

Sound production mechanism in carapid fish: first example with a slow sonic muscle

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Summary

Fish sonic swimbladder muscles are the fastest muscles in vertebrates and have fibers with numerous biochemical and structural adaptations for speed. Carapid fishes produce sounds with a complex swimbladder mechanism, including skeletal components and extrinsic sonic muscle fibers with an exceptional helical myofibrillar structure. To study this system we stimulated the sonic muscles, described their insertion and action and generated sounds by slowly pulling the sonic muscles. We find the sonic muscles contract slowly, pulling the anterior bladder and thereby stretching a thin fenestra. Sound is generated

when the tension trips a release system that causes the fenestra to snap back to its resting position. The sound frequency does not correspond to the calculated resonant frequency of the bladder, and we hypothesize that it is determined by the snapping fenestra interacting with an overlying bony swimbladder plate. To our knowledge this tension release mechanism is unique in animal sound generation.

Key words: Carapidae, sound production, sonic muscle, sonic mechanism, swimbladder.

Introduction

Many fish species have developed mechanisms allowing them to emit sounds for social communication (Ladich and Fine, 2006; Parmentier et al., 2006). Sounds are most frequently produced by the action of specialized fast-contracting striated muscles. Intrinsic sonic muscles completely attach to the wall of the swimbladder, as in the Batrachoididae *Opsanus* sp. (Demski et al., 1973; Fine et al., 1990) and *Porichthys notatus* (Greene, 1924; Bass and Marchaterre, 1989), the Triglidae *Prionotus* sp., *Bellator* sp. (Evans, 1973), *Eutrigla* sp. (Hawkins and Myrberg, 1983; Connaughton, 2004). Extrinsic sonic muscles have various origins and insertions (Jones and Marshall, 1953; Alexander, 1966, Demski et al., 1973). Generally speaking, these paired muscles insert on the swimbladder or a neighbouring structure, which attaches to the swimbladder *via* ligaments. Extrinsic muscles are found in different taxa including the Ophidiiformes (Howes, 1992), Holocentridae (Carlson and Bass, 2000) and Sciaenidae (Ono and Poss, 1982; Connaughton et al., 1997; Sprague, 2000).

Swimbladder sounds have a fundamental frequency ranging from 75 to 300 Hz, which corresponds to the muscular 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 sonic muscles of the swimbladder in *Opsanus tau* can generate power at 10 times the maximum frequency of white epaxial muscles (Young and Rome, 2001). Additional studies on sonic muscles in *Cynoscion regalis* (Sciaenidae), *Prionotus scitulus* (Triglidae), *Arius felis* (Ariidae), *Bagre marinus* (Ariidae) and *Terapon jarbua* (Terapontidae) place them among the 'champions' of contraction speed (Schneider, 1967; Tavolga, 1967; Sprague, 2000). This enormous speed of the swimbladder muscles is due in part to their extremely fast relaxation rate (Rome and Lindstedt, 1998). This characteristic results from numerous morphological and biochemical adaptations, including specialization of protein isoforms (Hamoir and Focant, 1981) and the high concentration of intracellular components (Pennypacker et al., 1985; Rome et al., 1999). The fibres and myofibrils of sonic muscles are also thinner (Evans, 1973; Fine et al., 1993), and possess a more developed sarcoplasmic reticulum (Hamoir and Focant, 1981; Appelt et al., 1991; Feher et al., 1998) than fast white fibres (Eichelberg, 1976). This set of characteristics could facilitate rapid flows of metabolites and calcium (Eichelberg, 1976; Fine et al., 1990; Rome et al., 1996; Feher et al., 1998).

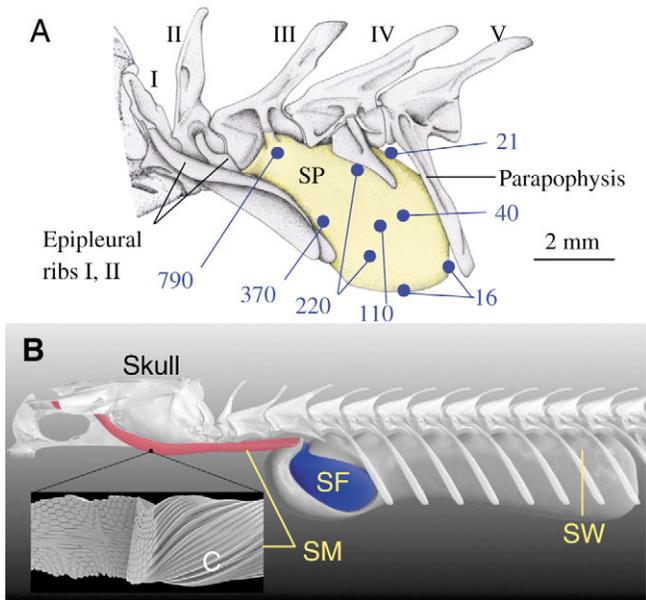


Fig. 1. Sonic mechanism of *Carapus boraborensis*. (A) Left lateral view of the first five vertebrae (I–V) with the associated epineural ribs and swimbladder plate. Numbers on the swimbladder plate indicate its thickness, which decreases laterally and toward its posterior margin. (B) Schematic left lateral view of the skeleton (epineural ribs and swimbladder plate removed), sonic muscles and swimbladder. (C) Inset in B illustrates the helical organisation of the myofibrils in a single sonic muscle fiber with straight central myofibrils. SF, swimbladder fenestra; SM, sonic muscle; SP, swimbladder plate, SW, swimbladder.

