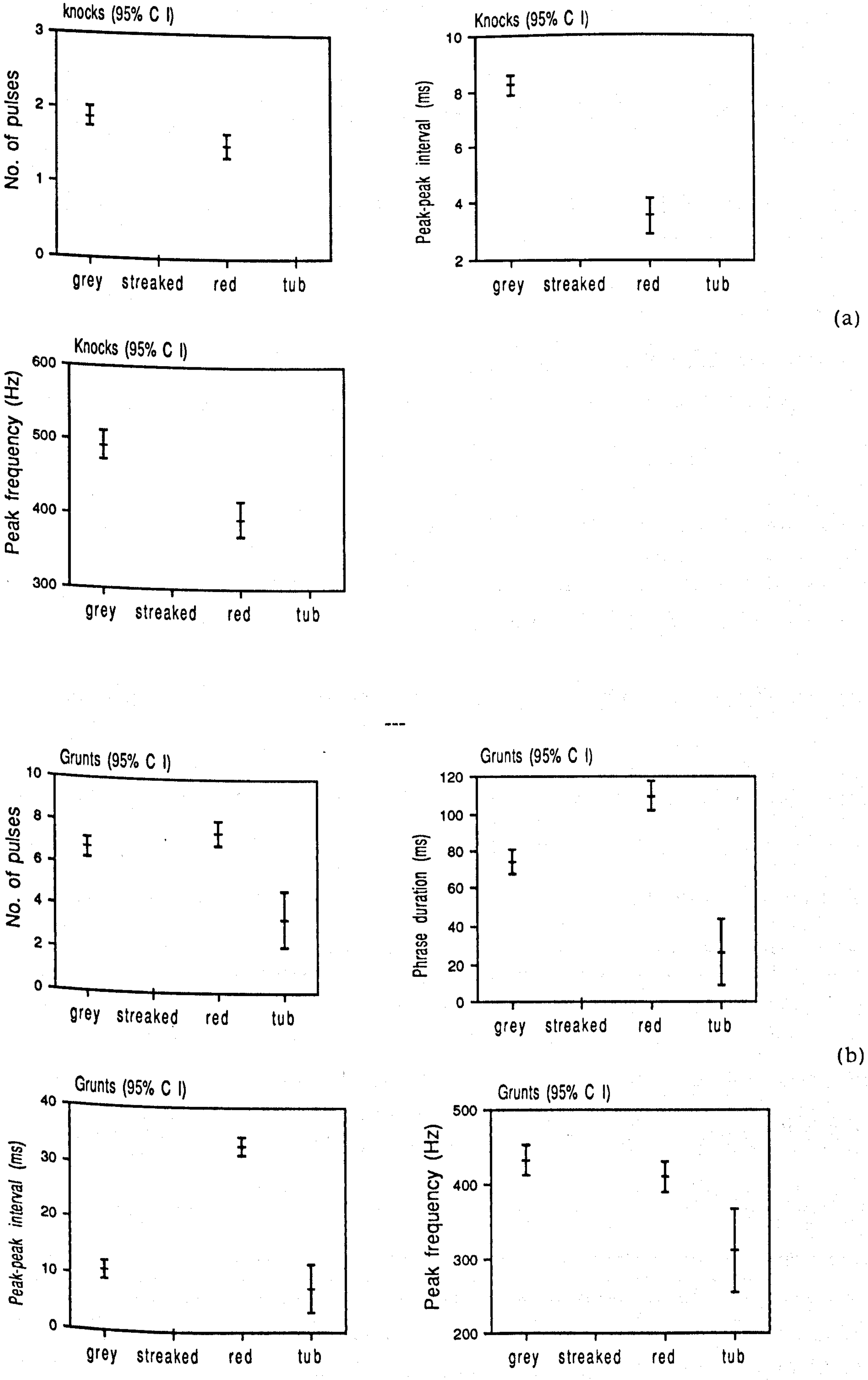
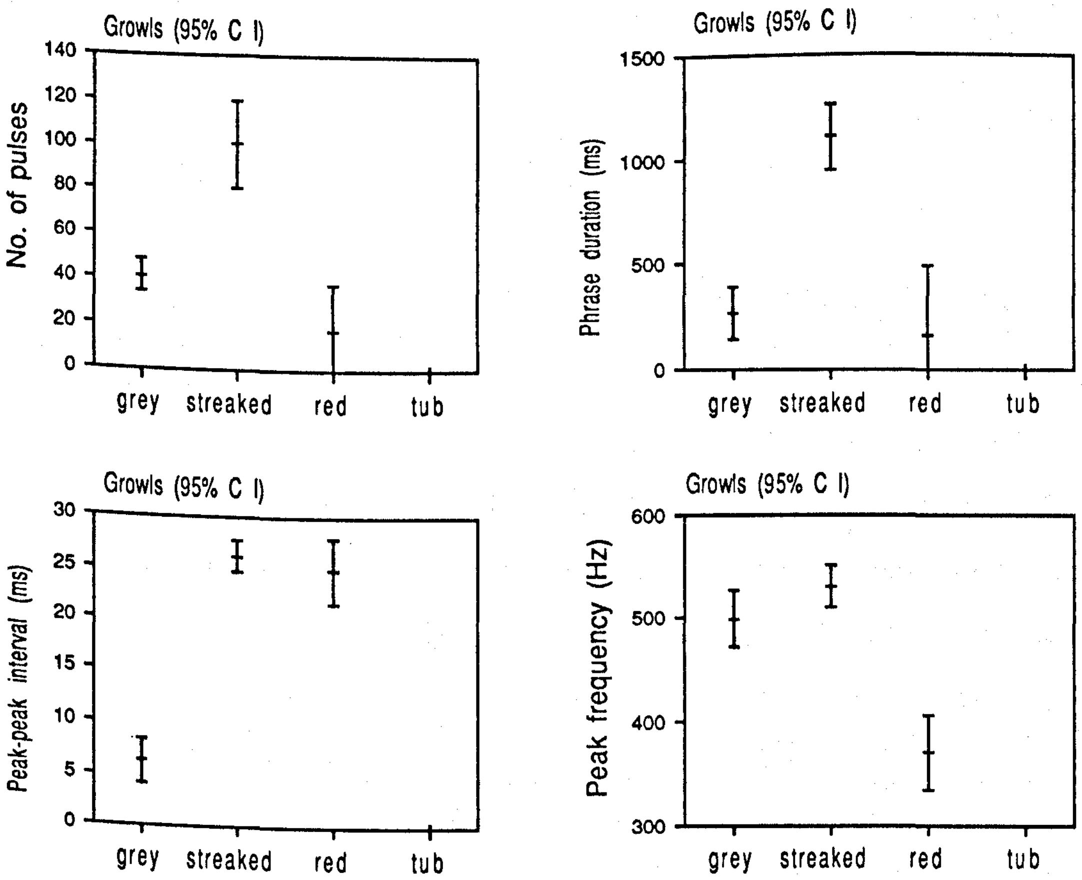
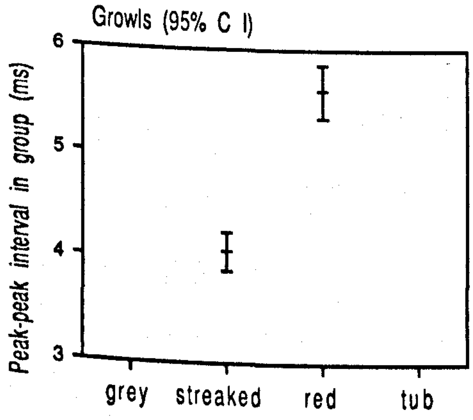

knocks (95% C I)
No. of pulses
0
1
2
3
grey
streaked
red
tub
Knocks (95% C I)
Peak-peak interval (ms)
2
4
6
8
10
grey
streaked
red
tub
(a)
Knocks (95% C I)
Peak frequency (Hz)
300
400
500
600
grey
streaked
red
tub
Grunts (95% C I)
No. of pulses
0
2
4
6
8
10
grey
streaked
red
tub
Grunts (95% C I)
Phrase duration (ms)
0
20
40
60
80
100
120
grey
streaked
red
tub
(b)
Grunts (95% C I)
Peak-peak interval (ms)
0
10
20
30
40
grey
streaked
red
tub
Grunts (95% C I)
Peak frequency (Hz)
200
300
400
500
grey
streaked
red
tub

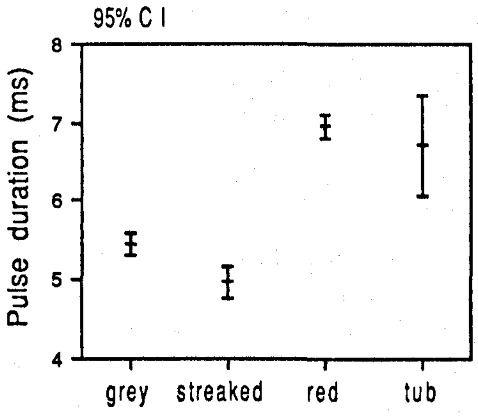
Figure 2.26.



(c)



(d)



(e)

Figure 2.26 - Differences in the structure of knocks (a), grunts (b) and growls (c) for all gurnards species studied. Vertical bars indicate 95% confidence intervals. For an explanation of the parameters listed see section 2.2.5. Peak to peak interval in graphs concerning grunts (b) and growls (c) refers to the group peak-peak interval, i.e. intervals between the peaks of the first pulses of consecutive groups; in the case of grey and tub gurnards the groups are of single pulses. (d) Concerns the peak-peak intervals between the pulses of the same group of growls. (e) Shows the differences in pulse duration between the 4 species of gurnards. For the grey and the red gurnards the data used in this comparison included data for all phrase types.

grey gurnard. The peak-peak interval between the first pulses of consecutive groups of pulses was likewise significantly longer for the red than for the grey and tub gurnards (Fig. 2.26b).

Growls uttered by the streaked gurnards were significantly longer and had a significantly higher total number of pulses than those of the grey and the red gurnards. There were no significant differences found between the growls of the grey and the red gurnards as far as duration and the total number of pulses were concerned. The peak-peak interval between groups of pulses was significantly higher in both streaked and red gurnards (with no significant difference found between the two) than in the grey gurnard (Fig. 2.26b). However, the peak-peak interval between pulses within the same group was signficantly longer in the red than in the streaked gurnards (Fig. 2.26d).

In general, the mean phrase peak frequency ranged between 300 - 600 Hz, but it was higher for the grey and the red gurnards and lower for the streaked and the tub gurnards, within this range (see Fig. 2.26a - c).

The red and the tub gurnards produced pulses significantly longer than the ones produced by the grey and the red gurnards. Grey gurnard pulses were also significantly longer than the red gurnard ones.

Sound production rate (number of sounds produced per 5 minutes of recording) was also compared between the grey and the streaked gurnards. The red and the tub gurnards were not considered for this comparison because the first species was not recorded under natural conditions and the second species only produced sounds during a very restricted period. The grey gurnards showed a significantly higher sound production rate than the streaked gurnards(results from a Mann-Whitney test: N (grey / streaked) = 43 / 12; W = 1458.0; $P < 0.001$).

Summarising, the differences in sound production between the different species of gurnards are based on the differences in size of the acoustic repertoire and differences in features of the phrases uttered. The latter consist mainly in variations in timing and grouping of the pulses. Differences in sound production rate may also be important, as seen for the grey and the streaked gurnards.

2.4 - DISCUSSION

2.4.1 - The grey gurnard

The acoustic repertoire

The grey gurnard uttered 3 types of phrases distinguishable by their duration, number of pulses and pulse peak to peak interval. Previous studies have only accounted for 2 phrase types (Hawkins 1968; Freytag 1964; see Table 2.1), and their description is also somewhat limited. None of these authors mentioned the number of phrases analysed nor fish length or group size. Hawkins (1968) described the calls emitted by this species as knocks and growls: respectively single pulses and several knock-like pulses repeated very rapidly. In the present study knocks presented a much more variable number of pulses, but typically ranging from 1 - 3 pulses. Hawkins (1968) did not mention the number of pulses found in a growl. Although the interval between pulses in growls as well as phrase duration found by Hawkins (1968) resembled the ones for the growl phrases presented in this Chapter, it might well be that this author was including both growls and grunts, as here classified, in the same category, especially since grunts appeared to be much more commonly uttered than growls. The pulse durations obtained here (2.6 - 9.8 ms; see Table 2.4) also contrasted with the ones found by the previous author for knocks (7 - 42 ms; Table 2.1) but resembling closer the pulse duration in growls (6.5 - 8.0 ms; Table 2.1). Freytag (1964) only described growl sounds and only provided information on their frequency. The frequency range found in the present study is again wider than that presented by either Hawkins (1968) or Freytag (1964).

It is possible that the acoustic repertoire of the grey gurnards is wider than that presented here since this species could not be recorded in its natural environment, nor in different contexts, such as courtship.

Diurnal and seasonal rhythms of sound production

The grey gurnard displayed an increase in locomotion activity and acoustic emissions during day time, consistent with Freytag's (1964) observations. In contrast, gurnards from the Black Sea (species not identified) showed a peak of sound production at dusk and / or at night time (Protasov & Romanenko 1962 in Protasov 1965). Other fish show a circadian rhythm of sound production, but vocalisations increased towards dusk or at night: e.g. the river bullhead *Cottus gobio* (Cottidae) (Ladich 1989); the scorpaenoid fish *Sebasticus marmoratus* (Miyagawa &Takemura 1986); the Japanese drum fish, *Nibea albiflora* and *Argyrosomus argentatus* (Sciaenidae) (Takemura *et al.* 1978); the weakfish *Cynoscion regalis* (Sciaenidae) (Connaughton & Taylor 1995b). Moreover, the marine catfish *A. felis* (Ariidae) (Breder 1968) and the squirrelfish *H. rufus* (Holocentridae) (Winn *et al.* 1964) produced more sounds at dawn and dusk whereas the satinfin shiner *Notropis analostanus* (Cyprinidae) (Winn 1964) only emitted sounds during the day. Sound production was usually correlated with the level of fish activity (Ladich 1988), as found in the present study for the grey gurnard, and was associated in many fish with competitive feeding, territory defence and breeding (Winn 1964).

The grey gurnard showed some seasonal variations in sound production. Although there was no seasonal variation in the sound emission rate (total number of sounds produced per 5 min of recording), the proportion of grunts increased (and therefore the proportion of knocks decreased) in the Spring-Summer period. Furthermore, some sound features varied seasonally: the interval between pulses decreased both in knock and grunt phrases, and the peak frequency increased in grunts likewise in the Spring-Summer period. These changes are probably correlated with the increase in hours of light and water temperature found during this period (see Fig. 2.11). Several authors have found a direct influence of temperature on the calls' characteristics in some fish species, such as in the northern sea robin *P. carolinus*, in the river bullhead, in the freshwater goby *Padogobius martensi*, and in the toadfish (Fine 1978; Kastberger 1981b; Ladich 1989; Torricelli *et al.* 1990b; Baker & Bass 1991). In general, it was found that the number of acoustic emissions, the pulse rate and the fundamental frequency tended to increase with rising temperatures, consistent with the seasonal variations found in the present study for the grey gurnards' acoustic emissions. Sound duration, in contrast, tended to decrease with increasing water temperatures. Indeed, muscle twitch contraction time is known to speed up by a factor of 2 for a temperature rise of 10°C (Videler & Wardle 1991). The fundamental frequency and the duration of sounds depend on the pattern of sonic muscle contraction (Bass & Baker 1991).

Other species of triglids such as the American sea robins *P. carolinus* and *P. evolans*, showed marked seasonal trends in their acoustic activity. These species produced squawks and clucks throughout the year, but they only utter their long staccato calls during the breeding season (Fish *et al.* 1952, Fish 1954, Moulton 1956, 1958a). Shiskhova (1963 in Protasov 1965) also suggested that the gurnards from the Black Sea uttered sounds related to spawning. Several authors have reported seasonal variations of sound production associated with breeding: e.g. the goby *Bathygobius soporator* (Tavolga 1956); the corvina *Cynoscion xanthulus* (Fish & Cummings 1972); the toadfish (Fine 1978); the red drum *Sciaenops ocellata* , the weakfish and other Sciaenidae (Guest 1978; Connaughton & Taylor 1995b). It is probable that in Nature, seasonal variations of sound production in the grey gurnard are associated not only with external physical factors, as seen here, but also with hormonal levels, i.e. with courtship and spawning behaviours.

Ontogenetic changes in sound production

Intraspecific differences in vocalisation are poorly investigated in fish although the biological significance of these differences have been widely assessed in other groups (e.g. Davies & Halliday 1978). In the grey gurnard sound production changed with fish size. The total number of phrases decreased with increasing fish size and the proportion of each phrase type in the fish's acoustic repertoire also varied. Furthermore, the physical features of phrases also showed ontogenetic variations: the number of pulses in a phrase and the duration of the temporal features increased, while the peak frequency decreased, with increasing fish length. Differences in pulse duration and pulse peak to peak interval were small and may perhaps result from differences in the motivation state of the individuals studied.

While for many species of fish, including the sea robins, it has been demonstrated that peak and/or fundamental frequency was negatively correlated with body size (Myrberg *et al.* 1965; Bayoumi 1970; Takemura 1984; Myrberg & Riggio 1985; Ladich 1990; Ladich *et al.* 1992a, b), variations in temporal features have only been reported for the croaking gourami *Trichopsis pumilus* Ladich (1992a): the interval between the first pulse of each double pulse in croaks was positively correlated with body mass. In contrast, for other species, temporal features of sounds remained extremely stereotyped in different-sized individuals (e.g. Myrberg *et al.* 1993), consistent with the fact that temporal patterning in calls is thought to mediate species recognition (Myrberg 1980).

Ontogenetic changes in the temporal patterns of the acoustic emissions of the grey gurnard may not have any biological importance if they cannot be resolved by the fish's ear. Myrberg *et al.* (1978) concluded that different species of damselfish (Pomacentridae) could distinguish sounds with differences in their inter-pulse interval as small as 5 - 10 ms at repetition rates of 23 - 32 times/s, which also seemed to match the temporal hearing sensitivity of the goldfish *Carassius auratus* (Fay 1980). On average, differences in pulse peak to peak interval and pulse duration between small and extra-large grey gurnards were smaller than 5 ms and hence might not be descriminated by the fish's ear. However, number of pulses, peak frequency and perhaps phrase duration may provide potential cues for intraspecific recognition. Sound frequency, for example, seems to be important for individual recognition and assessment in some fish (Myrberg & Riggio 1985, Ladich 1990, Ladich *et al.* 1992b, Myrberg *et al.* 1993) and other groups of animals (e.g. Davies & Halliday 1978), and may play a major role in, for example, territory defence and agonistic interactions.

2.4.2 - The acoustic repertoire of streaked, red and tub gurnards

The streaked gurnard

The sounds heard from this species were long growls made up of repetitive pulses organised within the phrase in groups of typically 1 - 3 pulses. The streaked gurnard's calls had only been studied previously by Hawkins (1968) (see Table 2.1). Hawkins (1968) described this species' calls as repetitive knocks and growls, similar to the ones uttered by the grey and the red gurnards, although no description of the sounds' physical features was given for either the streaked or the red gurnards. In the present study, the structure of the calls uttered by these 3 species were significantly different from each other, as shown in section 2.3.5. Differences in the results could be due to differences in recording contexts: in the present study recordings were made from groups of streaked gurnards while competing for food, whereas in Hawkins' study single fish produced sounds in a defensive context, when disturbed or approached by other species. Furthermore, geographical differences could be involved (Mediterranean *vs* North Sea). For example, the oyster toadfish *Opsanus tau,* showed geographical variations in the fundamental frequency and duration of its mating call (Fine 1978).

The red gurnard

The red gurnard produced 3 types of phrases: knocks, grunts and growls. Hawkins (1968), as previously mentioned, described 2 types of phrases: knocks and growls, without providing any further description.

In this study, some phrases produced by the red gurnards were intermediate between grunts and growls. This, and the fact that all growl phrases included grunt-like sections, leads one to believe that there is a continuum of sounds between typical grunts and growls. The same could be true for knock and grunt phrases, since the main difference between a train of knocks and a grunt seems to consist of the knock repetition rate.

The tub gurnard

Freytag (1961, 1964) described the tub gurnards' acoustic emissions as deep purring sounds. Hawkins (1968), however, described 2 types of phrases: knocks and grunts. Only grunt-like sounds were recorded in the present study and with much shorter phrase duration than the growls described by Hawkins (1968).

2.4.3 - Interspecific differences in call structure

Interspecific differences in the acoustic repertoire of gurnards were based on the number of phrase types produced, and on the temporal patterning of the calls. Several studies have compared the calls uttered by members of the same family and have consistently suggested that the temporal organisation of sounds is the main feature for species discrimination in vocalising fish (Gerald 1971; Hawkins & Rasmussen 1978; Myrberg *et al.* 1978; Spanier 1979; Chen & Mok 1988; Ladich *et al.* 1992a). Indeed, Myrberg *et al.* (1978) and Spanier (1979) among others, have played back natural and manipulated sounds of closely related species of damselfish (*Pomacentrus* sp.), that lived in close proximity, and have concluded that they can distinguish their own species' courtship sounds on the basis of the pulse interval; pulse number gave information on the fish's motivational state.

CHAPTER 3

THE MECHANISMS OF SOUND PRODUCTION IN GURNARDS

3 - MECHANISMS OF SOUND PRODUCTION IN GURNARDS

3.1 - INTRODUCTION

The swimbladder sound-producing apparatus of teleost fish reaches its highest development in the family Triglidae (Protasov 1965). Triglids have a true physoclist swimbladder associated with a pair of strongly developed intrinsic sonic muscles imbedded on either side of its wall (Rauther 1945, Fish 1954, Moulton 1960, Freytag 1964). Rapid contractions of the sonic muscles cause the swimbladder wall to vibrate rhythmically, producing a typical drumming noise (Hawkins 1968).

The swimbladder wall is composed of two layers: a thin, tough collagenous outer wall - the *tunica externa* - and a thick but softer and more elastic inner layer - the *tunica interna* (e.g. Rauther 1945). The former forms the mechanically strongest part of the swimbladder; it is made up of dense sheets of connective tissue with collagen fibres orientated in different directions. The second layer forms the loose mobile lining of the swimbladder, and consists of a network of connective tissue fibres imbedded in a mucosa containing elastic fibres, fibroblasts, smooth muscle fibres and blood vessels (Hawkins 1968). The *tunica interna* forms a sac composed of two chambers separated by a transverse constriction or diaphragm, pierced by a central aperture that is lined with connective tissue and smooth muscle (Fig. 3.1) (Rauther 1945, Hawkins 1968). The primary function of the swimbladder is as an hydrostatic organ maintaining the fish at neutral buoyancy by regulating its gas content. In the grey gurnard (and generally in the Triglidae family), as in many other teleosts (e.g. Labridae, Zeidae, Gadidae), the anterior chamber of the swimbladder corresponds to the secretory part of the swimbladder, with the gas gland (the secretory organ) and the *retia mirabilis* (an associated system of small parallel blood

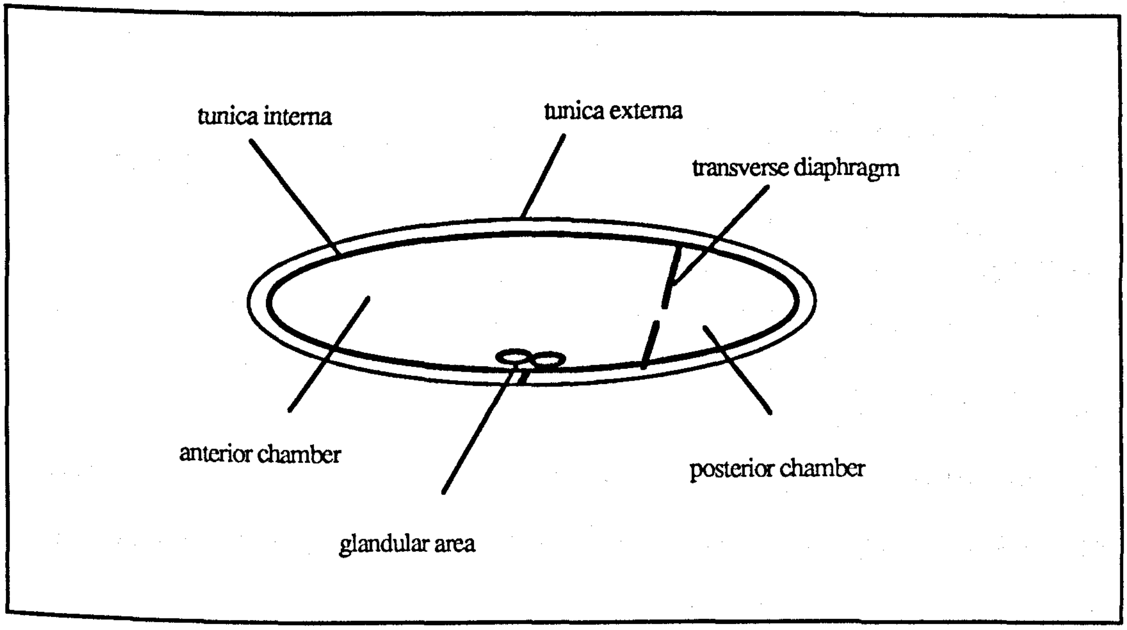


Figure 3.1 - Schematic view of the lateral section of the grey gurnard's swimbladder. Adapted from Hawkins (1968).

vessels) situated ventrally, the posterior chamber forming the resorbent part (Fig. 3.1) (Hawkins 1968).

Two straps of striated muscles can be found imbedded in the outer surface of the *tunica externa* at the lateral margins of the swimbladder, extending along the longitudinal axis of the organ from just behind the anterior lobes to the posterior end. The sonic muscles are derived embryologically from the body wall, rather than developing from the involuntary muscle of the gut wall; embryologically the swimbladder develops as a budding off from the gut. However, the muscles are able to contract independently of the body wall muscle and have separate innervation (e.g. Rauther 1945). These sonic muscles lie close to each other in the dorsal region of the swimbladder and are separated by a central sinew. They were found to be innervated by a branch of the occipital nerve complex, which represents a fused series of spinal nerves (Rauther 1945, Freytag 1964, Hawkins 1968). Other triglids (Rauther 1945, Evans 1973) and fish from other teleost families (e.g. Zeidae (Dufossé 1874), Sciaenidae (Schneider & Hasler 1960), Ariidae (Tavolga 1962), Gadidae (Hawkins 1968), Batrachoididae and Cottidae (Bass & Baker 1991)) possess similar muscles, supplied by anterior spinal nerves, or modified spinal nerves, suggesting that part of the anterior somatic musculature has independently assumed a sound producing function in different families (Hawkins & Myrberg 1983). A complete discussion on the evolution of these and other vocal control traits is made by Bass & Baker (1991).

The size of the sonic apparatus generally increases with the size of fish, but also varies seasonally within a length group, and with fish gender (Ross 1980). The size of the swimbladder and of the muscle system attached to it may affect the characteristics of the sounds produced (Fish 1954, Fine *et al.* 1977b). As a consequence, variations in the swimbladder and in the sonic muscles may be important to the understanding of the functional role of sound production in the grey and other gurnards.

But what is the relation of muscle contraction to the characteristics of the emitted sound? Several authors have recorded muscle action potentials and the mechanical contractions of the sonic muscles simultaneously with the acoustic emissions, and have also recorded sounds from deflated and normal swimbladders, as well as from a swimbladder filled with water (e.g. Skoglund 1961, Winn & Marshall 1963). They have shown that the fundamental frequency of the sounds depends on the contraction rate of the sonic muscles and on the synchrony of contraction. For example, the common sea robin can double the fundamental frequency of their sounds by vibrating the sonic muscles on either side of the swimbladder out of phase (Bass & Baker 1991). The peak frequency (where most of the energy concentrates) is, however, dependent upon the resonance properties of the swimbladder (Protasov 1965, Demski *et al.* 1973). In addition, the sound amplitude is affected by the volume of the swimbladder as well as by the number and dimension of the sonic muscle fibres (Bass & Baker 1991), and the sound duration is determined by the onset and offset of muscle contractions (Fine 1979). The relation between sonic muscle contraction and sound production is very poorly studied in triglids (see Bass & Baker 1991 and Hawkins 1968). In the European gurnards, only the grey gurnard has received some attention. Hawkins (1968) has shown that in electrically stimulated sonic muscles, a single twitch of the muscle was associated with a one pulse knock sound.

In this Chapter, a description of the sonic apparatus of the grey gurnard (*Eutrigla gurnardus*), the streaked gurnard (*Trigloporus lastoviza*), the red gurnard (*Aspitrigla lucerna*), the tub gurnard (*Trigla lucerna*), the large scaled gurnard (*Lepidotrigla cavillone*) and the piper (*Trigla lyra*) is given. The ontogeny of the sonic apparatus was studied for the grey, the streaked, the red, the tub and the large scaled gurnards. The effects of the time of the year (seasons) and fish sex and maturation state on the sonic apparatus were investigated in the grey gurnard. Sexual dimorphism of the sonic apparatus was also studied in the red gurnard. Ontogenetic, seasonal and sexual variations of the weight of the swimbladder and sonic muscles of European triglids were never studied previously.

In addition, the relation between the contraction of the sonic muscle and sound production was examined in the grey gurnard by relating electromyograms with naturally-produced sounds. This study also aimed to examine the synchrony of sonic muscle contraction.

3.2 - METHODS

3.2.1 - The sonic apparatus

Samples of the grey, red and tub gurnards were collected by trawling every three months from different points in the North Sea from May 1993 to February 1994, at depths of 20 - 150 m. Specimens of the streaked, the red, the large scaled gurnards and the piper were also collected in the Mediterranean, off the Bay of Iraklion, Crete (Greece) from May to June 1994, by trawling at depths from 10 to 260 m. All fish were frozen until being studied.

Measurements of standard length and total length, fish body weight, gonad weight, maximum oocyte diameter (grey gurnard females only), sonic muscles weight and swimbladder + sonic muscles weight were taken. When gonadal development allowed diagnosis, fish gender was also noted. The fish somatic and gonadal wet weights were measured with a balance (Mettler, model PC4400 and model PE1600) precise to the nearest 10 mg, and the swimbladder and the sonic muscles were weighed with a balance (Mettler, model P163 and model AJ100) precise to the nearest 1 mg. The weights of the swimbladder and the sonic muscles were chosen instead of size since the swimbladder varies in volume with pressure changes, thus making weight a more accurate measure of its dimensions. Swimbladder weight proved to be a good indicator of its volume (see Appendix 2).

In order to quantify gonadal development, gonadosomatic indexes (GSI = (gonad weight/somatic weight)× 100) were used. The maximum oocyte diameter found in a female gonad was also measured in the grey gurnard. Diameters of 30 of the bigger oocytes were measured with the eye-piece graticule of a microscope (Heerbrugg WILD, magnification of 25.2 times) precise to the nearest 0.1 mm. The greatest oocyte diameter of the 30 oocytes measured was considered the maximum oocyte diameter. Both parameters give indication of the fish maturation state (Kjesbu 1994), and they were related to variations in the sonic apparatus.

3.2.2 - Neuromuscular mechanisms of sound production in the grey gurnard

Three specimens of grey gurnards belonging to the medium size class (15 - 20 cm TL) were used to study the neuromuscular mechanisms of sound production in this species, by relating the timing of the electromyograms (EMG) with the timing of the produced sound waves. These fish were kept in a group of 7 individuals in a 1.5 m diameter fibreglass tank with running treated recirculated sea water at 10°C. Each subject fish was removed from the tank and anaesthetised in a 0.1 g l^{-1} sea water solution of MS222 (Sandoz) and then perfused through the gills with aerated cooled (7°C) anaesthetic solution (0.05 g l^{-1}) to maintain blood oxygen and the anaesthesia for the electrode implantation.

Two pairs of copper wire electrodes (0.18 mm wide × approximately 5 cm long) were inserted, one pair in each side of the fish body. Each individual electrode was previously insulated and labelled with colours, and soldered to very fine twin insulated stainless steel wires (50.8μ wide × 2m long) ending in labelled plugs. The electrodes were inserted into the fish body by hooking each electrode tip into the end of a hypodermic needle (0.5 × 16 mm). One electrode was placed very close to the sonic muscle and the other was put, as a reference electrode, in dorsal swimming muscle tissue near the skin surface (see Fig. 3.2). The intention was to record simultaneously the EMGs from both the left and the right sonic muscle. Previous dissections

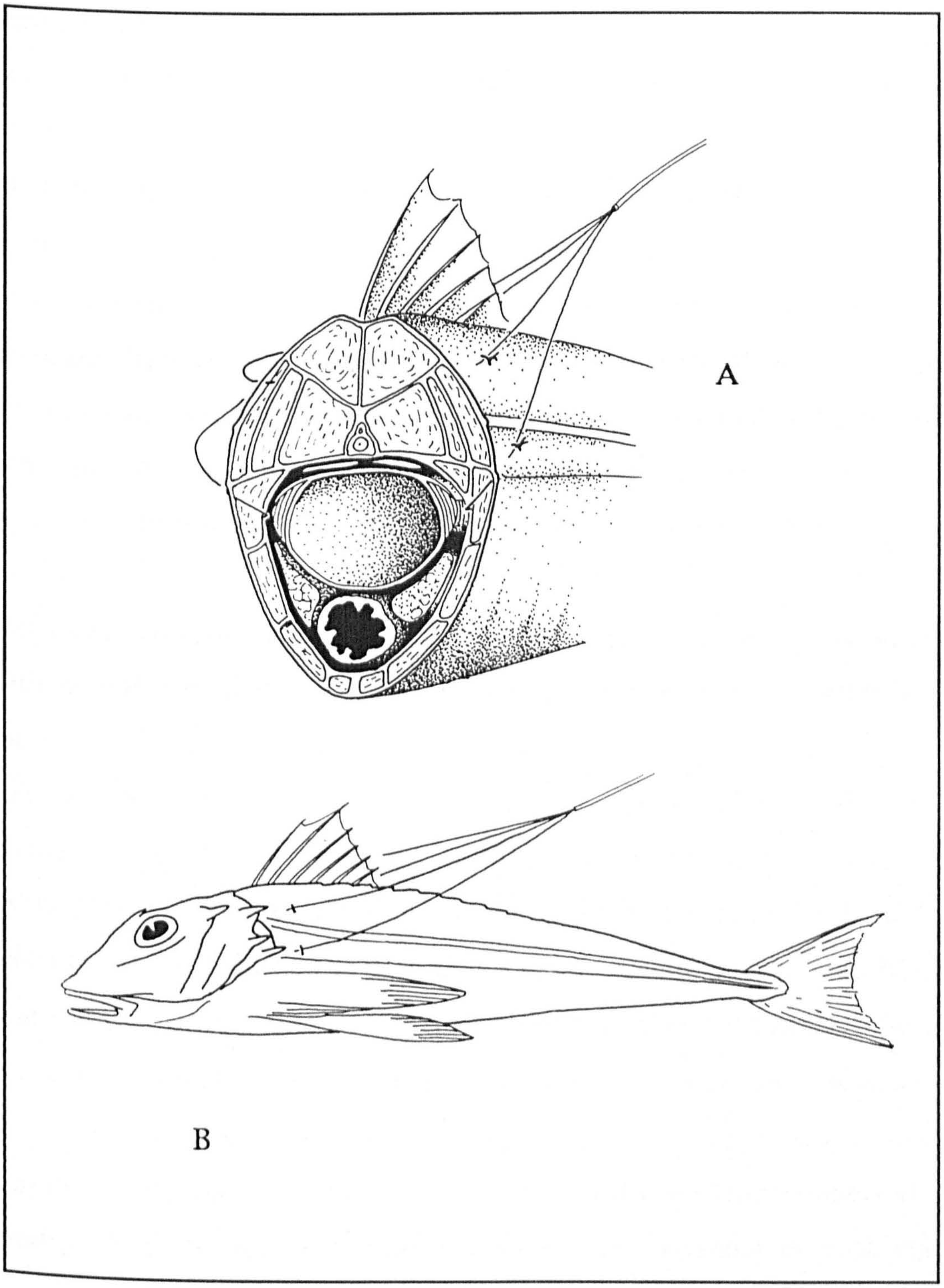


Figure 3.2 - Diagram of a section of a grey gurnard showing the position of the electrodes in relation to the sonic muscles (A). B shows the external position of the electrodes.

the EMGs from both the left and the right sonic muscle. Previous dissections were performed on similar-sized fish to improve the knowledge of the fish's anatomy in order to avoid piercing the swimbladder, or blood vessels, etc. when inserting the electrode. Once fitted, each of the electrodes was stitched to the fish's skin with a pre-threaded 16 mm round-bodied needle (Ethicon 5/0 coated vicryl) to prevent them being displaced. The whole procedure was completed in approximately 30 min, after which the fish was perfused through the gills with normal sea water to allow it to recover. Once almost recovered the fish was put back into its tank with the other fish and left undisturbed until complete recovery, recognised by the fish performing its normal behaviour. This generally took around 3 hours.

EMGs were recorded from the left and the right sonic muscle, simultaneous with sound production, on the same day of the above procedure and thereafter for the 2 following days. The EMG signals were filtered (30 - 300 HZ), amplified (Gould, Universal Amplifier and Preamplifier) and recorded on tape (Racal, 7DS, at 19.1 cm s^{-1} on FM channels with frequency response DC to 5000 Hz). Fish sounds were obtained with hydrophones and amplified and filtered as described in section 2.2.4, and simultaneously recorded on tape along with the EMG signals (in a third FM channel). The EMG and the acoustical signals were replayed and were visually and acoustically monitored with an oscilloscope (Gould, Datasys 760, 150 MHz) and with an amplifier (Neurolog System model NL900A, Digitimer Ltd.) connected to a loudspeaker. The sound emissions that were accompanied by EMG signals were identified and replayed to the channels of an analogue to digital computer interface (Cambridge Electronic Design, 1401 and SPIKE 2) at a speed of 2.4 cm s^{-1} to optimise the resolution of the signals.

The acoustic and the EMG signals from the left and the right sonic muscle were displayed simultaneously using the SPIKE2 software and the following measurements were taken:

- Number of spikes in an EMG. These data were compared with the data on sound production for medium grey gurnards (see section 2.3.1.4).
- Time intervals (measured to the nearest 0.001 ms) between the corresponding spikes of the left and the right sonic muscles. This measurement was made to check for synchrony of contraction between the 2 sonic muscles.
- Time lag (ms) between an EMG spike and sound emission (sound spike).

At the end of the experimental procedure, each fish was sacrified and dissected in order to confirm that the electrodes were well positioned.

3.3 - RESULTS

3.3.1 - Ontogenetic, seasonal and sexual changes in the sonic apparatus of different species

3.3.1.1 - The grey gurnard - *Eutrigla gurnardus*

The sonic apparatus

In the grey gurnard, the swimbladder occupies the dorsal part of the body cavity in the mid-line of the fish, immediately below the vertebral column and kidneys, and extends from the head region back to the anus at the level of the 14th vertebra (Hawkins 1968), being about 1/7 of the body length (Fig. 3.3a) (Dufossé 1874). It has an oval shape, is moderately bifurcated in the anterior region, and when viewed in cross section it is slightly flattened (Fig.3.3b).

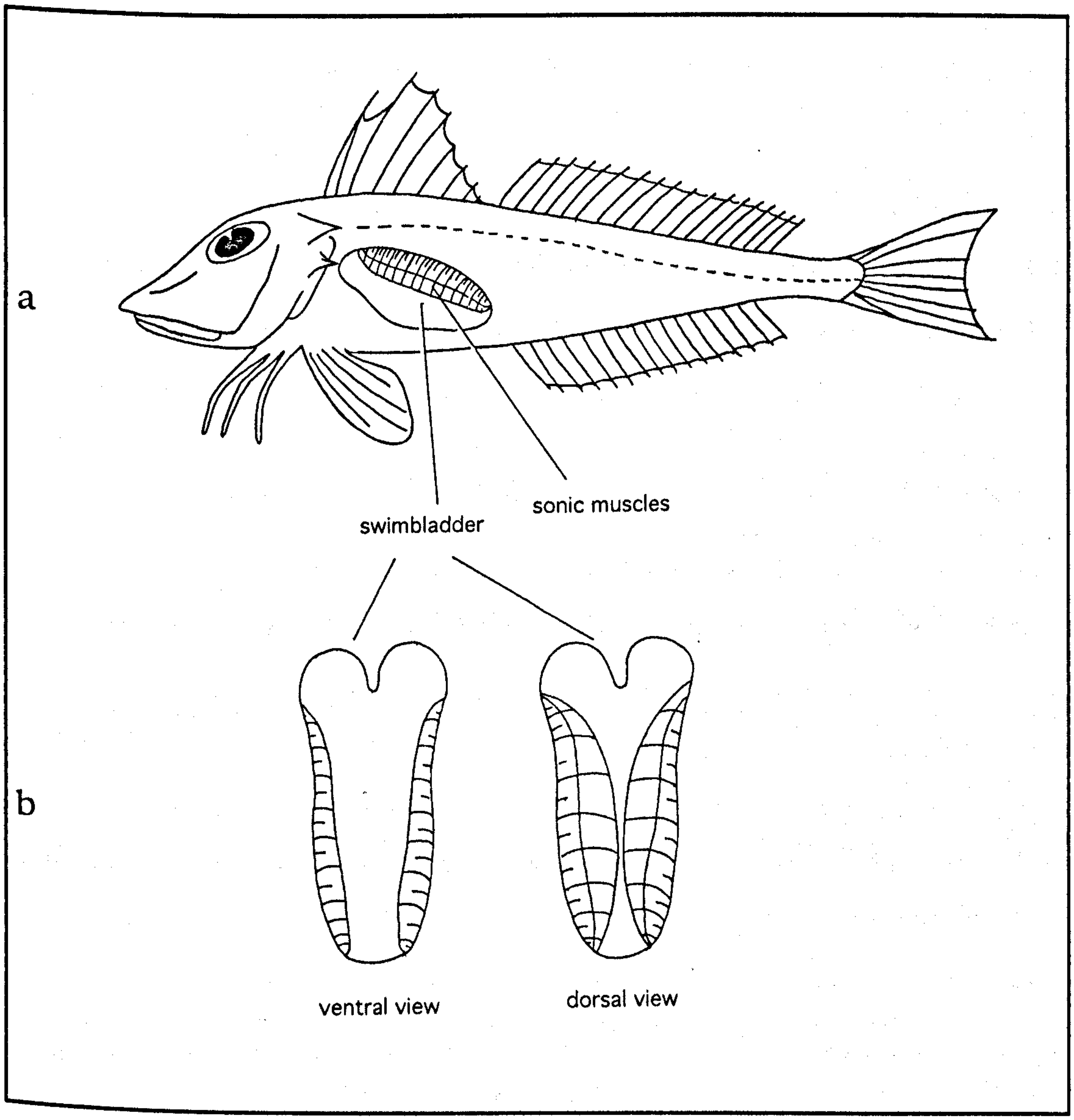


Figure 3.3 - The sonic apparatus of the grey gurnard. (a) shows the location of the swimbladder in the body and (b) presents a lateral and ventral view of this organ with a pair of intrinsic sonic muscles on each side of the swimbladder's wall.

The total swimbladder weight (including the sonic muscles) represents on average 0.008 of the grey gurnards' body weight (N = 229; s.d. = 0.002; min = 0.004 and max = 0.015) and the sonic muscles weight 0.636 of the total swimbladder weight (N = 229; s.d. = 0.088; min = 0.324 and max = 0.882).

Ontogenetic development

The total swimbladder (SB) weight (including the sonic muscles) and the sonic muscles (SM) weight increased significantly with fish total length (TL), as shown by regression analysis (see Figs. 3.4 and 3.5). SB weight and TL were transformed by $\log_{10}$ to fulfil the regression assumptions (Zar 1984), since weight has an exponential relation with length.

The sonic muscles weight, however, did not increase proportionally to the total swimbladder weight with fish growth. Figure 3.6 depicts the decrease of the SM/SB weight ratio with increasing total length and presents the results of a linear regression fitted to the data. Data were transformed by $\arcsin(\sqrt{x})$ as proportions usually follow a binomial rather then a normal distribution. Indeed, the regression's fit improved with the data transformation.

Sexual dimorphism

Sexual dimorphism for the total swimbladder (SB) weight (g) (including the sonic muscles) and for the sonic muscles weight (g) (SM) was tested with a Mann-Whitney test. As females were usually bigger than males (Fig. 3.7), sexual dimorphism of the sonic apparatus was tested in fish from a range of size classes in order to compare data from females and males with similar sizes. Fish smaller than 15 cm and bigger than 35 cm in total length (TL) were not considered because of the small sample size of females in the former and of males in the latter group.

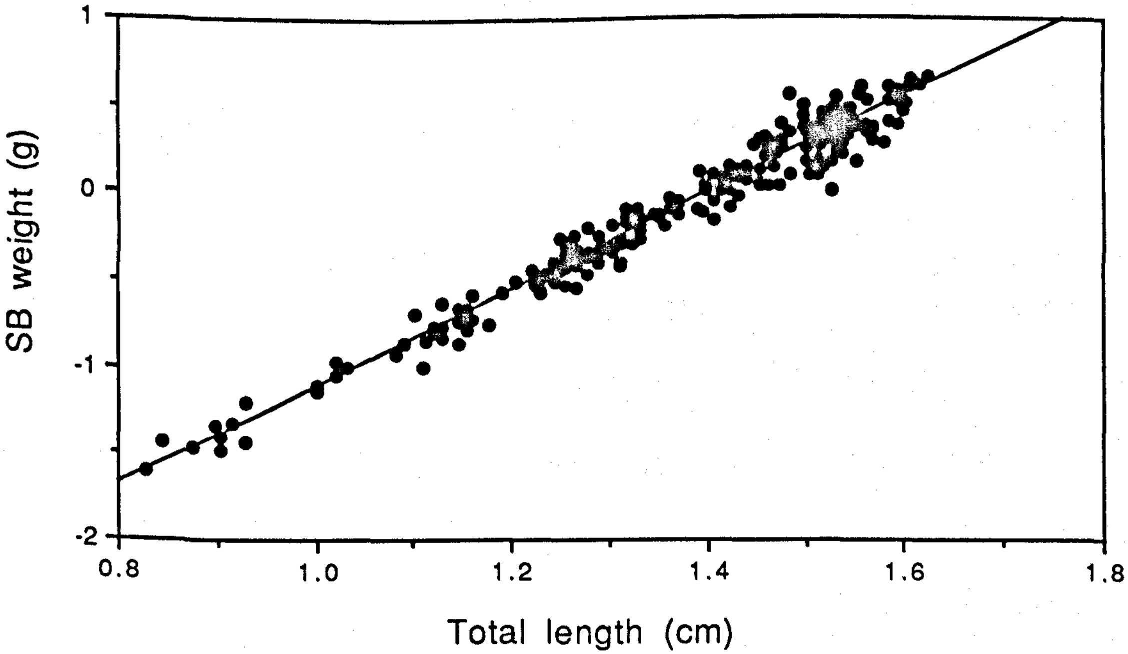


Figure 3.4 - Relationship between the swimbladder weight (SB) and grey gurnard total length (TL). SB weight and TL are transformed by $\log_{10}$. The regression equation fitted to the transformed data is $y = -3.90 + 2.78x$. Sample size = 229. The regression coefficient $r = 0.982$; $P < 0.001$.

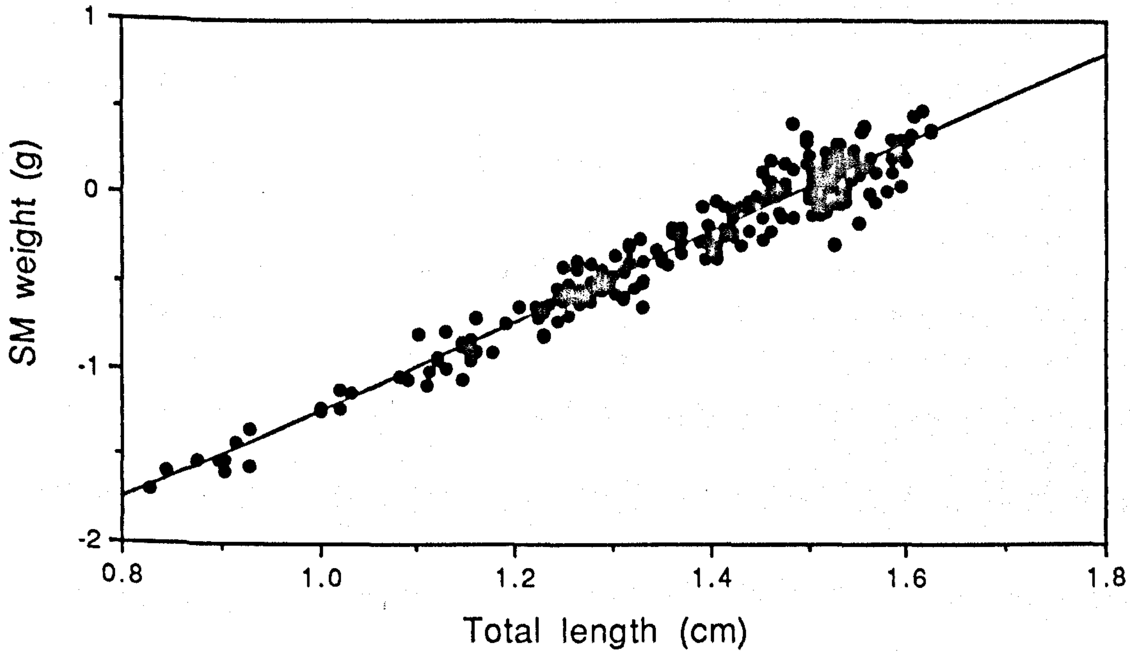


Figure 3.5 - Relationship between the sonic muscles weight (SM) and grey gurnard total length (TL). SM weight and TL are transformed by $\log_{10}$. The regression equation fitted to the transformed data is $y = -3.81 + 2.56x$. Sample size = 229. The regression coefficient $r = 0.971$; $P < 0.001$.

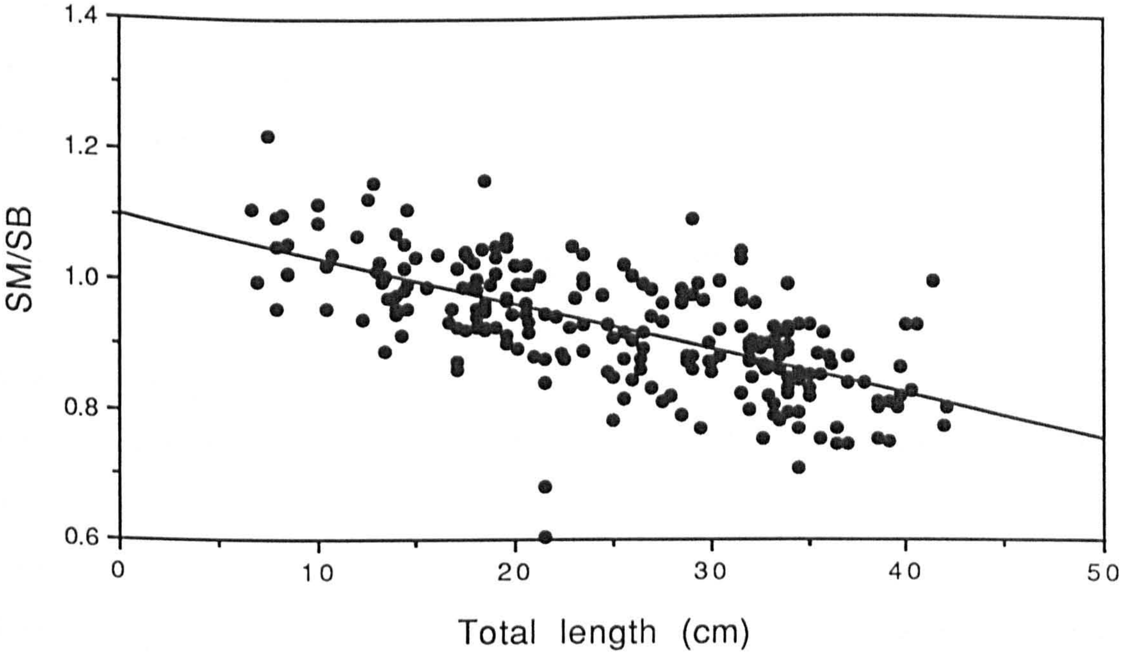


Figure 3.6 - Relationship between the sonic muscles weight (SM) / total swimbladder weight (SB) ratio and grey gurnard total length. SM/SB are transformed by arcsin$\sqrt{x}$. The regression equation fitted to the transformed data is $y = 1.10 - 6.88x$. Sample size = 229. The regression coefficient $r = 0.650$; $P < 0.001$.

