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Sound production and sonic apparatus in deep-living cusk-eels (*Genypterus chilensis* and *Genypterus maculatus*)

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ABSTRACT

Cusk-eels (Ophidiidae) are known sound producers, but many species live in deep water where sounds are difficult to record. For these species sonic ability has been inferred from inner anatomy. *Genypterus* (subfamily Ophidiinae) are demersal fishes inhabiting the continental shelf and slope at depths between 50 and 800 m. Males and females *G. maculatus* have been maintained together in a tank and 9 unsexed specimens of *G. chilensis* in a second tank, providing a valuable opportunity to record the sounds of living species usually found at great depths. *Genypterus chilensis* and *G. maculatus* respectively produced one and two sound types mainly between 7 and 10 pm. Sound 1 in *Genypterus maculatus* consists of trains of pulses that vary in amplitude and pulse period; call 2 sounded like a growl that results from the rapid emission of pulses that define sound 1. *Genypterus chilensis* produced a growl having an unusual feature since the first peak of the second pulse has always greater amplitude than all other peaks. These sounds are probably related to courtship behavior since floating eggs are found after night calls. The anatomical structures of the sound-producing organ in both species present an important panel of highly derived characters including three pairs of sonic muscles, a neural arch that pivots on the first vertebral body and a thick swimbladder with unusual features. Sonic structures are similar between species and between sexes. Therefore both biological sexes are capable of sound production although precedent from shallow ophidiids and sonic fishes in general suggests that males are more likely to produce courtship calls. This study reports two main types of information. It demonstrates that two deep-living species are capable of sound production, which is a pioneer step in the acoustic study of deep-sea fauna. Recorded sounds should also help to locate fish in open sea. As these species are currently used to diversify the aquaculture industry in Chile, deeper studies on their acoustic behavior should also help to target spawning period and to identify mature specimens.

1. Introduction

Sound production for social communication is a crucial part of behavior in an increasing number of fish species (Fine and Parmentier, 2015; Lobel et al., 2010). In most cases, species have been recorded in shallow water on coral reefs, estuaries, continental shelves or in lakes and rivers. However, the fish distribution at sea is not restricted to shallow areas, and many species live in bathyal zones. Fish recordings were already realized around 120 m (Ruppé et al., 2015), but potential fish sounds are also reported deeper, from 700 m to 1800 m (Mann and Jarvis, 2004; Rountree et al., 2012; Wall et al., 2014). The absence of recordings is frustrating since inner anatomy of many deep species clearly indicates they possess muscles attached to the swimbladder (Fine et al., 2018; Howes, 1992; Marshall, 1967; Nguyen et al., 2008), a

feature well known to evoke sound production (Fine and Parmentier, 2015). Although sounds may be the best mode of communication for fish active at night (Ruppé et al., 2015) and/or in a large dark environment like the deep-sea (Mann and Jarvis, 2004), the lack of data could be due to different factors such as being at the right place at the right moment, technical limitations or the fact deep-sea fish produce only low amplitude sounds (Wall et al., 2014). Additionally, increasing hydrostatic pressure would decrease sound amplitude using equations for a resonant bubble (Sprague, Fine and Cameron, pers. com.).

Among potential callers in deep environments, Ophidiiform species are good candidates for several reasons. Sound-producing mechanisms 1) are found in all but one species examined to date (Howes, 1992; Marshall, 1967), 2) are quite complex with up to 6 sonic muscles (3 pairs) in some species and deep modifications of the swimbladder,

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rostral vertebral bodies and associated epineurals (Parmentier et al., 2010, 2008a, 2006a; Rose, 1961), 3) are able to produce different sounds (Mann et al., 1997; Parmentier et al., 2016a, 2016b, 2018b, 2008b; Sprague and Luczkovich, 2001) and have sexually dimorphic sonic systems (Ali et al., 2016; Casadevall et al., 1996; Kéver et al., 2014a, 2014c; Nguyen et al., 2008; Rose, 1961). These features clearly support the importance of sonic communication in the Ophidiiformes (Fine et al., 2018, 2007; Nguyen et al., 2008). Since many species live in deep water (Nielsen et al., 1999) sound recordings have only been made for a few shallow species from the Carapidae (Kéver et al., 2014c; Parmentier et al., 2016a, 2016b, 2018b, 2003) and Ophidiidae (Kéver et al., 2016, 2015; Mooney et al., 2016; Rountree and Bowers-Altman, 2002).

Genypterus species are benthic-demersal Ophidiiformes inhabiting the continental shelf and slope in the southern hemisphere at depths between 50 and 800 m (Freijo et al., 2009; Smith and Paulin, 2003; Wiff et al., 2007). Within the genus, *Genypterus chilensis* (Guichenot, 1848) and *Genypterus maculatus* (Tschudi, 1846) have been highly exploited for human consumption and are candidates for aquaculture to counterbalance the effects of overfishing (Aedo et al., 2015; Vega et al., 2018, 2012). The Marine Research Center Quintay (CIMARQ, Chile) of Andrés Bello University was the first aquaculture center to spawn both species in captivity and maintain them for their entire life cycle. This situation has provided a valuable opportunity to study living species found at great depths. Our aim was to record and describe the sounds made by both species in order to know their acoustic features for future recording in the open sea and to describe their sonic anatomy. Showing these fish are capable of sound production is a pioneering step in the acoustic study of deep fauna.

2. Materials and methods

Individuals were collected from Bahía Quintay (33°11'S; 71°1'W), Región de Valparaíso, Chile. Wild fishes were stocked in large plastic tanks (3 m diameter X 0.65 m height) in the "Centro de Investigaciones Marinas de Quintay" (33°11' S, 71°1' W) belonging to the Universidad Andrés Bello (CIMARQ).

All procedures and all methods were approved by the ethical commission of the Universidad Andrés Bello, Quintay, Chile. All experiments were performed in accordance with the relevant guidelines and regulations.

2.1. Sound analysis

Recordings were made in October 2016 at CIMARQ. Both species were kept in separate fiberglass tanks under natural temperature ($13 \pm 1^\circ\text{C}$) and photoperiod (13L:11D). The first tank sheltered 11 specimens of the black cusk-eel *Genypterus maculatus* (4 females, 5 males and 2 with undetermined sex). The second tank sheltered 9 specimens of the red cusk-eel *Genypterus chilensis* whose sex was undetermined.