The sonic apparatus displays common characteristics in all known carapids (Fig. 1) (Parmentier et al., 2003a). The first two vertebrae display epineural ribs that are movable in all directions, and attach to the swimbladder by a distal ligament. The third vertebra bears a broad, ossified swimbladder plate, which attaches to the swimbladder and is fixed to the fourth epineural rib. The almost cylindrical swimbladder may be divided into three regions (Fig. 1B). The sonic muscles insert on the anterior region; the wall of the second region, the ‘swimbladder fenestra’, is situated just under the swim bladder plate and is thinner due to the lack of submucosa (Parmentier et al., 2003a); the posterior region is long and narrow and firmly attached to the abdominal vertebrae. The swimbladder fenestra is teardrop-shaped on each side, and both parts are connected dorsally by a segment of regular bladder tissue. Paired sonic muscles run from the upper wall of the orbit to the anterior face of the swimbladder forward of the swimbladder fenestra. These muscles present an unique helicoidal organization (Parmentier et al., 2003b) (Fig. 1C): central myofibrils are straight whereas peripheral ones are more and more twisted. Sounds in *C. boraborensis*, *C. homei*, *C. acus* and *C. mourlani* and *E. gracilis* are species-specific (Parmentier et al., 2003a; Parmentier et al., 2006; Lagardère et al., 2005) although all species have a similar sonic bauplan (Parmentier and Vandewalle, 2003).

Biochemical, histochemical and morphological methods have been used to examine sonic muscles and to compare them with epaxial muscles in *Carapus acus* (Parmentier et al., 2003b). Sonic muscles have features of both red (numerous mitochondria, high glycogen content) and white (alkali-stable ATPase) fibres. They differ also from the white epaxial muscle in the isoforms of the light chain and heavy chain, in the position of the T tubules and in a unique parvalbumin isoform that may aid relaxation.

In this study, the mechanism of sound production in carapids was examined by measuring twitch parameters of the sonic muscle, comparing sounds induced by slowly pulling on the sonic muscle to voluntary sounds, and finally by examining the motion of these artificially induced sounds on the swimbladder fenestra.

Materials and methods

The studies were conducted on two different carapid species, depending on availability.

Carapus boraborensis

Twenty *Carapus boraborensis* (Kaup 1856) (total length $TL=18\text{--}25$ cm) were collected by scuba diving in Opunohu Bay (Moorea, French Polynesia) in March 2005. They were found inside specimens of sea cucumber *Bohadschia argus*. They were stocked in tanks (15 m^3) filled with running seawater. Sounds were recorded in a smaller glass aquarium ($0.9\text{ m}\times 0.5\text{ m}\times 0.4\text{ m}$). Only females were used for the experiments because they are easier to obtain. *Carapus boraborensis* longer than 21 cm are females, and the sex ratio of this species is 3 females:1 male (Parmentier and Vandewalle, 2005). Males and females produce different sounds (Lagardère et al., 2005), and exclusive use of females allowed us to compare the same type of sound. Two experiments were performed.

Following published protocol (Lagardère et al., 2005), a specimen of the host was placed in the centre of the tank, and several individual *Carapus boraborensis* were introduced successively into the aquarium. Sounds were recorded using an Orca hydrophone (sensitivity: $-186\text{ dB re } 1\text{ V}/\mu\text{Pa}$) connected via an Orca-made amplifier (ORCA Instrumentation, Brest, France) connected to a Sony TCD-D8 digital audio tape-recorder (recording band width: $20\text{--}22000\text{ Hz } \pm 1.0\text{ dB}$). This system has a flat frequency response range ($\pm 3\text{ dB}$) between 10 Hz and 23.8 kHz. The hydrophone was placed above the sea cucumber.

Seven females were deeply anaesthetised with MS 222 and then rapidly dissected in order to expose the sonic mechanism. Manually generated sounds were obtained by pulling the sonic muscles forward with forceps. They were recorded in a small vessel ($38\text{ cm}\times 28\text{ cm}\times 5\text{ cm}$) full of seawater as described above. After recordings, head, body and swimbladder length were measured to the nearest millimetre with a vernier calliper.

There are potential hazards recording in small tanks because of reflections and tank resonance (Akamatsu et al., 2002). Akamatsu et al. provide formulae to calculate the resonant

frequency (Akamatsu et al., 2002) and empirically determined that sound spectrum shape can be measured without artefacts for a frequency range lower than the minimum resonant frequency of the tank if a hydrophone is placed within an attenuation length of the fish. The resonant frequencies of the tanks were 2560 Hz (0.9 m×0.5 m×0.4 m) and 3327 Hz (0.38×0.28×0.05 m), respectively, and the hydrophone was placed within 23 cm of the fish.