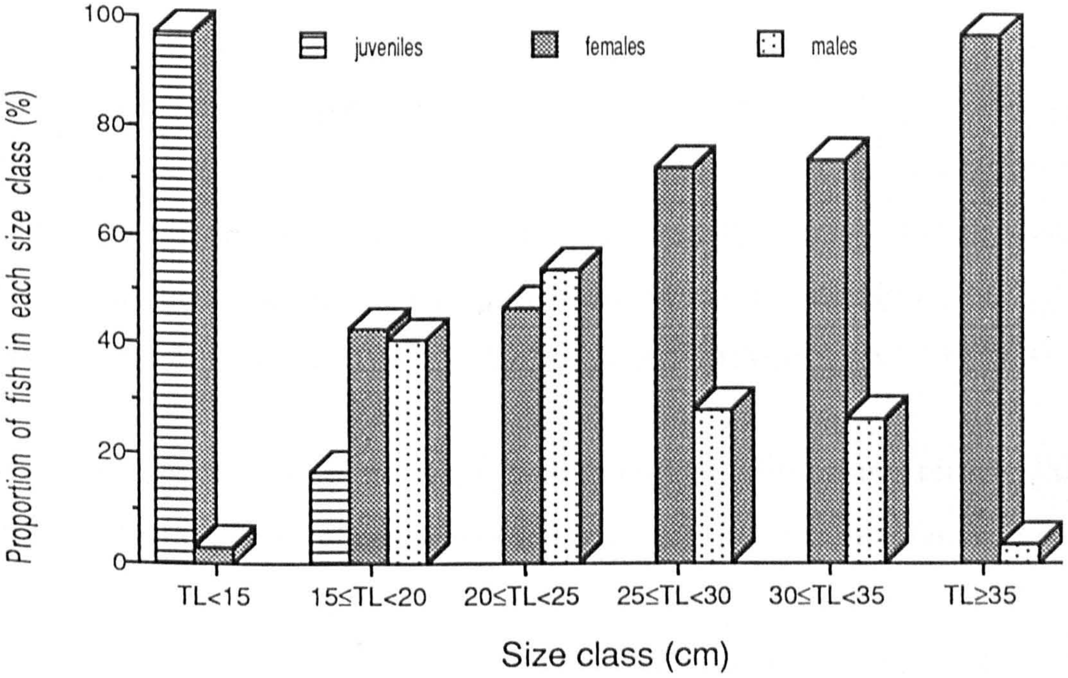


Figure 3.7 - Proportion of juvenile, female and male grey gurnards in each size class (percentages in each size class add up to 100). Juvenile fish are smaller individuals with no visible or with extremely small gonads. N (juveniles) = 41; N (females) = 129; N (males) = 59.

A tendency for males to have on average heavier swimbladders and sonic muscles than females was observed (Figs. 3.8 and 3.9 - note that there was only one male larger than 35 cm TL). Nevertheless, the differences between sexes were only significant for the swimbladder weight in one size class (fish ranging from 25 to 30 cm in total length) (Table 3.1).

Seasonal changes

Seasonal variations in the sound-producing apparatus of the grey gurnard were tested with a Kruskal-Wallis test. Data from both sexes were merged since there was no evident sexual dimorphism in the sonic apparatus. Only the length groups 25 - 30 cm and 30 - 35 cm (TL) were considered since only those contained a sufficient number of fish for all months of collection. Both total swimbladder and sonic muscle weight data sets were significantly different between these 2 length groups (Kruskal-Wallis test: swimbladder weight - $H = 53.08$; d.f. = 1; $N = 92$; $P < 0.001$ and sonic muscles weight $H = 42.80$; d.f. = 1; $N = 92$; $P < 0.001$, respectively), so the 2 size classes were studied separately.

The sound-producing apparatus of the grey gurnard did not show any evident pattern of seasonal variation (Figs. 3.10 and 3.11), although significant differences in the swimbladder and the sonic muscle weight were found between the different months of data collection ($P < 0.05$) respectively for the size classes $25 \leq TL < 30$ cm and $30 \leq TL < 35$ cm (Table 3.2).

The association between the swimbladder (SB) and the sonic muscle (SM) weight with either the gonodosomatic index (GSI) or the maximum oocyte diameter was tested with the non-parametric Spearman correlation test. In females, both the SB and the SM weight were significantly correlated with both the GSI and the maximum oocyte diameter (Table 3.3). In males, however, no significant relation was found between the SB and the SM weight with the GSI (Table 3.3).

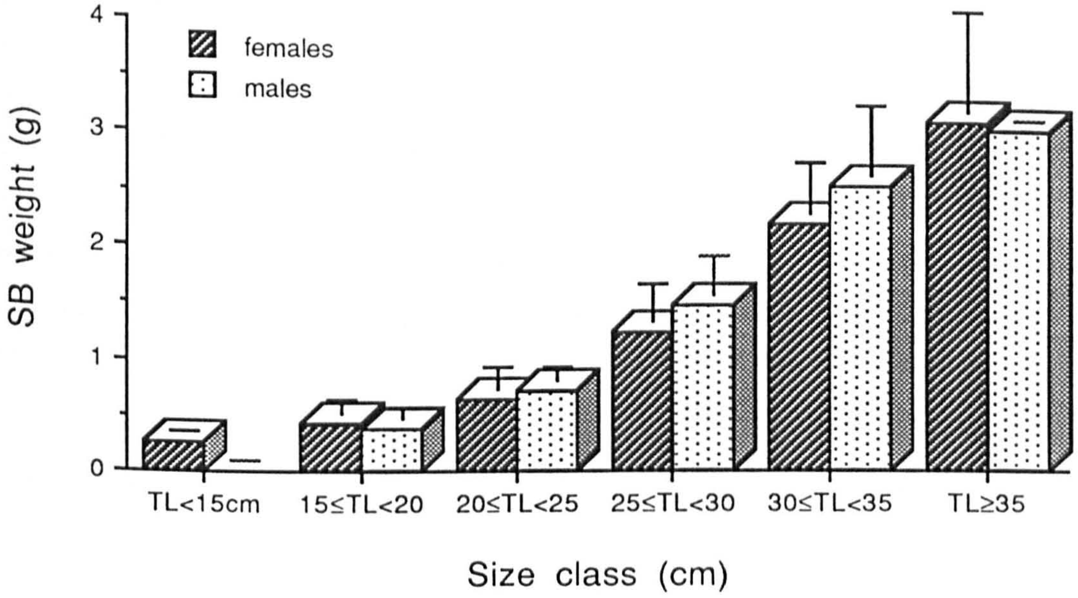


Figure 3.8 - Mean and standard deviation of the total swimbladder weight (g) (SB) for female and male grey gurnards from different size classes. TL = total length.

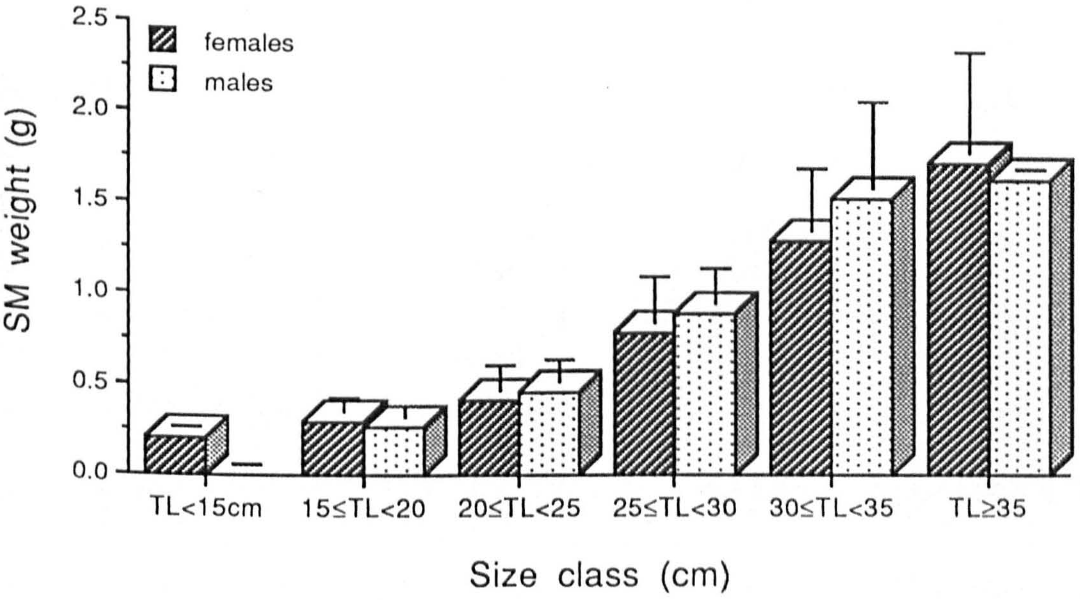


Figure 3.9 - Mean and standard deviation of the sonic muscles' weight (g) (SM) for female and male grey gurnards from different size classes. TL = total length.

Table 3.1 - Results of the Mann-Whitney test for sexual dimorphism of total swimbladder weight (g) (including the sonic muscles) (SB) and sonic muscles weight (g) (SM) for grey gurnards belonging to different size classes. Probabilities are adjusted for ties. N = sample size; n.s. = not significant; * = $P < 0.05$; TL = total length.

Size class (cm)	females / males	SB weight (g)		SM weight (g)	
	N	W	P	W	P
15≤TL<20	18 / 20	333.0	n.s.	342.0	n.s.
20≤TL<25	14 /16	200.5	n.s	191.5	n.s
25≤TL<30	31 / 12	603.0	*	625.5	n.s
30≤TL<35	36 /13	836.0	n.s	827.0	n.s

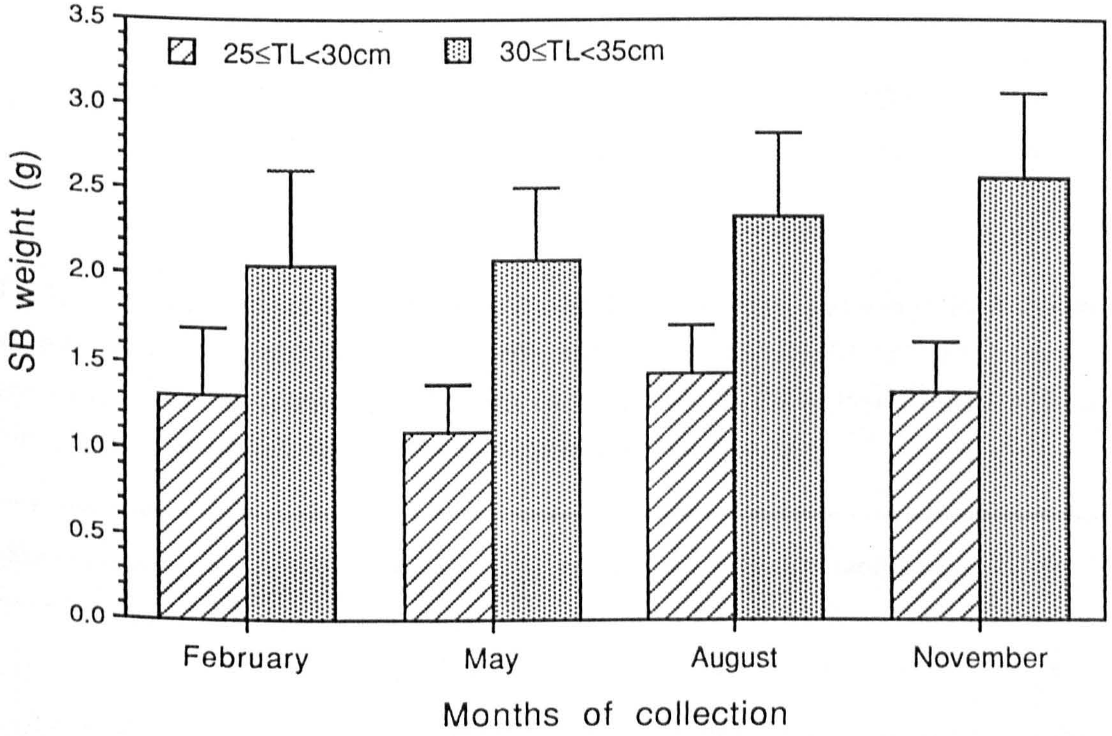


Figure 3.10 - Mean and standard deviation of the total swimbladder weight (g) (SB) of grey gurnards ranging in total length (TL) from 25 to 30 cm and from 30 to 35 cm collected at different times of the year.

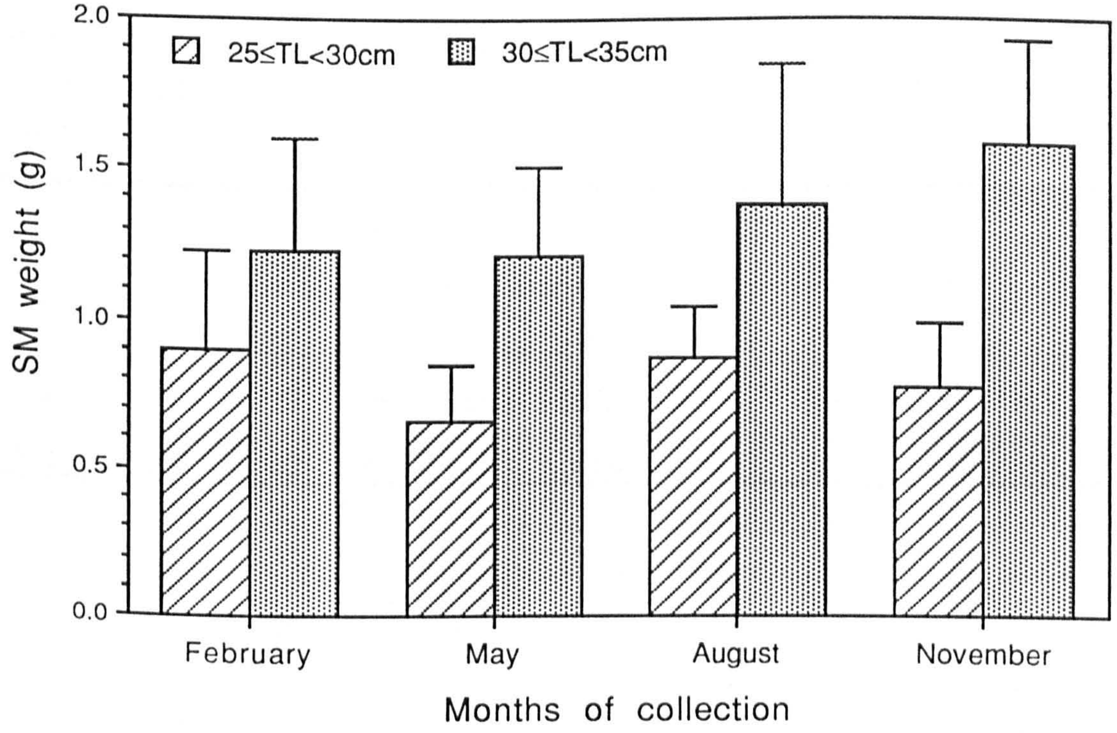


Figure 3.11 - Mean and standard deviation of the sonic muscle weight (g) (SM) of grey gurnards ranging in total length (TL) from 25 to 30 cm and from 30 to 35 cm collected at different times of the year.

Table 3.2 - Results of the Kruskal-Wallis test for seasonal variations of total swimbladder weight (g) (SB) and the sonic muscle weight (g) (SM) for grey gurnards ranging from 25 to 35 cm in total length (TL). Probability adjusted for ties. N = sample size; n.s. = not significant; * = P < 0.05.

Size class (cm)	SB weight (g)				SM weight (g)			
	N	d.f.	H	P	N	d.f.	H	P
25≤TL<30	43	3	8.47	*	43	3	6.74	n.s.
30≤TL<35	49	3	6.96	n.s.	49	3	8.15	*

Table 3.3 - Results of the Spearman correlation coefficient associating the total swimbladder weight (g) (SB) and the sonic muscle weight (g) (SM) with either the GSI or the maximum oocyte diameter in grey gurnards. Rho is corrected for ties. N = sample size; n.s. = not significant; * = $P < 0.05$; ** = $P < 0.01$.

	Females						Males		
		GSI			max oocyte diameter			GSI	
	N	Rho	P	N	Rho	P	N	Rho	P
SB	129	0.227	**	103	0.288	**	56	0.130	n.s.
SM	129	0.182	*	103	0.269	**	56	0.133	n.s.

3.3.1.2 - The streaked gurnard - *Trigloporus lastoviza*

The sonic apparatus

Anonymous (1938) and Rauther (1945) described the swimbladder of the streaked gurnard as an oval structure, roundish anteriorly, with a median internal diaphragm dividing it into an anterior large chamber and a small posterior one. Figure 3.12a depicts a section of the streaked gurnard's body showing the position of the swimbladder and its pair of intrinsic sonic muscles. A dorsal and ventral view of the swimbladder and sonic or drumming muscles is also shown in figure 3.12b.

The swimbladder occupies approximately 1/6 of the fish total length and extends from the 6th to the 13th vertebra of the vertebral column. Furthermore, the total swimbladder weight is on average 0.009 of the body weight (N = 34; s.d. = 0.002; min = 0.005 and max = 0.012) and the sonic muscles weight 0.790 of the total swimbladder weight (N = 34; s.d. = 0.061; min = 0.625 and max = 0.919).

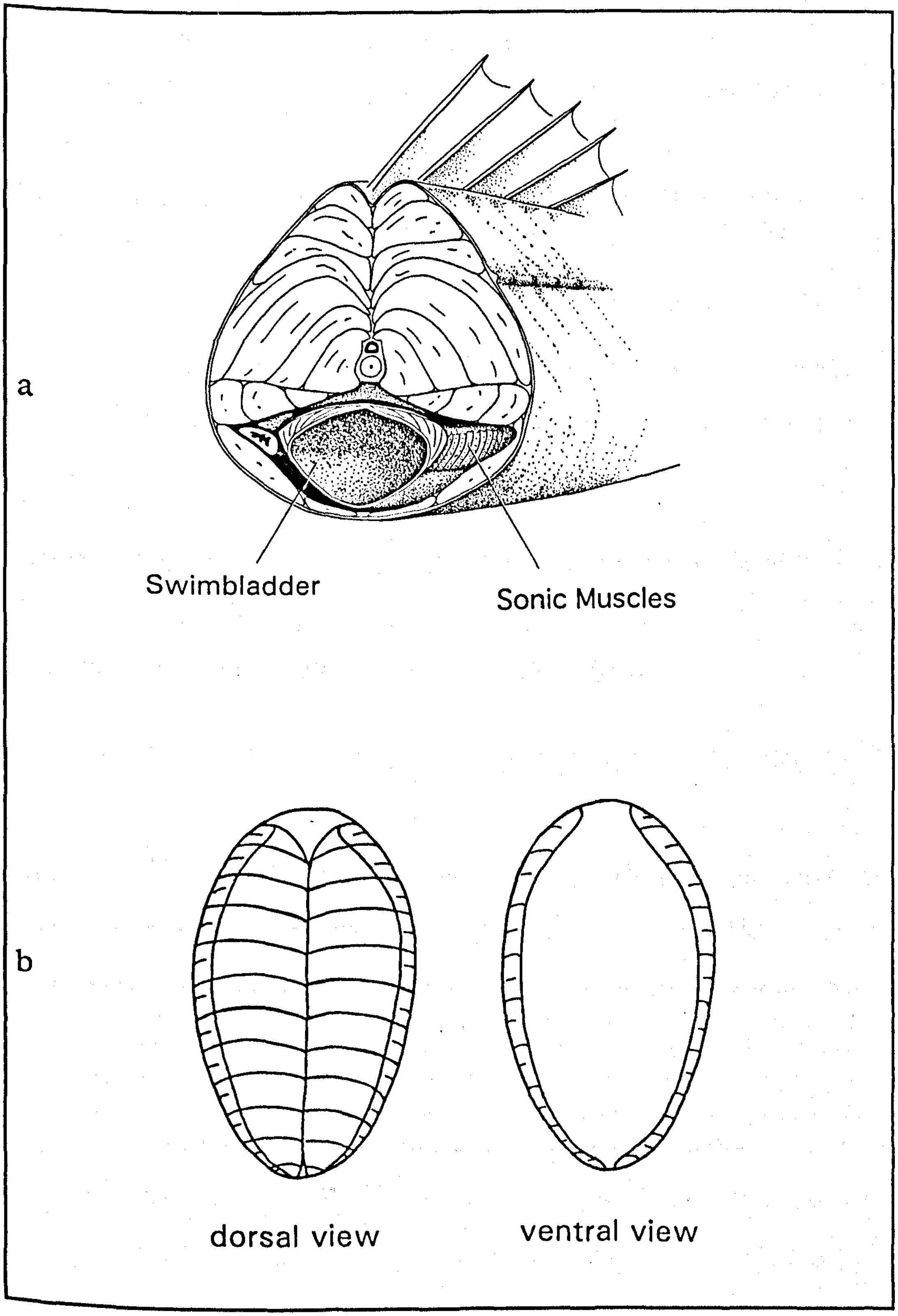


Figure 3.12 - (a) Cross section of a streaked gurnard showing the swimbladder and the sonic muscles (after Hawkins). (b) Dorsal and ventral view of the swimbladder and the imbedded pair of sonic muscles.

Ontogenetic development

Total swimbladder (SB) weight (including the sonic muscles) and the sonic muscles (SM) weight increased significantly with increasing fish size (Figs. 3.13 and 3.14). Simple linear regression was used for transformed data (see section 3.3.1.1 for explanations) (see Figs. 3.13 and 3.14). The sonic muscles / swimbladder weight ratio, however, did not seem to be influenced by fish size ($P > 0.05$).

3.3.1.3 - The red gurnard - *Aspitrigla cuculus*

The sonic apparatus

The swimbladder of the red gurnard is bifurcated anteriorly with two round short ends, and it has a pair of intrinsic sonic muscles imbedded in each side of its wall, as found in the grey gurnard (Fig. 3.15).

The swimbladder of red gurnards collected in the North Sea extends from the 6th to the 13th vertebra of the dorsal spine and weighted on average 0.011 of the body weight ($N = 61$; s.d. = 0.002; min = 0.007 and max = 0.015); the sonic muscles weighted on average 0.762 of the total swimbladder weight ($N = 61$; s.d. = 0.063; min = 0.541 and max = 0.853). The swimbladder of red gurnards collected in the Mediterranean weighted on average 0.007 of the body weight ($N = 20$; s.d. = 0.002; min = 0.004 and max = 0.011) and the sonic muscles 0.712 of the swimbladder weight ($N = 20$; s.d. = 0.100; min = 0.463 and max = 0.861).

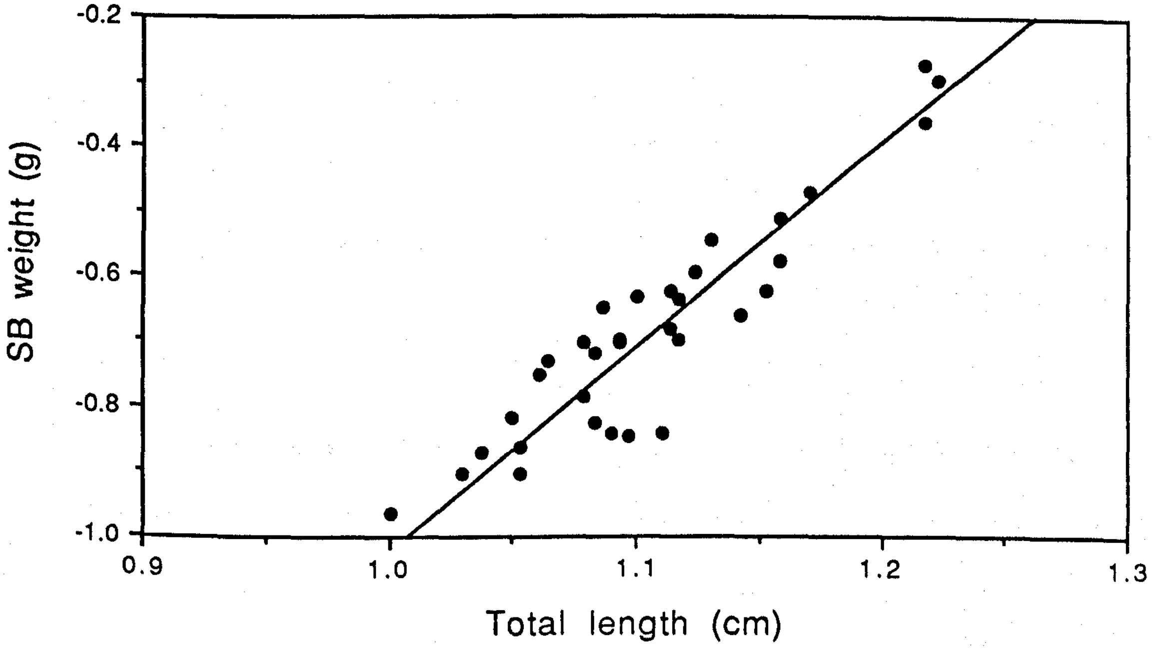


Figure 3.13 - Relationship between the swimbladder weight (SB) and streaked gurnard total length (TL). SB weight and TL are transformed by $\log_{10}$. The regression equation fitted to the transformed data is $y = -4.15 + 3.13x$. Sample size = 34. The regression coefficient $r = 0.888$; $P < 0.001$.

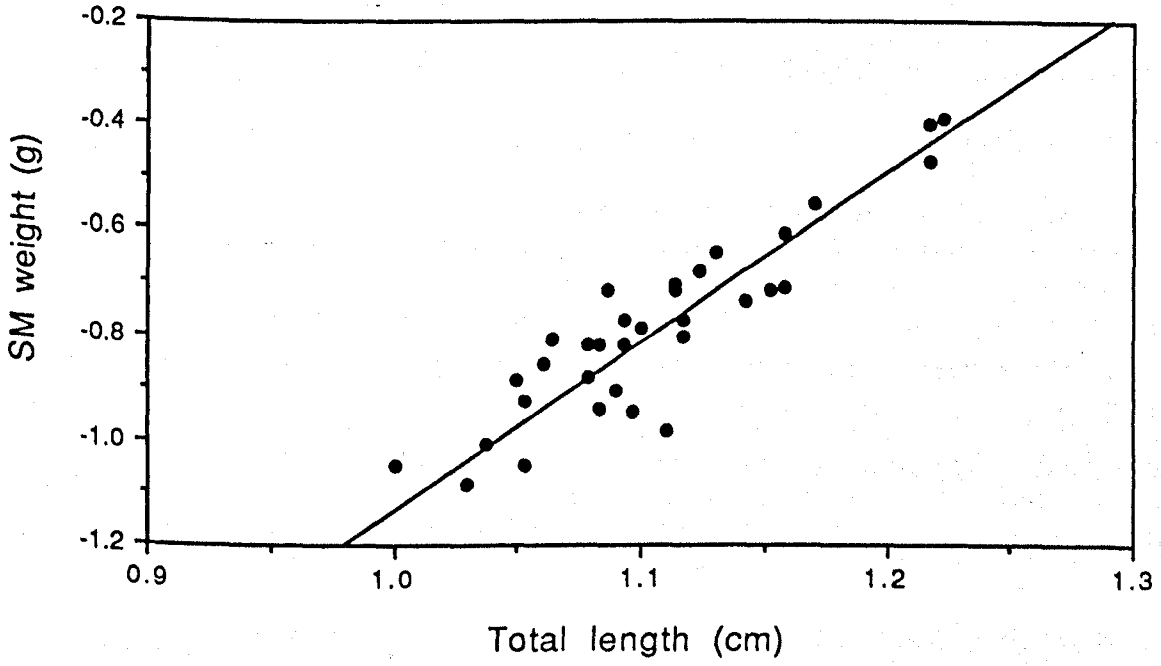


Figure 3.14 - Relationship between the sonic muscles weight (SM) and streaked gurnard total length (TL). SM weight and TL are transformed by $\log_{10}$. The regression equation fitted to the transformed data is $y = -4.36 + 3.22x$. Sample size = 34. The regression coefficient $r = 0.856$; $P < 0.001$.

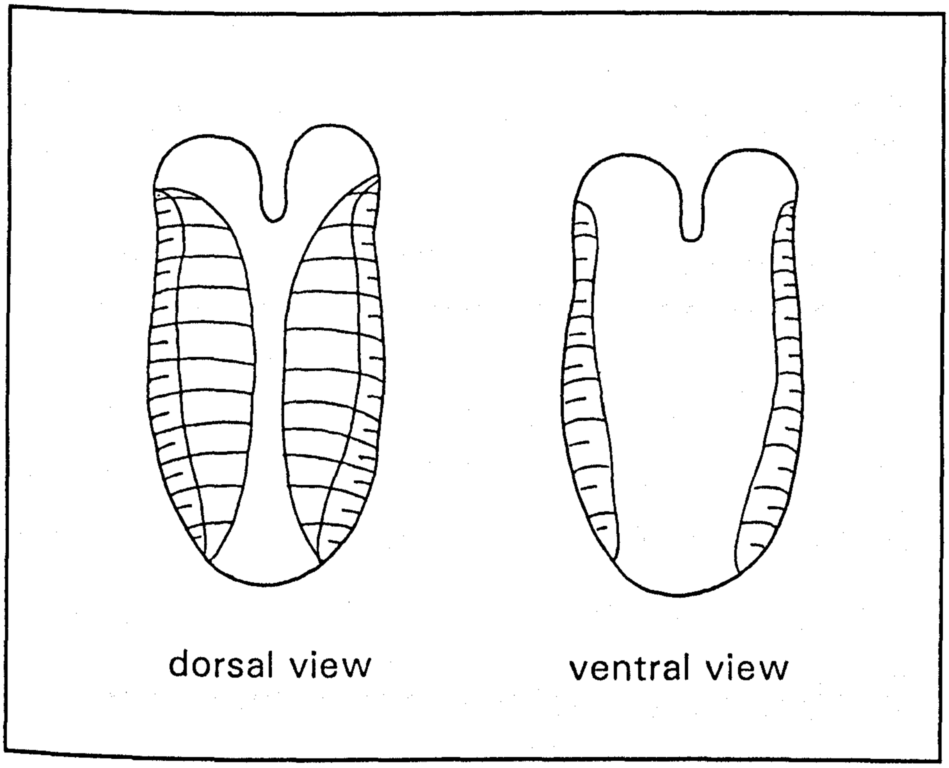


Figure 3.15 - Dorsal and ventral view of the the swimbladder and sonic muscles of the red gurnard.

Ontogenetic development

A non-parametric Mann-Whitney test was used to compare body, swimbladder and sonic muscles weight from fish collected in the Mediterranean and in the North Sea. As, on average, the North Sea red gurnards were larger than the Mediterranean ones, only data of fish belonging to a similar size range were considered. The results of the Mann-Whitney test are given in Table 3.4 and show that in spite of having similar length and body weight, the red gurnards collected in the North Sea have significantly heavier swimbladders and sonic muscles than red gurnards collected in the Mediterranean. Hence, data on the sonic apparatus of red gurnards of the Mediterranean and the North Sea were treated separately.

Table 3.4 - Results of the Mann-Whitney test for differences in the red gurnard total length, body, swimbladder and sonic muscles weight between samples collected in the Mediterranean and in the North Sea. Mediterranean red gurnards ranged in total length from 13.8 to 24.5 cm and North Sea red gurnards from 15.0 to 22 cm. The probabilities are adjusted for ties. N = sample size; n.s. = not significant; *** = P< 0.001.

	Med. / North Sea		
	N	W	P
Total length (cm)	14 / 27	241.0	n.s.
Body weight (g)	14 / 27	242.0	n.s.
SB weight (g)	14 / 27	159.0	***
SM weight (g)	14 / 27	146.0	***

The relationship between the total swimbladder and sonic muscle weight with fish length were tested with a linear regression test as described in section 3.3.1.1. The weight of the sonic apparatus increased significantly with fish size in both Mediterranean and North Sea red gurnards (Figs. 3.16 and 3.17).

The SM/SB weight ratio was significantly correlated with red gurnard size (TL) (North Sea) (Fig. 3.18). The arcsin$\sqrt{x}$ transformation did not improve the regression fit (see explanations in section 3.3.1.1) and consequently the original data were used. In the Mediterranean red gurnards, however, the SM/SB weight ratio did not seem to be influenced by the fish total length ($P > 0.05$) (Fig. 3.18).

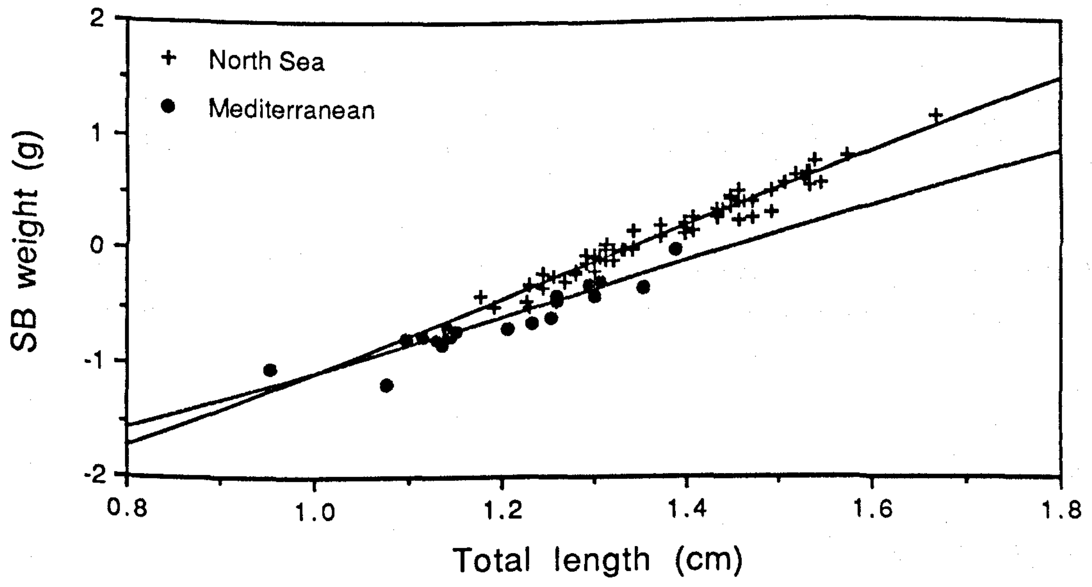


Figure 3.16 - Relationship between the swimbladder weight (SB) and red gurnard total length (TL). SB weight and TL are transformed by $\log_{10}$. The regression equation fitted to the transformed data regarding the North Sea red gurnards is $y = -4.12 + 3.00x$; sample size = 27; the regression coefficient $r = 0.974$; $P < 0.001$. The regression equation fitted to the transformed data regarding the Mediterranean red gurnards is $y = -3.90 + 2.61x$; sample size = 14; the regression coefficient $r = 0.894$; $P < 0.001$.

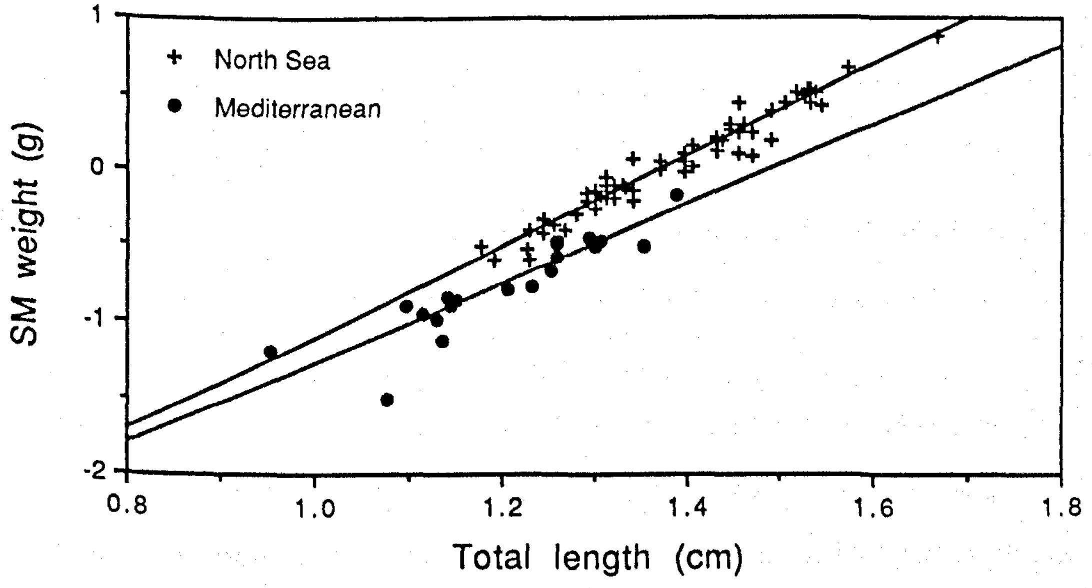


Figure 3.17 - Relationship between the sonic muscle weight (SM) and red gurnard total length (TL). SM weight and TL are transformed by $\log_{10}$. The regression equation fitted to the transformed data regarding the North Sea red gurnards is $y = -4.32 + 3.23x$; sample size = 27; the regression coefficient $r = 0.979$; $P < 0.001$. The regression equation fitted to the transformed data regarding the Mediterranean red gurnards is $y = -3.52 + 2.43x$; sample size = 14; the regression coefficient $r = 0.932$; $P < 0.001$.

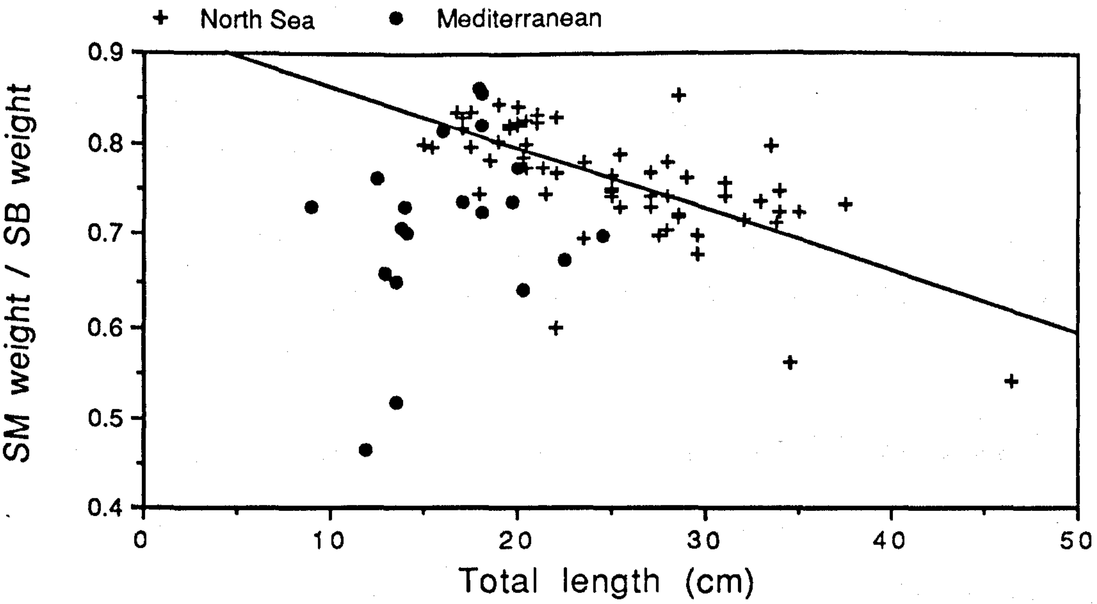


Figure 3.18 - Relationship between the sonic muscle weight (SM) / total swimbladder weight (SB) and red gurnard total length (TL). The regression equation fitted to the data regarding the North Sea red gurnards is $y = 0.93 - 0.007x$; sample size = 61; the regression coefficient $r = 0.674$; $P < 0.001$. Regarding the Mediterranean red gurnards, the sample size = 20; the regression coefficient $r = 0.272$; $P > 0.05$.

Sexual dimorphism

Sexual dimorphism in the sonic apparatus of the red gurnard was tested only for the specimens collected from the North Sea (the sample size for the Mediterranean red gurnards was too small). Only fish ranging in total length from 20 to 35 cm were considered since this size class provided an adequate sample size for both sexes and also no significant differences were found between male and female body weight (Mann-Whitney test, $P > 0.05$) within this size class.

Neither total swimbladder nor sonic muscles weight differed significantly between male and female (Mann-Whitney test, $P > 0.05$; Table 3.5).

Table 3.5 - Descriptive statistics for the total swimbladder weight (SB) (including the sonic muscles) and the sonic muscles weight (SM) for red gurnards of either gender.

	SB weight (g)		SM weight (g)	
	female	male	female	male
N	20	20	18	18
mean	2.443	2.213	1.817	1.585
s.d.	1.164	1.340	0.852	0.861
min	0.770	0.950	0.640	0.620
max	4.700	5.740	3.410	3.220

3.3.1.4 - The tub gurnard - *Trigla lucerna*

The sonic apparatus

The sonic apparatus of the tub gurnard consists of a large swimbladder with 2 lateral diverticula, and a pair of sonic muscles imbedded in the outer surface of the swimbladder as depicted in Figure 3.19.

The swimbladder represents on average 0.008 of the body weight (N = 13; s.d. = 0.005; min = 0.003 and max = 0.016) and the sonic muscles 0.527 of the total swimbladder weight (N = 13; s.d. = 0.077; min = 0.376 and max = 0.633).

Ontogenetic development

Total swimbladder (including the sonic muscles) (SB) and the sonic muscles (SM) weight were significantly correlated with fish total length (for explanations on the data transformations see section 3.3.1.1) (Figs. 3.20 and 3.21). The sonic muscles / swimbladder weight ratio did not show a significant trend with increasing fish size ($P > 0.05$).

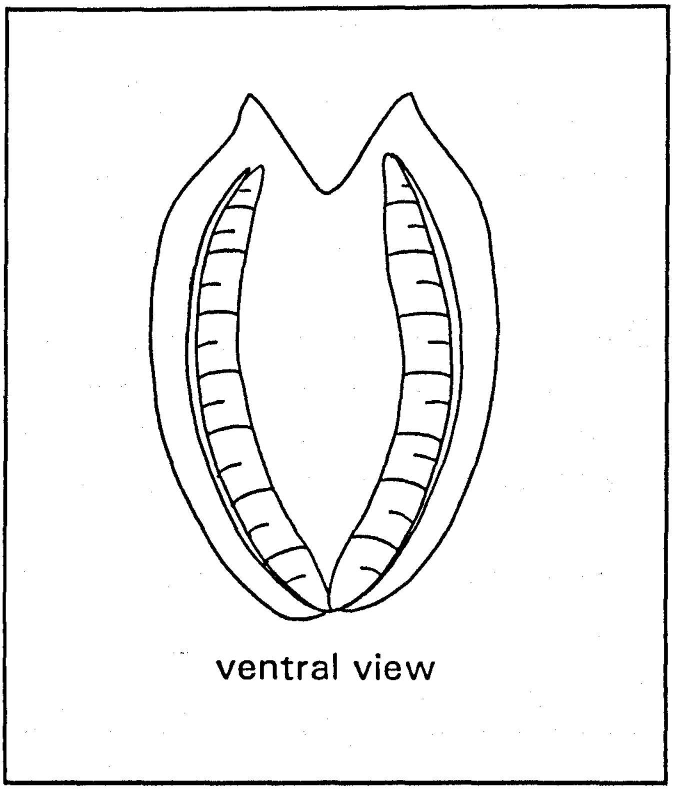


Figure 3.19 - Ventral view of the swimbladder of the tub gurnard showing its lateral diverticula and a pair of intrinsic sonic muscles.

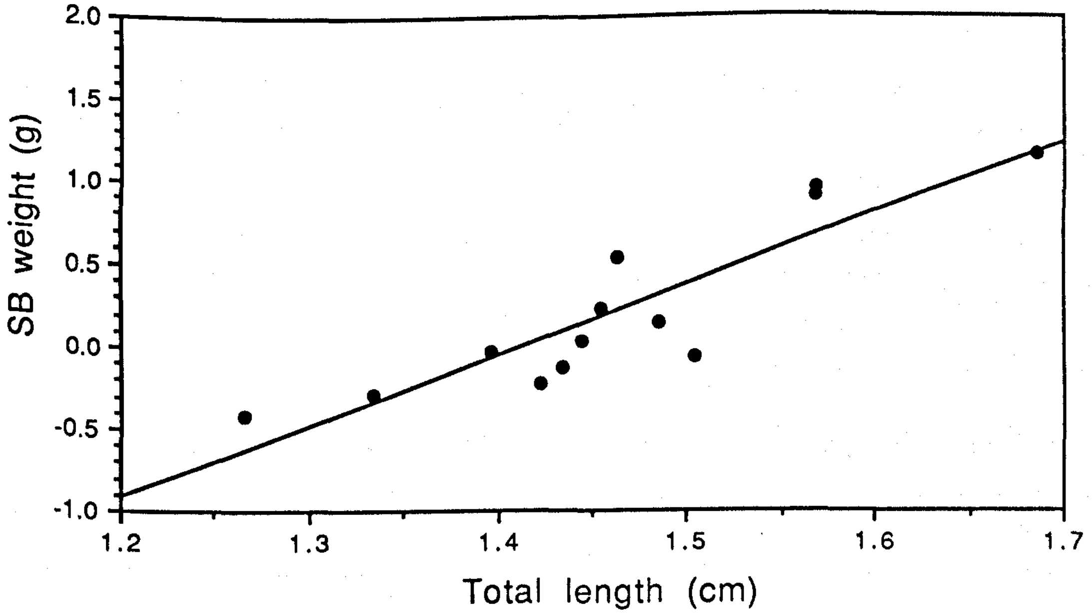


Figure 3.20 - Relationship between the total swimbladder weight (SB) and tub gurnard total length (TL). SB weight and TL are transformed by $\log_{10}$. The regression equation fitted to the transformed data is $y = -6.04 + 4.27x$. Sample size = 13. The regression coefficient $r = 0.891$; $P < 0.001$.

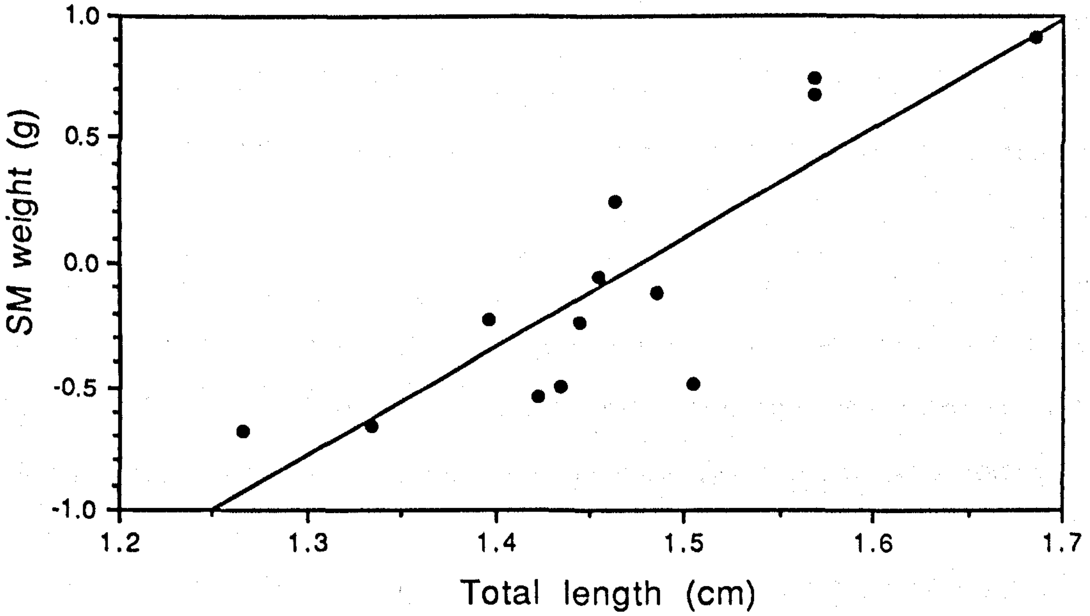


Figure 3.21 - Relationship between the sonic muscles weight (SM) and tub gurnard total length (TL). SM weight and TL are transformed by $\log_{10}$. The regression equation fitted to the transformed data is $y = -6.51 + 4.40x$. Sample size = 13. The regression coefficient $r = 0.859$; $P < 0.001$.

3.3.1.5 - The large scaled gurnard - *Lepidotrigla cavillone*

The sonic apparatus

Lepidotrigla cavillone has a well-developed oval swimbladder slightly bifurcated anteriorly, but with no marked lobes, and with a pair of intrinsic muscles imbedded in each side of its wall (pers. observ.). In contrast with the other European species, the swimbladder of this species possesses a medium septum dividing the anterior third of the bladder into right and left chambers as found in American triglids (Rauther 1945, Evans 1973). The swimbladder length is 1/6 of the fish total length.

The swimbladder weight represents on average 0.009 of the body weight (N = 42; s.d. = 0.004; min = 0.005 and max = 0.035) and the sonic muscles 0.707 of the total swimbladder weight (N = 42; s.d. = 0.069; min = 0.500 and max = 0.833).

Ontogenetic development

The sonic apparatus (swimbladder and sonic muscles) weight of the large scaled gurnard increased significantly with increasing fish total length (Figs. 3.22 and 3.23). Data transformations are as described in section 3.3.1.1.

The sonic muscles' weight, however, did not increase proportionally to the total swimbladder weight (including the sonic muscles weight) with the increase of fish size. Figure 3.24 illustrates the significant trend of the SM/SB weight ratio with fish total length. Data were transformed as described in section 3.3.1.1.

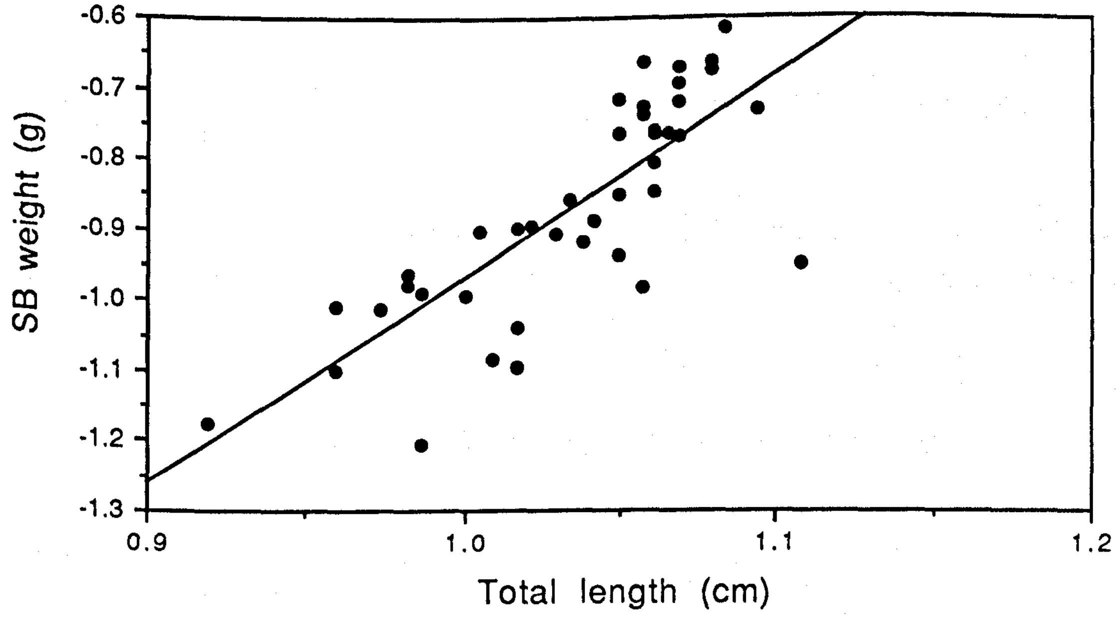


Figure 3.22 - Relationship between the total swimbladder weight (SB) and large scaled gurnard total length (TL). SB weight and TL are transformed by $\log_{10}$. The regression equation fitted to the transformed data is $y = -3.87 + 2.90x$. Sample size = 41. The regression coefficient r = 0.787; P < 0.001.

