Variations in sound-producing mechanism in the pearlfish Carapini (Carapidae)

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Abstract

Sound production in *Carapus boraborensis* results from the action of different sonic muscles terminating in complex tendons, which have hooks that fit over a tubercle on the swimbladder wall. The primary sonic muscles (PSM) draw progressively the forepart of the swimbladder until the hook releases the tubercle. This allows the swimbladder to snap back to its resting position, which initiates the onset of the sound. In the present study, the morphology of the *C. boraborensis* sound-producing apparatus and the resulting sounds were compared with *Encheliophis gracilis* and *Carapus homei*. The main difference concerns the direct insertion of the PSM on the swimbladder in *C. homei* and *E. gracilis* and, concurrent sonic characteristics. These morphological features also allow both fish to produce an additional kind of sound with more compact pulses, each being clearly composed of two parts and each having a different frequency. The sound-producing system could be compared with a guitarist who makes a sound in releasing a guitar string and modulates it by moving his/her finger along the string. However, *E. gracilis* possess more filtered sounds than *C. boraborensis* and *C. homei*, probably because of the unusual shape of its swimbladder. This study highlights the diversity and plasticity of sonic mechanisms and their implication in the development of sonic repertoire in evolving species.

Introduction

Certain Carapidae, known as pearlfish, are small, eel-like fishes that live inside invertebrate hosts (Trott, 1970). Species of the Carapini tribe (*Carapus* and *Encheliophis*) are mainly found in the respiratory trees or inside the body cavity of different holothurian species (Arnold, 1956; Smith, 1964; Shen & Yeh, 1987). *Carapus* species are commensals and use their hosts as shelters, while *Encheliophis* species are parasitic and eat the host's gonads (Parmentier & Vandewalle, 2003; Parmentier & Das, 2004).

Sounds have been recorded in different *Carapus* species (Parmentier, Vandewalle & Lagardère, 2003; Lagardère, Millot & Parmentier, 2005; Parmentier *et al.*, 2006*a,b*): *Carapus boraborensis*, *Carapus homei*, *Carapus mourlani* and *Carapus acus*. All recorded sounds appear to be species specific. Carapini display specializations of the anterior vertebrae, swimbladder and associated muscles (Emery, 1880; Courtenay & McKittrick, 1970; Markle & Olney, 1990; Parmentier *et al.*, 2000). These structures appear to be a sound-producing device. In *C. boraborensis*, it was recently shown that the primary sonic muscles (PSM) terminate in a complex tendon, the 'tendon–hook' system (THS), which includes a 'hook' that fits over a tubercle on

the dorso-anterior wall of the swimbladder. The muscles contract slowly, pulling the anterior bladder. The sound is produced when the swimbladder is released, snapping back to its resting position, and this sets the swimbladder plate into motion. Energy from plate motion would then be transferred through the fenestra and would excite the bladder to produce sound. This tension-release mechanism seems unique in animal sound generation, and it is also the first example of sound production with a slow sonic muscle (Parmentier *et al.*, 2006*a*). The THS has been found in *C. boraborensis* (Parmentier *et al.*, 2006*a*), *C. acus* and *C. mourlani* (pers. obs.). Another important feature of this *Carapus* sound-producing system lies in the unique myofibrillar helical organization providing the muscle with spring-like mechanical properties (Parmentier *et al.*, 2003). The uncoiling of the helix during relaxation would help in the lengthening of the muscle. Additionally, a second pair of sonic muscles, the secondary sonic muscles (SSM), could also influence sound production (Courtenay & McKittrick, 1970). These muscles originate on the posterior part of the skull and insert on the two first epineural ribs, which are connected to the anterior part of the swimbladder by ligaments. In contrast to the PSM, these muscles have never been closely examined.

It is widely assumed that temporal parameters (sound length, period, etc.) are the main features in acoustic fish communication (Winn, 1964; Myrberg, Spanier & Ha, 1978; Hawkins & Myrberg, 1983; Hawkins, 1993; Mann & Lobel, 1998). On the other hand, spectral frequencies, and perhaps sound intensity, might also have a biological importance for inter- and intra-specific recognition in fish (Ladich, Brittinger & Kratochvil, 1992). This spectral and temporal variability is based at minimum on morphological characteristics. The best example in fish sound variation due to morphology is linked to swimbladder size: the pulse duration increases and the dominant frequency decreases in larger fishes (Ladich *et al.*, 1992; Lobel & Mann, 1995; Connaughton, Taylor & Fine, 2000). Parmentier *et al.* (2006*a*) demonstrated in *C. boraborensis* that peak frequency of manually generated sounds decreased over an octave from almost 500 Hz in a female with a 25.3-mm-long bladder to about 170 Hz in a female with a 38 mm bladder.

Other morphological studies are sparse. *Carapus mourlani* and *C. acus* possess a shorter pulse duration, a higher peak frequency and a less sharp tuning than is the case in *C. boraborensis* and *C. homei* (Parmentier *et al.*, 2006*b*). It has been suggested that these differences could be due to the presence of a central constriction of the swimbladder in the former group, which is not present in *C. homei* and *C. boraborensis*. Moreover, fishes of the *C. mourlani* group can only have conspecific encounters in their host, whereas they can be heterospecific for *C. homei* and *C. boraborensis* (Markle & Olney, 1990; Parmentier *et al.*, 2000). The latter difference could explain why the pulses are shorter and less repetitive in *C. acus* and *C. mourlani*. As an individual entering a sea cucumber, which is already inhabited by the same or a different species, can lead to eviction or death (Lagardère *et al.*, 2005; Parmentier & Vandewalle, 2005), it would be adaptive to produce repetitive calls. This constraint could be less important in *C. acus* and *C. mourlani* because they have no chance of encountering another fish species in the host. On the other hand, *C. boraborensis*, *C. homei* and *Encheliophis gracilis* can be found in the same host species, at the same depth and in similar waters (Parmentier & Vandewalle, 2005).