Sounds were recorded at random times with a hydrophone (HTI Min-96, $-164.4\text{ dB re } 1\text{ V for a sound pressure of } 1\mu\text{Pa}$; High-Tech Industries, Long Beach, MS, USA) connected to a Tascam DR-05 recorder (44.1 kHz sampling rate; TEAC, Wiesbaden, Germany). The hydrophone was placed close to the center of the tank, 5–10 cm from the bottom. The black wall of the tank did not allow us to observe fish behavior. A mini-DSG, a low-power acoustic recorder ($-180\text{ dB re } 1\text{ V.IPa}^{-1}$) designed to sample at different rates, was deployed in the *G. maculatus* tank for four days and for 3 days for *G. chilensis*. Because previous random recordings showed that sounds are made after sunset, the DSG was deployed from 1800 to 0800 h. It was set to record 8 min per half-hour at 20 kHz, and the memory was unloaded each day.

Different calls were randomly selected for analysis. Sounds from HTI recordings were digitized at 44.1 kHz (16-bit resolution) and analyzed using Avisoft-SAS Lab Pro 4.33 software. Only sounds with a good

signal to noise ratio were analyzed. Temporal features were measured from oscillograms and frequency parameters from power spectra (Hamming window) and double-checked with spectrograms (FFT size 256 points, time overlap 96.87%, and a Flat Top window). The sound (or call) is made of one to multiple units (pulses), and pulses are made of one to several cycles. The following sound parameters were measured: sound duration; number of pulses per sound; pulse period (measured as the average peak-to-peak interval between consecutive pulses determined for the entire sound, ms) and dominant frequency (the peak frequency with the most energy, Hz). With continuous tonal sounds the dominant frequency was the fundamental frequency. Sounds of both species were powerful enough so that one could feel tank vibration with the hands of the tank wall. Unfortunately wall vibrations, which may cause some distortion, is a common problem in tank studies (Akamatsu et al., 2002; Parmentier et al., 2014).

2.2. Dissection

The study of the morphology has been realized in two steps. First, one male and one female of each species were dissected to describe the swimbladder and associated muscles. Head lengths of the male and female *Genypterus maculatus* were 17.3 and 14.5 cm respectively and 14.2 and 10.2 cm for *Genypterus chilensis*. The morphology of the sonic apparatus was examined with a Wild M10 (Leica) binocular microscope equipped with a camera lucida.

The anterior skeletons of the four specimens were investigated with computed tomography (μCT) imaging systems. Structural images of the sound-producing apparatus were obtained using the X-ray Computed Tomography scanner *eXplore 120 microCT* (TriFoil Imaging, USA). The physical characteristics of this scanner have been described previously (Bahri et al., 2010; Bretin et al., 2013). This system acquires a set of images of samples with a high resolution ($100\mu\text{m}$) that can be reconstructed to a three-dimensional volume. We used a customised protocol "Fast-scan 360" (70 kV, 0.512 mAs, 360 views over 360° , continuous rotation). Segmentation and surface rendering of the skull, anterior vertebrae and associated bony structures were done in Amira 5.4.0 (FEI, Hillsboro, OR, USA). Automatic thresholding was used to identify bones.

3. Results

Sounds were only recorded after sunset (Fig. 1). In these dark conditions inside the black tank wall, it was impossible to associate sounds with behavior. However, egg masses were floating in the tank in the morning indicating spawning took place during the night and suggesting that acoustic behavior is associated with courtship and reproduction.

A total of 248 sounds was analyzed in *Genypterus maculatus*, which produces two sound types. Sound 1 consists of trains of pulses that vary in amplitude and pacing (Fig. 2). Trains varied from 0.65 s to 4 s and were made of 4–17 pulses. Pulses were emitted irregularly with a period ranging from 54 to 850 ms (mean \pm s.d.: $309 \pm 171\text{ ms}$, $n = 153$), but there was a significant correlation ($r = 0.83$, $p = 0.007$) between the call duration and number of pulses. Pulse duration was $52 \pm 13\text{ ms}$ ($n = 174$). The fundamental frequency (Fig. 3A) was particularly low at $62 \pm 8\text{ Hz}$ ($n = 176$).

Genypterus maculatus call 2 sounded like a growl (Fig. 3B). Its duration ranged from 80 to 615 ms ($332 \pm 87\text{ ms}$, $n = 74$) and duration was strongly related to the number of pulses ($r = 0.97$, $p < 0.001$). Growls resulted from the rapid emission of pulses that defined sound 1. During the growl, the pulse emission rate accelerates but individual pulse waveforms can be clearly distinguishable at the beginning of the growl (Fig. 4A, B). As the pulse repetition rate increased during the call, portions of the cycle waveforms were more and more truncated and the oscillation became restricted to single peaks (Fig. 4C, D). Our hypothesis is supported by the similarity of the shape

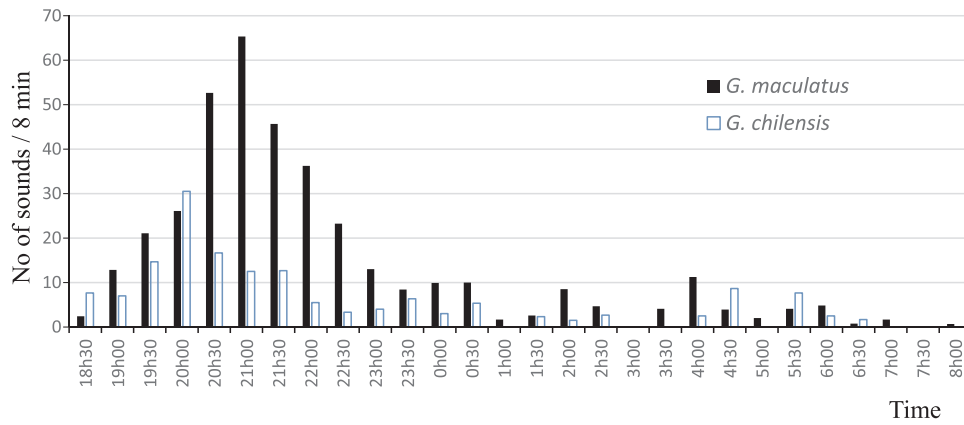


Fig. 1. Number of calls per 8 min by *Genypterus maculatus* (n = 4 days) and *Genypterus chilensis* (n = 3 days).

