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RESEARCH ARTICLE

Sound production in *Onuxodon fowleri* (Carapidae) and its amplification by the host shell

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ABSTRACT

Onuxodon species are well known for living inside pearl oysters. As in other carapids, their anatomy highlights their ability to make sounds but sound production has never been documented in *Onuxodon*. This paper describes sound production in *Onuxodon fowleri* as well as the anatomy of the sound production apparatus. Single-pulsed sounds and multiple-pulsed sounds that sometimes last more than 3 s were recorded in the field and in captivity (Makemo Island, French Polynesia). These pulses are characterized by a broadband frequency spectrum from 100 to 1000 Hz. *Onuxodon fowleri* is mainly characterized by its ability to modulate the pulse period, meaning that this species can produce pulsed sounds and tonal-like sounds using the same mechanism. In addition, the sound can be remarkably amplified by the shell cavity (peak gain can exceed 10 dB for some frequencies). The sonic apparatus of *O. fowleri* is characterized by a rocker bone in front of the swimbladder, modified vertebrae and epineurals, and two pairs of sonic muscles, one of which (primary sonic muscle) inserts on the rocker bone. The latter structure, which is absent in other carapid genera, appears to be sexually dimorphic suggesting differences in sound production in males and females. Sound production in *O. fowleri* could be an example of adaptation where an animal exploits features of its environment to enhance communication.

KEY WORDS: Carapidae, Morphology, Pearl Oyster, Rocker bone, Sound

INTRODUCTION

Coral reefs are recognized as noisy marine environments. Acoustic communication has been reported in dozens of coral reef species (e.g. Moulton, 1958; Myrberg, 1997; Parmentier et al., 2009; Parmentier et al., 2011) and those sounds constitute the dominant component of low-frequency biotic sounds in seas (Cato, 2008). In this context, coral reef fish sounds need to be conspicuous and species-specific to have a high communicative value, which is especially true for species active in the dark, where acoustic cues cannot be reinforced with visual signals. The fact that acoustic cues are used to support visual cues has been demonstrated in some cichlid fish such as *Metriaclima zebra* (Bertucci et al., 2010).

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Carapidae is a monophyletic ophidiiform family that includes eight genera (Markle and Olney, 1990; Nielsen et al., 1999; Anderson and Satria, 2007). The 33 carapid species are equipped with a sonic apparatus and, depending on the species, adults are free-living, commensal or parasitic (Trott, 1981; Parmentier et al., 2002). Adults from the genus *Carapus* and *Encheliophis* generally live in symbiosis with sea cucumbers (Parmentier and Michel, 2013) but some of them inhabit starfishes, bivalves or ascidians (Trott, 1981; Parmentier and Vandewalle, 2003). Sounds have been recorded in captivity for *Carapus acus*, *Carapus homei*, *Carapus boraborensis*, *Carapus mourlani* and *Encheliophis gracilis* (Parmentier et al., 2003; Lagardère et al., 2005; Parmentier et al., 2006b). The associated behaviors are, however, not clearly determined because the calls are mainly produced during intra- or interspecific encounters inside their holothurian host. Sounds have not yet been recorded in other carapid genera, although all species examined have a sound production apparatus (Parmentier et al., 2002).

Despite the differences in their sound production mechanisms (Courtenay and McKittrick, 1970; Parmentier et al., 2002; Parmentier et al., 2008), all carapid species have at least in common: (1) a bilateral pair of primary sonic muscles (PSMs) that originate on the upper wall of the orbit and insert on the anterior part of the swimbladder and (2) specializations in the epineurals of the first three vertebrae (Courtenay and McKittrick, 1970; Parmentier et al., 2002). Many of them are also equipped with a bilateral pair of secondary sonic muscles (SSMs) that originate on the caudal part of the skull and insert on the first epineurals (Courtenay and McKittrick, 1970; Parmentier et al., 2002). In non-ophidiiform teleosts, sexual dimorphism in the sound production apparatus seems to be commonly related to the size of sonic muscles and swimbladder (Kratochvil, 1980; Fine et al., 1990; Brantley et al., 1993) or the absence or presence of sonic muscles (Hill et al., 1987; Tellechea and Norbis, 2012). However, the recent work of Conway et al. (Conway et al., 2014) highlighted extreme (shape and size) internal dimorphisms in some minnows, which suggest that pronounced intersexual differences may have been missed, even in well-studied groups. In the past, sex-related differences in the sound-production apparatus (and sounds) of carapids may have been overlooked because they are inconspicuous, especially when compared with the sexual dimorphism observed in the sister family Ophidiidae (Rose, 1961; Courtenay, 1971; Kéver et al., 2012).

The physiology and histology of fish sonic muscles has been investigated in some fish species because it can help to understand the sound-production mechanism (Fawcett and Revel, 1961; Tavolga, 1964; Gainer et al., 1965; Eichelberg, 1977; Fine et al., 1990; Fine et al., 2001; Connaughton, 2004; Parmentier and Diogo, 2006; Parmentier et al., 2006a; Rome, 2006; Kéver et al., 2014). A key finding is that all superfast sonic muscles show structural similarities such as thin fibers, a high proportion of sarcoplasmic

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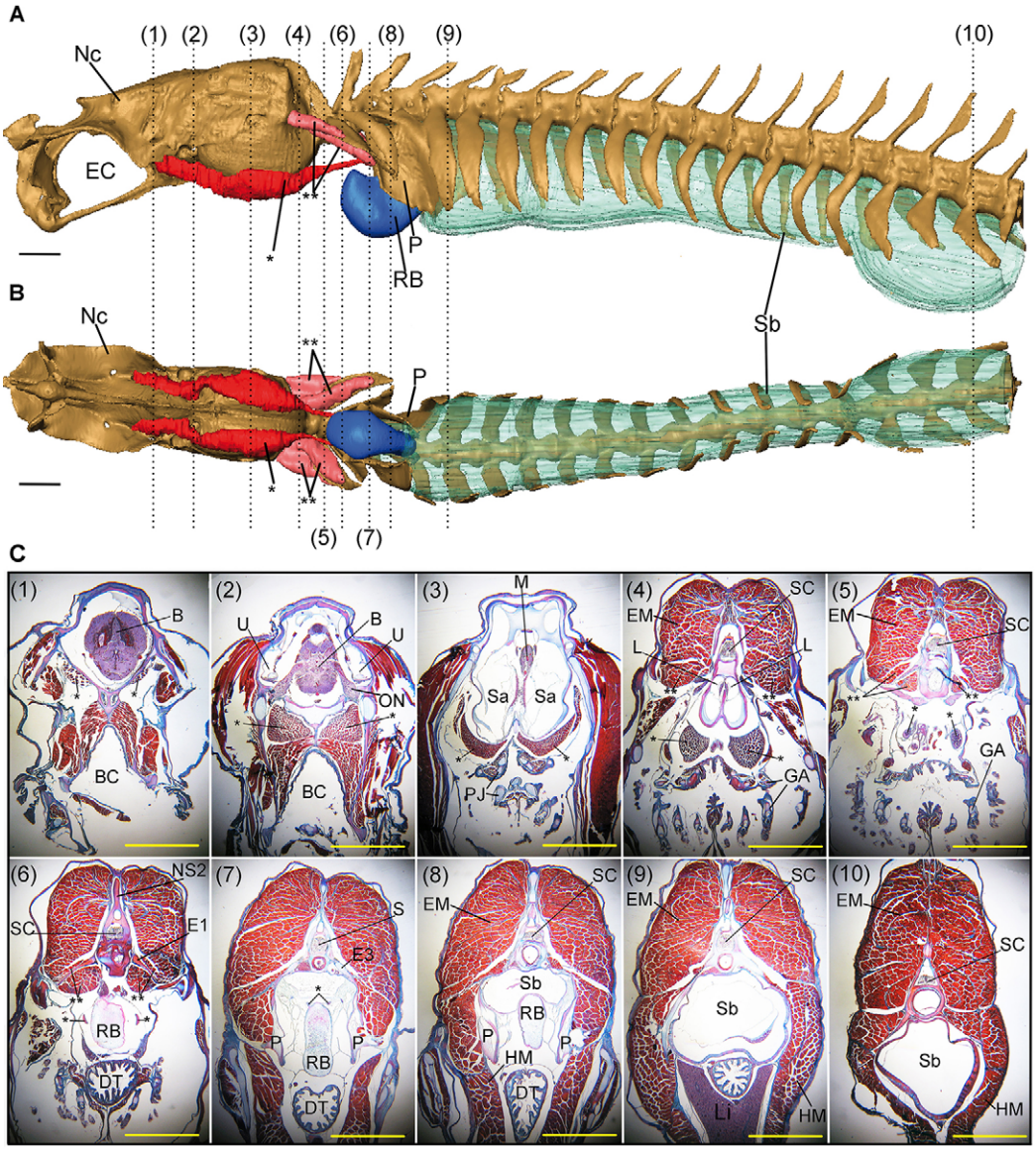

