

RESEARCH ARTICLE

Sound production and mechanism in *Heniochus chrysostomus* (Chaetodontidae)

Eric Parmentier^{1,*}, Kelly S. Boyle^{2,3}, Laetitia Berten¹, Christophe Brié⁴ and David Lecchini⁵

¹Laboratoire de Morphologie Fonctionnelle et Evolutive, Institut de Chimie, Bât. B6C, Université de Liège, B-4000 Liège, Belgium, ²Department of Zoology, University of Hawai'i at Manoa, 2538 McCarthy Mall, Honolulu, HI 96822, USA, ³Hawai'i Institute of Marine Biology, 46-007 Lilipuna Road, Kane'ohe, HI 96744, USA, ⁴Tropical Fish Tahiti, Avatoru, 98729 Rangiroa, French Polynesia and ⁵CRILOBE, USR 3278 – CNRS / EPHE, Centre de Recherche Insulaire et Observatoire de l'Environnement, CBETM – Université de Perpignan, BP 1013 Moorea, French Polynesia

*Author for correspondence (e.parmentier@ulg.ac.be)

Accepted 12 May 2011

SUMMARY

The diversity in calls and sonic mechanisms appears to be important in Chaetodontidae. Calls in *Chaetodon multicinctus* seem to include tail slap, jump, pelvic fin flick and dorsal–anal fin erection behaviors. Pulsatile sounds are associated with dorsal elevation of the head, anterior extension of the ventral pectoral girdle and dorsal elevation of the caudal skeleton in *Forcipiger flavissimus*. In *Hemitaurichthys polylepis*, extrinsic swimbladder muscles could be involved in sounds originating from the swimbladder and correspond to the inward buckling of tissues situated dorsally in front of the swimbladder. These examples suggest that this mode of communication could be present in other members of the family. Sounds made by the pennant bannerfish (*Heniochus chrysostomus*) were recorded for the first time on coral reefs and when fish were hand held. In hand-held fishes, three types of calls were recorded: isolated pulses (51%), trains of four to 11 pulses (19%) and trains preceded by an isolated pulse (29%). Call frequencies were harmonic and had a fundamental frequency between 130 and 180 Hz. The fundamental frequency, sound amplitude and sound duration were not related to fish size. Data from morphology, sound analysis and electromyography recordings highlight that the calls are made by extrinsic sonic drumming muscles in association with the articulated bones of the ribcage. The pennant bannerfish system differs from other Chaetodontidae in terms of sound characteristics, associated body movements and, consequently, mechanism.

Key words: acoustic, electromyography, sonic muscle, swimbladder.

INTRODUCTION

In teleosts, the ability to emit sounds was developed independently in distant phylogenetic taxa (Hawkins, 1993; Ladich, 2001). Different types of sonic mechanisms may be listed in fishes but there exists no commonly accepted classification of sound-generating (sonic) mechanisms in fishes (Ladich and Fine, 2006). Among the various mechanisms, swimbladders can be utilized in various ways in sound production. Swimbladder muscles for sound production are classified as either intrinsic or extrinsic on the basis of their association with the swimbladder (Tavolga, 1971). Intrinsic sonic muscles completely attach to the wall of the swimbladder, as in the Batrachoididae (Demski et al., 1973; Bass and Marchaterre, 1989; Rice and Bass, 2009) and the Triglidae (Evans, 1973; Hawkins and Myrberg, 1983; Connaughton, 2004). Extrinsic sonic muscles have various origins and insertions (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; Sprague, 2000). 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

(Connaughton et al., 2000; Fine et al., 2001). Recently, slow sonic swimbladder muscles have been discovered in carapids (Parmentier et al., 2003; Parmentier et al., 2006a) and appear to occur in various ophidiiform fishes (e.g. Fine et al., 2007; Nguyen et al., 2008; Parmentier et al., 2006b). The muscles produce sounds using a different principle, namely a mechanical decoupling in which the slowly stretched swimbladder is released and snaps back to its resting position (Parmentier et al., 2006a; Parmentier et al., 2010).

Many fish families (Gobiidae, Pomacentridae, Labridae, Holocentridae, Ariidae, Ostraciidae, Carapidae, Scaridae, Serranidae, Balistidae, Haemulidae, etc.) living on coral reefs include at least some species known to make sounds for communication (see Ladich and Fine, 2006). However, few published accounts of sound production in butterflyfishes exist. The Chaetodontidae (approximately 122 species) are social inhabitants of coral reefs living often in pairs or small shoals (Helfman et al., 2009). Members of the genus *Chaetodon* have swimbladder horns and a morphological feature known as the 'laterophysic connection', which may be a unique vertebrate adaptation for processing acoustic stimuli (Tricas et al., 2006; Webb et al., 2006), suggesting that acoustic stimuli may be used during behavioral interactions. Calls were recently recorded in *Chaetodon multicinctus* (Tricas et al., 2006), *Forcipiger flavissimus* (Boyle and Tricas, 2009) and *Hemitaurichthys polylepis* (Boyle and Tricas, 2010), suggesting that this mode of communication could be present in other members of the family. Moreover, these studies seem to indicate that sound

production mechanisms may be quite variable within the butterflyfish family and may have evolved independently within the family (Boyle and Tricas, 2010). In *C. multinctus*, territory defence includes the production of agonistic sounds and hydrodynamic stimuli that are associated with tail slap, jump, pelvic fin flick and dorsal–anal fin erection behaviors. The interpretation of the communicative nature of these sounds requires, however, some caution because they could be byproduct of swimming behaviours. In addition, grunt pulse trains were tentatively interpreted to function as an alert call among pair mates in *C. multinctus* (Tricas et al., 2006). In a second species, *F. flavissimus*, pulsatile sounds are associated with dorsal elevation of the head, anterior extension of the ventral pectoral girdle and dorsal elevation of the caudal skeleton (Boyle and Tricas, 2009), but the exact mechanism remains unknown. During sound production in *H. polylepis*, a strong relationship was found between the production of each pulse and the inward buckling of tissues situated dorsally in front of the swimbladder, showing that extrinsic swimbladder muscles could be involved in sounds originating from the swimbladder (Boyle and Tricas, 2010).