in isolated pulses (Fig. 4A) and the ones at beginning of the growl (Fig. 4C). They are both made of two cycles (Fig. 4): the first is always shorter and of greater amplitude than the second one that possesses an additional small peak (Fig. 4). Depending on the pulse period, some oscillations (Fig. 4B) were intermediately shaped between the isolated pulse (Fig. 4A) and the growl (Fig. 4D), supporting our hypothesis. This configuration also supports the emission rate is increasing from the beginning of the call towards its end: the pulse period decreases from ca 20–10 ms depending to the number of pulses. The mean dominant frequency (Fig. 3C) was 61 ± 5 Hz (n = 73), which is not significantly different from sound 1 (t-student $t = 1.004$ df = 247, $p = 0.31$). Interestingly, the period between two peaks in isolated pulses (14.2 ± 1 ms, n = 58) and between peaks in tonal sounds (14.7 ± 2 ms, n = 58) was not significantly different (Mann Whitney, $p = 0.44$). The growl of *Genypterus maculatus* sound reveals up to 9 harmonics with a fundamental frequency (corresponding to the dominant frequency) of approximately 60 Hz.

Genypterus chilensis produced one sound type (Fig. 5). The waveform is not easy to describe because of overlap between the pulses. One unusual feature can however be found at the onset of the call where the first peak of the second pulse has greater amplitude than all other peaks. At the end of the call, pulses were further apart, making it easier to distinguish different pulses, each made of two peaks; the first peak had a greater amplitude and higher pitch than the second one. Many calls end with an alternation between short and long pulse periods, which could be an acoustic signature for *G. chilensis*. Moreover, peak amplitude within each pulse can vary throughout the call, and the peak with greater amplitude can be either the first or the second (Fig. 5).

Calls varied from 164 to 285 ms (221 ± 31 ms, n = 32) and were made of 7–19 units (12 ± 3 ms, n = 13). The pulse period ranged from 7 to 28 ms (14 ± 4 ms, n = 137). The pulse duration was 16 ± 5 ms (n = 41) whereas the call dominant frequency (Fig. 3) was 102 ± 34 Hz (n = 31). Contrary to *G. maculatus*, pulses are not truncated in *G. chilensis*; it means all the pulses in the call are still distinguishable.

3.1. Sound-producing mechanism

Females clearly have robust sonic muscles meaning also the sound-producing mechanism is roughly similar between species and between sexes. Differences may be present in the relative weights of muscles, but the small number of dissected fish does not allow a quantitative conclusion.

The description is based mainly on a female *Genypterus chilensis*. The sonic apparatus is composed of modified vertebra, three pairs of sonic muscles and the swimbladder.

The first neural arch (termed the neural rocker) is highly specialized and has no neural spine. The neural arch is shaped like a triangle above the vertebra with both left and right branches articulating with the vertebral body so that it pivots in the anteroposterior plane (Fig. 6). Just above its articulation on the vertebral body, the neural arch possesses a cavity allowing the attachment by connective fibres from the first epineural, classically called the wing-like process (Fine et al., 2007). This structure is shaped like a board disposed on its edge, offering a vertical plane for sonic muscle insertion. The second vertebra has two rod-like epineurals (Fig. 6). Both first and second epineurals are

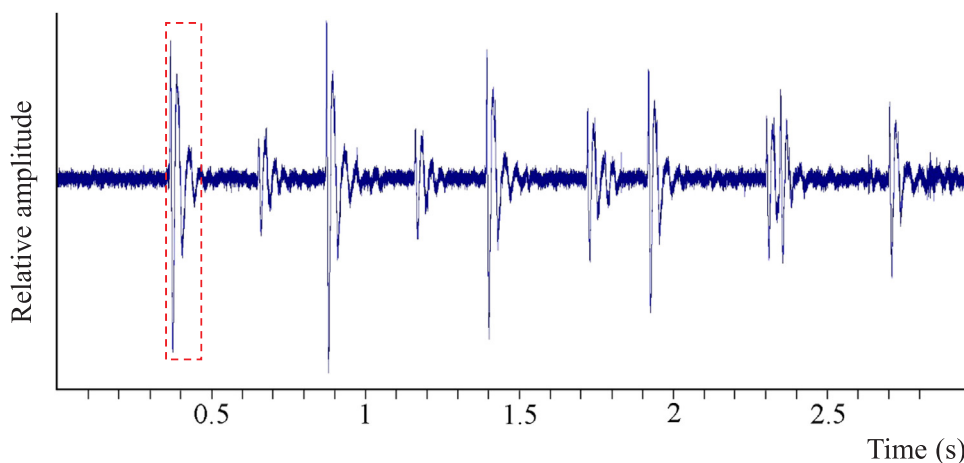


Fig. 2. Oscillogram of *Genypterus maculatus* call 1 with irregularly emitted pulses that vary in amplitude. Red rectangle indicates a single pulse. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