Fig. 1. Morphology of the sound-production apparatus in *Onuxodon fowleri*. (A) Left lateral and (B) ventral views of the neurocranium, the rocker bone, the anterior part of the vertebral column, the swimbladder and the sonic muscles of *O. fowleri*. Lateral and ventral views were obtained from 3D reconstructions based on µCT scans. (C) Transversal histological sections from a male *O. fowleri* (74 mm TL). The numbers 1–10 give the location of each cut in A and B. *primary sonic muscle; **secondary sonic muscle. B, brain; BC, buccal cavity; DT, digestive tract; E1, epineural 1; E3, epineural 3; EM, epaxial muscles; GA, gill arch; HM, hypaxial muscles; L, lagena; Li, liver; M, medulla oblongata; Nc, neurocranium; NS2, neural spine 2; ON, optic nerve; P, swimbladder plate formed by epineural 3; PJ, pharyngeal jaws; RB, rocker bone; Sa, sagitta; Sb, swimbladder; SC, spinal cord; U, utricule. Yellow and black scale bars: 1 mm.

reticulum and a low proportion of myofibrils. These characteristics appear to be compulsory to allow fast contraction in vertebrate muscles (Rome and Lindstedt, 1998). Thus, the study of fiber morphology should enable further insights into muscle kinetics.

Onuxodon spp. live exclusively in association with bivalves (Tyler, 1970). To date, three *Onuxodon* species have been described (Nielsen et al., 1999), all found between 1 m and 30 m deep in the tropical to temperate Indo-West Pacific, from East Africa to Hawaii (Nielsen et al., 1999). Although they live in shallow habitats, their biology remains poorly documented. Among carapids, the sound-producing mechanism of *Onuxodon* (Fig. 1) is unique because the rostral end of the swimbladder forms a mineralized structure (called the rocker bone) where the PSMs insert (Courtenay and McKittrick, 1970; Parmentier et al., 2002).

This paper describes the sounds of *Onuxodon fowleri* Smith 1955, provides insights into the sound-production apparatus, and sheds light on the sound production mechanism(s). Habitat has been demonstrated to impact acoustic communication in some gobies (Lugli, 2012; Lugli, 2013). We therefore also explored the effect of habitat on sound production because *Onuxodon* species live inside bivalves. The acoustic properties of the shell of their most common host (i.e. *Pinctada margaritifera*) were examined to determine whether bivalve cavities can alter the sound produced by fishes.

RESULTS

Sound production

In captivity

Sounds were only produced, and recorded, after sunset. In these dark conditions, and because the fish emitted sounds inside the oyster, it was impossible to associate sounds with behavior. Because there is no external sexual dimorphism in *Onuxodon* species, it was not possible to record males and females separately. The recorded sounds were composed of 1 to 40 pulses with a broadband frequency spectrum (Fig. 2A,C). In multiple-pulsed sounds (Fig. 2A,C), the mean pulse period was 99.5±13.7 ms (N=40) and the longest call lasted more than 3 s. Pulse duration (7.9±1.9 ms, N=56) was very short compared with the pulse period (Table 1; Fig. 3A). Inspection of the sound power spectrum showed in most cases the presence of three partially overlapping peaks in the frequency range 100–1000 Hz (Fig. 3B). The first, second and third mean peak frequencies were respectively at 259±96 Hz, 600±105 Hz and 961±132 Hz (Table 1). These sounds had no

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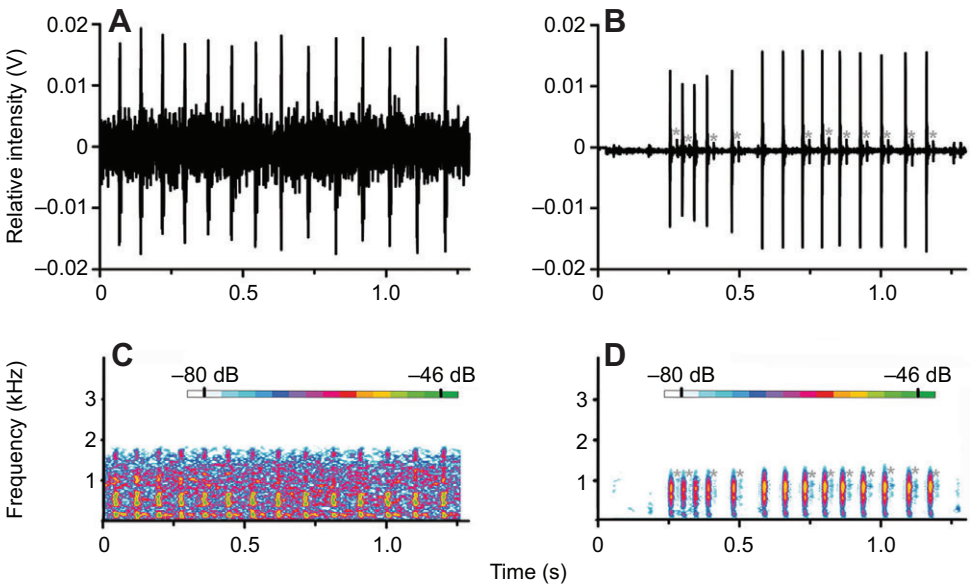

Fig. 2. Multiple-pulsed sounds of *Onuxodon fowleri*. (A,B) Waveforms and (C,D) spectrograms (FFT size, 512; Flat top) of two multiple-pulsed sounds. The first sound (A,C) was recorded in captivity whereas the second (B,D) was recorded in the field. Both sounds were band-passed between 0.05 and 1.5 kHz and down sampled at 8 kHz. Asterisks indicate the echo peaks of *O. fowleri* pulses. Color scale: relative intensity in dB.

harmonic pattern (Fig. 2C). However, additional smaller peaks were observed in some cases.

In the field

Hundreds of pulsed sounds were recorded in the field. These sounds were principally recorded after sunset. The bulk of sound production was between 17:00 h and 00:00 h (Fig. 4) and consisted of single-pulse sounds: less than 20% of sounds consisted of two or more pulses. Pulses were considered to be isolated when the time duration between two pulses was longer than the longest pulse period measured in pulse trains with a relatively constant pulse rate. The longest call contained 42 pulses and lasted almost 3.5 s. Pulses were short (8.2±2.5 ms) and their waveform consisted of a highly damped oscillation (Table 1; Fig. 3C,D). Each pulse had a broadband frequency spectrum (Figs 2 and 3) generally dominated by peaks of energy at 212±46 Hz (first peak frequency), 520±72 Hz (second peak frequency) and 787±68 Hz (third peak frequency). The pulse period (87±31 ms) was mainly characterized by its marked variability. Modulations of the pulse rate were observed within many calls (Fig. 5). In few cases, the inter-pulse interval was so short that the sound had a harmonic-like pattern on the spectrograms [fast Fourier transform (FFT) length, 512 Hz] and power spectra (Fig. 5). However, pulses within a call never overlapped and were therefore not 'true' tonal sounds.

In our field recordings, we also noted the presence of weaker peaks, each found ~24.8±0.2 ms (N=50) after pulses. These weak peaks are most probably the echoes of the *O. fowleri* pulses. The sound speed in the water is ~1500 m s^{-1}. The delay between each pulse and the following weak peak corresponds to the duration of sound propagation on a distance of 37.2 m. Hydrophones were placed close to the pearl oysters on the sea bed at ~18 m from the water surface. In this case, weak peaks most probably result from original pulses that traveled towards the water surface, bounced back on water–air interface and returned to the hydrophone. This explanation is supported by the fact that packs of multiple pulses with very short periodicity are followed by packs of several echoes having the same periodicity. Moreover, it shows that sounds can be detected at a distance of 40 m.