In this study we describe for the first time some sounds made by the threeband pennant butterflyfish *Heniochus chrysostomus* Cuvier 1831 in French Polynesia. These sounds were recorded from fish hand held in the laboratory and from fish in a lagoon. On the basis of the sonic data and electromyography (EMG), the mechanism of sound production is also proposed.

MATERIALS AND METHODS

Five specimens of *H. chrysostomus* (standard length: 112–134 mm) were caught with the help of a seine net (4 cm mesh) at 1 m depth in the lagoon of Rangiroa, French Polynesia (14°56'S, 147°42'W), during May 2008. Fish were placed in a 400 l tank (1.40×0.7×0.4 m) with running seawater (28°C).

In order to elicit sound emission, fish were held in the hand with the fins and the spines blocked along the body. They were placed at a distance of 5 cm from the hydrophone. This recording method was chosen in order to elicit sounds from the same behavioral context and to ensure that sounds would be produced at the same distance to the hydrophone in order to account for differences in signal loss. Sounds were recorded with an ORCA hydrophone (sensitivity: –186 dBV μPa^{-1} ; flat frequency response range between 10 Hz and 23.8 kHz) connected via an ORCA amplifier (ORCA Instrumentation, Brest, France) to a Tascam HD-P2 stereo audio recorder (recording bandwidth: 20 Hz to 20 kHz; Montebello, CA, USA). Sounds were digitized at 44.1 kHz (16 bit resolution) and analysed with Avisoft-SASLab Pro 4.33 software (Avisoft Bioacoustics, Berlin, Germany). Low-pass filters were not applied during sound analysis because the resonant frequency of the tank was estimated to be 2.35 kHz (Akamatsu et al., 2002).

Some sounds of *H. chrysostomus* were recorded in the lagoon of Moorea (French Polynesia) while six specimens were gathered below a coral patch. Recordings of sound production were made using a SONY HDD video camera placed in a housing (HC3 series, Ocean Images, Cape Coral, FL, USA) and coupled with an external hydrophone (High Tech. Inc., Gulfport, MS, USA) with a flat response of 20 Hz to 20 kHz and a nominal calibration of –164 dBV μPa^{-1} (Loggerhead Instruments Inc., Sarasota, FL, USA).

The following features were measured from sound recordings: sound duration (ms; duration from the beginning of the first pulse to the end of the last pulse), number of pulses in a series, pulse duration (time interval between the onset of one pulse and its end), pulse interval (time interval between the end of one pulse and the

beginning of the next), pulse period (measured as the mean peak-to-peak interval between consecutive pulse units in a series) and dominant frequency (Hz). Temporal features were measured from the oscillograms, and frequency parameters were obtained from power spectra.

EMG experiments followed the methods described in Boyle and Tricas (Boyle and Tricas, 2010). Briefly, contraction activity of the obliquus superior hypaxial musculature was determined by EMG recordings in three (42–59 mm standard length) free-swimming subjects in a 110 l aquarium filled to approximately 40% capacity. Bipolar recording electrodes, made from pairs of 0.05 mm insulated tungsten wire (California Fine Wire, Grover Beach, CA, USA) with insulation removed at the tip (1 mm) and bent back to form hooks, were inserted with 28 gauge hypodermic needles. Fish were anesthetized with 100 mg l⁻¹ of tricaine methanesulfonate (MS-222, Argent Labs, Redmond, WA, USA) and ventilated with seawater and anesthetic solution while electrodes were implanted and the hypodermic needle tips were removed. EMG electrodes were placed in the left and right sides in the white bar posterior to the pectoral girdle, at the same height as the lower part of the eye. A loop of surgical silk was inserted in the dorsal trunk musculature, tied and glued with cyanoacrylate around both electrodes for strain relief and to prevent electrode dislodgement. EMGs were amplified with a differential amplifier (AM Systems, Sequim, WA, USA) with 10,000× gain, band-pass filtered between 100 and 5000 Hz, with a 60 Hz notch filter.

During EMG experiments, sounds were measured with a Brüel and Kjær 8103 hydrophone (sensitivity: –211 dBV μPa^{-1} ; Nærum, Denmark) positioned ~3 cm from the end of the aquarium and amplified with a Nexus conditioning amplifier (gain of 31.6 mV Pa^{-1} ; Nærum, Denmark), and fish movements were recorded with a Casio Ex-F1 Exilim camera at 300 and 600 frames s⁻¹. Sounds and EMGs recorded simultaneously from one or two recording sites were digitized with a CED Power 1401 life science data acquisition system with Spike 2 software (Cambridge Electronic Design, Cambridge, UK) at 10 kHz. Video segments were synchronized with sound and EMG recordings with a flasher circuit in which LEDs were recorded by the camera and square pulses were recorded on the hydrophone channel in Spike 2.

In addition, muscles were stimulated with square pulse stimuli generated from Spike 2 software and a CED box connected to a BAK BSI-2 constant current stimulus isolator (BAK Electronics, Inc., Mount Airy, MD, USA). EMGs were first recorded, and then fish were euthanized with an overdose of MS-222, preserved in 10% formalin for 10 days and then transferred to 70% alcohol. They were dissected and examined with a Wild M10 (Leica Camera, Solms, Germany) binocular microscope equipped with a camera lucida to assist in the illustration process. Obliquus superioris hypaxial muscles were then stimulated through the EMG electrodes. Stimulation consisted of sets of seven pulses (each lasting 5 ms) at different frequencies (10, 30, 70, 100 and 120 Hz) at different current amplitudes (200, 300, 400, 900, 950 and 1000 μA) and across a voltage range (5–90 V).

Data are presented as means \pm s.e.m.

