

This item is the archived peer-reviewed author-version of:

Unusual sound production mechanism in the triggerfish *Rhinecanthus aculeatus* (Balistidae)

Reference:

Parmentier Eric, Raick Xavier, Lecchini David, Boyle Kelly, Van Wassenbergh Sam, Bertucci Frédéric, Kéver Loïc.- Unusual sound production mechanism in the triggerfish *Rhinecanthus aculeatus* (Balistidae)
The journal of experimental biology - ISSN 0022-0949 - 220:2(2017), p. 186-193
Full text (Publisher's DOI): <https://doi.org/doi:10.1242/JEB.146514>
To cite this reference: <http://hdl.handle.net/10067/1404320151162165141>

CORRECTION

Correction: Unusual sound production mechanism in the triggerfish *Rhinecanthus aculeatus* (Balistidae)

Eric Parmentier, Xavier Raick, David Lecchini, Kelly Boyle, Sam Van Wassenbergh, Frédéric Bertucci and Loïc Kéver

There was an error published in *J. Exp. Biol.* 220, 186-193.

The surname of Sam Van Wassenbergh was incorrectly displayed. This has been corrected in the online full-text and PDF versions.

The authors apologise for any inconvenience this may have caused.

Unusual sound production mechanism in the triggerfish

Rhinecanthus aculeatus (Balistidae)

Eric Parmentier^{1*}, Xavier Raick¹, David Lecchini^{2,3}, Kelly Boyle⁴, Sam Vanwassenbergh^{4,5}, Frédéric Bertucci¹, Loïc Kéver¹

¹Université de Liège, Laboratoire de Morphologie Fonctionnelle et Evolutive, AFFISH-RC, Institut de Chimie - B6C, Sart Tilman, 4000 Liège, Belgium

²USR 3278 CNRS-EPHE-UPVD, CRIOBE, Paris Sciences Lettres (PSL), Moorea, French Polynesia

³Laboratoire d'Excellence "CORAIL", Moorea, French Polynesia

⁴Département d'Ecologie et de Gestion de la Biodiversité, Muséum National d'Histoire Naturelle, 57 rue Cuvier, Case postale 55, Paris 75231, Cedex 5, France.

⁵Department of Biology, Universiteit Antwerpen, Universiteitsplein 1, B-2610 Antwerpen, Belgium.

* Corresponding author: Tel : ++3243665024; E.Parmentier@ulg.ac.be

Abstract

The ability to produce sounds has been known for decades in Balistidae. Sounds of many species have been recorded and a variety of sound producing mechanisms have been proposed including teeth stridulation, collision of buccal teeth, and movements of the fins. The best supported hypothesis involves movements of the pectoral fins against the lateral parts of the swimbladder called a drumming membrane. In this study, we describe for the first time the sounds made by the Blackbar triggerfish *Rhinecanthus aculeatus* that sound like short drum rolls with an average duration of 85 ms, 193 Hz dominant frequency and 136 dB SPL level at 3 cm distance. Sounds are a series of pulses that result from alternate sweeping movements of the right and left pectoral fins, which push a system of three scutes that are forced against the swimbladder wall. Pulses from each fin occur in consecutive pairs. High-speed videos indicate that each pulse consists in two cycles. The first part of each cycle corresponds to the inward buckling of the scutes whereas the second part of the cycle corresponds to an apparent passive recoil of the scutes and swimbladder wall. This novel sound production mechanism is likely found in many members of Balistidae because these peculiar scutes are found in other species in the family. Comparison of sound characteristics from fishes of different sizes shows that dominant frequency decreases with size in juveniles but not in adults.

Keyword: Acoustic, sound production, swimbladder

1. Introduction

In the field of fish acoustic communication, triggerfishes (Balistidae) are particularly well-known because their ability to produce sounds has been reported for decades (Moseley, 1879; Cunningham, 1910; Fish, 1954a). However, the sound descriptions and associated mechanisms are incomplete and research on sound production in this important reef fish family has been neglected for over 50 years. Triggerfish are thought to use different sonic mechanisms, but many of them are not clearly described. 1) Sounds are produced during feeding activities. They result from collision of buccal teeth *e.g.* in *Balistes capriscus* (Vincent, 1963) or during grating of pharyngeal teeth in *B. capriscus*, *B. vetula* and *Odonus niger* (Norman, 1931 ; Fish, 1948; Fish *et al.*, 1952; Moulton, 1958). Because these sounds are mainly heard while crushing mollusks and hard corals (Fish, 1948, 1954 ; Tavolga, 1965 ; Tricas & Boyle, 2014), it is unclear if they are used for communication. 2) In some triggerfishes (*O. niger*, *B. capriscus*) sounds could also result from movements of the spine of the first dorsal fin that can be locked into an erect position (Fish *et al.*, 1952; Schneider, 1961) but this sound producing mechanism deserves deeper description and an associated behavior has not been discovered. 3) The last mechanism involves the swimbladder and the pectoral fins. Pectoral sounds are produced during agonistic interactions in different species such as *Rhinecanthus rectangulus*, *Melichthys niger*, *M. vidua*, *Sufflamen bursa* and *S. fraenatum* (Salmon *et al.*, 1968). The swimbladder is large and in close contact to the skull, the pectoral girdle and the vertebral column. It also has lateral expansions that join the body wall dorsal to the pectoral fins. This part of the swimbladder constitutes a drumming membrane covered by skin and connective tissue and some enlarged, plate-like scales (Moulton, 1958) or scutes. Ablation experiments showed the pectoral fin and the drumming membrane are responsible for sound production (Salmon *et al.*, 1968). Details of the movement, however, were missing and it is not known whether the fin rubs or strikes the scutes. A rubbing mechanism should provoke stridulatory sounds with different parameters such as broad band, high (> 2000 Hz) and variable dominant frequency (Fine and Parmentier, 2015), which is not the case (Moulton, 1958; Salmon *et al.*, 1968). Historically, the swimbladder has been modeled as an underwater resonant structure, meaning that hits on its wall were thought to be sufficient to generate sounds (Fine and Parmentier, 2015). As a result, the updated hypothesis was: “movements of the stiff pectoral fin spine across the drumming membrane contribute to most of the resultant sound pressure”. This hypothesis is not sufficient to explain sound production because the sound does not rely upon prolonged contact between the fin and the tympanum. Previous authors have argued the sounds should result from pectoral fin strikes on the swimbladder (Cunningham, 1910; Fish *et al.*, 1952; Moulton, 1958; Sørensen, 1895). Two observations contradict this assumption. First, it has been experimentally shown in different distantly related species the