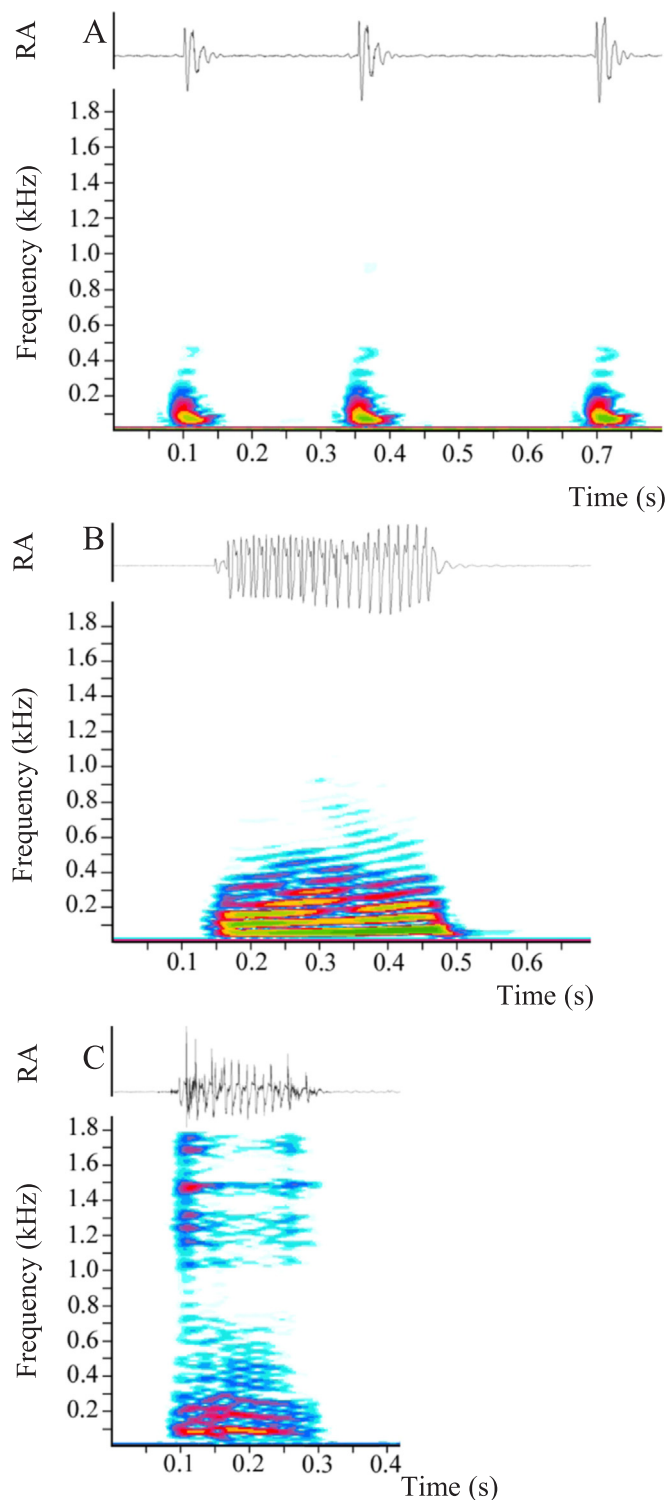


Fig. 3. Oscillograms and corresponding spectrograms for two sound types in *Genypterus maculatus* (A, B) and the growl in *Genypterus chilensis* (C). Spectral analysis information: sampling frequency 44,100 Hz; Windows: Flat Top; FFT length: 512. RA: Relative Amplitude.

firmly connected by ligaments along their entire length. A short ligament connects the distal tips of both epineurals with the lateral part of the anterior swimbladder (Fig. 7). The third, fourth and fifth epineurals are shaped like an arch and articulate directly on their corresponding vertebral bodies. First parapophyses are found on the sixth vertebra. The third epineurals is enlarged and corresponds to the swimbladder plate found in different Ophidiiform species (Howes, 1992; Parmentier et al.,

2010, 2016a, 2016b; Parmentier et al., 2010, 2010, 2003)

The bladder lies under the first through thirteenth vertebrae and is tightly coupled to the vertebral column through the ninth vertebra. The swimbladder is a slender sac consisting of a single chamber, which possesses two lateral tubercles anteriorly. The swimbladder ends in a short tail (Fig. 8). In lateral view, the anterior part of swimbladder has a step-like shape which tread extends up to the third epineurals. The white outer swimbladder walls, the tunica externa, are thick (ca. 2.5 mm), rigid and relatively resistant to deformation. A thinner tunica interna surrounds the swimbladder lumen. At the level of the anterior tubercles, this inner tissue had two mucus masses (Fig. 6) whose function is unclear. Anterior and posterior margins of the third epineurals possess intimate connections with the swimbladder. The tunica externa inserts on the rostral margin of epineural 3 and descends vertically, corresponding to the riser of the “swimbladder step” previously mentioned. Caudally, the tunica externa attaches along the posterior margin of epineural 3.

The bladder may be divided into three regions. The anterior region is notably thickened and possesses vertical trabeculae joining the dorsal to ventral side of the swimbladder. The second region, termed the swimbladder fenestra in Ophidiiform fishes (Fine et al., 2007; Parmentier et al., 2006b), is straight and thin because it is deprived of the tunica externa. In both species, this region is hidden by the vertebrae and is not easy to see. The third region is the largest and corresponds to the remainder of the bladder. It begins at the level of epineural 3 and is intimately coupled to the vertebral bodies and parapophyses.

Three pairs of sonic muscles are involved in the sound-producing system (Fig. 8). The dorsal sonic muscle (DSM) originates on the neurocranium (exoccipital, supraoccipital and epiotic) and inserts on the neural rocker of the first vertebra. Manually pulling on the DSM causes the first neural arch to pivot rostrally, causing the tips of the wing-like process to pivot posterodorsally, thereby stretching the anterior part of the swimbladder. The ventral sonic muscle (VSM) originates on the rostral margin of the basioccipital; this bone has also developed a ventral crest allowing the attachment of numerous muscular fibres. It ends on the riser of the “swimbladder step”, at the level of the third epineurals. Manually pulling the VSM causes the rostral displacement of the anterior part of the swimbladder. Between the 3rd and 4th epineurals, dorsal folding in the swimbladder allows rostral extension of the swimbladder because muscle contraction unfolds this part of the swimbladder. The intermediate sonic muscle inserts on the rostral vertical plane of the wing-like process. Baudelot’s ligament, which connects the basioccipital to the pectoral girdle, penetrates this muscle. The contraction of the intermediate muscles displaces the wing-like process forward and outward, stretching the anterior part of the swimbladder laterally. Rostrally, the origin is quite unusual. Muscle fibres originate on a tendon that attaches to an area extending from the intercalar to the pterotic. In addition, a ventral enlarged tendon covers parts of the anterior VSM and attaches at the border between the basioccipital and the intercalar.

We did not notice important differences between species and sexes. There may be some differences in the size of the muscles, but the number of specimens is too small to permit solid conclusions.