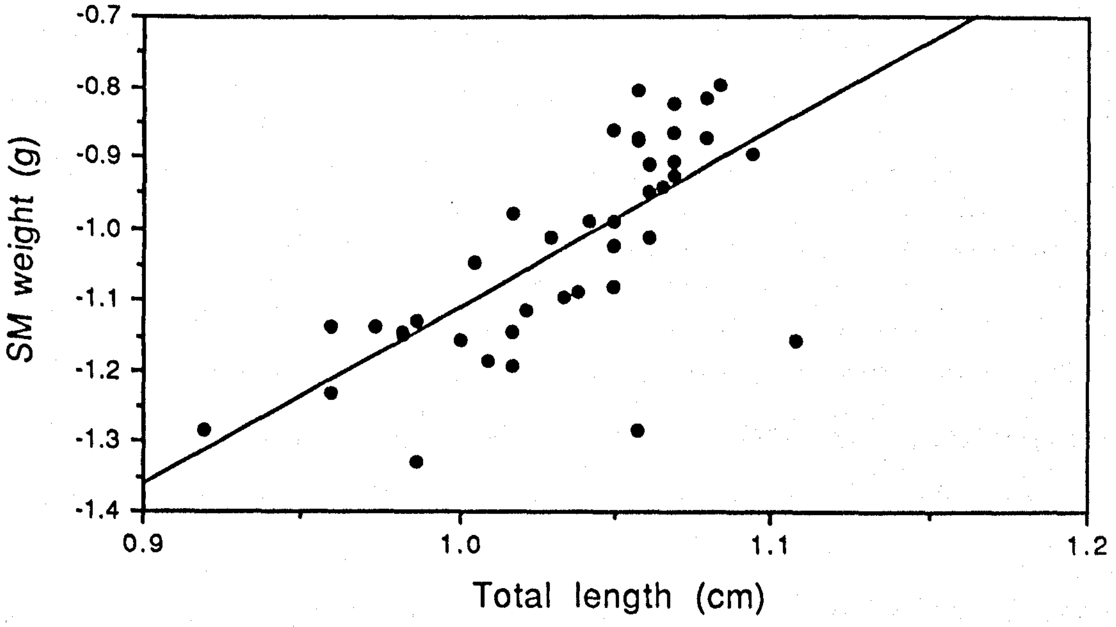


Figure 3.23 - Relationship between the sonic muscles weight (SM) and large scaled gurnard total length (TL). SM weight and TL are transformed by $\log_{10}$. The regression equation fitted to the transformed data is $y = -3.61 + 2.50x$. Sample size = 41. The regression coefficient r = 0.711; P < 0.001.

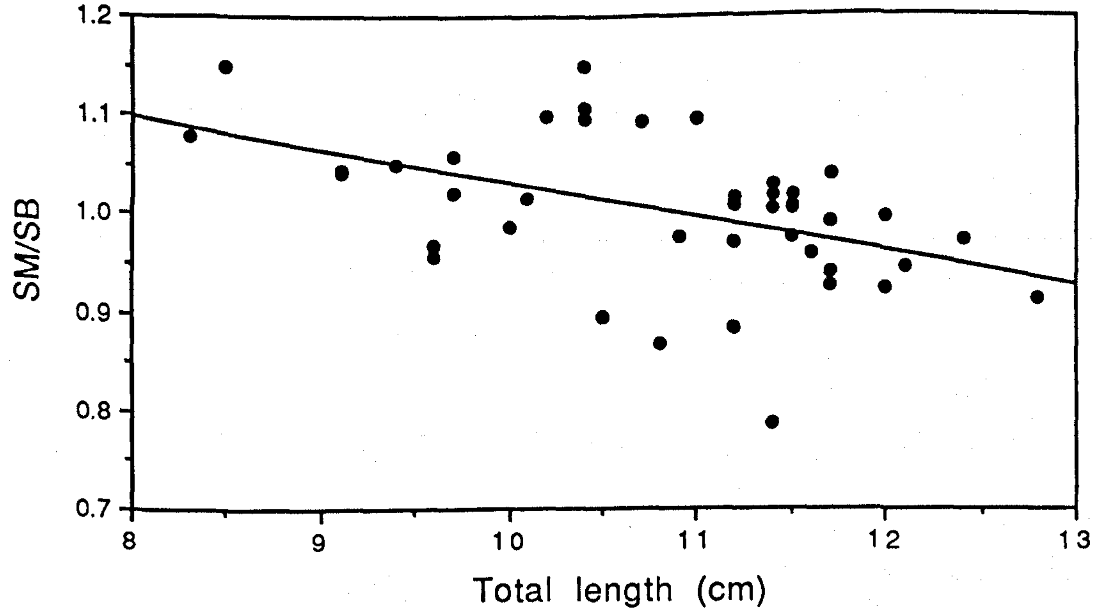


Figure 3.24 - Relationship between the sonic muscles weight (SM) / total swimbladder weight (SB) ratio and large scaled gurnard total length. SM/SB are transformed by arcsin$\sqrt{x}$. The regression equation fitted to the transformed data is $y = 1.37 - 0.034x$. Sample size = 42. The regression coefficient $r = 0.475$; $P < 0.01$.

3.3.1.6 - The piper - *Trigla lyra*

The swimbladder of *Trigla lyra* is oval, small, with a pointed anterior end and a round posterior one. This species, unlike other gurnards from shallower waters, possesses extrinsic sound muscles, as illustrated in Figure 3.25. The swimbladder extends from the 1st to the 13th vertebra of the dorsal spine (Fig. 3.25). The extrinsic muscles are attached posteriorly by 4 ligaments to the 7th, 8th, 9th and the 10th vertebra and anteriorly to the pectoral girdle.

The swimbladder weight (with no sonic muscles) weights approximately 0.003 of the body weight (N = 3; s.d. = 0.001).

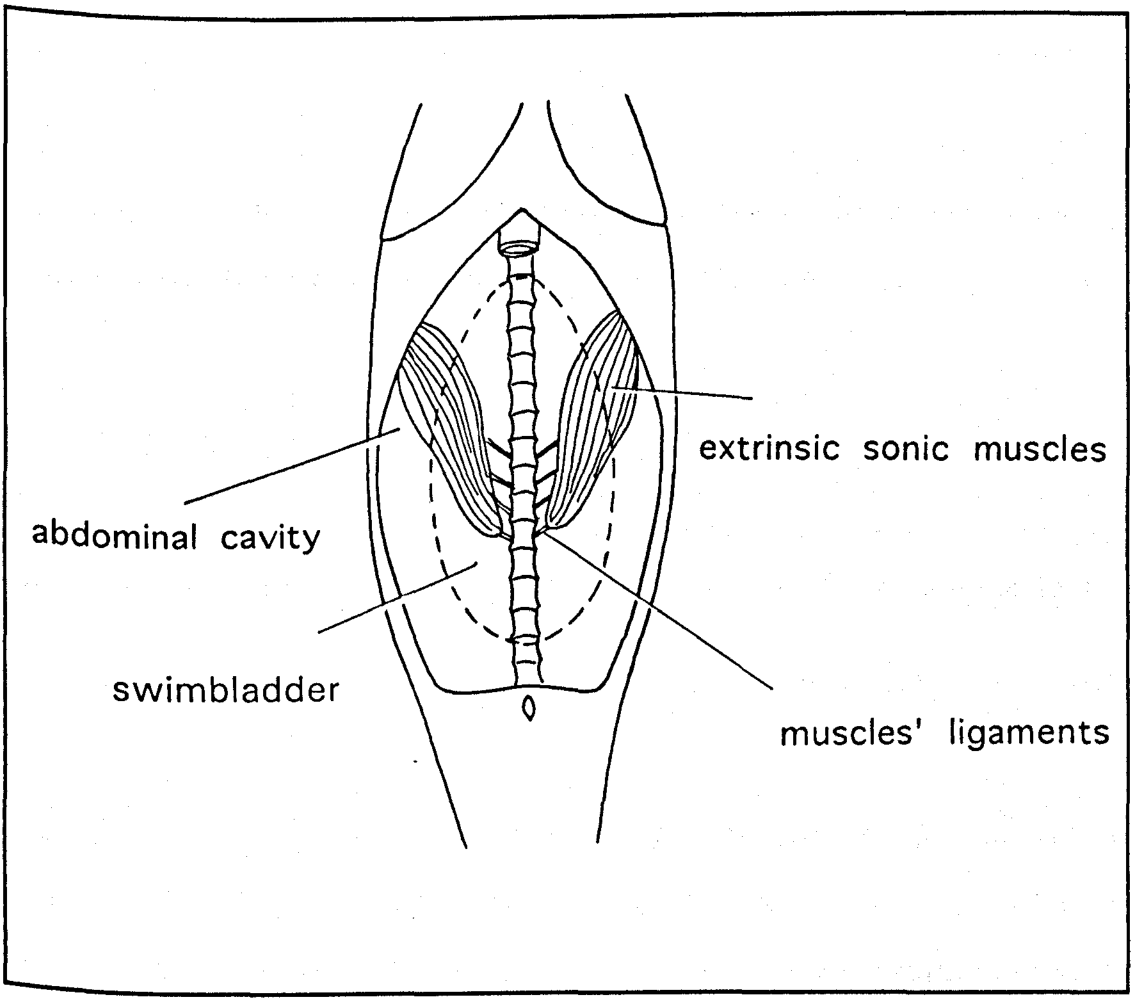


Figure 3.25 - Ventral aspect of a dissected piper depicting an oval swimbladder and a pair of extrinsic sonic muscles.

3.3.1.7 - Variations in the sonic apparatus of different species

The sonic apparatus of the studied species of gurnards varied in shape, structure and weight. The streaked, the large scaled and the piper gurnards have oval swimbladders. The grey and the red gurnards also have oval swimbladders but bifurcated anteriorly. In contrast with the previous species, the swimbladder of the tub gurnard possesses 2 lateral diverticula. Furthermore, all species have a pair of instrinsic sonic muscles except for the piper which has a pair of extrinsic sonic muscles.

Body weight, total swimbladder weight (including the sonic muscles) (SB) and the sonic muscles weight (SM) were compared between the studied species. Sample sizes ranged from N = 229 for the grey gurnard to N = 13 for the tub gurnard. In addition, as found in the previous sections (3.3.1.1, 3.3.1.2, 3.3.1.3, 3.3.1.4 and 3.3.1.5), body, SB and SM weight is a function of fish length. Furthermore, length range varied considerably between species. Comparisons of weight variables between species are only meaningful with similar sizes of fish. As the grey gurnard length range effectively covered the length ranges of the other triglids, all individual values of each species' body, SB and SM weights were compared with the grey gurnard body, SB and SM weights at a given length. A regression line and 95% confidence intervals for the fitted regression were calculated for the body weight/total length (TL), the SB weight/TL and the SM weight/TL relationships of the grey gurnard. For the rest of the species, the individual values of body, SB and SM weights at a given length were superimposed on the corresponding regression line and 95% confidence intervals (weight/TL relationships) for the grey gurnard, and comparisons were then made. When most of the superimposed values of either body, SB or SM weights, were within the 95% confidence bands, then they were considered to be similar to those of the grey gurnard; otherwise they were considered larger, smaller or "with no trend", as appropriate.

The body weights of both the streaked and the large scaled gurnard were greater than the body weight of the grey gurnard at a given length. The tub gurnards and the North Sea and the Mediterranean red gurnards had similar body weights to the grey gurnard at a given length (Fig. 3.26). Only the North Sea red gurnards had greater SB and SM weights than the grey gurnard at a given length; all other species showed similar values to the grey gurnard (Fig. 3.27 and 3.28). Both SB and SM weights of the tub gurnard were very variable, falling within, below and above the 95% confidence bands, and hence showing no particular trend in relation to the SB and SM weights of the grey gurnard at various length. Also, not all the SB and SM weight values of the North Sea red gurnards lay above the upper 95% interval. Nevertheless, as most of them lay above the upper 95% confidence intervals, both SB and SM weights were considered to be greater in this species than in the grey gurnard at a given length.

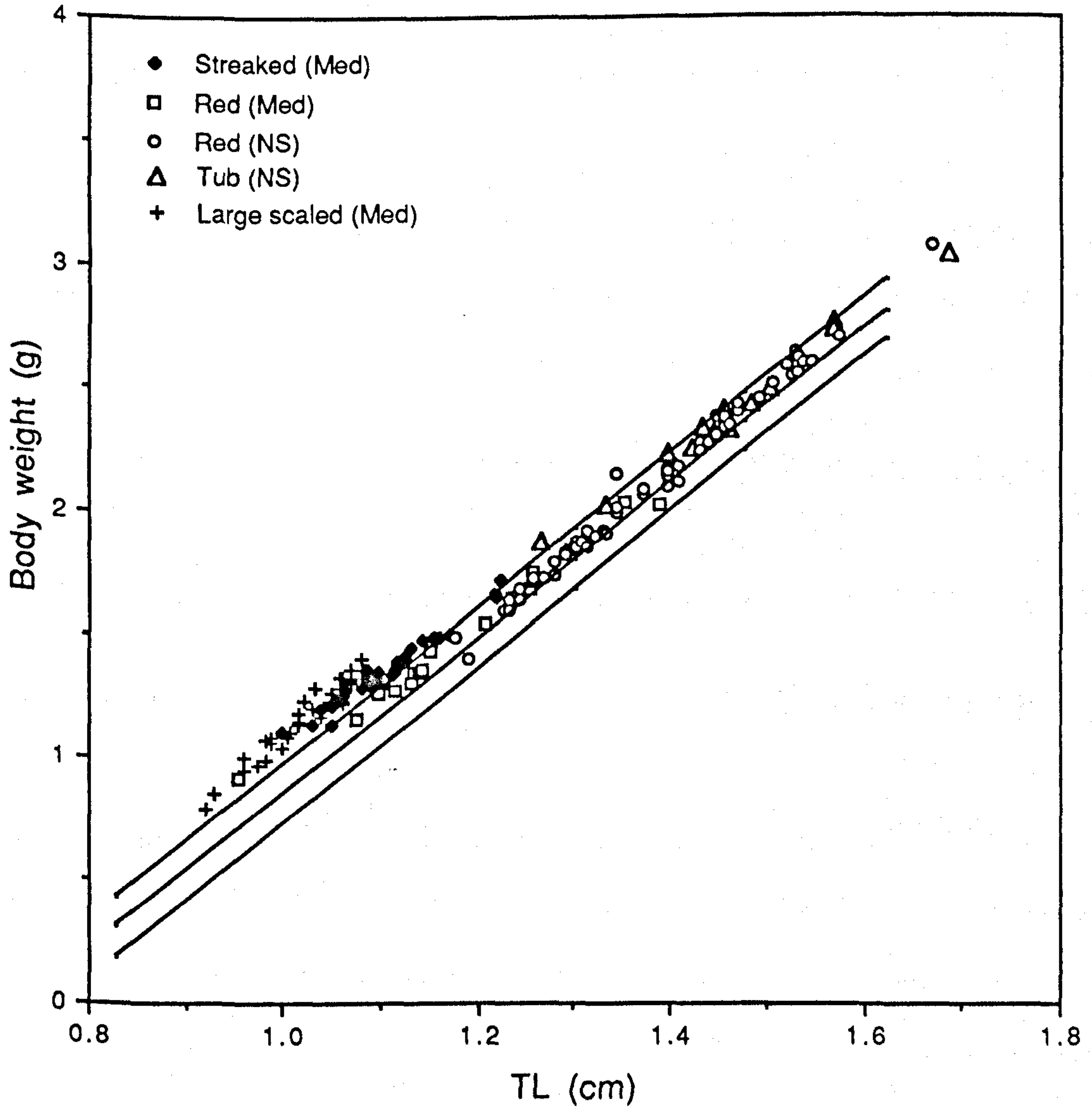


Figure 3.26 - Relationship between the body weight and fish total length (TL) for the streaked, the red, the tub and the large scaled gurnards. NS = North Sea; Med = Mediterranean. Body weight and TL are transformed by $\log_{10}$. The 3 lines represent the fitted regression (middle line) and the 95% confidence intervals (upper and lower lines) for the relationship between the body weight and TL for the grey gurnard.

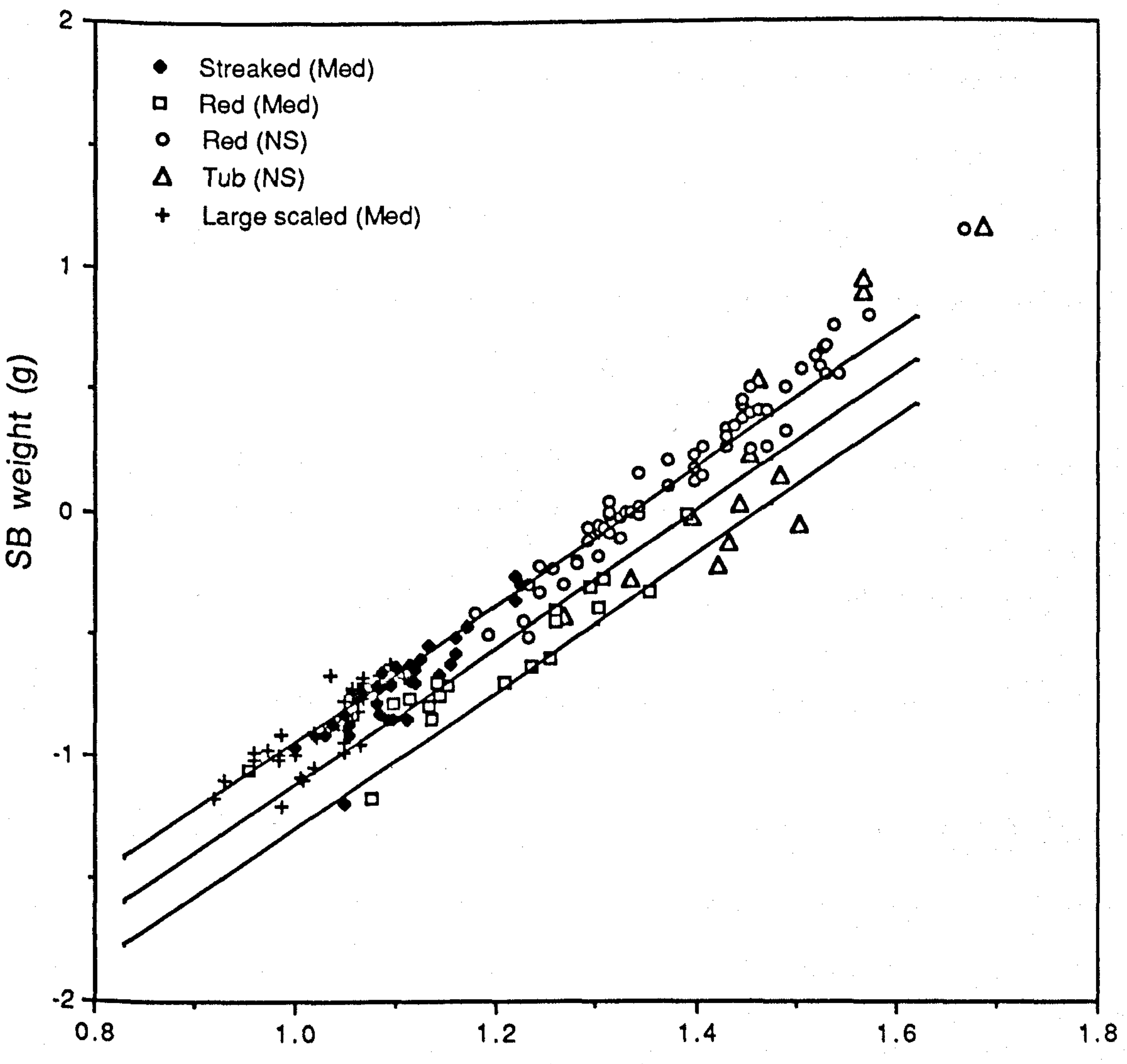


Figure 3.27 - Relationship between the total swimbladder weight (including the sonic muscles) (SB) and fish total length (TL) for the streaked, the red, the tub and the large scaled gurnards. NS = North Sea; Med = Mediterranean. SB weight and TL are transformed by $\log_{10}$. The 3 lines represent the fitted regression (middle line) and the 95% confidence intervals (upper and lower lines) for the relationship between the SB weight and TL for the grey gurnard.

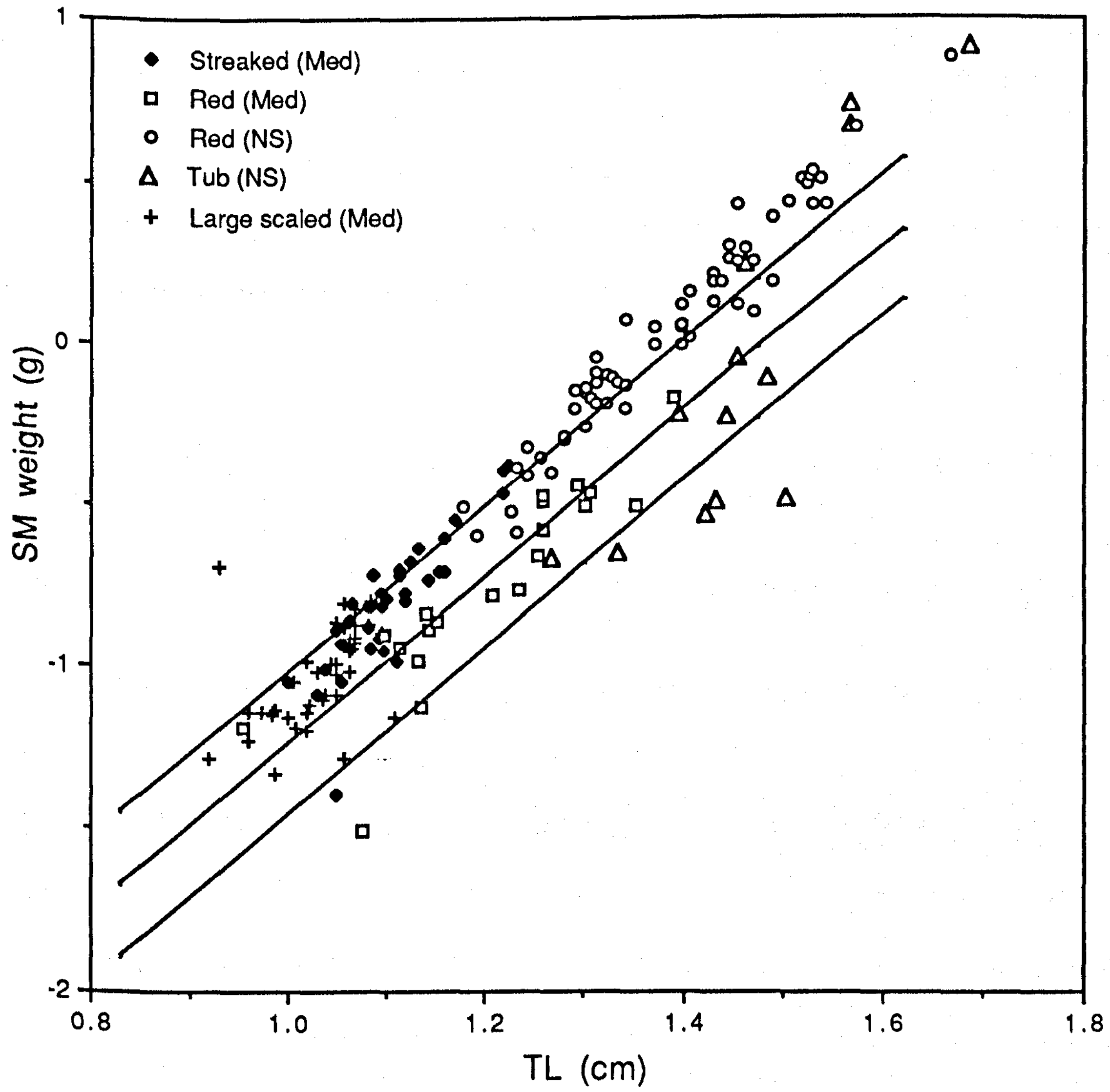


Figure 3.28 - Relationship between the sonic muscles weight (SM) and fish total length (TL) for the streaked, the red, the tub and the large scaled gurnards. NS = North Sea; Med = Mediterranean. SM weight and TL are transformed by $\log_{10}$. The 3 lines represent the fitted regression (middle line) and the 95% confidence intervals (upper and lower lines) for the relationship between the SM weight and TL for the grey gurnard.

3.3.2 - Neuromuscular mechanisms of sound production in the grey gurnard

3.3.2.1 - Relationship of electromyograms to sound production

Only 2 of the 3 grey gurnards studied emitted sounds naturally. The third was silent and was latter found to have a completely shrunken swimbladder. Only 1 of the 2 individuals that uttered sounds (only grunts) provided recordings with sufficient quality for analysis, although not all the pulses of each grunt were clear (Fig 3.29).

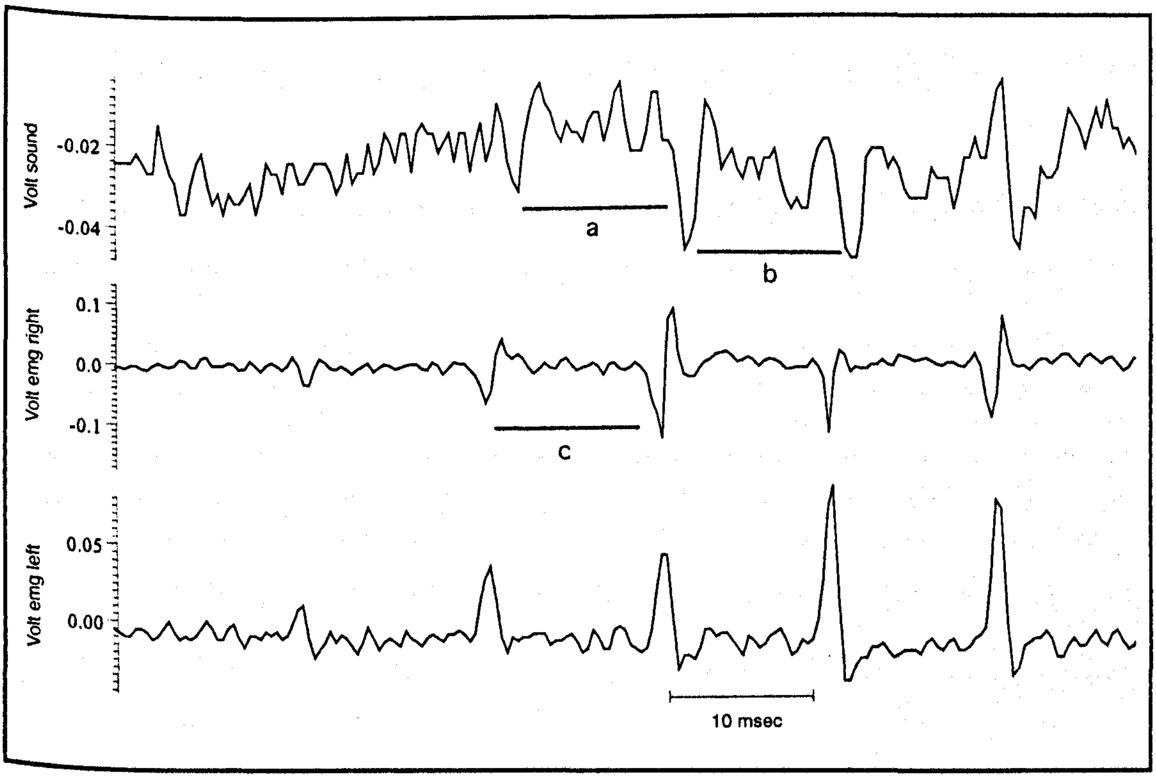


Figure 3.29 - An example of a grunt obtained from the experimental grey gurnard (top trace) and corresponding EMG signals from the right (middle trace) and the left (bottom trace) sonic muscles. **a**, **b** and **c** are peak to peak time intervals of EMGs and sound spikes.

Each muscle elecromyogram (EMG) corresponded to a single pulse of sound. The time lag between EMGs and sound production (the resulting pulse of sound) was obtained by measuring the time interval between EMG spikes and sound pulse spikes. Because only some of the pulses were clear in a grunt it was difficult to decide whether a muscle contraction (resulting from 1 EMG spike) caused the immediately subsequent pulse of sound or the one after (Fig.3.29) (i.e. if EMG spike 1 caused sound spike 1 or 2). In order to overcome this problem, the time intervals between 2 consecutive EMG spikes (measured between peaks) was correlated with the time intervals between 2 consecutive sound spikes or pulses (also measured between peaks) with a time lag0 and a time lag1 (i.e. time intervals **a** were correlated with time intervals **b** (lag0) and **c** (lag1); see Figure 3.29). The results of the Pearson correlation were: lag0 - N = 98, r = 0.783; lag1 - N = 66, r = 0.311. As these 2 correlation coefficients are significantly different (Z = 4.51, $P < 0.01$) (see Zar 1984), it is therefore concluded that an EMG spike causes the immediately succeeding sound spike (with a lag0 time interval).

The time lapse (measured peak to peak) between an EMG and a sound spike was on average 1.85 ms (N = 128; s.d. = 1.09; min = 0.13 and max = 6.25).

The number of spikes in an EMG was on average 6.94 (N = 50; s.d. = 1.58; min = 5 and max = 13) and the spike interval (also measured peak to peak) was on average 12.49 ms (N = 200; s.d. = 1.49; min = 6.75 and max = 17.63), which falls in the same range of the number of pulses and pulse peak to peak interval measured for grunts produced by the medium grey gurnards (Table 3.6; see also Tables 2.4 and 2.10 and Fig. 2.20).

Table 3.6 - Descriptive statistics for the number of pulses and the pulse peak to peak interval of grunts produced by medium grey gurnads.

Grunts - medium fish	N	mean	s.d.	min	max
No. of pulses	413	5.92	1.92	2	19
Pulse interval (ms)	413	10.64	1.84	7.30	18.60

3.3.2.2 - Synchrony of muscle contraction

Time intervals (measured from peak to peak) between the right and left EMG spikes were measured to ascertain the synchrony of the sonic muscles' contraction. The time interval (ms) between the right and the left EMG spikes was on average 0.12 ms (N = 100; s.d. = 0.16; min = 0.00 and max = 0.63), which falls into the recording and analysis error. Therefore it can be concluded that the 2 sonic muscles contract synchronously.

3.4 - DISCUSSION

3.4.1 - The sonic apparatus

Diversity amongst different species

In triglids, the swimbladder and its associated musculature have long been known as a sound-producing mechanism (Evans 1973). In the present work, a great diversity of size and shape of the swimbladder was observed in the species of triglids studied. The size and shape of the sonic apparatus have been reported to affect the qualities of the sounds emitted (e.g. Fine *et al.* 1977b, Bass & Baker 1991, Brantley & Bass 1994, also see section 3.1). Also, both extrinsic and intrinsic sonic muscles were found, although the piper was the only species that possessed extrinsic muscles. Rauther (1945) studied the development of the sonic muscles in Triglidae. He investigated the ontogeny of the swimbladder and its intrinsic muscles in various species, relating it to the structure found in other fish. He stated that in larval stages of the European triglids, the external intracostal muscles are attached by tendons running between the skull and the cleithrum in the pectoral region, lying loosely against the swimbladder. In the course of the post-embryonic development these muscles become intimately joined to the swimbladder, assuming their differentiated adult form when the body length is over 4 cm. The piper possesses probably the more primitive form of sonic muscles, since in the adults they are attached to the cleithrum, as found in larval stages. When comparing the body, total swimbladder (including the sonic muscles) (SB) and sonic muscles (SM) weights of other triglids with those of the grey gurnard, few differences were found. Only the North Sea red gurnard showed greater SB and SM weight at a given length than the grey gurnard.

In general, a great diversity of size and shape of the sonic apparatus can be found amongst different species of triglids. In American triglids (sea robins) of the genus *Bellator*, the swimbladder consists of two elongated sacs united by a narrow duct and with a diaphragm present in one of them. In contrast to the European gurnards, a pair of intrinsic sonic muscles and a pair of extrinsic sonic muscles are associated with the swimbladder (Evans 1973). Triglids of the genus *Prionotus* have similar swimbladders and musculature to *Bellator* although some species do not possess extrinsic sonic muscles (Tower 1908, Dufossé 1874, Fish 1954, Evans 1973, Ross 1980). In the Japanese gurnard, *C. kumu*, the swimbladder shows great variability in size and shape - it is a large elongated thick-walled sac with well-developed lateral diverticula. These horn-like lateral diverticula are not found in the

European and American species (though they are paralleled in the tub gurnard) and it has been proposed that they act as resonators during sound production (Bayoumi 1970).

Ontogenetic development

In all species studied there were significant positive relationships between both total swimbladder weight (SB) and the sonic muscles' weight (SM), and fish total length (TL). The SM/SB weight ratio had a significant negative relationship with TL in the grey, the red (North Sea) and the large scaled gurnards, which means that for these species, the sonic muscles did not grow proportionally to swimbladder. In the streaked, the red (Mediterranean) and the tub gurnards, however, the sonic muscles grew in proportion to the swimbladder's growth. It was interesting to verify that individuals belonging to the same species (the red gurnard), but coming from distinct geographical areas (the North Sea and the Mediterranean), showed different growth patterns of the sonic apparatus. The swimbladder and the sonic muscles were significantly lighter in the Mediterranean red gurnards when comparing with specimens from the North Sea with similar TLs and body weight. Also, the growth rate of the sonic muscles in relation to the whole swimbladder differed between red gurnards from the 2 different populations. These differences in the growth of the sonic apparatus may be related to differences in water temperature or other abiotic factors, to differences of the role of sound production in the social behaviour of these 2 populations, or perhaps to genetic differences.

In contrast with the present results, the swimbladder of the leopard sea robin, *Prionotus scitulus*, may be longer in smaller fish than in larger ones, since the swimbladder length increases linearly with fish length until approximately 8.5 - 9.0 cm (standard length), after which fish length only explains 1% of the variation in swimbladder length (the swimbladder length was significantly correlated with weight) (Ross 1980). In most other fish species, such as the oyster toadfish or the haddock, the swimbladder and the sonic muscles grow throughout life (e.g. Templeman & Hodder 1958, Fine 1975).

In sciaenids, in contrast with triglids and the oyster toadfish in which the sonic muscles originate in the embryological stages, the sonic muscles develop as a secondary sexual character; the development of the sonic muscles is concurrent with gonadal development although it may occur long before complete gonadal maturation (Hill *et al.* 1987).

Templeman & Hodder (1958) and Odense *et al.* (1978) studied in detail the ontogenetic changes in morphology of the sonic muscles of haddock. In this species, the increase in size of the sonic muscles with maturity is due to an increase in size of the muscle fibres. Similarly, Fine (1989) stated that the number and diameter of fibres increase throughout life, and further suggested that fibre splitting could provide a possible mode of fibre addition.

Sexual dimorphism

No evidence of sexual dimorphism of the sonic apparatus was found for either the grey or the red gurnards. This suggests that both genders are equally active sound producers in these 2 species. Ross (1980) found a weak sexual dimorphism in the swimbladder size of the leopard sea robin. In many other species of fish, however, it is common to find that only males produce sounds (e.g. gobies (Tavolga 1958a, Torricelli *et al.* 1990a, b), most sciaenid species (Hill *et al.* 1987)) or that males are much more active sound producers than females (e.g. gadoids (Hawkins & Rasmussen 1978), batrachoidids (Fine 1975)). This sexual dimorphism in sound production activity usually reflects the existence of a sexually dimorphic sound -

producing apparatus (e.g. Templeman & Hodder (1958), Hill *et al.* (1987), Brantley & Bass 1994). At one extreme, the plainfin midshipman fish is found to be sexually polymorphic, with 2 different reproductive morphotype males, distinguishable by somatic (including the sonic muscles) and neurobiological features which are paralleled by their different reproductive tactics and the characteristics of their acoustic signals (e.g. Brantley & Bass 1994).

Fine & Pennypacker (1986) demonstrated that sexually dimorphic sonic muscle growth in the oyster toadfish is controlled primarily by androgenic steroids, consistent with the findings of Hill *et al.* (1987) for sciaenid fish and of Bass *et al.* (1990) for the plainfin midshipman.

Sexual dimorphism has not only been found in the size of the sonic muscles, but also at a finer morphological level. Indeed, the larger sonic muscles of male oyster toadfish have a greater number of smaller fibres (caused by hypertrophy) than those of females (Fine *et al.* 1995). The smaller fibres in males have been interpreted as an adaptation for speed and fatigue resistance (Fine & Pennypacker 1986, Fine *et al.* 1993)

Seasonal changes

Although there was no evident seasonal trend in the sonic apparatus weight, the positive correlations found with GSI (females) and maximum oocyte diameter suggest that there may be an increase in the females' swimbladder and sonic muscle mass associated with reproduction. In contrast, Bayoumi (1970) and Ross (1980) noted an inverse relationship between swimbladder size and gonadal development for, respectively, the Japanese gurnard and the leopard sea robin. The increase of the swimbladder and sonic muscle weight with spawning readiness in the grey gurnard may mean that sound production, as in other fish species, could play a fundamental role in courtship and spawning of the grey and perhaps other gurnards. Indeed, it is during the reproductive period (summer period) that the acoustic activity of triglids is especially evident (Protasov 1965). Shiskhova (1963 in Protasov 1965) suggested that the increase in sound production of gurnards during the summer in the Black Sea is associated with spawning. Likewise, the staccato calls of the sea robins are thought to characterize the breeding season of these fish (Moulton 1958a).

Sound production varies seasonally in several fish. Breder (1968) observed that the catfish and the toadfish were more active sound producers during the spring and summer months than throughout the rest of the year, temperature being one of the major controlling factors. Generally, seasonal trends in sound production occur due to the increase in acoustic emissions associated with reproductive activity of numerous fish. Here, sounds usually accompany complex visual displays and have an important role in the successful accomplishment of courtship, culminating in spawning (e.g. Fine *et al.* 1977b, Myrberg 1981, Hawkins & Myrberg 1983, Torricelli *et al.* 1986, Bass & Andersen 1991). For example, acoustic emissions produced by male toadfish and male gobies may attract gravid females (Tavolga 1956, Gray & Winn 1961). In male serranids, acoustic emissions may announce spawning readiness and help coordinate gamete release (Lobel 1992). In addition, sound production may be used in nest guarding (Gray & Winn 1961) or agonistic interactions amongst males (Hawkins *et al.* 1967).

The role of male sound production in courtship and spawning of fish is often reflected in the existence of sexual dimorphism in the sonic apparatus. The increased role of sound production during the spawning season is correlated with the increase in the sonic muscle size in the male haddock (Templeman & Hodder 1958). In the male weakfish (Sciaenidae), the sonic muscles' mass increases roughly three-fold during the spawning season, to which sound production is restricted. These seasonal changes in the mass of the sonic muscles are related to variations in androgen levels (Connaughton & Taylor 1994, 1995a).

It would be interesting to investigate whether an increase circulating androgens would affect the sonic muscles' mass and structure as well as acoustic activity, in the grey and other gurnards.

3.4.2 - Neuromuscular mechanisms of sound production

All EMG signals obtained from the experimental fish were very clear but their sounds were weak. It is not clear why the operated fish gave poor sound signals to noise ratio but the insertion of the electrodes into the fish's body may have somehow affected the capability of the swimbladder to vibrate. The sounds recorded from the other non-experimental fish in the tank showed a very good signal to noise ratio (i.e. the problem did not arise from the sound recording equipment).

Only Hawkins (1968) has previously studied the pattern of contraction of the sonic muscles of the grey gurnard during sound emission, in response to single or repetitive stimulation. He reported that the sonic muscles' cycle of contraction was extremely brief, the maximum tension developing within 6.0 ms, and the full cycle being completed within 13.0 ms.

In the present work, a 1:1 relation between muscle contraction and sound production was found in naturally produced grunts. Consistently, Hawkins (1968) observed that in the grey gurnard, a single twitch of the muscle was associated with a single pulse in a knock sound, although he did not prove that a similar 1:1 relation was found in longer sounds. Indeed, the fundamental frequency of the sound of other fish species is known to be a direct translation of the muscle contraction, with a 1:1 relation between each muscle contraction and each sound pulse (e.g. the sculpin *Myoxocephalus octodecimspinosus* (Barber & Mowbray 1956), the pigfish *Congiopodus leucopaecilus* (Packard 1960), the catfish *Bagre marinus* (Tavolga 1962), the squirrelfish (Winn & Marshall 1963), the tigerfish *Therapon jarbua* (Schneider 1967), the plain midshipman, the oyster toadfish, the longhorn sculpin *Myoxocephalus scorpius*, the Pacific staghorn sculpin *Leptocottus armatus*, and the northern sea robin (Bass & Baker 1991)).

Packard (1960) and Skoglund (1961) observed a delay of approximately 1.5 ms between the incoming motor nerve volley and the arise of the sonic muscle action potential. In addition, an average time interval of 0.5 s was found between the peak of the action potential and the onset of the sonic muscle contraction (Skoglund 1961). Consistent with the results in the present work, Skoglund (1961) reported a time lag of approximately 1.6 ms between the spike peaks of the EMG and sound signals (experiment carried out at 21°C, see Fig. 4E in Skoglund 1961).

The sonic muscles of the grey gurnard contracted synchronously. Synchrony of muscle contraction is determined by the synchronous firing of pacemaker neurons, which regulate the discharge rate of the motoneurons which, in turn, innervate the sonic muscles (Bass & Baker 1991). Highly synchronous contractions of the sonic muscles have been documented for several species of fish (e.g. Packard 1960, Skoglund 1961, Cohen & Winn 1967, Kastberger 1981a, b, Bennett *et al.* 1985, Bass & Baker 1991). In the sea robins, however, the sonic muscles contract asynchronously, creating double pulsed structure in their staccato calls (Bass & Baker 1991).

CHAPTER 4

BEHAVIOURAL CONTEXT OF SOUND PRODUCTION IN GURNARDS

4 - BEHAVIOURAL CONTEXT OF SOUND PRODUCTION IN GURNARDS

4.1 - INTRODUCTION

Acoustic signalling is known to play an important role in social contexts in fish, that is, in interactions between individuals. However, very few studies have dealt with the correlation of sound production with specific behavioural categories. Only a small number of laboratory and field studies have shown that sound production in fish may have a communicative function and contribute to both survival and reproductive success (see Hawkins & Myrberg (1983) and Bass (1990) for a review).

Most studies of sound production have dealt with the importance of conspecific sounds during territorial defence (e.g. Gray & Winn 1961, Winn *et al.* 1964, Ballantyne & Colgan 1978a, Myrberg & Riggio 1985, Miyagawa & Takemura 1986, Ladich 1989, Bass 1990, Torricelli *et al.* 1990a) and reproductive interactions (e.g. Tavolga 1956, 1958a, b, Gray & Winn 1961, Gerald 1971, Ballantyne & Colgan 1978a, Myrberg *et al.* 1978, Bass 1990, Torricelli *et al.* 1990a, Lobel 1992), but very few studies have investigated the significance of acoustic emissions in agonistic contexts, other than in territorial defence (e.g. Ladich 1988, Hawkins 1993). One example is the female haddock that, although silent during courtship, utters sounds outside the breeding season during competitive feeding interactions (Hawkins 1993).

Among triglids, only very general descriptions of the behavioural context in which sound occurs are given in the literature (see Table 2.2). The sounds of triglids are produced mainly in aggressive and alarm contexts (Table 2.2), but the relationship between calls produced and fish behaviour has not been quantified or described in detail. In general, calls are emitted simultaneously with visual displays, such as extension of pectorals (which are sometimes brightly coloured), and erection of the dorsal fins (Hawkins 1968).

This Chapter aims at studying the significance of sound production in triglids, with particular regard to the grey gurnard. The competitive feeding interactions of the grey gurnard (*Eutrigla gurnardus*), the streaked gurnard (*Trigloporus lastoviza*) and the tub gurnard (*Trigla lucerna*), are characterised and comparisons are made between species. In addition, the different sound types studied in Chapter 2, uttered by the grey and the streaked gurnards, are associated with the different behavioural categories observed during competitive feeding interactions. For the grey gurnard, the correlation of sounds with competitive feeding behavioural categories is achieved for 3 different size classes, which are then compared. Furthermore, experiments with sound playback were also carried out with grey gurnards, in an attempt to further ascertain the significance of knocks and grunts in an agonistic context, and to investigate which of the sound features are relevant in terms of acoustic communication.

4.2 - METHODS

4.2.1 - Preliminary behavioural observations

The behavioural repertoires of the grey, streaked and tub gurnards were scored by means of direct *ad libitum* observations (i.e. with no systematic constraints) (Martin & Bateson 1993), outside, or during, feeding bouts, while fish sounds were recorded to describe the species' acoustical repertoire (Ch. 2). These preliminary observation periods spanned over a year for the grey gurnard, and 3 and 2 months for, respectively, the streaked and the tub gurnards. The behavioural categories described for all species are as follows:

- *Swim*: swimming in the water column or on the bottom
- *Search*: The fish moves slowly on the bottom exploring the substrate with the free pectoral fin rays
- *Wait*: stay underneath or swim around the opening of the feeding tube, waiting for food to fall
- *Dash*: swim rapidly towards the food items and suddenly stopping just before reaching them, without attempting to *grasp* any. This behaviour is observed when there are other fish simultaneously rushing towards the food items or already grasping a food item
- *Circle*: circling the feeding area without attempting to *grasp* any food item, usually because of the presence of conspecifics in the vicinity or because the subject fish is *feeding*
- *Grasp*: catching a food item
- *Feed*: handling and ingesting a food item with noticeable gill movements.
- *Orient*: turning the head towards a conspecific
- *Approach*: swimming or moving towards a conspecific, decreasing the distance between the performer and the recipient
- *Chase*: swimming rapidly towards a conspecific. The behaviour *chase* is distinguishable from the behaviour *approach*, because in the former the recipient is *fleeing* while in the latter it is not
- *Frontal display*: spreading out the paired and the dorsal fins with the head directed towards the opponent's head
- *Touch*: body contact between the performer and the recipient fish. In the streaked gurnard, where this behaviour was very frequent, the performer most commonly *touched* the recipient with its free rays, but sometimes also with its snout or with its body
- *Bite*: opening and closing the jaws on the opponent's body
- *Flee*: swimming rapidly away from a conspecific

4.2.2 - Video analysis

The characterisation of competitive feeding interactions and the association between sound production and the fish behaviour, was carried out by means of video analysis.

4.2.2.1 - Video recording

The grey gurnard - *Eutrigla gurnardus*

The context of sound production was studied in small (10≤TL<15 cm), medium (15≤TL<20 cm) and extra-large (30≤TL≤40 cm) grey gurnards, in groups of respectively, 6, 4 and 8 individuals. Small and medium-sized grey gurnards were maintained respectively in 1.5 m and 3 m fibreglass; extra-large fish were kept in a swimming-pool (3.5 m width × 7.0 m length × 1.5 m depth), as described in section 2.2.1.

Video recordings were taken with a Sony video 8 camcorder (CCD-FX500E Pal 8) that was placed above the tank in order to record all the events in the feeding area (the area where the food was dropped and the fish came to eat) (see Fig. 2.1). The camcorder was also connected to the sound recording system described in section 2.2.4, so that the images could be synchronised with sound. For the small fish, as the experimental tank was small, the video covered the full area of the tank. Because the tanks containing the medium and extra-large grey gurnards were larger, the video only covered part of the tank, which was either the feeding area or a larger area including it. The area of the tank covered and image resolution were optimised, so that the larger area could be seen without losing details of the fish behaviour.

10 sessions of video recording lasting approximately 15 min each were obtained for each grey gurnard size class. Only 9 out of the 10 sessions

recorded with extra-large fish were analysed since in the 10th session the fish appeared to be disturbed.

For both small and medium grey gurnards, food was given through feeding tubes that were placed at one edge of the tank. Extra-large grey gurnards were fed by throwing the food items in the water, always at the same spot. All fish were fed (and recorded) 3 times a week. Food was dropped every minute throughout the filming session (from minute 0 to minute 14).

The streaked gurnard - *Trigloporus lastoviza*

A group of 8 streaked gurnards (7 in the last 2 filming sessions), ranging in length from 10 to 15 cm total length, was studied. Fish were maintained in a 1.5 m fibreglass tank, as described in section 2.2.1.

Synchronised image / sound video recordings were obtained as described above for the grey gurnard, the image recorded covering practically all the tank area.

10 sessions of video recording lasting approximately 20 min each were obtained for the streaked gurnard on consecutive days. Only 8 out of the 10 sessions recorded were analysed because in the first 2 sessions the fish seemed still to be getting accustomed to the experimental procedure. Also, the 8 sessions analysed provided an adequate data sample size for statistical analysis.

Food was dropped at the water surface, always in the same area, every 2 minutes throughout the filming session (from minute 0 to minute 19). Because video recordings and hence feeding were performed on consecutive days (due to the short stay in the Institute of Marine Biology in Crete, Greece, where the study took place), food was given more sparingly than for the grey and the tub gurnards, in order to prevent the fish becoming satiated in the course of the recording.

The tub gurnard - *Trigla lucerna*

Feeding interactions were studied in a group of four 20 to 30 cm (total length) individuals, kept in a concrete tank with a glass front (0.82 m width × 1.50 m length × 0.60 m water depth), as described in Chapter 2.2.1.

Video recordings were obtained as described above for the grey gurnard, with the video 8 camcorder placed in front of the glass front of the tank, covering the full area of the tank. Sound production was not recorded simultaneously because sound production only occurred for a very restricted period of the study and it was not possible to connect the sound recording equipment to the video recorder for practical reasons.

Ten sessions of video recording lasting from 10 to 20 min each were obtained. The duration of recording varied so much because fish behaviour (e.g. number of fish interactions per recording session) was very variable.

Food was given every minute throughout the filming session. Fish were fed (and recorded) 3 times a week.

4.2.2.2 - Recording feeding interactions

Fish feeding interactions were studied in the grey, the streaked and the tub gurnards. For the grey gurnard, the following behavioural categories were considered: *dash*, *circle*, *grasp + feed*, *orient*, *approach*, *chase*, *frontal display* and *flee*. The behavioural category *grasp + feed* is here redefined, because the behavioural category *feed*, as defined in section 4.2.1, was difficult to measure in small grey gurnards. As far as fish feeding interactions are concerned, *grasp + feed* includes, apart from the action of *grasping* a food item, quite exaggerated gill movements associated with handling a food item plus the act of spitting out food and *grasping* it again. The use of this new behavioural category allowed comparisons between different sizes of grey gurnards as well as between species. All the behavioural categories observed for the streaked and the tub gurnards were considered: streaked gurnard - *swim*, *search*, *dash*, *circle*, *grasp*, *feed*, *orient*, *approach*, *chase*, *frontal display*, *touch* and *flee*; tub gurnard - *circle*, *grasp*, *orient*, *approach*, *frontal display* and *flee*.