Effects of oyster shell cavity on sounds

When the hydrophone was placed inside the shell cavity, the three shells covered with polydimethylsiloxane (PDMS) to mimic the mantel tissues of living oyster (further details in the Materials and methods) showed amplitude gains (i.e. higher intensity compared to the 'no cavity condition') in the frequency range investigated (Fig. 6). The three shells fixed on the rock (oyster shells are fixed on the reef in the field) all featured two major peaks of gain, between 200 and 300 Hz and between 990 and 1500 Hz. The two larger shells also featured a third gain peak between 500 and 600 Hz (see example in Fig. 6A). The presence of three peaks of gain, at 200 Hz, 600 Hz and 1 kHz, respectively, also characterized the shell average frequency response (Fig. 6B). The frequency response of individual shells did not show remarkable changes after the PDMS lining was removed (not shown), or when the shell was tested on sand (Fig. 6). Frequencies with the highest amplification depended on the shell and the way it was tested. The amplification magnitude

Table 1. Characteristics of sounds produced by *Onuxodon fowleri*

	Captivity			Field			
	N	Mean	s.d.	*N*	Mean	s.d.	*P*-value
Call duration (ms)	56	652.6	699.4	100	272.1	462.0	**<0.001**
Pulse number	56	7.7	7.9	100	4.6	5.9	**0.002**
Pulse period (ms)	40	99.5	13.7	66	86.8	30.8	**0.012**
Pulse duration (ms)	56	7.9	1.9	100	8.2	2.5	0.948
First peak frequency (Hz)	53	259	96	92	212	46	0.091
Second peak frequency (Hz)	53	600	105	59	520	72	**<0.001**
Third peak frequency (Hz)	53	961	132	96	787	68	**<0.001**

Sounds were recorded in captivity and in the field (*P*-values are the results of a Mann–Whitney U-test of differences between distributions).

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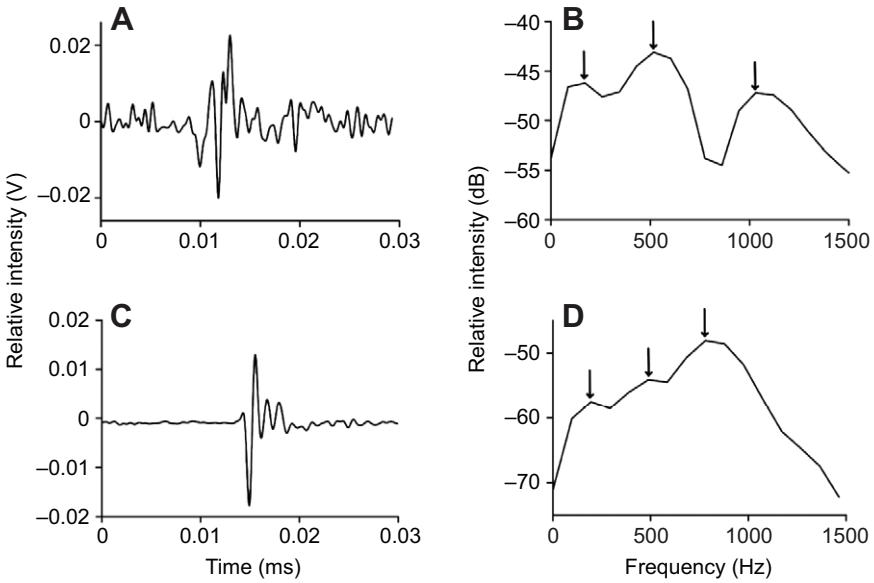

Fig. 3. Pulses of *Onuxodon fowleri* sounds. (A) Waveform and (B) power spectrum of a pulse isolated from a multiple-pulsed sound recorded in captivity. (C) Waveform and (D) power spectrum of a single-pulsed sound recorded in the field. Each sound was band-passed between 0.05 and 1.5 kHz. Arrows indicate the three peak frequencies

could exceed 10 dB for some frequencies. When the hydrophone was placed outside the cavity of the shell (tested on sand) gain disappeared at frequencies below 600 Hz (Fig. 6). When the shell was tested in such a configuration, gain at higher frequencies was always higher in the front of the opening than laterally (not shown).

Morphology

Morphology of the sound-production apparatus

The sonic apparatus of *O. fowleri* is composed of the primary sonic muscle (PSM), the secondary sonic muscle (SSM), the rocker bone, the swimbladder, four modified vertebrae and three highly modified epineurals (Figs 1, 7, 8 and 9). A comparison of all specimens indicates that the rocker bone shapes can be grouped into two categories that correspond to the sex of the fish. Type 1 rocker bones are characterized by an almost spherical shape surmounted by a cone in its posterodorsal region (Fig. 7). This cone divides into two small and lateral horns. Type 2 rocker bones appear more laterally compressed and are kidney shaped (Figs 7 and 8). Their dorsal face is characterized by an anterior deep groove at the front of a strong process called the knob (see Courtenay and McKittrick, 1970). This knob is followed caudally by a second groove (Fig. 7).

In both rocker bone types, the caudal part is narrower than the cranial part. Types 1 and 2 shown in Fig. 7 were found in two fish having the same total length (TL; 79 mm), similar head length (HL; 9.4 mm versus 10.6 mm), and head height (HH, 7 mm versus 7.3 mm). However, their rocker bones greatly differ in shape and size (Fig. 7): type 2 is almost three times more voluminous than type 1 (2.2 mm^3 and 0.8 mm^3, respectively). This observation refutes the hypothesis that both types are successive steps of the ontogeny. The plot of HL against TL reinforced this assumption because fish with Type 1 or Type 2 rocker bone widely overlapped (Fig. 10). The examination of gonads and histological sections revealed that the type 1 rocker bone was observed in females whereas the Type 2 rocker bone corresponded to males. Thus, the rocker bone of *O. fowleri* is sexually dimorphic.

Remarkable modifications of the anterior vertebral column were observed in both sexes. The body of the first two vertebrae is much shorter than the body of vertebrae 3 and 4 (Fig. 7). Moreover, the neural arch of the first vertebra is unusually dorsally opened (Fig. 7). The caudal part of the exoccipital process extends between the reduced lateral arms of this neural arch (Fig. 1A). Markle and Olney (Markle and Olney, 1990) considered this characteristic as a synapomorphy of the *Onuxodon*. The first three epineurals are strongly modified whereas the fourth vertebra is completely devoid of any kind of rib or parapophysis (Fig. 7). The first epineural is wing-shaped and its distal extremity is close to the tip of the enlarged second epineural (Fig. 7). The third epineural showed the most striking modifications and consists of two parts: the posterior part looks like an epineural and is curved similarly to epineural 2 whereas the anterior part forms a large plate called the swimbladder plate (Parmentier et al., 2002). The left and right swimbladder plates delimit a longitudinal and anterodorsally closed (medial arch) corridor for the rocker bone (Figs 7 and 9). The dorsal face of the medial arch adjoins the ventral face of the third vertebra. In both

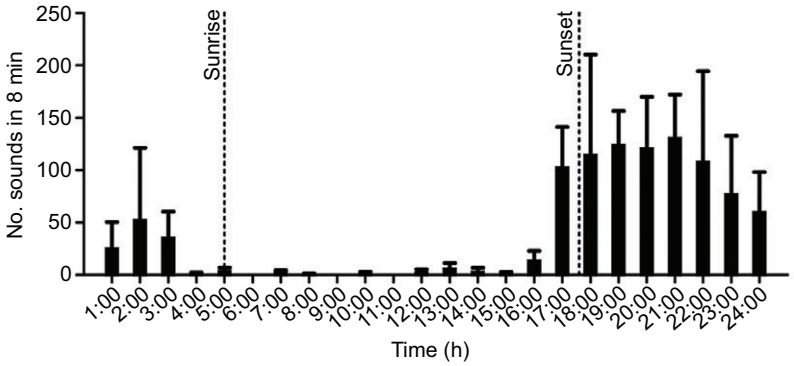

Fig. 4. Variation in sound production of *Onuxodon fowleri* during the day. Averaged number of sounds recorded in the field per 8 min from 31 October 2013 to 4 November 2013 for each hour of the day. Values are means ± s.d.

