

Call properties and morphology of the sound-producing organ in *Ophidion rochei* (Ophidiidae)

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SUMMARY

The anatomical structures of the sound-producing organ in *Ophidion rochei* males present an important panel of highly derived characters: three pairs of putatively slow sonic muscles; a neural arch that pivots; a rocker bone at the front pole of the swimbladder; a stretchable swimbladder fenestra; a swimbladder plate; and an internal cone that terminates in a pair of membranes in the caudal swimbladder. Male courtship calls are produced nocturnally and consist of trains of 10 to 40 pulses that increase in amplitude and decrease in rate before exhibiting alternating periods of ca. 84 and 111 ms. Each pulse includes an unusual waveform with two parts. Pulse part 1 is a single cycle followed by a longer duration pulse part that exhibits gradual damping. Sounds and morphology suggest two hypotheses on the sound-producing mechanism. The ‘pulley’ hypothesis would require an alternate contraction of the ventral and dorsal muscles to form the two parts of each pulse. The ‘bow’ hypothesis involves a release mechanism with the sustained contraction of the dorsal muscle during all of the call, and the rapid contraction/relaxation of the ventral muscle to form each pulse.

Key words: acoustic, Ophidiiforme, rocker bone, sonic muscles, swim bladder.

INTRODUCTION

Classically, swimbladder sound production in many fish is evoked as a forced response by the contraction of specialized sonic or drumming muscles (Ladich and Fine, 2006). Generally speaking, swimbladder sounds have a fundamental frequency ranging from 75 to 300 Hz, which would correspond to the muscle contraction rate, placing sonic muscles among the fastest in vertebrates (Rome et al., 1996; Loesser et al., 1997; Connaughton et al., 2000; Fine et al., 2001). The average dominant frequency of pulsed sounds in several carapid species varies between 40 and 340 Hz (Parmentier et al., 2003; Lagardère et al., 2005; Parmentier et al., 2006a). We recently discovered that unlike sounds generated by fast sonic swimbladder muscles, sounds in a carapid fish (Ophidiiformes) are generated with slow muscles that require 490 ms for a twitch and that tetanize above 10 Hz (Parmentier et al., 2006a). The carapid mechanism depends on a mechanical decoupling in which the slowly stretched swimbladder is released and snaps back to its resting position. Unlike examples with fast muscles, muscle contraction rate in slow muscles determines the pulse period but not the sound frequency. In the cusk-eel *Ophidion marginatum*, sounds are composed of one to 27 pulses with a peak frequency of about 1200 Hz (Mann et al., 1997; Sprague and Luczkovich, 2001; Rountree and Bowers-Altman, 2001). A typical fish mechanism of sound generation would require opposite movements of the muscle antagonists to occur in less than 1 ms to produce a sound with a dominant frequency above 1 kHz. Such speed is unlikely for muscle contraction or motoneuron control (Ladich and Fine, 2006). The frequency of contraction is about 23 Hz in this species, reinforcing the assumption that, in Ophidiiformes, there is no correspondence between sonic muscle contraction and sound frequency. Because sounds have been recorded only in one ophidiid (*O. marginatum*),

investigators have inferred the sonic mechanism based on functional morphology in other species (Parmentier and Diogo, 2006; Parmentier et al., 2006b; Fine et al., 2007; Nguyen et al., 2008).

Ophidiiformes present a variety of highly specialized sound-production mechanisms. In some species the anterior part of the swimbladder forms a so-called ‘rocker bone’ (Rose, 1961; Parmentier et al., 2006b; Parmentier et al., 2008). This structure is likely to stimulate sound production, as its movement by muscles will deform the swimbladder (Courtenay and McKittrick, 1970; Parmentier and Diogo, 2006). This assumption is reinforced by sexual dimorphism in the sound-producing apparatus of different members of the Ophidiidae (Rose, 1961; Courtenay, 1971; Casadevall et al., 1996; Fine et al., 2007).

Ophidion rochei are Mediterranean inhabitants that live in the sand between the intertidal region and 150 m depth (Matallanas, 1980; Dulčić et al., 2002). They are nocturnal carnivores that eat mainly decapods and small teleosts (Matallanas, 1980; Dulčić et al., 2004), and they spawn from June through the end of September (Dulčić et al., 2002). The aim of this paper is to present the extraordinary specialized sound-producing system in this fish, which possesses a rocker bone, and to examine its sonic mechanism based on morphology and call analysis.

MATERIALS AND METHODS

Calls were recorded in a sandy area in the Cetina Estuary near the town of Omis in Croatia (43°26'N, 16°41'E). Water depth was about 2 m and temperature about 23°C. Fish were recorded at three locations about 300 m apart during three consecutive nights between 18:10 and 07:30 h (UTC + 1) from 1st September to 4th September, 2008. These dates are within the spawning period. Recordings commenced 90 min before sunset and ended 90 min after sunrise.