swimbladder is not a drumhead (Fine and Parmentier, 2015). Numerous studies have demonstrated that swimbladders damp rapidly, meaning they are poor resonators (Batzler and Pickwell, 1970; McCartney and Stubbs, 1970; Sand and Hawkins, 1973; Weston, 1967). In the oyster toadfish *Opsanus tau* and the red piranha *Pygocentrus nattereri*, the swimbladder wall rather acts as a shock absorber. Therefore, a single strike cannot generate a resonant sound because of damping: the swimbladder does not continue to vibrate after being struck. In both species at least, sounds are dictated by contraction dynamics of superfast sonic muscles (Fine et al., 2001; Millot et al., 2011). Studies in these other fishes suggest that long sounds recorded in different members of the Balistidae are unlikely the result of a single strike (Salmon et al., 1968). Second, in his study on three balistid species, Salmon et al. (1968) also reported that the swimbladder did not appear to emphasize any particular frequencies and thus does not function as a resonator (Salmon et al., 1968). The Blackbar triggerfish *Rhinecanthus aculeatus* (Linnaeus, 1758) is an omnivorous species found on coral reefs in the Indo-Pacific Ocean (Chen et al., 2001 ; Lobel & Johanne, 1980). The male is polygynous and actively defends a territory housing two to five females (Künzli & Tachihara, 2012; Kuwamura, 1997). Calls of the species were reported, but not described 130 years ago, as a throbbing, drumming or whirring sound (Mobius, 1889). Calls are made during encounters with predators or when fish are startled (Hutchins et al., 2003). According to Mobius (1889), the mechanism is not due to the teeth, dorsal fin, pectoral fins or opercles because sounds were still produced while these skeletal pieces were held immobile. Mobius believed drumming sounds resulted from stridulation between the postcleithrum and the inner surface of the cleithrum (Mobius, 1889). The frictional sound would then be transferred to the swimbladder wall (Schneider, 1961) or radiated directly to the surrounding water medium without transfer to the swimbladder (Cunningham, 1910). Substantive data are obviously missing because, although sounds have been reported, they have not yet been clearly described. Further, the proposed mechanism is only inferred from preserved museum specimens, which is problematic because preservation prevents free movements of structure.

The aim of this study is to provide a first quantitative description of the sound produced by the triggerfish *Rhinecanthus aculeatus* (Linnaeus, 1758) and to unravel its sound producing mechanism. Moreover, sounds were recorded in specimens having different lengths to determine the effect of size on acoustic characteristics.

2. Materials and Methods

(a) Biological material

A field campaign was conducted at the CRIOBE research station (www.criobe.pf) in Moorea Island (French Polynesia) between February 2015 and April 2015. Sixteen juvenile *R. aculeatus* (Total Length, TL: 4,8 - 9,8 cm) were caught at Ta'ahiamanu beach (17° 29' 23" S, 149° 50' 59" O) and Pihaena beach (17° 29' 06" S, 149° 49' 50" O). They were found hiding inside reef rocky crevices from 0.4 to 1.2 m depth.

In *R. aculeatus*, fish are sexually mature at > 14 cm TL (Myers, 1991). Eleven adult specimens (TL: 15.9 - 20.6 cm) were caught at 3 m depth at Ta'ahiamanu beach with a gillnet (25 m long and mesh of 2.5 cm). They were stocked in tanks with running seawater (temperature 28 - 29°C) on a natural light cycle (12 h light: 12 h dark). Nine individuals (TL: 6.4 – 10.1 cm) were also purchased from a commercial tropical fish wholesaler (Tropic Nguyen, France) for additional studies in Liège (Belgium). In the laboratory, triggerfish were kept in individual salt-water aquaria (with sand and hiding places) at 26 - 28°C and on a 10 h light: 14 h dark cycle. Fish were fed daily with bivalves (*Mytilus* sp. and *Cerastoderma* sp.).