4. Discussion

Both species produce sounds that are probably involved with courtship behavior since egg masses were found floating in the tanks. Moreover, the sonic behavior is similar to that of Ophidiiforms living in shallow water including *Ophidion marginatum* (Mann et al., 1997; Mann and Grothues, 2009; Rountree and Bowers-Altman, 2002), *Ophidion rochei* (Parmentier et al., 2010) and *Onuxodon fowleri* (Kéver et al., 2016, 2014c). Similar to these shallow species, sound production starts approximately 1 h after dusk, peaks 1–3 h after sunset and can last for the whole night. Qualitative association between sound production and

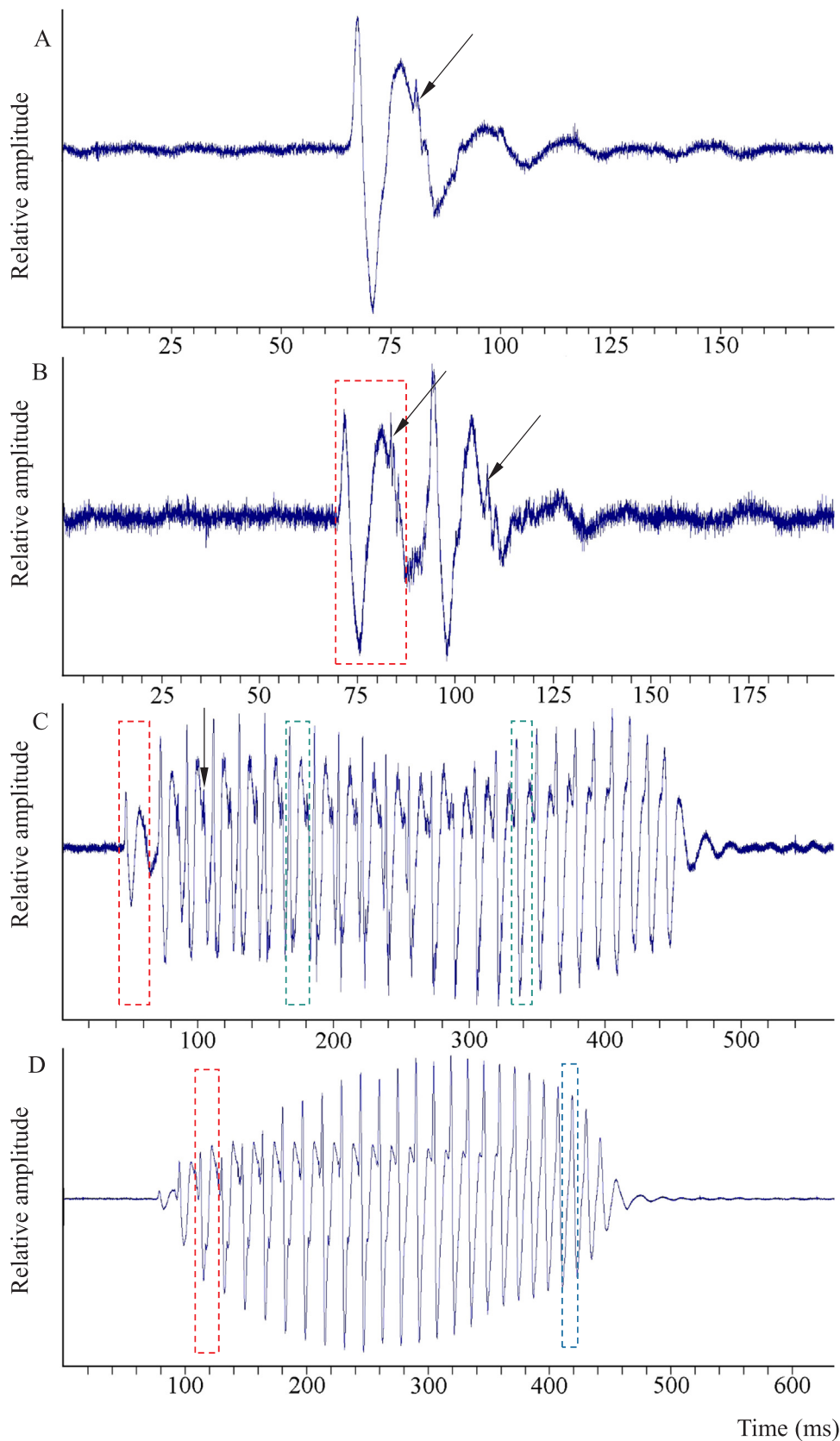


Fig. 4. Oscillograms illustrating different calls of type 2 (growl, 4B to 4D) produced by *Genypterus maculatus*. The different panels support the hypothesis that growls result from repetition of the individual pulse shown in 4A. They are two pulses in 4B. In 4C and 4D, pulse rate accelerates so that individual pulse waveforms occur at the beginning of the growl but subsequent pulses are less clearly defined. Red dotted rectangles delineate a single pulse corresponding to 4A. Green dotted rectangles show pulses can have different shapes according to their positioning in the call. At the end of the call in 4C and 4D, each pulse is restricted to a single cycle (blue dotted rectangle). Black arrows show the extra-peak on the second main peak of the pulse. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

spawning has also been shown in some Sciaenidae (Connaughton and Taylor, 1996; Locascio et al., 2012; Lowerre-Barbieri et al., 2008; Montie et al., 2016), Gadidae (Hawkins and Amorim, 2000; Rowe and Hutchings, 2006), Gobiidae (Lugli et al., 1997), etc. Functions of

soniferous behavior related to spawning include the attraction of gravid females, the supply of information on the male (and thus intersexual selection), the formation of spawning aggregations on a specific location or the synchronisation of the reproductive behavior of the male