Sounds were recorded with a hydrophone (186 dBV/ μ Pa) for 10 min every 30 min (sample rate 40 kHz) onto a Digital Spectrogram Long-Term Acoustic Recorder (DSG, Loggerhead Instruments, Sarasota, FL, USA). DSG is a low-power acoustic recorder designed to sample at different rates, continuously or on a duty cycle to Secure Digital High Capacity (SDHC) memory.

Fish were caught in the Cetina estuary from 23:00 to 04:00 h with a beach seine (22 m long, mesh size of 4 mm at the outer wing and 2 mm at the central part), transported to the laboratory (Liège, Belgium), and kept in a 250 l tank with seawater at 20°C and a 10 cm high sandy bottom.

Sounds from field 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 were obtained from power spectra (Hamming window) and double-checked with spectrogram (FFT size 256 points, time overlap 96.87%, and a FlaTop window). 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 in the entire sound); pulse length (measured as the time from the beginning of one pulse and its end); and dominant frequency.

For morphological examination and histology, fish were deeply anesthetized with MS 222 (500 mg l⁻¹). A freshly dead specimen was manipulated to assess the action of the various muscles, skeletal components, and the rocker bone on swimbladder movement. Forty-eight fish were fixed in 7% formaldehyde or in 100% ethanol for dissection and future studies. Morphology of the sonic apparatus was examined with a Wild M10 (Leica) binocular microscope equipped with a camera lucida and a digital camera (Canon Power Shot S50).

Two *O. rochei* specimens were fixed in Bouin's solution for serial histological sections. They were dehydrated in butanol, decalcified and embedded in paraffin, and serially sectioned at 10 μ m using a Reichert microtome. Sections were stained with Romer's cartilage and bone distinctive stain, Gallego's ferric fushin stain for elastic fibers, and Masson's trichrome stain for collagen (Gabe, 1976). Sections were observed using a polarizing Olympus microscope (Leica DM 100) coupled with a digital camera (Canon Power Shot S50).

RESULTS

Sounds

Chorus began 30–45 min after sunset and ended 30–45 min before sunrise (Fig. 1). In each case, sound production peaked for 120 min from 20:00 to 22:00 h. Calling diminished to low levels or even silence during the night and increased to a smaller second peak before sunrise, around 05:00 h. In these conditions, it was impossible to give a precise account of the number of callers. However, sounds were recorded during different nights at different places, and we obtained sounds having different intensities. This allows us to be confident that the recordings correspond to the calls of different specimens.

Calls consisted of between nine and 43 broad-band pulses (Fig. 2), and number of pulses per call correlated with call duration ($R^2=0.96$, $P<0.001$; Fig. 3). The pulse period and amplitude increased gradually during the first eight pulses, separating the call into two parts: call part 1 (cp1) and call part 2 (cp2; Fig. 4). The pulse period in cp2 exhibited a saw-like profile (Fig. 4B, Fig. 5), alternating between short (85.8 ± 0.2 ms) and long (113.7 ± 0.4 ms) periods that differed significantly ($t_{345}=60.46$, $P<0.001$). Pulse length was

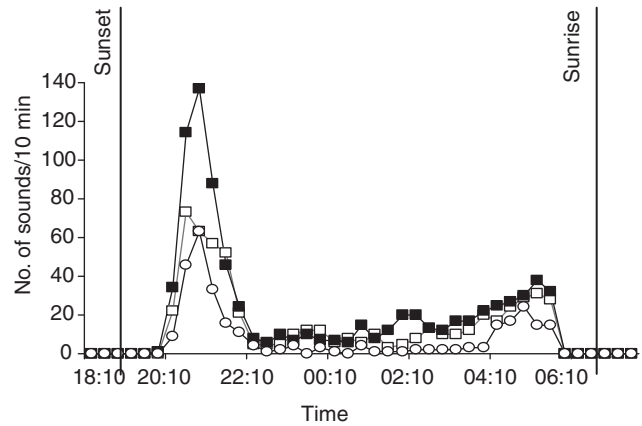


Fig. 1. Number of calls per 10 min by *Ophidion rochei* males at three different sites in the Cetina Estuary, Croatia.

25.5 ± 4.6 ms ($N=237$). The pulse waveform consisted of two parts, pulse part 1 (pp1) and pulse part 2 (pp2; Fig. 4). The pp1 waveform consisted of one major cycle that lasted 6.9 ± 0.2 ms ($N=81$), and pp2 exhibited a sinusoidal waveform with an exponential decay that lasted 21.4 ± 2 ms ($N=77$). The duration of pp1 was more fixed ($CV=3.2\%$) than for pp2 ($CV=12.4\%$).