After an acclimatization period of 10 days, fish were recorded with a hydrophone (HTI Min-96, sensitivity: -163.9 dB re. 1 V μ Pa⁻¹; flat frequency response range between 2 Hz and 30 kHz) (Long Beach, MS, USA) placed in the center of the aquarium and connected to a Tascam DR-07 recorder (TEAC, Wiesbaden, Germany) at a 44.1 kHz sampling rate. Fish were hand-held in the tank approximately 3 cm from the hydrophone, with the dorsal fin blocked. Ten sounds were recorded for each fish.

(b) Sound analysis

Sound description is based on the nine subadult fish from Liège, but the intraspecific comparison is based on all specimens. Sounds were digitized at 44.1 kHz (16-bit resolution) and analyzed with Avisoft-SAS Lab Pro 5.2 software (Avisoft Bioacoustics, Glienicke, Germany). Recordings in small tanks induce potential artifacts because of reflections and tank resonance. In Liège, the recording tank was 99 cm x 39 cm x 25 cm whereas it was 83 cm x 53 cm x 23 cm in French Polynesia with estimated minimum resonance frequencies of 3640 Hz and 3670 Hz respectively (Akamatsu et al., 2002). A band-pass filter (0.05 to 3.5 kHz) was applied to all recordings. Only sounds with a high signal to noise ratio

were analyzed. According to the sounds, the following temporal acoustic variables were measured on the oscillogram: call duration (duration from the beginning to the end of the sound, ms), pulse period (duration between the onset of two consecutive pulses, ms), pulse duration (duration from the beginning to the end of the pulse, ms) and the number of peaks in the sound. Spectral characteristics of sounds, obtained from power spectra (Hamming window, FFT: 512 points) allowed measurement of dominant frequency (frequency component with the most energy, Hz) and its associated amplitude (Sound Pressure Level, dB SPL at 3 cm).

(c) Morphological study

Six specimens (TL: 4.4 - 18.4 cm) were euthanized with an overdose of MS 222, fixed in 7% formalin and transferred to 70% alcohol. Three specimens (TL: 4,4 - 7,2 cm) were stained with Alizarin Red S (Taylor and Van Dyke, 1985) to visualize osseous structures. The other individuals were carefully dissected under a stereoscopic microscope (Leica, Wild M10) coupled to a *camera lucida* to study mineralized and soft tissues of the sonic apparatus.

In order to visualize the arrangement of the pectoral fin, girdle, and swimbladder, a museum specimen MNHN IC.1954-0039, 205 mm SL, 233 mm TL was scanned at the AST-RX technical platform at the Muséum National d'Histoire Naturelle with a μ CT scanner (v|tome|x 240 L, GE Sensing & Inspection Technologies phoenix|x-ray) with the imaging system set to 115 kV. The isotropic voxel size was 84.8 μ m. Segmentation and surface rendering of the cleithrum, postcleithrum, radials, scutes, and swimbladder were done in Amira 5.4.0 (VSG, GEI, Company). Automatic thresholding was used to identify bones while the swimbladder membrane was outlined by hand.

(d) Functional study

Pectoral fin (spine and rays) movements of five fish were recorded at 500 fps with a high speed camera (Model NX4-S1, IDT, Tallahassee, USA; 640 x 456 pixels) coupled with a 19-LED light. The camera was connected to a computer (video chart: Asus v9280S, San Diego, CA, USA), making it possible to visualize the fish movements in detail. This imaging system was connected to an IDT data acquisition box 01-XS-DAS (Tallahassee, FL, USA) allowing the synchronization with a HTI Min-96 hydrophone (Long Beach, MS, USA). Fish were anesthetized with MS 222 (20 - 100 mg l⁻¹) and cyanoacrylate adhesives (Scotch-Weld, 3M, USA) were used to fix colored pieces of paper (Northwest Marine Technology, USA) on the pectoral fin. Movements were observed with the MotionStudio software (Integrated Design Tools©, USA).

To highlight the sound-producing mechanism, recordings were made during three different experiments, each using a set of five specimens. 1) After immobilization of one (or both) pectoral fin(s). 2) After cutting of the pectoral spine of each pectoral fin in anesthetized specimens. 3) After puncturing the swimbladder wall of anesthetized specimens, gas was removed from the swimbladder using a 1 ml syringe. Sounds of treated specimens were compared with controls.

(e) Statistics

Juveniles and adults sounds were plotted in a two dimensional space using the first two components (PC1 and PC2) of a principal components analysis (PCA) using all the variables measured in sound analysis. Sounds of juveniles and adults were compared with Wilcoxon signed-rank tests. The correlation between body size and acoustic features was examined with a Spearman correlation matrix associated to a p value (Holm-Bonferroni adjusted method) matrix. The sounds produced by one or both pectoral fins were compared with the nonparametric multivariate test of Oja and the Wilcoxon signed-rank test for comparison between each variable. Sounds made before and after the manipulation experiments (cutting of the pectoral spines or deflating the swimbladder) were compared with Friedman tests, followed by corrected Wilcoxon *post hoc* tests. All the statistical analyses were carried out with R 3.0.2.