Feeding interactions were characterised by registering the succession of behavioural categories for each individual fish participating in the interaction. For the streaked gurnard the emission of growls was also noted, specifying which behavioural categories were accompanied by sound emission in order to correlate behaviour with sound production. For the grey gurnard, only the presence or absence of knock and grunt production in an interaction was registered. For all species, the number of fish participating in an interaction was scored, as well as the time when the interaction occurred (i.e. if it occurred in the first, second, etc minute of the video session). Interaction duration was also scored. A total of 6, 6 and 9 video sessions were analysed, for respectively, the small, medium and extra-large grey gurnards. Eight and 10 video sessions were analysed, for respectively, the streaked and the tub gurnards.

The following variables were quantified for each species, and the different size classes in the grey gurnard:

1 - **Proportion of each behavioural category in interactions**

2 - **No. of behavioural categories per interaction** (per fish)

3 - **Frequency of behaviour category x per interaction** (per fish); i.e. the total number of times a particular behaviour occurred in all

interactions observed in a video session divided by the number of interactions for that video session. The number of interactions is considered per fish, i.e. if there is an interaction with 3 fish involved and the sequence of behavioural categories are registered for each fish, then 3 interactions are counted

4 - **No. of fish involved in an interaction**

5 - **No. of interactions / min** (here, if 3 fish are involved in a interaction only one interaction is counted, irrespectively of the number of fish involved)

6 - **Interaction duration** (s)

7 - **% of interactions accompanied by sound production** (if 3 fish are involved in an interaction 3 interactions are counted)

In addition, the **sequences of behaviour (8)** during feeding interactions were also characterised for each species and for each size class of the grey gurnard. The data, as previously mentioned, were represented as sequences of behavioural acts. By definition, only interactions involving more than 1 behavioural category were considered. It is assumed that a behavioural act cannot follow itself. Whether successive acts can be the same or not is an important matter because it affects the statistical procedure used, since transitions between identical acts will dominate the analysis and hide more interesting associations (between different acts) (see Slater 1973, Fagen & Young 1978, Bakeman & Gottman 1986).

There are 2 common approaches when studying sequential analysis of observational data. The first is descriptive, where transitional probabilities are computed, and the second is modelling, where it is determined if these transitional probabilities fit, in the present case, a first-order Markov chain model (Slater 1973, Bakeman & Gottman 1986). The transitional probability is the probability with which a particular behavioural event occurred immediately after another given event. If all data are represented in a contigency table where each cell indicates the number of times a particular transition occurred, and columns refer to the preceeding acts and rows to the following acts, then the transitional probability is the frequency of a particular cell divided by the frequency for that row (Bakeman & Gottman 1986). A first-order Markov chain model assumes that behavioural acts are mutually exclusive and exhaustive as well as stationary in time, and tests for dependence (on the previous act) in the observations; i.e. it compares observed frequencies with those expected if the observations were independent (Fagen & Young 1978).

4.2.2.3 - Associating behaviour with sound production

The grey gurnard - ***Eutrigla gurnardus***

All behavioural categories observed for the grey gurnard were scored during video analysis: *swim*, *search*, *wait*, *dash*, *circle*, *grasp*, *feed*, *orient*, *approach*, *chase*, *frontal display*, *bite* and *flee*. *Wait* was not considered for the extra-large gurnards since this behaviour was related to the presence of the feeding tube. In addition, *feed* was not considered for the small fish because quite often it was difficult to distinguish whether a fish was *feeding*, due to its small size in relation to the area filmed. All video sessions obtained for each size class were analysed.

Two main problems arose when planning the video analysis procedure for the grey gurnard. The first was that, as grey gurnards do not perform any particular body movement when emitting sounds, it was difficult to attribute sound production to a particular individual. The second was that, for the medium and extra-large fish, focal observations could not be carried out, since a particular fish could not be seen at all times. Taking into consideration these 2 restrictive factors, fish behaviour was analysed with a one-zero sampling (time sampling) recording technique (see Martin & Bateson 1993). Each video session was divided into 5 second intervals, and at the end of each sample interval it was noted whether each behavioural category had occurred. In addition, the number of fish involved per interaction and the number of grunt and knock calls were also registered for each 5 second interval. The size of the sample interval, 5 second, was chosen, in order to optimise the accuracy of the time-sample record and to compromise as little as possible the reliability of recording several categories of behaviour at once (see Martin & Bateson 1993).

The streaked gurnard - *Trigloporus lastoviza*

All behavioural categories observed for the streaked gurnards were scored during video analysis: *swim*, *search*, *dash*, *circle*, *grasp*, *feed*, *orient*, *approach*, *chase*, *frontal display*, *touch* and *flee*.

A different video analysis methodology was chosen for the streaked gurnards since it did not present the same problems found during the video analysis of the grey gurnard. As practically all the tank was included in the filming, and the video camera was tilted to view any feeding interaction, it was assumed that all fish could be observed at all times. Although the streaked gurnards did not perform any specific body movement when uttering sounds, it was easy to identify the sound-producing fish because, as previously mentioned, these fish acted very slowly and did not charge in groups towards a food item uttering sounds as grey gurnards did. Furthermore, identification of the sound-producing individual was facilitated because sound production was highly associated with the behaviour *grasp* (see section 4.3.2.2), sound production rate was low and only one sound type was involved.

The association of sound production with the behavioural context was accomplished by counting, for each video session, the number of times each of the behavioural categories was accompanied by sound emission, after characterising interactions occurring during competitive feeding (see section 4.2.2.2). The behavioural categories which accompanied sound emissions outside fish feeding interactions were also registered. In addition, because the behaviour *grasp* was clearly highly associated with sound production, all occurrences of this behaviour outside feeding interactions were noted down, also registering whether each occurrence was accompanied by sound emission.

4.2.3 - Sound playback - experimental design

Grey gurnards produced mainly 2 types of sounds during interactions: knocks and grunts (see section 2.3.1.2). Video behavioural analysis has suggested that knocks are produced during low levels of aggression or during feeding arousal, and grunts accompany higher levels of aggression. In order to find out whether these 2 sound types have different communicative values, 3 experiments were planned.

Two groups each of 4 grey gurnards ($18 \leq TL < 23$ cm), were tagged with different coloured Floy tags after being anaesthetised in a 0.1 g l^{-1} sea water solution of MS 222 (Sandoz), to enable individual identification. In each group, one individual was left untagged to check for tag effect. Each group was maintained in a round 2 m diameter fibreglass tank, with flowing sea water and a sandy bottom and left to acclimatise to laboratory conditions for a minimum of 15 days. Aeration was also provided but was stopped during trials.

Typical sounds were selected for the recordings based on clarity and structure of the sounds, as well as having a high signal / noise ratio. Because there can be uncontrolled factors which may cause differences in the fish response to different sound types when a single exemplar is used (McGregor *et al.* 1992), 15 exemplars of both knocks and grunts were used in the playback recordings to assure that the results obtained were appropriate for testing the hypothesis formulated. The sound playback aimed at presenting the fish with a series of natural sound bursts. Therefore, the call features (the interval between calls and between phrases within a call, as well as the number of phrases per call) and the phrase features (number of pulses, pulse repetition rate, etc.) used in the playback presentations, represented typical values of the grey gurnard's calls / phrases (see section 2.3.1.2) (Table 4.1). Sound level of playback was also adjusted to resemble the natural sound emission level of the fish. Edited calls were recorded with a digital audiotape recorder (DAT) (Casio, model DA-1) connected to an amplifier (Deritron Electronics Ltd., 25W), and played back with an underwater sound projector (USRD type J9 Audio-frequency transducer) placed in each tank. Some fish may respond vocally to sound playback (eg. Winn 1972). Any sound reactions to playback were detected with hydrophones (one in each tank) (see section 2.2.4), amplified and filtered with a low-noise amplifier (Brookdeal, model 450, see section 2.2.4), and recorded with a DAT (Casio, model DA-2).

Behaviour reactions to playback were recorded through direct observation. The behavioural categories checked for were: *dash*, *circle*, *grasp*, *orient*, *frontal display* and *flee*.

Table 4.1 - Features of the calls and phrases used in the sound playback presentations. The values shown are approximately the mean ± the standard deviation, except for the ones marked with *, that vary around the median. The characteristics of the sound playback presented to the fish attempted to simulate natural calls.

Call features	**Range of values**
no. of phrases in a call	2 - 6*
interval between calls (ms)	4 - 12*
grunt features	----
interval between grunts (ms)	50 - 250*
grunt - no. of pulses	4 - 8
grunt - pulse duration (ms)	4.5 - 5.5
pulse peak - peak interval (ms)	8.8 - 12.4
grunt - peak frequency (Hz)	400 - 700
Knock features	----
interval between knocks (ms)	100 - 300*
knock - no. of pulses	1 - 3
knock - pulse duration (ms)	4.9 - 6.5
interval between pulses (ms)	0.5 - 3.0
knock - peak frequency (Hz)	400 - 700

Experiment I - Playback of sounds during feeding bouts

Each trial started with a control period (I) which consisted of a 4 min silence observation period, followed by an 8 min playback session. The 8 min playback session consisted of 2 presentations of knock playbacks and 2 presentations of grunt playbacks (1 min each) alternated with 1 min silence control periods (control II) (Table 4.2). The 8 min playback sequence always began with periods of sound; the first presentation was alternately knock or grunt playbacks, to avoid habituation to the playback sequence (Table 4.2). Also, the first and second presentations of both knocks and grunts were different from each other. The experimental design included 2 control observation periods since behavioural data collected during silence control II could present a carry-over effect from the preceding sound playback presentation. Control I allowed behavioural measurements to be taken in a period when the fish were definitely outside the influence of the sound playback presentations. Food (chopped fish or squid; one piece per fish) was given through a feeding tube placed beside the loudspeaker, and was provided at the onset of each sound / silence period of the playback sequence. Food supply was included in the experimental design, since all sounds analysed were recorded during feeding bouts. Furthermore, because grey gurnards are more likely to produce sounds during feeding, possibly they are also more prone to respond to sounds, including played back ones during feeding.

A trial (control I + playback sequence) was carried out 3 days a week. A total of 8 trials were carried out for each fish group.

Fish behaviour was monitored through direct observations during sound playback and silence controls (I and II). During the silence control I presentation, 1 min focal observations were achieved for each of the 4 fish (see Table 4.2). Only 1 individual was monitored for each sound / silence

Table 4.2 - Experiment I design: each trial is divided into 2 observation periods, a 4 min silence period (control I), where each of the 4 fish (A, B, C and D) are observed for 1 min, and a 8 min playback sequence where 1 min presentations of sound and silence (control II) are carried out alternately. G1,2 and K1,2 represent respectively the first and the second of the grunt and the knock presentations. The order of the letters indicate the order in which the 4 fish were observed (1 + 1 min behavioural observations). The same procedure was carried out for the 2 experimental fish groups.

Trial day	Control I: silence				Sound and silence (control II) presentations			
					G1 + Sil	K1 + Sil	G2 + Sil	K2 + Sil
1	A	B	C	D	A	B	C	D
					K1 + Sil	G1 + Sil	K2 + Sil	G2 + Sil
2	B	C	D	A	C	B	A	D
					G1 + Sil	K1 + Sil	G2 + Sil	K2 + Sil
3	C	D	A	B	C	D	A	B
					K1 + Sil	G1 + Sil	K2 + Sil	G2 + Sil
4	D	A	B	C	A	D	C	B
					G1 + Sil	K1 + Sil	G2 + Sil	K2 + Sil
5	A	B	C	D	A	B	C	D
					K1 + Sil	G1 + Sil	K2 + Sil	G2 + Sil
6	B	C	D	A	C	B	A	D
					G1 + Sil	K1 + Sil	G2 + Sil	K2 + Sil
7	C	D	A	B	C	D	A	B
					K1 + Sil	G1 + Sil	K2 + Sil	G2 + Sil
8	D	A	B	C	A	D	C	B

presentation of the playback sequence (1 + 1 min); the order of observing the 4 different fish was pseudo-randomised, so that at the end of the experiment an equal number of observations was obtained for each individual (see Table 4.2). A sample size of 8 was obtained (since the effect of sound playback was monitored for 8 different individual fish in total) (see McGregor *et al.* 1992), with 4 replicates for the fish behavioural response to each sound type presentation, and 8 replicates for the behavioural monitoring during the experimental control I and control II. This experimental design allows group effect to be assessed by comparing the results obtained for the 2 groups.

Experiment II - Feeding bouts with no playback

This experiment was similar to experiment I (shown in Table 4.2), except that there were no sound playback presentations; only food was presented. Also, only 4 trials were conducted for both fish groups (3 a week).

Experiment III - Playback of sounds outside feeding bouts

The experimental procedure used in experiment III was also similar to the one used in experiment I (Table 4.2), except that the playback sequence was carried out outside feeding periods (i.e. food was not given during trials). Also, only the behavioural categories *orient* and *frontal display* were scored, as well as the frequency of sound emission. Four trials were conducted for both fish groups in consecutive days.

A minimum of 9 days rest was given to the fish between experiments.

Experiments II and III aimed at clarifying the effect of feeding and of sound playback on the fish behavioural data obtained in experiment I.

4.2.4 - Data analysis

4.2.4.1 - Feeding interactions

In the grey gurnard, fish size effect was tested for the variables: no. of behavioural categories per interaction (2), frequency of behaviour x per interaction (3) and number of interactions / min (5) (see section 4.2.2.2), with linear regression analysis. The median total length for each size class was considered. Data transformations were used as appropriate to fulfil the regression analysis assumptions (Zar 1984): $\sqrt{x}$ transformation for counts (variable 2). The arcsin$\sqrt{x}$ and the ln transformations were not used for, respectively, the variables 3 and 5, since they did not seem to improve the regression fit. Descriptive statistics are given for the number of fish involved in an interaction (4) for each fish size class. For the streaked and the tub gurnards, descriptive statistics are given for variables 2 - 6.

The previous variables (2 - 5) plus interaction duration (6) and the % of interactions accompanied by sound production (7) were compared between species. Differences between species were investigated with a Kruskal-Wallis non-parametric test. When the p-value of the Kruskal-Wallis was similar to the p-value of the parametric ANOVA, 95% confidence intervals were used as an *a posteriori* test (see section 2.3.1.3).

For each of the 3 gurnard species the variable 2 was correlated with variable 4 with a non-parametric Spearman correlation.

As previously mentioned (section 4.2.2.2), in order to test for temporal dependence of behavioral acts, observed frequencies are compared with those expected if the observations were independent. The method used for calculating expected frequencies in a test of sequential dependence is the same as that used for testing independence between rows and columns in a contigency table with a χ^2 test (Fagen & Young 1978). The data from the present work, however, did not follow the χ^2 assumptions (Siegel & Castellan 1988), since the expected values cannot be less than 1 and not more than 20% less than 5. Hence, a simulation analysis for tests of independence of contigency tables (programme ACTUS - Estabrook & Estabrook 1989) was preferred. The programme ACTUS creates 1000 simulated tables of observed values, with independent rows and columns and with equal expected values to the original observed values. Each of the simulated tables is compared with the original one, indicating possible significant differences between individual cells of the contigency table: if they are significantly too small or too large. This programme also calculates the χ^2 value for each simulated table and compares it to the χ^2 of the original table producing a level of significance for the χ^2 of the original table.

Regression analysis was used to test whether the behavioural acts observed for each species were stationary in time. $\sqrt{x}$ transformations were used.

The number of video sessions analysed for each species of gurnards, was chosen in order to provide a sufficiently large data set to study the sequential dependence of behavioural categories of their feeding interactions. According to Fagen & Young (1978), $10R^2$ behavioural acts provide an adequate sample size, whereas $5R^2$ behavioural acts is just on the border line for statistical analysis; R is the behavioural repertoire size. Seven behavioural categories were considered for the grey gurnard - *approach* and *chase* were pooled together (see section 4.2.2.2). Nine behavioural categories were considered for the streaked gurnard - *swim* was pooled with *search* and *approach* with *chase*. All 6 behavioural categories concerning the tub gurnard were used to test for temporal dependence of behavioral acts. As for the tub gurnard the sample size (number of acts observed in all interactions) was not large enough, and only transitional probabilities were calculated.

4.2.4.2 - Behavioural context of sound production

The grey gurnard - *Eutrigla gurnardus*

Generalised additive models (GAMs) were used to associate the behavioural categories scored during video analysis for each 5 second sample interval with the knock and grunt calls heard during the same time span for the different fish size classes.

GAMs are non-parametric generalisations of generalised linear models (see section 2.3.1.1), which provide a powerful tool for modelling variations in the response variable, since they can use smooth functions of any shape and find the one that best explains the data (Hastie & Tibshirani 1990). The explanatory variables included the behavioural categories, session, time, and the number of fish involved in an interaction. All behavioural categories were considered for data analysis except for *wait* and *bite*, since they were not frequent enough. The number of explanatory variables needed to be kept to a manageable size and therefore "similar" behavioural categories were pooled together. The behaviour *swim* was pooled with *search*, *circle* was pooled with *dash* and *approach* with *chase*, reducing the number of behavioural categories to 8. The variable session was considered as a factor in contrast with the others which were continuous variables. Non-linear effects for the variables time and number of fish were considered. Time was given in minutes and data were pooled at the minute level. Therefore, the occurrence of each behavioural category (scored for each 5 s interval) was summed for every minute. The number of fish present in an interaction and the number of knock and grunt calls uttered were also summed for every minute (note that in this case it was the actual frequency that was summed up and not the number of 5 s interval in which they occurred).

Because the response variables consisted of counts, a Poisson distribution was assumed (Crawley 1993) and model selection was based on a stepwise elimination procedure (Draper & Smith 1981). In the first stage of the analysis a full model is fitted containing all explanatory variables. Subsequently, several simpler models were constructed by dropping each term of the current model one at a time. In addition, all variables previously dropped at earlier steps, were re-introduced one at a time to the current model. The whole procedure was continued until the simplest model, that best described the variability of the response variables, was found. The whole procedure was repeated until no further term could either be dropped or added to the current model. This procedure provides a way of reducing one initial large set of variables into a smaller more meaningful one.

The streaked gurnard - *Trigloporus lastoviza*

A χ^2 statistic was used to test for dependence of behavioural categories and sound production.

4.2.4.3 - Sound playback

The effect of sound playback on the frequency of the behavioural categories performed and on the frequency of sound production was examined with a non-parametric Kruskal-Wallis test. Parametric 95% confidence intervals were considered as a valid *a posteriori* test when both the Kuskal-Wallis and the one-way ANOVA had equivalent results (see section 2.3.1.3).

4.3. - RESULTS

4.3.1 - The grey gurnard - *Eutrigla gurnardus*

4.3.1.1 - Feeding interactions

1 - Proportion of each behavioural category in interactions

The proportions of the different behavioural categories considered during the analysis of competitive feeding interactions of the grey gurnard are shown in Figure 4.1. The data are pooled for the small, medium and extra-large grey gurnards.

2 - Number of behavioural categories per interaction

The total number of behavioural categories observed per interaction is described for each of the studied grey gurnard size classes in Table 4.3.

Ontogenetic changes in this variable were checked for with a linear regression test. Data were transformed by $\sqrt{x}$ (see section 4.2.4.1). Because the number of fish involved in an interaction may influence the number of behavioural categories per interaction (for all fish sizes - Spearman correlation: $N = 698$, $Rho = 0.226$, $P < 0.001$), interactions with 2, 3 and 4 fish were discriminated and tested separately. Fish size did not have a significant effect on the number of behavioural categories in an interaction (2 fish: $N = 353$; 3 fish: $N = 243$; 4 fish: $N = 90$; $P > 0.05$).

3 - Frequency of behavioural category x per interaction

The frequencies of each behavioural category in interactions of competitive small, medium and extra-large grey gurnards are described in Table 4.4.

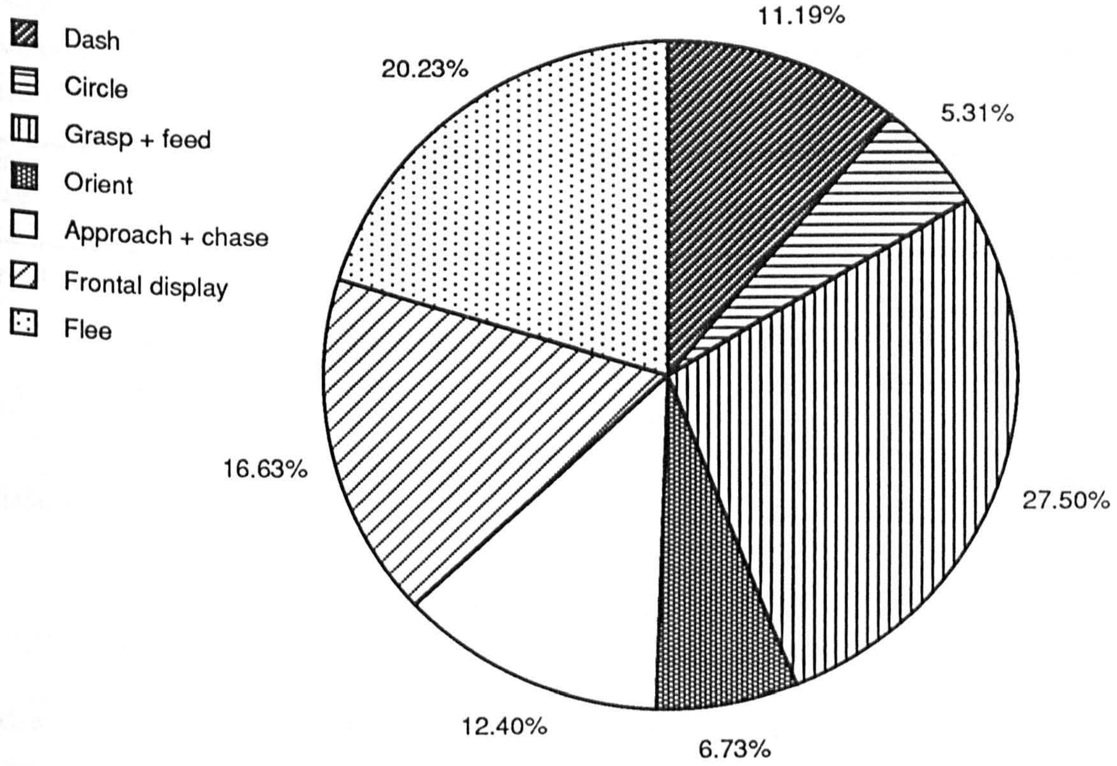


Figure 4.1 - Proportions of the different behavioural categories observed during competitive feeding interactions in the small, medium and extra-large grey gurnards. N = 2084.

Table 4.3 - Descriptive statistics for the number of behavioural categories observed per interaction for the small, the medium and the extra-large grey gurnards. N = number of interactions analysed.

Size class	N	mean	s.d.	min	max
Small	280	2.86	1.18	2	8
Medium	216	3.39	1.47	2	10
Extra-large	202	2.73	1.04	2	7

Table 4.4 - Descriptive statistics for the frequency of the behavioural categories observed for the small, the medium and the extra-large grey gurnards. The sample size are 6, 6, 9 for respectively the small, the medium and the extra-large grey gurnards.

Fish size		Dash	Circle	Grasp + Feed	Orient	Approach + chase	Frontal display	Flee
Small	mean	0.34	0.13	0.60	0.20	0.48	0.55	0.59
	s.d.	0.10	0.06	0.04	0.06	0.09	0.08	0.09
	min	0.18	0.07	0.56	0.12	0.37	0.46	0.47
	max	0.48	0.23	0.65	0.27	0.61	0.67	0.72
Medium	mean	0.41	0.21	1.17	0.20	0.23	0.50	0.67
	s.d.	0.13	0.11	0.18	0.06	0.12	0.11	0.18
	min	0.19	0.09	0.97	0.13	0.09	0.36	0.48
	max	0.59	0.39	1.42	0.29	0.42	0.66	0.97
Extra - large	mean	0.32	0.14	0.70	0.20	0.35	0.38	0.61
	s.d.	0.15	0.10	0.08	0.09	0.15	0.18	0.13
	min	0.13	0.00	0.60	0.08	0.14	0.14	0.46
	max	0.57	0.28	0.82	0.35	0.52	0.61	0.86

The effect of fish size was tested for the frequency of each behavioural category. Fish size did not seem to have a significant effect on any of the behavioural categories (regression test, $P > 0.05$) except for the behaviour *frontal display* ($N = 21$, $y = 0.637 - 0.008x$, $r = 0.477$, $P < 0.05$). However, this result was largely influenced by 3 very low data points from the extra-large gurnards (Fig. 4.2), and was no longer significant when these were removed.

4 - Number of fish involved in an interaction

The number of fish involved in feeding interactions was similar for each size class of the grey gurnard (Table 4.5).

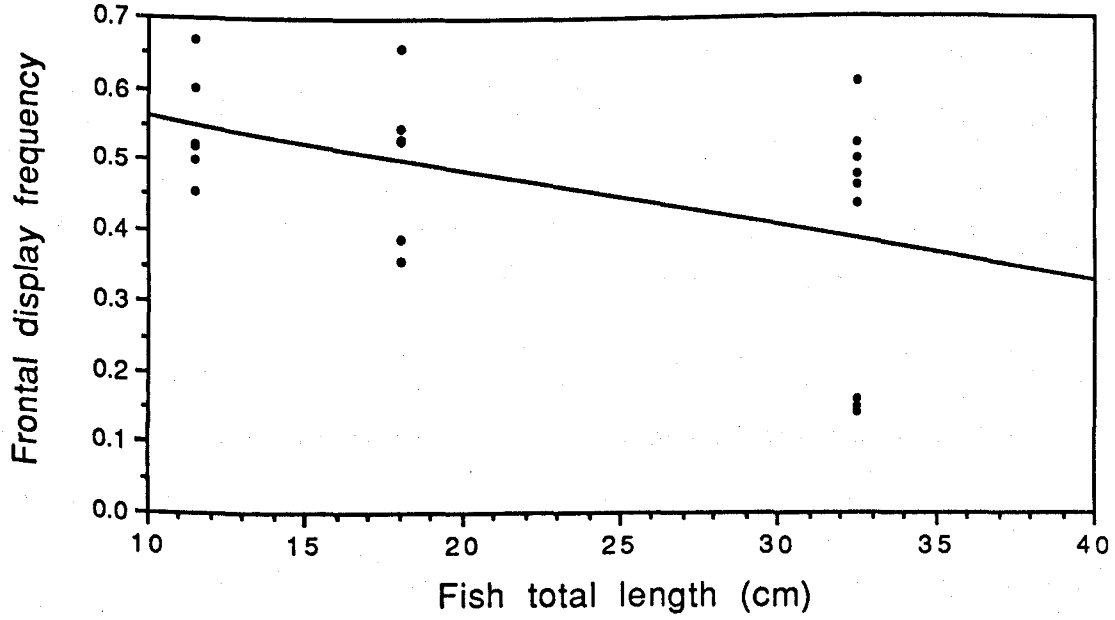


Figure 4.2 - Relationship between the frequency of behaviour *frontal display* (section 4.2.4.1) and grey gurnard total length. The regression equation fitted to the data is $y = 0.637 - 0.008x$. Sample size = 21. The regression coefficient $r = 0.477$; $P < 0.05$.

Table 4.5 - Number of fish participating in feeding interactions for the small, the medium and the extra-large size classes of the grey gurnard. N = number of interactions analysed.

Size class	N	mean	s.d.	min	max
Small	280	2.56	0.72	2	5
Medium	216	2.94	0.77	2	4
Extra-large	202	2.50	0.74	2	5

5 - Number of interactions / min

Interaction rate observed for the small, medium and extra-large grey gurnards is depicted in Figure 4.3. As shown by regression analysis, fish size has a significant negative effect on this variable ($N = 21$, $y = 3.64 - 0.062x$, $r = 0.588$, $P < 0.01$) (Fig. 4.4).

6 - Interaction duration

The duration (s) of 50 interactions were measured at random for the 3 grey gurnard size classes. The average interaction duration for this species was 4.16 s (s.d. = 1.98, min = 2, max = 10).

7 - % of interactions accompanied by sound production

The % of feeding interactions accompanied by grunt calls decreased with increasing fish size, whereas the % of those that occurred with knock calls showed an opposite trend (Fig. 4.5). Overall, the proportion of interactions accompanied by sound production was similar in all fish sizes (Fig. 4.5).

8 - Sequences of behaviour

The sequences of behavioural acts observed for the different sizes of grey gurnard are given in Table 4.6. As the significance of the behavioural transitions did not show a trend with fish size, the data for the 3 fish size classes were pooled together. Figure 4.6 depicts the behavioural sequences that were more frequent than expected (probabilities given by the programme ACTUS; see section 4.2.4.1), while Figure 4.7 shows the transitions that were significantly less frequent. Feeding interactions typically start with *dash, approach* or *grasp* and end up with *flee.*

Time did not have a significant effect on the number of behavioural acts in an interaction (data pooled for all fish and transformed by $\sqrt{x}$) (regression test, $P > 0.05$), therefore stationary of the behavioural acts can be assumed.

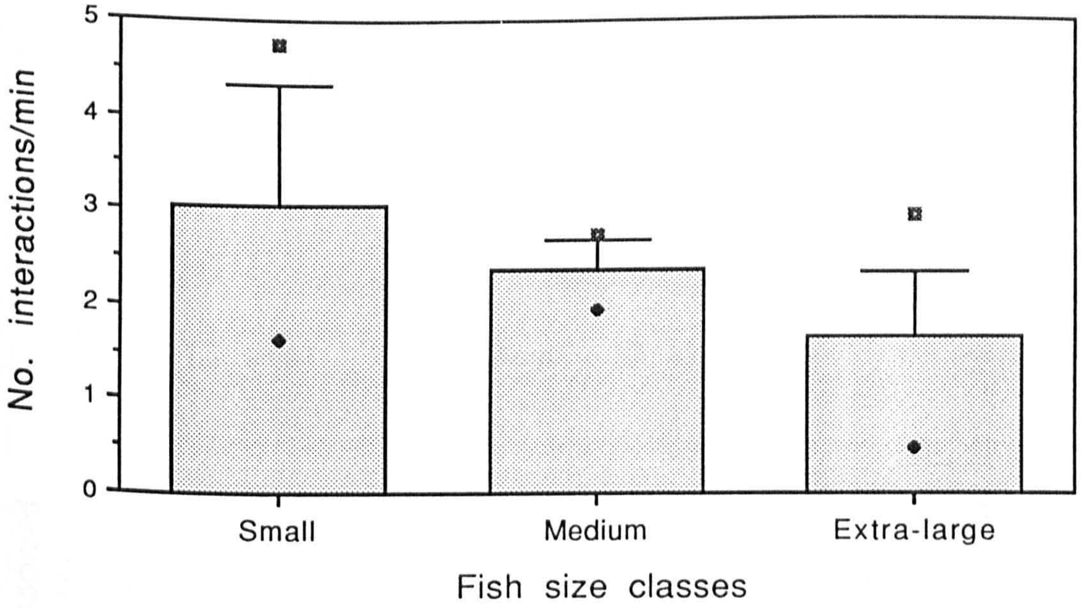


Figure 4.3 - Mean and standard deviation of the number of feeding interactions per minute for small, medium and extra-large grey gurnards. The lozenge and the square represent the minimum and the maximum values observed. The sample sizes are 6, 6 and 9 respectively for the small, medium and extra-large grey gurnards.

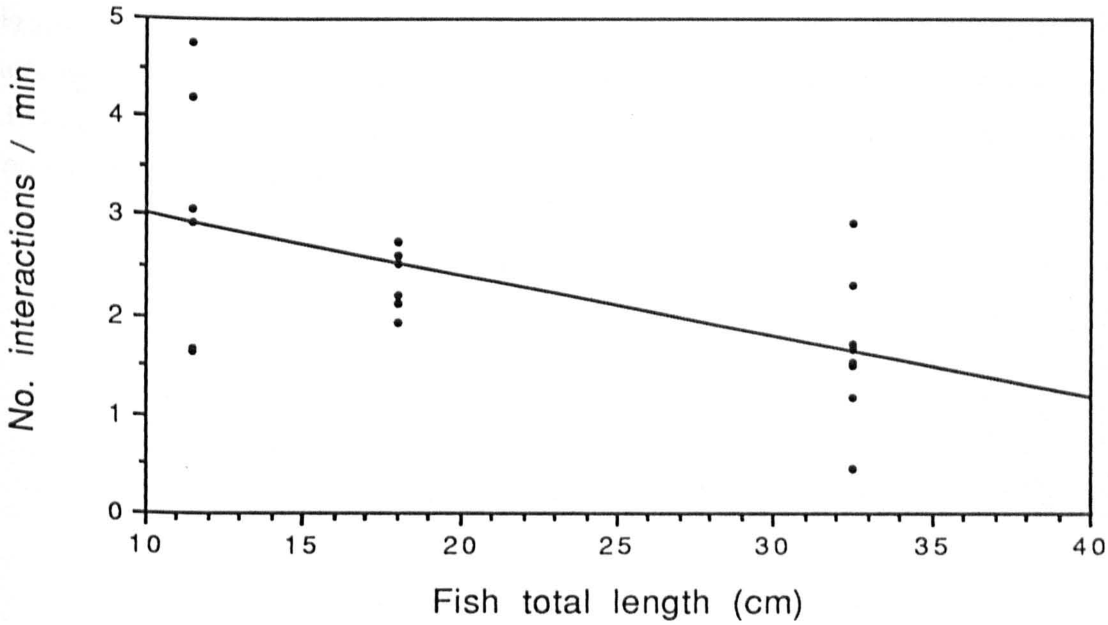


Figure 4.4 - Relationship between the number of interactions / min and grey gurnard total length. The regression equation fitted to the data is $y = 3.64 - 0.062x$. Sample size = 21. The regression coefficient $r = 0.588$; $P < 0.01$.

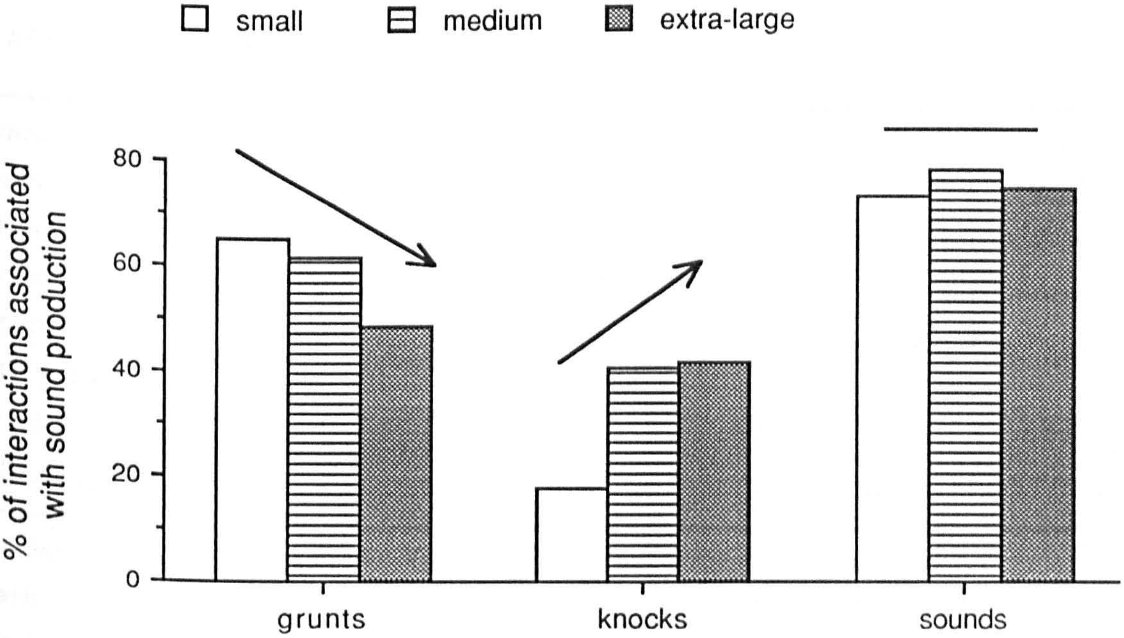


Figure 4.5 - Percentage of interactions accompanied with grunt calls, knock calls or with total sound production, for the small (N = 280), medium (N = 216) and extra-large (N = 202) grey gurnards. The arrows and the horizontal line show the trend with fish size.

Table 4.6 - Transition frequency matrices of the behavioural sequences observed during feeding interactions of the small, medium and extra-large (x-large) grey gurnards. Columns indicate the preceeding behavioural acts and rows the following behavioural acts. The number in the cells shows the number of times one act was immediately followed by another act. "Grasp +" = *grasp + feed*; "App + ch" = *approach + chase*; "Front d." = *frontal display*.

Small	Dash	Circle	Grasp +	Orient	App + ch	Front d.	Flee
Dash		5	6	5	7	56	8
Circle	3		6	4	4	4	2
Grasp +	1	2		12	2	14	99
Orient	3	2	8		7	2	17
App + ch	2	2	14	12		71	3
Front d.	0	1	28	10	42		23
Flee	0	3	9	4	4	6	

Medium	Dash	Circle	Grasp +	Orient	App + ch	Front d.	Flee
Dash		9	23	3	2	45	6
Circle	7		20	5	1	0	4
Grasp +	2	13		17	5	25	122
Orient	0	4	10		4	2	6
App + ch	0	0	12	2		25	1
Front d.	0	3	65	10	8		9
Flee	5	9	24	3	3	3	

X-large	Dash	Circle	Grasp +	Orient	App + ch	Front d.	Flee
Dash		3	7	3	3	17	24
Circle	3		7	5	1	1	10
Grasp +	2	3		10	5	11	60
Orient	0	0	7		9	0	4
App + ch	0	0	8	6		42	5
Front d.	0	1	28	4	15		11
Flee	0	1	17	6	1	6	

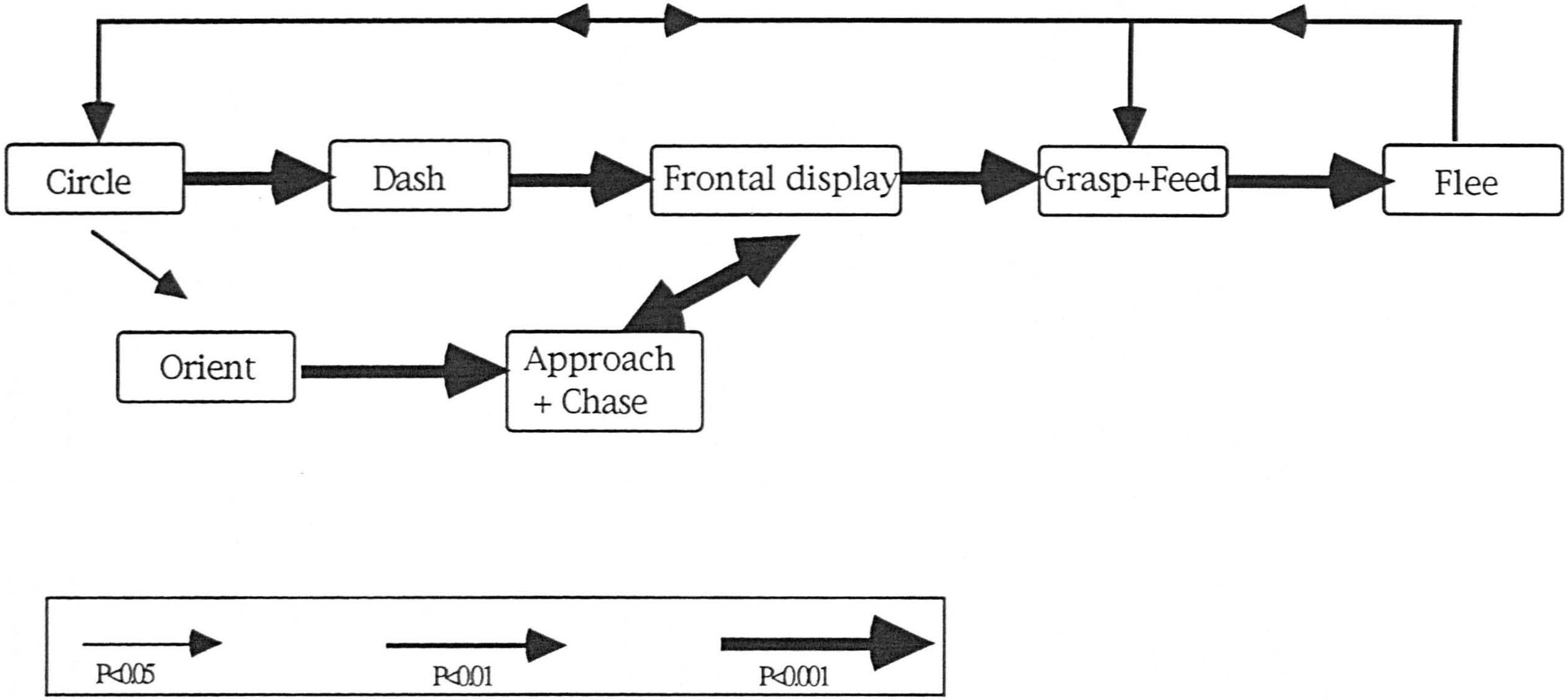


Figure 4.6 - Behavioural sequences observed during competitive feeding interactions of the grey gurnard occurring more frequently than expected by chance alone. Sequential dependence of behavioural acts was tested by comparing observed frequencies with those expected if the observations were independent. The probabilities were determined by the programme ACTUS - a simulation analysis for tests of independence of contingency tables (see section 4.2.4.1). Data concern all 3 sizes of the grey gurnard. N = 1372.

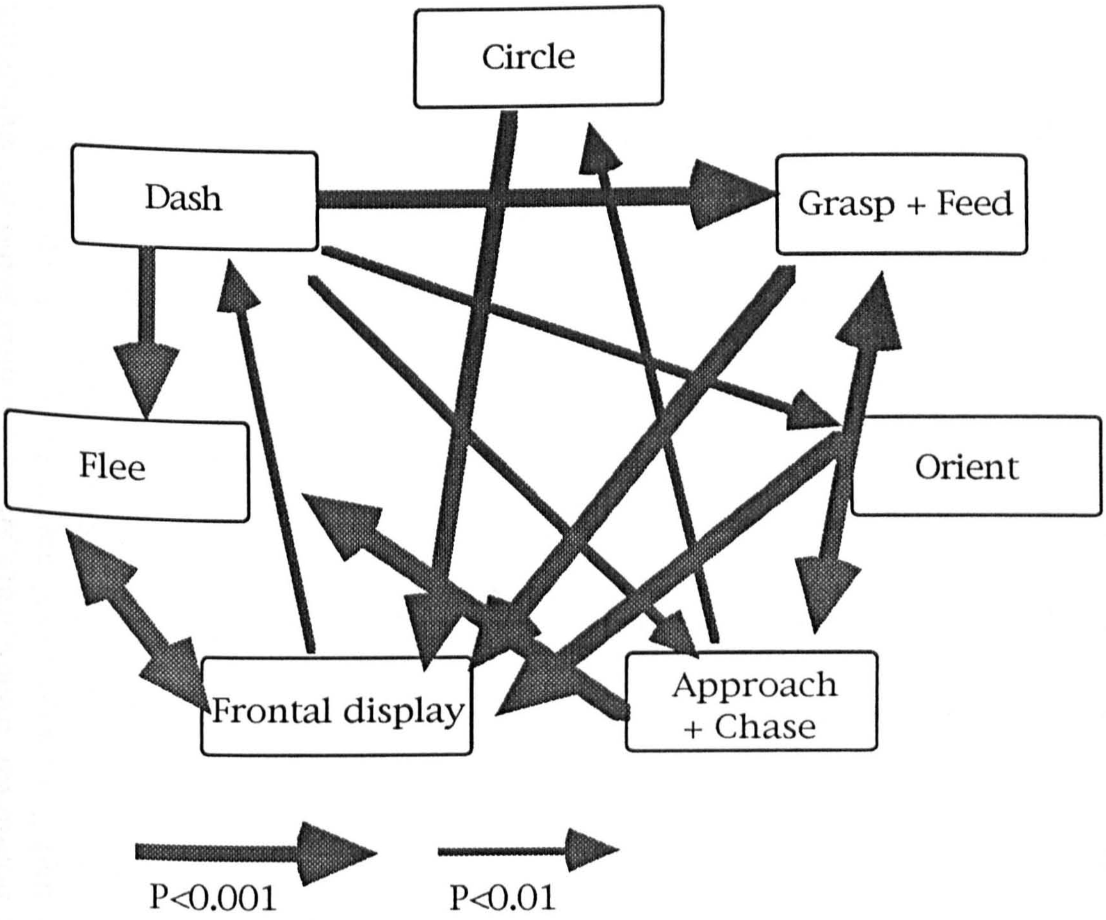


Figure 4.7 - Behavioural sequences observed during competitive feeding interactions of the grey gurnard occurring less frequently than expected by chance alone. Sequential dependence of behavioural acts was tested by comparing observed frequencies with those expected if the observations were independent. The probabilities were determined by the programme ACTUS - a simulation analysis for tests of independence of contingency tables (see section 4.2.4.1). Data concern all 3 sizes of the grey gurnard. N = 1372.

4.3.1.2 - The context of sound production

The results of the GAMs (see section 4.2.4.2) fitted to the data are summarised in Table 4.7. Overall, there is a positive association between the number of grunt calls with the behavioural categories *approach + chase* and *frontal*

Table 4.7 - GAM results for grunt and knock calls uttered by small (S), medium (M) and extra-large (XL) grey gurnards. Straight lines depict positive or negative linear trends. Curvy lines depict non-linear trends. Thin and thick lines stand for significance levels at 5% and 1% level, respectively. * = factor significant at 5% and * = factor significant at 1%. – = not significant; n.a. = not applicable. Dev_0 and $d.f._0$ stand respectively for deviance and the degrees of freedom of the original data set. Dev and d.f. are the deviance and degrees of freedom after fitting the final model. R^2 is the determination coefficient. "S+S" = *swim* + *search*; "C+D" = *circle* + *dash*; "App+ch" = *approach* + *chase*; "Front d." = *frontal display*.

Response var/ Fish size	Explanatory variables											Statistics				
	Session	Time	No. fish	S+S	C+D	Grasp	Feed	Orient	App+ch	Front d.	Flee	Dev_0	$d.f._0$	Dev	d.f.	R^2
Grunt / S	*	****	–	n.a.	**/**	–	n.a.	–	**/**	**/**	–	291.5	148	116.1	135	0.60
Grunt / M	–	–	**╭**	–	–	–	–	–	–	**/**	–	255.1	141	105.4	136	0.59
Grunt / XL	–	~	╭	–	–	–	–	–	**/**	**/**	–	197.9	122	106.0	116	0.46
Knock / S	*	~	**/**	n.a.	–	**/**	n.a.	–	–	\\	/	274.9	147	155.5	132	0.43
Knock / M	–	\\	**╭**	–	**/**	–	–	–	–	–	–	233.2	141	155.8	137	0.33
Knock / XL	*	\\	/	–	–	**/**	–	–	–	–	/	232.6	122	152.8	110	0.34

display. For the small fish grunt calls were also correlated with *circle + dash*. In this case, grunts are probably associated with *dash* since this behaviour is usually followed by *frontal display* (see Fig. 4.6). Knock calls are positively correlated with the behavioural categories *grasp* and *flee*. For the medium grey gurnards, knock calls also showed a positive trend with the behavioural category *circle + dash*. *Circle* is probably associated with knock calls because this behaviour is significantly associated with *grasp* (see Fig. 4.6). *Frontal display* decreased with increasing numbers of knock calls in the small fish. It seems that knocks are more associated with feeding and less with aggressive behaviour (Fig. 4.8). In turn, grunt calls are mainly uttered during aggressive behaviour (Fig. 4.8).

The selected models for grunts explain more of the total variation in the data than the selected models for knocks (see R^2 values in Table 4.7).

Grunt and knock calls decreased with time probably due to increasing levels of satiation. The number of grunt and knock calls emitted per interaction also increased with increasing number of fish present in an interaction.

4.3.1.3 - Sound playback

Effect of sound playback on fish behaviour

The total number of behavioural categories performed every minute throughout the playback experiment I (PBK1) was compared between fish from the 2 experimental tanks to check for a tank effect. Fish from one tank performed a significantly larger number of behavioural categories than the fish in the other tank (tank 1: N = 96, mean = 2.35, s.d. = 1.28; tank 2: N = 96, mean = 1.95, s.d. 1.68) (Kruskal-Wallis test: N = 192, d.f. = 1. H = 5.38, $P < 0.05$). However, as the differences were not very big and only at the 5%

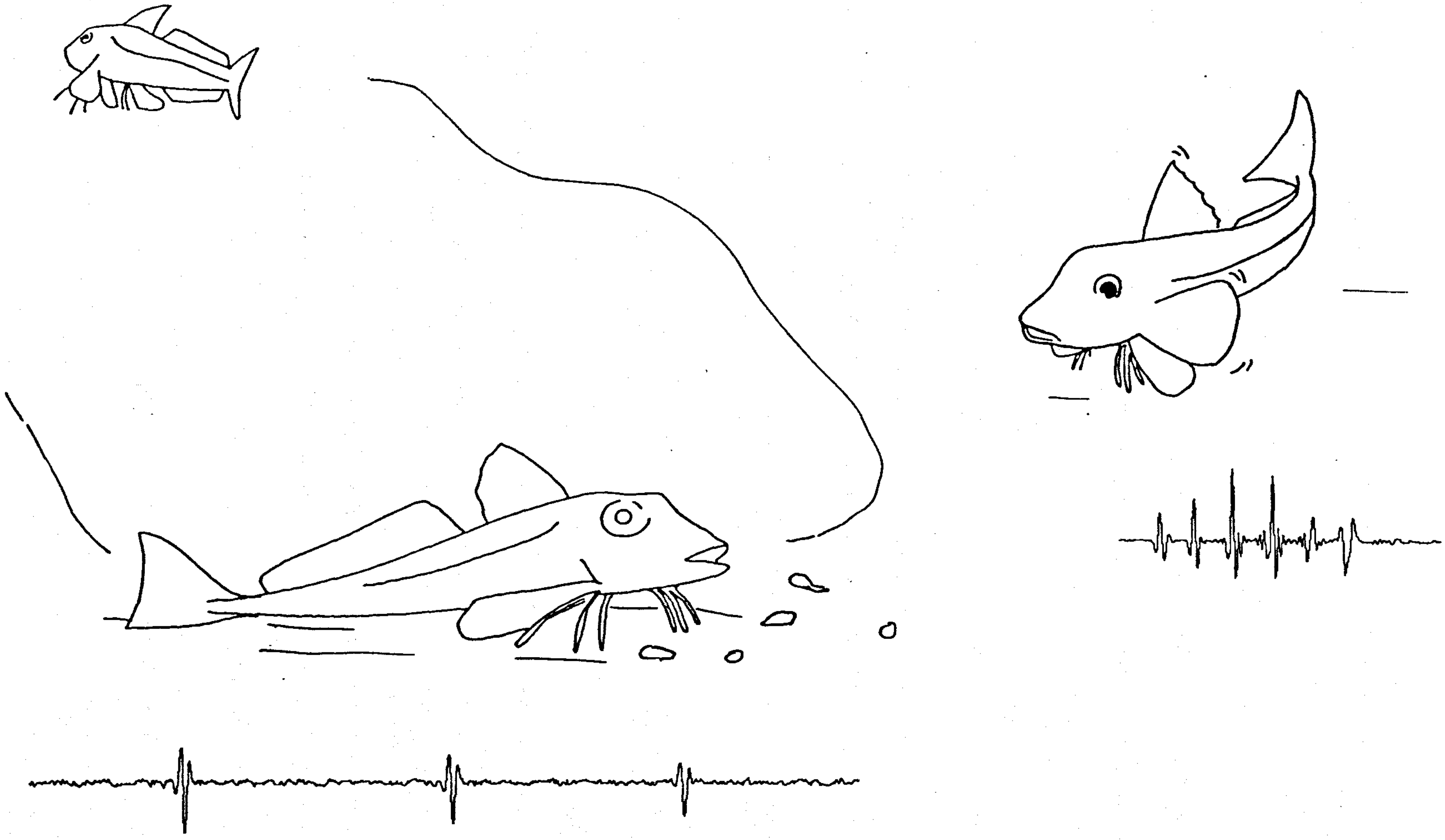


Figure 4.8 - This Figure represents a typical interaction between 2 grey gurnards and depicts the association of the behavioural categories with sound emission. *Grasp* and *flee* are associated with the production of knocks (left) whereas the behaviour *frontal* display is correlated with the emission of grunt calls (right).

level, tank or individual fish were not discriminated.