After the sound recordings, the swimbladder plate was removed to expose the swimbladder fenestra and the insertions of the sonic muscle. The movement of the swimbladder was then filmed (Wild M10 binocular microscope, Heerbrugge, Switzerland, equipped with a Canon PowerShot S45 camera, Tokyo, Japan) while the sonic muscles were pulled with forceps. The microscope was coupled with a camera lucida that allowed measurement of the thickness of the swimbladder plate. The plate was progressively measured and then carved away from its posterior edge allowing a series of thickness measurements. Relative movements of the fenestra were determined by marking it with ink dots and taking a picture. A second photograph was then taken of the stretched fenestra. By superimposing the two pictures, it was possible to quantify relative movements of different parts of the fenestra.

The swimbladders of 14 females were removed, and their diameters and lengths were measured with the Wild M10 binocular microscope and camera lucida. Their calculated volume was used to obtain the equivalent radius of a sphere. This radius was used to calculate the resonant frequency of the swimbladder according to the formula for an underwater bubble (Weston, 1967):

$$F = \left(\frac{1}{2\pi R} \right) \sqrt{\frac{3\gamma P}{\rho}},$$

where F is resonant frequency calculated for an underwater bubble; R , radius (cm); γ , ratio of specific heats (=1.4); P , pressure (atmospheric pressure + hydrostatic pressure); ρ , water density. To compare with the manually generated sounds, the resonant frequency was calculated for a depth of 5 cm. Note that elongation of the bladder causes only minor increases in resonant frequency (Weston, 1967).

Carapus acus

Five *Carapus acus* Ratinesque (Brünnich 1768) ($TL=8-12$ cm) were found in specimens of *Holothuria tubulosa* obtained in front of the STA.RE.SO station (Calvi Bay, Corsica) in April 2004. Fish were stored in a community tank (0.6 m×0.6 m×0.4 m) with running seawater at 19°C. Recordings were made in a smaller tank (0.4 m×0.4 m×0.31 m). These fish were transported to the Aquarium Dubuisson (Liège, Belgium) where their sonic muscles were stimulated electrically.

The five fish were deeply anaesthetised with MS 222 (500 mg l⁻¹) and rapidly dissected to expose the sonic mechanism. The right and left ventral sonic muscles were ligatured together at their anterior and posterior ends, and the

posterior ends were tied to a force transducer (P K30 type 351, Hugo Sach Elektronik-Harvard apparatus GmbH, Hugstretten, Germany). The opposite extremity was fixed, respecting the original *in vivo* length of muscles. Both muscles were stimulated simultaneously (0.1 ms pulses of 4 V and frequencies of 2, 5, 6, 8, 10, 13 and 20 Hz) with metal electrodes connected to an electrical stimulator (Stimulator P type 201, Hugo Sach Elektronik-Harvard apparatus GmbH).

Results

Twitch parameters

Stimulation experiments on *Carapus acus* indicate that unlike other fish sonic muscles, the sonic muscle was surprisingly slow: a twitch averaged 490±3 ms (mean ± s.e.m., $N=17$) with contraction and relaxation times of approximately 110 ms and 380 ms, respectively. Temporal summation was evident at 4 Hz, and the muscle exhibited an unfused tetanus at 12–13 Hz (Fig. 2). Rather than exhibiting a typical plateau at the approach of tetanus, muscle force increased exponentially (Fig. 3). Moreover, the wave summation appears to be irregular. During a stimulus train, force increased rapidly, leveled off and then increased again with a lower slope to a peak. The facilitated force during tetanic stimulation could be caused by recruiting myofibrils in two phases (13 Hz and 20 Hz, Fig. 2). This capability could be due to the helical organization of the sonic muscles fibres and myofibrils that allow the twisted and straight myofibrils to contract at different moments.

Reexamination of the (functional) morphology

The swimbladder plate is intimately coupled to the fenestra in *Carapus boraborensis*; the posterior half of the plate forms a semicircular seal with the bladder surrounding the fenestra leaving the anterior edge free. The plate decreases in thickness from about 800 µm anteriomedially to <20 µm posteriorly (Fig. 1A), and therefore rapid movement of the fenestra is likely to excite vibrations, particularly in the posterior region of the swimbladder plate.

The sonic muscle terminates in a complex tendon, which includes a 'hook' that fits over a tubercle on the dorsoanterior wall of the swimbladder (Fig. 4). Tendons of the right and left sonic muscles are connected by connective tissue within the anterior wall of the swimbladder. At rest, the hook is anchored over the bladder tubercle (Fig. 5). Muscle contraction pulls the anterior part of the swimbladder forward stretching the swimbladder fenestra, particularly at its forward end (Fig. 5). The caudal part of the bladder does not appear to move because it is decoupled from the anterior wall by the fenestra. Additionally, it is attached to the posterior margin of the third epineural rib, and its dorsal surface is firmly attached to the ventral face of the vertebrae. Contraction continues until the hook releases the tubercle allowing the anterior swimbladder and fenestra to snap back to their resting positions. Apparently guided by attached connective tissue, the hook returns to its locked position on the tubercle during muscle relaxation.