Pulse energy was concentrated in two frequency bands of relatively similar amplitude (Fig. 6): 226 ± 1 Hz ($N=209$) and 410 ± 1 Hz ($N=209$). Above the second band, energy decreased rapidly from about 450 Hz to about 900 Hz.

Morphology

The sound-producing mechanism is constituted from modified vertebra, three pairs of sonic muscles and a highly modified swimbladder, which possesses a rocker bone, a swimbladder fenestra and three vibrating membranes (Figs 7 and 8).

Vertebral modifications

The first neural arch (also termed the neural rocker) is highly specialized and has no neural spine (Fig. 7). It is shaped like a horseshoe above the vertebra, and both branches articulate with the vertebral body so that it pivots in the anteroposterior plane. It has two large transverse plates firmly attached by connective fibres to the first epineural, called the wing-like process (Fine et al., 2007). These epineurals are connected to the rocker bone by a ligament (Fig. 7B). The second epineurals are rod-like, articulate on the second vertebra, do not contact the swimbladder and appear to have no role in sound production. On the third vertebra there is an osseous stem that articulates with the vertebral body proximally and a distal part

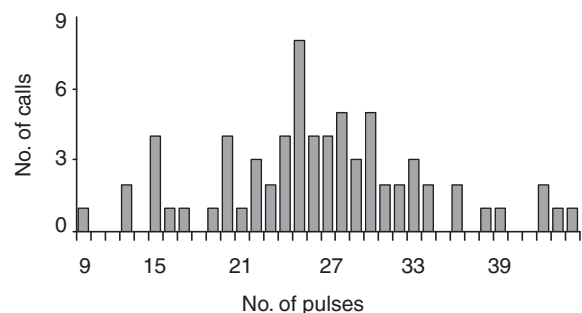


Fig. 2. Histogram of the number of pulses in the call of *O. rochei*.

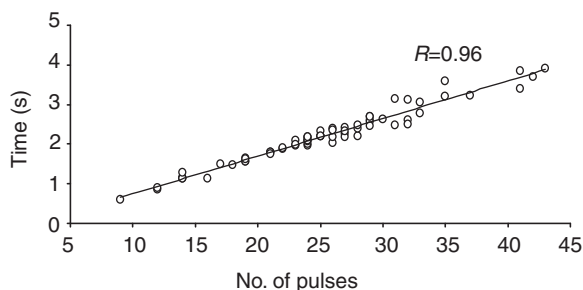


Fig. 3. Relationship of call duration to number of pulses in a call from *O. rochei*.

that expands into a widespread convex bony plate (the swimbladder plate). The plate surrounds part of the rocker bone dorsolaterally, and its margins creep within the tissues of the swimbladder (Fig. 7). Extending backwards from the dorsal part of the swimbladder plate, the fourth and fifth vertebrae possess two ventral plates (Fig. 7A) that support the dorsal swimbladder. More caudally, the dorsal part of the swimbladder is firmly applied against the vertebral bodies by the means of connective fibres.

Rocker bone

The rocker bone (Figs 7 and 8) is a kidney-shaped structure situated in the anterior wall of the swimbladder. Its anterior edge is large, and it narrows into a posterior hilum. The hilum is the insertion site of the ventral muscle tendon and the ligaments of the wing-like process (Figs 7 and 8). The insertion sites of the right and left epineural ligaments define a transverse axis ventrally, around which the rocker bone can pivot (Fig. 7).

Swimbladder

The swimbladder (Fig. 8) is shaped like a bottle with a short neck surmounted by a cork, i.e. the rocker bone. Moreover, there is dorsally a thinner layer between the rocker bone and the neck, the swimbladder fenestra. Sagittal sections reveal three compartments. The first is the neck, the second is the main part of the bladder, and the third is an internal cone-shaped tube. Membranes are present between the neck and the main compartment, and at both ends of the cone (Fig. 8A). In freshly dead specimens, manual back and forth movements of the rocker bone automatically drive membrane 3 back and forth, respectively.

The swimbladder walls are thick and rigid, and are relatively resistant to deformation. Sagittal and transverse sections show several tissue layers (Fig. 8). From inside out, the main chamber is composed of (1) an epithelial tissue layer (the mucosa), (2) a thick layer of elastic and collagen fibres (the submucosa), and (3) a second epithelial tissue (the serosa). The submucosa is composed of a network of interlaced fibres with longitudinal and transverse orientations.