The frequency of each behavioural act observed (see section 4.2.4.3) during PBK1 was compared between sound and silence presentations to check for the effect of sound playback. Sound playback did not seem to have any significant effect on the frequency of any of the behavioural categories studied (for each behavioural category - Kruskal-Wallis test: $N = 128$, $P > 0.05$). In addition, the frequency of each behaviour observed for each minute throughout the experiment PBK1 was compared with that observed in playback experiment II (PBK2). The data were pooled for all fish. There were no significant differences found for any of the behavioural categories studied or for the total number of behavioural categories between PBK1 and PBK2 (for each behavioural category - Kruskal-Wallis test: $N = 192$, $P > 0.05$). Since during the silence (control I) periods, only the behavioural categories *frontal display* and *orient* were observed, only these 2 behavioural categories were considered in comparing behaviour between PBK 1, PBK 2, PBK 3 and silence (control I). For this comparison, data from PBK 1 and PBK 2 were pooled for both behavioural categories since they were not significantly different. The frequency of *frontal display* was significantly higher during PBK 1 and 2 than in PBK 3 and silence (control I), which were similar (Kruskal-Wallis test: $N = 376$, d.f. = 2, $H = 27.32$, $P < 0.001$) (Fig. 4.9). The frequency of *orient* did not vary significantly between the different experiments and the silence period (Kruskal-Wallis test: $N = 376$, $P > 0.05$).

In conclusion, sound playback did not seem to have a significant effect on the behaviour of the grey gurnard.

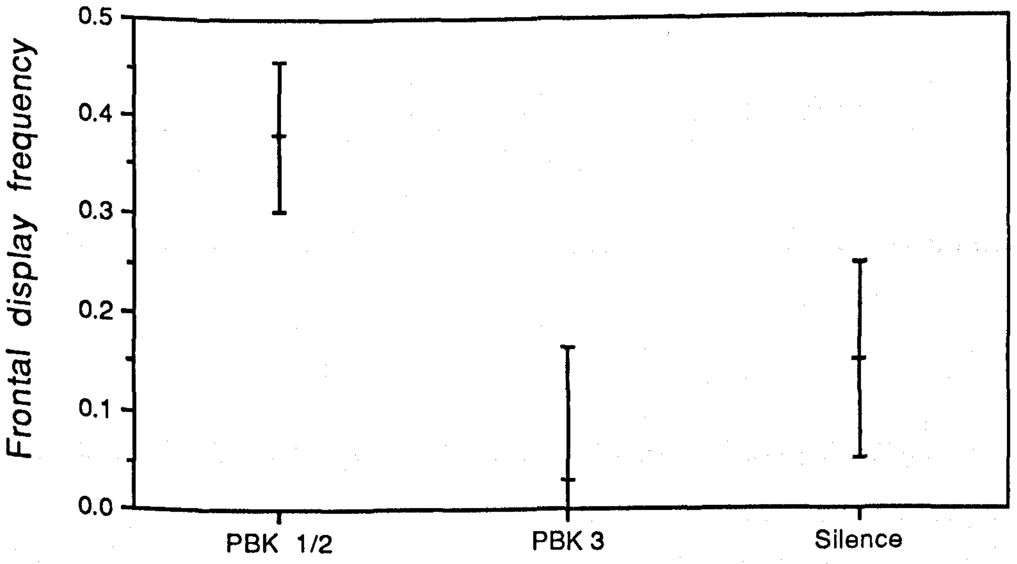


Figure 4.9 - Mean frequency of the behaviour *frontal display* observed during PBK 1 and 2, PBK 3 and silence (control I). Bars indicate 95% confidence intervals. N (PBK 1/2) = 192; N (PBK 3) = 64; N (silence) = 120.

Individual differences

Individual behavioural fish differences were also examined since in the previous tests data were pooled for all fish. This also allowed checking for differences between tagged and untagged fish. Because the data sets from PBK1 and PBK2 were similar they were pooled for the subsequent analysis. There were significant individual differences in the frequency of each behavioural act performed (except for *circle* and *frontal display*) as well as for the total number of behavioural categories (all behavioural categories lumped together) (Fig. 4.10, Table 4.8). However, fish did not seem to be affected by tagging (Fig. 4.10), as the frequencies of each behaviour observed for tagged fish were not significantly different from those observed for untagged ones (for each behavioural category - Kruskal-Wallis test: N = 192, P > 0.05).

Figure 4.10 - Observed frequencies (mean and standard deviation) for each and for all behavioural categories observed during experiments PBK1 and PBK2 (see text for explanation). All experimental fish except fish 7 and 8 are tagged. The fish 1, 2, 3 and 7, and the fish 4, 5, 6 and 8 were kept in 2 different tanks.

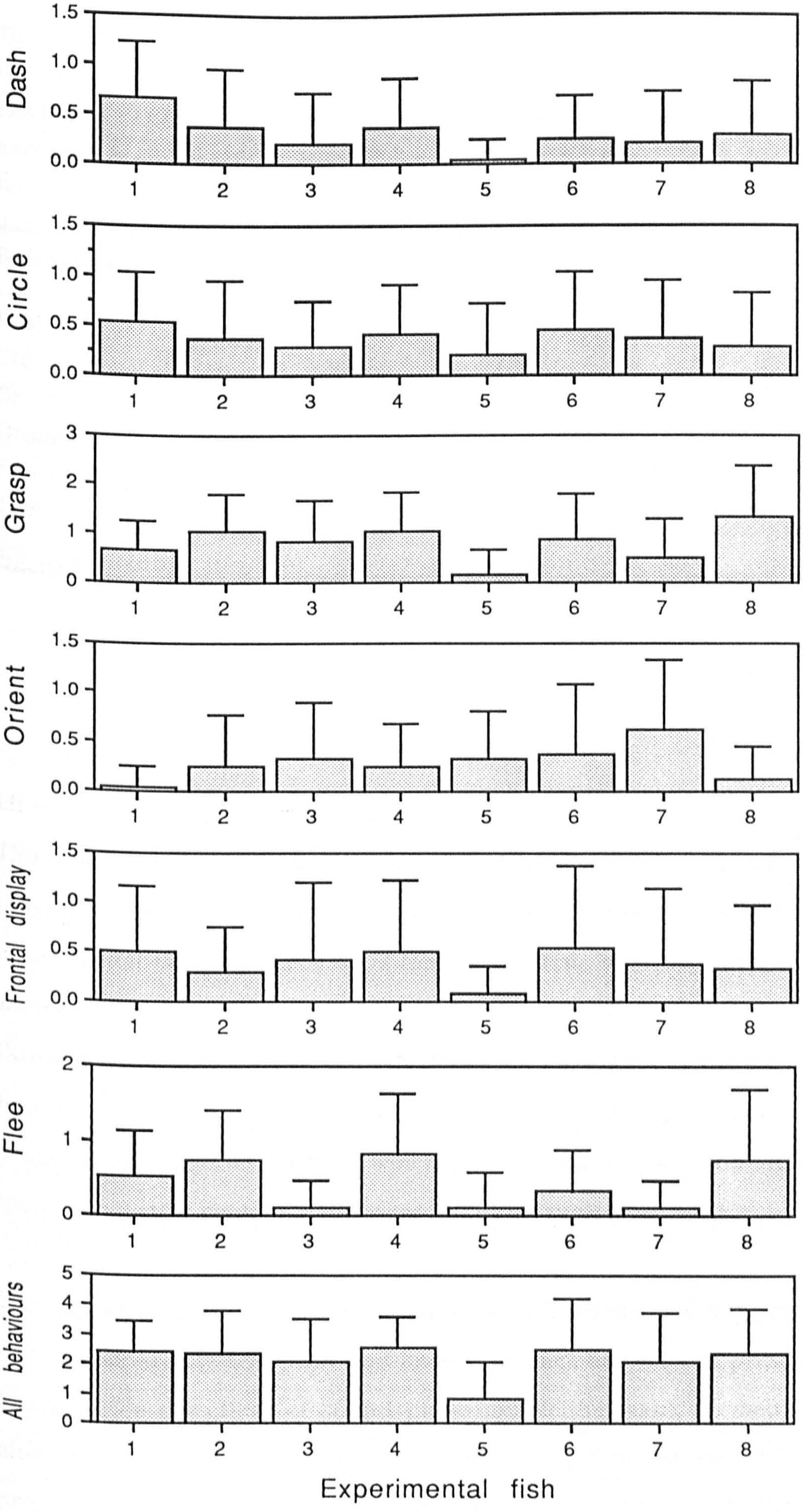

Dash
0.0
0.5
1.0
1.5
Circle
0.0
0.5
1.0
1.5
Grasp
0
1
2
3
Orient
0.0
0.5
1.0
1.5
Frontal display
0.0
0.5
1.0
1.5
Flee
0
1
2
All behaviours
0
1
2
3
4
5
1
2
3
4
5
6
7
8
Experimental fish

Table 4.8 - Results of the Kruskal-Wallis test comparing the frequency of the behavioural categories scored for each minute during experiments PBK1 and PBK2 (see text for explanations) between the 8 experimental grey gurnards. N = total sample size. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$; n.s. = not significative.

Behaviour	N	d.f.	H	P
Dash	192	7	24.61	**
Circle	"	"	8.61	n.s.
Grasp	"	"	33.94	***
Orient	"	"	17.30	*
Frontal display	"	"	9.09	n.s.
Flee	"	"	37.74	***
All behavioural categories	"	"	25.33	**

Effect of sound playback on sound production

The total number of sounds produced every minute throughout the playback experiment I (PBK1) were compared between fish from the 2 experimental tanks to check for a tank effect. Fish from one tank emitted a significantly greater number of sounds than those from the other tank (Kruskal-Wallis test: N = 128, d.f. = 1, H = 28.21, $P < 0.001$), so the 2 tanks were treated separately in subsequent statistics. Note that, in contrast with the behavioural categories studied, which were scored for each individual fish, sound production could only be examined at the tank level.

The total number of sounds produced by each fish for every minute of PBK1 was compared between grunt and knock playback and silence presentations to check for the effect of sound playback. Neither tank seemed to have altered the rate of sound production with the different sound / silence presentations (for each tank - Kruskal-Wallis test: N = 64, $P > 0.05$). A similar analysis, but with knock and grunt production discriminated, was also carried out with identical results (for each tank - Kruskal-Wallis test: N = 64, P > 0.05).

Sound production by the fish from both tanks was further tested for differences between PBK1, PBK2, PBK3 and silence control I (from all experiments). Knock and grunt production was similar in PBK1 and PBK2, and significantly higher than in PBK3 and silence control I (Table 4.9, Fig. 4.11).

In conclusion, sound playback did not seem to have any effect on fish sound production.

In addition to the experiments described, sounds from the streaked gurnard and noise were also played back to the grey gurnards with no apparent response. Further experiments with the playback of artificially altered sounds (varying either pulse repetition rate or number of pulses in a phrase) were planned but not carried out due to the negative response of grey gurnards to sound playback.

Table 4.9 - Results of the Kruskal-Wallis test comparing the knocks and grunts produced during each minute in each experimental tank (1 and 2) between experiments PBK1, PBK2, PBK3 and silence control I (see text for explanations). N = total sample size. *** = $P < 0.001$; * = $P < 0.05$.

Sound production	N	d.f.	H	P
Tank 1 - knocks	192	3	149.62	***
Tank 1 - grunts	"	"	126.10	***
Tank 2 - knocks	"	"	31.03	***
Tank 2 - grunts	"	"	8.63	*

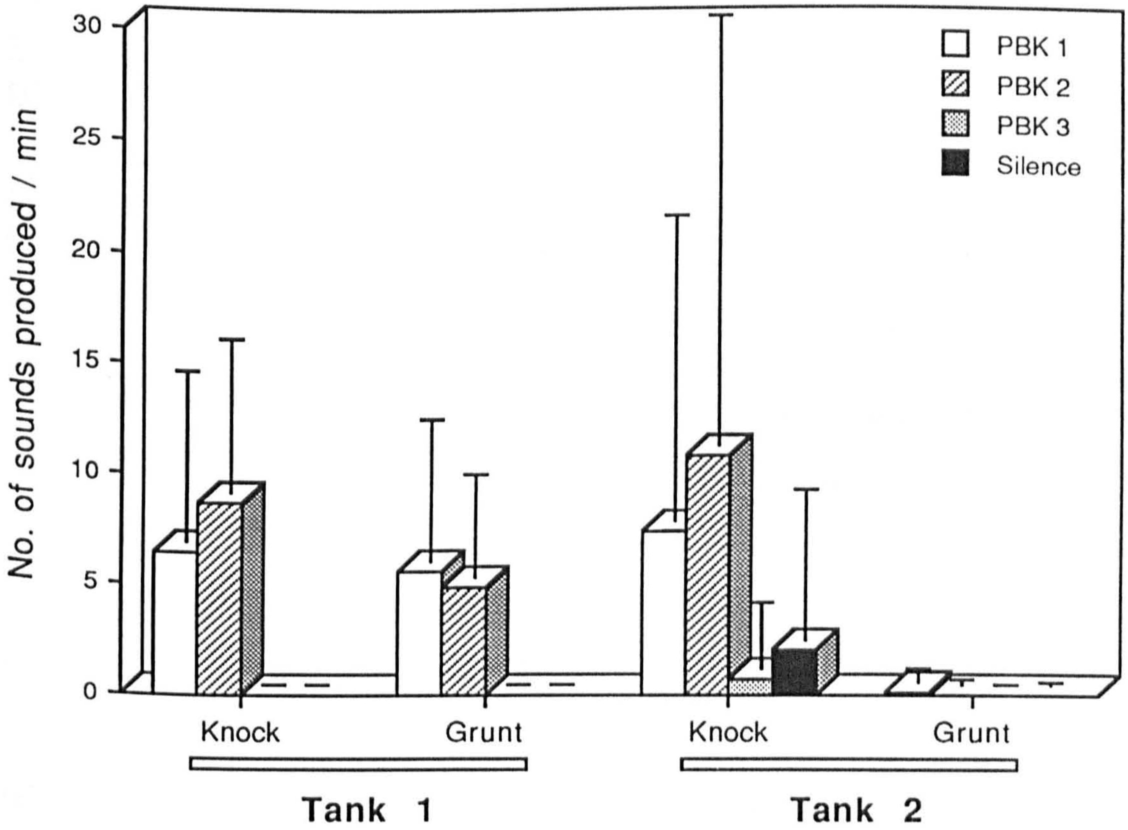


Figure 4.11 - Mean number of knocks and grunts produced by fish from the experimentals tanks 1 and 2, during the experiments PBK 1, PBK 2, PBK 3, and silence (control I). The bars indicate standard deviations. N (PBK 1) = 64, N (PBK 2) = N (PBK 3) = 32, N (silence) = 64 for knock and grunt production in each tank.

4.3.2 - The streaked gurnard - *Trigloporus lastoviza*

4.3.2.1 - Feeding interactions

1 - Proportion of each behavioural category in interactions

The proportions of the different behavioural categories considered during the analysis of competitive feeding interactions of the streaked gurnard are depicted in Figure 4.12.

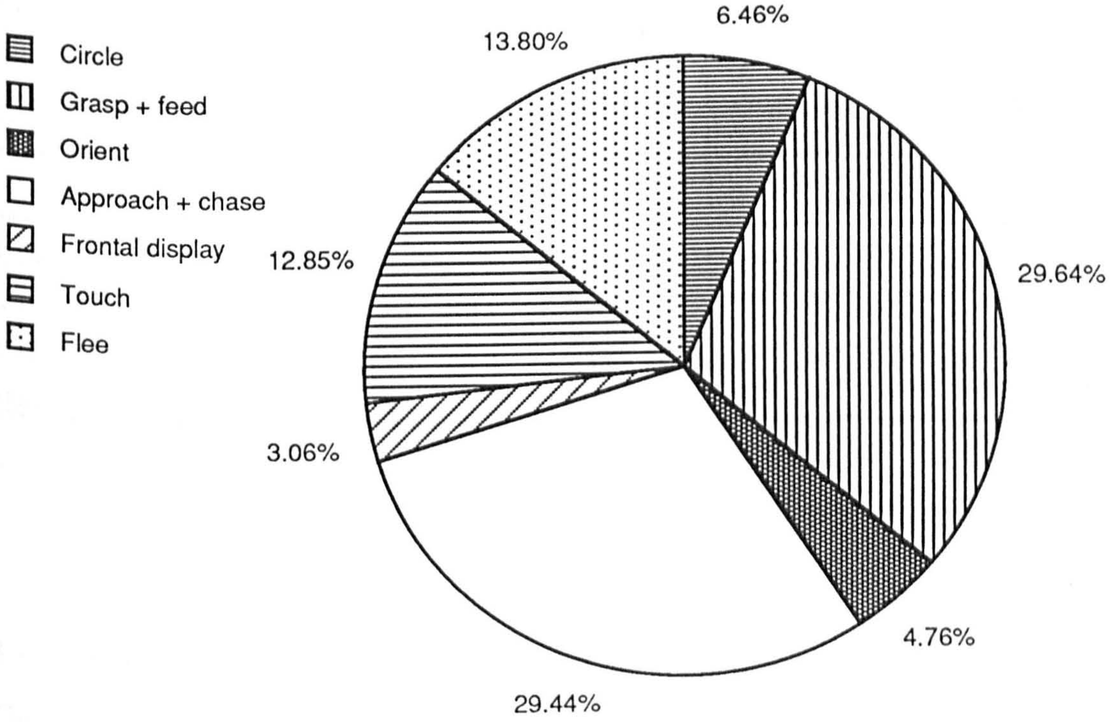


Figure 4.12 - Proportions of the different behavioural categories observed during competitive feeding interactions in the streaked gurnards. N = 1902.

2 - Number of behavioural categories per interaction

The number of behavioural categories observed per interaction was on average 4.08 (N = 465, s.d. = 3.02, min = 2 and max = 27). The number of fish present in an interaction had a significant effect on the number of behavioural categories observed per interaction (Spearman correlation: N = 465, Rho = 0.542, P < 0.001).

3 - Frequency of behavioural category x per interaction

The frequencies of each behavioural category in competitive interactions of streaked gurnards are described in Table 4.10.

Table 4.10 - Descriptive statistics for the frequency of the behavioural categories: *swim + search, circle, grasp, feed, orient, approach + chase, frontal display, touch* and *flee* observed for the streaked gurnards. N = 8.

	Swim+ search	Circle	Grasp	Feed	Orient	Approach +chase	Frontal display	Touch	Flee
mean	0.86	0.21	0.35	0.55	0.15	0.90	0.09	0.39	0.43
s.d.	0.46	0.05	0.17	0.31	0.07	0.32	0.04	0.16	0.13
min	0.49	0.15	0.11	0.18	0.08	0.55	0.05	0.21	0.22
max	1.66	0.27	0.65	1.16	0.30	1.56	0.16	0.61	0.67

4 - Number of fish involved in an interaction

The mean number of fish involved in an interaction was on average 3.37 (N = 465, s.d. = 1.41, min = 2 and max = 7).

5 - Number of interactions / min

In the streaked gurnard, the rate of feeding interactions averaged 1.20 interactions /min (N = 8, s.d. = 0.30, min = 0.67 and max = 1.60).

6 - Interaction duration

The duration of 46 interactions was measured at random. The mean duration was 42.76 s (s.d. = 40.40, min = 4 and max = 151).

7 - % of interactions accompanied by sound production

Approximately one quarter (24.3%, N = 465) of the total number of interactions observed during 163 min of videos analysed (8 recording sessions) were accompanied by sound production.

8 - Sequences of behaviour

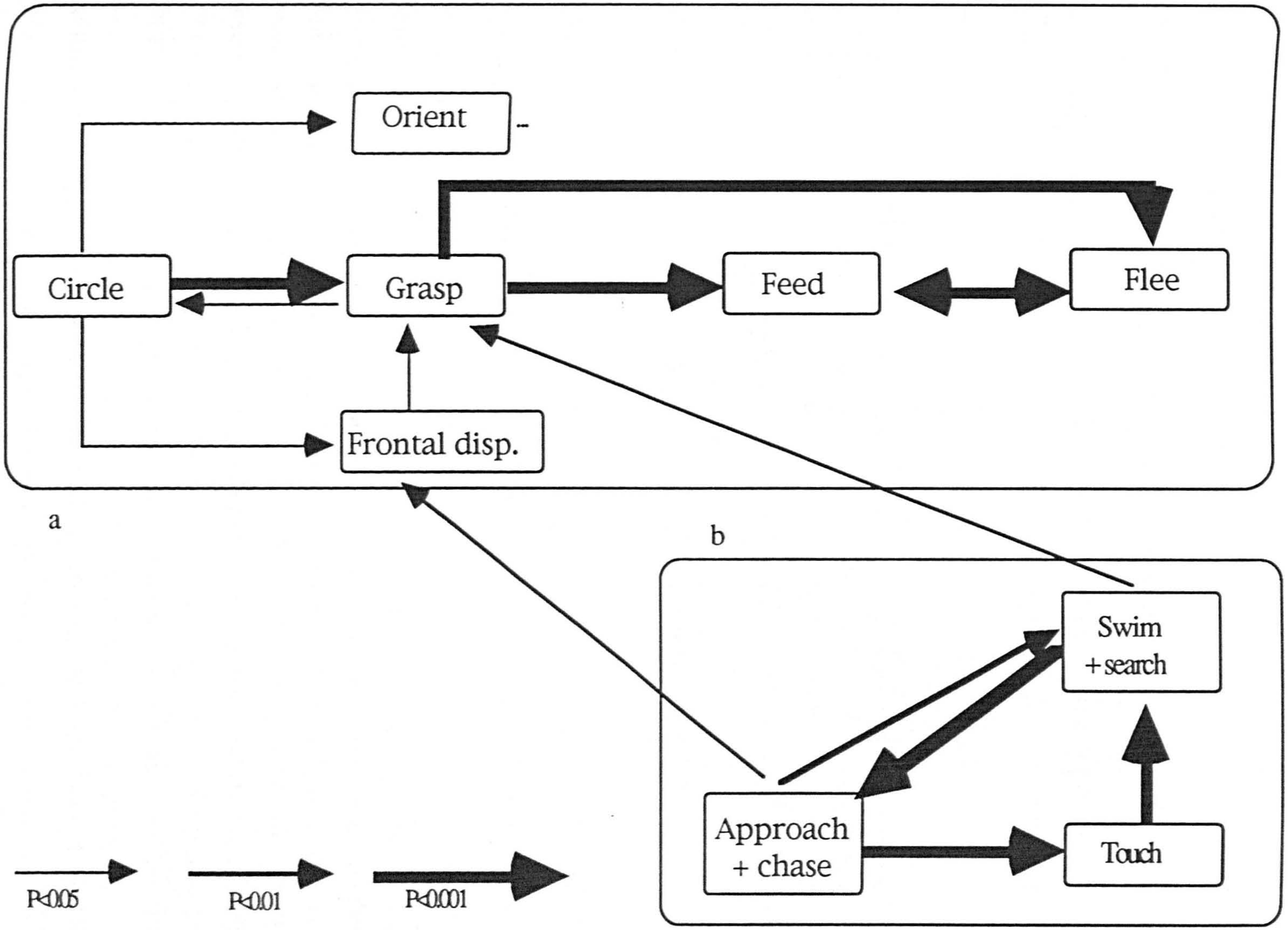
The number of transitions between behavioural acts observed for the streaked gurnard is given in Table 4.11. Figure 4.13 and 4.14 depict, respectively, the behavioural sequences that were more frequent than expected and less frequent than expected (probabilities given by the programme ACTUS; see section 4.2.4.1). The streaked gurnards perform 2 groups of behavioural sequences (Fig. 4.13): one when they are *grasping* or handling food, and the other when they are *searching* for food because they have either stopped *feeding* or have not *grasped* a food item when food was dropped. In the latter group, fish *search* for food and *approach* other fish that are *feeding*, very often *touching* them. Sometimes these fish managed to *grasp* food items either because they found one or because they *grasped* food another individual had spat out while handling it. It is common for streaked gurnards to spit out and *grasp* food consecutively when *feeding*.

Time did not have a significant effect on the number of behavioural categories in an interaction (regression test, $P > 0.05$), hence stationarity can be assumed. Data was transformed by $\sqrt{x}$.

Table 4.11 - Transition frequency matrix of behavioural sequences observed during feeding interactions of the streaked gurnard. Columns indicate the preceeding behavioural act and rows the following behavioural acts. The number in the cells shows the number of times one act (column) was immediately followed by another act (row). "Search+s" = *swim* + *search*; "App+ch" = *approach* + *chase*; "Front d." = *frontal display*.

	Search+s	Circle	Grasp	Feed	Orient	App+ch	Touch	Front d.	Flee
Search+s		1	36	30	10	121	24	7	14
Circle	9		52	1	9	11	1	7	8
Grasp	9	4		51	5	9	7	4	50
Feed	52	1	3		12	31	7	1	104
Orient	15	0	6	5		14	6	1	3
App+ch	118	1	21	22	13		132	19	14
Touch	81	0	5	11	3	49		2	6
Front d.	11	1	10	1	0	10	5		2
Flee	13	0	3	67	1	7	2	1	

Figure 4.13 - Behavioural sequences observed during competitive feeding interactions of the streaked gurnard occurring more frequently than expected by chance alone. Sequential dependence of behavioural acts was tested by comparing observed frequencies with those expected if the observations were independent. The probabilities of each of the sequences occurring were determined by the programme ACTUS - a simulation analysis for tests of independence of contingency tables (see section 4.2.4.1). a and b depict 2 different groups of behavioural sequences; a is performed by fish that grasped a food item, whereas b is performed by fish that either have stopped feeding or did not catch any food. N = 1372.


Orient
Circle
Grasp
Feed
Flee
Frontal disp.
a
b
Swim
+ search
Approach
+ chase
Touch
P<0.05
P<0.01
P<0.001

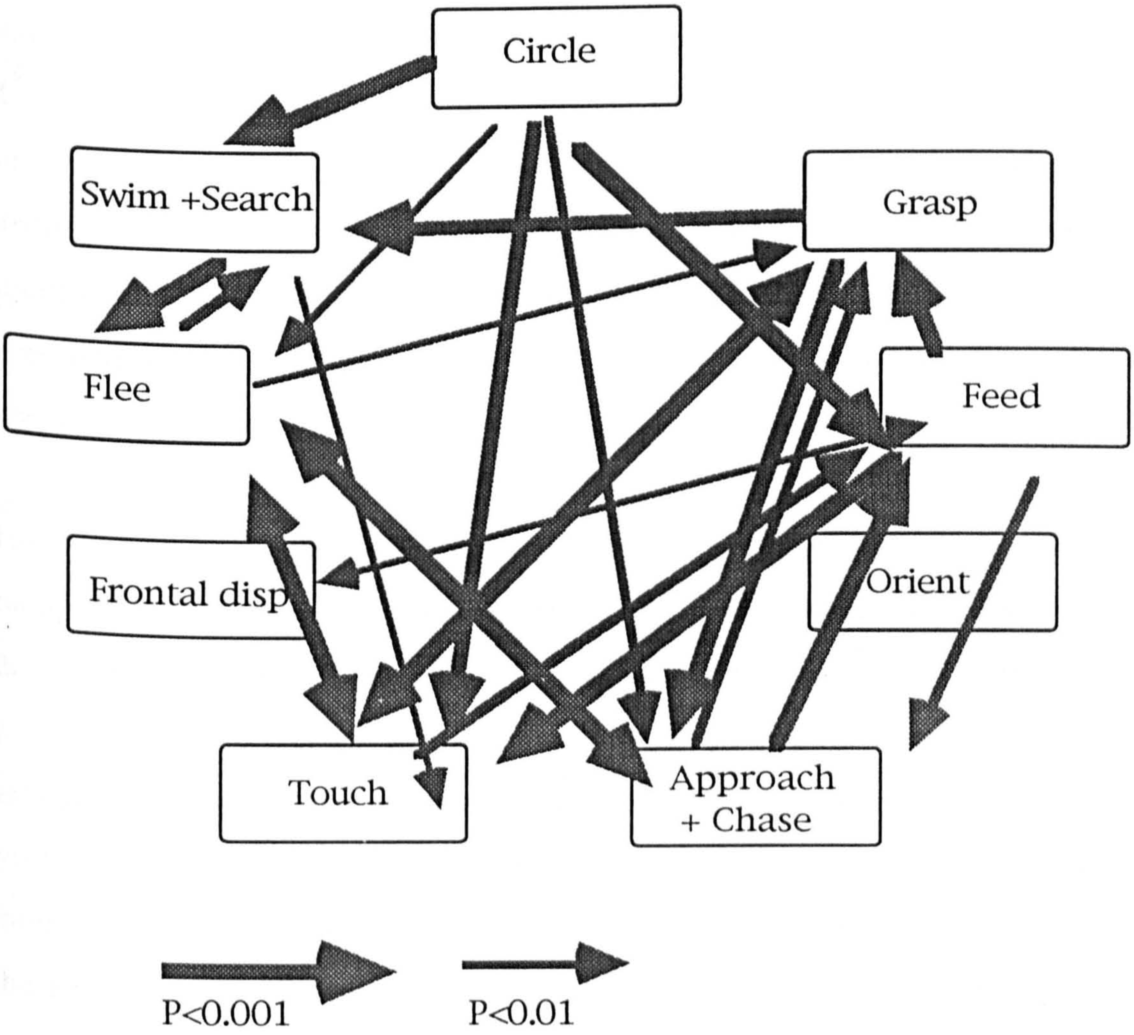


Figure 4.14 - Behavioural sequences observed during competitive feeding interactions of the streaked gurnard occurring less frequently than expected by chance alone. Sequential dependence of behavioural acts was tested by comparing observed frequencies with those expected if the observations were independent. The probabilities of each of the sequences occurring were determined by the programme ACTUS - a simulation analysis for tests of independence of contingency tables (see section 4.2.4.1). **a** and **b** depict 2 different groups of behavioural sequences; a is performed by fish that grasped a food item, whereas b is performed by fish that either have stopped feeding or did not catch any food. N = 1372.

4.3.2.2 - The context of sound production

The number of behavioural categories that occurred with and without sound production was scored for all interactions analysed (section 4.3.2.1). A χ^2 statistics was used to test for dependence of behavioural categories and sound production. In the streaked gurnard behavioural acts and sound production occurring during competitive feeding interactions were significantly dependent ($\chi^2 = 612.85$, $N = 1901$, d.f. = 10, $P < 0.001$). Sound production was mainly associated with the behavioural categories *circle*, *grasp*, *feed* and *flee* (Fig. 4.15).

The context of sound production was further studied for behavioural categories occurring outside fish interactions. All behavioural categories that were accompanied by sound production were noted. Furthermore, as *grasp* was the behaviour with which most of the growls were emitted, all *grasps* observed outside interactions were scored, and the number of *grasps* with and without sound production was counted. Outside fish feeding interactions only 36 growls were heard, of which 29 (80.6%) accompanied the behaviour *grasp*, 3 (8.3%) *circle*, 2 (5.6%) *feed*, 1 (2.8%) *flee* and 1(2.8%) *swim*. In general, only 16.7% of the total number of sounds emitted (36 + 180 = 216) took place outside feeding interactions. The behaviour *grasp* occurred 39 times outside interactions (18.4%), of which 29 (74.4%) were accompanied with sound emission. Overall, 62.7% of *grasps* (occurring during or outside interactions) were accompanied with sound emission. *Grasp* seems to be the most important behavioural category related to growl emission during feeding interactions as well as outside them.

The number of growls in an interaction were not significantly correlated with the number of fish involved in the interaction (Spearman correlation: $N = 465$, $P > 0.05$).

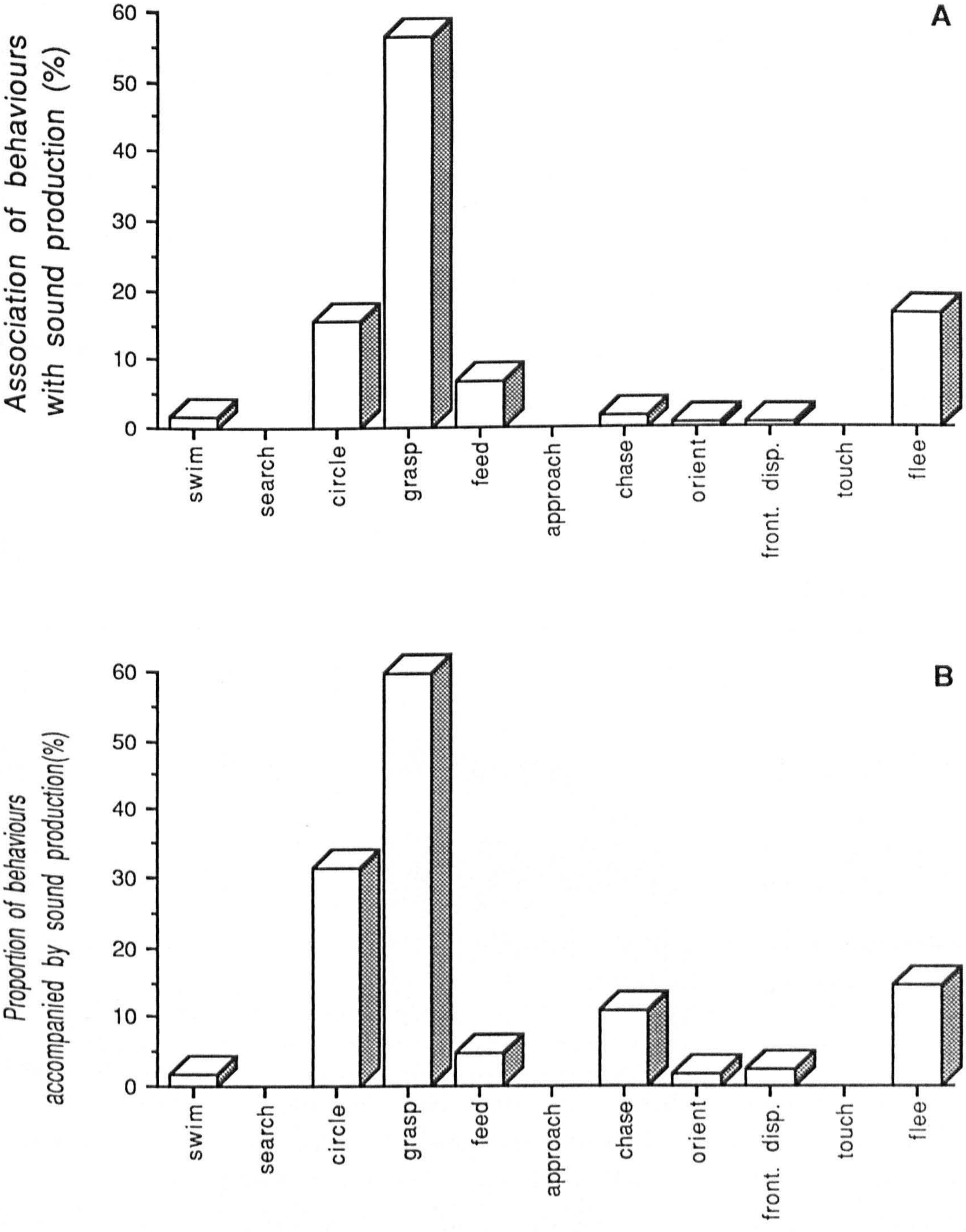


Figure 4.15 - **A** depicts the proportion of behavioural categories accompanied by sound production; the values of all behavioural categories sum up to 100%. B shows, for each behaviour, the percentage that occurred with the emission of growling sounds. All behavioural categories and sounds occurred during fish feeding interactions.

4.3.3 - The tub gurnard - *Trigla lucerna*

4.3.3.1 - Feeding interactions

1 - Proportion of each behavioural category in interactions

The proportions of the different behavioural categories observed during the competitive feeding interactions of the tub gurnard are depicted in Figure 4.16.

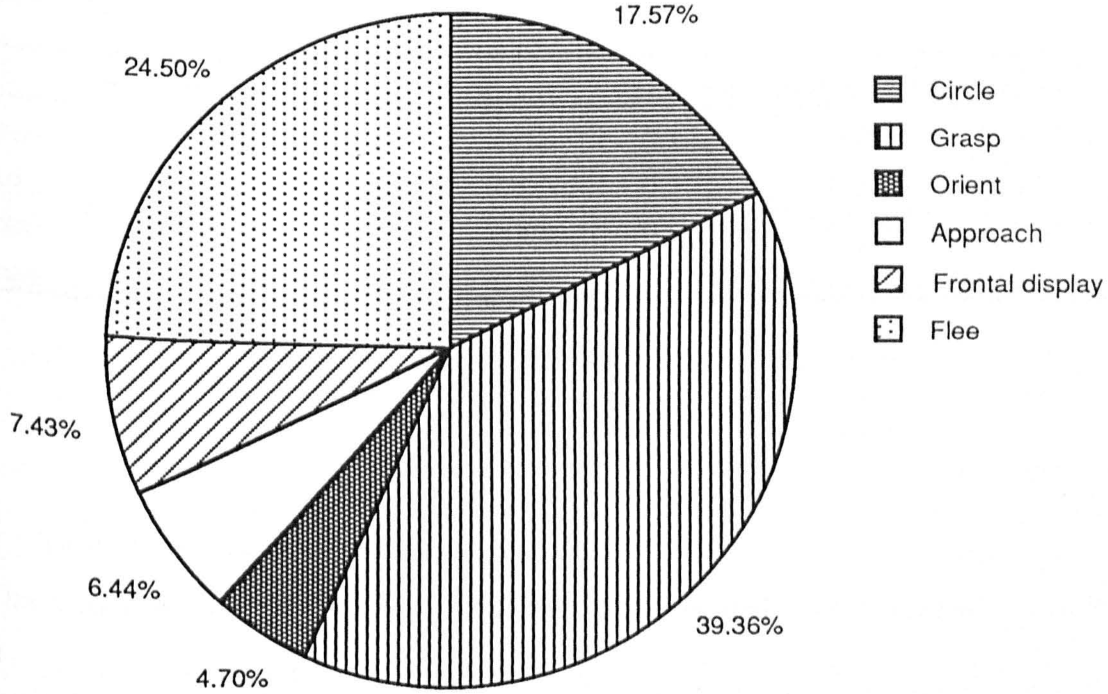


Figure 4.16 - Proportions of the different behavioural categories observed during competitive feeding interactions in the tub gurnard. N = 404.

2 - Number of behavioural categories per interaction

The tub gurnards performed on average 3.21 behavioural categories per interaction (N = 125, s.d. = 1.62, min = 2 and max = 9). As seen in the grey and the streaked gurnards, the number of behavioural categories per interaction was significantly correlated with the number of fish involved in an interaction (Pearson correlation: N = 125, Rho = 0.197, P < 0.05).

3 - Frequency of behavioural category x per interaction

The frequencies of each behavioural category observed during feeding interactions of competitive tub gurnards are described in Table 4.12.

Table 4.12 - Descriptive statistics for the frequency of the behavioural categories: *circle, grasp, orient, approach, frontal display* and *flee* observed for the tub gurnards. N = 9.

	Circle	Grasp	Orient	Approach	Frontal display	Flee
mean	0.55	1.24	0.16	0.21	0.23	0.83
s.d.	0.19	0.52	0.09	0.12	0.21	0.22
min	0.25	0.50	0.00	0.00	0.00	0.50
max	0.78	2.14	0.29	0.38	0.50	1.18

4 - Number of fish involved in an interaction

The mean number of tub gurnards participating in an interaction was 2.54 (N = 125, s.d. = 0.50, min = 2 and max = 3).

5 - Number of interactions / min

Tub gurnards performed on average 0.38 interactions / min (N = 58, s.d. = 0.14, min = 0.25 and max = 0.71).

6 - Interaction duration

The duration of 56 competitive feeding interactions was measured and averaged 13.61 s (s.d. = 9.21, min = 3 and max = 48).

7 - Sequences of behaviour

The number of behavioural transitions observed for the tub gurnard is given in Table 4.13. Figure 4.17 depicts the behavioural sequences which occurred during feeding interactions with a transitional probability greater than 0.30. All interactions except one involved *grasping* a food item.

4.3.3.2 - The context of sound production

As mentioned in section 4.2.2, video recordings of the tub gurnard did not include sound recordings, but audio monitoring was possible through head phones. Nonetheless, few direct observations were carried out (note that the tub gurnards only uttered sounds for a very restricted time span). Sound emissions were accompanied mostly by *frontal display* and also by *approach* and *orient* (direct observations).

4.3.4 - Comparisons between species

4.3.4.2 - Feeding interactions

1 - Proportion of each behavioural category in interactions

Since not all behavioural categories were considered for the grey (see Fig. 4.1), the streaked (see Fig. 4.12) and the tub (see Fig. 4.16) gurnards, only broad comparisons will be made. In general, the streaked gurnard performs (proportionally) a greater number of the behavioural categories *approach + chase*, and *touch* (which is exclusive of this species) and *flees* less than the other 2 gurnard species. The grey gurnard, in turn, does more *frontal displays* than the streaked and tub gurnards (Fig. 4.18). *Dash* is also an

Table 4.13 - Transition frequency matrix of behavioural sequences observed during feeding interactions of the tub gurnard. Columns indicate the preceeding and rows the following behavioural acts. The number in the cells shows the number of times one act (column) was immediately followed by another act (row). "Front d." = *frontal display.* N = 275.

	Circle	Grasp	Orient	Approach	Front d.	Flee
Circle		27	4	1	9	19
Grasp	3		9	6	9	72
Orient	0	12		1	0	3
Approach	1	10	3		4	1
Front d.	3	20	1	2		3
Flee	12	28	3	3	6	

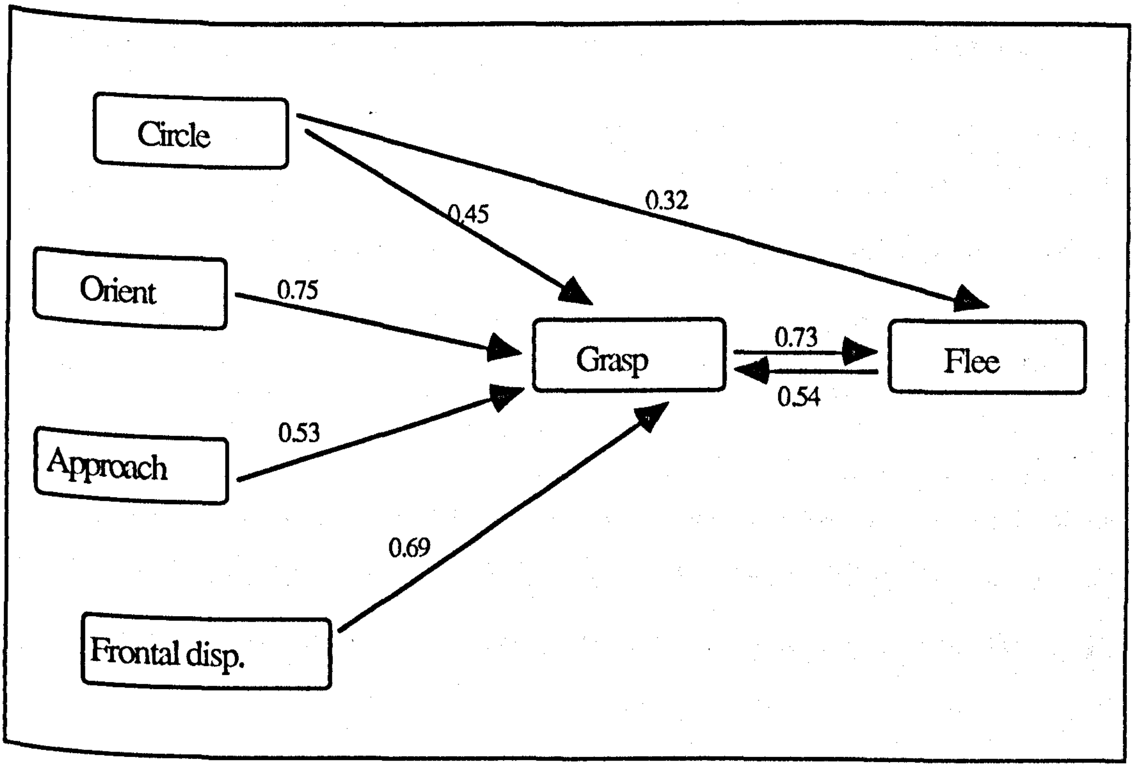


Fig. 4.17 - Behavioural sequences observed during competitive feeding interactions in the tub gurnard. The probabilities of each of the sequences occurring are transitional probabilities (see section 4.2.2.2). Only the transitional probabilities greater than 0.3 are depicted in this Figure.

important behaviour in the grey gurnard's feeding interactions, in contrast with the other gurnard species (Fig. 4.18). The most important components of the competitive feeding interactions of the tub gurnard were *circle*, *grasp* and *flee*. *Grasp* and especially *circle* were observed in a much higher proportion in the tub than in the grey and the streaked gurnards. Social behaviour did not play such an important role in the behaviour of the 4 tub gurnards studied as in the other 2 species.

2 - Number of behavioural categories per interaction

The number of behavioural categories per interaction were significantly higher for the streaked than for the grey and the tub gurnards, which were not significantly different from each other (Kruskal-Wallis test: $N = 1019$, d.f. $= 2$, $H = 29.56$, $P < 0.001$) (Fig. 4.19).

3 - Frequency of behavioural category x per interaction

The frequency of the behavioural categories *circle*, *grasp* + *feed*, *orient*, *approach* + *chase*, *frontal display* and *flee* were compared between the 3 species of gurnards. Because in the streaked gurnard a higher number of individuals were involved in feeding interactions in relation to the other 2 species (see sub-section 4 of this section) and this variable is known to have a significant effect on the number of behavioural categories per interactions, interactions with more than 5 individuals were not considered for this species in the subsequent comparisons. The results of the Kruskal-Wallis tests are shown in Table 4.14. The frequency of *circle* was significantly higher for the tub gurnard than for the grey and the streaked gurnards (Fig. 4.20a). The tub gurnard also *grasped* significantly more often than the grey gurnard, which in turn *grasped* significantly more than the streaked gurnard (Fig. 4.20b). The frequency of the behaviour *orient* was significantly higher in the grey than in the streaked gurnard. The tub gurnard displayed this behaviour with intermediate

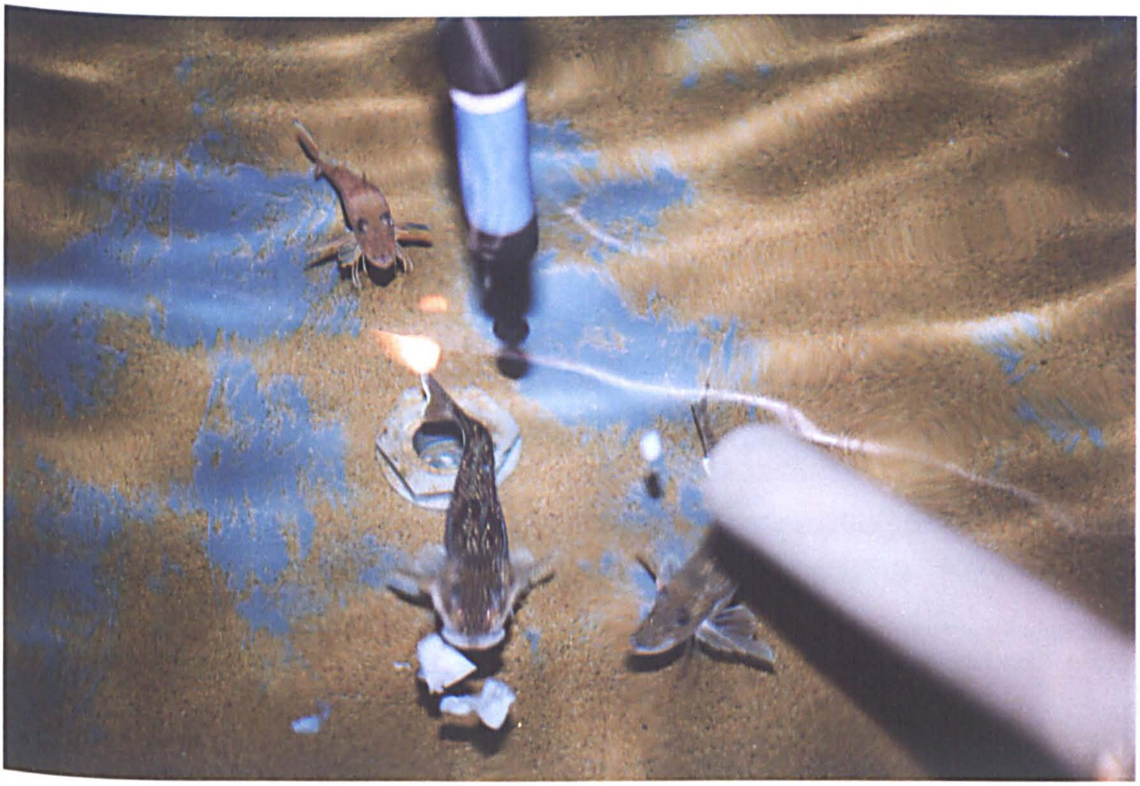


Figure 4. 18 - Illustration of 3 grey gurnards in a feeding interaction, just after a few pieces of squid were dropped through the feeding tube. The fish on the bottom right has just dashed and is performing a frontal display directed to the fish on the bottom left, that is just about to grasp a food item. On the background is another fish approaching the feeding area, and a hydrophone to detect sound production.

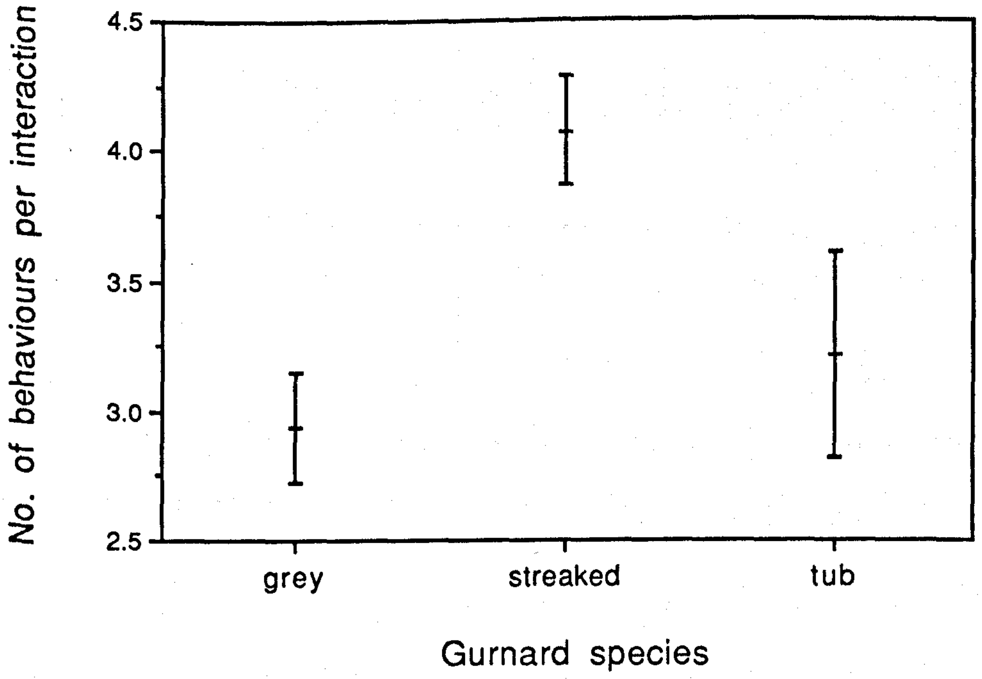


Figure 4.19 - Mean number of behavioural categories performed per interaction by the grey, the streaked and the tub gurnards. Bars indicate 95% confidence intervals.