RESULTS

Sounds

Sonic characters recorded in tanks result from the analysis of 801 calls and 1098 pulses ($N=5$ fish). *Heniochus chrysostomus* calls (Fig. 1) consisted of different combinations of isolated pulses (51%) and pulsed trains (18.9%). Moreover, trains were preceded by a single pulse (Fig. 1B) in 29.1% of the calls. For this combination,

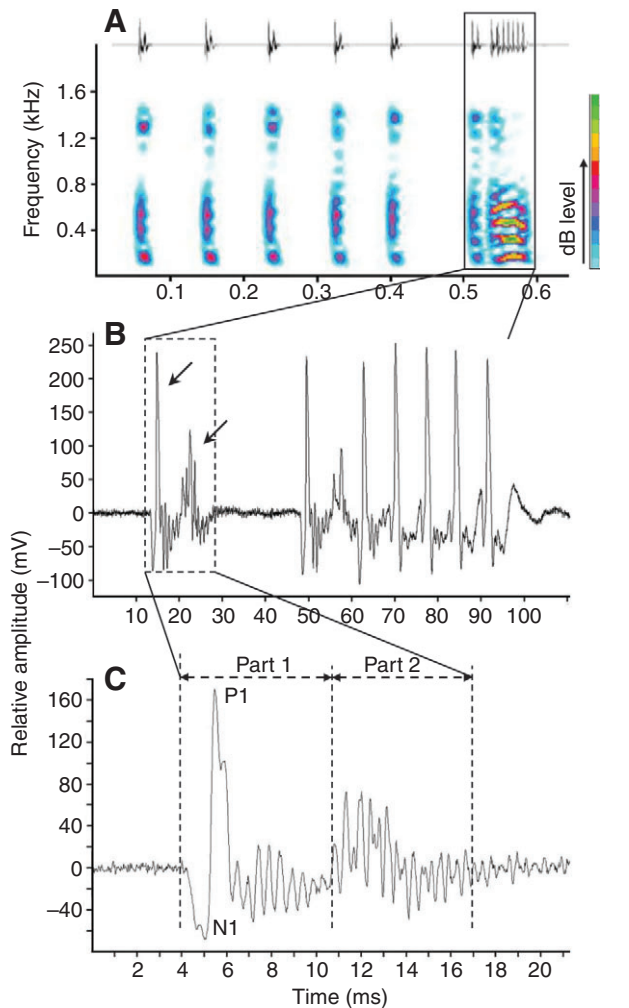


Fig. 1. Oscillograms of sounds produced by hand-held *Heniochus chrysostomus* with two successive enlargements to highlight the pulse waveforms. (A) Five isolated pulses and one combination with one isolated pulse and one train of six pulses; the combination is enlarged in B. The bottom part of A also shows a spectrogram of the corresponding top oscillogram. The different horizontal bands show the different harmonics of the call. (C) Detailed view of one pulse. P1, positive peak; N1, negative peak. Arrows indicate the two parts of the pulse.

the time period between the isolated pulse and the train was 36 ± 1.6 ms ($N=35$). Trains were made of four to 11 pulses (mean = 6.68 ± 0.17 pulses) lasting between 33 and 103 ms (mean = 56.6 ± 1 ms, $N=140$). The number of pulses in a call was not related to the fish size ($P=0.78$).

All pulses had the same structure and were made of two parts (Fig. 1C). Each pulse waveform presented a small negative peak (N1) preceding a larger positive one (P1). This waveform is then followed by a set of decaying peaks (Fig. 1C). Duration of decaying pulses varied because it was punctuated and masked by successive pulses (Fig. 1). In isolated pulses, the first part (part 1) was followed by a second set of smaller peaks (part 2; Fig. 1). The recording of sound in aquaria may present pitfalls because sounds can be reflected by tank walls, which generate additional peaks. Sound reflections can be recognized by more energetic peaks that appear predictably in time among the successive decaying peaks that follow P1. However, the delay of 7 ms between the first and the second

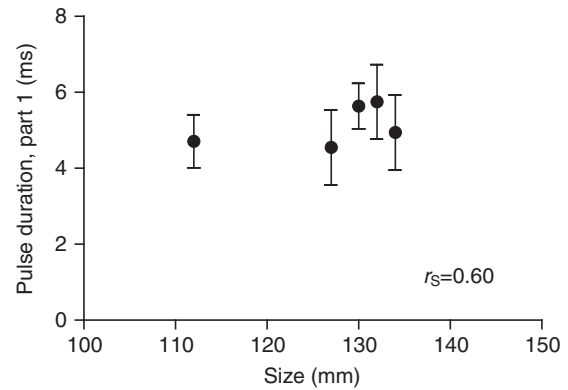


Fig. 2. Mean \pm s.d. pulse duration (part 1) according to fish size in calls of five hand-held *Heniochus chrysostomus*. r_s , Spearman correlation coefficient.

part of the pulse is too long to be an echo, and the sounds we recorded in the field were also made of two parts, as in the tank.

In the isolated, and thus complete pulses, of the five *H. chrysostomus*, the mean duration of part 1 was 5.5 ± 0.03 ms ($N=275$) and part 1 + part 2 was equivalent to 11.6 ± 0.1 ms ($N=144$). In both cases, the durations were not significantly related with fish size (Fig. 2). The fundamental frequency was between 130 and 145 Hz and showed two to five harmonics (Fig. 1A). The fundamental frequency was not related to fish size ($P=0.35$).

In the pulse train, the mean pulse period was 7.96 ± 0.06 ms ($N=821$) or 125 Hz. However, the period was not constant. The pulse rate increased to a maximum (pulse period decreased to a minimum) before decreasing towards the end of the call (Fig. 3) and pulse period was correlated with pulse position within a pulse train ($r_s=0.77$). The relative amplitude (dB) of pulses in a call was not related to fish size ($P=0.93$).