The parasitic species *E. gracilis* possess a wide geographical distribution in the Indo-Pacific, from East Africa to Hawaii. This widespread distribution is likely due to the dispersive ability of the coral-reef larvae (Leis, 1991, 2002). Larval dispersal is dependent on the duration of larval life (between hatching and settlement on the reef), current strength and direction, swimming behaviour and the mortality rates of the larvae (Munro & Williams, 1985). In *E. gracilis*, the pelagic larval period is particularly long: more than 70 days (Parmentier, Lagardère & Vandewalle, 2002; Colleye *et al.*, 2007). *Encheliophis gracilis* has been reported to produce two kinds of sounds in French Polynesia: single beats or drum rolls (Parmentier *et al.*, 2003). However, the description of the sounds was only given for the single beats, and was not accurate enough for the drum rolls.

The aim of this study is to understand more deeply the links in Carapini between morphology and the resulting ability to make sounds. The mechanism of sound production in the parasitic *E. gracilis* is compared with that of commensal species (*C. homei* and *C. boraborensis*) in order to discover how morphological differences can influence sound parameters in closely related species.

The aim of this study is to understand more deeply the links in Carapini between morphology and the resulting ability to make sounds. The sound and the mechanism of sound production were described in *C. boraborensis*; only the sounds were presented in *C. homei* and data are lacking for the sound and the mechanism in the parasitic *E. gracilis*. This study completes the published data in order to show how morphological differences can influence sound parameters in closely related species.

Material and methods

Four specimens of the sea cucumber *Bohadshia argus* were collected by scuba diving in the lagoon in front of the coast of Tulear (Mozambique Channel, west coast of Madagascar, 23°220S; 46°390E) in November 2005. The sea cucumbers were placed in a tank (3.5 × 0.7 × 0.2 m) filled with seawater (26 °C). Three holothurians were cut longitudinally and two *E. gracilis* (TL: 18.5–22 cm) were found inside each specimen. The sex was impossible to determine using external features. These fish were stored in a glass tank (1 × 0.5 × 0.6 m, 26 °C) in natural day–night cycle and were not fed for a period of 24 h before the experiments. Two specimens of *E. gracilis* (TL: 20–24 cm), 11 specimens of *C. homei* (TL: 8–15 cm) and 11 specimens of *C. boraborensis* (TL: 8–23 cm) were also collected by scuba diving in front of Opunohu Bay, Moorea, French Polynesia. These fishes, used for morphology examination, were found in specimens of the sea cucumbers *Thelenotas ananas* and *B. argus*, respectively.

The sounds were recorded under the same conditions as those used by Parmentier *et al.* (2003, 2006*b*) for *C. homei* and *C. boraborensis*. Sounds were recorded in a glass aquarium (1 × 0.5 × 0.6 m) containing the sea cucumber, above which a hydrophone was placed at a distance of < 20 cm. For each experiment, individuals were introduced one after the other into the aquarium in order to allow enough time for each one to enter the sea cucumber host. Six fish were used for the experiments. However, the sounds were made once the fish was inside the sea cucumber; it was thus impossible to identify the emitting fish. Sounds were recorded with an Orca hydrophone (sensitivity: −186 dB and 1 V µPa^{-1}; flat frequency response range between 10 Hz and 23.8 kHz) connected via an Orca-produced amplifier (ORCA Instrumentation, Brest, France) to a Sony TCD-D8 digital audio tape-recorder (Liège, Belgium) (recording bandwidth: 20–22 000 Hz ± 1.0 dB). Sounds were digitized at 44.1 kHz (16 bit resolution) and analysed with AvisSoft-SAS Lab Pro 4.33 software (Avisoft Bioacoustics, Berlin, Germany). The resonant frequency of the tank was 2.1 kHz and the corresponding low-pass filters were applied (Akamatsu *et al.*, 2002). Temporal features were measured from the oscillograms, and frequency parameters were obtained from power spectra (filter bandwidth 300 Hz,
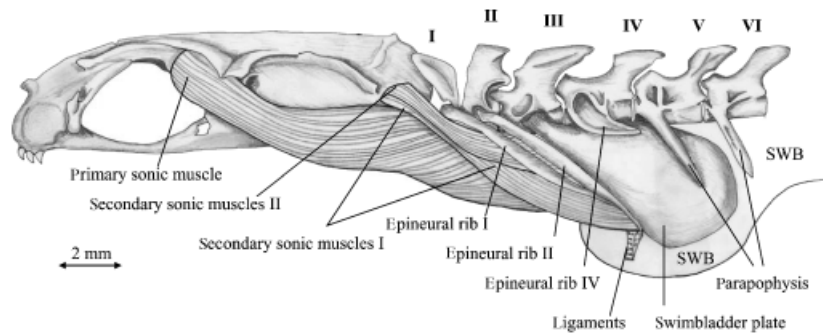
Figure 1 Left lateral view of the anterior part of the sound-producing system in *Encheliophis gracilis*. SWB, swimbladder.

FFT size 256 points, time overlap 96.87% overlap and a Hamming window). The following sound parameters were measured: (1) sound duration [time elapsed from the start of the first pulse to the end of the last pulse (ms)]; (2) number of pulses in a sound; (3) pulse period [average peak-to-peak interval between consecutive pulse units in the entire sound (ms)]; (4) pulse duration (time elapsed from the start and the end of the pulse); (5) the pulse interval (time elapsed from the end of the pulse and the start of the next pulse).