Table 4.14 - Results of the Kruskal-Wallis test comparing the frequencies of the behavioural categories *circle*, *grasp* + *feed*, *orient*, *approach* + *chase*, *frontal display* and *flee*. N = total sample size. *** = P < 0.001; ** = P < 0.01. (1) - *feed* and *chase* not considered for the tub gurnard.

Behaviour	N	d.f.	H	P
Circle	38	2	19.81	***
Grasp + Feed (1)	"	"	22.02	***
Orient	"	"	11.83	**
Approach + Chase(1)	"	"	20.95	***
Frontal display	"	"	19.60	***
Flee	"	"	19.46	***

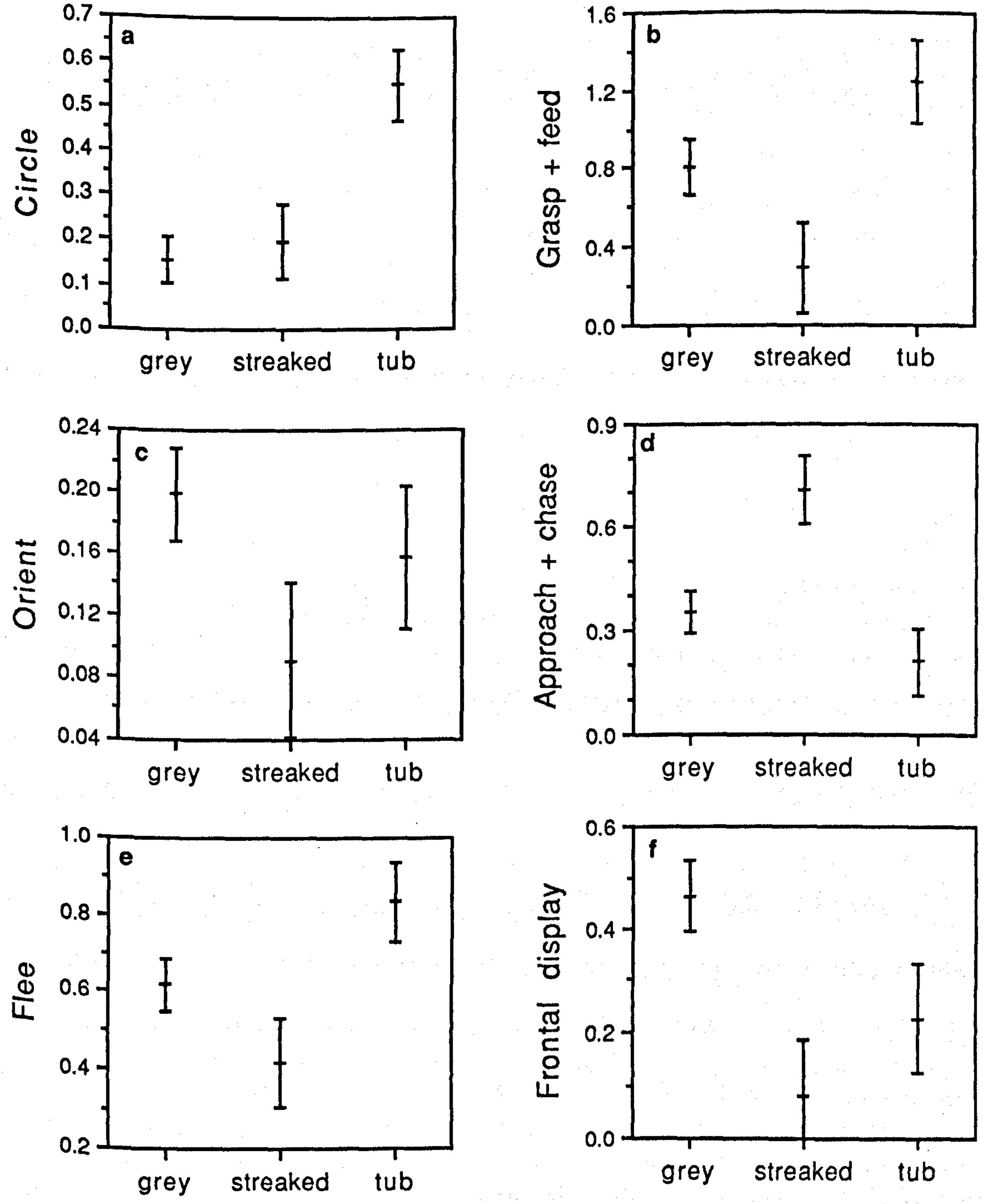


Figure 4.20 - Mean frequency of the behavioural categories: *circle* (a), *grasp + feed* (b), *orient* (c), *approach + chase* (d), *flee* (e) and *frontal display* (f) observed for the grey, the streaked and the tub gurnards. The behavioural categories *feed* and *chase* concerning the tub gurnard were not considered in respectively (b) and (d). Bars indicate 95% confidence intervals.

frequencies to the other 2 species (not significantly different from the number of *orients* performed by the grey and the streaked gurnards) (Fig. 4.20c). *Approach* and *flee* followed the same trend as the behaviour *grasp* (Figs. 4.20d and 4.20e), i.e. the frequencies observed for these behavioural acts were significantly higher in the streaked than in the grey gurnards, and in turn, significantly higher in the grey than in the tub gurnards. The grey gurnard showed a significantly higher frequency of *frontal displays* than the streaked and the tub gurnards, which did not seem to be significantly different from each other (Fig. 4.20f).

4 - Number of fish involved in an interaction

The number of fish participating in an interaction was significantly higher in the streaked than in the grey and the tub gurnards (Kruskal-Wallis test: $N = 1019$, d.f. $= 2$, $H = 64.59$, $P < 0.001$) (Fig. 4.21).

5 - Number of interactions / min

The interaction rate was significantly higher in the grey (data pooled for the 3 size classes) than in the other gurnard species (Kruskal-Wallis test: $N = 38$, d.f. $= 2$, $H = 26.22$, $P < 0.001$) (Fig. 4.22). Although on average the number of interactions / min was greater in the streaked than in the tub gurnard, this difference was not significant (Fig. 4.22). Furthermore, since fish size had a significant effect in this variable in the grey gurnard (see Fig. 4.4), this test was repeated descriminating the data for the different size classes of this species. The number of interactions / min was not significantly different between the extra-large grey gurnard and the streaked gurnard (95% Confidence Intervals, $P > 0.05$).

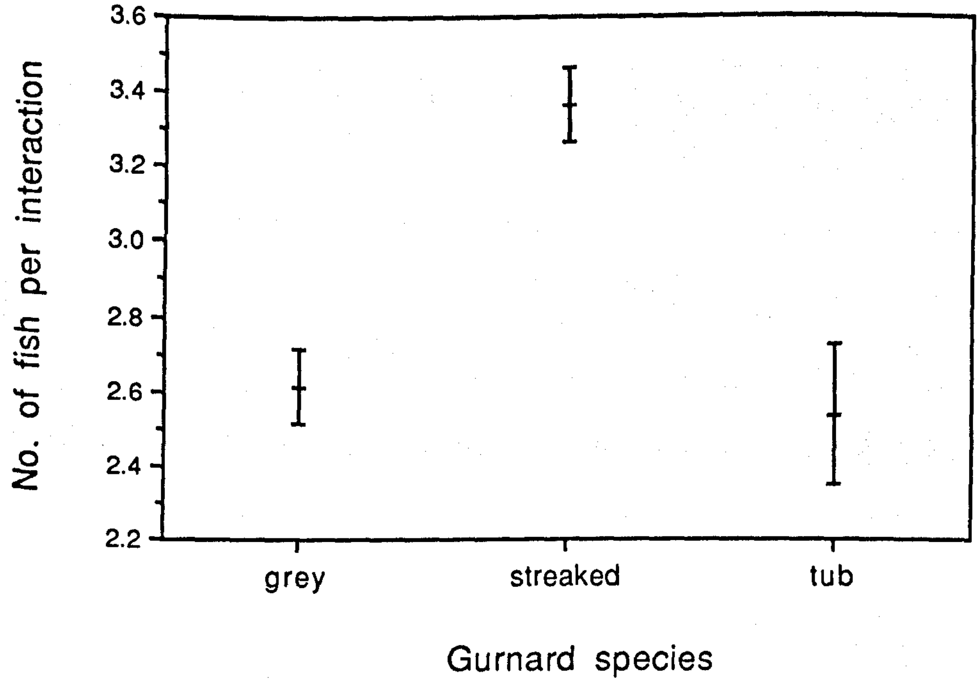


Figure 4.21 - Mean number of fish involved per interaction for the grey, the streaked and the tub gurnards. Bars indicate 95% confidence intervals.

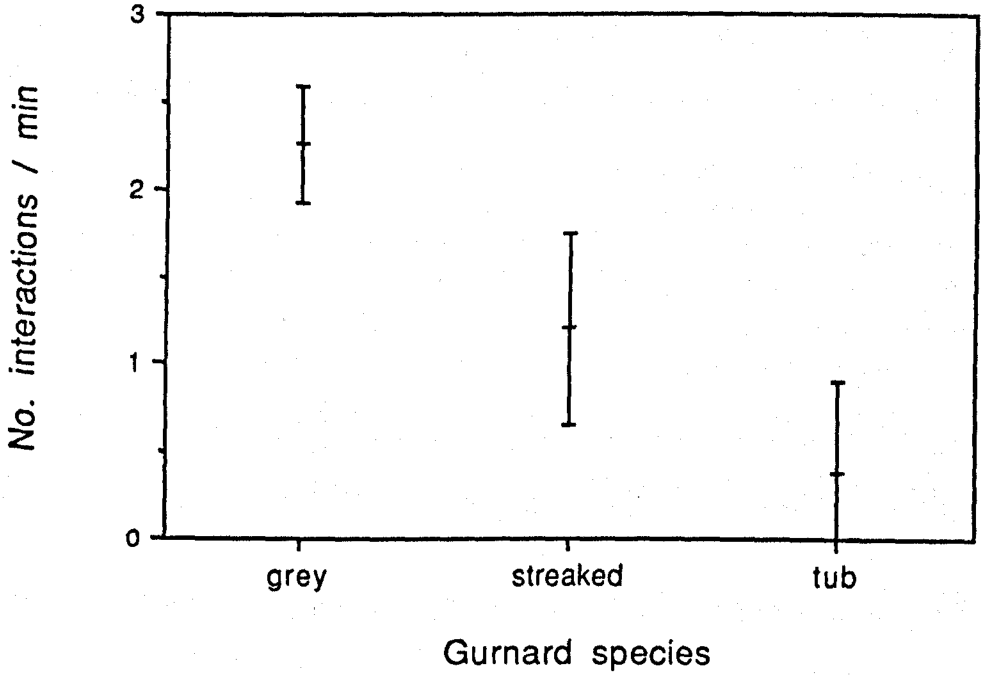


Figure 4.22 - Mean number of interactions / min for the grey, the streaked and the tub gurnards. Bars indicate 95% confidence intervals.

6 - Interaction duration

Interactions were significantly longer in the streaked than in the grey and the tub gurnards (Kruskal-Wallis test: N = 152, d.f. = 2, H = 91.71, P < 0.001) (Fig. 4.23).

7 - % of interactions accompanied by sound production

The proportion of interactions associated with sound production is greater in the grey than in the streaked gurnard (Fig. 4.24).

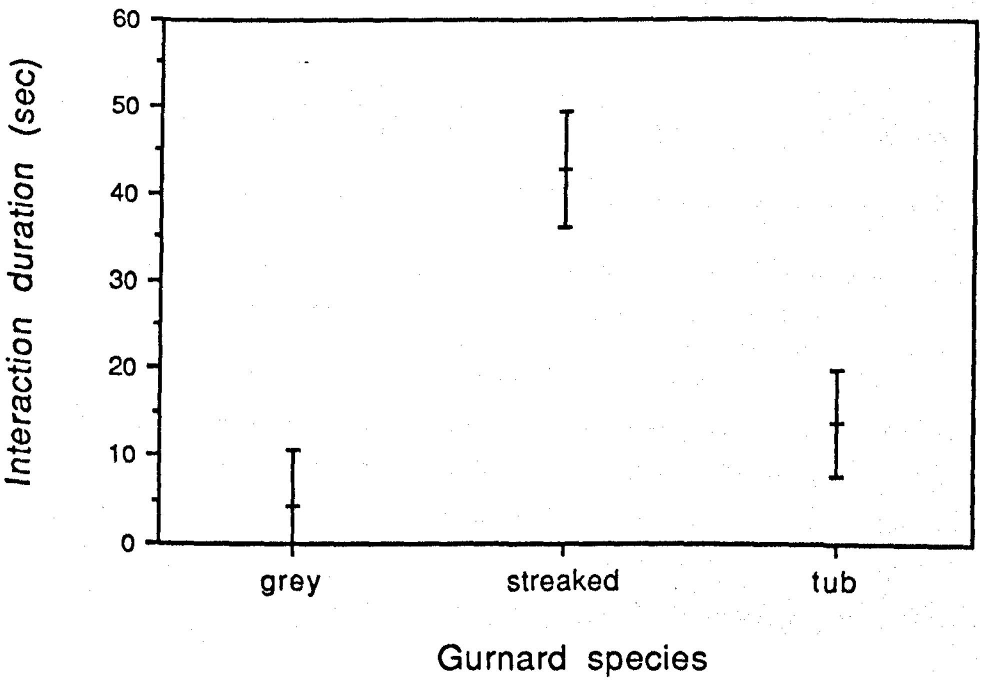


Figure 4.23 - Mean interaction duration for the grey, the streaked and the tub gurnards. Bars indicate 95% confidence intervals.

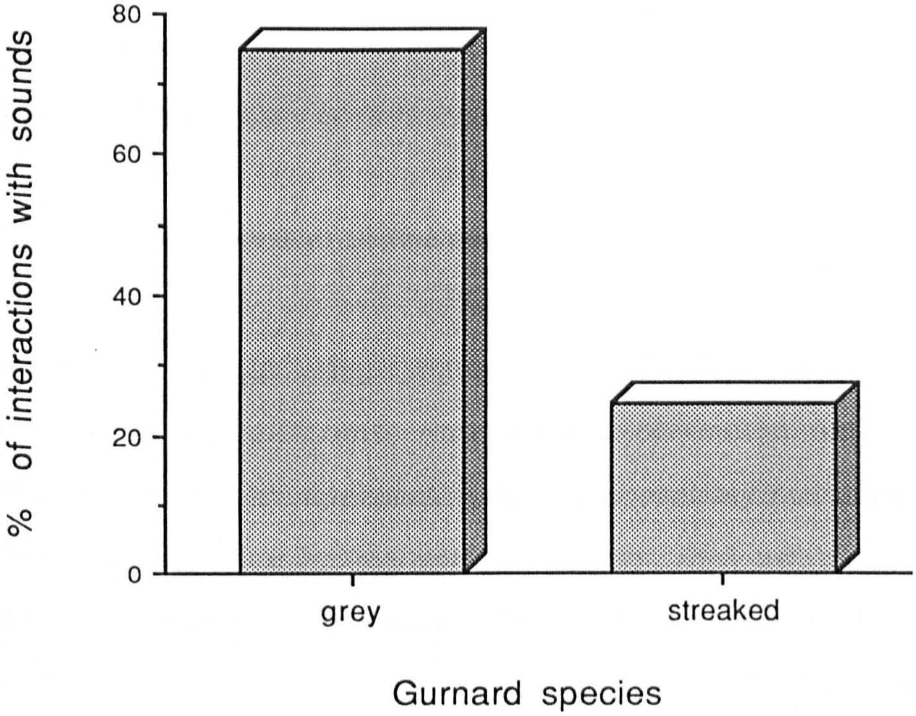


Figure 4.24 - Percentage of interactions accompanied by sounds for the grey and the streaked gurnards.

8 - Sequences of behaviour

The sequences of behavioural acts observed during competitive feeding interactions for the grey, the streaked and the tub gurnards showed some similarities. For individual fish of all 3 species, an item of food was either obtained directly by *grasping* it (e.g. by charging towards the food item and being the first to snap it, by *circling* around the feeding area until it is clear of other fish) and then usually *fleeing*, or *grasping* after performing agonistic behaviour. In the grey gurnard, the most probable behaviour leading to *grasp* was the agonistic behaviour *frontal display*. In the streaked gurnard it was *circling* that most often resulted in *grasping*. In the tub gurnard, all behavioural categories had similar probabilities of being immediately followed by a *grasp*. For this species, probably more interesting sequential relations would have been picked up if the analysed data set was bigger.

4.3.4.2 - The context of sound production

In the grey gurnard, the 2 sound types produced were associated with 2 different contexts. Grunts were mainly correlated with the behaviour *frontal display*, with *approach* + *chase* and possibly with *dash*. Knocks were associated with the behavioural acts *grasp*, *flee* and possibly *circle*. The growls uttered by the streaked gurnards were, like the grey gurnard knocks, uttered in a feeding context since growl production accompanied the behavioural categories *circle*, *feed*, *flee* and mainly *grasp*.

4.4 - DISCUSSION

4.4.1 - Feeding interactions

Ontogenetic changes in the grey gurnard

The ontogenetic changes in competitive feeding interactions of grey gurnards were based on variations of the interaction rate and on the type of acoustic emissions accompanying feeding interactions. Smaller fish interacted more and a larger proportion of their interactions were accompanied by grunt calls, probably associated with the larger frequencies of *frontal displays* observed per interaction. Indeed, grunt calls were highly correlated with this behaviour in all fish sizes (Table 4.7). Knock calls, however, played a greater role in the feeding interactions of larger fish, but, on the whole, the percentage of interactions accompanied by acoustic emissions was similar in all size classes.

Comparisons between species

A comparison of the feeding interactions between the grey, the streaked and the tub gurnards is given in section 4.3.4.2. Social behaviour played the least important role in feeding interactions in the tub gurnard (less than 20% of the total occurrence of behavioural categories), probably because the specimens studied were not showing their full repertoire. However, in the grey and the streaked gurnards more than half of the behavioural categories performed during interactions were social behavioural categories, of which *frontal displays* and *approach + chase* were the most frequent acts, performed by, respectively, the grey and the streaked gurnards. Overall, the grey gurnards seemed to be more vocal (see section 2.3) and aggressive than the other 2 species, consistent with the fact that this species, in comparison with the others, performed more *frontal displays* with which sound production was associated. The grey gurnard also showed a greater interaction rate than the streaked and the tub gurnards. In contrast, a greater number of individuals were involved per interaction in the streaked gurnards. Interactions were also longer (in duration and in the number of behavioural categories) in the streaked gurnards than in the other 2 species.

Overall, the range and the sequences of the behavioural categories observed for the 3 species showed similarities (see Figs. 4.6, 4.13 and 4.17). A foraging individual succeeded in *grasping* a food item by either avoiding confrontations (by avoiding periods when there were competitors in the feeding area or by being the fastest to reach a food item) or by performing agonistic behaviour such as *approaching, chasing* and *frontal displays.* The behavioural categories *approach* in the grey and the streaked gurnards, and *touch* in the latter species probably have the same function as was found in the stoneloach (*Noemacheilus barbatulus*) (Amorim 1994). The stoneloach seem to use these behavioural acts to assess whether conspecifics are *feeding* and to take advantage of the conspecific's ability to find food. Furthermore, stoneloach, like the streaked and the grey gurnards, were also observed to *grasp* food items spat out by their conspecifics, thus gaining from *approaching* conspecifics when they are handling food (Amorim 1994).

4.4.2 - Acoustic communication in gurnards

The main ways of examining the communicative values of fish sounds are (1) correlating different sound types with different behavioural categories or sound contexts (e.g. Torricelli *et al.* 1986), (2) playback techniques (e.g. Tavolga 1958b) and (3) comparisons between mute fish and unaltered controls (e.g. Valinsky & Rigley 1981, Ladich *et al.* 1992b).

In the present work, techniques 1 and 2 were carried out. Sound playback did not elicit any response from the grey gurnard. Freytag (1964) mentions that playback of grunts attracted the grey gurnards to the sound source and they emitted sounds at it, losing interest after some time, while Hawkins (1968) reports that this species did not react to sound playback, as found in the present work. Indeed, some workers have emphasised the limited response obtained with playback alone, and have suggested that sound production is only a part of a more complex signalling system (Hawkins & Myrberg 1983). It would be interesting to carry out experiments to disentangle the role of visual, acoustic, tactile and olfactory stimuli in gurnard's communication system. Many studies have however shown that various species of fish do react to sound playback alone (see review by Fine *et al.* 1977b). When sounds are played back without any other stimuli, there may be an increase in calling rates (e.g. in sea robins (Moulton 1956); toadfish (Winn 1972); squirrelfish (Horch & Salmon 1973)), in nest building behaviour (e.g. *Colisa lalia* (Schuster 1986)), in agonistic behaviour (e.g. in the satinfin shiner (Stout 1963), croaking gourami, *Thrichopsis vittatus* (Marshall 1965), skunk loach, *Botia horae* (Valinsky & Rigley 1981)), and in courtship displays (e.g. in the satinfin shiner (Stout 1963), sunfish (*Lepomis* sp.) (Gerald 1971)). There can also be an attraction to the loudspeaker (e.g. in males of *B. soporator* (Tavolga 1956, 1958b), toadfish (Winn 1967), sunfish (Gerald 1971), plainfish midshipman fish (Ibara *et al.* 1983) and bicolour damselfish, *Pomacentrus partitus* (Myrberg *et al.* 1986)). Agonistic sounds played back in addition to visual stimuli resulted in an increase in aggressive behaviour in the cichlid fish *Hemichromis bimaculatus* (Rowland 1978, Jeppesen 1981) and a decrease in the cichlid fish *Cichlasoma centrarchus* (Schwarz 1974b). Females of *B. soporator* are only attracted to the loudspeaker when a visual stimulus is also present (Tavolga 1956, 1958b). Spanier (1979) verified that sound playback increased courtship displays in bicolour damselfish, but sound was played back to a colony of fish, so visual stimuli were already present. When male courtship calls were played back to females in the absence of males no response was observed.

All species of gurnards studied uttered sounds during competitive feeding. The different sound types uttered by the grey and the streaked gurnard were correlated with the different behavioural categories observed in this context. Knocks and grunts uttered by different-sized grey gurnards were correlated with similar behavioural categories. The emission of growls by the streaked gurnards seems to have the same communicative function as knocks in the grey gurnard. These sounds were associated with the feeding behavioural categories *circle* and *grasp* in both species, and also *feed* in the streaked gurnard. Knocks and growls were also uttered by either species during the agonistic behaviour *flee*. Grunts produced by the grey gurnard were mainly associated with *approach + chase* and with *frontal display*, both aggressive behavioural categories. *Dash* (in fact *dash + circle* - see section 4.3.1.2) was also significantly correlated with grunt calls in the small grey gurnards. Although the association of sound production and behaviour was not studied in the tub gurnard, these fish were observed to utter grunts during the behavioural categories *orient* and *approach*, but mostly during *frontal displays*, as with the grey gurnard.

The behavioural categories correlated with each sound type in both species are highly associated in time as shown by the sequences of the behavioural categories observed for each species (see Figs. 4.6 and 4.13). This result was expected, as calls may be uttered for a longer time span than a particular behaviour takes to be performed. Indeed, a call that is associated with a particular behaviour may start before and end after the occurrence of that behaviour. This is especially true for the streaked gurnard which may emit calls in the order of seconds (see section 2.3.2). Hence, the observed association of growls with *circle* and *flee* may only reflect the fact that this species utters sounds in association with the behaviour *grasp*, as the sequence *circle* -> *grasp* -> *flee* was extremely frequent.

Sound production seems to have a communicative role in these gurnard species. Knock production in the grey gurnard, and also growls in the streaked, may reflect a state of feeding arousal caused by the sight of food or be associated with low levels of aggression, such as warning a possible competitor of its presence. This hypothesis is consistent with the fact that, in both species, knock and growl production was also correlated with *fleeing*, a defensive agonistic behaviour. On the other hand, the emission of grunts by the grey gurnard seemed to be associated with higher levels of aggression. In the grey gurnard, grunts seemed to be better correlated with the related behaviour than were knocks, which were less behaviour-specific. Growls emitted by the streaked gurnards were well associated with feeding behaviour, *grasp* in particular.

The emission of sounds seems to confer advantages to a foraging fish. When charging simultaneously with other individuals towards an item of food, sound production may deter the competitor fish and make it (them) *dash*, for example. The spread-out of fins in a *frontal display* may also be enhanced by grunting, especially bearing in mind that the interacting fish are often very close together.

Intra- and interspecific differences in sound production (based on the temporal patterning of the calls emitted, Chapter 2), and the association of different sound types with particular behavioural categories in the 3 gurnard species, provided some evidence of acoustic communication in these gurnards. It has been shown for some fish that temporal features of sounds, such as pulse repetition rate and phrase duration, enable species and individual recognition (Spanier 1979, Myrberg & Riggio 1985), and territorial defence (Ladich *et. al.* 1992b).

The role of sound production in feeding interactions has been rarely mentioned in the literature. Sound production by the croakers has been related to feeding activity (Fish 1954). In the Black Sea, increases in sound production by gurnards (species not identified, probably red and tub), in the evening and at night have been attributed to feeding and defensive activities (Protasov & Romanenko 1962 in Protasov 1965). Moulton (1958b) states that the intensive acoustic activity of *H. rufus* observed during the day can be explained by their feeding and defensive relationships. Also, sound production outside the breeding season in the haddock has been linked to competitive feeding interactions (Hawkins 1993). Furthermore, few studies on the role of sound emissions in agonistic behaviour concern non-territorial fish. For example, the menpachi (*Myripristis berndti*) is a schooling fish, and produces grunts and stacatto calls that act as warning calls and 2 other call types that serve to maintain distance between fish in the school (Salmon 1967). The river bullhead displays agonistic behaviour all year round and utters sounds during threatening and *chasing* (Ladich 1989). Croaking gouramis achieve dominance through fighting in which sound production plays an important role. Ladich *et al.* (1992b) have demonstrated that soniferous males with similar sizes had a higher probability of winning contests than did mute fish. *Chromis viridis* utters click-like sounds associated with *chasing* behaviour (Amorim 1996). The present work provides the first detailed quantitative information on the use of acoustic emissions during competitive feeding in fish. Also, it adds to the information available regarding the significance of acoustical signals in non-territorial fish species.

Although the role of growls uttered by the grey gurnard was not studied in the present work, it may differ from that of the grunt and knock calls. Furthermore, since the grey and the tub gurnards utter sounds during social behaviour, it is possible that sound production might be used in different contexts, other than feeding, but associated with the same behavioural categories. In addition, it is possible that the species studied utter these sounds associated with other behavioural categories when produced in different contexts, and also emit other types of phrases. It would be very useful to study the role of sound production in the courtship of gurnards since it is likely that European triglids are very noisy during the reproductive season as has been reported for the American species (Moulton 1956). If European triglids do make sounds during the reproductive season it would be reasonable to expect these sounds to be growling sounds, as other species, such as the haddock, utter longer sounds and with a faster pulse repetition rate as spawning approaches (Hawkins 1993).

CHAPTER 5

METABOLIC COSTS OF SOUND PRODUCTION IN FISH -

A THEORETICAL APPROACH

5 - METABOLIC COSTS OF SOUND PRODUCTION IN FISH - A THEORETICAL APPROACH

5.1 - INTRODUCTION

It is usually accepted that the costs of producing acoustic signals underwater are high (Ladich 1990), although the costs of sound production have never actually been studied in teleosts. In general, the costs of producing signals may include ecological costs such as the attraction of predators (e.g. Ryan *et al.* 1982) or retaliation by conspecifics (e.g. Enquist 1985, Møller 1987), and physiological energy costs (e.g. Ryan 1988), which also have ecological implications related to survival (Priede 1977).

Metabolic costs of sound production are mainly related to the energy expenditure associated with muscle contraction. It is possible to approach this by looking at the actual work done by the musculature associated with sound production. However, the rate of oxygen consumption has been usually used to estimate the costs of particular activities such as swimming in fish (Videler 1993), the flight of insects (Ellington 1985) sound production in different groups of animals such as insects (e.g. MacNally & Young 1981), amphibians (e.g. Bucher *et al.* 1982), birds (e.g. Eberhardt 1994) and bats (Speakman *et al.* 1989). It is easy to measure oxygen consumption and direct conversion can be made to energy consumption. Any activity undertaken by an animal should fall within the metabolic scope, that is the difference in energy consumption between maximum activity and rest, in order to minimize the probability of mortality (Priede 1985). In some fish, for example, feeding to satiation seems to leave little metabolic scope for any other activity, such as swimming (Soofiani & Hawkins 1985). On the other hand, sustained swimming activity may be capable of using up to the maximum of the metabolic scope, leaving fish with a permanent conflict between the needs of locomotion and feeding (Priede 1985). In this context, the costs of sound production in fish may be especially important since aggressive and courtship behaviour (during which vocalisations are most common) are already very costly involving as they do active and often sudden movements of the body and fins, often lasting for extended periods (Brett & Groves 1979).

In this Chapter, a theoretical calculation of the metabolic costs of sound production in fish was made, based on background information on fish energetics. In addition comparisons were made for vocalisation costs between different groups of animals

5.2 - METHODS

There is no information available on metabolism in triglid fish. Their metabolism was assumed to be intermediate between that of cod, having a very small proportion of red sustained swimming muscle, and of salmon, which has more. Oxygen consumption during standard metabolic rate (SMR) and active metabolic rate (AMR) in a 150 g cod is respectively 132 and 322 $mgO_2Kg^{-1}h^{-1}$ (15°C), where SMR is the oxygen consumption of an unfed fish at rest and AMR is the oxygen consumption at a sustained maximum level of activity, usually swimming (Soofiani & Priede 1985). SMR and AMR for a 100g sockeye salmon (*Oncorhynchus nerka*) are respectively 75 and 790 $mgO_2Kg^{-1}h^{-1}$ (15°C) (Brett 1964, Brett & Groves 1979). An oxicalorific value of 13.6 $J.mg^{-1}O_2$ (Elliot & Davidson 1975) was used to transform oxygen consumption into energy values. Thus, it can be assumed that an "intermediate" fish has a standard metabolic rate of about 50 mW and an active metabolic rate of 240 mW (note that 1 $Js^{-1} = 1$ W).

Altringham & Johnston (1990) found a maximum power output for fast (glycolytic) muscle fibres of 25 - 35 WKg^{-1} (5 - 7 Hz; 5°C), and for slow (oxidative) fibres of 5 - 8 WKg^{-1} (2 Hz; 5°C) for a teleost fish, the bullrout (see Peters (1989) for a review on the structure of vertebrate skeletal muscles). Since fish sonic muscles are fast oxidative glycolytic fibres (Fine & Pennypacker 1988) an intermediate power output of 20 WKg^{-1} was assumed. This is in agreement with Walsh *et al.* (1995) who stated that the sonic muscles, although capable of repeated superfast contraction, may have a much lower work load than a typical white muscle used in an escape response to power the fish against the drag of water. The actual energy consumption of the muscle, however, should be about five times more than the muscle power output since muscles are only about 20% efficient (Webb 1971a, b). An expected energy expenditure for these muscles would be therefore about 100 WKg^{-1}.

The common techniques to measure aerobic metabolism (such as determining oxygen consumption) found in the literature, give average metabolic power (in contrast with peak metabolic power, see Fig. 5.1), that is, the average energy usage during all phases of contraction and relaxation of the muscles (Prestwich 1994). In order to make comparisons with other animals, average metabolic power during sound production by fish should be calculated. Knowing the mass of the sonic muscles of triglids (section 3.3.1) and the expected chemical energy consumption (power input) for the assumed power output of the muscle, it is possible to calculate the energy expenditure of sound production per second of continuous work by multiplying the 2 values. Average energy expenditure during calling bouts can be calculated by adding the muscle energy expenditure during rest periods. Metabolic costs were extrapolated for different activity rates of sound production.

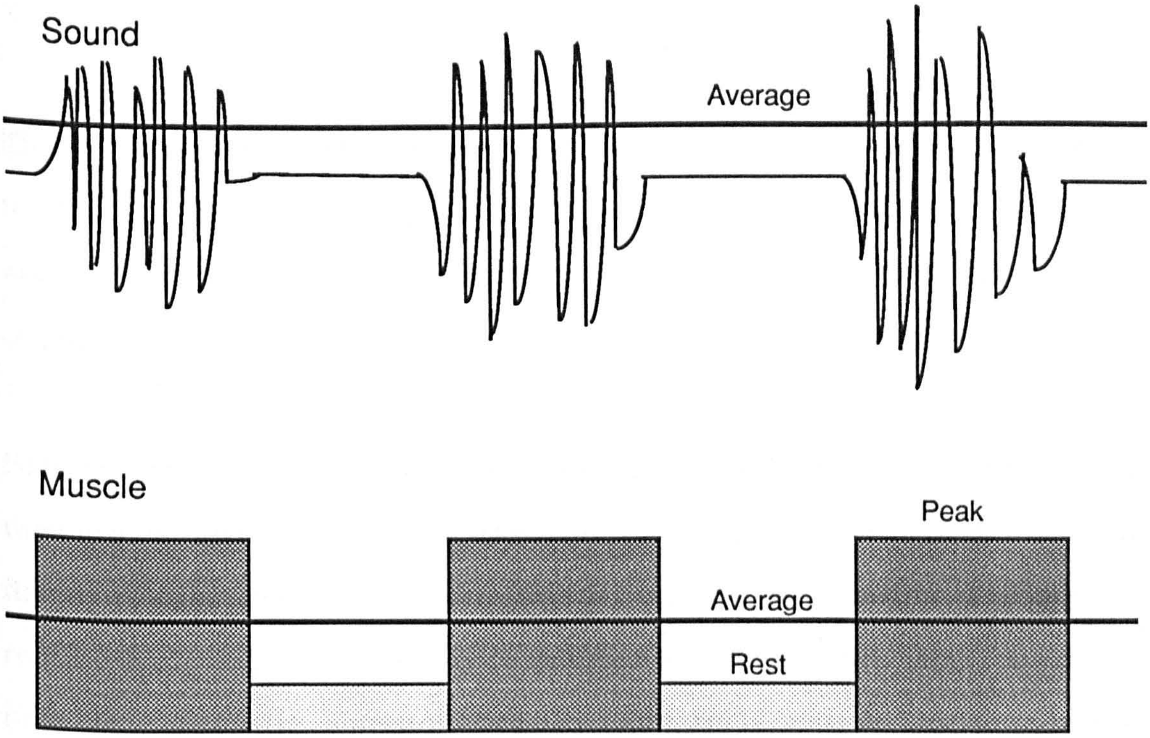


Figure 5.1 - Representation of the peak and average metabolic power of the sonic muscle (modified after Prestwich 1994). Fish calls are made up of phrases and silence periods. When the fish is producing a sound, the sonic muscle is at maximum activity (peak) and the energy expenditure increases (high bars); between phrases the muscle is resting and the energy expenditure decreases (low bars).

One way to determine cost of sound production in terms of energy expenditure is to compare the cost to the standard metabolic expenditure (the SMR). The factorial metabolic scope (the number of times metabolism must be raised during calling or during any particular activity as compared to rest (e.g. Ryan 1988)) was used to compare the theoretical costs of sound production in fish, with other activities and with the costs of sound production in other groups of animals.

5.3 - RESULTS

The typical sonic muscle mass of a 125 g grey gurnard (with an approximate total length of 25 cm) is approximately 0.6 g (see section 3.3.1.1). As the assumed power input of the muscle is 100 WKg^{-1} then the metabolic power of the sonic muscles is 0.0006 Kg times 100 W, which equals 60 mW.

Fish can produce sounds at different rates depending upon which activity they are engaged in. Three different calling states are considered here. The first is routine calling, which considers that fish are uttering sounds on a routine basis (e.g. upon fish encounters, etc.) but are not engaged in any prolonged acoustic emission activity. For such routine calling, sound production rates obtained during the study of the diel rhythms of sound production in the grey gurnard (see section 2.3.1.1) were used. The second state of sound production activity is associated with fish calling during aggressive bouts. The rate of sound production observed for medium and large grey gurnards during competitive feeding bouts were used for these calculations. The third state considered calling rates observed for the toadfish during courtship. During the mating season, males from this species can vocalise for hours at a time (Walsh *et al.* 1995).

Routine calling

The mean duration of knock and grunt calls in the grey gurnard is approximately 1.3 s; a knock or grunt call is made up on average of 3.3 phrases and the mean knock / grunt phrase duration is about 41 ms (see Tables 2.4 and 2.5). In a 1.3 s call only 135 ms (3.3 * 41 = 135.3 ms) corresponds to sound production. This means that only 104 ms (135/1.3) per second of calling involves actual sound production. This will correspond to an energy expenditure of 6.24 mJ (60 mJs^{-1} * 0.104 s) per second through contraction of the sonic muscles. During the 0.896 s of the second (1 - 0.104 =

0.896), while the muscle is resting, an energy expenditure of 44.8 mJ (50 mJs^{-1} (assumed SMR) * 0.896 s) is expected. The total average energy expenditure per second of calling will be 6.24 + 44.8 = 51.04 mJ. The factorial metabolic scope in a second of calling will then be 51.04 / 50 = 1.02.

The grey gurnard uttered on average 0.39 calls per 10 min of elapsed time sampled at random during 144 hours (see section 2.3.1.1), which means that they emitted on average 0.039 calls per min or 2.3 calls per hour. Grey gurnards calling in a routine fashion only spend 50.7 ms (0.039 * 1.3 s) per min or 2.99 s (2.3 * 1.3 s) per hour calling. The increase of energy expenditure due to sound emission is therefore negligible during routine calling.

Calling during competitive feeding bouts

Medium and large grey gurnards produce on average 63 sounds or 13 calls per 5 min interval, while being fed. Using an average call duration of 1.3 s (see Table 2.5), then they would spend 16.9 s calling during this 5 min period. The average energy expenditure associated with calling would be 51.04 mJs^{-1} * 16.9 = 862.5 mJ. If rest is assumed for the rest of the feeding period (300 s - 16.9 s = 283.1 s), than approximately 14.155 J (50 mJs^{-1} (assumed SMR) * 283.1 s) would be spent. The total energy expenditure during this 5 min would be 0.863 + 14.155 = 15.018 J. If the fish was at rest it would spend 15 J of energy (50 mJs^{-1} (assumed SMR) * 300 s) in the same period. Therefore, the factorial metabolic scope would be 15.018/15 = 1.00. Again the energy expenditure in sound production during feeding bouts is expected to be negligible.

Calling during courtship

Male toadfish (*Opsanus tau*) emit boatwhistles during the mating season. Typically, these fish utter 300 ms calls about 15 times a minute (Fine pers.

com.), which means that they spend, on average, 4.5 s per min calling. Assuming a weight of 2 g for the sonic muscles (Fine *et al.* 1990) and also a power input of 100 Wkg^{-1}, then the metabolic power of these muscles is 0.002 * 100 = 0.2 W or 0.2 Js^{-1}. The amount of energy spent per min calling would be therefore, 4.5 s * 0.2 Js^{-1} = 0.9 J (associated with calling) plus the energy spent resting during 55.5 s (60 s - 4.5 s), 55.5 s * 50 mJs^{-1} (assumed SMR) = 2.8 J. If the toadfish was resting during the whole min, it would spend energy resting: 60 s * 50 mJs^{-1} (assumed SMR) = 3 J. The factorial metabolic scope would then be (0.9 + 2.8) / 3 = 3.7 / 3 = 1.2, which is still very small.

A comparison of the costs of sound production between different groups of animals is given in Table 5.1.

5.4 - DISCUSSION

The energy cost of sound production is the increment in metabolism caused mainly by the muscles used in calling (Prestwich 1994). Costs of sound production are usually thought to be very high. Indeed, in insects and in anurans, that have received considerable attention, the factorial metabolic scope (the ratio of active to resting metabolism) ranges from 2 to 21 during sound production bouts (see Table 5.1). In these groups, advertisement calls are produced aerobically and the costs of calling are similar or exceed the aerobic costs of terrestrial locomotion (Prestwitch 1994). In Carolina wrens the cost of singing (3-9 times the SMR) is higher than the costs of other common activities of passerines (including perching, eating, gliding and preening) except flight (Eberhardt 1994). By contrast, the costs of sound production of other birds, the rooster and the red junglefowl, and of bats are negligible. Since birds and bats are endotherms, their basal metabolism

Table 5.1 - Factorial metabolic scope (FMS) during calling in different animals. n.d. = not detectable. (1) - Ryan (1988); (2) - Prestwich (1994); (3) - MacNally & Young (1981); (4) - Eberhardt (1994); (5) - Chappell *et al.* (1995); (6) - Horn *et al.* (1995); (8) - Speakman & Racey (1991); * - present study. sps = species.

Group	Species	FMS (call/rest)	Reference
Insects	Crickets (6 sps.)	4 - 13	(1); (2)
	Katydids (2 sps.)	7 - 8	"
	Chirpers (2 sps.)	2 - 4	"
	Cicadas (1 sps.)	21	(3)
Anurans	Frogs (8 sps.)	6 - 21	(1); (2)
Fish	Triglids / toadfish agonistic courtship	0 / 1.2	*
Birds	Carolina wren	3 - 9	(4)
	Red junglefowl	n.d.	(5)
	Rooster	1.15	(6)
Bats	*Pipistrellus*	n.d. during flight	(7)
	Plecotus	"	"

is high and the metabolic costs of sound production are probably more difficult to detect (Chappell *et al.* 1995). The costs of emitting sounds seem also to be negligible in fish, if all assumptions considered here hold.

Estimates of the energetic efficiency of sound production (acoustic power / net metabolic power) in animals indicate it is very inefficient (0.05% - 6% in insects and anurans), and large amounts of energy must be spent to generate noises (McNally & Young 1981, Prestwich 1994, Ryan 1988). However, the favourable properties of water as a medium for conductance of sound (Hawkins 1993), especially if sounds are of low frequency (Urick 1983) reduce the required intensity of the initial call signal, and hence probably the costs of its generation (Speakman *et al.* 1989). This may explain why sound production seems so "cheap" in fish. Furthermore, it is possible that the efficiency of sound production in fish is higher than in other animals, also decreasing the expected costs of vocalisations. Indeed, the pulsations of the swimbladder due to the contraction of the sonic muscles are transmitted through the tissues of the fish to the outside with little loss (De 1981). However, studies on the metabolic costs of sound production in different fish species uttering call at different rates rates would be required in order to make further conclusions.

Costs of sound production may be put into perspective by comparing them with the costs of other activities, such as locomotion. In insects, for instance, terrestrial locomotion involves an increase of metabolism of 4 to 6 times while insect flight amounts to an increase in the order of 30 to 200 times the resting metabolic rate (Prestwich 1994). In birds, sustained flight may result in an increase of metabolism in the order of 11 - 23 times (Eberhardt 1994), while in bats the increase of the metabolic rate during flight averages 21 to 14.6 times respectively in small and large species (Carpenter 1985, Racey & Speakman 1987). Typically, the metabolic scope is more restricted in fish than in birds or mammals. In fish, swimming activity is capable of taking up all the metabolic scope, which represents, for example, an increase in the standard metabolism of the order of 2.4 in the cod and of 7.3 in the brown trout (*Salmo trutta*) (Priede 1985). Other activities in fish may also be quite costly. In a study on the juvenile cod, Soofiani & Hawkins (1985) showed that the increase in oxygen consumption due to feeding may take as much as 98% of the metabolic scope. Likewise, swimming activity in fish is capable of taking up all the metabolic scope (Priede 1985). Furthermore, studies carried out with pumpkinseed fish (*Lepomis gibbosus*) and young sockeye salmon engaged in aggressive contests, showed that energy expenditure amounted to one half of the active metabolic rate when territory dispute was intense (Brett & Groves 1979). It seems, therefore, that for triglids engaged in competitive feeding, when the fish are feeding, swimming and engaged in aggressive displays, may benefit greatly from the low costs of sound production, since increased time spent at high-activity levels approaching the active metabolic rate may imply death (Priede 1977).

The acoustic features that contribute to determine total energetic costs include repetition rate, call duration and intensity (Prestwich 1994). In this study, both call repetition rate and call duration were taken into account in the theoretical calculations of the costs of sound production in fish. Differences in sound intensity between triglids and toadfish were not considered but may be quite large, the boatwhistles of the latter species being probably much loader. Furthermore, differences in the costs of uttering sounds with different pulse repetition rate were not considered but are probably higher for growls than for grunts or knocks of similar duration. The call parameters considered for the calculations took values intermediate between those for knocks and grunts.

CHAPTER 6

SOUND PRODUCTION IN OTHER SPECIES

6 - SOUND PRODUCTION IN OTHER SPECIES

6.1 - INTRODUCTION

Myrberg (1981) listed over 50 families of fish which included soniferous species. Many attempts at describing fish sounds have been made in the past, but it is still not known how widespread sound production is among teleosts. Also, little is known about the biological significance of the sounds uttered in different contexts (for a review see Hawkins & Myrberg 1983). In this Chapter, 13 fish species other than gurnards were examined for sound production. When a species was found to emit sounds, the accompanying behaviour was also noted. The species studied are listed in Table 6.1. Acoustic emissions from the first 8 species mentioned in Table 6.1 were recorded successfully, but no sounds were heard from the last 5 species of fish, during the period of study.

Only the tadpole fish, the armed bullhead and the short-spined sea scorpion were studied previously and their sounds have been described by Hawkins & Rasmussen (1978), Hawkins (1968) and Bass & Baker (1991), respectively. The acoustic repertoire of the parental species of the *Amphiprion* hybrids was studied by Schneider (1964), Takemura (1983) and Chen & Mok (1988). Fourmanoir & Laboute (1976) and Thresher (1982) mentioned that the emperor angelfish grunted during agonistic encounters and courtship, but they did not provide any description of these sounds.

In this study, the sound-producing mechanism was examined in the shore rockling, the armed bullhead and the horse mackerel. The mechanism of sound production in short-spined sea scorpion was described by Barber & Mowbray (1956) and Bass & Baker (1991). The sound-producing apparatus of the tadpole fish was described by Hawkins & Rasmussen (1978).

Table 6.1 - Species examined for sound production in the present study.
* - Clown fish hybrids (first generation) of the fire clown *Amphiprion frenatus* Bervoort, 1856 and the yellow tailed clown *A. clarkii* (Bennett, 1830).

	Species	Family
1	Shore rockling, *Gaidropsarus mediterraneus* (L., 1758)	Gadidae
2	Tadpole fish, *Ranicepus raninus* (L., 1758)	Ranicipiteridae
3	The blenny, *Parablennius pilicornis* (Cuvier, 1829)	Blenniidae
4	Two-banded sea bream, *Diplodus vulgaris* (E. Geoffroy Saint Hilaire, 1817)	Sparidae
5	Gold, cobalt and black-barred (BB) morphs of the *Pseudotropheus zebra* complex	Cichlidae
6	Emperor angelfish, *Pomacanthus imperator* (Bloch, 1787)	Pomacanthidae
7	Blue-green damselfish, *Chromis viridis* (Cuvier, 1830)	Pomacentridae
8	Clown fish, *Amphiprion* sp.*	Pomacentridae
9	Gilthead sea bream, *Sparus aurata* L., 1758	Sparidae
10	Sea bass, *Dicentrarchus labrax* (L., 1758)	Serranidae
11	Armed bullhead, *Agonus cataphractus* (L., 1758)	Agonidae
12	Horse mackerel, *Trachurus trachurus* (L., 1758)	Carangidae
13	Short-spined sea scorpion, *Myoxocephalus scorpius* (L., 1758)	Cottidae

6.2 - GENERAL METHODS

Fish calls were monitored, recorded and analysed as described in sections 2.2.4 and 2.2.5. Before the beginning of each recording session running water and aeration was stopped. In the case of species 3, 4, 6, 7 and 8 (see Table 6.1), which were recorded in the Vasco da Gama Aquarium Exhibition Centre in Lisbon (Portugal), recordings were carried out when the Aquarium was closed to the public or at periods of low public attendance (i.e. when the extraneous noise conditions allowed recordings). Furthermore, because these species shared their aquaria with other fish species, which included sound producers, only the sounds that could be unequivocally attributed to the fish in study were included for analysis. Particular behavioural acts usually identified the individual producing sounds.

Species 1, 2 and 9 - 13 (see Table 6.1) were fed during the recordings to increase the probability of observing sounds related to competitive feeding and to agonistic encounters.

The following sound features were measured: pulse and phrase duration (ms); number of pulses in a phrase; pulse peak to peak interval (interval between the peak amplitude of 2 pulses, ms); peak frequency (frequency at the highest sound amplitude, Hz) (filter bandwidth - 125 Hz); minimum and maximum frequency (frequency range, Hz) (filter bandwidth - 7 or 15 Hz); interval between phrases (ms) when produced in bursts; call duration (ms). For definitions of call, phrase and pulse see section 2.2.5. Because recordings were carried out most often in small tanks, possible reverberation from the tank walls may have affected the sounds analysed (Parvulescu 1967).

6.3 - THE SHORE ROCKLING - *Gaidropsarus mediterraneus*

Two specimens of *G. mediterraneus* (13.8 and 20.0 cm SL) were collected at Parede (9° 22' W, 38° 41' N) and Arrábida (8° 59' W, 38° 28' N) on the west coast of Portugal in December 1994. They were captured underwater at night and in tide pools, with the help of hand nets. Fish were kept one in each half of a 0.70 m (l) × 0.40 m (w) × 0.38 m (h) glass aquarium, separated by an opaque partition. The aquarium was supplied with biological filtration and aeration. Rocks were provided for shelter. The photoperiod was natural and the water temperature was 15.5°C. Fish were fed with fish and mussels. This study was carried out in Vasco da Gama Aquarium Exhibition Centre in Lisbon, Portugal, during December 94 - January 95. Before the beginning of each recording session, the partition that separated the shore rocklings was removed to allow the 2 fish to interact.

Only thump-like sounds were heard from the 2 specimens studied. A description of the physical features of the 64 thumps analysed is presented in Table 6.2 and a sonogram and oscillogram of a typical thump is presented in Figure 6.1. The number of pulses and the pulse peak to peak interval are not specified in Table 6.2 since the pulse structure was not always clear. Pulse duration was only measured from pulses that presented a clear structure.

Sounds were uttered during *chasing* and *biting*. These aggressive interactions and acoustic emissions were always a result of the smaller fish *approaching* or *intruding* upon the larger fish's shelter. Part of the interactions (16.9%) could not be observed because they took place behind the shelter. It was not clear which of the 2 participants in an encounter produced the sounds recorded.