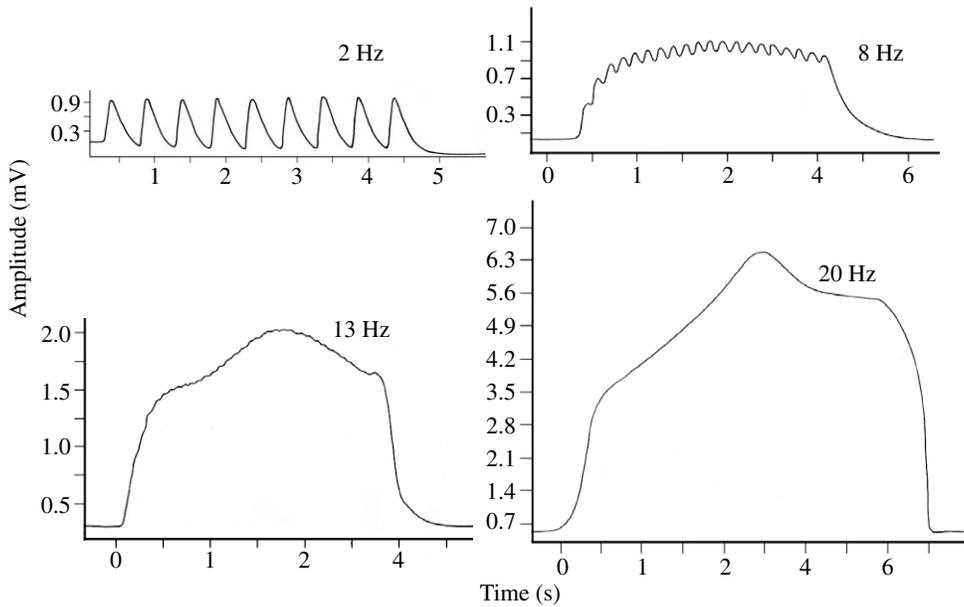


Fig. 2. Contraction of *Carapus acus* sonic muscle stimulated at 2, 8, 13 and 20 Hz.

Slowly pulling the sonic muscles with forceps in deeply anesthetized specimens was sufficient to generate sound pulses (Fig. 6). The sound was produced during the manual extension when the hook released the tubercle, and the swimbladder snapped back to its resting position (Fig. 5). Therefore, the contraction speed is likely not important in generating a sound pulse; the role of the muscle is to provoke the decoupling of the hook anchored on the swimbladder. Manually generated sounds exhibited many similarities to voluntary sounds although they were shorter (pulse duration: 30.9 ± 2.6 ms, $N=37$) than voluntary sounds (pulse duration: 73.5 ± 0.05 ms, $N=24$) ($t=13.40$, d.f.=59, $P<0.0001$) and exhibited a slow exponential decay not previously seen in fish swimbladder sounds. Manually generated sounds had between 7 and 14 peaks compared to around 15 for natural sounds, but amplitude of the voluntary sound maintained a steadier level without the exponential decay. Sound onset was rapid with several short duration high frequency cycles in both cases. After the initial onset, the periods between cycles became steadier but still varied in duration, particularly in the voluntary sounds (Fig. 7). The high frequency onset of the pulse could result from the immediate recoil of the fenestra, and the variable pulse durations from a complex interaction of the fenestra with the swimbladder plate.

Calculation of the resonant frequency of the bladder

Peak frequency of voluntary sounds was between 80 and 203 Hz. Voluntary sounds were not compared against fish size since they are made by at least two unseen fish within the body cavity of a sea cucumber. Peak frequency of manually generated sounds in a 50 mm deep vessel of seawater decreased over an octave ($r^2=0.28$, $P<0.001$) from almost

500 Hz in a 19.7 mm TL female with a 25.3 mm long bladder to about 170 Hz in a 25.2 mm female with a 38 mm bladder (Fig. 8). These values were compared with the resonant frequency calculated for the radius of swimbladders of an equivalent volume (Weston, 1967). The calculated resonant frequency values were about twice the measured values. This finding strongly suggests that sound frequency is not determined by the natural frequency of the swimbladder. Since the swimbladder is more likely to radiate sounds with its lateral than its antero-posterior surfaces; a calculated frequency from swimbladder radius, instead of equivalent radius, would make this discrepancy

even greater. Voluntary *Carapus boraborensis* sounds were more sharply tuned ($Q_{3dB}=15$, $N=25$; Q_{3dB} =quotient of the dominant frequency divided by the bandwidth 3 dB down from the peak frequency) than manually generated sounds (4.3 ± 0.3 ; $P<0.001$), but it is likely that opening the fish could have changed its vibratory properties.