Six *E. gracilis* specimens were deeply anaesthetized with the anaesthetic MS 222 (Sigma-Aldrich, St. Louis, Missouri, USA). Two were fixed in Bouin's solution for the production of serial histological sections. The general morphology of muscles was observed on 6–7 µm sections stained using Masson's trichrome. Small samples (1 cm^3) of the PSM, SSM and epaxial muscle were taken from two specimens and were fixed in glutaraldehyde 2.5% for electronic microscopy (TEM). The cellular ultrastructure was examined on ultrathin sections (60–80 nm) stained with uranyl acetate and lead citrate. The sections were viewed with a JEOL JEM 100SX electron microscope (Jeol Ltd, Tokyo, Japan). Two fishes were placed in 75% alcohol. They were dissected and examined with a Wild M10 (Leica Camera) binocular microscope (Leica, Wetzlar, Germany) equipped with a camera lucida. Morphological observations related to *C. homei* concerned the dissection of preserved specimens. The morphology of the sonic apparatus was compared with the data obtained in specimens of *C. boraborensis* (Parmentier *et al.*, 2002, 2003, 2006*a*).

This study led to results that needed deeper investigations of published data on the carapid species *C. homei* and *C. boraborensis* (Parmentier *et al.*, 2003, 2006*a*; Lagardère *et al.*, 2005). Sounds emitted by these fishes in the same kind of experiment were examined deeply to provide more precise comparisons with *E. gracilis*.

Results

Morphology

Different parts of the Carapini sound-producing system have already been described in different studies (Parmentier *et al.*, 2003, 2006*a*). Some information is, however, given here for the clarity of the text. The first two vertebrae display
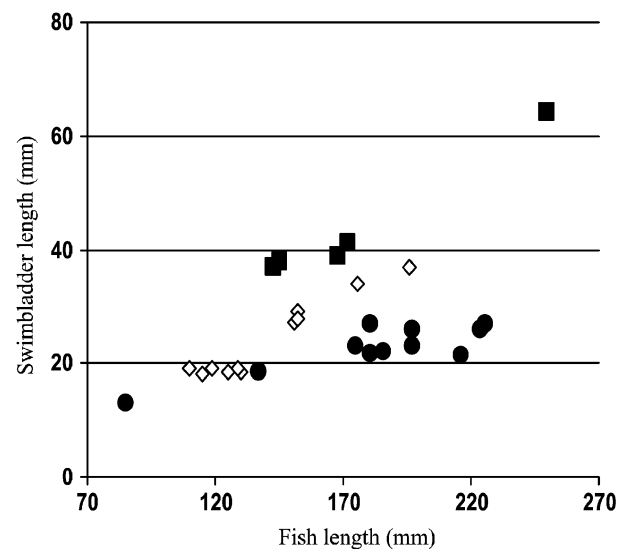
Figure 2 Relationship between the fish and the swimbladder length in *Encheliophis gracilis* (■, *r* = 0.98), *Carapus homei* (◇, *r* = 0.96) and *Carapus boraborensis* (●, *r* = 0.84).

epineural ribs, which are movable in all directions and are attached to the swimbladder by a ligament inserting on their distal tip. The ventral (α) SSM is inserted on the epiotic and on the distal portion of the first epineural rib. The dorsal SSM (β) has its anterior part attached to the epiotic and joins the ventral part of the second epineural rib. The third vertebra bears a broad, ossified swimbladder plate, which attaches to the swimbladder and is fixed to the fourth epineural rib. In *E. gracilis*, the swimbladder plate reaches the sixth vertebra instead of the fifth vertebra in *C. boraborensis* and in *C. homei* (Fig. 1).

The almost cylindrical swimbladder occupies 90% of the general cavity in all three species. However, its size is 24.8 ± 1.3% (*n* = 5) of the total length in *E. gracilis*, 17 ± 2% (*n* = 11) in *C. homei* and 12.6 ± 1.4% (*n* = 6) in *C. boraborensis*. This results in the swimbladder extending beyond the 23rd–26th, 16th–17th and 11th–12th vertebrae, respectively. The swimbladder size appears to be related to the fish size, the relationship being, however, weaker in *C. boraborensis* (Fig. 2). The swimbladder may be divided into three zones (Fig. 3). The PSM insert on the anterior
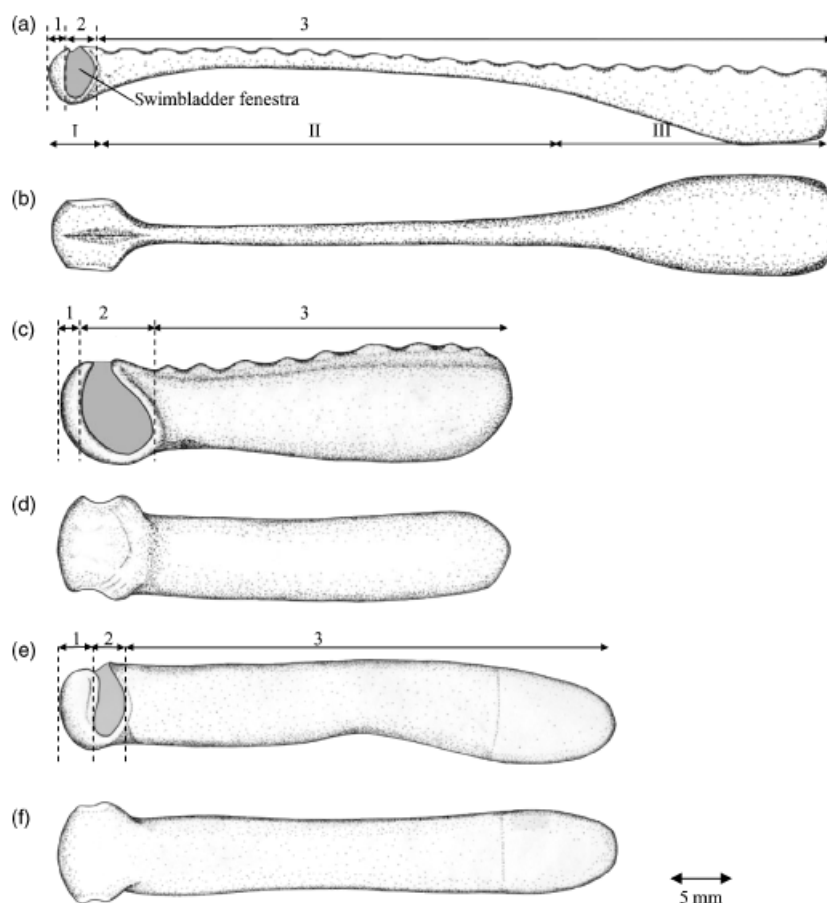
Figure 3 Left lateral (a, c, e) and ventral (b, d, f) views of the swimbladder in *Encheliophis gracilis* (a, b), *Carapus boraborensis* (c, d) and *Carapus homei* (e, f). Arabic numbers refer to the functional parts of the sound-producing system. In *E. gracilis*, in comparison with the Helmolthz resonator, I, external air volume; II, neck; III, chamber.