Table 6.2 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features (see section 6.2 for explanations) of the thump sounds produced by *G. mediterraneus.*

Sound parameters	N	mean	s.d.	min	max
Phrase duration (ms)	64	80.969	30.515	27.5	197.9
Pulse duration (ms)	44	6.859	1.691	4.6	13.1
Peak frequency (Hz)	64	180.953	75.901	82.0	409.0
Min frequency (Hz)	61	47.951	16.092	23.0	105.0
Max frequency (Hz)	61	736.279	249.604	251.0	1673.0
Interval between phrases (ms)	36	1004.981	923.013	51.4	3178.9

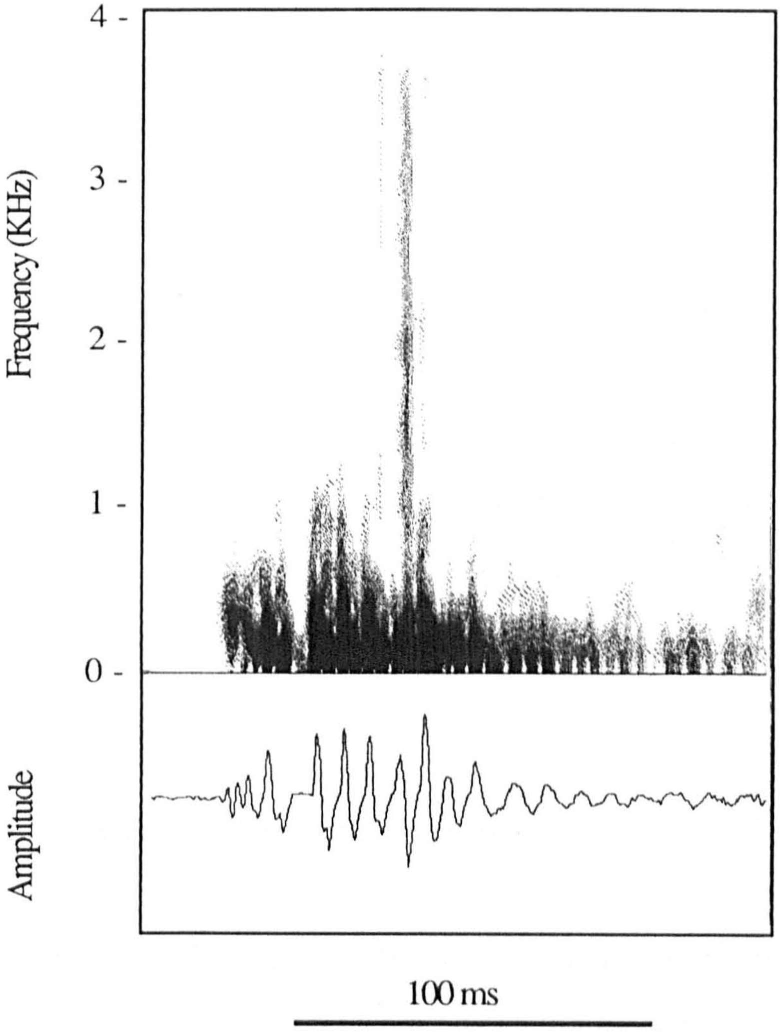


Figure 6.1 - Sonogram and oscillogram of a thump produced by *G. mediterraneus* (filter bandwidth = 125 Hz).

Examination for a sound-producing apparatus showed that this species possesses a swimbladder, but with no intrinsic sonic muscles attached to it, unlike other Gadidae which are sound producers (Hawkins & Rasmussen 1978). The swimbladder may still be involved in sound production through being vibrated under the action of body muscle contraction (Tavolga 1971).

Many gadoid species show well-developed agonistic behaviour, and a few species emit sounds in both agonistic and courtship contexts (for a review see Hawkins & Rasmussen 1978). Although Hawkins & Rasmussen (1978) suggested that neither the 3- nor the 5-bearded rockling were vocal (*Gaidropsarus vulgaris* and *Ciliata mustela*, respectively), the present study showed that the shore rockling is capable of producing sounds. The agonistic behaviour of the shore rockling is described by Almada *et al.* (1996). Sound emission in this species occurs during *chases* associated with disputes over a shelter. Consistently, other benthic resident fish of the rocky littoral also present agonistic behaviour related to patterns of space occupancy and disputes over access to shelter sites (Gibson 1968).

Hawkins & Rasmussen (1978) concluded that the smaller species of gadoids were not vocal, probably to lessen vulnerability to predation. The shore rockling is nocturnal (Almada *et al.* 1996), a situation where acoustic communication becomes especially advantageous.

6.4 - THE TADPOLE FISH - *Ranicepus raninus*

Two tadpole fish of approximately 12.0 and 18.0 cm TL were captured in crab traps in the subtidal zone of Stonehaven Bay, Scotland, and were kept at the Marine Laboratory in Aberdeen in a glass aquarium with the dimensions of 0.70 m (l) × 0.35 m (w) × 0.40 m (h) with running sea water, aeration and a sandy bottom. PVC tubes were provided as shelter. The photoperiod was controlled by a computer to resemble the natural cycle. Water temperatures ranged from 7° to 11°C. Krill was given as food 3 times a week. Fish were monitored for sound production from January to March 94.

The tadpole fish studied produced low frequency growl-like sounds during agonistic encounters. Fish spent most of their time hiding in their shelters. However, when food was given, they swam out of the shelters to forage. Agonistic encounters resulted from the fish getting too close when feeding and consisted of sound emission, which resulted in one or both fish *fleeing*. This situation did not occur many times during this study, probably because of the different sizes of the fish, and therefore the outcome of any encounter was predetermined. Most often, when the small fish came out of the shelter and saw the larger fish already feeding, it retreated back to its shelter, where it stayed until the other fish had stopped feeding.

Only 8 growls were heard and analysed, and the description of their physical features is presented in Table 6.3. Figure 6.2 depicts a sonogram and oscillogram of a growl.

Only Hawkins & Rasmussen (1978) had previously reported and described sound production in this species. These authors only studied single specimens from this species that uttered sounds when alarmed. The sounds reported were shorter than the ones analysed in the present study, but with a similar frequency range and peak frequency.

Hawkins & Rasmussen (1978) studied the sound apparatus of the tadpole fish and stated that it possessed very large extrinsic sonic muscles running around the swimbladder.

Table 6.3 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features (see section 6.2 for explanations) of the growls produced by the tadpole fish.

Sound parameters	N	mean	s.d.	min	max
Phrase duration (ms)	8	167.70	95.31	64.0	378.9
Pulse duration (ms)	8	9.12	0.66	8.3	10.1
Peak - peak interval (ms)	8	9.91	1.15	8.0	11.4
Peak frequency (Hz)	8	253.23	15.08	222	275
Min frequency (Hz)	8	94.63	31.75	52	275
Max frequency (Hz)	8	682.38	298.71	155	961

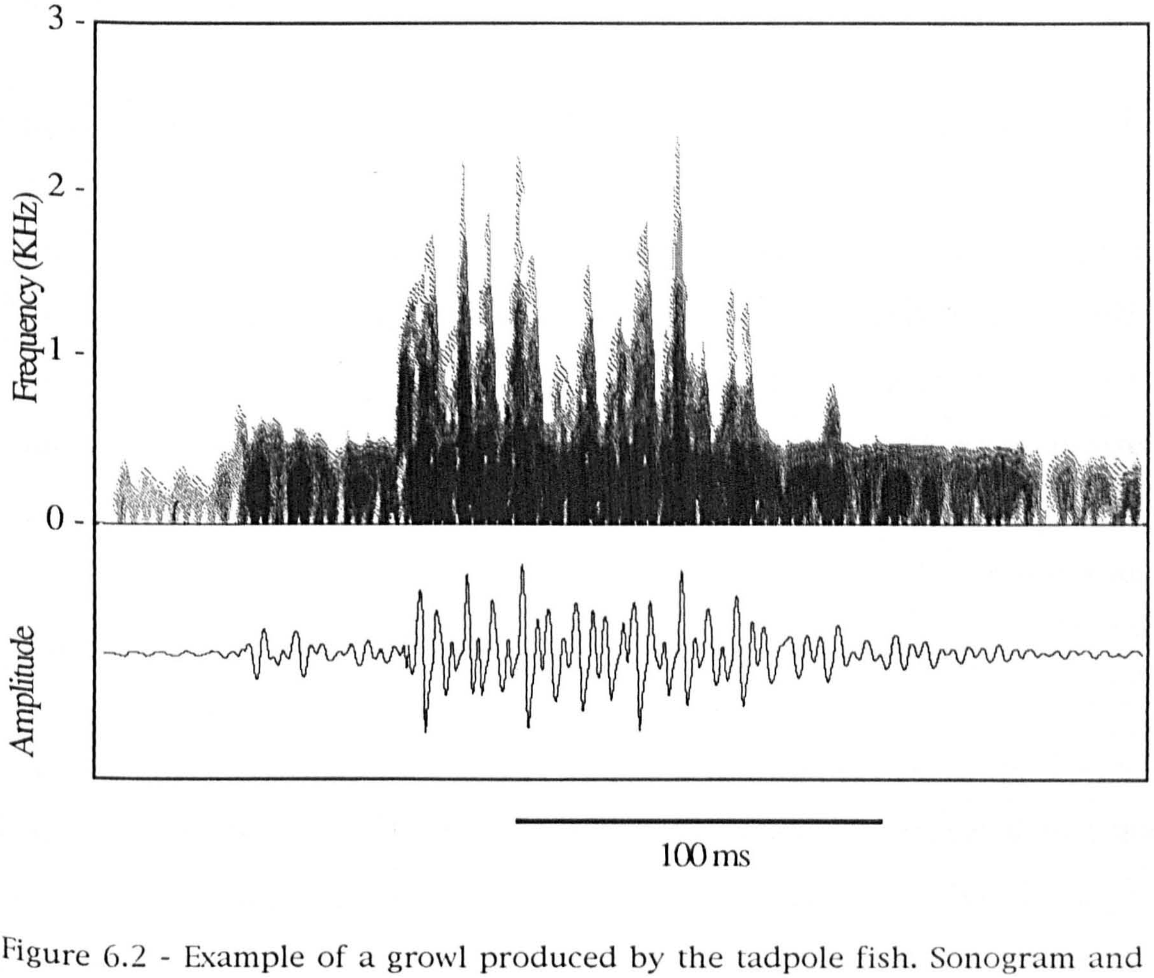


Figure 6.2 - Example of a growl produced by the tadpole fish. Sonogram and oscillogram of the growl sound (filter bandwidth = 125 Hz).

6.5 - THE BLENNY - *Parablennius pilicornis*

A male and a female of *P. pilicornis* (≈ 12 cm TL) were examined for sound production during their breeding period. The blennies were already kept in a glass display aquarium (1.23 m (l) × 0.50 m (w) × 0.64 m (h)) in Vasco da Gama Aquarium Exhibition Centre in Lisbon, Portugal, where they had just recently spawned. Because the blennies were kept for exhibition, the aquarium was shared with other fish species: *Dicentrarchus labrax* (Serranidae); *Symphodus (Crenilabrus) bailloni* (Labridae); *Coryphoblennius galerita* (Blenniidae) and *Lepadogaster* sp. (Gobiesocidae). The aquarium was provided with aeration, biological filters, running water from a closed circuit, and also a sandy bottom, rocks, coral and anemones. The water temperature ranged from 15.5° to 17.5°C during the period of study: December 94 - January 95.

Nineteen knocks in total were heard from the male and female blennies. The male *P. pilicornis* produced the great majority of the sounds heard (79%). The male and female blennies occupied territories at opposite sides of the aquarium, which they defended by *chasing* any intruder away (either the other blenny or any other fish in the aquarium). The area that the male blenny defended included a recently laid batch of eggs. Sound emission always occurred during *chases* but not all *chases* were accompanied by knocks. The characteristics of these sounds are depicted in Table 6.4 and Figure 6.3.

Sound production has been described for a few other blenny species (see Tavolga 1958b and Tavolga 1960), but only males were reported to make sounds and solely during courtship (while attracting the female to the nest). In the present study, the female of *P. pilicornis* also uttered knock sounds, although the male was heard more frequently. Furthermore, all

Table 6.4 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features (see section 6.2 for explanations) of the knocks produced by *P. pilicornis*.

Sound parameters	N	mean	s.d.	min	max
Phrase duration (ms)	19	10.437	4.302	5.3	19.5
No. of pulses	19	1.667	0.597	1	3
Pulse duration (ms)	19	6.047	1.033	4.9	9
Peak - peak interval (ms)	9	6.943	2.586	4.1	10.9
Peak frequency (Hz)	19	504.80	128.749	304	690
Min frequency (Hz)	19	162.93	79.635	70	351
Max frequency (Hz)	19	1318.53	781.723	363	3614

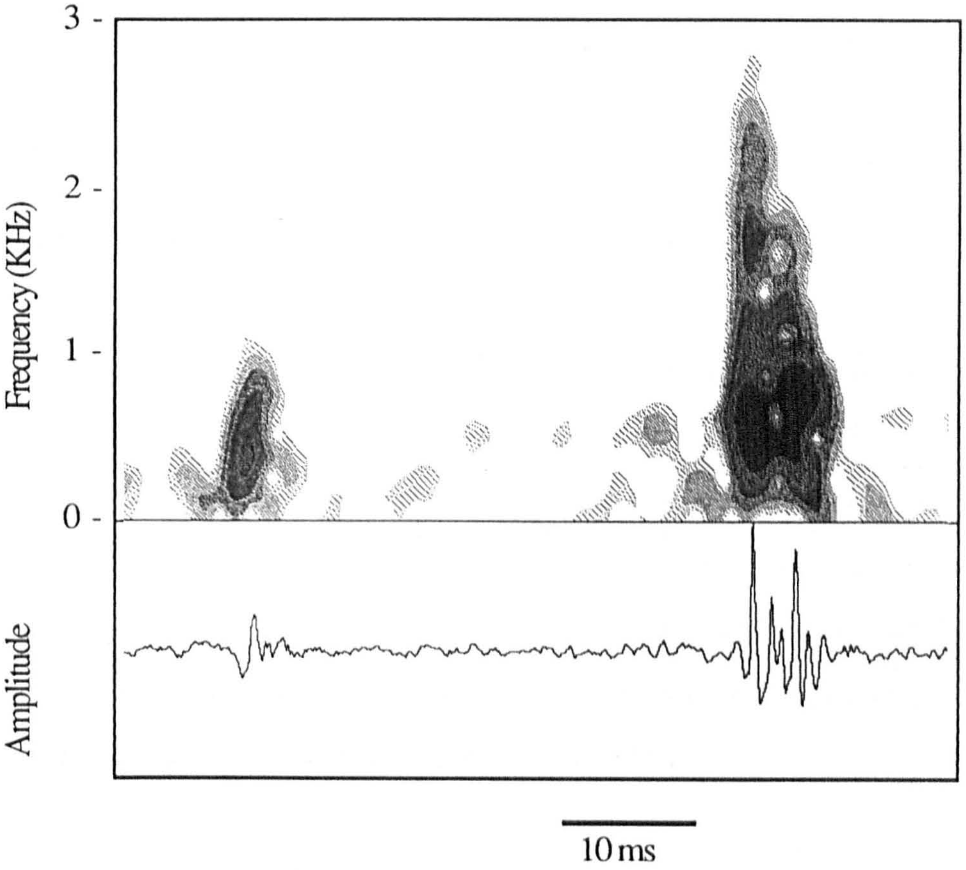


Figure 6.3 - Sonogram and oscillogram of 2 knocks produced by *P. pilicornis* (filter bandwidth = 125 Hz), made up of 1 and 2 pulses.

sounds recorded were emitted in an agonistic context, related to territorial defence, and not during courtship. The male was defending a nest site with recently laid eggs, a behaviour well documented for other blennies (e.g. Marraro & Nursall 1983, Hastings 1988). Tavolga (1960) described the sounds of other blennies (*Bathygobius soporator, Hypleurocheilus geminatus* and *Hypsoblennius hentz*) as dull thumps with a much longer duration and also with a lower peak frequency than the knocks of *P. pilicornis.*

Tavolga (1960) reported that sound production in 3 blenny species was accompanied by a quick sidewise shake of the head, a movement possibly related to the mechanism of sound production. These fish did not possess a swimbladder. No similar head movement accompanying sound production was observed in *P. pilicornis* and so presumably a different sound production method occurs in this species.

6.6 - THE TWO-BANDED SEA BREAM - *Diplodus vulgaris*

The 8 individuals (≈ 15 cm TL) examined for sound production were already kept in a concrete display tank with a glass front 4.10 m (l) × 2.00 m (w) × 1.45 m (h)), in Vasco da Gama Aquarium Exhibition Centre in Lisbon, together with the file fish *Balistes carolinenses* (Balistidae). The tank was provided with a sandy bottom, rocks, aeration, biological filtration and running water (closed circuit). The water temperature ranged from 15.5° to 17.5°C during the period of study: Dec. 94 - Jan. 95.

The two-banded sea bream uttered knock sounds during the *chasing* of conspecifics. Ten knocks were recorded and analysed. A description of the physical features and a sonogram and oscillogram of knocks is shown in Table 6.5 and Figure 6.4 respectively.

Table 6.5 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features (see section 6.2 for explanations) of the knocks produced by the two- banded sea bream.

Sound parameters	N	mean	s.d.	min	max
Phrase duration (ms)	10	16.75	5.87	9.0	28.5
No. of pulses	10	1.9	0.74	1	3
Pulse duration (ms)	10	8.80	1.22	7.5	11.5
Peak - peak interval (ms)	7	7.95	0.81	7.1	9.1
Peak frequency (Hz)	10	306.4	39.699	246	351
Min frequency (Hz)	10	148.4	20.576	117	175
Max frequency (Hz)	10	635.1	177.520	363	842

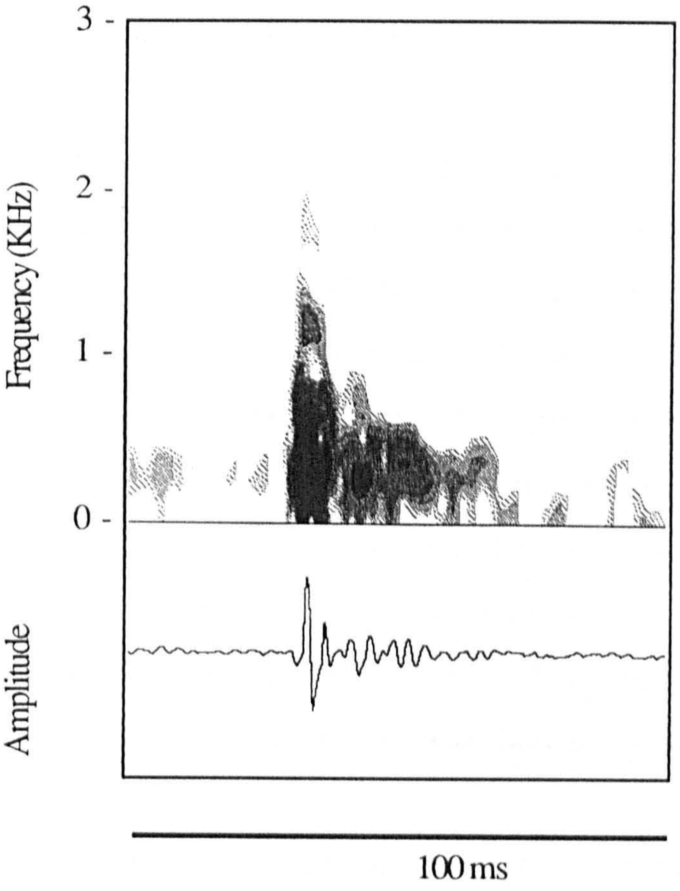


Figure 6.4 - Sonogram and oscillogram of a knock produced by the two-banded sea bream (filter bandwidth = 125 Hz).

The literature on sound production by fish belonging to the Sparidae family is very sparse. Fish *et al.* (1952) refer to single thumps (swimbladder sounds with peak frequency at 200 - 225 Hz) and stridulation sounds uttered by the scup, *Stenotomus chrysops.* Tavolga (1964) mentioned that the porgies emitted stridulation sounds as an alarm call. Caldwell & Caldwell (1967) stated that the pinfish, *Lagodon rhomboides,* produced sharp click sounds while vigorously defending a territory against intruders. From their pulse and frequency structure, it is likely that the sounds produced by the two-banded sea bream are swimbladder-related. Both stridulation and swimbladder sounds have been reported from fish of the Sparidae family (Fish *et al.* 1952, Tavolga 1964).

6.7 - THE CICHLID - *Pseudotropheus zebra*

Specimens of the gold, the cobalt and the black-barred (BB) *P. zebra* morphs were captured at Lake Malawi by professional divers and then transported to the Zoology Department (Aberdeen University) aquarium facilities. Both stock and experimental fish were kept in glass aquaria provided with a bottom layer of gravel, aeration and biological filtration. The 2 experimental aquaria measured: 0.6 m (l) × 0.6 m (w) × 0.4 m (h). Calcareous rocks were provided for shelter and as a pH buffer. The water temperature was maintained at 25° - 27°C with the use of thermostats. All fish were fed with tropical fish flake food. The whole experimental period extended from October to November 94 and from January to April 95.

Sound production in the gold, BB and cobalt morphs was studied following 2 different experimental designs. These experiments were planned after several preliminary observations had indicated that only male fish produced sounds, and only during courtship:

- In the first experiment, the 2 experimental glass aquaria were divided into 2 halves by a transparent partition. A rock was provided in each half. A subject male was put in one of the halves of each aquarium and allowed to acclimatise for 3 to 7 days. To help the fish's acclimatisation another male, usually from the same morph, was put in the other half of the tank, so that the fish was kept in visual contact with a conspecific. Prior to each sound recording session, the aeration and the filtration pumps were stopped. The second male from each tank was then replaced with a female of the same morph as the subject male, after which recordings started. Each recording session lasted for approximately 15 min for each female presented to the subject male. Usually, a total of 3 females were presented to each of the males. Each male was tested for sound production usually for more than 1 experimental day. An interval of 3 days was allowed between experimental days.

- In the second experiment, one half of each of the experimental aquaria was further divided in two by means of another transparent partition. In each of these new smaller divisions a subject male was kept, allowing 2 subject males per aquarium. The larger division (half of the aquarium) was used to keep females. Only one fish morph was used in each tank. All fish were allowed at least 3 to 7 days to acclimatise. Outside recording periods, fish were isolated visually from each other by means of mirrors that were placed against the transparent partitions. These mirrors also facilitated the fish's acclimatisation. Before recordings the filter pumps and the aeration were switched off and all mirrors removed to allow visual contact between males, and between males and females. Experiment 2 resulted in an improvement to the first experimental design, since sound production could be monitored simultaneously for 2 males, and both inter-male agonistic behaviour and courtship could also be simultaneously assessed. Furthermore, by maintaining females permanently in the aquarium, fish were handled less and therefore less stressed. Recording sessions lasted 20 min and only one was achieved per experimental day and per aquarium. Again, 2 - 3 days were allowed between experimental days. A total of 6 gold, 5 BB and 3 cobalt males were studied during these 2 experiments.

Only 1 gold and 2 BB males produced sounds throughout experiments 1 and 2. No sound was ever heard during inter-male or male-female agonistic interactions although fish were always quite aggressive. Calls uttered during courtship coincided with male *quivering* (a male courting behaviour of the early stages of courtship, McElroy & Kornfield 1990). Calls were made up of a sequence of knocks and are described in Table 6.6. Each knock seemed to coincide with a body quiver. Less intensive *quivering* was also observed in agonistic contexts. However, agonistic *quivering* was never accompanied by sound production. Oscillograms of calls produced by the gold and the 2 BB fish are shown in Figure 6.5.

Call parameters were compared between morphs (gold *vs* BB) with a Kruskal-Wallis non-parametric test. Knock duration was significantly greater for the gold than for the BB fish (N = 506, d.f. = 1, H = 9.26, $P < 0.01$), because of the differences in the number of pulses in a knock (N = 808, d.f. = 1, H = 190.80, $P < 0.001$). Indeed, 27% of the knocks emitted by the fish Gold3 were made up of 2 pulses, whereas each of the 2 BBs knocks always consisted of only 1 pulse (Fig. 6.5). No significant differences were found between the 2 morphs for call duration, for the number of knocks in a call or for knock frequency.

Although the mean interval between knocks did not increase or decrease with the number of knocks in a call, that is with call duration, for either fish (Fig. 6.6), regression analysis showed that the interval between knocks increased throughout call emission (i.e. with increasing knock order)

Table 6.6 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features (see section 6.2 for explanations) of the calls produced by gold (gold3) and BB (BB3 and BB4) morphs of *P. zebra* during the early stages of courtship. The number of courtship calls analysed for respectively the gold3, BB3 and BB4 fish were 12, 59 and 13. * - In this case it is equivalent to the interval between knocks in a call.

Sound parameters	N	mean	s.d.	min	max
Gold3					
Call duration (ms)	10	478.21	393.71	8.0	1180.0
No. of knocks in a call	10	10.40	6.77	1	22
Knock duration (ms)	84	8.91	3.26	5.1	16.5
No. of pulses in a knock	120	1.27	0.44	1	2
Pulse duration (ms)	84	6.62	0.95	4.0	11.3
Pulse peak-peak interval (ms) (within knock)	30	7.48	1.10	5.9	10.8
knock peak-peak interval (ms)*	111	51.03	21.51	17.5	191.8
Peak frequency (Hz)	98	353.00	96.30	164	573
Minimum frequency (Hz)	83	102.73	21.28	69	175
Maximum frequency (Hz)	84	634.80	186.17	269	971
BB3					
Call duration (ms)	59	545.19	407.92	6.5	2112.9
No. of knocks in a call	59	8.56	5.43	1	28
Knock (= pulse) duration (ms)	324	6.41	0.50	4.9	8.6
No. of pulses in a knock	505	1	_	1	1
Knock peak-peak interval (ms)*	445	72.30	33.89	25.4	256.4
Peak frequency (Hz)	217	273.73	57.54	155	498
Minimum frequency (Hz)	269	111.47	17.81	69	206
Maximum frequency (Hz)	66	799.08	185.93	321	1200
BB4					
Call duration (ms)	13	809.58	407.06	156.5	1492.3
No. of knocks in a call	13	14.38	5.88	5	23
Knock (= pulse) duration (ms)	98	6.09	0.70	4.6	8.3
No. of pulses in a knock	183	1	_	1	1
Knock peak-peak interval (ms)*	171	59.11	32.17	19.3	206.9
Peak frequency (Hz)	130	411.07	72.60	211	585
Minimum frequency (Hz)	162	139.28	36.76	103	189
Maximum frequency (Hz)	162	639.26	93.51	327	1146

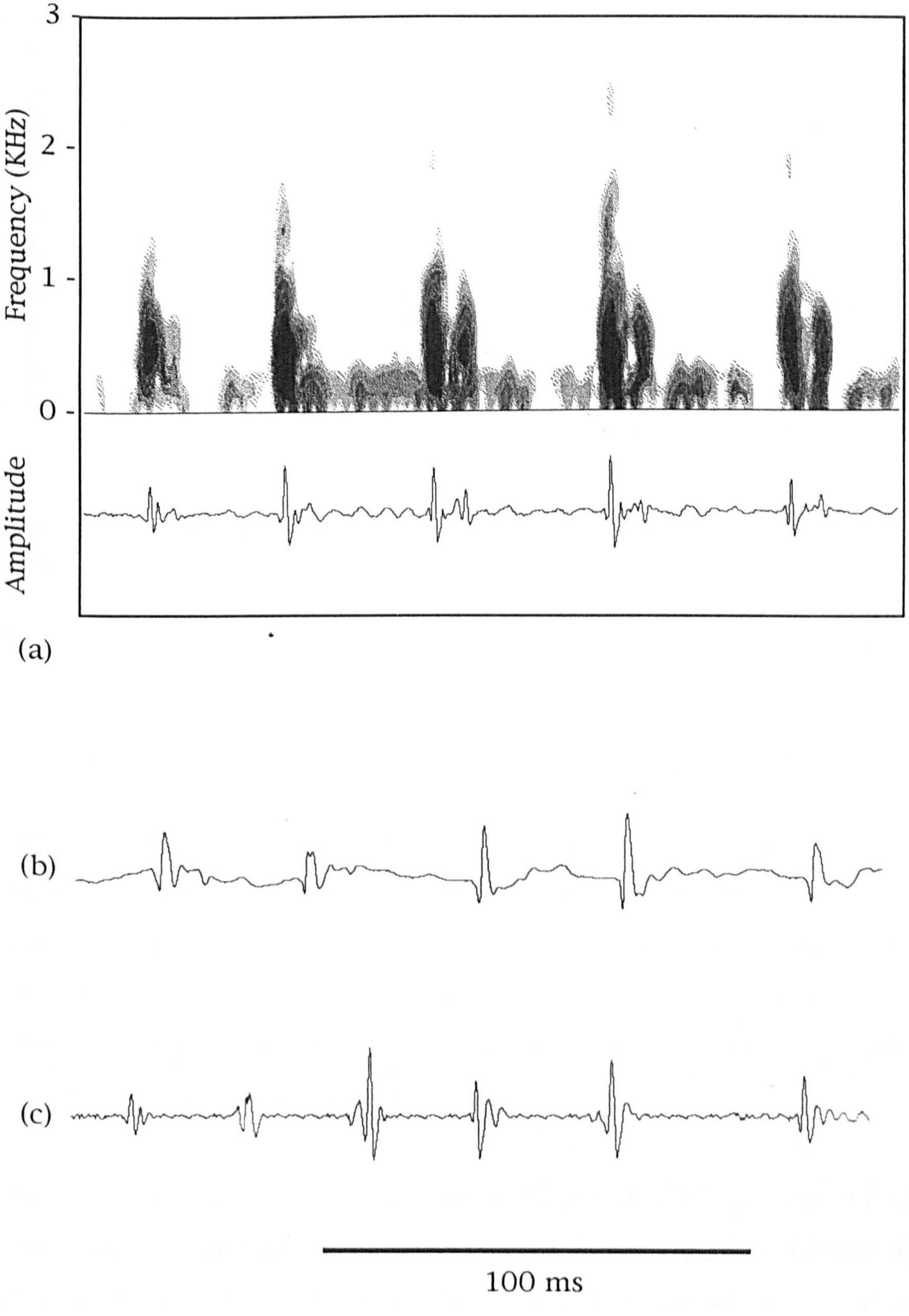


Figure 6.5 - (a) Sonogram and oscillogram of a portion of a courtship call made up of repetitive knocks uttered by a specimen of the gold morph of *P. zebra* (filter bandwidth = 125 Hz). (b) and (c) are oscillograms of portions of calls emitted by respectively the fish BB3 and BB4 (BB morph).

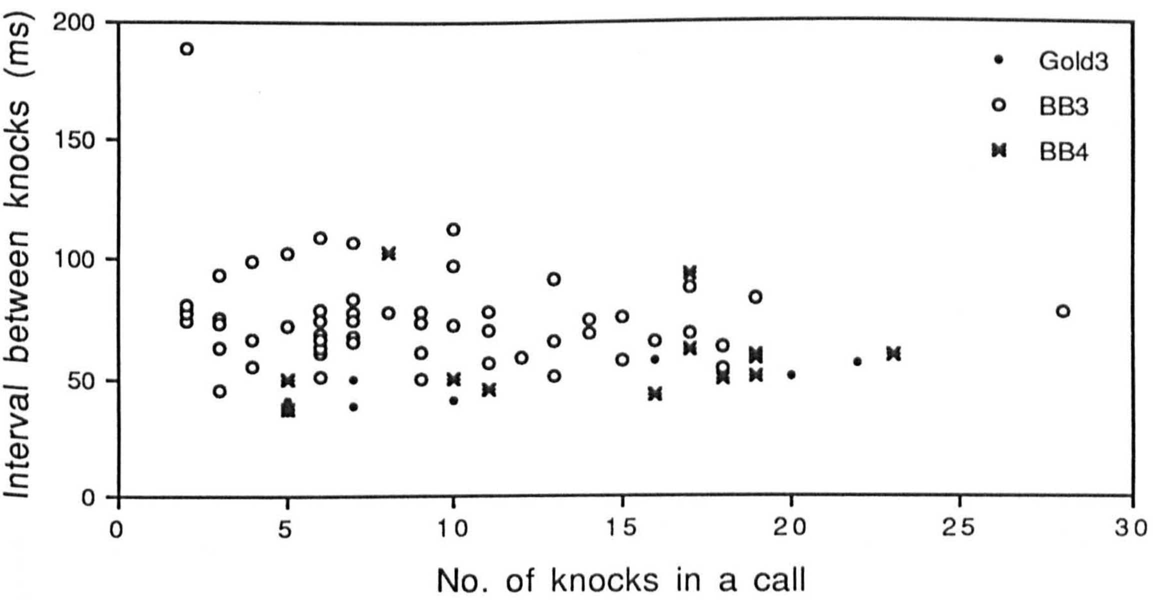


Figure 6.6 - Interval between knocks against the number of knocks in a call.

(Fig. 6.7). Because the distribution of the data was deviating from the normality assumption required for the simple linear regression, the natural logarithms of the original data were used instead. The slopes of the regression curves on the transformed data obtained for each individual fish were compared with an analysis of covariance which showed that the three slopes did not differ significantly ($P > 0.05$).

Cichlids from Lake Malawi and other African Lakes have fascinated evolutionary biologists for many years since there is a great number of cichlids endemic to this lake (probably 500 to 2000 species or forms (Turner 1994)), that belong to taxonomically and ecologically complex species flocks (Greenwood 1991), such as the *P. zebra* complex. Many of these cichlids are known to have restricted geographical distributions within the lake (Ribbink *et al.* 1983) and it has been suggested that these species or forms

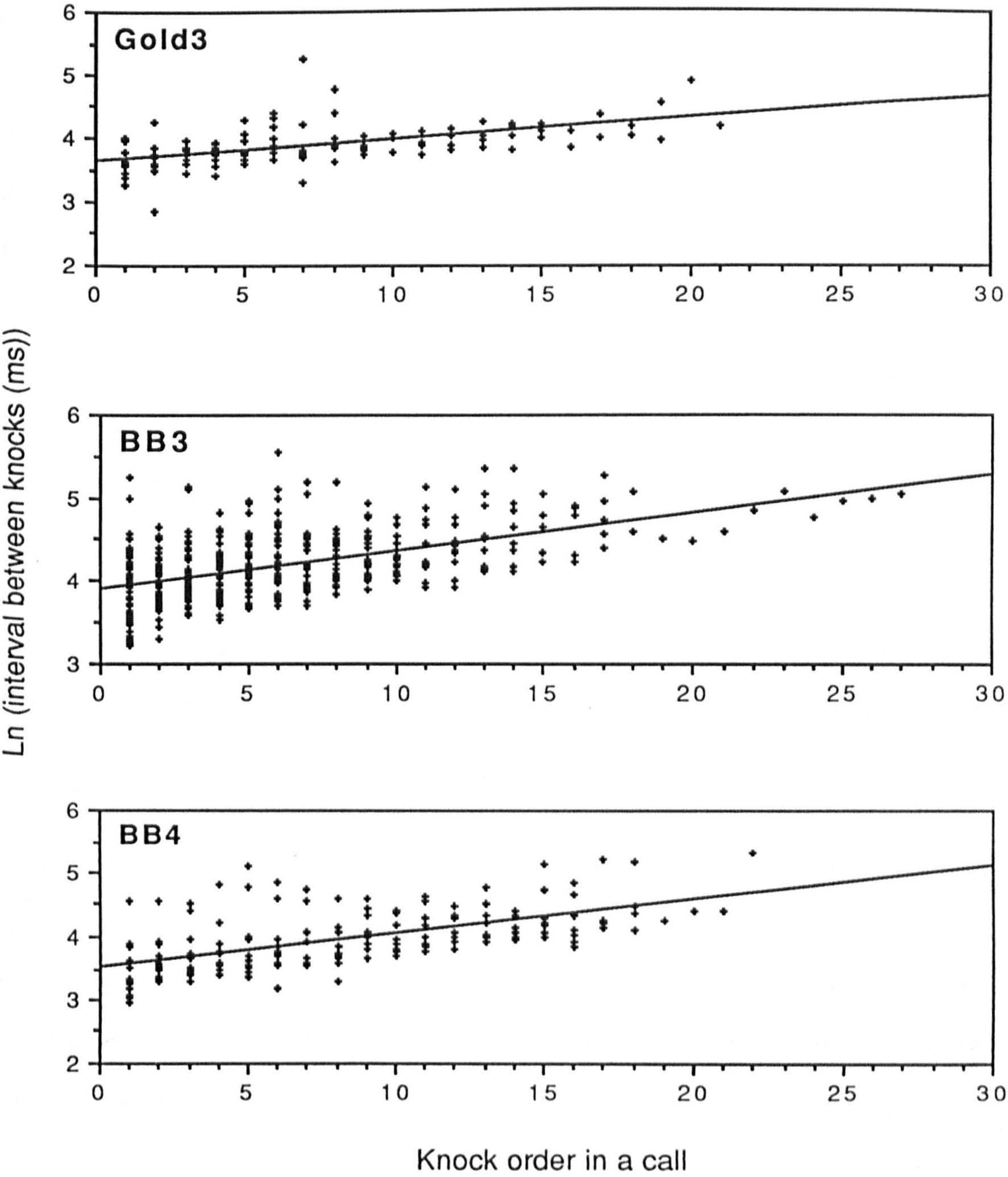


Figure 6.7 - Increase in the interval between knocks with the increasing knock order in calls produced by fish from the gold (gold3) and the BB morphs (BB3 and BB4) of the *P. zebra* complex. The regression equation on the transformed data (see text), the sample size (N), the correlation coefficient (r), and the probability are for each case: gold3, $\ln(y) = 3.618 + 0.035x$ ($N = 111$, $r = 0.544$, $P < 0.001$); BB3, $\ln(y) = 3.902 + 0.046x$ ($N = 445$, $r = 0.538$, $P < 0.001$), BB4, $\ln(y) = 3.518 + 0.053x$ ($N = 171$, $r = 0.592$, $P < 0.001$).

originated within the last few hundred years (Turner 1994). Molecular evidence suggests an age for the entire species flock of 700 000 to 2 000 000 years (Meyer *et al.* 1990). Many mechanisms have been proposed to explain the presumed speed at which cichlid speciation has occurred in the great African Lakes, such as species recognition (Paterson 1985), sexual selection (Dominey 1984) and behavioural mechanisms (Wyles *et al.* 1983). Supporters of the species recognition concept have mainly focused on species-specific male colouration (Greenwood 1991). Other elements of the specific-mate recognition system, such as pheromones, courtship behaviour patterns and sound production have not been studied in so much detail, and rarely in the context of speciation (Greenwood 1991). In this context, the study of species-specific courtship calls is especially interesting in cichlids since vocalisation can perhaps play a fundamental part in cichlid speciation through species recognition.

Sound production has been studied in several genera of cichlids: *Cichlasoma, Pterophyllum, Sarotherodon, Herotilapia, Oreochromis, Simochromis, Hemichromis, Haplochromis, Tropheus, Aequidens* (Myrberg *et al.* 1965, Schwarz, 1974a, b, 1980, Nelissen 1975, 1977b, 1978, Brown & Marshall 1978, Rowland 1978), but no report has been published for the genus *Pseudotropheus.*

Males from the gold and BB morphs of *P. zebra* produced sounds while *quivering* during the early stages of courtship. Reports of sound production during *quivering*, a typical display of many cichlids performing agonistic or courtship behaviour (Nelissen 1977a), have been made for *Simochromis diagramma* (Nelissen 1975), *Haplochromis burtoni* and *Tropheus moorii* (Nelissen 1977b), *Herotilapia multispinosa* (Brown & Marshall 1978) and *Hemichromis bimaculatus* (Rowland 1978). The quivering calls described for the listed species are similar to the ones recorded for *P. zebra* (see Table 6.6 and Table 6.7). Typically, it was the male that produced sounds during courtship *quivering*, as in *P. zebra*. The growl sound emitted during *quivering* by *H. multispinosa* was produced by either the male or the female while in the spawning area, but it was the male that mainly (90% of the total occurrences) uttered this quivering sound (Brown & Marshall 1978). Nelissen (1975) did not specify the sex of *S. diagramma* producing the "brrr" sound that occurred during the courtship *quivering*, but which also occurred in aggressive contexts. Other cichlid species also utter sounds during courtship, although not associated with *quivering*: *Oreochromis mossambicus*, *Sarotherodon galilaeus* and *Oreochromis* (=*Tilapia*) *niloticus* (Marshall 1971, Schwarz 1974a, Brown & Marshall 1978).

The courtship calls of the gold and BB individuals studied differed in the duration and number of pulses in knocks. These temporal characteristics could perhaps play an important role in mate recognition by *P. zebra*. Molecular studies in progress indicate that the gold and BB forms are reproductively isolated in Lake Malawi (M. van Oppen, pers. com.). Temporal and pulse grouping patterns in fish calls have been shown to have a fundamental role in acoustic communication in fish, including species recognition (Gerald 1971, Hawkins & Rasmussen 1978, Myrberg *et al.* 1978). For example, the female of the bicolour damselfish (*P. partitus*) is able to locate the male's nest by listening to their calls and distinguish the calls uttered by different males (Myrberg *et al.* 1986). It would be interesting to record from more individuals of each morph and to look further into intra- and inter-morph variability of *P. zebra* courtship calls. Furthermore, it would be of fundamental importance to test whether sound production is involved in species recognition in the *P. zebra* complex by carrying out experiments where male courtship calls of different morphs were played-back to females. It is possible that the differences observed

Table 6.7 - Description of the calls uttered during *quivering* in cichlids.

Cichlid species	Call duration (ms)	No. of pulses in a call	Pulse duration (ms)	Pulse interval	Peak frequency (Hz)
H. burtoni (Nelissen 1977b)	370 (230-1410)	13 (5 - 37)	---	---	320 (120-630)
T. moorii (Nelissen 1977b)	"	"	---	---	"
H. bimaculatus (Rowland 1978)	---	---	---	<10-15/s	< 100
H. multispinosa (Brown & Marshall 1978)	200 (108-1600)	---	5 (3-10)	121 ms (76-310)	150-300
S. diagramma (Nelissen 1975)	500-2000	---	---	3 pulses/ 100ms	200

may have been the result of individual variation rather than species-specific traits.

Myrberg *et al.* (1965) suggested that the sounds produced by the cichlids, *H. bimaculatus*, *Cichlasoma nigrofasciatum* and *Pterophyllum* sp. were swimbladder sounds due to their pulse and frequency structure, consistent with Rowland's (1978) suggestion for *H. bimaculatus*. As *P. zebra* produced similarly structured sounds, it is likely that they are also swimbladder-related.

6.8 - THE EMPEROR ANGELFISH - *Pomacanthus imperator*

One individual emperor angelfish (≈22 cm TL) was available for study in a concrete display tank with a glass front (2.0 m (l) × 0.7 m (w) × 0.8 m (h)), in Vasco da Gama Aquarium Exhibition Centre in Lisbon. This fish was kept together with other species: *Platax orbicularis* (Ephippidae); *Parachanturus hepatus* (Achanturidae); *Rhinecanthus aculeatus* (Balistidae); *Amphiprion sp.* (Pomacentridae); *Centropyge biopinosus* (Chaetodontidae). The tank was provided with a gravel bottom and rocks, aeration and running water (closed circuit). The water temperature ranged from 15.5° to 17.5°C during the period of study: Dec. 94 - Jan. 95.

The one individual of *P. imperator* available for study uttered very loud grunts that could be clearly heard outside the aquarium without using any equipment. These sounds were directed at other fish, accompanying agonistic visual displays, such as *approaching* or *chasing* other fish and during *lateral displays*, or at people passing near the front glass of the aquarium. The emperor angelfish grunted at people while it was swimming back and forth in the aquarium, always presenting the colourful sides of its body, and usually at times of low public attendance.

Fourteen grunts were recorded and analysed. The descriptive statistics of the grunts' physical features is presented in Table 6.8. A sonogram and oscillogram of a grunt is presented in Figure 6.8.

Only Fourmanoir & Laboute (1976) and Thresher (1982) referred to sound production in *P. imperator* but without giving any description of the physical features. Fourmanoir & Laboute (1976) stated that *P. imperator* grunted during agonistic encounters and Thresher (1982) reported that males of this species produced clearly audible grunts when chasing the

Table 6.8 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features (see section 6.2 for explanations) of the grunts uttered by the emperor angelfish.

Sound parameters	N	mean	s.d.	min	max
Phrase duration (ms)	14	61.150	25.368	30.8	128.3
No. of pulses	12	6.083	2.353	3	12
Pulse duration (ms)	12	9.917	0.490	8.7	10.7
Peak - peak interval (ms)	12	9.70	0.524	8.7	10.4
Peak frequency (Hz)	14	303.79	43.718	211	339
Min frequency (Hz)	14	129.14	26.100	101	199
Max frequency (Hz)	14	2372.71	1232.442	713	3778

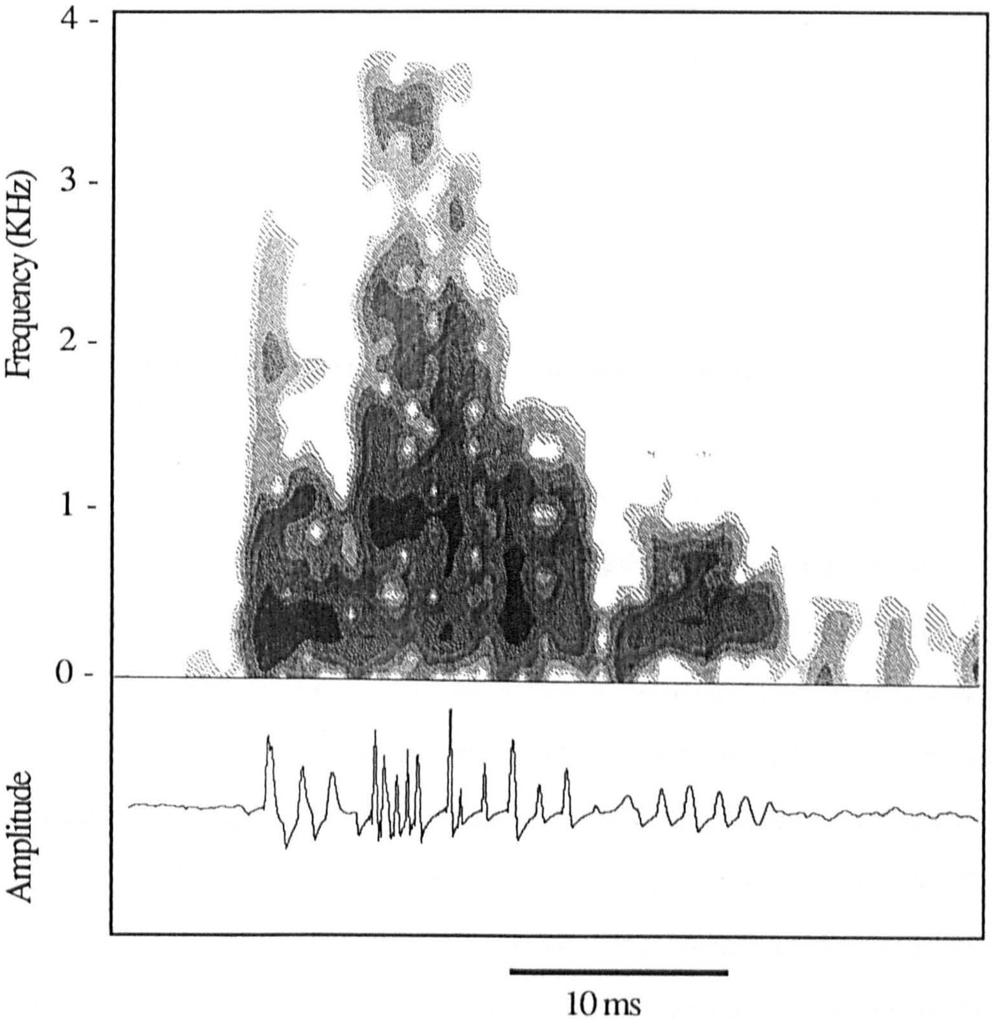


Figure 6.8 - Sonogram and oscillogram of a grunt uttered by a specimen of the emperor angelfish (filter bandwidth = 125 Hz).

female, just after spawning. Thresher (1982) suggested that this "*after-chase*" behaviour is more an agonistic display than courtship *per se*. Moyer *et al.* (1983) also mentioned "audible grunting" occurring frequently during courtship (possibly likewise involved with agonistic interactions) of *P. arcuatus*. Moulton (1958b) briefly described the grunts of *P. arcuatus* with durations ranging from 40 - 200 ms and frequencies extending up to 1500 Hz, but with predominant energy below 500 Hz, similarly to the grunts of *P. imperator*. *P. arcuatus* grunts were produced during feeding or when the fish was startled (Moulton 1958b).

From their pulse and frequency structure, it is likely that the sounds produced by the emperor angelfish are also swimbladder-related. Indeed, Moulton (1958b) suggested that the grunts of the closely-related angelfish, *P. arcuatus*, resulted from the action of the axial musculature adjacent to the swimbladder (this fish did not possess intrinsic muscles).

6.9 - THE BLUE-GREEN DAMSELFISH - *Chromis viridis*

The 5 blue-green damselfish examined included both males and females, measuring between 8 and 9 cm in total length. Fish were kept together with other tropical fish (*Amphiprion ocellaris* (Pomacentridae); *Amphiprion* hybrids (Pomacentridae); *Halichoeres trispilus* (Labridae); *Anampses chrysocephalus* (Labridae); *Pseudanthias squamipinnis* (Serranidae); *Nemateleotris magnificus* (Eleotridae)) in a glass display tank (1.6 m (l) × 0.35 m (w) × 0.68 m (h)) in Vasco da Gama Aquarium Exhibition Centre in Lisbon. The aquarium was supplied with hard coral, rocks and anemones, and also with biological filters, aeration and running water (closed circuit). The water temperature ranged from 15.5° to 17.5°C during the period of study: December 94 - January 95. Fish were fed with brine shrimp.

The blue-green damselfish produced click-like sounds (Table 6.9) during agonistic interactions. The calls were produced in bursts of 1 to 22 clicks (N = 12; mean = 5.58; s.d. = 5.68), during *chases*, most frequently directed at conspecifics (93.3%). Figure 6.9 shows a portion of one such burst of clicks. Very often these *chases* ended in mutual *parallel displays* or in the *fleeing* of the chased fish.

Clicks were usually single pulses, but they could be made up of 2 or more pulses (Figures 6.10 and 6.11). Multiple-pulsed clicks presented a typical pulse period (peak-peak interval) of approximately 7 ms (Fig. 6.12).

Sound production in Pomacentridae has received considerable attention and has been shown to play a fundamental role in courtship, principally in mate choice (Myrberg 1972, Myrberg & Spires 1972, Spanier 1979, Myrberg *et al.* 1986), and in other social contexts such as aggressive territorial behaviour (Myrberg 1972, Myrberg *et al.* 1978, Myrberg & Riggio 1985), and in species and individual recognition (Myrberg & Spires 1972, Myrberg *et al.* 1978, Spanier 1979, Myrberg & Riggio 1985). However, most of these studies have concentrated on the genus *Pomacentrus* and none of the previous research has focused on fish of the *Chromis* genus.

C. viridis emitted click sounds during agonistic interactions as reported for other damselfish (for a review see Amorim 1996). Agonistic sounds in damselfish have been reported for several species of *Amphiprion* (e.g. Schneider 1964, Allen 1975, Takemura 1983, Chen & Mok 1988), for *Pomacentrus partitus* (Myrberg 1972) and *Dascillus trimaculatus* (Luh & Mok 1986). The sounds uttered by various species of *Amphiprion* labelled by Schneider (1964), Allen (1975), and Chen & Mok (1988) respectively as threatening sounds, clicks and chirps, probably belong to the same

Table 6.9 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features (see section 6.2 for explanations) of the click sounds uttered by the blue-green damselfish.