Discussion

Historically, teleost swimbladders have been considered resonant bubbles damped by surrounding fish tissue (Weston, 1967; Sand and Hawkins, 1973; Bradbury and Vehrenkamp, 1998). An alternative explanation is that swimbladders are inefficient low Q sources with minimal dependence on resonance because of rapid damping by the swimbladder wall (Fine et al., 2001; Connaughton et al., 2002). In this view, rapid speed in fish sonic muscles is necessary because sound frequency is determined as a forced response to muscle contraction. As a result, the rate of sonic muscle contraction

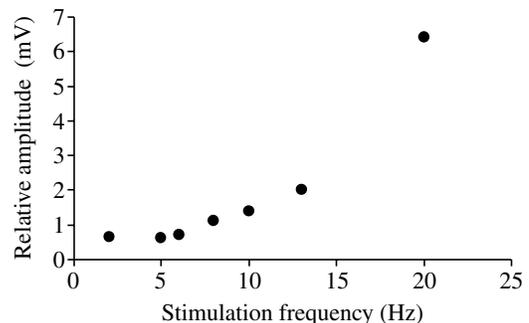


Fig. 3. Amplitude of sonic muscle contractions at different stimulation frequencies in *Carapus acus*.

determines fundamental frequency in long duration calls (Fine et al., 2001; Connaughton, 2004). In the oyster toadfish, for instance, a 250 Hz contraction rate generates a sound with a 250 Hz fundamental frequency, and slow bladder movements do not generate sound (Barimo and Fine, 1998; Fine et al., 2001). Sonic muscles of *Bagre marinus*, *Galeichthys felis*, *Prionotus scitiulus*, *Prionotus carolinus*, *Epinephalus guttatus* and *Holocentrus* sp. have been observed to tetanize between 150 and 400 Hz (Tavolga, 1962; Tavolga, 1964; Gainer et al., 1965; Connaughton, 2004). In weakfish, which produce each sound pulse with a single muscle twitch, sound frequency similarly appears to relate to the timing of bladder movement forced by the sonic muscle contraction–relaxation cycle (Connaughton et al., 1997; Connaughton et al., 2002).

The carapid mechanism uniquely does not utilize fast-contracting muscles, depending instead on a mechanical decoupling. Carapid fishes drive the bladder with a slow muscle that puts the anterior bladder under tension and then trips a release system that excites sound production. The muscle contraction rate is dramatically slower than in other drumming muscles. The average dominant frequency of the sounds in five different carapid species varies between 40 and 340 Hz (Parmentier et al., 2003a; Parmentier et al., 2006; Lagardère et al., 2005). In *C. acus*, the dominant frequency of 340 Hz in each isolated pulse (Parmentier et al., 2006) does not correspond to the contraction rate of its sonic muscle, which tetanizes in the vicinity of 10 Hz. Moreover, the manually generated sound clearly demonstrates that the sound does not depend on the contraction–relaxation cycle speed. On the other hand, the pulse period was between 4.5 and 6.9 Hz in *C. boraborensis* (Parmentier et al., 2003a; Lagardère et al., 2005), which likely corresponds to the muscle contraction rate. In this case, the contraction rate of the muscle does not determine the main frequency of the pulse but the pulse period. In the

Ophidiiforme *Ophidion marginatum*, sounds are composed of 1–27 pulses with a peak frequency of 1200 Hz (Mann et al., 1997; Sprague and Luczkovich, 2001), and a contraction–relaxation cycle at this rate would be physiologically impossible. However, the pulse period is about 23 Hz, reinforcing the assumption that, in this group, there is not a correspondence between sonic muscle contraction and main sound frequency, but with the pulse period.

In carapids, calculations of the resonant frequency of the swimbladder indicate that swimbladder resonance does not explain the recorded frequencies (Fig. 7). The peak frequency is lower than the calculated swimbladder resonant frequency, but it is higher than dictated by the timing of a muscle twitch. Therefore, some other factor must be driving the swimbladder. Cicada insects produce sound by rapid buckling of a pair of