region; the wall of the second region, the 'swimbladder fenestra', is situated just under the swimbladder plate and is thinner; the posterior region is long and narrow. The shape of the swimbladder is quite regular along its entire length in *C. boraborensis* and in *C. homei*. In *E. gracilis*, the anterior part of the third region is at first narrow and is then posteriorly enlarged, the caudal part being twofolds larger (Fig. 3).

The primary sound-producing muscles (PSM) are attached to the orbital roof, on the inner face of the frontal, and to the antero-dorsal portion of the swimbladder (Fig. 1). Important differences are found between species. In *C. boraborensis*, the sonic muscle terminates in a complex tendon, which includes a 'hook' that fits over a tubercle on the dorso-anterior wall of the swimbladder (Parmentier *et al.*, 2006*a*,*b*). In *C. homei* and *E. gracilis*, the anterior part of the swimbladder does not possess small tubercles, and the PSM tendon is very short and deprived of a hook (Fig. 4). In *E. gracilis* PSM, the fine morphology of the sonic fibres and myofibrils is identical to that of *C. boraborensis*: central ones are straight, whereas peripheral ones become increasingly twisted giving to the whole a helicoidal organization

(Fig. 5a, see also Parmentier *et al.*, 2003). In the PSM, the tubule system/sarcoplasmic reticulum (T system/SR) are found at the Z line level and at the A/I junctions. The mitochondria are numerous and are concentrated in packs under the sarcolem, around the myofibrils.

The organization of the myofibrils and the cellular content of the SSM correspond to the epaxial musculature. Myofibrils are parallel and occur in a straight line (Fig. 5b). The T system/SR (one T tubule + two terminal system of the sarcoplasmic reticulum) only surrounds the sarcomere at the Z line level. The mitochondria are sparser and are distributed in the muscles, between the myofibrils.
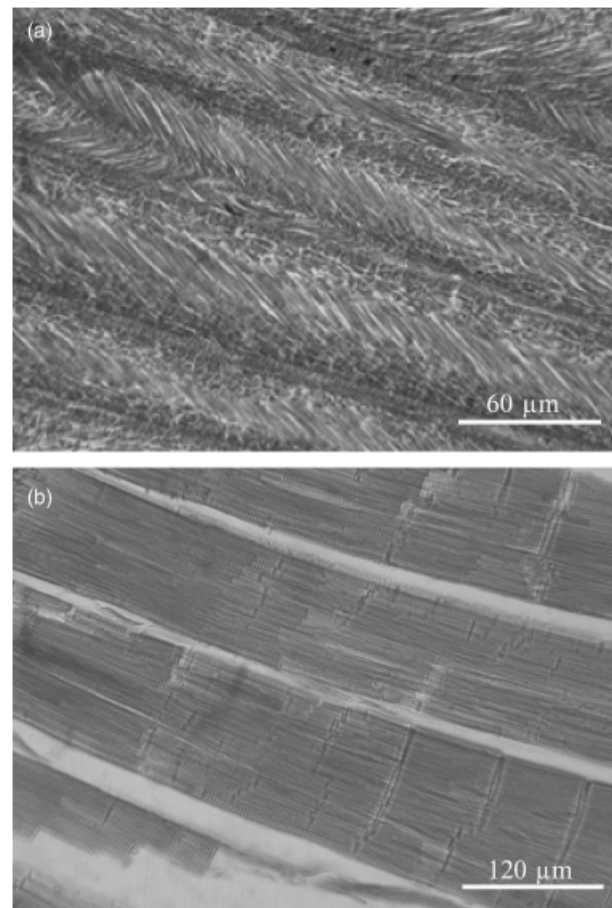
In *E. gracilis*, the PSM fibre diameter is significantly ($P<0.001$) thinner than the SSM fibre and the white epaxial fibres (Fig. 6). There is no significant difference between the epaxial and SSM diameter (Student's *t*-test, $P = 0.632$). The PSM myofibril diameter ($0.55 \pm 0.09\,\mu$m, $n = 47$) is significantly ($F_{2,101} = 96$, $P<0.05$) thinner than the SSM myofibrils ($1.08 \pm 0.21\,\mu$m, $n = 25$) and the white epaxial myofibrils ($1.02 \pm 0.25\,\mu$m, $n = 30$). There is no significant difference between the epaxial and SSM diameter (Student's *t*-test, $P = 0.632$, Fig. 6).
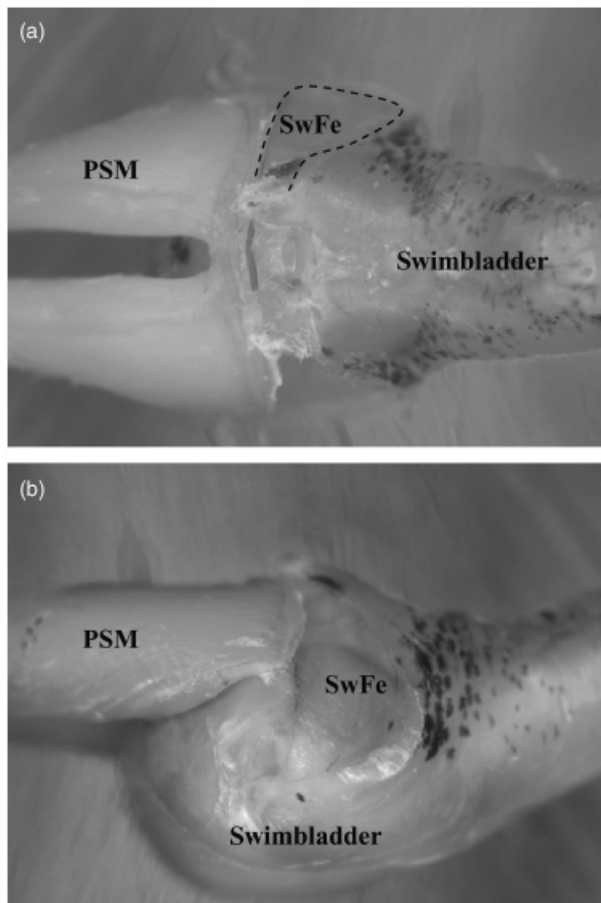
Figure 4 Insertion of the primary sonic muscle (PSM) on the swimbladder in *Encheliophis gracilis*. (a) dorsal view, (b) lateral view. SwFe, swimbladder fenestra.