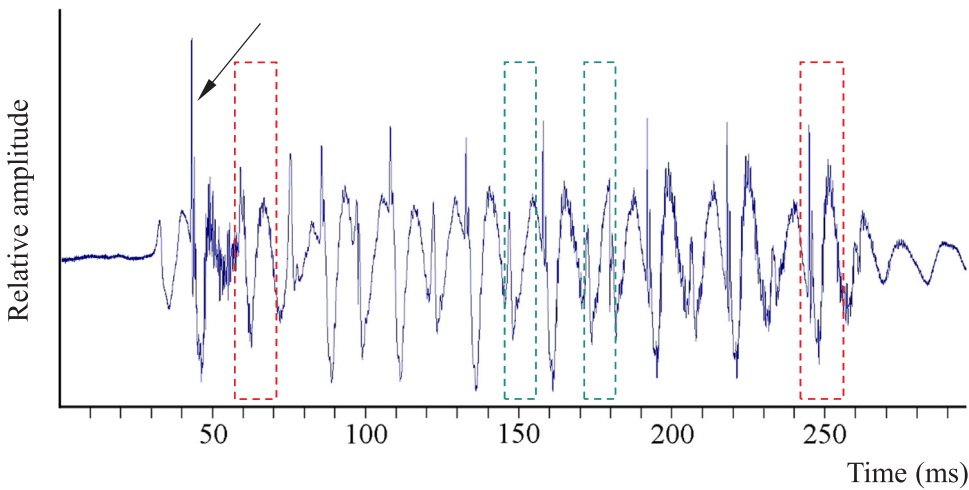


Fig. 5. Oscillogram of a call produced by *Genypterus chilensis*. Red and green dotted rectangles delineate a single pulse. In green, the first peak of the pulse is weaker than the second peak whereas this first peak is higher than the second peak in red sections. Arrow highlights the cycle of the first pulse is always higher than others. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and female. (Connaughton and Taylor, 1996; Gilmore, 2003; Mok and Gilmore, 1983). In the case of *Genypterus* species, more research is needed since males and females both possess sound-producing mechanisms which is not the case in most sciaenid species (Parmentier et al., 2017, 2014; Ramcharitar et al., 2006).

Results on both species can be developed into two research fields. First, sounds could be used to improve rearing of *Genypterus* in aquaculture because their acoustic behavior will target the spawning period and can be used to identify mature specimens. It could also be used to select prolific breeding pairs. In captive spotted seatrout (*Cynoscion nebulosus*), a positive relationship exists between the sound level and the number of eggs (Montie et al., 2017).

The recording in fiberglass tanks has probably modified some acoustic features in both species (Parmentier et al., 2014), complicating the analysis (Parmentier et al., 2018b). However, it is quite impossible to study these fish in the field without information on the sounds they produce, and these problems occur ubiquitously in tank studies

(Akamatsu et al., 2002; Parmentier et al., 2014). This study furnishes features that should help future fish-sound identifications in the open sea since potential distortion does not affect the pulse period equivalent to the fundamental frequency or, the number of pulses or the period between consecutive calls (Parmentier et al., 2014). Interestingly these features are also used by pomacentrid fishes for acoustic communication (Myrberg et al., 1978; Myrberg and Spires, 1972). Although they are closely related, the oscillogram shapes of both *G. chilensis* and *G. maculatus* clearly differ (Fig. 3B and C), which should help to discriminate the species in the field. The growl of *G. maculatus* possesses a declining pulse period (Fig. 4C and D) whereas the pulse period in *G. chilensis* is irregular and mostly presents a saw-tooth pattern (Fig. 5). The sound-producing apparatus of both species shows many similarities that support a common mechanism. This assumption is reinforced by the shape of a single pulse in each species. It is made of two peaks, the first one being narrower indicative of faster swimbladder movement than the second one. Moreover, the sounds in *G. maculatus* nicely show

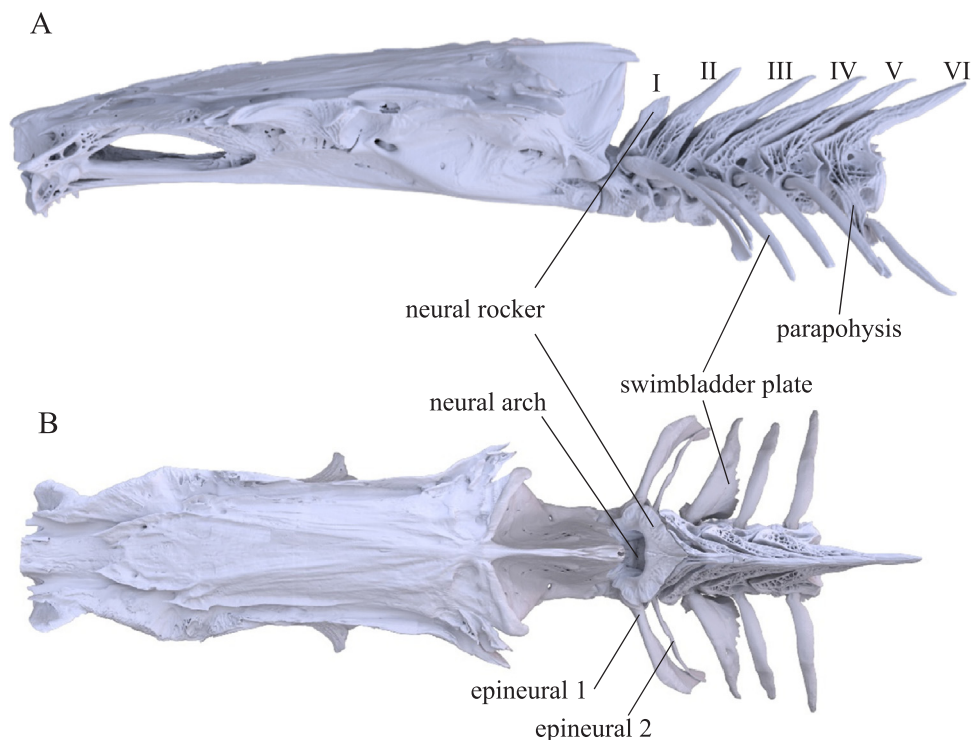


Fig. 6. Lateral (A) and dorsal B view of the skull and first vertebrae in a *Genypterus chilensis* female.