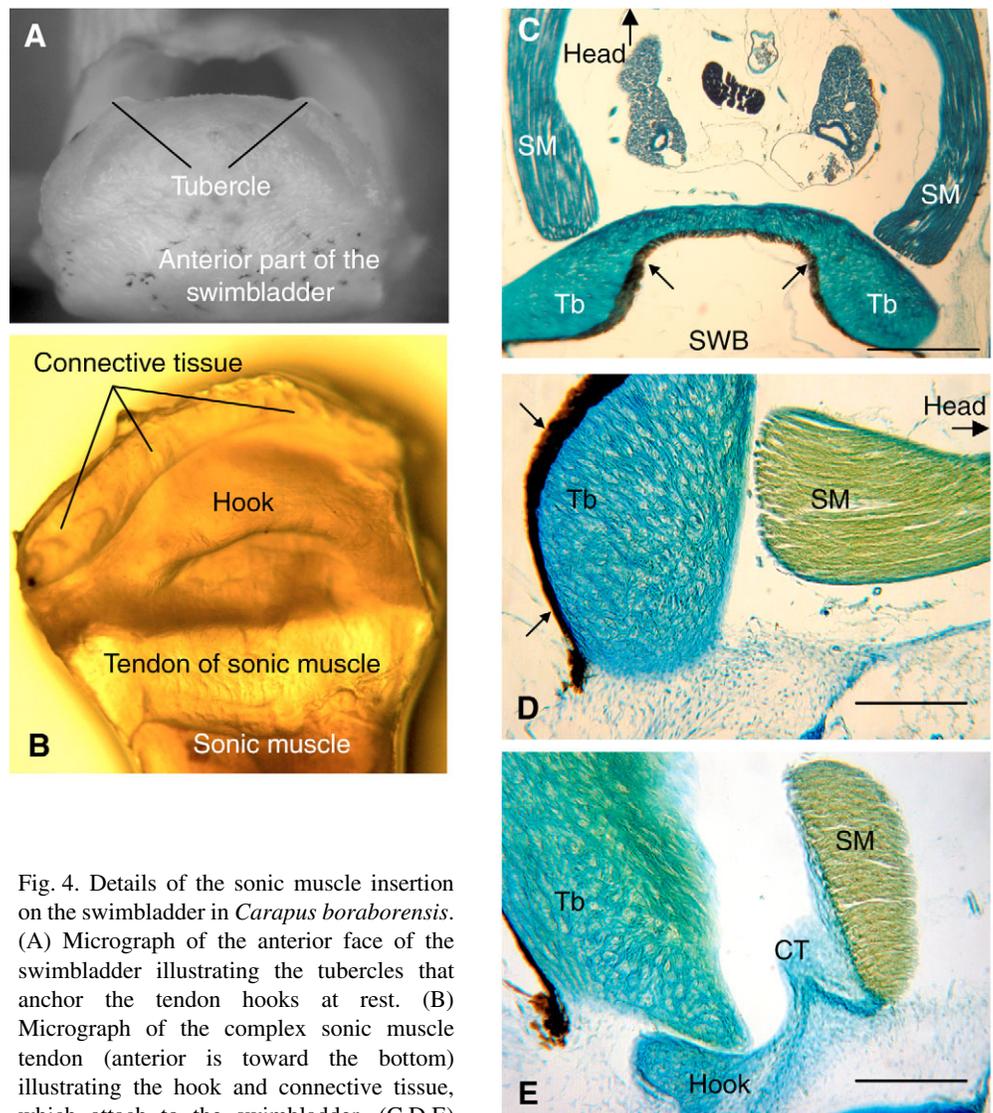


Fig. 4. Details of the sonic muscle insertion on the swimbladder in *Carapus boraborensis*. (A) Micrograph of the anterior face of the swimbladder illustrating the tubercles that anchor the tendon hooks at rest. (B) Micrograph of the complex sonic muscle tendon (anterior is toward the bottom) illustrating the hook and connective tissue, which attach to the swimbladder. (C,D,E) Frontal sections of the anterior part of the swimbladder illustrating the tubercles that anchor the tendon hooks. CT, connective tissue; SWB, swimbladder; Tb, tubercule; black arrows, tissue of the swimbladder fenestra. Scale bars, 50 μ m.

domed tymbals situated on the sides of the first abdominal segment (Pringle, 1954). This sound-producing system acts as a frequency multiplier that converts the 117 Hz contraction frequency of each of the paired tymbal muscles into the 4.3 kHz frequency of the insect's song (Bennet-Clark, 1997; Bennet-Clark and Daws, 1999). In these insects, transduction of muscle energy into sound energy is a two-stage process. Stage one uses muscle power to drive a small mechanical

resonator (=the primary resonator), which acts as the frequency-multiplier that determines the song frequency (Bennet-Clark, 1999). This motion may then drive a second, larger acoustic resonator, from which the sound is radiated. This second resonator was modelled in insects as a Helmholtz resonator (Young, 1990; Bennet-Clark and Young, 1992), which consists of a cavity open to the outside *via* a hole that has a real or notional neck (Bennet-Clark and Young, 1994).