The anterior swimbladder exhibits extensive modification to house the rocker bone (Fig. 8B, compartment 1) (see also Parmentier et al., 2008). The submucosa thickens and forms a Z-shaped fold that terminates rostrally in a horizontal strip that penetrates and ramifies within the rocker bone (Fig. 8C). This strip of the elastic submucosa appears to have a dual function: it acts as a hinge around which the rocker can pivot and as a spring enabling rapid return of the displaced rocker bone. The serosa thins and completely surrounds the anterior face of the rocker bone. Posteriorly, the serosa develops numerous thin layers that enter the swimbladder neck. At the caudal-most end of the neck, the serosa extends across the mucosa, and

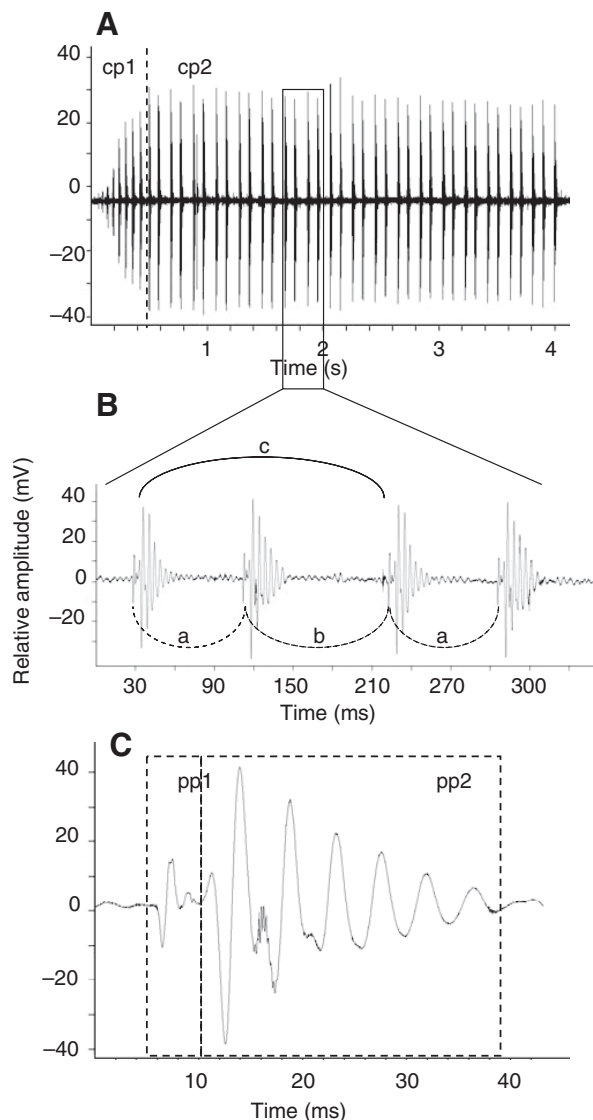


Fig. 4. (A) Oscillograms of a given call by *O. rochei*. (B,C) Two successive enlargements to highlight the pulse waveforms. The pulse in C was enlarged from the second pulse illustrated in B. The two kinds of arc show the two ways used to measure the periods between pulses. Dotted arch corresponds to the measure from pulse to pulse, giving two periods: a and b. Solid arcs measure one pulse of out two and gives the period c (see Discussion). cp1, call part 1; cp2, call part 2; pp1, pulse part 1; pp2, pulse part 2.

the juxtaposition of both tissues forms membrane 1 (Fig. 8Cii). The submucosa is not present at the level of the posterior part of the rocker bone: it inserts dorsally onto the triangular shape of the fourth and fifth vertebrae. As a result, the anterodorsal part of the swimbladder is made only of the thin serosa, forming the so-called swimbladder fenestra. Because the swimbladder fenestra is very thin, it allows the movement of the rocker bone.

The inner cone forms from an invagination of the posterior part of the swimbladder (Fig. 8D). The serosa introverts in the swimbladder and forms a loose fibrous and vascularized tissue that has numerous elastic fibres elongated in the longitudinal plane. Surrounding the serosa, the submucosa forms the main part of the tube wall. At the front of the tube, the mucosa is thickened and develops villi. Membrane 2 results from the apposition of the serosa

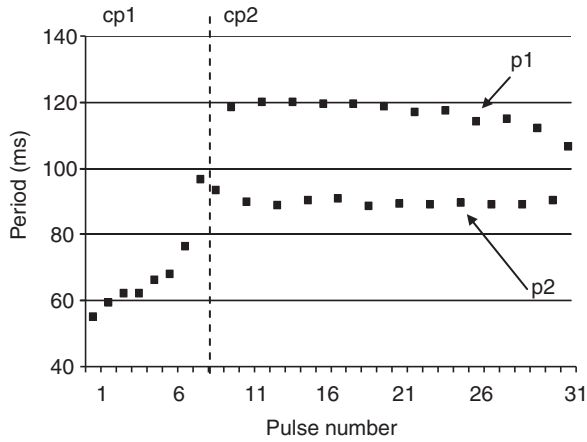


Fig. 5. Graph of the periods of successive pulses within a given call in *O. rochei*. The first part of the call (cp1) shows a decreasing speed of the pulse apparition. In the second part of the call (cp2), two kinds of periods (p1 and p2) follow one after the other.

and mucosa. Elastic membrane 3, capping the posterior end of the swimbladder, is thickened, derived from serosa only, and is highly vascularized. It consists of a more compact fibrous tissue and is oriented perpendicularly to the fibres of the cone.