(f) Ethics

All experimental procedures followed a protocol that was approved by the local ethics committee of the University of Liège. *Rhinecanthus aculeatus* is not an endangered or protected species and specimens were not caught in protected areas.

3. Results

(a) Sounds

Sounds were recorded in both juveniles (4.8 – 9.8 cm) and adults (15.9 – 20.6 cm). The calls are clearly similar in both groups (Table 1) and sound like short drum rolls (Figure 1). These sounds are made of (mean \pm SD) 13.7 ± 3.5 peaks (9 specimens, 90 sounds, TL: 6.4 – 10.1 cm). They last 85 ± 22 ms, have a dominant frequency of 193 ± 38 Hz and their amplitude is *ca* 136 ± 16 dB re $1\mu\text{Pa}$. A principal components analysis (PCA) was conducted to determine whether variation in mean sound characteristics among individual fish is related to sexual maturity. PCA that incorporated the spectral and duration features of individual sound events indicates broad acoustic similarity between juveniles and adults (Figure 2). However, although overlapping, juvenile sounds differed statistically from adults

in call duration, dominant frequency and sound level (Wilcoxon test, all $p < 0.01$) but not number of peaks ($p = 0.014$).

Size was positively correlated with sound pressure level ($r_s = 0.49$, $p < 0.01$) but was inversely correlated with dominant frequency ($r_s = -0.87$, $p < 0.01$) and the number of peaks ($r_s = -0.42$, $p < 0.01$). No relationship was found between size and call duration ($r_s = -0.04$, $p = 1$).

However, the relationship between size and dominant frequency is not linear (Figure 3). Dominant frequency decreases with fish size before reaching a plateau (between 6 and 85 mm TL).

(b) Morphology

Previous dissections on the sound-producing mechanism (Salmon et al., 1968) focused principally on the pectoral girdle and associated structures. In teleosts at least, the swimbladder is made of two main layers: the tunica externa and the tunica interna. A portion of the swimbladder that does not have a tunica externa is usually called a swimbladder fenestra (Mok et al., 2011; Parmentier et al., 2003; Parmentier et al., 2016). The swimbladder is conical-shaped, enlarged at the anterior end and tapers sharply posteriorly (Fig. 4). Dorsally, it fits closely with the vertebral column and the posterior part of the neurocranium. Laterally, it joins and fits the cleithrum and post-cleithrum. These bones define a set square bordering the frontal and ventral sides of the swimbladder fenestra. The caudal and dorsal borders of the fenestra are not facing bones. Externally, the fenestra is covered by three scutes (rostral, ventral and caudal) that have numerous deep radial wrinkles that form a bulge on each scute (Fig. 4). All scutes are in contact with each other. The ventral scute is half the size of the others. External rims of these three plates lie on the border of the swimbladder fenestra whereas their inner junctions are found at the zone of high flexibility of the fenestra. This configuration between the scutes allows rapid buckling at the level of the inner junctions as soon as there is a compressive stress on the fenestra.

The pectoral girdle comprises the usual bones: the posttemporal, supracleithrum, cleithrum, scapula, coracoid and post-cleithrum. Three osseous protrusions can be distinguished. The first is found on the cleithrum (Fig. 4A), between the basis of the rostral and ventral scutes. The second and third protrusions are found on the post-cleithrum (Fig. 4B), close to the ventral part of the caudal scute. The base of the pectoral fin contains five radials, and the dorsal most radial has a tiny protrusion (Fig. 4A). The fin is composed of a dorsal spine and *ca* 13 lepidotrichia (Fig. 4).

(c) Study of the sound production mechanism

No drumming sounds were detected when the pectoral fins were held immobile against the side of the body. However, the immobilization of one pectoral fin reveals sounds that consist of a suite of pulses produced in alternation by movements of the left and right pectoral fins (Fig. 5). Holding one fin does not change the sound duration but reduces by half the number of peaks (Fig. 5; Table 2) and highlights that a pulse is made of two consecutive cycles (Fig. 5). In this case, the pulse period is 23 ± 10 ms (Table 2) which indicates it is highly variable, from 14 to 40 ms (Fig. 6). The period usually increases towards the end of the sound. Comparisons of sound characteristics (call duration, dominant frequency, dB SPL, pulse period) produced by immobilization of either pectoral fin were not statistically different (Fig. 6; Oja, $p = 0.08$) meaning the mechanism is the same for both fins. Removing the dorsal pectoral spine (Fig. 4) decreases sound amplitude by 33 dB but does not change sound duration or dominant frequency ($p = 0.55$ and 0.45 respectively; Friedman test). Moreover, the number of peaks was similar in both cases ($p = 0.86$, paired student t-test).

Similarly, swimbladder deflation decreased sound amplitude by 28 dB (Wilcoxon, $p < 0.01$), dominant frequency increased significantly (Wilcoxon, $p < 0.01$) from 143 ± 23 Hz to 273 ± 78 Hz ($n = 49$), but sound duration was not significantly different (Wilcoxon, $p = 0.49$).