A few sounds of *H. chrysostomus* were also recorded in the field in the lagoon of Moorea when six specimens were gathered below a coral patch (Fig. 4). Our short recording sample (10 min) did not allow us to precisely describe the behaviour(s) associated with the sounds. These sounds seemed to be emitted when a specimen chased a conspecific. The fish produced isolated pulses and trains of three to five pulses. The pulse duration was in the same range as recordings made in the tank (part 1 = 6.1 ± 0.1 ms, $N=22$; part 1 + part 2 = 11.9 ± 0.3 ms, $N=18$). The mean fundamental frequency (\pm s.d.) was 150 ± 11 Hz ($N=7$) and multiple harmonics were present.

Morphology

When fishes were hand held during laboratory sound production, vibration was felt in the region of the flanks behind the pectoral girdle, which aided in location of the sonic muscles.

The swimbladder is a simple sac situated dorsally in the abdominal cavity (Fig. 5). The swimbladder wall is divided into two main regions. Dorsally, the walls are thin and not pressed against the vertebral bodies, whereas the layers are clearly thicker (with a silver coloration) on the lateral and ventral sides. The swimbladder does not have any muscle, ligament or tendon that inserts on its wall. The abdominal cavity is covered by 12 vertebrae showing different types of intermuscular bones. The two first vertebrae have two small articulated epineurals that do not come into contact with the swimbladder, vertebrae 3 to 9 have long articulated ribs and vertebrae 10 to 12 possess parapophyses. Vertebrae 3 to 9 are united by pairs of ligaments at the level of the proximal tips of the ribs

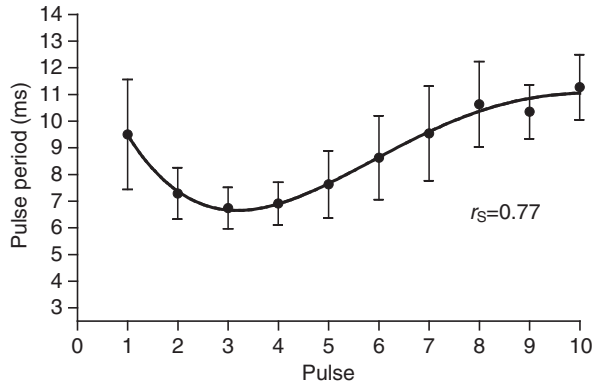


Fig. 3. Pulse period (measured as the mean peak-to-peak interval between consecutive pulse units in a series) in a given call in *Heniochus chrysostomus*. r_s , Spearman correlation coefficient.

(Fig. 5). The lateral wall of the swimbladder is united to the medial surface of intermuscular bones of vertebrae 3 to 12. The upper thinner wall, however, is not attached to the ventral surfaces of the vertebrae.

Two kinds of putative sonic muscles were found in *H. chrysostomus*. An important superficial muscle (obliquus superioris 1 α) is inserted on the skull, at the level of the occipital region and on the rostral part of the third rib (Fig. 5). A second muscle (obliquus superioris 1 β) is thinner and deeper (Fig. 5). It inserts dorsally to the insertion point of Baudelot's ligament and on the rostral part of the third rib. Manual traction of these muscles pulls the third ribs rostrally. This movement can be propagated to the posterior ribs because of the small dorsal ligaments between ribs (Fig. 5).

Another interesting set of muscle fibres is found at the level of the hypaxial musculature, which will not be described in detail here. Medial to the superficial musculature and ventral to the ligaments of the ribs, six massive muscles (=obliquus superioris 2) unite ribs 3 to 9. They can be clearly distinguished from the superficial hypaxial musculature because they are isolated by aponeuroses. These muscles face the thicker part of the swimbladder lateral walls. Dorsal to this zone, the swimbladder is thinner and, ventrally, the remainder of the ribcage is united by connective fibres passing from anteroventral to posterodorsal at an angle of 60 deg. These fibres belong not to the swimbladder but to the somatopleura of the abdominal cavity.

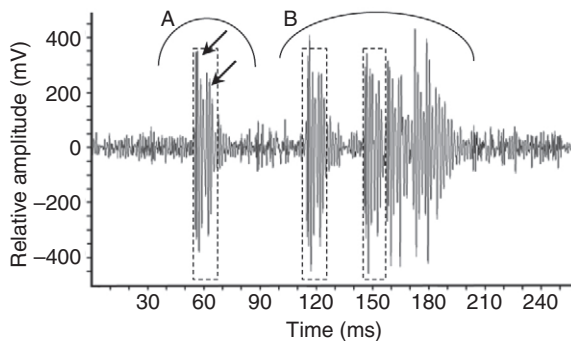


Fig. 4. Oscillograms of sounds produced by *Heniochus chrysostomus* in the field (1 m depth). A corresponds to an isolated pulse and B to a combination. Arrows indicate the two parts of the pulse. Dotted lines correspond to the pulse length.