Sonic muscles

Three pairs of sonic muscles are involved in the sound-producing system (Figs 7 and 8). The dorsal sonic muscle (DM) originates on the neurocranium (exoccipital, supraoccipital and epiotic) and inserts on the neural rocker of the first vertebra. Manually pulling on the DM causes the first neural arch (neural rocker) to pivot rostrally, causing the tips of the wing-like process to pivot posterodorsally, thereby rotating the rocker bone clockwise. The distance between the insertion of the dorsal muscle on the arch and its pivotal point is shorter than that between the tip of the rib and its pivotal point. In terms of a lever arm, a short displacement of the neural arch will generate a movement of greater amplitude at the distal tip of the rib. The ventral sonic muscle (VM) originates

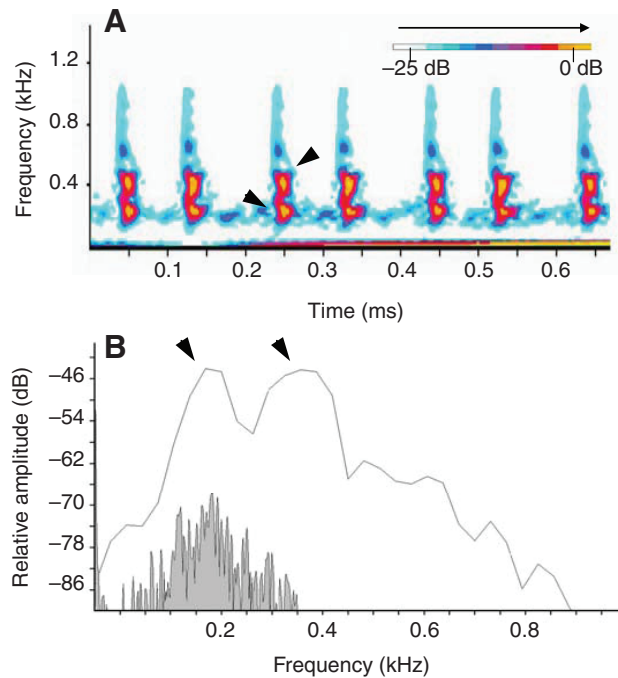


Fig. 6. (A) Spectrogram of seven following pulses and (B) frequency spectrum of one pulse in *O. rochei*. The grey part corresponds to the power spectrum of the background noise. In B, the background noise was calculated on the basis of samples lasting 1 s, whereas the power spectrum is obtained from a duration corresponding to the pulse length (ca. 25 ms). In both figures, two main frequencies are present (arrowhead).

on the basioccipital, the intercalarium and the prootic. It ends in a tendon that inserts on the rocker bone (Fig. 7), behind the insertion of the epineural ligament 1. Manually pulling the VM causes the rocker bone to swivel counter clockwise. The intermediate sonic muscle originates on the exoccipital and inserts on the ventral face of the wing-like process. This muscle is crossed by the Baudelot's ligament, which connects the basioccipital to the pectoral girdle. The contraction of the intermediate muscles displaces the wing-like