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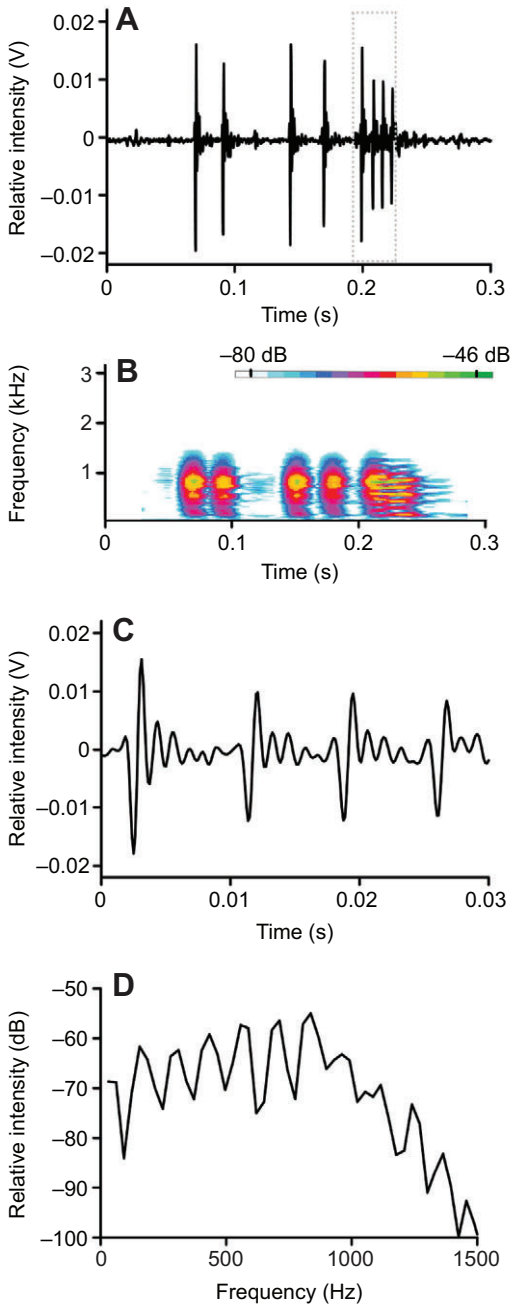

Fig. 5. A multiple-pulsed sound of *Onuxodon fowleri* with a modulated pulse period. (A) Waveform and (B) spectrogram (FFT size: 512; Flat top) of the multiple-pulsed sound recorded in the field that shows much shorter inter-pulse intervals between the final pulses. (C) Waveform and (D) power spectrum of the last four pulses of the sound (boxed region in A). Color scale shows relative intensity in dB.

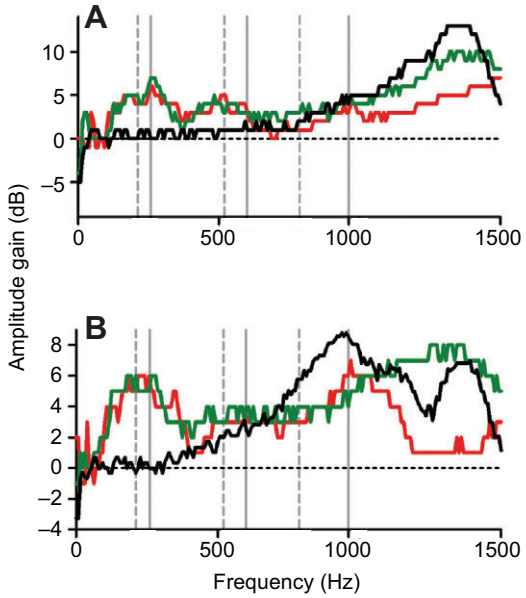

Fig. 6. Amplitude gain by the cavity of pearl oyster shells. (A,B) Differences in power spectra (amplitude versus frequency) of S^+ (cavity) and S^- (no cavity) conditions. (A) Gains when the oyster was fixed on a rock with the hydrophone inside the cavity (red), the oyster was placed on sand with the hydrophone inside the cavity (green) and when the oyster was placed on sand with the hydrophone at the front of the cavity opening (black). (B) Mean amplitude gains measured for the three pearl oysters when the oyster was fixed on a rock with the hydrophone inside the cavity (red), the oyster was placed on sand with the hydrophone inside the cavity (green) and when the oyster was placed on sand with the hydrophone at the front of the cavity opening (black). Vertical lines correspond to the mean peak frequencies of *Onuxodon fowleri* sounds (see Table 1). The solid vertical lines represent the three peak frequencies of sounds recorded in captivity whereas the dotted lines correspond to the three peak frequencies of sounds recorded in the field.

sexes, the posterodorsal area of the rocker bone is located between the swimbladder plates. In Fig. 7, the swimbladder plates of the female are less developed than in the male but it was not the case in µCT scans of the other individuals investigated.

The PSM originates on the upper wall of the orbit, runs along the ventral part of the neurocranium, and inserts through a tendon on the dorsolateral parts of the rocker bone (Figs 1, 7 and 9). Note that the PSM tendon inserts on the top of the small horns in females and close to the dorsocaudal tip in males (Figs 7 and 9). The SSM originates on the exoccipital and inserts on the first epineural in both sexes. The superior part of the SSM inserts on the dorsal face of the epineural whereas the inferior part of the SSM inserts on the ventral side of the epineural (Fig. 1).

A ligament (ligament 1, Fig. 9) connects the distal tip of first epineural to the rocker bone in males but was absent in females (Fig. 9). In both cases, epineurals 1 and 2 are held together by muscle fibers and connective tissue. The second epineural is connected by a ligament (ligament 2, Fig. 9) to the external face of the third epineural in both males and females (Fig. 9). Because the distal tips of the first two epineurals lie very close to one another, the second ligament (ligament 2, Fig. 9) is located just caudally to the first one (ligament 1, Fig. 9) in males.

The swimbladder is elongated and extends from the arch formed by swimbladder plates (3rd vertebra) to the level of the 20th or 21st vertebra (Figs 1, 8 and 9). Its caudal part is expanded, and surrounded by large vertebral parapophyses and the hypaxial musculature (Fig. 1). Its anterior region is attached to the swimbladder plates and the dorso-caudal part of the rocker bone (Figs 8 and 9). At the front of the knob of the male rocker bone (level of the rocker bone groove), the swimbladder wall is much thicker and resembles a large ligament which connects left and right internal face of the swimbladder plates (Fig. 8 and Fig. 9B). In the female, the contact between the rocker bone and swimbladder wall differed slightly because of the rocker bone shape (Fig. 9). However, a thickened part of the swimbladder runs horizontally at the frontal base of the rocker bone horns and also connects left and right swimbladder plates (Fig. 9). Interestingly, the PSM is ventral to the thickened part of the swimbladder wall in males

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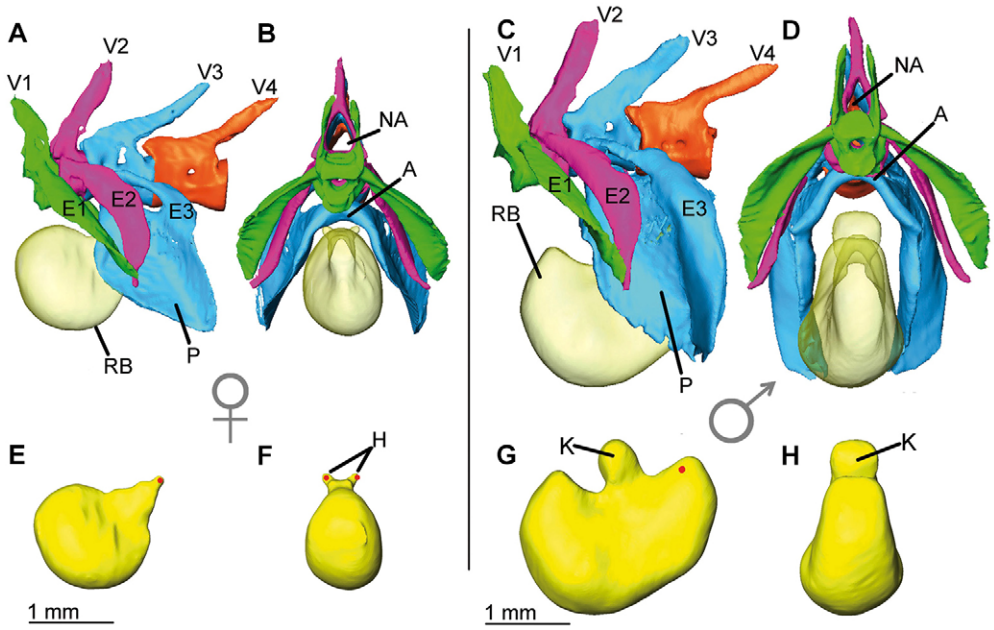

Fig. 7. 3D reconstructions of the rocker bone, the first four vertebrae and the associated pairs of epineurals of male and female *Onuxodon fowleri* using µCT scan data. (A,C,E,G) Left lateral and (B,D,F,H) frontal views. The insertion points of the primary sonic muscle are shown in red. Both specimens measured 79 mm (TL). A, bony arch between the left and right swimbladder plates; E1–E3, epineurals 1–3; H, horn; K, knob of the rocker bone; NA, neural arch; P, swimbladder plate formed by epineurals 3; RB, rocker bone; V1–V4, vertebrae 1–4.

(Figs 8 and 9) whereas it lies dorsal to it in females (Fig. 9). In females, pulling on the PSM caused a counterclockwise rotation (in a left lateral view) of the rocker bone (antero-ventral part of the rocker bone moving caudally). Movements of the male rocker bone appear more restricted because of the large thickened part of the swimbladder wall and the ligament 1. Thus, it was not possible to determine on fixed tissue whether PSM contractions would only pull the rocker bone more cranially or whether the forward movement is associated with a small rotation.