High-speed video shows that pulses are associated with inward and outward movements of the scutes. During sound production, the pectoral fin is first placed along the fish with the pectoral spine, against the body wall (see supplementary material Movie 1). The fin is then lowered, allowing the spine to push the bulges of the scutes successively. As a result, the scutes perform two back and forth movements that correspond to sound production (blue boxes, Fig. 6). Comparisons of high speed movies with the waveform in oscillograms showed the first cycle corresponded to the first contact of the scutes with the swimbladder and the second cycle corresponded to the second contact. During each contact, the negative peak of the cycle (A_1 or B_1 on Fig. 6) corresponds to the buckling (i.e. inward displacement of the scutes) of the body wall and the positive part of the cycle (A_2 or B_2 on Fig. 6) corresponds to the recoil (i.e. outward displacement of the scutes). As a whole, double back and forth movements of the scutes during sound production correspond to the pulse duration (12 ± 7 ms, Table 2). Once the fin has been lowered, it can be elevated back to its initial position, producing again two cycles when touching the scutes. In summary, the pulse is made of two consecutive cycles and correspond to one fin movement.

4. Discussion

The present study provides the first physical description of the drumming sounds in *R. aculeatus*. Although some data are lacking from other balistids (*B. capricus*, *B. undulatus*, *R. rectangulus*, *M. niger*) that appear to use a similar pectoral fins-swimbladder mechanism to produce sounds (Salmon et al., 1968; Vincent, 1963), sounds of *R. rectangulus* appear similar, e.g. sound duration and frequency spectra are in the same range.

Relationships between fish size and different acoustic characteristics have been shown in many species (Parmentier and Fine, 2016). Sound level changes in a sciaenid (Connaughton et al., 2000) and in *Forcipiger* sp. (Boyle and Tricas, 2011), dominant frequency in most species studied (Amorim and Hawkins, 2005; Amorim et al., 2003; Bertucci et al., 2012; Colleye et al., 2011; Malavasi et al., 2003; Myrberg et al., 1993) and pulse duration in clownfish (Colleye et al., 2009; Colleye et al., 2012) and *Forcipiger* sp. (Boyle and Tricas, 2011). In these studies, the linear relationships between size and acoustic characters support the notion that sound can carry information about the emitter's size. In *R. aculeatus* however, dominant frequency is related to size in juveniles but not in adults. Deeper comparative studies on at least the swimbladder, scutes and pectoral girdles are required to understand these data. In the whitemouth croaker *Micropogonias furnieri*, sound characteristics correlated with size in smaller fish, but were independent among larger size classes (Tellechea et al., 2010). The sound production mechanism in whitemouth croaker involves sonic muscles acting directly on the swimbladder and thus the reason for the similar size-ontogeny relationship with sound may be different from *R. rectangulus*.

In most of the fishes using a swimbladder for sound production, calls result from high speed muscles whose contraction rate corresponds to the fundamental frequency of the call (Boyle et al., 2015; Fine et al., 2001; Parmentier et al., 2011). Alternatively, swimbladder walls can be stretched before rapid release in order to provoke sound production (Kéver et al., 2014; Parmentier et al., 2003; Parmentier et al., 2010). Some species (in the Terapontidae, Glaucosomatidae, Pempheridae) can possess both systems: the recoiling system is thought to help relaxation of sound-producing muscles in this case (Mok et al., 2011; Parmentier et al., 2016).

Although there are many differences within and between these sound production systems, high velocity is always required to generate swimbladder sounds. In *R. aculeatus*, videos show that the pectoral fins do not rapidly or violently strike the scutes (supplementary material Movie 1). During sound production from a single pectoral fin, each pulse consists of two main cycles (Fig. 5). Each cycle corresponds to the back and forth back movements of the scutes. The pectoral fin actively deforms the scutes that rapidly bend inward, pushing the wall of the swimbladder, increasing its internal pressure and creating the first negative peak (A_1 , Fig. 6). The first positive peak (A_2 , Fig. 6) is produced by passive recoil of the fenestra. Also, the buckling is feasible because the scutes are mobile where they contact each other and are solidly supported by the post-cleithrum along the ventral periphery and the cleithrum along the rostral rim. There is less support dorsally and caudally, but more support than in the center of the scutes, which contact the area where the swimbladder wall is extremely thin.

Sound production requires contact between the spine and the scutes. Each sweeping of the spine results in a pulse made of two cycles. It is, however, not possible to distinguish the pulses when right and left pectoral fins participate together to produce sounds. During sound production, pectorals complete a cycle between 15 and 40 ms, corresponding to about 25 - 60 beats per second. It appears quite higher than typical rates observed for locomotion because pectoral fin beat frequencies can be from 20 – 30 Hz in larval and juvenile fishes (Green et al., 2011; Hale et al., 2006) but are generally below 10 Hz in most adult species during swimming (Gibb et al., 1994; Mussi et al., 2002; Tudorache et al., 2008). Alternating pectoral fin motions also have been shown to function during station holding, maneuvering, turning, and deceleration in many species (Drucker and Lauder, 2003; Hove et al., 2001). Detailed description of all these complex movements are required to determine if acoustic related movements of the fins correspond to a new motor pattern or are coopted from locomotion movements. Balistiform locomotion is the swimming mode in triggerfishes that use simultaneous undulations of the dorsal and anal fins. This way of swimming may have allowed more freedom for these fish to evolve a pectoral fin sonic mechanism. It is worth mentioning the high variability in pulse period, which indicates that synchronization of motion between the two pectoral fins is not perfect.