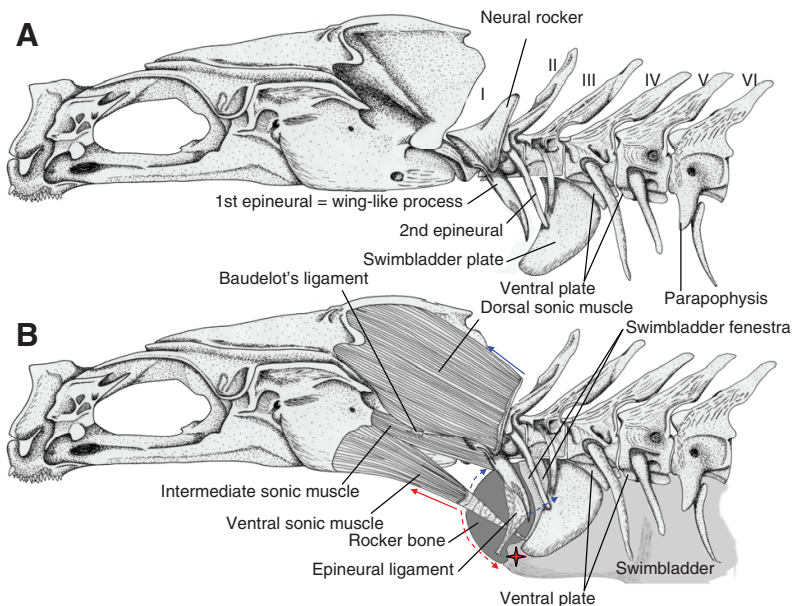


Fig. 7. Left lateral view of the sound-producing apparatus of male *O. rochei* showing (A) the skull and the modified first vertebrae, and (B) the muscles and the rocker bone. The red solid line shows the contraction of the ventral sonic muscle (VM) and the red dotted line shows the resulting frontward and clockwise movement of the rocker bone. The blue solid line shows the contraction of the dorsal sonic muscle (DM); the blue dotted line shows the resulting rise of the tip of the wing-like process, and the backward and counter-clockwise movement of the rocker bone. The star indicates the rotation axis.

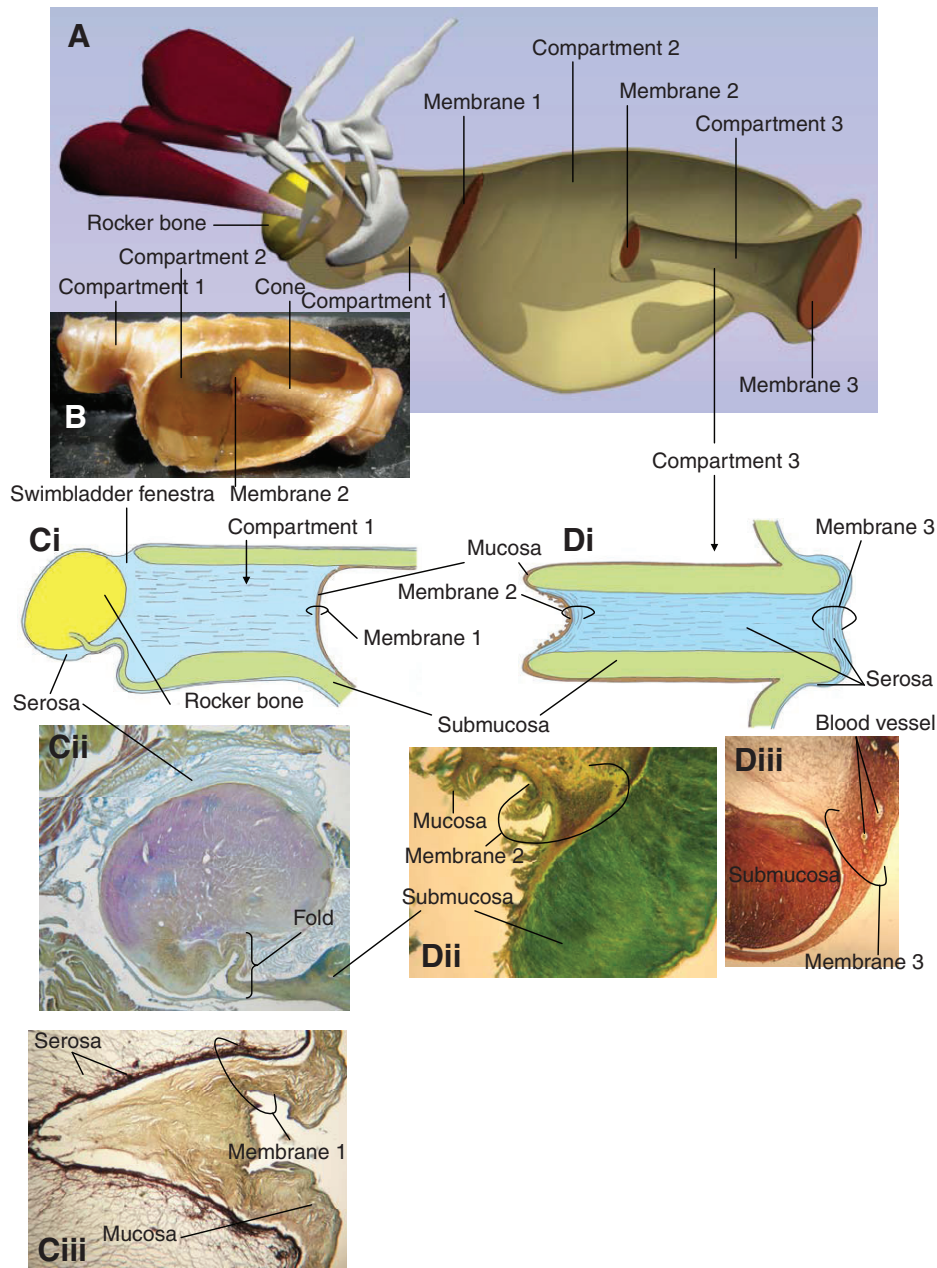


Fig. 8. Left diagrammatic view of the male *O. rochei* sound-producing apparatus (A) and of the isolated swimbladder (B), in which a window has been cut to see the inner cone. Schematic view of a sagittal section through compartment 1 (Ci) and compartment 3 (Di) corresponding to the inner cone. Sagittal section at the level of the rocker bone (Cii), membrane 1 (Ciii), and the anterior (Dii) and posterior (Diii) part of the inner cone with membranes 2 and 3, respectively.

process forwards and outwards, appearing to act as a stay that would minimize movement of the anterior swimbladder wall during rotation of the rocker bone.