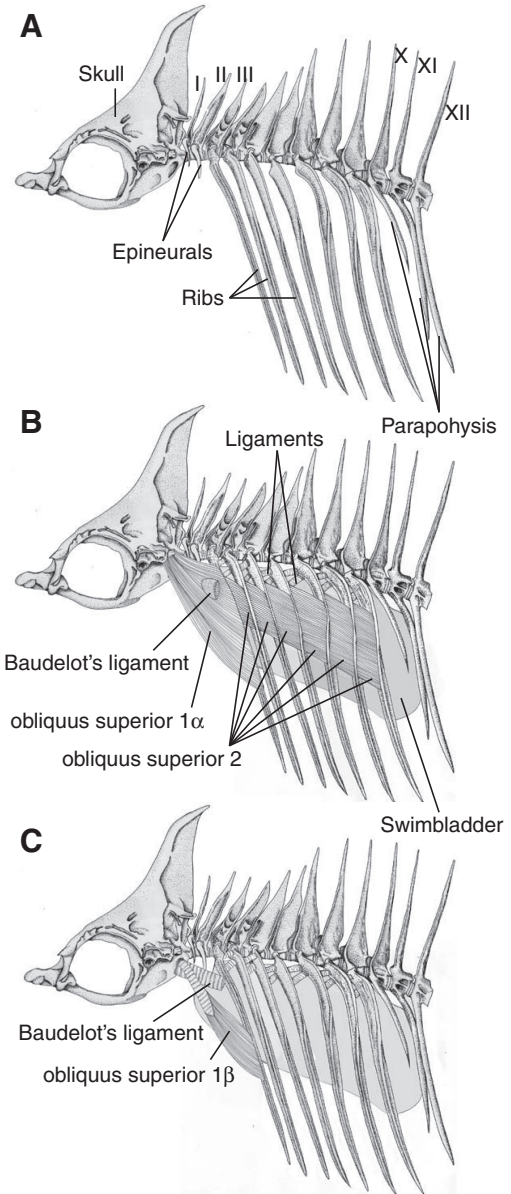


Fig. 5. (A) Left lateral view of the skull and first vertebrae in *Heniochus chrysostomus*. Roman numerals corresponds to vertebrae number. (B,C) The putative sonic muscles, the ligaments between the intermuscular bones and the swimbladder.

EMG and video

EMGs were recorded from electrodes placed in the lateral superficial fibres of the obliquus superioris hypaxial muscle (Fig. 6). Simultaneous sound and EMG recordings revealed a spike prior to each sound pulse (Fig. 6). Muscle firing estimated by EMGs was highly synchronous between the right and left sides of the body. On each side, occurrence of muscle activity started 2 to 3 ms before the onset of sounds. EMGs were characterized by strong amplitude with a short duration (between 3 and 4ms) whereas the pulse duration was approximately 12ms. Moreover, the periodicity of EMG onsets corresponded to the onset of sound pulses, which indicates that muscle contraction is approximately 85 Hz. These data also indicate that the onset of each pulse results from a single muscle contraction, and that a resonating structure is probably used to sustain

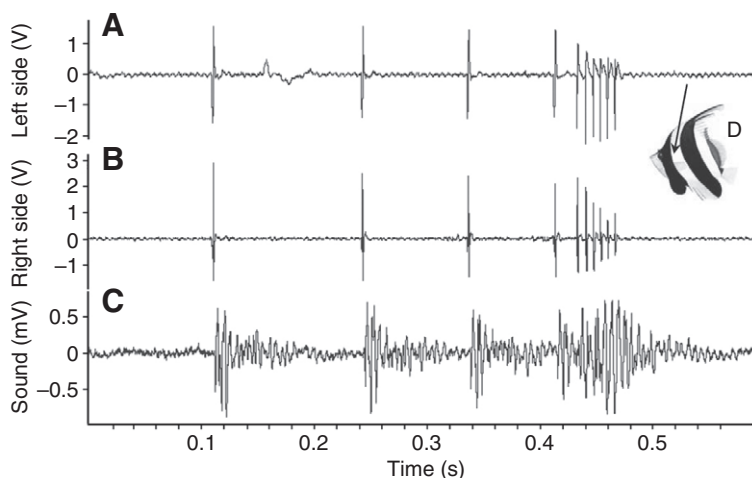


Fig. 6. Electromyography (EMG) recording from the (A) left and (B) right side of *Heniochus chrysostomus*, and (C) corresponding oscillogram of the calls. In isolated pulses and in trains, each pulse corresponds to a muscular contraction. (D) Left lateral view of *Heniochus chrysostomus*. The arrow indicates the position of the recording electrode.

the sound. In some EMG, however, the waveform clearly showed two main peaks with different amplitudes and an interval of *ca.* 7 ms (arrows, Fig. 7). These characteristics were also found in the pulse waveform (arrows, Fig. 2), supporting the hypothesis that the second part of the sound is not an artefact.

Stimulation of the hypaxial musculature, close to the pectoral girdle, induced the production of sounds (Fig. 8). These data indicate that other parts of the body (buccal cavity, fins, etc.) were not involved. However, it is difficult to determine the precise role of individual muscles involved in natural sound production. Differences in the acoustic structure of stimulated and natural sounds could be attributed to differences in the motor pattern or additional muscles involved in sound production.

At 300 and 600 frames⁻¹, a new image is made every 3.33 or 1.66 ms, respectively. Analysis of trains indicated a new pulse appeared every 8 ms, meaning these speeds were appropriate to see movements at the level of the body during sound production. However, no visible movement over the anterior swimbladder region was observed from high-speed video sequences of sound emission, indicating little movement of musculature near the surface of the body.

DISCUSSION

This study provided a first description of the acoustic call in the threeband pennantfish (*H. chrysostomus*). These fish sounds were recorded in the field and were also obtained with fish held in the water while the fins were blocked, ruling out a mechanism involving multiple kinds of swimming movements, as has been hypothesized in *C. multicoloratus* (Tricas et al., 2006). According to

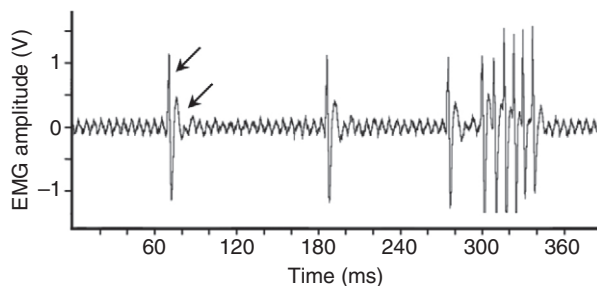


Fig. 7. Muscle activity during sound production by *Heniochus chrysostomus*. EMG recording from the fish's left side. Arrows coincide with the two parts of the pulse (see Fig. 1).