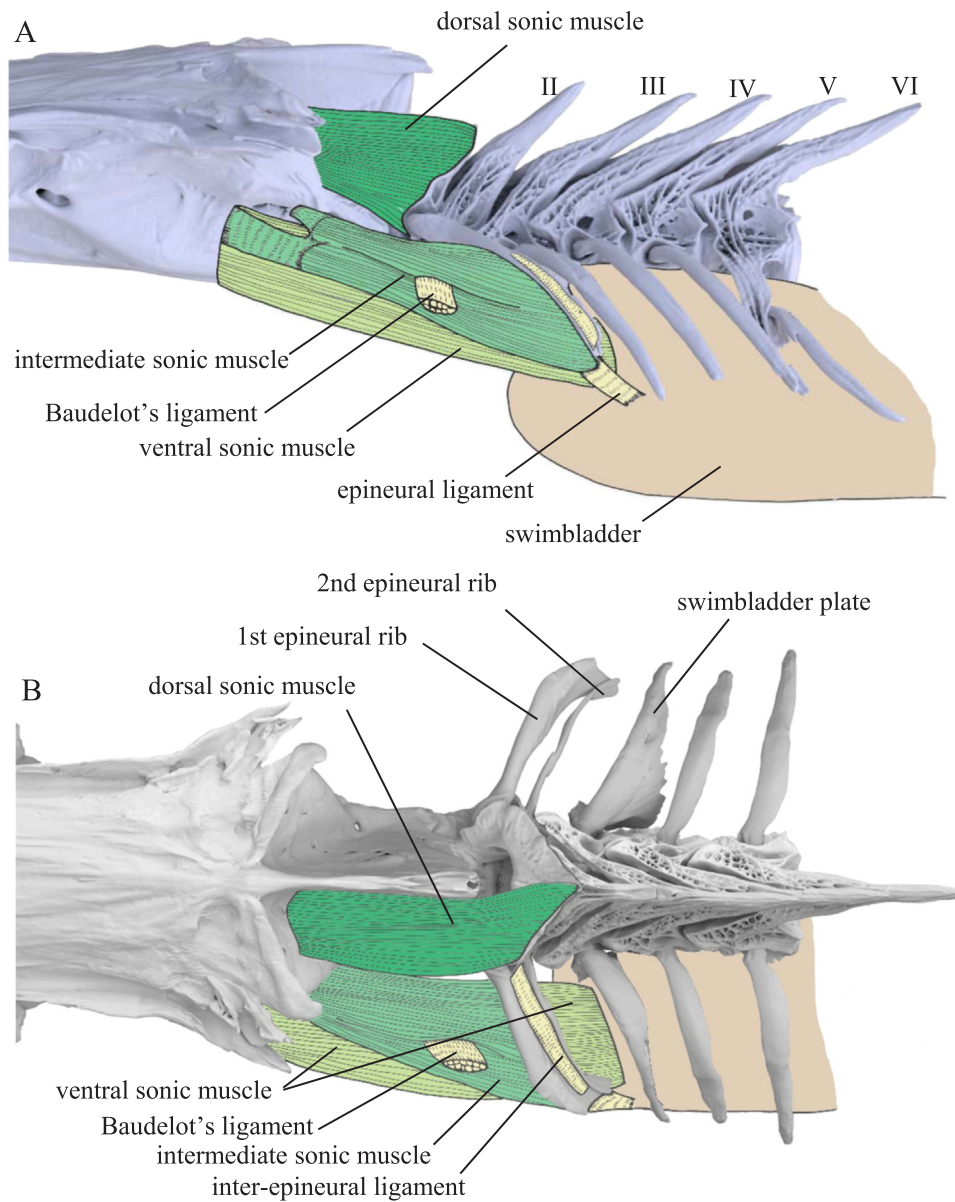


Fig. 7. In female *Genypterus chilensis*, left lateral (A) and dorsal (B) view of the sound producing apparatus showing the skull, the first vertebrae and sound producing muscles. In B, muscles and swimbladder are not shown on the right part.

the transition from pulsed sounds to tonal-like sounds. The different pulses are simply emitted in increased number and higher speed causing the second part of the pulse to be directly incorporated in the first part of the next one (Fine et al., 2001). The time period between two peaks corresponds to ca 14.5 ms (or 68 Hz) which corresponds to the fundamental frequency of these sounds. In *G. chilensis*, pulses are emitted at a period of ca 13.7 ms whereas the mean sound frequency is around 102 Hz. This small discrepancy can be easily explained by the variation in the pulse period within the call, ranging from 8 to 27 ms. Therefore the fundamental frequency is related to the pulse repetition rate. Experimental studies should however be conducted to confirm this because this kind of relationship has not been found in males of *Ophidion rochei* and *Ophidion marginatum*. In *O. rochei*, the dominant frequency is between 226 and 410 Hz and thus does not correspond to the pulse period that varies from 84 to 111 ms (Parmentier et al., 2010). In *O. marginatum*, sounds are broad-frequency pulses of 1–2 kHz and again does not correspond to the mean pulse period of 43.5 ms (Mann et al., 1997). It is worth mentioning males of both species possess unique morphological characteristics with a rocker bone in *O. rochei*

(Parmentier et al., 2010) and a cartilage cap in front of the swimbladder in *O. marginatum* (Courtenay, 1971). These kinds of features are not found in *Genypterus* species. In fact, the sonic mechanism of both *Genypterus* species is quite similar to *O. rochei* females that also possess a direct insertion of the VSM of the swimbladder. In *Ophidion rochei* females, there is a direct relationship between the fundamental frequency and the pulse period (Kéver et al., 2012). In summary, despite similar morphology, there appear to be major differences in frequency generation with the subfamily: in some species the frequency spectrum is determined by individual oscillations of the swimbladder and in others by the pulse-repetition rate.

Both *Genypterus* species share many common characters with other Ophidiiformes belonging to the group 1 proposed by Howes (Howes, 1992) such as *Neobythites* (Ali et al., 2016; Fine et al., 2018; Parmentier et al., 2018a), *Ophidion* sp. (Kéver et al., 2014b; Parmentier et al., 2010, 2006a) and *Lepophidium* sp. (Fine et al., 2007; Nguyen et al., 2008). These fishes possess somewhat different sonic morphology, but it seems they are all based on the same basic principle. A pair of ventral muscles originates on the skull and inserts directly on the rostral swimbladder.