DISCUSSION

The definitive identification of the sounds in *O. rochei* is based on different concordant and conclusive elements. Calls were produced only during the night, and the fish were caught at the place of recordings, in 75 cm of water on a sandy bottom. These characters match to the nocturnal activities (Bacescu et al., 1957; Letourneur et al., 2001) and ecology of the species (Bacescu et al., 1957; Matallanas, 1980; Matallanas and Riba, 1980; Dulčić et al., 2004). Moreover, calling corresponds to the call pattern of the North American *O. marginatum* that live in a similar environment: *O. marginatum* call with a crepuscular pattern, with a strong peak at dusk, less sound production during the night, and a lesser peak in sound production at dawn (Mann and Grothues, 2009). The call

pattern of *O. rochei* was similar to that of *O. marginatum*, i.e. long trains of long-duration pulses (25 ms) with multiple peaks, and a pulse repetition rate below 20 Hz (Mann et al., 1997; Sprague and Luczkovitch, 2001). It was not possible to see the behaviour corresponding to the calls. However, fishes that produce courtship calls in choruses (Takemura et al., 1978; Lagardère and Mariani, 2006; Ramcharitar et al., 2006) do so during the spawning season.

Basic mechanism

On the basis of sonic morphology in *O. barbatum*, Parmentier et al. (Parmentier et al., 2006b) proposed the sounds resulted from the alternate contraction of two antagonistic pairs of muscles, the VMs and the DMs. However, the recording of the sounds and the resulting oscillograms were more precise in *O. rochei* and allowed the elaboration of another hypothesis.

Classically in fish sound production, each contraction of the sonic muscles generates a sound pulse. In *O. rochei*, alternate contraction

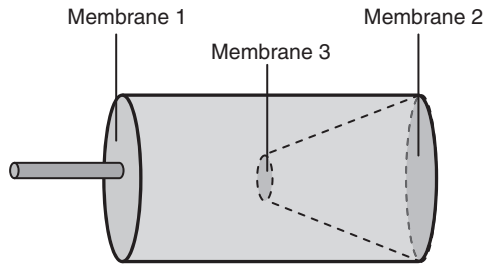


Fig. 9. Model used to roughly mimic the sound behaviour in the swimbladder of the *O. rochei* male.

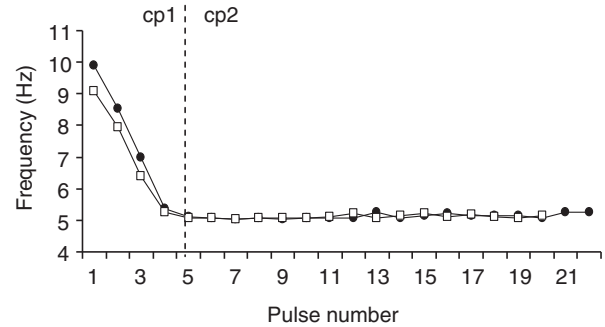


Fig. 10. Variation in the contraction rate of the right and left VMs during a given call in a *O. rochei* male. The rate was inferred from a sound file; it cannot be determined which is the right or the left muscle. cp1, call part 1; cp2, call part 2.

of the DM and the VM could provoke back-and-forth movement of the rocker bone, generating each successive pulse. However, the back-and-forth movement of the rocker bone should generate successive pulses that have different shapes, which is not the case. In *O. rochei*, all the pulses are identical, which suggests they are all formed by the same mechanism. Moreover, the shape of the pulse oscillogram is unusual because it does not present the classical high-amplitude onset followed by rapid attenuation. The pulse of *O. rochei* is made of two parts, starting with a low-amplitude onset before a longer high-amplitude part (Fig. 4C). The first part of the pulse (pp1, Fig. 4) could correspond to the rostral (counter clockwise) displacement of the rocker bone. The VMs pull on the rocker bone and create an aspiration at the swimbladder plate, generating a small oscillation and a short pulse. Acting as a lever-arm, the contraction of the dorsal muscles generates a movement of greater amplitude of the distal tip of the epineural and a fast backward (clockwise) displacement of the rocker bone. This movement increases pressure in the anterior chamber of the swimbladder, generating high amplitude lateral movements of the swimbladder plate and, consequently, the second part of the pulse (pp2, Fig. 4). This system could be roughly compared to a pulley in which the alternated contraction of VM and DM change the direction of the applied force. We produced a model consisting of two plastic bag membranes stretched on both extremities of a plastic bottle, with a truncated conical neck ended by a third internal small membrane (Fig. 9). A stem was fixed on the anterior membrane and was repeatedly pulled and released. In each case, it produced waveforms with two parts. The first corresponded to the pulling and possessed a smaller duration than the second one, obtained during the release of the stem. The rapid increase in pressure could potentially damage the swimbladder. However, the thick elastic layer and the internal cone with two membranes united by elastic fibres could act as pressure-release valve. An understanding of the precise role of the three membranes in the swimbladder awaits further research and modelling. Indeed, the results of this study cannot actually admit or reject the possible role of the cone in the sound production. *O. rochei* shows specifically two sets of non-harmonically related frequencies that could result from the dynamic vibratory properties of the paired oscillators (the membranes 2 and 3).