Figure 5 Longitudinal sections in the primary (a) and secondary (b) sonic muscles in *Encheliophis gracilis*.

Sounds

In each trial, sounds were only recorded when one individual entered the sea cucumber that was already occupied. So, it is impossible to observe the interactions between fishes. *Encheliophis gracilis* drum rolls consisted of a train of five to six pulses, each pulse lasting ($X \pm$ SE) 23.69 ± 0.35 ms ($n = 59$). The average of the pulse period and pulse interval was 39.89 ± 0.79 ms ($n = 58$) and 16.15 ± 0.70 ms ($n = 59$), respectively. The frequency of these sounds ranged between 40 and 600 Hz with an average peak frequency of 157 ± 2 Hz ($n = 58$). The oscillogram shows that each pulse can be divided into two parts (Fig. 7a and b). The beginning of the sound generally has three to four cycles, and is followed by a shorter sequence in which a higher number of cycles is found. As a result, the spectrogram (Fig. 7c) shows that this second part possesses a higher frequency than the first part: ± 3800 versus ± 200 Hz. However, the sound pressure is always more pronounced in the first part of the sound, giving to the whole pulse its main energy. According to Akamatsu *et al.* (2002), true characteristics of sounds are hard to assess in small tanks due to their physical constraints. If the resonant frequency is close to the frequency of the sounds produced by the fish, the original spectrum can be distorted. Pulsed sounds as in *E. gracilis* exhibit a broadband spectrum, enhancing the probability of a fish frequency component being close to the minimum resonant frequency of the tank. In this case, the resonant component of 3800 Hz could be overemphasized (Akamatsu *et al.*, 2002). In the present study, the resonant frequency of the tank was 2.1 kHz and the corresponding low-pass filters were applied (Fig. 7b): the second part of the sound still has had higher frequencies than the first part.

Discussion

The sound-producing mechanism has only been closely examined in two *Carapus* species: *C. acus* and *C. boraborensis* (Parmentier *et al.*, 2003, 2006*a*,*b*). In these species, slowly pulling the sonic muscles with forceps was shown to be sufficient to generate sound pulses. The sound was produced when the tendinous hook of the PSM released the tubercle of the swimbladder, and the latter snapped back to its resting position. Apparently guided by attached connective tissue, the hook was shown to return to its locked
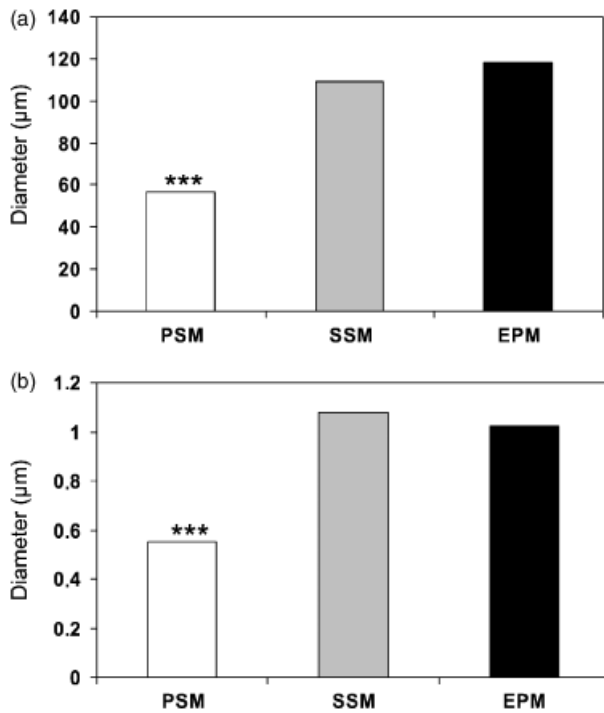
Figure 6 Fibre (a) and myofibril (b) diameters of the primary sonic muscle (PSM), secondary sonic muscle (SSM) and epaxial muscle (EPM) in *Encheliophis gracilis*. ***$P < 0.001$.

position on the tubercle during muscle relaxation (Parmentier *et al.*, 2006*a*,*b*).