Sound parameters	N	mean	s.d.	min	max
Phrase duration (ms)	62	15.687	7.350	5.8	33.3
No. of pulses	62	2.113	1.190	1	5
Pulse duration (ms)	62	7.942	2.713	4.9	20.8
Peak - peak interval (ms)	29	7.031	1.635	4.7	9.8
Peak frequency (Hz)	62	709.02	92.83	573	936
Interval between clicks (ms)	45	251.880	398.566	14.9	1693.5

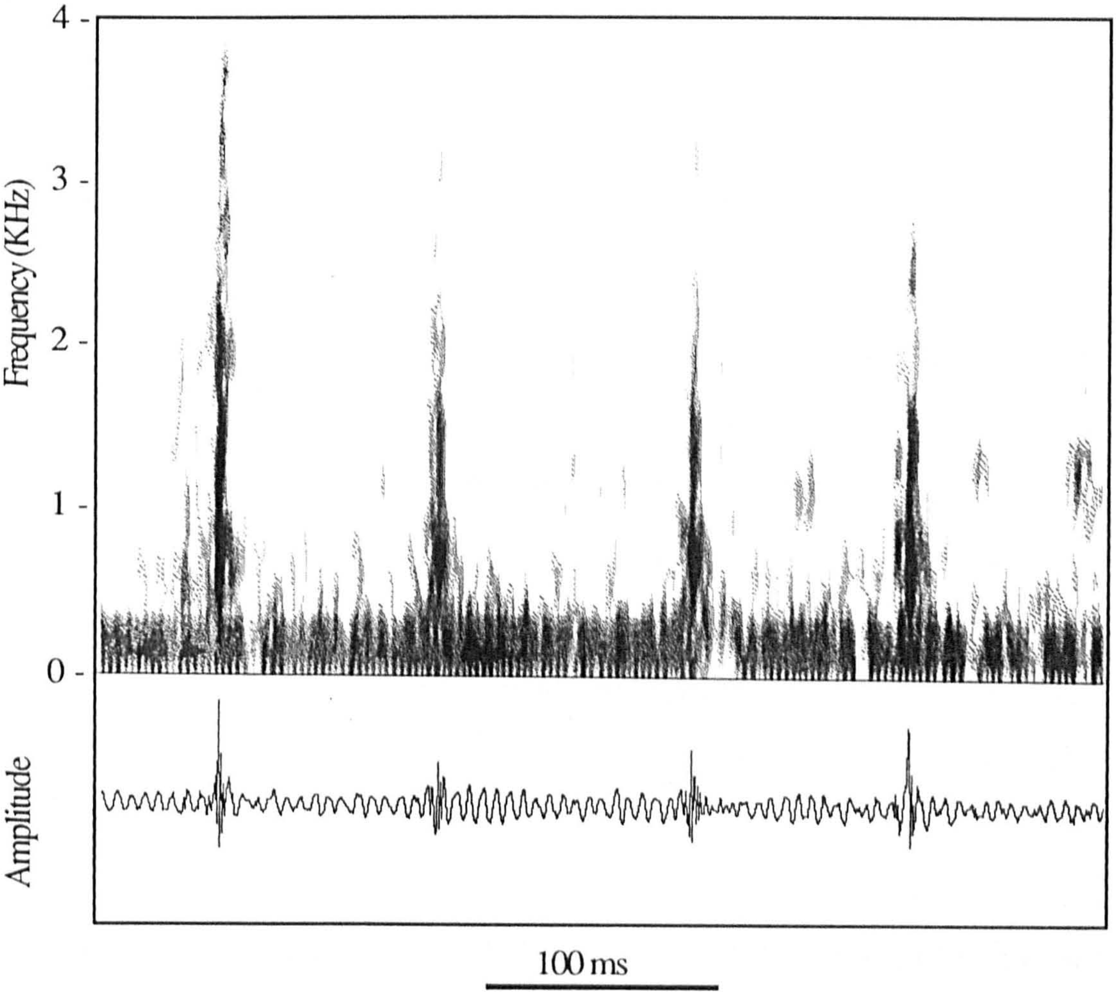


Figure 6.9 - Sonogram and oscillogram of a series of clicks produced by the blue-green damselfish (filter bandwidth = 125 Hz).

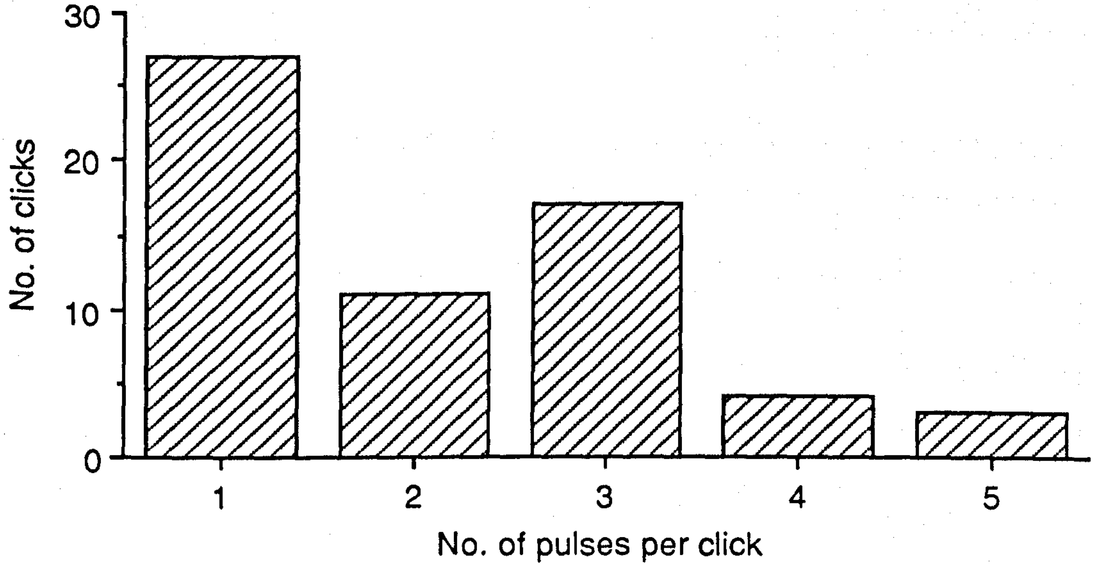


Figure 6.10 - Distribution of pulse numbers in click sounds.

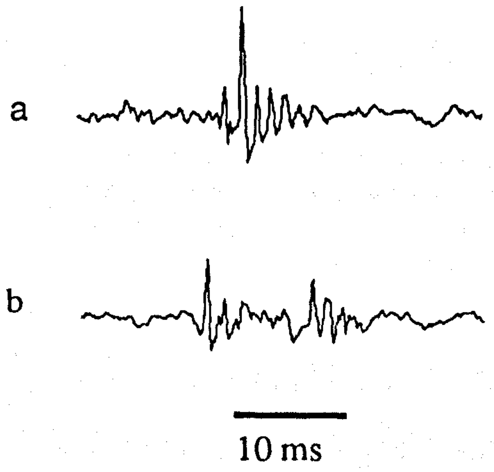


Figure 6.11 - Oscillograms of two clicks made up of 1 and 2 pulses (a and b respectively).

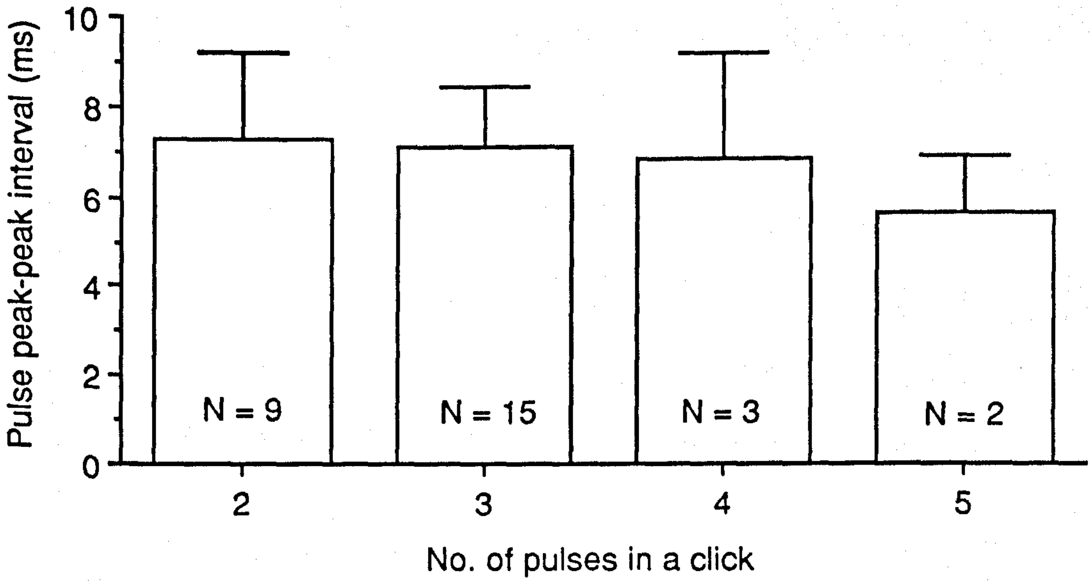


Figure 6.12 - Pulse peak to peak interval (ms) of clicks with 2, 3, 4 and 5 pulses.

category of sounds as the clicks described in the present study for *C. viridis*, when taking into account the sound's frequency structure and the number of sounds in a call. However, the pulse duration of the blue-green damselfish's clicks was considerably shorter than that reported for the *Amphiprion*'s agonistic sounds, and also, unlike the previous sounds, the clicks of *C. viridis* may present more than 1 pulse. Multi-pulse aggressive acoustic emissions have only been described for *D. trimaculatus* (Luh & Mok 1986). Most of the multi-pulsed damselfish sounds are emitted in a courtship context and present a much larger pulse interval than the blue-green damselfish's clicks.

Members of the family Pomacentridae also seem to emit sounds that are amplified by the swimbladder. For example, the swimbladder plays an important role in the sound features of *P. partitus* (Myrberg *et al.* 1993).

6.10 - THE CLOWN FISH - *Amphiprion* sp.

The 5 clown fish hybrids of the fire clown *A. frenatus* and the yellow tailed clown *A. clarkii* (first generation) (≈8 cm TL) were kept in the same display aquarium as the blue-green damselfish (Vasco da Gama Aquarium Exhibition Centre in Lisbon), and therefore in the same conditions as described in section 6.9.

Amphiprion hybrids emitted click-like sounds during agonistic interactions (Table 6.10 and Figure 6.13) in a series of 3 to 16 clicks (N = 8; mean = 6.5; s.d. = 4.5). All sounds analysed were produced during *chasing* (one fish swam very rapidly after another), or during *frontal threat displays* (a fish positioned itself with its head directed at the opponent, with extended fins); this behaviour was usually preceded by *chasing* or *approaching* (slowly swimming towards another individual). During *frontal threat displays*, the fish vocalised while producing a head jerking motion. The frequency of this jerking head movement seemed to coincide with the emission of each click. Very often the threatened fish *fled*, or responded with a similar *frontal threat display* and sound production, or also positioned their bodies perpendicularly to the threatening fish. The latter behaviour is probably what Allen (1975) defines as *lateral dorsal leaning*, *i.e.* a behaviour where a fish adopts a lateral posture to another (usually the threatener) and quivers its head. Allen (1975) states that this behaviour appears to have a dual role in threat and submission situations Clown fish (genus *Amphiprion*), like other damselfish, are very active

Table 6.10 - Sample size (N), mean, standard deviation (s.d.), minimum (min) and maximum (max) of the physical features (see section 6.2 for explanations) of the click sounds uttered by the *Amphiprion* hybrids.

Sound parameters	N	mean	s.d.	min	max
Click (= pulse) duration (ms)	50	10.938	5.037	5.8	21.9
No. of pulses in a click	50	1	_	1	1
Peak frequency (Hz)	50	666.220	106.881	468	865
Interval between clicks (ms)	43	158.386	228.271	11.4	1538.6

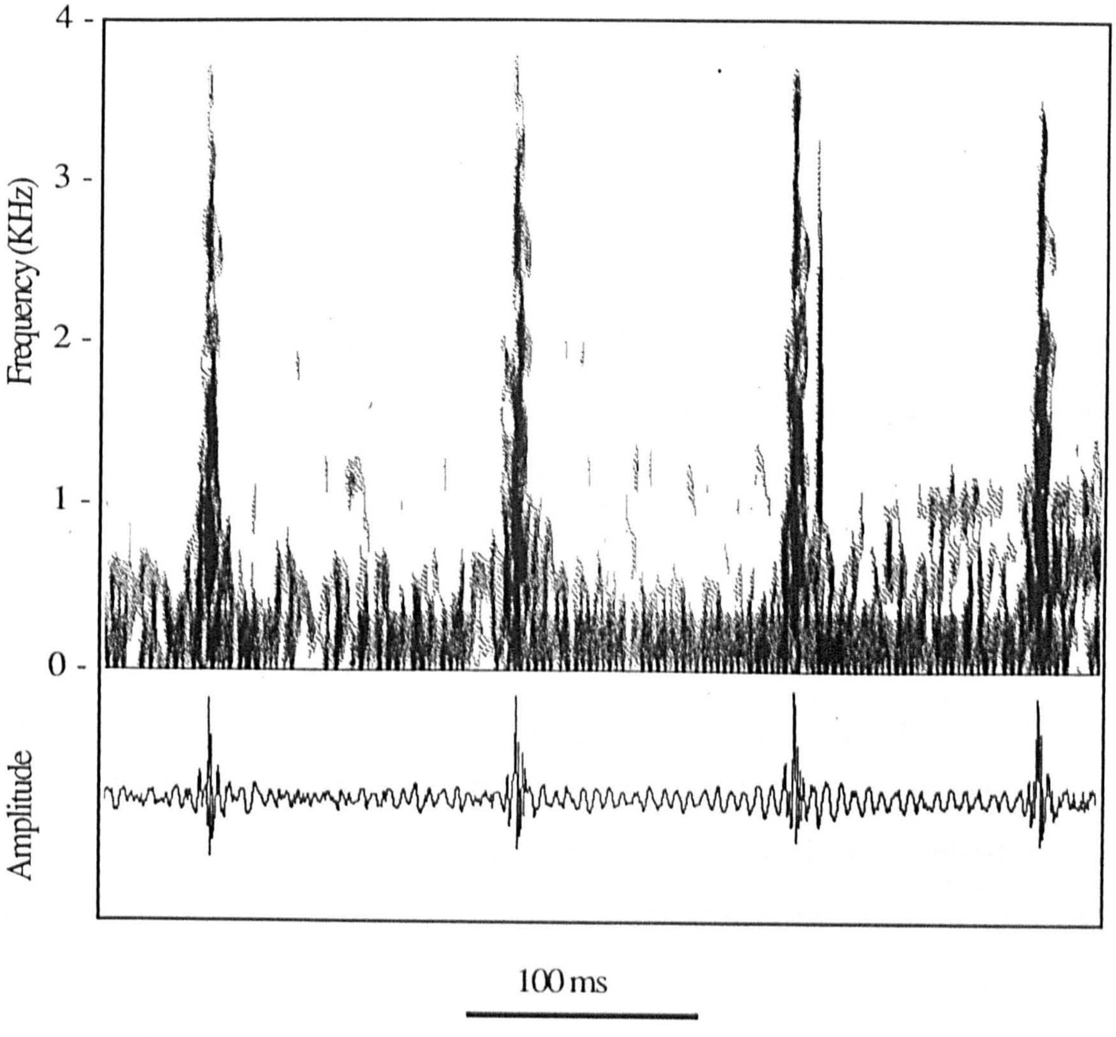


Figure 6.13 - Sonogram and oscillogram of a series of clicks produced by the *Amphiprion* hybrids (filter bandwidth = 125 Hz).

sound producers. Verwey (1930), Schneider (1964), Allen (1975), Takemura (1983) and Chen & Mok (1988) have reported acoustic emissions in this group, but none of these studies has provided a quantitative insight into the communicative system of this genus. There is also a considerable lack of information on sound production by hybrid species. Sounds described for *A. frenatus* × *A. clarkii* hybrids are similar to the ones reported for their parental species (Table 6.11) as well as to the sounds of other clownfish (e.g. *A. chrysopterus*, Allen 1975). *Amphiprion* hybrids, like other fish of the same genus, often produce click-like sounds during agonistic interactions, either in threatening or submissive situations, accompanied by head jerking movements, usually during fights over an anemone (Schneider 1964, Allen 1975, Takemura 1983). *Amphiprion* species are known to interbreed and produce fertile offspring (Marliave 1985). Indeed, Marliave (1985) has proposed that different *Amphiprion* species, which also included *A. frenatus*, that were observed to hybridise and produce a viable F_2 offspring, were probably stable polymorphs of a single species. The sounds and associated behaviour reported in the present study were broadly similar to those cited by other authors for the different species of clown fish (*Amphiprion*) probably due to their genetic similarity.

Other sound types, such as grunts, have also been reported for this genus. It is likely that these hybrids also have a more complex sound repertoire, since the sounds analysed here were taken over a very limited time of recording. Furthermore, Takemura (1983) reported that *A. frenatus*, and also *A. ocellaris* and *A. sandaracinos*, produce sounds during courtship and spawning, and none of the hybrids in the present study were in breeding condition.

Table 6.11 - Sounds produced by *Amphiprion clarkii*, *A. frenatus* and their hybrid. (a) Threatening sounds (Schneider 1964); (b) Chirps (Chen & Mok 1988); (c) ? (Takemura 1983).

Sound parameters	*A. clarkii*	*A. frenatus*	hybrid
Sound (= pulse) duration (ms)	25-30 (a); 50 (b)	50 (b); 56 (c)	11 (6-22)
Main frequency (Hz)	600 (a); < 1500 (b); < 8000 (c)	< 1000 (b); 500 (c)	670 (470-870)
No. of sounds / call	4-12 (a); 1-17 (b)	1-17 (b)	6.5 (3-16)
Sound repetition rate (no. sounds / s)	16-20 (b)	12 (b)	12 (7-23)

Although the sound-producing mechanism has not been investigated in *Amphiprion*, it is possible that sound production is associated with the mouth and opercular movements accompanying sound production which is then amplified by the swimbladder, as suggested by Verwey (1930), Allen (1975) and Takemura (1983).

6.11 - NON SOUND-PRODUCING SPECIES?

The gilthead sea bream and the sea bass (≈ 20 cm TL) were obtained from the aquaculture division of the Institute of Marine Biology of Crete (IMBC), Greece. Shoals of both species were kept separately in two 1.5 m diameter fibreglass tank in the IMBC. The photoperiod was natural and the tanks were provided with recirculated sea water (filtered and sterilised) running in both closed and open circuits. The water temperature ranged from 17° to

24°C, similar to the sea temperature. The study was carried out during June - July 94.

Seven armed bullheads were trawled with an otter demersal trawl off Stonehaven bay, in Scotland, and were kept at the Marine Laboratory in Aberdeen in a glass aquarium (1.83 m (l) × 0.58 m (w) × 0.62 m (h)), provided with running sea water, aeration, a sandy bottom, rocks and *Laminaria* stems. The photoperiod was controlled by a computer to resemble the natural cycle. Water temperatures ranged from 7° to 12°C. Fish were monitored for sound production from January to June 93. Fish size ranged between 10 and 15 cm in total length.

A large shoal of horse mackerel, ranging in total length from 26 to 34 cm, was captured with a purse seine off the Minch (Scotland) and transferred to the Marine Laboratory in Aberdeen, where it was kept in a large tank (6.1 m (l) × 3.6 m (w) × 1.5 m (h)), provided with running sea water and aeration. The photoperiod was controlled by a computer to resemble the natural cycle. Water temperature was approximately 11°C. Examination for sound production was conducted during the month of April 1993.

Five short-spined sea scorpions (20 - 30 cm TL) were collected and maintained as described for the armed bullhead. Monitoring for sound production took place during the month of October 93.

For both the armed bullhead and the short-spined sea scorpion the outcome of the investigation for sound production was negative, although evidence that these are soniferous species had previously been presented (Hawkins 1968, Bass & Baker 1991). Likewise, the specimens of the horse mackerel, the gilthead sea bream and the sea bass did not emit sounds during the period of study. However, Fish & Mowbray (1970) have reported that species related to the horse mackerel produce sounds. Moreover, in the present study the sounds uttered by the two-banded sea bream (Sparidae) are described. Failure to observe acoustic emissions from the armed bullhead and the short-spined sea scorpion may be due to several factors including the conditions under which they were kept in captivity, the specimens' sex etc. However, agonistic interactions, courtship and spawning were observed for the armed bullhead, leading one to the belief that a great part of this species' behavioural repertoire was observed without being accompanied by acoustic emissions. Furthermore, Hawkins (1968) stated that sounds from the armed bullhead were only heard with fish which were chased with a stick, or disturbed by other species, and did not appear to play any part in the social behaviour of the species.

The existence of a sound-producing apparatus was examined for the armed bullhead and the horse mackerel. Only the horse mackerel possessed a swimbladder, but no muscles attached to the swimbladder which might produce sounds were found.

6.12 - FINAL REMARKS

In this Chapter, sound production was reported for the first time in 8 "species" of fish. Of these species, only 1 uttered sounds in a courtship context; all the others produced sounds while performing agonistic behaviour. Furthermore, only in the two-banded sea bream, the emperor angelfish and in the blue-green damselfish was sound production not associated with a territory or a defended area. Clown fish usually fought over an anemone; the blenny, *P. pilicornis*, fiercely *chased* intruders away from its territory, which included eggs in the case of the male; even the *P.*

zebra male uttered courtship calls when close to the nest, which was promptly defended from other males (Vodegel 1978).

All the sound-producing species studied appeared to have uttered swimbladder-associated sounds, because of the latter's pulse and frequency structure. The tadpole fish, for example, possesses very well-developed specialized sonic muscles imbedded in the swimbladder wall (Hawkins & Rasmussen 1978). In other species, such as the shore rockling or the clown fish hybrids, the swimbladder probably only acts as a resonator. Indeed, the swimbladder may amplify and impart a hollow resonant quality to sounds produced, for example, by stridulatory mechanisms (Hawkins 1993).

The results presented in this Chapter increased the information available on the number of fish species that produce sounds in a social context. The study of sound production in fish may give a different insight into the species' behaviour or to broader aspects of its biology, as in the case of *P. zebra* and other cichlids, where courtship calls may play an important role in mate recognition and perhaps in their rapid speciation.

CHAPTER 7

GENERAL DISCUSSION

7 - GENERAL DISCUSSION

This project studied sound produced by fish in different behavioural contexts: agonistic behaviour (Chapter 6), agonistic behaviour related to competitive feeding (Chapter 2) and territorial defence (Chapter 6), and courtship (Chapter 6). The results obtained during this study will be discussed with special reference to the role of sounds in the communication system of gurnards and fish in general, bearing in mind the definition of communication proposed by Krebs & Davies (1987) (see Chapter 1). This Chapter will first focus on the sounds produced by gurnards and on the possible features relevant for acoustic communication. Secondly, the mechanism of sound production in gurnards and its relation to the sounds produced by each species will be discussed. Further, it is argued whether this sound-producing mechanism is capable of producing signals with a communicatory function. Thirdly, the behavioural context of sound production is compared between the grey, the streaked and the tub gurnards, and then the existence of sound communication in gurnards and other fish is debated. Finally, the possible low costs of sound production is related to the use of acoustic displays in fish agonistic behaviour.

Sound production in gurnards

This study has shown that there are interspecific differences in the acoustic repertoire of the grey, the streaked, the red and the tub gurnards. These differences were based on the number of phrase types, and on the timing and grouping of pulses in their phrases. Indeed, while the grey and the streaked gurnards produced 3 different phrase types, the streaked and the tub gurnards only emitted 1 phrase type. The phrase unit was a single pulse in both the grey and the tub, but it was a group of pulses in the phrases of the streaked and the red gurnards. Also, the temporal parameters of knocks, grunts and growls varied between species (see Fig. 2.26).

Furthermore, within a species (the grey gurnard) different phrase types could be discriminated based on the temporal patterning of pulses (see Fig. 2.20). Such inter- and intraspecific differences in sound production probably encode information relevant to the social communication system of the triglid family since interspecific differences in sounds produced by other families of fish also lie in the temporal organisation of pulses (e.g. Gerald 1971, Myrberg *et al.* 1978, Ladich *et al.* 1992a) and field work has shown temporal cues to be the basis for sound discrimination by fish (e.g. Spanier 1979). Indeed, Winn (1964, 1972) has suggested that fish acoustical signals encode information through temporal patterning since, with few exceptions, they show weak frequency modulation and are made up of brief low frequency pulses. This suggestion is also consistent with the present belief that hearing in fish is specialised in extracting information in the time domain (Fay 1985).

The production of sounds changed ontogenetically in the grey gurnard. Sound production rate was lower in larger grey gurnards, as was the proportion of knocks and growls uttered during feeding bouts. Grunts, on the other hand, were found to be more frequently produced by larger individuals of this species. The analysis of the feeding interactions showed that interaction rate (the number of interactions per minute) decreased significantly with increasing fish size, which explains the decrease in sound production rate. There was a striking decrease in the percentage of interactions accompanied by grunt, and an increase in knock calls with increasing fish size. Since grunts were associated with aggressive behaviour, these results show that grey gurnards tend to become less aggressive in the disputes over food, and probably generally in agonistic contests, as they grow. This is consistent with the fact that the frequency of the behaviour *frontal display* in an interaction decreased in larger fish.

Variations in the sounds uttered by different size classes of the grey gurnard are likewise based on differences in the temporal features and also in the peak frequency of their sounds. However, acoustic temporal features may not be relevant to intraspecific communication in grey gurnards since they may fall beyond the resolution capabilities of fish. Indeed, within any one species, the temporal patterning of sounds of calls is found to be quite stereotyped (Myrberg *et al.* 1993). Alternatively, the peak frequency and the number of pulses in a phrase could provide relevant information for intraspecific communication in the grey gurnard. Peak frequency is commonly used in individual recognition and assessment in fish (e.g. Myrberg & Riggio 1985, Ladich *et al.* 1992b) and other groups (Davies & Halliday 1978), since it may provide information on the individual's size. In the bicolour damselfish (*P. partitus*), the number of pulses in a sound gives indication of the fish's motivational state: 1-pulse sounds occur in an agonistic context, whereas 3- and 4-pulse sounds are courtship sounds; 2-pulse sounds are heard in intermediate states between aggression and courtship (Myrberg *et al.* 1978).

The mechanism of sound production in gurnards

The swimbladder of the different species of triglids studied varied fundamentally in their shape. The piper possesses a pair of extrinsic sonic muscles in contrast with the other species of gurnards which have intrinsic sonic muscles. The structure, size and shape of the swimbladder and attached muscle system is known to affect the characteristics of the sounds produced (Fish 1954, Fine *et al.* 1977b). The species of gurnards whose sound repertoire were studied (the grey, the streaked, the red (Mediterranean) and the tub) presented similar swimbladder and sonic muscles weights at a given length. These gurnards also have swimbladders with similar shapes, with the exception of the tub gurnard swimbladder which possesses 2 lateral diverticula. As the size and shape of the swimbladder affect the frequency structure of the sounds produced (Bass & Baker 1991), it would be expected that the frequency structure of the sounds uttered by the grey, the streaked and the red gurnards would be very similar. This was confirmed in the acoustic recordings (see Figs. 2.6 - 2.8 and Figs. 2.21 - 2.24). The few sounds emitted by the tub gurnard during the present study also resembled the sounds of the other species as far as the frequency structure is concerned. It might be expected that the diverticula of the tub gurnard's swimbladder would alter the resonance characteristics of the swimbladder, perhaps in adding more harmonics to the sounds. However, as very few sounds from this species were analysed and as the tank where fish were held proved to affect the characteristics of sounds recorded from this species, no conclusions can be drawn on the influence of the diverticula on the sound structure.

The grey gurnard was found to contract the pair of sonic muscles synchronously, with each simultaneous muscle contraction resulting in a pulse of sound, as found for other species (e.g. Packard 1960, Winn & Marshall 1963, Bass & Baker 1991). The time lag between muscle contraction and sound production was also similar to those found in the literature (Skoglund 1961). Similarities found in the neuromuscular mechanisms of sound production and, at a higher level in the sonic motor system in various species of fish, suggest that these vocal control traits are homologous (Bass & Baker 1991).

If sound production is to have a communicatory function, than acoustic signals should have been shaped by natural selection (Krebs & Davies 1987) and so should the mechanisms to produce them. The mechanisms to produce sounds appear to have evolved independently in various fish taxa (Fine *et al.* 1977b, Hawkins 1993). While the original function of the swimbladder is the control of buoyancy, it has been secondarily modified not only as a sound production organ, but also as a respiratory, auditory, or accessory luminescent structure (Blaxter 1981, McFall-Ngai 1983, Liem 1989). The sonic muscles of some fish, including gurnards, have also been secondarily modified from body wall muscles (Rauther 1945).

Behavioural context of sound production in gurnards

The sequences of behavioural categories observed during feeding bouts were broadly similar amongst the grey, the streaked and the tub gurnards. Agonistic behaviour was related to disputes over food items. A successful forager either avoided confrontations by being the fastest to reach a piece of food or displayed aggressive behaviour, such as *approaches*, *chases*, *frontal displays*, often accompanied by sound production. Poorer foragers could also obtain food by avoiding periods when there were competitors in the feeding area. Overall, the grey gurnard was the species that disputed food most aggressively. This species interacted more often, was more vocal and made *frontal displays* more frequently during feeding disputes than the other two species.

In addition, there were also broad similarities in the use of sounds by the grey, the streaked and the tub gurnards. Although no quantitative study was conducted to relate the sounds produced by the tub gurnard with particular behavioural categories, direct observations showed that grunts emitted by this species accompanied aggressive behaviour, as observed in the grey gurnard. The knocks emitted by the grey gurnard and the growls emitted by the streaked gurnard were related to feeding arousal and low levels of aggression. It is not surprising that the growls of the streaked gurnard are related to a less aggressive context than the grunts of the grey and the tub gurnards, since in spite of being very long phrases with many pulses, the sound units (groups of pulses) of the growls are repeated at a much lower rate than the units of the grunts of the latter species. Indeed, the growls of the streaked gurnards resembled the knocks of the grey gurnards when repeated rapidly. It is therefore probable that in triglids, pulse (or the sound unit) repetition rate is the most relevant cue in giving information on the level of aggressiveness of the sender, although this hypothesis could not be proved with playback experiments.

Sound production in gurnards is frequently accompanied by visual displays, as mentioned above. *Frontal displays*, for example, involve extending the pectoral fins and erecting the dorsal, which can result in a quite striking visual effect. It is interesting to note that the species that showed the ability to produce a smaller range of phrase types (the tub and streaked gurnards) also have much brighter coloured pectorals, thus probably producing a more effective visual stimulus than the other species when performing the same display. In cichlids, a group that has both well developed visual and acoustic communication, Nelissen (1978) observed that, in studies on 6 related species, the more sound types a species produced the fewer colour patterns it showed. This author related the degree of specialisation in either acoustical or visual communication with their diel rhythms of activity; thus fish that were mostly active at night solved the problem of the lack of visibility with the development of sound production. In triglids other than the grey gurnard, diel rhythms of activity were not studied, but the streaked and tub gurnards usually inhabit shallower waters than the red and the grey gurnards (Wheeler 1969, Papaconstantinou 1983, Hureau 1986, Tsimenides *et al.* 1992). This would suggest that also in triglids, acoustic and visual communication are complementary and acoustic communication is more developed when visual communication is restricted. Similarly, Torricelli *et al.* (1986) showed that in the freshwater goby, *P. martensi*, acoustic signals have an increasing role as visual displays become ineffectual when males enter the nest.

Few studies other than the present one have made a comparison of the behavioural context of sound production in different species of fish of the same family. Indeed, comparative behavioural and acoustical studies have only been previously carried out for the Pomacentridae family (see Myrberg *et al.* 1978 and Spanier 1979). In addition, research on the sound production and on the behaviour of several species of gadoids have also been carried out, but no quantitative comparisons were made between species (see Brawn 1961, Hawkins & Chapman 1966, Hawkins 1968, Hawkins & Rasmussen 1978, Almada *et al.* 1996).

Do gurnards communicate when they produce sounds?

The association of sounds with particular behavioural categories in the species of gurnards studied was quite stereotyped in both the intra- and the interspecific level, indicating that sound production probably has a communicatory function in triglids in at least feeding and agonistic contexts. Indeed, communication is said to occur when behavioural displays or signals are ritualised (Dawkins 1986). Temporal features, and probably mainly the pulse repetition rate, may indicate the motivational state of the emitter, such as its level of aggressiveness. Further, in agreement with previous work on sound communication in fish and on fish hearing, the results obtained in the present work showed that temporal features of sounds emitted by triglids are potentially capable of promoting species recognition. Likewise, peak frequency and perhaps the number of pulses in a phrase are able to promote intraspecific communication, such as individual recognition. Unfortunately, the playback experiments performed in this project did not help in clarifying the acoustic communicatory system of the grey gurnard.

It would be interesting to investigate whether other species of gurnards react to played back sounds and if so, to carry out experiments with them.

Muting fish would also provide an effective technique to investigate the role of sound production in the social behaviour of these species (see for example Valinsky & Rigley 1981), although the removal of the sound-producing apparatus in triglids would probably cause severe damage to the operated animals. As an alternative, studying sound production in other behavioural contexts, such as courtship, and studying a wider range of triglid species and other closely related species of other families, would improve the knowledge of the acoustic communication system of this family. Another interesting option would be to compare the behaviour of 2 or more closely related species, which differ in the extent to which they produce sounds, to further understand the adaptive significance of sound production in fish. One of the major problems encountered in this project was the difficulty in identifying the individual fish that was emitting sounds. Working with more "vocally conspicuous" species would help in planning more detailed experiments that would help in understanding the biological significance of fish sounds.

Several studies have shown that sound production has a communicative function in fish. Acoustic emissions may serve the following functions:

- as an alarm signal (Hawkins 1968)
- to ensure synchronization of gamete release (Lobel 1992)
- to advertise male quality and resource ownership (Lugli *et al.* 1995)
- to announce reproductive readiness (Winn 1964, 1967, 1972, Hawkins & Chapman 1966)
- to attract the female to the nest (Tavolga 1956, 1958a, Torricelli *et al.* 1986)
- in mate selection (Myrberg *et al.* 1983, 1986)
- to stimulate reproductive maturation in the female (Marshall 1972),
- in species (Myrberg *et al.* 1978, Spanier 1979) and individual recognition (Myrberg & Riggio 1985, Myrberg *et al.* 1993)

- in the facilitation of schooling (Moulton 1960)
- to gain dominance or to win fights (Valinsky & Rigley 1981, Ladich 1990, Ladich *et al.* 1992b)
- and in the inhibition of the opponent's aggressive behaviour (Schwarz 1974b).

While some of the above-listed studies only suggested the existence of acoustic communication in fish (e.g. Moulton 1960) or produced scientific work without sufficient controls (e.g. Tavolga 1956, 1958a), others have provided conclusive evidence that acoustic communication takes place in teleosts (see the communication definition given by Krebs & Davies (1987) presented in section 1.3.1) (e.g. Myrberg *et al.* 1978, Valinsky & Rigley 1981, Ladich *et al.* 1992b). In the latter case, it was clearly demonstrated that the receivers' behaviour was altered through the emission of sounds by the sender.

The costs of sound production in fish

Unlike in other groups, sound production in fish seems to be cheap in energy terms, if all assumptions considered for the theoretical calculations in the present work hold. This is probably because the mechanism of sound production (with the swimbladder) in fish seems to be efficient (De 1981, Speakman *et al.* 1989, Hawkins 1993) in contrast with those of insects and higher vertebrates (e.g. McNally & Young 1981, Ryan 1988). The main disadvantages of sound production in fish are probably related to the interception of acoustic signals by undesired receivers, which may increase for example, the risk of predation. However, many fish produce low intensity sounds within close range of the receiver which may have low probability of interception. In triglids, acoustic signals emitted during competitive feeding may be intercepted and attract competitors to the exploited food patch, decreasing the food resources available for the signallers.

It is interesting to note that the species of triglids studied did not get involved in serious fighting, but instead made use of visual displays and acoustic signals to solve disputes over food items. Indeed, biting was observed very rarely, especially in larger gurnards, and never led to injury. Since fighting is both costly and disadvantageous (not only because it causes depletion of energy but also because it can cause severe injuries and ultimately result in death), the resource value disputed for should exceed the cost of injury in order for an animal to engage in a serious fight (Huntingford & Turner 1987). Escalation of fighting should be expected when competing for high value resources, such as the opportunity to pass on genes to the next generation (Krebs & Davies 1987). In the present study, fish were competing for food. As all animals were fed frequently and fish may stay for long periods unfed, the resources contested probably did not have such a high value and it was more advantageous for the fish to make lower cost visual and acoustic displays than to engage in a fight. Enquist *et al.* (1985) and Enquist (1985), for example, have studied variable threat displays in the fulmar (*Fulmaris glacialis*), a sea bird, competing for food (fish). Fulmars choose between low cost (less effective) and high cost (more effective) displays, depending on how hungry they are, that is, depending on the resource value.

In the case of gurnards, competing foragers probably benefit from responding to each other's acoustic signals, since it may avoid fights. When both signaller(s) and receiver(s) benefit from the signal (cooperative signalling), receivers may evolve specialised receptors or, in other cases, the signaller and the receiver may interact close to each other, in order to reduce noise (competing stimuli) (Harper 1991). Fish seem to have chosen both strategies because not only is their hearing abilities specialised for the sounds they produced (Fay 1985), but they usually interact in close proximity to each other (Hawkins & Myrberg 1983), In these cases, signals evolve to become as cheap as possible (Harper 1991) and this may explain why sound production in fish seems to be especially cheap. Cooperative signals, however, should be quiet or inconspicuous (Krebs & Davies 1987), which does not seem to be the case for many fish sounds, especially courtship calls (e.g. Winn 1967, 1972).

Final remarks

One of the major themes in ethology has been the interpretation of signals and communication amongst various animals including humans. One reason why animal communication has caught the interest of ethologists is that many signals produced by animals are very striking to humans. The distinctive smell of a tomcat, the outstanding colours of tropical fish and birds, the monotonous sounds of insects and the varied songs of birds are easily observed and challenge human curiosity. While acoustic communication has received considerable attention in mammals, birds, anurans and insects, acoustic communication in fish remains to a large extent unexplored. The present work has contributed to our understanding of the role of acoustic signals in gurnards and in other species of fish. It is suggested that these fish species use acoustic signals to communicate, especially when other channels of communication are impaired. Many questions remain to be answered, however, not only in relation to the species studied here but also in relation to teleosts in general.

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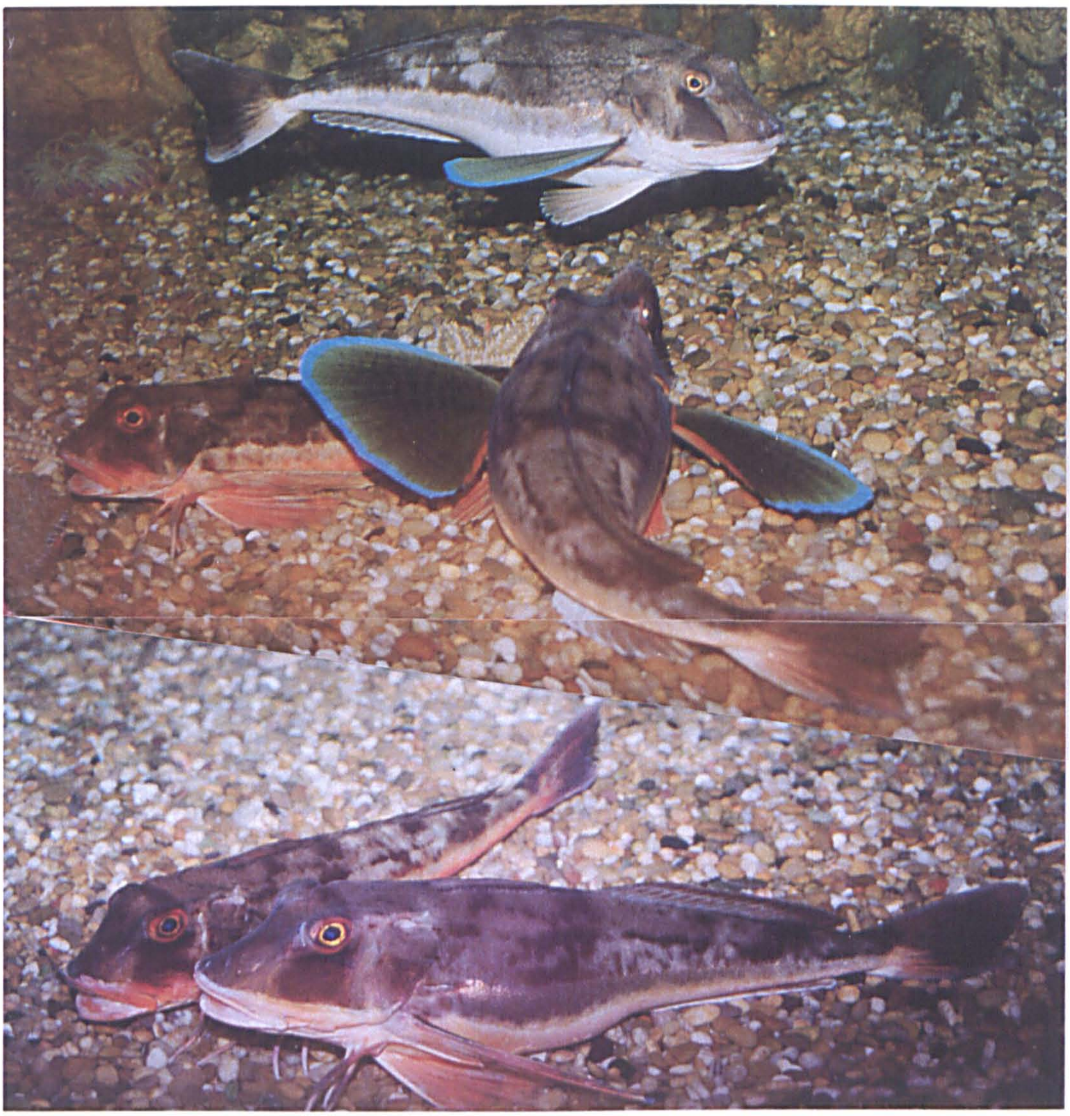
APPENDIX 1

A



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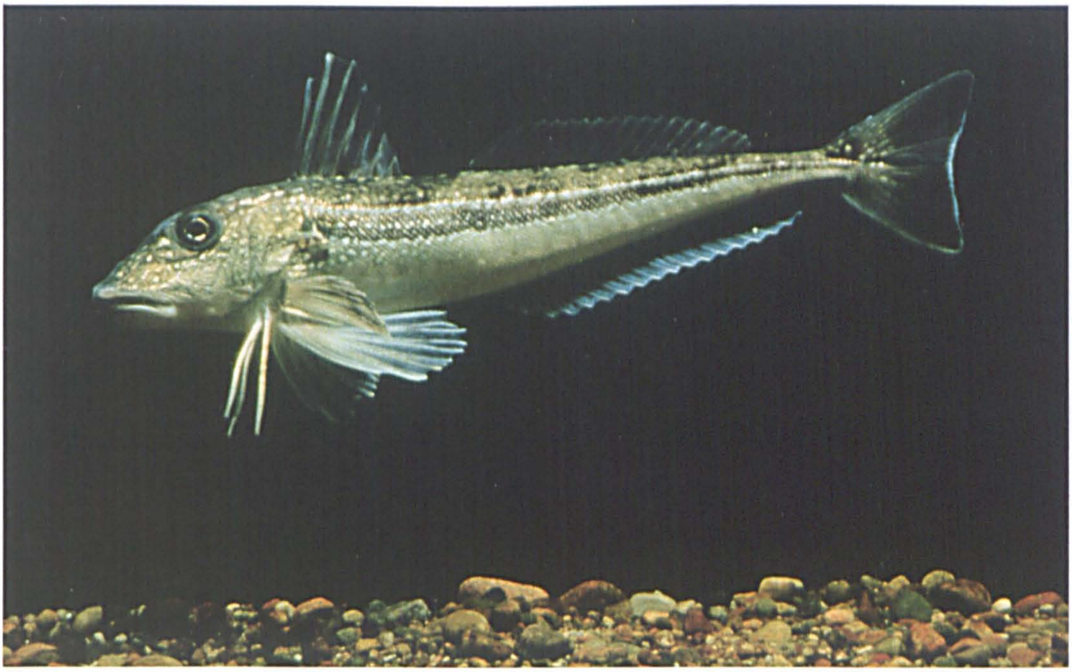

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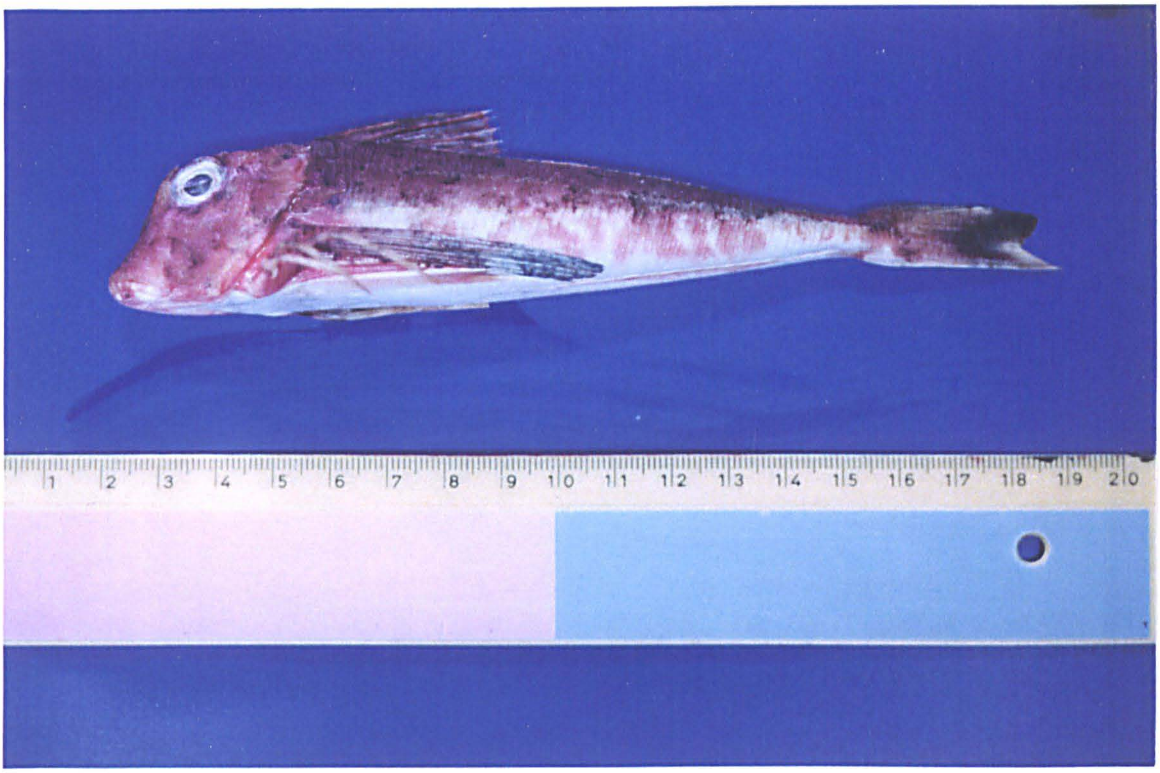

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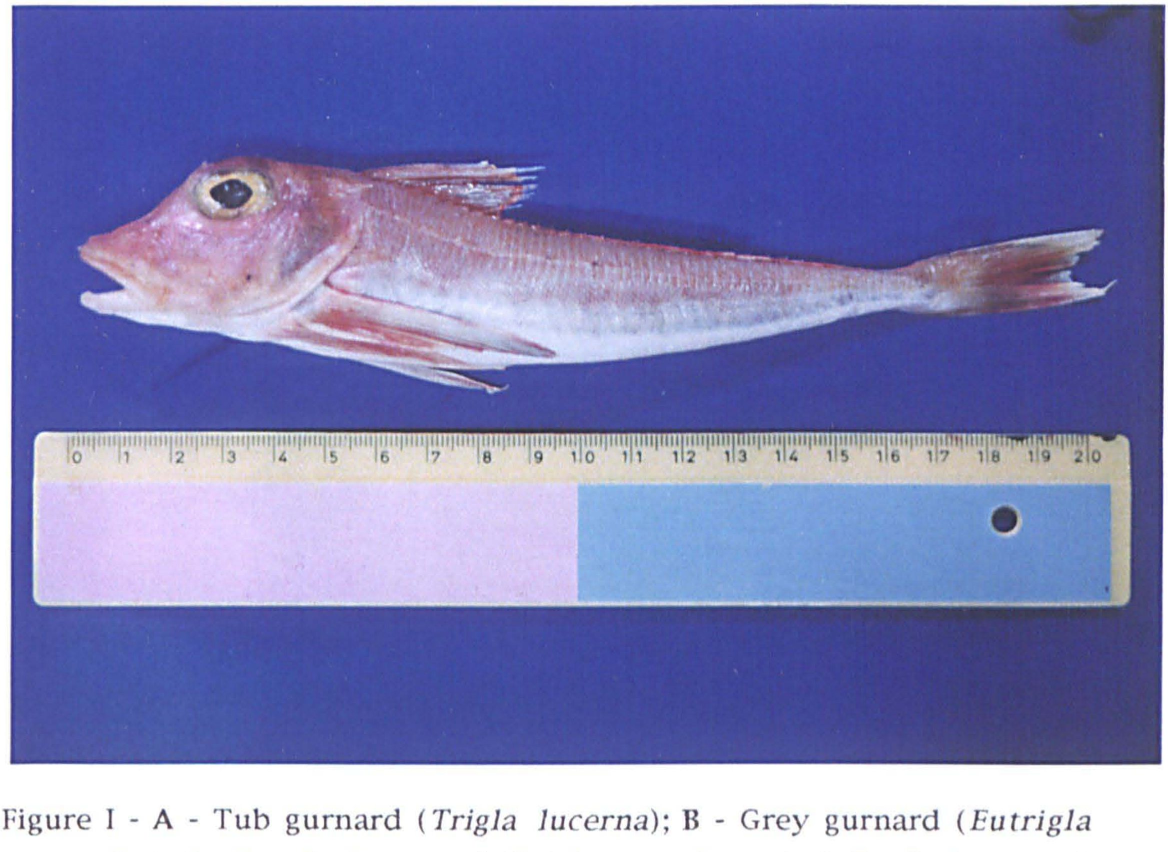

Figure I - A - Tub gurnard (*Trigla lucerna*); B - Grey gurnard (*Eutrigla gurnardus*); C - Streaked gurnard (*Trigloporus lastoviza*); D - Red gurnard (*Aspitrigla cuculus*).

APPENDIX 2

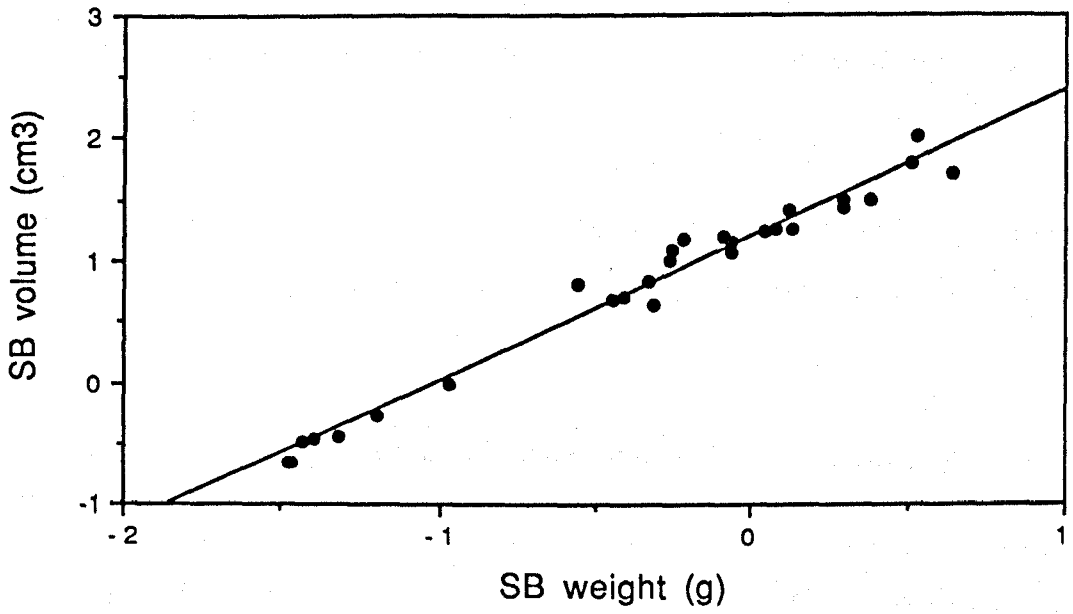

Figure II - Relation between the swimbladder (SB) volume and weight in the grey gurnard. SB weight and volume are transformed by $\log_{10}$. The regression equation fitted to the transformed data is $y = 1.20 + 1.19x$. Sample size = 28. The regression coefficient $r = 0.988$; $P < 0.001$. Swimbladder volumes were measured using milimetric paper to determine length, width and height; the swimbladder was assumed to be close to a parallelepiped.

APPENDIX 3