Muscle fiber diameter and ultrastructure

The morphology of the two presumed sonic muscles was compared with the epaxial musculature (EM) in order to infer some information about the kinetics of these muscles. The rmGLM highlighted that there are significant differences (P<0.001) in fiber diameter between muscle types. The Tukey HSD (honest significant difference) gave significant differences (P<0.05) between each pair of muscle types except between the inferior and superior part of the SSM, and between the superior part of the SSM and the PSM. The lowest mean value was found for the PSM (13±2.7 µm, N=5) whereas the EM showed the largest fiber diameter (37±8.5 µm, N=5). The superior and inferior SSM had an intermediate fiber diameter (19.9±5.5 µm for the superior part of SSM and 23.1±3 µm for the inferior part of SSM, N=5).

No radial architecture or central core of sarcoplasm was observed in the PSM, SSM or EM fibers. The most striking differences in fiber ultrastructure were seen between the PSM (Fig. 11A–D) and the other muscle types (Fig. 11E,F). This distinction is based on two obvious features of the PSM fibers. First, myofibrils are small and separated by large areas of sarcoplasm and an extensive sarcotubule network. Second, mitochondria and extensions of the sarcoplasmic reticulum were densely concentrated along the fiber periphery. In some places, generally near the periphery of the cell, aggregated membrane formed circular structures called whorl bodies (Fig. 11C,D). Compared with the fibers of epaxial musculature and SSM, those of the PSM also showed more circular nuclei.

DISCUSSION

Sexual dimorphism

Although Courtenay and McKittrick (Courtenay and McKittrick, 1970) stated that there is no sexual dimorphism in the *Onuxodon* sound production apparatus, they made an interesting comment about *Onuxodon parvibrachium*. They considered that the paratype (ANSP 91016) had a mature but abnormal rocker bone and wrote: '… in the paratype the insertions are at the tips of distinct rounded lateral projections on the knob which are not present in the other specimens'. In the present study, a similar morphotype was seen in 11 *O. fowleri* specimens and attributed to females. It seems reasonable to suggest that a sexual dimorphism in the rocker bone is present in other *Onuxodon* species and that the 'abnormal' *O. parvibrachium* was a female.

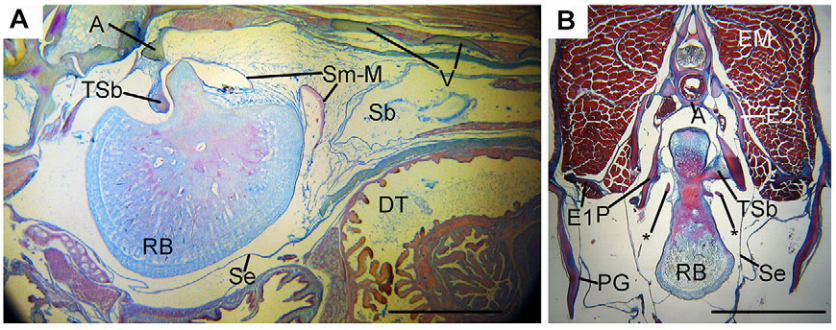

Fig. 8. Histological sections of the rocker bone area of a male *Onuxodon fowleri*. (A) Sagittal and (B) transverse sections through the rocker bone. *primary sonic muscle; A, bony arch formed by the left and right plates; DT, digestive tract; E1,E2, epineural 1 and 2; EM, epaxial muscles; RB, rocker bone; P, swimbladder plate formed by epineurals 3; PG, pectoral girdle; Sb, swimbladder; Se, serosa of the swimbladder wall; Sm-M, submucosa and mucosa of the swimbladder wall; TSb, thickened part of the swimbladder wall; V, ventral part of two vertebrae. Scale bars: 1 mm.

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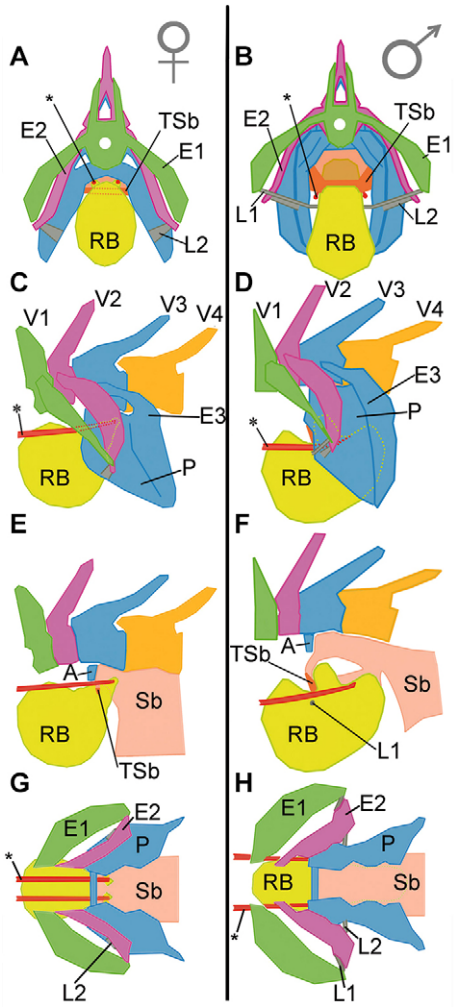

Fig. 9. Schematic representations of the rocker bone area in female and male *Onuxodon fowleri*. (A,B) Frontal, (C–F) left lateral and (G,H) dorsal views of (A,C,E,G) a female and (B,D,F,H) a male *O. fowleri*. In E and F, left epineurals were removed. In G and H, vertebrae were removed. Insertions of the secondary sonic muscle on the first epineural are not shown. *primary sonic muscle; A, bony arch formed by the left and right swimbladder plates; E1–3, epineural 1–3; L1, ligament that joins 1st epineural and rocker bone; L2, ligament between epineural 2 and swimbladder plate; P, swimbladder plate formed by epineurals 3; RB, rocker bone; Sb, swimbladder; TSb, thickened part of the swimbladder wall; V1–V4, vertebrae 1–4.

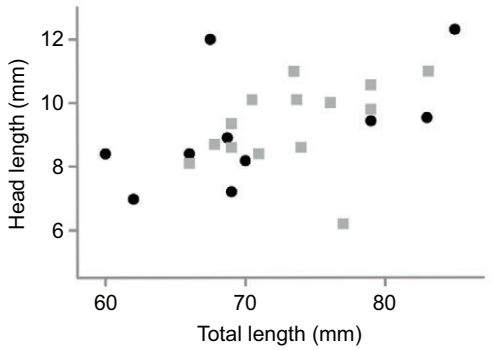

Fig. 10. Two types of rocker bones in *Onuxodon fowleri* with different total length and head length. Black circles, female rocker bone; gray squares, male rocker bone.

To date, the sexual dimorphism in the rocker bone of *O. fowleri* can be considered as the most pronounced in carapids. However, sexual dimorphism was not investigated in many genera (e.g. *Echiodon*, *Pyramodon* and *Snyderdia*). The unique example previously reported is a moderate difference in swimbladders between male and female *C. boraborensis* (Parmentier and Vandewalle, 2005). This dimorphism was associated with differences in some acoustic features. Thus, the sounds of male and female *O. fowleri* probably show some sex-related differences. However, the sex of the sound producer was unknown in this study and no obvious dichotomy in sound characteristics was observed.

Potential mechanism(s)

The PSM inserts on the rocker bone which is located at the front of the swimbladder close to the lateral swimbladder plates (Fig. 1). Its role in sound production was experimentally demonstrated in some *Carapus* (Parmentier et al., 2006c). In *O. fowleri,* the PSM is characterized by thin fibers and myofibrils. The latter being separated by large spaces of sarcoplasm mostly filled with the sarcotubule network. In addition, the myofibrils do not reach the sarcolemma: the periphery is mostly filled with high concentrations of mitochondria and extension of the sarcoplasmic reticulum. Such fibers are generally considered to be adapted for fast contractions (Rome and Lindstedt, 1998; Boyle et al., 2013). A pulse of *O. fowleri* sounds most probably results from a single twitch of the PSM (see below). An important characteristic of *O. fowleri* sonic activity is the ability to modulate the pulse rhythm. In most of the sound-producing fishes, the pulse period is usually fixed and is beyond neuronal control. The modulation of the pulse rate from a pulsed to tone-like sound within or between calls is relatively rare in fish but was described in some gobiids (Lugli et al., 1997; Parmentier et al., 2013). This ability to modulate the pulse repetition rate enables the production of different calls with the same basic mechanism. At low pulse rates the call is a typical pulsed sound whereas at higher rates the discrete pulses start to show characteristics of tonal-like sounds (Fig. 5). In the goby *Padogobius bonelli* (formerly *P. martensii*), sounds with differences in pulse rate (tonal, complex pulsed to tonal-like and pulsed sounds) are associated to the different stages of reproduction behavior (Lugli et al., 1997).