Ladich and Fine, drumming muscles vibrate the swimbladder at high rates (90–250 Hz), which results in emission of low-frequency harmonic sounds in which the contraction rates constitutes the fundamental frequency (Ladich and Fine, 2006). Calls in *H. chrysostomus* are harmonic sounds (Fig. 1A). Moreover, the vibrations at the level of the trunk and the EMG and stimulation data suggest that the calls resulted from the action of sonic muscles associated with the swimbladder. A close analogous organisation of the obliquus superior 1 fibres can be found in *Holocentrus rufus* and *Myripristis berndti*, in which a bilateral pair of extrinsic muscles also originates on the skull and extends across the third vertebra ribs, which are firmly attached to the swimbladder (Winn and Marshall, 1963; Salmon, 1967). Analysis dealing with muscle ablation (Winn and Marshall, 1963) and physiology (Gainer et al., 1965) clearly demonstrated the role of these muscles in sound production in *H. rufus*.

The calls of *H. chrysostomus* also showed common features with some Holocentridae (Moulton, 1958; Winn and Marshall, 1963; Salmon, 1967; Fish and Mowbray, 1970; Parmentier et al., 2011): the call series presented a variable time period between the pulses (Fig. 3), the calls are harmonic, the number and timing of muscle potentials correlated with the number and timing of pulses in the sound (Fig. 6), the onset of the sound corresponded to its bigger peak (Fig. 1), the pulse period is approximately 125 Hz (Fig. 1), and they do not make any external visible movements during sound production. Moreover, some Holocentridae, such as *Neoniphon sammara*, can also present a single isolated pulse in front of the pulsed calls (Parmentier et al., 2011).

Fish drumming muscles are often considered the fastest muscles in vertebrates (Tavolga, 1964). Their cycle time (contraction and relaxation) determines sound fundamental frequency (Fine et al., 2001; Bass and McKibben, 2003; Connaughton, 2004). The fundamental frequency in *H. rufus* was 85 Hz, but drumming muscles are capable of contracting at a frequency of 112 Hz with no mechanical summation (Gainer et al., 1965). In *H. chrysostomus*, the correlation between the potentials and the pulses highlight that these muscles are also able to contract at high speed and that the contractions are synchronous between the right and left sides. These properties were also found in other fish taxa that also have a sound-producing mechanism involving the swimbladder and associated muscles (e.g. Skoglund, 1961; Cohen and Winn, 1967; Connaughton et al., 2000). However, no muscles were found directly on the swimbladder in *H. chrysostomus*, indicating that the mechanism uses extrinsic sonic drumming muscles.

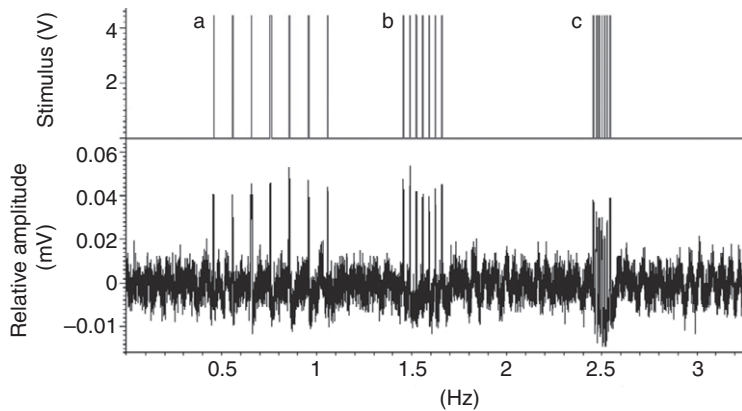


Fig. 8. Stimulation of *Heniochus chrysostomus* muscle (cf. Fig. 1) at 10 Hz (a), 30 Hz (b) and 70 Hz (c), and corresponding oscillogram of the calls. Each pulse corresponds to a muscular stimulation.

The proposed mechanism is based on different morphological observations: the ribs are articulated on the vertebrae and are intimately related laterally to the swimbladder walls. Movements of articulated ribs can provoke volume modifications in the swimbladder. These deformations are possible because of the upper part of the swimbladder, which possesses a thinner wall that is not in contact with the ribs. According to abovementioned acoustic similarities with the Holocentridae, the sonic muscles of *H. chrysostomus* could be the muscles between the skull and the third ribs. Muscle stimulations positively reinforce this assumption. However, there is an important difference between the pulse waveform of Holocentridae and that of *H. chrysostomus*, which possesses decaying peaks after the onset of the main peak and a second set of peaks (part 2, Fig. 1). EMG indicates this additional set of peaks could be due to a second muscle stimulation and/or to a second set of muscles. Intercostal sonic muscles (=obliquus superior 2) were found in the curimatid *Semaprochilodus insignis* (Schaller, 1971) and in the cichlid *Oreochromis niloticus* (Longrie et al., 2009). These muscles could also play a role in sound production in *H. chrysostomus*. From this point of view, differences in acoustic structure of stimulated and natural sounds could be attributed to differences in the motor pattern or additional muscles involved in the sound production.

The multiple smaller peaks that are found in both parts of the sound can, however, not be explained by the single muscle contraction, meaning that there is a vibration of the swimbladder or the ribcage. For decades, the swimbladder has been modeled as an underwater resonant bubble, an acoustic monopole (Bergeijk, 1964; Harris, 1964). However, recent studies have indicated, at least in toadfish, that the swimbladder has a high intrinsic damping that inhibits the expression of resonance (Fine et al., 2009). In this species, peak sound amplitude overlaps fundamental frequencies of the call because of muscle mechanics and not the natural frequency of the bladder (Fine et al., 2001). Although this has not been demonstrated in all species, it could be the case for many fishes with fast-contracting muscles, such as Scianidae (Connaughton et al., 2000). In these fish, however, sonic muscles are intrinsic and the swimbladder is not closely associated with the ribcage. Damping could explain why sounds do not show long series of decaying peaks (e.g. Connaughton, 2004; Rice and Bass, 2009). In *H. chrysostomus*, there are no intrinsic muscles and the multiple decaying peaks suggest that there is a resonating structure. Because the obliquus superior bundles act directly on the ribs, the ribcage could be this resonating structure.