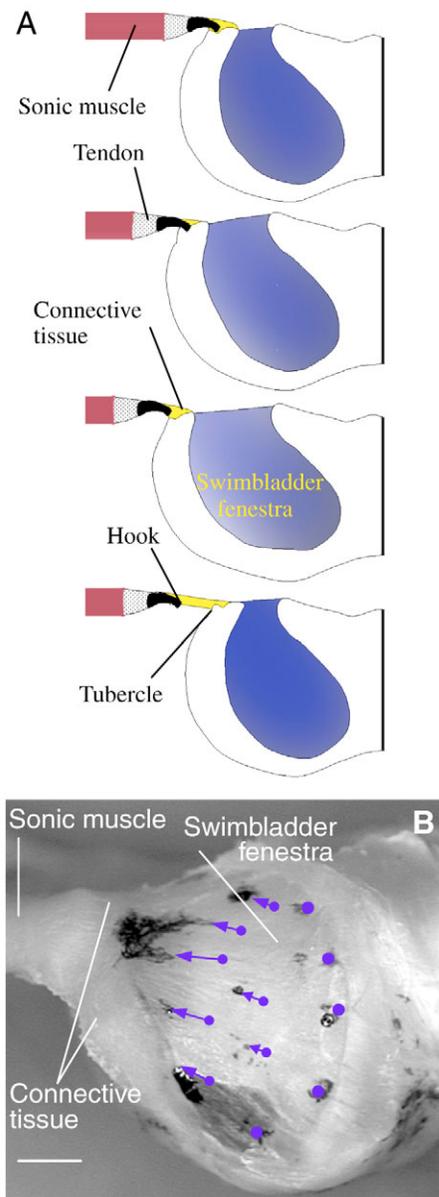


Fig. 5. (A) Diagram of sound pulse generation progressing in time from top to bottom. Contraction or pulling on the sonic muscle in a fresh specimen extends the anterior bladder and swimbladder fenestra. In the third panel the hook has disengaged from the bladder tubercle, and the system then snaps back to its resting state generating sound. (B) Left lateral view of the anterior part of the swimbladder. Blue arrows represent the amplitude of the displacement of different points of the swimbladder fenestra when stretched.

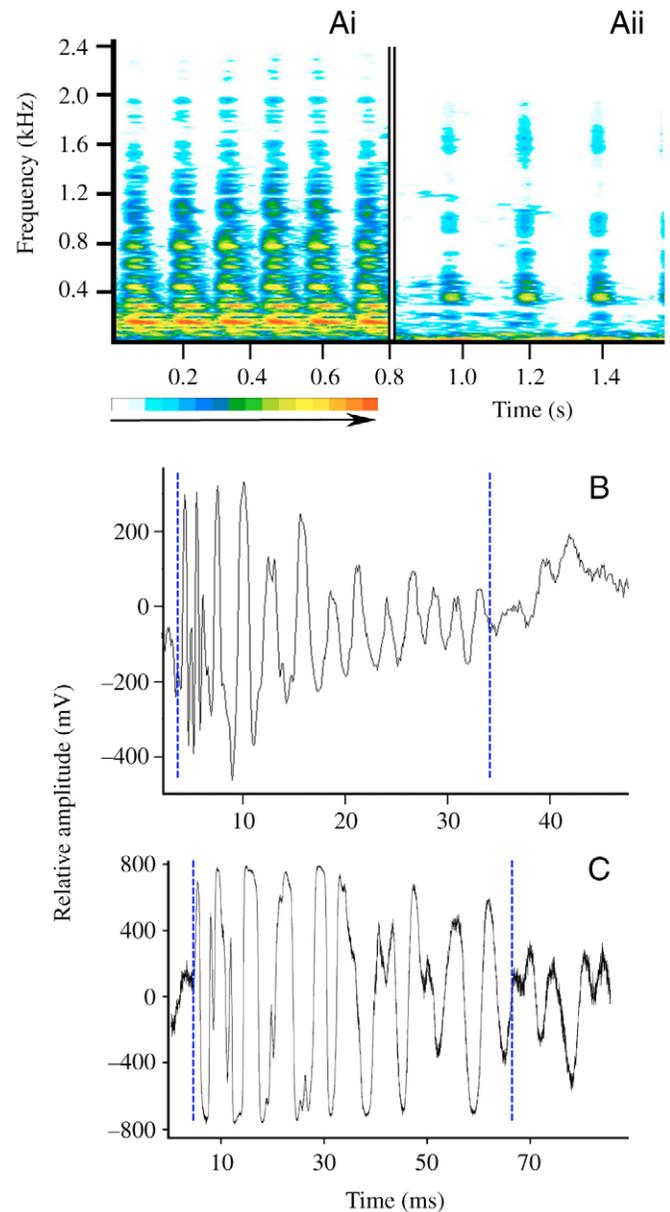


Fig. 6. Voluntary and manually induced sound pulses in *Carapus boraborensis*. Sonogram of 6 voluntary pulses (Ai) produced by a fish within a sea cucumber and 3 pulses (Aii) produced by 3 pulls on the sonic muscle of a fish with its sonic apparatus exposed. Oscillograms of a single pulse produced voluntarily (B) and by manual manipulation (C). Broken lines indicate termination of the pulse. The color ramp in A shows the scale of the relative dB, from white (lowest) to red (highest).