Having a skeletal element (rocker bone) at the rostral part of the swimbladder highlights the presence of important constraints that act on this structure during sound production in *Onuxodon spp.* In male *O. fowleri*, the insertions of the ligaments of the first epineurals on the rocker bone (Fig. 8) provide a perpendicular axis around which the rocker bone may rotate. During PSM contraction, the rocker bone is pulled frontally and may rotate in a counterclockwise direction for a fish in a left lateral view (Fig. 8). In this situation, PSM contraction would mainly decrease the pressure in the swimbladder (explaining the first negative pulse in the oscillogram) and increase tension in the thickened part of the swimbladder (TSb in Figs 8 and 9). The continuation of the mechanism can be envisaged in two ways. (1) During muscle relaxation, the thickened part of the TSb energy is released and the low swimbladder pressure causes the rapid snap back of rocker bone, causing raised swimbladder pressure and TSb and swimbladder plate vibrations. This probably corresponds to the first positive pulse in the oscillogram. (2) During muscle contraction, the rocker bone knob goes beyond the TSb, which pops over this structure provoking TSb vibration and consequently the vibration of the swimbladder plate on which it is attached. The TSb can be compared with a guitar string and shows similarities with other sound production mechanisms such as the tendon hook system of *Carapus*

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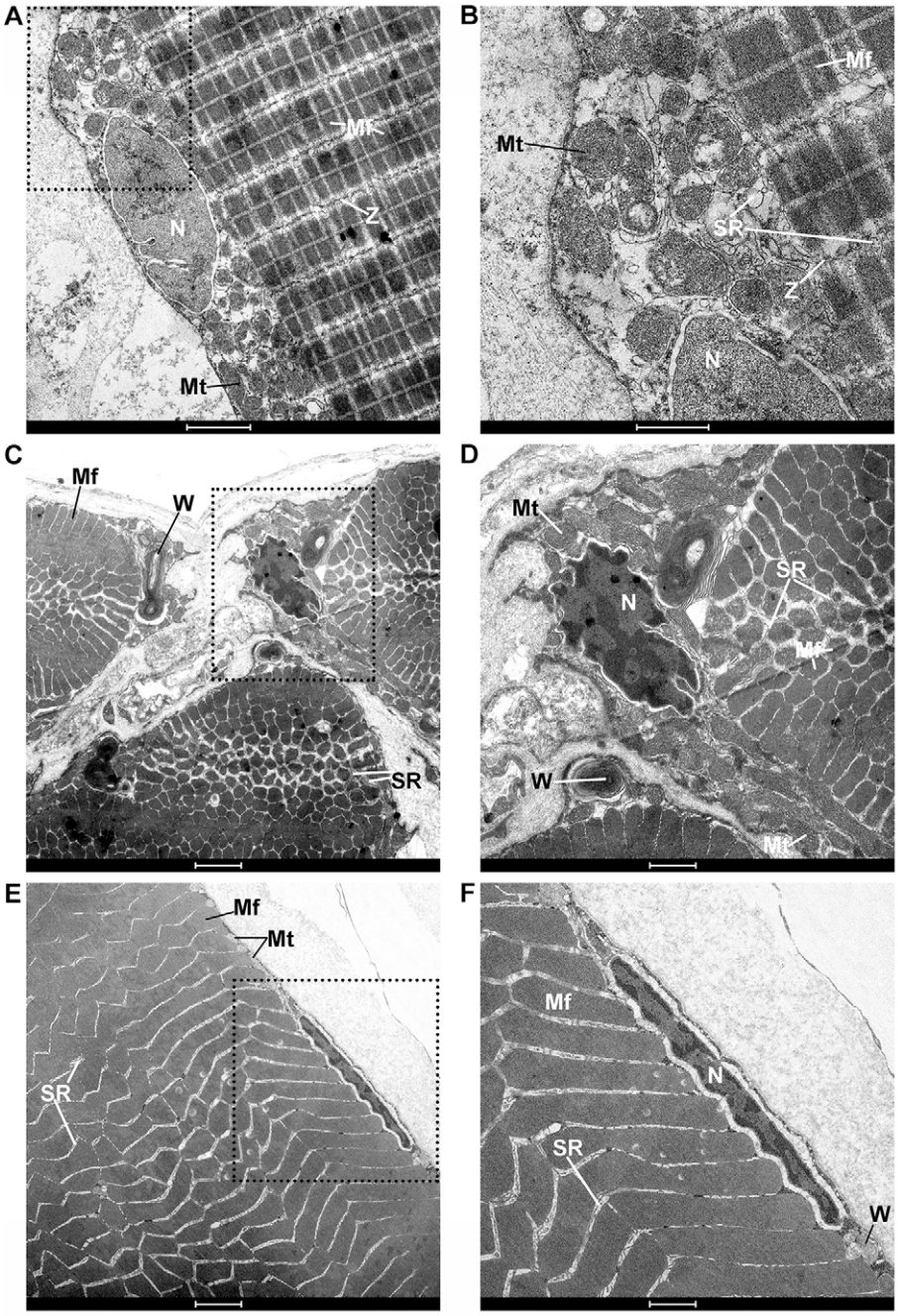

Fig. 11. Fiber ultrastructure of the primary sonic muscle and the epaxial musculature from *Onuxodon fowleri*. (A,B) Longitudinal and (C,D) transverse sections of the primary sonic muscle of *O. fowleri*. (E,F) Transverse sections of the epaxial musculature of *O. fowleri*. The micrographs shown on the right correspond to the boxed regions of micrographs on the left. Mf, myofibrils; Mt, mitochondria; N, nucleus; SR, sarcoplasmic reticulum; W, whorls; Z, Z-line. Scale bars: 2 µm (left) and 1 µm (right).

boraborensis (Parmentier et al., 2006c) or the pectoral fin system of *Trichopsis vittata* (Kratochvil, 1978). The rostral rocker bone displacement should be limited by the swimbladder wall and the ligament 1. Finally, the expanded caudal part of the swimbladder could work as a pressure-release mechanism. In females, pulling on the PSM rotates the rocker bone rostrally. This suggests that the thinner TSb could be used as a transverse axis for the rotation in females. Sounds should be of lower amplitude in females because the rocker bone is smaller, the TSb is thinner and ligament 1 is absent (Fig. 9).

The role of SSM in sound production needs to be confirmed because its fibers do not show any specialization. As suggest by Courtenay and McKittrick (Courtenay and McKittrick, 1970), the role of the SSM is probably limited to a modification of the tension in the sonic apparatus.

Comparison with the rocker bone of *Ophidion*

The genus *Ophidion* belongs to the Ophidiidae, which is the sister family of Carapidae. The comparison between the anatomy of the sound-producing apparatus in *Onuxodon fowleri*, *Ophidion rochei* (Ophidiidae) and *Ophidion barbatum* is interesting. These species have in common the presence of a rocker bone but differ in the swimbladder anatomy, in the muscles involved in sound production and in the shape of the first four vertebrae (Parmentier et al., 2006a; Parmentier et al., 2010). In *O. rochei* at least, the mineralized rocker bone is a secondary sexual character present only in males, which explains why male and female sounds differ radically (Kéver et al., 2012). In *O. rochei* and *O. barbatum*, the submucosa of the swimbladder wall thickens and forms a Z-shaped fold that terminates rostrally in a horizontal strip that penetrates and ramifies within the rocker bone (Parmentier

et al., 2006a; Parmentier et al., 2010). This is not the case in *Onuxodon*, highlighting that its mode of development is likely to be different (our unpublished observations). However, the skeletal element described in some species of both families probably evolved under similar constraints: a mechanical stress caused by the sonic muscle traction on the swimbladder wall (Parmentier and Diogo, 2006). This suggests that rocker bones in carapids and ophidiids are homoplastic features. In other words, the rocker bones of *Onuxodon* spp. and *Ophidion* spp. are not homologous, but probably evolved independently (i.e. convergence hypothesis).