The six massive muscles between the ribs (obliquus superioris 2) should be able to compress the swimbladder, and the movements

of a total of 10 articulated ribs (ribs 3 to 7 on each side) could play a role. This kind of mechanism was recently described in the tilapia *Oreochromis niloticus* (Longrie et al., 2009), but the calls of this cichlid do not present the same kind of waveform: they do not have, as in holocentrids (Winn and Marshall, 1963; Salmon, 1967) or *H. chrysostomus*, a strong peak at the beginning of each pulse. Also, the contraction of the intercostal muscles provokes a discernable body movement in the tilapia and not in *H. chrysostomus*. Consequently, the intercostal muscle cannot explain all the pulse waveform but could intervene in the smaller acoustic cycles at the end of the pulse. Additional studies are needed.

Chaetodontidae are divided in two main groups, bannerfishes and butterflyfishes (Fessler and Westneat, 2007). The sound-producing mechanism seems to be different in the three bannerfish genera able to make sounds. Members of the genus *Forcipiger* produce sounds that are associated with rapid dorsal elevation of the head, anterodorsal motion of the ventral pectoral girdle and dorsal elevation of the caudal skeleton, which could elongate the body cavity and stimulate sound emission from the swimbladder (Boyle and Tricas, 2009). Sound characteristics, body movements and EMG are also different between *H. chrysostomus* and *Hemitaurichthys polylepis*: the calls of *H. chrysostomus* are harmonic (Fig. 1), they do not show any external body movement and the calls have a different pulse waveform. More investigations are necessary to determine whether the sonic mechanism evolved from a common pattern or developed independently many times. For example, there may be a relationship between the EMG doublets of *H. polylepis* (Boyle and Tricas, 2010) and the double set of peaks that characterizes the sounds produced by *H. chrysostomus*.

ACKNOWLEDGEMENTS

E.P. is a research associate of the Fonds National de la Recherche Scientifique of Belgium (FRS-FNRS). This study was supported by 'starting grants' from the University of Liège and by grants from the CRISP program, ANR (ANR-06-JCJC-0012-01) and MOM (06 PF 15). T. Tricas kindly provided equipment and laboratory supplies for EMG experiments. Remarks of two anonymous referees improved this manuscript. Methods were approved by the Institutional Animal Care of the University of Hawaii (protocol number 02-007).

REFERENCES

- Akamatsu, T., Okumura, T., Novarini, N. and Yan, H. Y. (2002). Empirical refinements applicable to the recording of fish sounds in small tanks. *J. Acoust. Soc. Am.* **112**, 3073-3082.
- Bass, A. H. and Marchaterre, M. A. (1989). Sound-generating (sonic) motor system in a teleost fish (*Porichthys notatus*): sexual polymorphism in the ultrastructure of myofibrils. *J. Comp. Neurol.* **286**, 141-153.
- Bass, A. H. and McKibben, J. R. (2003). Neural mechanisms and behaviors for acoustic communication in teleost fish. *Prog. Neurobiol.* **69**, 1-26.
- Bergeijk, W. A. V. (1964). Directional and nondirectional hearing in fish. In *Marine Bioacoustics* (ed. W. N. Tavolga), pp. 281-299. New York: Pergamon Press.