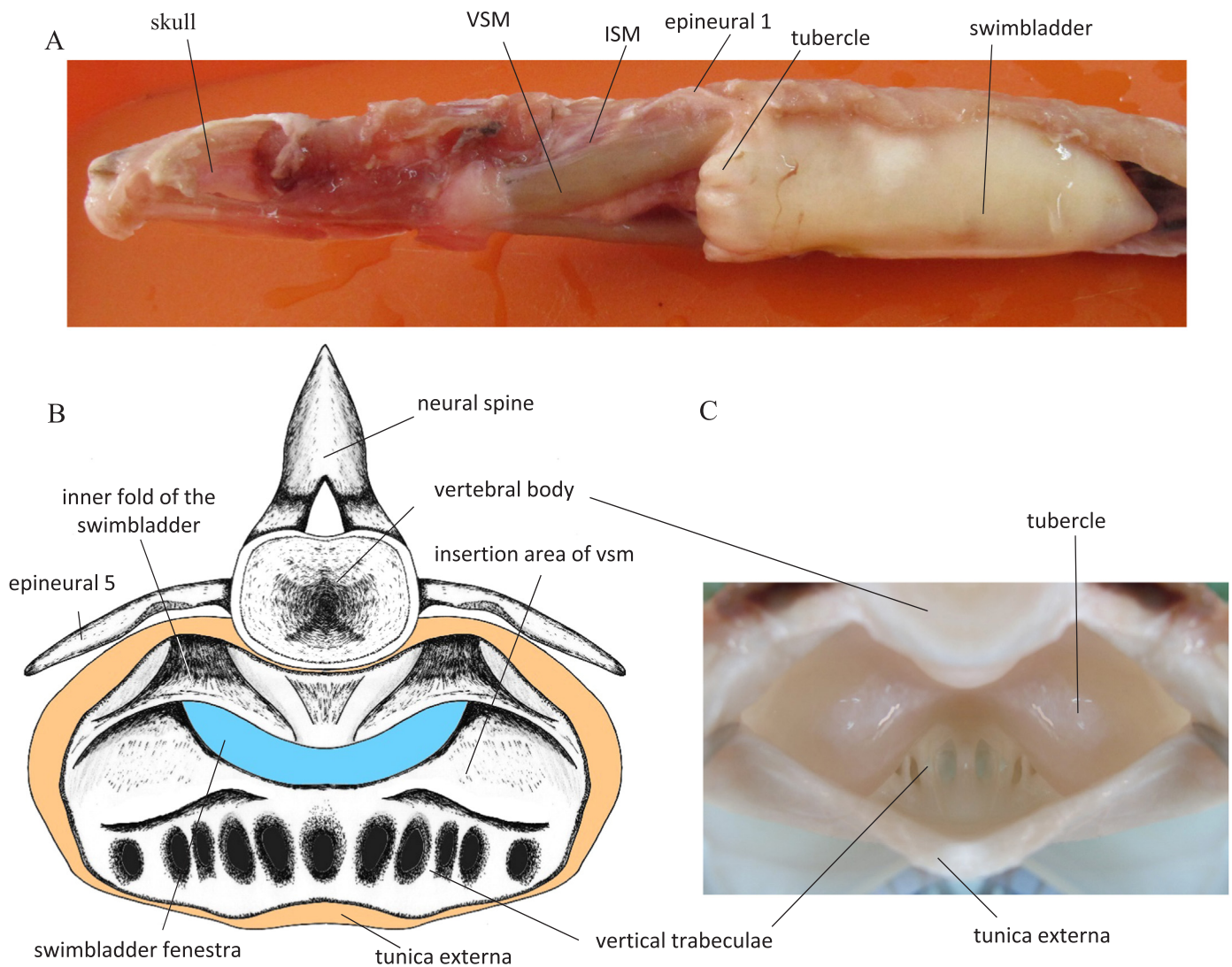


Fig. 8. Left lateral view of the anterior region in *Genypterus maculatus* male showing the swimbladder and main sound producing muscles (A). Diagram (B) and picture (C) show in cross section the inside swimbladder at the level of its rostral part in *Genypterus chilensis* female. The inner tubercle is delineated by the tunica interna. VSM: ventral sound-producing muscle; ISM: intermediate sound-producing muscle.

The ventral muscle appears to be the chief driver for sound production. A second pair of muscles originates on the skull, above the ventral muscles, and these muscles are close to or penetrated by Baudelot's ligament and insert on the first epineurals. Within this group, some species such as *Ophidion* sp. (Courtenay, 1971; Parmentier et al., 2010, 2006a) and *Lepophidium* (Fine et al., 2007; Nguyen et al., 2008) possess a pair of dorsal muscles that originates on the skull and inserts on the first neural arch. Intermediate and dorsal muscles are thought to adjust the swimbladder tightness.

Among these different species, the morphology of the sound-producing apparatus is close to that of *Ophidion* species (Parmentier et al., 2010, 2006a), supporting close phyletic relationships and helping to understand the mechanism. In *O. rochei*, the dorsal muscle contracts for the duration of the call, thereby placing the wing-like process in a dorso-caudal position. During that sustained muscle work, rhythmic contraction of the ventral sound producing muscle corresponds to the production of individual pulses (Kéver et al., 2014b; Parmentier et al., 2010). Because they possess many similarities, we infer the mechanism is similar in both *Genypterus* species. Within each species, all the pulses are identical, which suggests they are all formed in the same way. The contraction of the dorsal muscles pulls the neural rocker rostrally and consequently generates an upward movement of the distal tip of the

epineural and the resulting lateral extension of the anterior part of the swimbladder. During the sustained contraction of the dorsal muscles, rhythmic contractions of the ventral muscles provoke pulse emission. Pulses should be produced at the termination of the contraction, when the anterior part of the swimbladder snaps back and rebounds. *O. rochei* pulses are longer than in *G. chilensis* and *G. maculatus* probably because both species lack transverse membranes within the swimbladder (Parmentier et al., 2010, 2006a). In *O. rochei*, these membranes could allow the rebounds of acoustic waves stimulating longer pulses. Having the same kind of sonic mechanism, differences in sound features between *G. maculatus* and *G. chilensis* could be related to at least differences in neural firing (Bass et al., 2015).

5. Conclusion

This study indicates two deep-living species are capable of sound production, which is a pioneer step in acoustic studies. It also suggests we should be able to record sounds in many other deep-living Ophidiiformes because they show a similar sound-producing apparatus. Moreover, sounds could be used to improve rearing of *Genypterus* species in aquaculture.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EP and JME designed the experiments. EP carried out the recordings and analysed the data. MAB and AP realised the CTscan study EP studied the sonic mechanisms. EP, MLF and JME wrote the manuscript. All authors gave final approval for submission.

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