Effects of the environment on sound characteristics

Several characteristics of fish sounds are affected by the environment in which they are produced (e.g. Mann and Lobel, 1997; Connaughton et al., 2000; Akamatsu et al., 2002). The most intriguing part of this study concerns the probable effects of the host on the *O. fowleri* calls because peak frequencies were amplified in the oyster cavity. Using white noise as a stimulus, three frequency bands were amplified by the shell. Two frequency bands (around 250 and 500 Hz) are amplified only within the shell, whereas amplification of the third (around 1000 Hz) can also be detected outside the shell. The energy peaks of the fish sound matched well with the gain peaks of the shell (Fig. 6). This is particularly striking when the mean spectrum of laboratory sounds is compared with the mean frequency response of the shells tested on rock (Fig. 6). These findings suggest that, inside the host, the low frequency (<600 Hz) of *O. fowleri* sounds is affected by the amplification proprieties of the shell cavity. This suggests that one possible function of the *O. fowleri* sounds might be the acoustic communication inside the oyster, as shown for sand gobies (Lugli, 2013). However, our field recordings also indicate that the whole stimulus can be detected at some distance from the shell, meaning that conspecifics should be able to intercept the sounds produced inside the host. Because some parts of the frequency band can be amplified outside the shell (Fig. 6), it suggests fish calls could be also adapted to amplifications outside the shell.

How the presence of the cavity affects the external sound field needs to be tested in an environment where sounds can propagate. However, preliminary tests suggest that the amplification of higher frequencies of the sounds can be detected outside the shell, especially in the front of the opening. Thus, a receiver listening in the front of, or along a path aligned with, the cavity opening could better benefit from the amplification effect of the shell (see also Lugli, 2012). Furthermore, a gradient of sound intensity near the shell opening might help the receiver to localize the host and consequently the caller. Many animals are able to exploit or modify the surrounding environment to increase the effectiveness of signals used for communication. We postulate that the fish uses the host shells as a tool to amplify its calls, providing an example of an adaptation where an animal incorporates features of its environment to enhance communication.

In summary, the marked modulations of pulse rate, the dimorphism in sound production apparatus and the amplification of low sound frequencies in the oyster cavity bring new insights into the complexity of acoustic communication in carapids. Furthermore, the sound-production mechanisms observed in Ophidiiformes raise many questions about the evolution of sound production in this order. The rocker bone, for example, probably evolved independently (i.e. convergence hypothesis) in *Ophidion* and *Onuxodon*.

MATERIALS AND METHODS

Sample collection

Three field campaigns were conducted to Makemo Island (French Polynesia): April 2011, November–December 2011 and October–November 2013. The lagoon (length, 62 km and width, 15 km) of Makemo atoll (16°36 S, 143°42 W) has two large connections with the ocean: the Arikitamori Pass on the northern side and a second pass located on the western side. In April 2011, 10 *P. margaritifera* were collected in the lagoon near the western pass. During the second and the third field campaigns, wild pearl oysters (*P. magaritifera*) were collected in the lagoon near Arikitamori Pass. In each case, pearl oysters were found while scuba diving on pinnacles between 1 and 30 m deep and opened in a tank to look for specimens of *Onuxodon* spp. All experimental procedures were approved by the local ethics committee of the University of Liège. *Onuxodon fowleri* are not endangered or protected species and they were not caught in protected areas.

Recordings and analysis of *O. fowleri* sounds

Recordings in captivity

These recordings took place in November and December 2011 in Makemo. Pearl oysters and *O. fowleri* were placed in a circular plastic tank (radius, 86.5 cm and water height, 50 cm). The number of fish in the tank varied depending on new captures and deaths. Sounds were recorded with an Orca hydrophone (sensitivity: −186 dB re. 1 V μPa^{-1}) connected with an Orca-made amplifier (ORCA Instrumentation) and digitized at 44.1 kHz with a Tascam DR-07 recorder (TEAC America, Inc.).

Recordings in the field

Field recordings were carried out from 31 October 2013 to 4 November 2013. Eight *P. margaritifera* were collected in the lagoon at a sampling site where 70% of the oysters had fish living inside of them. These oysters were grouped on a small sandy area (<1 m^2) at 18 m depth. A mini-digital spectrogram long-term acoustic recorder (DSG, Loggerhead Instruments, Sarasota, FL, USA) was then deployed close to the oysters for 4 days. It recorded 8 min in every 50 min and its sampling rate was set at 50 kHz.

Sound analysis

Sounds recorded in the field and in captivity were digitized at 44.1 kHz (16-bit resolution) and analyzed with AviSoft-SAS Lab Pro 4.33 software. Recordings in small tanks induce potential artifacts because of reflections and tank resonance. The computed resonant frequency of the recording tank was 1.64 kHz (Akamatsu et al., 2002). A band-pass filter from 0.05 to 1.5 kHz was therefore applied to all recordings. Overall, 56 sounds showing a high signal to noise ratio (SNR) were analyzed.

For comparative purposes, field sounds were also band-pass filtered from 0.05 to 1.5 kHz. Only sounds that had a high SNR (because of the closeness of the hydrophone to the shell housing the fish) and similar spectral characteristics to those of sounds recorded in captivity were analyzed and used to assess the daily variation of sound production in *O. fowleri* in the field. Because the hydrophone recorded at intervals of 50 min, two records could be obtained for a single hour (e.g. 14:00–14:08 h and 14:50–14:58 h). In such cases, the number of sounds obtained from the two recording files was averaged. One hundred sounds with a high SNR and attributed to *O. fowleri* were selected for the analysis of their characteristics.

The following temporal acoustic variables were measured on the oscillogram: call duration (duration from the beginning to the end of the sound), pulse number (number of pulses in a call), pulse period (measured as the peak-to-peak interval between consecutive pulses) and pulse duration (measured as the time interval from the beginning of a pulse to its end). For the last three variables, measurements were done on each pulse (averaged per call in multiple-pulsed sounds). Spectral characteristics of sounds were obtained from power spectra (Hamming window) and spectrograms (Flat Top window). The sounds of *O. fowleri* had broadband spectra characterized by the presence of three main peaks of energy.

Statistical analyses were performed with STATISTICA 10. The non-parametric U-test of Mann–Whitney was used to compare sounds recorded in the field and in captivity.

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Effects of oyster shell cavity on sounds

Preparation of the shell

Transportation of live oysters to the laboratory in Italy was not feasible. Therefore, only the shells were shipped and used for the tests. Three complete oysters were tested. The acoustic properties of the soft tissues of the oyster are unknown. However, because density of these soft tissues is similar to that of water, they are likely to be transparent to the sound. Therefore, their presence should be irrelevant to the response properties of the shell cavity to sound stimulation. Nonetheless, a correct testing procedure of the gain properties of the oyster shell must take into account the reconstruction of at least the mantle cavity with an organic material showing a density and mechanical properties similar to those of live tissues. It was assumed that the polydimethylsiloxane (PDMS), a viscoelastic polymer with density 0.97 g cm^{-3}, exhibit such properties. Thus, each valve was lined with a ~3-millimeter-thick layer of PDMS. Before testing, the two valves were coupled and pressed together to seal the rim of the shell. Just before testing the shell was slightly opened by manually spacing the two valves out by a distance of ~2 cm (measured at the side of the shell opposite to hinge). During the opening process, the organic polymer formed a continuous membrane especially along the rim in the hinge region thus keeping the two valves loosely coupled mechanically to each other at the base of the shell.

Test environment

All measurements were conducted in the laboratory in a 650 l plastic tank (112×74×79 cm, Cargopallet 600-plus). The bottom substrate was a 40-cm-thick layer of coarse sand. The tank was filled with tap water to a depth of 35 cm above the substrate. At such water depth no frequencies below 1.5 kHz propagate (Rogers and Cox, 1988) (M.L., unpublished results): the energy decays exponentially with theoretically and empirically determined losses of about 20 dB per 20 cm (Akamatsu et al., 2002). Because all the test frequencies were below 1.5 kHz (see below), experiments were conducted under conditions of no sound propagation. The tank was set on vibration-absorbing material (rectangles of foam rubber) to minimize the transmission of background vibrations from the floor to the tank.

Sound sources and probe stimulus

The speaker and the probe stimulus are described in a previous paper (Lugli, 2012). Briefly, in order to acoustically stimulate the shell by simulating a fish calling inside the shell cavity, a specially constructed hemispheric transducer of very small size was used. The frequency response of the shelter was determined using white noise as probe stimulus. A band-pass filter from 0.1 to 1.5 kHz (i.e. frequencies outside that range are rejected) was applied to the probe sequence. The filtered white noise was equalized (−4.5 dB per octave) to compensate for the high-frequency bias of the speaker's amplitude response. The probe stimulus was amplified via a stereo amplifier [REVAC, 101 integrated classic stereo amplifier (REVAC, Turin, Italy)] connected to a specially adapted output transformer (model PU-024) with a fixed gain (+28 dB). The level of sound stimulation was set to produce total band pressure levels of 119–134 dB [root mean square (rms) re. 1 µPa] measured at 3 cm from the source. Probe stimuli were recorded with a small size hydrophone (Bruel & Kjær, type 8103; Nærum, Denmark).