- Boyle, K. S. and Tricas, T. C. (2009). Head and body kinematics of pulse sound generation and feeding in longnose butterflyfishes (genus *Forcipiger*). *J. Acoust. Soc. Am.* **125**, 2487.
- Boyle, K. S. and Tricas, T. C. (2010). Pulse sound generation, anterior swimbladder buckling and associated muscle activity in the pyramid butterflyfish, *Hemitaenichthys polylepis*. *J. Exp. Biol.* **213**, 3881-3893.
- Carlson, B. A. and Bass, A. H. (2000). Sonic/vocal motor pathways in squirrelfish (Teleostei, Holocentridae). *Brain Behav. Evol.* **56**, 14-28.
- Cohen, M. J. and Winn, H. E. (1967). Electrophysiological observations on hearing and sound production in the fish, *Porichthys notatus*. *J. Exp. Zool.* **165**, 355-370.
- Connaughton, M. A. (2004). Sound generation in the searobin (*Prionotus carolinus*), a fish with alternate sonic muscle contraction. *J. Exp. Biol.* **207**, 1643-1654.
- Connaughton, M. A., Taylor, M. H. and Fine, M. L. (2000). Effects of fish size and temperature on weakfish disturbance calls: implications for the mechanism of sound generation. *J. Exp. Biol.* **203**, 1503-1512.
- Demski, L. S., Gerald, J. W. and Popper, A. N. (1973). Central and peripheral mechanisms of teleost sound production. *Am. Zool.* **13**, 1141-1167.
- Evans, R. R. (1973). The swimbladder and associated structures in western Atlantic searobins (Triglidae). *Copeia* **1973**, 315-321.
- Fessler, J. L. and Westneat, M. W. (2007). Molecular phylogenetics of the butterflyfishes (Chaetodontidae): taxonomy and biogeography of a global coral reef fish family. *Mol. Phylogenet. Evol.* **45**, 50-68.
- Fine, M. L., Malloy, K. L., King, C. B., Mitchell, S. L. and Cameron, T. M. (2001). Movement and sound generation by the toadfish swimbladder. *J. Comp. Physiol. A* **187**, 371-379.
- Fine, M. L., Lin, H., Nguyen, B. B., Rountree, R. A., Cameron, T. M. and Parmentier, E. (2007). Functional morphology of the sonic apparatus in the fawn cusk-eel *Lepophidium profundorum* (Gill, 1863). *J. Morphol.* **268**, 953-966.
- Fine, M. L., King, C. B. and Cameron, T. M. (2009). Acoustical properties of the swimbladder in the oyster toadfish *Opsanus tau*. *J. Exp. Biol.* **212**, 3542-3552.
- Fish, M. P. and Mowbray, H. M. (1970). *Sounds of Western North Atlantic Fishes*, 106 pp. Baltimore, MD: The Johns Hopkins Press.
- Gainer, H., Kusano, K. and Mathewson, R. F. (1965). Electrophysiological and mechanical properties of squirrelfish sound-producing muscle. *Comp. Biochem. Physiol.* **14**, 661-671.
- Harris, G. G. (1964). Considerations on the physics of sound production by fishes. In *Marine Bio-acoustics* (ed. W. N. Tavolga), pp. 233-247. New York: Pergamon Press.
- Hawkins, A. D. (1993). Underwater sound and fish behaviour. In *Behaviour of Teleost Fishes* (ed. T. J. Pitcher), pp. 129-169. London: Chapman & Hall.
- Hawkins, A. D. and Myrberg, A. A. (1983). Hearing and sound communication under water. In *Bioacoustics, A Comparative Approach* (ed. B. Lewis), pp. 347-405. London: Academic Press.
- Helfman, G. S., Collette, B. B., Facey, D. E. and Bowen, B. W. (2009). *The Diversity of Fishes: Biology, Evolution and Ecology* (2nd edn). West Sussex: John Wiley & Sons.
- Howes, G. J. (1992). Notes on the anatomy and classification of ophidiiform fishes with particular reference to the abyssal genus *Acanthonus* Günther, 1878. *Bull. Br. Mus. Nat. Hist. Zool.* **58**, 95-131.
- Ladich, F. (2001). Sound production and acoustic communication. In *Senses of Fishes* (ed. G. von der Emde and J. Mogdans), pp. 210-230. New Delhi: Narosa Publishing House.
- Ladich, F. and Fine, M. L. (2006). Sound-generating mechanisms in fishes: a unique diversity in vertebrates. In *Communication in Fishes*, Vol. 1 (ed. F. Ladich, S. P. Collin, P. Moller and B. G. Kapoor), pp. 1-42. Enfield, NH: Science Publishers.
- Longie, N., Van Wassenbergh, S., Vandewalle, P., Manguith, Q. and Parmentier, E. (2009). Potential mechanism of sound production in *Oreochromis niloticus* (Cichlidae). *J. Exp. Biol.* **212**, 3395-3402.
- Moulton, J. M. (1958). The acoustical behavior of some fishes in the Bimini area. *Biol. Bull.* **114**, 357-374.
- Nguyen, T. K., Lin, H., Parmentier, E. and Fine, M. L. (2008). Seasonal variation in sonic muscles in the fawn cusk-eel *Lepophidium profundorum*. *Biol. Lett.* **4**, 707-710.
- Ono, R. D. and Poss, S. G. (1982). Structure and innervation of the swimbladder musculature in the weakfish, *Cynoscion regalis* (Teleostei: Sciaenidae). *Can. J. Zool.* **60**, 1955-1967.
- Parmentier, E., Gennotte, V., Focant, B., Goffinet, G. and Vandewalle, P. (2003). Characterization of the primary sonic muscles in *Carapus acus* (Carapidae): a multidisciplinary approach. *Proc. R. Soc. Lond. B* **270**, 2301-2308.
- Parmentier, E., Lagardère, J. P., Braquenier, J. B., Vandewalle, P. and Fine, M. L. (2006a). Sound production mechanism in carapid fish: first example with a slow sonic muscle. *J. Exp. Biol.* **209**, 2952-2960.
- Parmentier, E., Fontenelle, N., Fine, M. L., Vandewalle, P. and Henrist, C. (2006b). Functional morphology of the sonic apparatus in *Ophidion barbatum* (Teleostei, Ophidiidae). *J. Morphol.* **267**, 1461-1468.
- Parmentier, E., Bouillac, G., Dragicevic, B., Dulcic, J. and Fine, M. L. (2010). Call properties and morphology of the sound-producing organ in *Ophidion rochei* (Ophidiidae). *J. Exp. Biol.* **213**, 3230-3236.
- Parmentier, E., Vandewalle, P., Brié, C., Dinraths, L. and Lecchini, D. (2011). Comparative study on sound production in different Holocentridae species. *Front. Zool.* **8**, 12.
- Rice, A. N. and Bass, A. H. (2009). Novel vocal repertoire and paired swimbladders of the three-spined toadfish, *Batrachomoeus trispinosus*: insights into the diversity of the Batrachoididae. *J. Exp. Biol.* **212**, 1377-1391.
- Salmon, M. (1967). Acoustical behavior of the menpachi, *Myripristis berndti*, in Hawaii. *Pac. Sci.* **21**, 364-381.
- Schaller, F. (1971). Über den Lautapparat von Amazonas-Fischen. *Naturwissenschaften* **58**, 573-574.
- Skoglund, C. R. (1961). Functional analysis of swimbladder muscles engaged in sound production of the toadfish. *J. Biophys. Biochem. Cytol.* **10**, 187-200.
- Sprague, M. (2000). The single sonic muscle twitch model for the sound-production mechanism in the weakfish, *Cynoscion regalis*. *J. Acoust. Soc. Am.* **108**, 2430-2437.
- Tavolga, W. N. (1964). Sonic characteristics and mechanisms in marine fishes. In *Marine Bio-acoustics* (ed. W. N. Tavolga), pp. 195-211. New York: Pergamon Press.
- Tavolga, W. N. (1971). Sound production and detection. In *Fish Physiology*, Vol. 5 (ed. W. S. Hoar and D. J. Randall), pp. 135-205. New York: Academic Press.
- Tricas, T. C., Kajjura, S. M. and Kosaki, R. K. (2006). Acoustic communication in territorial butterflyfish: test of the sound production hypothesis. *J. Exp. Biol.* **209**, 4994-5004.
- Webb, J. F., Smith, W. L. and Ketten, D. R. (2006). The laterophysic connection and swimbladder of butterflyfishes in the genus *Chaetodon* (Perciformes: Chaetodontidae). *J. Morphol.* **267**, 1338-1355.
- Winn, H. E. and Marshall, J. A. (1963). Sound-producing organ of the squirrelfish, *Holocentrus rufus*. *Physiol. Zool.* **36**, 34-44.