We propose that the buckling movements should generate the required velocity for swimbladder movement and resulting sound production. The folding of the plate formed by the three scutes corresponds to the beginning of the cycle and the return to the resting position at the end of the cycle. So, the sound would result from fast movements of the scutes that are set into motion when the pectoral spine passes along their bulging areas. From an evolutionary point of view, the novelty and

advantage of the scute mechanism is that the pectoral spine can quickly impact a large area of the swimbladder and consequently sets in motion a larger volume. Incidentally, the weaker intensity when deflating the swimbladder could be due to the movement of a smaller volume of gas meaning less energy is transferred to the medium. The important role of the pectoral spine is confirmed since its ablation corresponds to a lowering of 26 dB. The weaker intensity due to the cutting of the pectoral spine was already observed in *B. undulatus*, *R. rectangulus* and *M. niger* (Salmon et al., 1968). However, the ablated fish is still able to make sounds meaning the pectoral rays could also be involved in sound production. The sound is less loud because it probably corresponds to a weaker impact on the scutes and lower volume change in the swimbladder. The rays of the pectoral fin are made of a suite of bony elements and are thus more flexible than the spine.

A system also involving deformation of structures in the skin is found in the Senegal bichir *Polypterus senegalus* where deformation and recoil of the bony integument allows air-breathing (Brainerd et al., 1989). The mechanical system is however different because many scales are involved during bichir ventilation and they have peg and sockets articulations (Pearson, 1981), which are not found in the Blackbar triggerfish. In the Pyramid butterflyfish (*Hemitaurichthys polylepis*), sound production is related to a buckling mechanism that involves a small area of tissue located lateral to the anterior swimbladder. However, the area that buckles does not have specialized scales or scutes. The mechanism is also different since EMG experiments show that sounds were correlated with the contraction of some hypaxial muscles (Boyle and Tricas, 2010), meaning the body wall movements could be a by-product of the muscle activity. In parallel to *R. aculeatus* however, the sound production relies on fast movements of the swimbladder wall and the recoil should be due to the internal pressure of the swimbladder.

Acknowledgments

Marie Bournonville and the team from the Museum-Aquarium (Liège) kindly helped in fish maintenance. We thank the CRIOBE team for their assistance during the study in French Polynesia, Miguel Garcia Sanz for CT scanning the triggerfish specimen at the CT scan facility (AST-RX, plateforme d'accès scientifique à la tomographie à rayons X du MNHN, UMS 2700, CNRS-MNHN) and Steven Braine for 3D reconstruction. Michael Fine and Jacqueline Webb made interesting and constructive comments on a previous version. Research was funded by a grant from the Agence Nationale de la Recherche ANR-16-ACHN-0006-01 to Sam Vanwassenbergh and ANR-11-JSV7-012-01 to Xavier Raïck and David Lecchini and by a grant from the F.R.S-FNRS (T.0056.13) to Eric Parmentier.

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.
- Amorim, M. C. P. and Hawkins, A. D.** (2005). Ontogeny of acoustic and feeding behaviour in the grey gurnard, *Eutrigla gurnardus*. *Ethology* **111**, 255–269.
- Amorim, M. C. P., Fonseca, P. J. and Almada, V. C.** (2003). Sound production during courtship and spawning of *Oreochromis mossambicus* : male – female and male – male interactions. *J. Fish Biol.* **62**, 658–672.
- Batzler, W. E. and Pickwell, G. V** (1970). Resonant acoustic scattering from gasbladder fishes. In *Proceedings of an international symposium on biological sound scattering in the ocean* (ed. Farquhar, G. B.), pp. 168–179. Washington, D.C.: U.S. Government Printing Office.
- Bertucci, F., Attia, J., Beauchaud, M. and Mathevon, N.** (2012). Sounds produced by the cichlid fish *Metriaclyma zebra* allow reliable estimation of size and provide information on individual identity. *J. Fish Biol.* **80**, 752–766.
- Boyle, K. S. and Tricas, T. C.** (2010). Pulse sound generation, anterior swim bladder buckling and associated muscle activity in the pyramid butterflyfish, *Hemitaenichthys polylepis*. *J. Exp. Biol.* **213**, 3881–3893.
- Boyle, K. S. and Tricas, T. C.** (2011). Sound production in the longnose butterflyfishes (genus *Forcipiger*): cranial kinematics, muscle activity and honest signals. *J. Exp. Biol.* **214**, 3829–3842.
- Boyle, K. S., Riepe, S., Bolen, G. and Parmentier, E.** (2015). Variation in swim bladder drumming sounds from three doradid catfish species with similar sonic morphologies. *J. Exp. Biol.* **218**, 2881–2891.
- Brainerd, E. L., Liem, K. F. and Samper, C. T.** (1989). Air ventilation by recoil aspiration in polypterid fishes. *Science* (80-.). **246**, 1593–1595.
- Chen, T.-C., Ormond, R. F. G. and Mok, H.-K.** (2001). Feeding and territorial behaviour in juveniles of three co-existing triggerfishes. *J. Fish Biol.* **59**, 524–532.
- Colleye, O., Frederich, B., Vandewalle, P., Casadevall, M. and Parmentier, E.** (2009). Agonistic sounds in the skunk clownfish *Amphiprion akallopisos*: size-related variation in acoustic features. *J. Fish Biol.* **75**, 908–916.
- Colleye, O., Vandewalle, P., Lanterbecq, D., Lecchini, D. and Parmentier, E.** (2011). Interspecific variation of calls in clownfishes: degree of similarity in closely related species. *BMC Evol. Biol.* **11**, 365.
- Colleye, O., Nakamura, M., Frederich, B. and Parmentier, E.** (2012). Further insight into the sound-producing mechanism of clownfishes: what structure is involved in sound radiation? *J. Exp. Biol.* **215**, 2192–2202.
- 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.
- Cunningham, J. T.** (1910). On the marine fishes and invertebrates of St. Helena. *Proc. Zool. Soc. London* **1**, 86–130.
- Drucker, E. G. and Lauder, G. V** (2003). Function of pectoral fins in rainbow trout: behavioral repertoire and hydrodynamic forces. *J. Exp. Biol.* **206**, 813–826.
- Fine, M. L. and Parmentier, E.** (2015). Mechanisms of sound production. In *Sound Communication in Fishes* (ed. Ladich, F.), pp. 77–126. Wien: Springer.
- Fine, M. L., Malloy, K. L., King, C., Mitchell, S. L. and Cameron, T. M.** (2001).