Experimental procedure and acoustical testing

Individual shells, with the two valves lined with PDMS and closed, were tested in three different conditions: attached to a rock, held in the hands and laid on the substrate. In the first case, the shell was glued to a hard support (a small irregular 1.5 kg rock, Fig. 12) with a hot melt adhesive in order to best mimic natural conditions. The shell was placed vertically on the rock and held in place by positioning the hot glue of the glue gun between the rock and the umbo of one valve. After the glue was hardened (after 2–3 min) the shell was slightly opened. The rock and the attached shell with the two valves half closed were placed on the substrate in the middle of the tank. A first measurement was made with both the buzzer and the hydrophone placed vertically inside the shell cavity (S^+ condition, Fig. 12A), the two being held in place close together (2 cm apart from each other) by means of a tripod placed on the floor (see Lugli, 2012). Then, the shell cavity was eliminated by opening the shell completely (Fig. 12B). Care was taken not to alter the buzzer–hydrophone configuration. The probe stimulus was re-measured a second time with the shell in the new arrangement, i.e. without a cavity (S^- condition). In both cases the probe stimulus was played for 5 s. The same shell was re-tested after the PDMS lining was removed from all parts except along the hinge line (this was necessary to keep the two valves glued together for the short period of time required to accomplish the acoustic test). For comparison, the test was repeated with the shell manually held mid-water in the upright position, or laid on the substrate in the upright position with the base slightly buried in the sand. Despite the 'no propagation condition', the hydrophone was also placed outside the shell (a few centimeters in front of the cavity opening and laterally to the shell) to investigate effect of the cavity on sound intensity near the shell.

Sound analysis

Measurements were acquired with a PC (sampling rate: 44,100 Hz) and stored as wave files for later analysis. Fast Fourier transforms (FFTs) of the probe stimulus were computed using Avisoft SASLab Pro™ software package for sound analysis (Raimund Specht, Berlin, Germany) (FFT length, 1024 Hz;

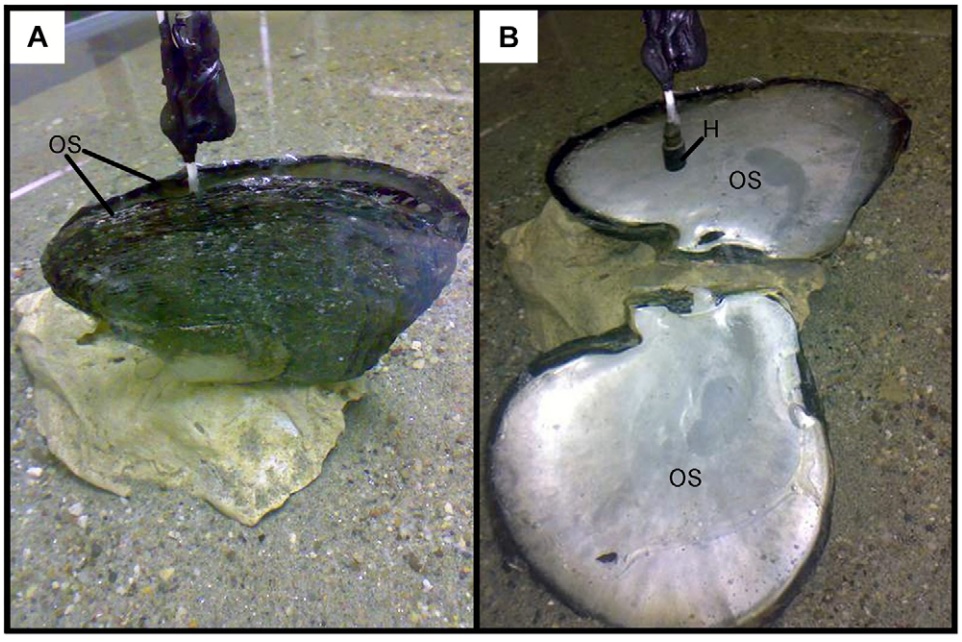

Fig. 12. Experimental setup used to test the effects of the oyster shell cavity on sounds. (A) The valves of the oyster form a cavity in the S^+ condition. (B) The valves of the oyster are opened and there is no cavity in S^- condition. H, hydrophone; OS, oyster shell. Buzzer is not shown.

frame size, 100%; Hamming window). The frequency response of the shell cavity (with and without the PDMS) was estimated by computing the pressure amplitude difference (AD, dB) between S^+ and S^- conditions. Sounds were analyzed within 8-Hz-wide bands in the range 0.05 to 1.5 kHz. A positive value of AD would mean amplitude gain by the shell cavity, whereas a negative value would mean amplitude loss (see Lugli, 2012).

Morphology

Morphology of the sonic apparatus

Thirty five specimens (supplementary material Table S1) were killed with an overdose of MS 222, fixed in solutions of formalin (7%) or glutaraldehyde (1%) and transferred in alcohol (70%) or in seawater with NaN_3 (1.3 g l^{-1}, 20 mmol l^{-1}), respectively. Two (total length; TL, 63 and 78 mm) were stained with Alizarin Red following the method of Taylor and Van Dyke (Taylor and Van Dyke, 1985) to visualize osseous structures. Twenty four individuals (60 to 85 mm TL) were carefully dissected under a stereoscopic microscope (Leica, Wild M10) coupled to a *camera lucida* to study mineralized and soft tissues of the sonic apparatus. Structures of interest were photographed and/or drawn.

Mineralized and soft tissues of the sonic apparatus were also identified on histological sections and µCT scans. Transverse sections from two specimens (74 and 79 mm TL) were stained with Kostowiecki trichrome following the protocol of Gray (Gray, 1954). For the specimen of 74 mm TL, serial histological sections (10 µm sections were cut, but only every third section was mounted) were made from the eye to the end of the swimbladder. Sagittal sections were obtained from a third specimen (73 mm TL) and stained with Gallego's ferric fushin stain (Gabe, 1968).

Finally, six fish (62 to 83 mm TL) were scanned at the National Museum of Natural History in Paris with a µCT scan (v-tome-x 240 L, GE Sensing & Inspection Technologies Phoenix X-ray). Prior to being scanned, specimens were submerged in a 5% phosphomolybdic acid solution for 10 days to improve contrast between the different tissues. The imaging system was set between 42 and 250 kV and specimens were scanned at isotropic resolutions between 6 and 22.4 µm. Volume and surface rendering was performed with AMIRA 5.4.0 (VSG, FEI company).

Morphology of muscle fibers

PSM, SSM and a sample of the epaxial musculature (EM) were collected from six *O. fowleri* (60 to 85 mm TL). All the samples came from individuals that were fixed in glutaraldehyde (1%) and conserved in seawater with NaN_3 (1.3 g l^{-1}, 20 mmol l^{-1}) to prevent bacterial growth. They were dehydrated in series of ethanol–propylene oxide and embedded in epoxy resin (SPIPON 812). Transverse (longitudinal for one fish) semi-thin sections (0.5 µm) were stained with toluidine blue (0.5% in a 1% borax solution) following the protocol of Richardson (Richardson et al., 1960), and photographed under a binocular microscope. For each sample, the mean fiber diameter (d) of 25 randomly selected fibers (grids) was calculated using $d=2*\sqrt{(A/\pi)}$, where A is fiber area measured in Adobe Photoshop CS4 (Adobe, San Jose, CA, USA). The qualitative fiber description is based on ultrathin sections (60–80 nm) stained with uranyl acetate and lead citrate and observed with a transmission electron microscope (JEOL JEM 100SX) with 80 kV accelerating voltage. The Kolmogorov–Smirnov test was used to determine whether parametric tests were appropriate. A general linear model with repeated measurements (rmGLM) was run in STATISTICA 10 to compare mean fiber diameter (dependent variable) obtained for the different muscle types (repeated measures). Tukey HSD *post hoc* test allowed comparisons between the four muscle types.

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Competing interests

The authors declare no competing financial interests.

Author contributions

L.K., O.C., M.L. and E.P. conceived and designed the experiments. L.K., O.C., F.L., D.L. and E.P. collected the fish and performed field recordings. M.L. performed the 'oyster shell cavity' experiment. A.H. performed the µCT-scans. K.L. studied the muscle histology, analyzed the data and wrote the manuscript. O.C., M.L., F.L., D.L., A.H. and E.P. revised the manuscript. E.P. gave final approval for submission.

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Supplementary material

Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.109363/-/DC1

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