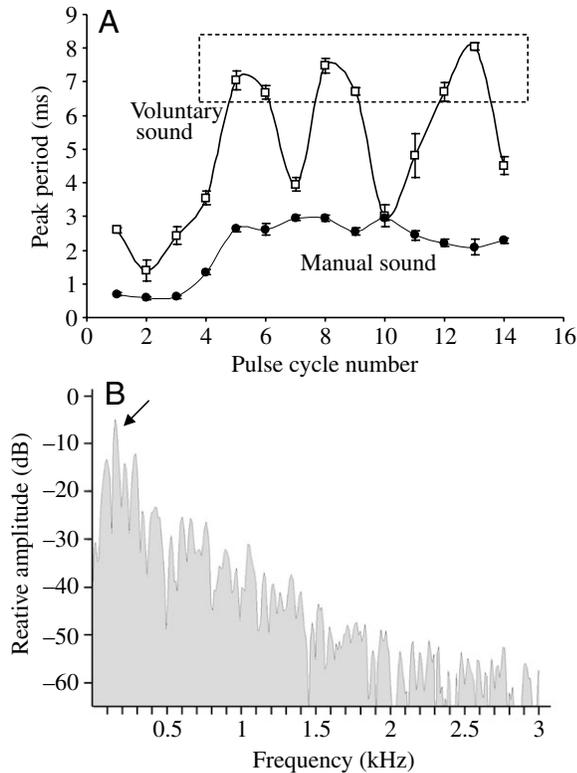


Fig. 7. (A) Periods of successive peaks in the voluntary (open squares) and manually induced (filled circles) sound pulses in *Carapus boraborensis*. ($N=25$ in both cases). (B) FFT of voluntary sound in *Carapus boraborensis*. The peak frequency (black arrow) corresponds to the part of the waveform within the rectangular broken line in A above.

Carapid sound production presents analogies with the insect process.

The mechanical (=primary) resonator can correspond to the mechanical decoupling, allowing the release of strain energy built up in the fenestra by slow muscle contraction to be converted into acoustic energy. Movements of the anterior edge and fenestra of the swimbladder appear to be decoupled from the remainder of the bladder, which is rigidly attached to the vertebral column. *Carapus boraborensis* sounds are sharply tuned; a Q_{3dB} of 15 is considerably higher than values recorded for typical bladder sounds: it is 1.9 in the searobin (*Prionotus carolinus*), 1.45 in the toadfish (*Opsanus tau*), 0.89 in *Galeichthys felis* and 0.33 in *Bagre marinus*, and 3 in the croaker (*Micropogonius undulates*) (Connaughton, 2004; Fine et al., 2004). Therefore, given the sharp tuning of carapid calls and their slow muscles, we hypothesize that carapid sounds are generated by a rapid release of the fenestra, which sets the swimbladder plate into motion; energy from plate motion would then be transferred through the fenestra and excite the bladder to produce sound. This forced vibration is possible due to the modifications in the thickness of the swimbladder plate. The plate is thicker at the anterior part with around 800 μm near its point of articulation on the third vertebra, and it

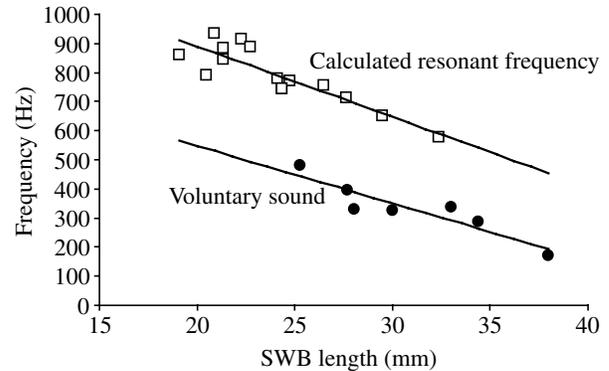


Fig. 8. Peak frequency of manually generated sounds (filled circles) and calculated resonant frequency (open squares) in a 5 cm deep vessel of seawater.

becomes progressively thinner toward the posterior part; the posterior region of the swimbladder plate is only about 20 μm at its connection with the swimbladder fenestra.

Despite numerous similarities between voluntary and manually generated sounds, differences exist mainly in pulse duration and in Q_{3dB} . These differences could be due to tanks that can be subject to various artifacts (Akamatsu et al., 2002; Parvulescu, 1964), to differences in acoustic loading that would occur with depth (Fine et al., 2004) or to experimental manipulations: opening the fish could change the vibratory coupling of the fenestra and swimbladder plate. Additionally, a second pair of sonic muscles, the secondary sonic muscles (Courtenay and McKittrick, 1970), could also influence sound production. These muscles originate on the posterior part of the skull and insert on the two first epipleural ribs (Fig. 1A), which are connected to the anterior part of the swim bladder by ligaments. According to Parmentier et al., the contraction of these muscles causes forward and outward displacement of the posterior tips of the epipleural ribs, stretching the swimbladder and making it more rigid (Parmentier et al., 2003a). The increased stiffness of the swimbladder should help to sustain the sound. These muscles were not tensed in the manually generated sounds.

This study demonstrates for the first time that rapid muscle speed is not required to generate sounds in a teleost fish. However, carapid sonic muscles have characters of both red and white fibres (Parmentier et al., 2003b) as is the case in fast sonic muscles (Ladich and Fine, 2006; Parmentier and Diogo, 2006). It appears that these sets of characters should be carefully interpreted.

Superfast muscles generate low forces (Rome et al., 1999). The production of high forces by 'superslow' muscles is not yet shown. An important feature lies in the unique myofibrillar helical organisation providing the muscle with spring-like mechanical properties (Parmentier et al., 2003b). The uncoiling of the helix during relaxation should help in the lengthening of the muscle during relaxation and may aid in the recoupling between the hook of the muscle tendon and the tubercle of the swimbladder.

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