- Movement and sound generation by the toadfish swimbladder. *J. Comp. Physiol. - A Sensory, Neural, Behav. Physiol.* **187**, 371–379.
- 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.** (1948). *Sonic Fishes of the Pacific (technical report n°2)*. Woods Hole, Massachusetts.
- Fish, M. P.** (1954a). The character and significance of sound production among fishes of the western North Atlantic. *Bull. Bingham Oceanogr. Collect.* **14**, 1–109.
- Fish, M. P.** (1954b). The character and significance of sound production among fishes of the Western North Atlantic. *Bingham Oceanogr. Collect.* **14**, 1–109.
- Fish, M. P., Kelsey, A. S. J. and Mowbray, W. H.** (1952). Studies on the production of underwater sound by North Atlantic coastal fishes. *J. Mar. Res.* **11**, 180–193.
- Gibb, A., Jayne, B. and Lauder, G.** (1994). Kinematics of pectoral fin locomotion in the bluegill sunfish *Lepomis macrochirus*. *J. Exp. Biol.* **189**, 133–161.
- Green, M. H., Ho, R. K. and Hale, M. E.** (2011). Movement and function of the pectoral fins of the larval zebrafish (*Danio rerio*) during slow swimming. *J. Exp. Biol.* **214**, 3111–3123.
- Hale, M. E., Day, R. D., Thorsen, D. H. and Westneat, M. W.** (2006). Pectoral fin coordination and gait transitions in steadily swimming juvenile reef fishes. *J. Exp. Biol.* **209**, 3708–3718.
- Hove, J. R., O’Byrne, L. M., Gordon, M. S., Webb, P. W. and Weihs, D.** (2001). Boxfishes (Teleostei: Ostraciidae) as a model system for fishes swimming with many fins: kinematics. *J. Exp. Biol.* **204**, 1459–1471.
- Hutchins, M., Thoney, D. A. and Loiselle, P. V.** (2003). Fishes II. In *Grzimek’s Animal Life Encyclopedia (Volume 5)*, Canada.
- Kéver, L., Boyle, K. S., Dragi, B., Dul, J. and Parmentier, E.** (2014). A superfast muscle in the complex sonic apparatus of *Ophidion rochei* (Ophidiiformes): histological and physiological approaches. **1**, 3432–3440.
- Künzli, F. and Tachihara, K.** (2012). Validation of age and growth of the Picasso triggerfish (Balistidae: *Rhinecanthus aculeatus*) from Okinawa Island, Japan, using sectioned vertebrae and dorsal spines. *J. Oceanogr.* **68**, 817–829.
- Kuwamura, T.** (1997). Evolution of female egg care in harem triggerfish, *Rhinecanthus aculeatus*. *Ethology* **103**, 1015–1023.
- Lobel, P. S. and Johanne, R. E.** (1980). Nesting, eggs and larvae of triggerfishes (Balistidae). *Environ. Biol. Fishes* **5**, 251–252.
- Malavasi, S., Torricelli, P., Lugli, M., Pravoni, F. and Mainardi, D.** (2003). Male courtship sounds in a teleost with alternative reproductive tactics, the grass goby, *Zosterisessor ophiocephalus*. *Environ. Biol. Fishes* **66**, 231–236.
- McCartney, B. S. and Stubbs, A. R.** (1970). Measurement of the target strength of fish in dorsal aspect, including swimbladder resonance. In *Proceedings of an International Symposium on Biological Sound Scattering in the Ocean* (ed. Farquhar, G. B.), pp. 180–211. Washington, D.C.: U.S. Government Printing Office.
- Millot, S., Vandewalle, P. and Parmentier, E.** (2011). Sound production in red-bellied piranhas (*Pygocentrus nattereri*, Kner): an acoustical, behavioural and morphofunctional study. *J. Exp. Biol.* **214**, 3613–3618.
- Mobius, K. A.** (1889). *Balistes aculeatus*, ein trommelnder Fisch. *Sitzungsberichte Der Königlich Preuss. Akad. Der Wissenschaften Zu Berlin* **14**, 999–1007.
- Mok, H.-K., Parmentier, E., Chiu, K.-H., Tsai, K.-E., Chiu, P.-H. and Fine, M. L.** (2011). An intermediate in the evolution of superfast sonic muscles. *Front. Zool.* **8**, 31.
- Moseley, H. N.** (1879). *Notes by a naturalist on the “Challenger” being an account of*