As in *Carapus*, the sonic fibres and myofibrils of *E. gracilis* exhibit the following characteristics: they have an unusual helicoidal organization, are thinner than white epaxial fibres, have numerous mitochondria in the muscle cell periphery and have T tubules at the A–I junctions. They also possess a swimbladder plate and a swimbladder fenestra. All these features highlight the fact that these sonic muscles are also highly specialized, and that they could work as in *Carapus* species (Parmentier *et al.*, 2003, 2006*a*,*b*). However, the fine sonic mechanism appears to be different in *E. gracilis* and, more surprisingly, in *C. homei* because they do not have the THS. In these fish, muscle contraction pulls the anterior part of the swimbladder forward, stretching the swimbladder fenestra. During relaxation of the muscle, spring-like mechanical properties due to the muscle helical organization (Parmentier *et al.*, 2003) should allow the swimbladder fenestra to snap back rapidly, originating sound. We assume that this flapping of the swimbladder fenestra corresponds to the first cycles at the onset of the pulse. The same kind of onset has, furthermore, also been found in *C. boraborensis* and in *C. homei* (Parmentier *et al.*, 2003, 2006*a*). The second part of the sound is however different between *C. boraborensis* on the one hand, and *C. homei* and *E. gracilis* on the other hand. In *C. boraborensis*, the onset is followed by cycles exhibiting a slow exponential decay (Fig. 8). The second part of the sound presents cycles with a shorter period (Figs 7 and 8) in
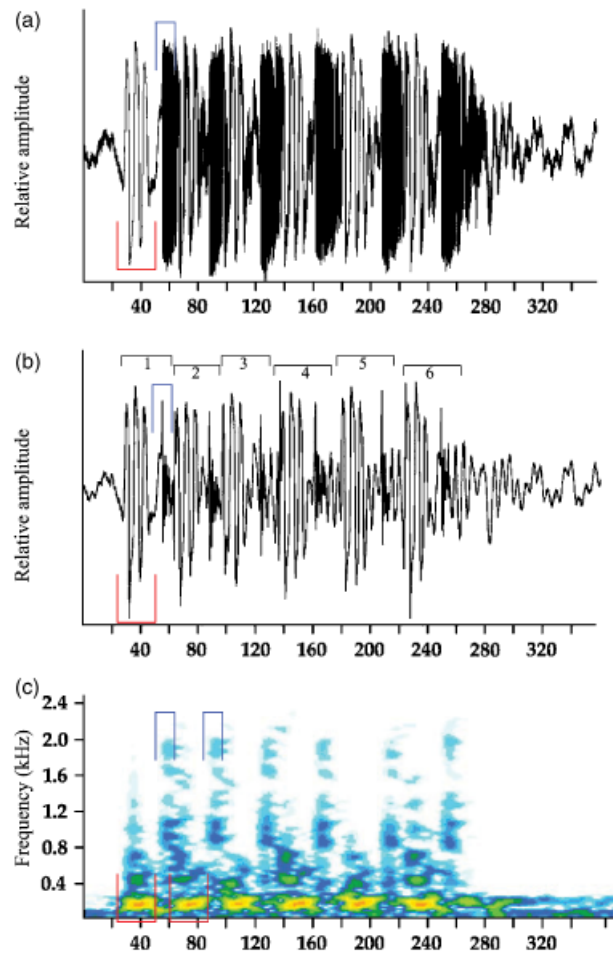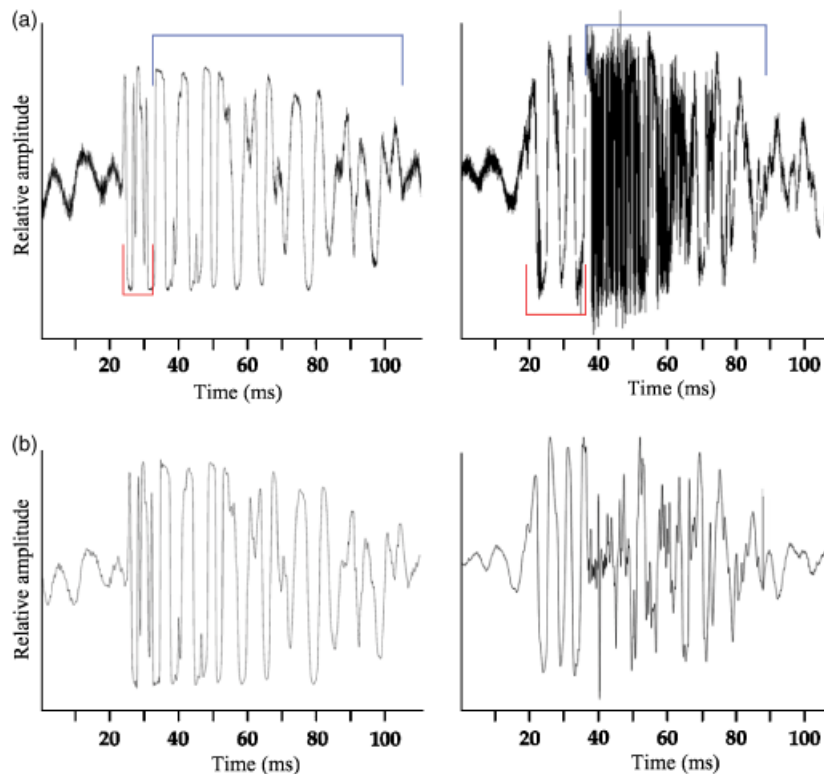

Figure 7 (a) Oscillograms of the sounds produced by *Encheliophis gracilis*. (b) Oscillogram of the same sound on which a low-pass filter of 3 kHz was applied. (c) Spectrogram of the same sound. Red lines delimit the first part of the sound; blue lines delimit the second part of the sound. Spectral analysis information: sampling frequency 44 100 Hz; Windows: Hamming; bandwith: 2.18 Hz.