An alternative hypothesis on sound generation is based on the tension-release mechanism observed in the Carapini sister-group (Parmentier et al., 2006a). It postulates the DM contracts first, pulling backwards the epineural, epineural ligament and rocker bone. During the sustained contraction of the DM, the contraction of the VM creates the first part of the pulse (pp1) and the storage of energy in the epineural and ligament. The second part of the pulse (pp2) would be created when the VMs relax. This system can be compared to a bow. At rest, the string and the rod are separated, the contraction

of the DM would tense the rod with the string to stretch the bow, and the contraction and relaxation of the VM would correspond to the pulling and releasing of the string.

Pulse period

The pulse rate presents two periods in alternation in the second part of the sound train (cp2 in Fig. 4): 84 and 111 ms. It could be encoded as follows: ababab, where 'a' corresponds to the first period of 84 ms and 'b' to the second period of 111 ms. A way to have a constant period (c) is to add a and b. If first hypothesis (the pulley) were correct, *O. rochei* would be the first example in which fish sound production is based on a pacemaker having at least two different firing rates. According to the second hypothesis (the bow), two successive pulses in a call could in fact result from the alternate contraction of left and right VMs. In this case, the pulse period is measured every two pulses giving a constant value of ca. 190 ms (the constant period c) for each muscle (Fig. 10). The searobin (*Prionotus carolinus*) shows an alternate contraction of right and left sonic muscles (Connaughton, 2004). The bow hypothesis would cause the production of a highly stereotyped acoustic signal, depending on the operation of a rhythmically active vocal neuron network. In fish, neurophysiological studies show a pacemaker-motoneuron circuit that establishes the rhythmic firing frequency of the vocal motoneurons that determine the contraction rate of sonic muscles (Bass and Clark, 2003).

Warm up

However, these considerations apply to the second part of the call in *O. rochei*. The onset of the period (cp1) shows seven to eight pulses that have shorter length duration and greater amplitudes and periods (Figs 4 and 10). The pulse rate decreases gradually and rapidly until a plateau (cp2, Fig. 10), indicating it is not a case of muscle fatigue during sound production. In *O. rochei*, everything takes place as though the system needs time to find a cruising speed. In some bushcrickets and cicadas, there are also differences in the basic rhythm of muscle activity, and also in the frequency of muscle potentials within individual chirps (Heller, 1986; Josephson and Halverson, 1971; Sanborn, 2001). This phenomenon has been called a warm-up period that is necessary so that the mechanical properties of the muscles match the mechanical resonance of their system (Pringle, 1978). As the muscle temperature increases, the muscle can contract more rapidly and the insect begins to produce its normal call. In *O. rochei*, the system is completely different, the warm-up period corresponding to a decreasing cycle period. The system seems

closer to a tuning-up period to find the adequate maintained oscillatory movement of the rocker bone. The pulse rate could depend on the drag of the rocker bone, which itself depends on the work of the muscles. In this case, the period could be shorter at the beginning of the call (Fig. 10) because the DM is not at the maximum of its contraction ability and the VMs would accomplish their contraction/relaxation cycle faster.

CONCLUSION

In *O. rochei*, the sound-producing mechanism and the sound characters both show a huge panel of highly specialized characters. The back and forth movement of the rocker bone originates the pulse and requires, as in the carapids (Parmentier et al., 2006a), the action of slow-contracting muscles. However, the present data do not enable us to decide between the 'pulley' and the 'bow' hypotheses. The first involves an alternate contraction of the VM and the DM to excite the pulse. The second involves the sustained contraction of the DM during the entire call to place the rocker under tension and a suite of rapid contraction/relaxation of the VM to create the successive pulses. Further studies are necessary to clarify the situation.

LIST OF ABBREVIATIONS

cp1	call part 1
cp2	call part 2
DM	dorsal sonic muscle
VM	ventral sonic muscle
pp1	pulse part 1
pp2	pulse part 2

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