C. homei and *E. gracilis*, giving to the call a metallic aspect. Both species have in common the absence of THS and have, consequently, direct insertion of their PSM into the swimbladder. The more high-pitched part of the sound could be linked to this common morphological trait. The direct insertion of the sonic muscle implies that the forepart of the swimbladder does not possess the ability to vibrate freely because direct muscular insertions can provoke a greater tension in the bladder walls, giving the higher-pitched sound.

The mechanism can be summarized as follows. The contraction of the sonic muscles extends the swimbladder fenestra; the relaxation of the muscle (and the elasticity of the swimbladder) causes the snapping back and the generation of the first part of the sound (lower frequency); the next contraction of the sonic muscle (or the return to its resting position after its elongation) provokes a new extension of the bladder and the second part of the sound with the higher

Figure 8 Oscillograms of the sounds produced by *Carapus boraborensis* (a) and *Carapus homei* (b). Right column: oscillogram of the same sound on which a low-pass filter of 3 kHz was applied. Red lines delimit the first part of the sound; blue lines delimit the second part of the sound.

frequency. The system could be compared with a guitarist who makes a sound in releasing a guitar string (first part of the sound) and modulates it in moving his/her finger along the string (second part of the sound).

The interpulses have been shown to last 76 ms in *C. boraborensis* (Lagardère *et al.*, 2005) versus 27 ms in *E. gracilis*, while the pulse has been shown to last on average 83 ms in *C. boraborensis* (Lagardère *et al.*, 2005) versus 30–36 ms in *E. gracilis*. On the other hand, the interpulses and pulses have been shown to be shorter in *C. boraborensis* than in *C. homei* (Lagardère *et al.*, 2005), showing that the absence of THS is not automatically linked with more compact sounds. However, this could be related to the ability to produce grouped pulses. The pulses are in a single unit or in long trains in *C. boraborensis*; they can be single, in trains or in groups of two to three in *C. homei*; they are single or in groups of five to six in *E. gracilis*. Lagardère *et al.* (2005) did not separate grouped pulses and pulses in train in their analyses dealing with *C. homei*. We have re-examined their data. The pulse length does not differ ($P = 0.93$, *t*-test) whether the pulses are grouped or in trains. However, the period and interpulse length are, respectively, 1.8 times and 3.3 times shorter ($P < 0.001$) when the pulses are grouped in pairs or in threes. Having these shorter interval times seem possible because the sound-producing system of *C. homei* and *E. gracilis* is deprived of THS; the mechanism is not restricted by the time the muscle needs to

lock onto the swimbladder again. The pulse intervals are likely to be important for recognition (Myrberg, Mohler & Catala, 1986; Bass & McKibben, 2003). In some carapid species such as *C. homei* and *E. gracilis*, the ability to compact pulses could be a way of aiming towards the elaboration of different and/or new kinds of message.

The swimbladder in *C. homei* and *C. boraborensis* is deprived of a long 'neck', as is the case in *E. gracilis* (Fig. 2). In all Carapini, the snapping back of the anterior part of the swimbladder creates a pressure wave moving in the swimbladder from the front to the back. The vibration here is due to the 'springiness' of the air. The Helmholtz resonator is a narrow-bandwidth device, designed to target specific frequency. It is an acoustic system consisting of an oscillating air plug connected to an air volume. In *E. gracilis*, the swimbladder could be compared with a bottle with a chamber and a neck. The forepart of the swimbladder, in front of the neck (Fig. 3) can be considered as the external air volume. This parallelism between the swimbladder shape and the Helmholtz resonator could explain why the resulting sound in *E. gracilis* is naturally more filtered than in *C. homei* and *C. boraborensis* (Fig. 9).

The parasitic *Encheliophis* species are thought to have evolved from species with a commensal way of life (Markle & Olney, 1990; Parmentier *et al.*, 2000). The THS found in *C. boraborensis*, *C. acus* and *C. mourlani* constitutes a highly complicated biological structure for which we do not know
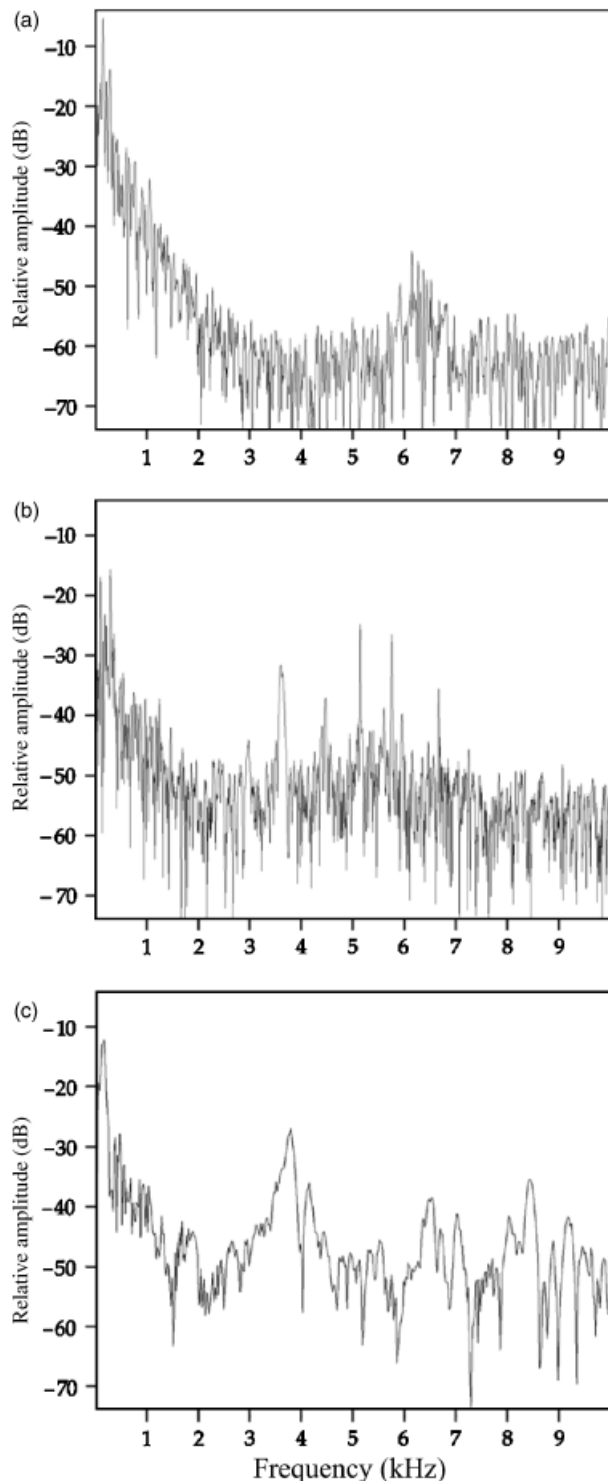
Figure 9 Spectrograms of an isolated pulse in *Carapus boraborensis* (a), *Carapus homei* (b) and *Encheliophis gracilis* (c). The pulses of *C. boraborensis* and *C. homei* are from the study of Lagardère *et al.* (2005). Spectral analysis information: sampling frequency 5500 Hz; Windows: Flat Top; bandwith: 81 Hz.

of an equivalent morpho-functional system. Consequently, it is difficult to believe that it represents the primitive condition. The absence of THS in *C. homei* and *E. gracilis* would thus correspond to a reversal character. This assumption is reinforced by the insertion of the PSM directly on the swimbladder in the sister group *Echiodon* (pers. obs.). However, the absence of THS also seems to allow the fish to produce a greater variety of sounds, and it is difficult to believe that the fishes have lost this ability. Further studies are needed to understand the advantage provided by the THS. The case of *C. homei* should be investigated in greater depth because of its dual position. This species is deprived of THS but the shape of its swimbladder is closer to the commensal species. Several studies supported the hypothesis that the environmental conditions play a role in sound divergence between closely related species (e.g. Slabbekoorn & Smith, 2002). However, all three Carapini species live in the same habitat. Variations in sounds can be considered prezygotic isolating mechanisms leading to speciation (Crocroft & Ryan, 1995). The morpho-functional aspect of the THS is very particular and it is difficult to imagine intermediate states: the muscle is directly inserted on the swimbladder or it is not the case. Its presence or absence appears automatically related to sound variation as it is the case between *C. boraborensis* and *C. homei*, which conserves, however, the same kind of swimbladder shape and other morphological structures linked to their way of life (Parmentier *et al.*, 2000). The evolution from commensal to parasitic way of life is more complicated from the communication point of view. The difference between the swimbladder shape of both *Carapus* and *E. gracilis* can easily find a great number of intermediate states, and the gradual modification can hardly be a barrier because the frequency spectrum is less subject to carry information (Myrberg *et al.*, 1978).

According to McCauley & Cato (2000), the fishes have precise control over the muscle contraction rate, whereas they have less control over the swimbladder resonant frequency (this is a function of the depth, the swimbladder volume, the swimbladder wall characteristics and the applied damping). So, it is possible that, for some fishes, the muscle contraction rate conveys more information than the swimbladder resonant frequency. In teleosts, swimbladder sounds have a fundamental frequency ranging from 75 to 300 Hz, which usually corresponds to the muscular contraction rate, placing sonic muscles among the fastest in vertebrates (Rome *et al.*, 1996; Loesser, Rafi & Fine, 1997; Connaughton *et al.*, 2000; Fine *et al.*, 2001). The speed of the fast-twitch swimbladder muscles could be due in part to their extremely fast relaxation rate (Rome & Lindstedt, 1998). However, the system is quite different in *Carapus* in which the sonic muscle tetanizes at 8 Hz (Parmentier *et al.*, 2006*a*). In this fish, the muscular contraction rate does not correspond to the sound frequency but seems to be related to the pulse period (Parmentier *et al.*, 2006*a*,*b*). In *E. gracilis*, the pulse period of 39.89 ms on an average indicates that the muscle contraction rate is around 25 Hz, three to four times faster than in *C. boraborensis*. We did not

find muscular morphological characters in relation to this difference. However, this higher rate could also be due to alternate muscle contraction (Connaughton, 2004).

The secondary sound-producing muscle (SSM) does not present the adaptations found in the primary sound production muscle (PSM). Fibres and myofibrils of the SSM appear to be morphologically similar to white epaxial fibres. The orientation of the epaxial musculature fibres and of their myofibrils is parallel and in a straight line, the T system/SR surrounds the sarcomere at the Z-line level, and mitochondria are isolated between the myofibrils. As a result, the SSM is highly similar to epaxial muscles. The contraction of the SSM causes a forward and outward displacement of the posterior tips of the epineural ribs, stretching the swimbladder transversally (Parmentier *et al.*, 2003). Depending on the time of contraction, this movement could have different rules. The SSM contraction occurring before the PSM stretches the swimbladder and makes it more rigid, modulating the sound frequency and pulse length. The SSM contraction occurring after PSM contraction could facilitate the snapping back of the anterior part of the swimbladder, modulating the period or the interpulse length.

Acknowledgements

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