various observations made during the voyage of H.M.S “Challenger” round the world, in the years 1872-1876. McMillan. London.

Moulton, J. M. (1958). The acoustical behavior of some fishes in the Bimini area. *Biol. Bull.* **114**, 357–374.

Mussi, M., Summers, A. P. and Domenici, P. (2002). Gait transition speed, pectoral fin-beat frequency and amplitude in *Cymatogaster aggregata*, *Embiotoca lateralis* and *Damalichthys vacca*. *J. Fish Biol.* **61**, 1282–1293.

Myrberg, A. A. J., Ha, S. J. and Shamblott, M. J. (1993). The sounds of bicolor damselfish (*Pomacentrus partitus*): predictors of body size and a spectral basis for individual recognition and assessment. *J. Acoust. Soc. Am.* **94**, 3067–3070.

Norman, J. R. (1931). *A history of fishes*. Third edit. (ed. Greenwood, P. H.) London.

Parmentier, E. and Fine, M. L. (2016). Fish Sound Production: Insight. In *Vertebrate sound production and acoustic communication* (ed. Suthers, R.), Tecumseh, F.), Popper, A. N.), and Fay, R. R.), New York: Springer.

Parmentier, E., Vandewalle, P. and Lagardère, J. P. (2003). Sound-producing mechanisms and recordings in Carapini species (Teleostei, Pisces). *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.* **189**, 283–292.

Parmentier, E., Bouillac, G., Dragicevic, B., Dulcic, J. and Fine, M. (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.

Parmentier, E., Fine, M. L. and Mok, H.-K. (2016). Sound production by a recoiling system in the Pempheridae and Terapontidae. *J. Morphol.* **277**, 717–724.

Pearson, D. M. (1981). Functional aspects of the integument in polypterid fishes. *Zool. J. Linn. Soc.* **72**, 93–106.

Salmon, M., Winn, H. E. and Sorgente, N. (1968). Sound production and associated behavior in triggerfishes. *Pacific Sci.* **22**, 11–20.

Sand, O. and Hawkins, A. D. (1973). Acoustic properties of the cod swimbladder. *J. Exp. Biol.* **58**, 797–820.

Schneider, H. (1961). Neuere Ergebnisse der Lautforschung bei Fischen. *Naturwissenschaften* **48**, 513–518.

Sörensen, W. (1895). Are the extrinsic muscles of the air-bladder in some Siluroidae and the “elastic spring” apparatus of others subordinate to the voluntary production of sounds? What is, according to our present knowledge, the function of the Weberian ossicles? *J. Anat. Physiol.* **29**, 109–139.

Taylor, W. R. and Van Dyke, G. C. (1985). Revised procedure for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium* **9**, 107–121.

Tellechea, J. S., Martinez, C., Fine, M. L. and Norbis, W. (2010). Sound production in the whitemouth croaker and relationship between fish size and disturbance call characteristics. *Environ. Biol. Fishes* **89**, 163–172.

Tricas, T. and Boyle, K. (2014). Acoustic behaviors in Hawaiian coral reef fish communities. *Mar. Ecol. Prog. Ser.* **511**, 1–16.

Tudorache, C., Jordan, A. D., Svendsen, J. C., Domenici, P., DeBoeck, G. and Steffensen, J. F. (2008). Pectoral fin beat frequency predicts oxygen consumption during spontaneous activity in a labriform swimming fish (*Embiotoca lateralis*). *Environ. Biol. Fishes* **84**, 121–127.

van Bergeijk, W. A. (1964). Directional and nondirectional hearing in fish. In *Marine Bioacoustics* (ed. Tavolga, W. N.), pp. 281–299. New York: Pergamon Press.

Journal of Experimental Biology • Advance article

- Vincent, F.** (1963). Note préliminaire sur des émissions acoustiques chez *Balistes capriscus*. *Bull. Cent. Etud. Rech. Sci. Biarritz* **4**, 307–316.
- Weston, D.** (1967). Sound propagation in the presence of bladder fish. In *Underwater Acoustics* (ed. Albers, V.), pp. 55–88. New York: Plenum.

Table 1. Acoustic characteristics of sounds recorded in *Rhinecanthus aculeatus*. Eleven adults (TL: 15.9 – 20.6 cm) and 25 juveniles (TL: 4.8 – 10.1 cm) were used. Mean \pm SD

Calls	Adults	Juveniles
	n = 110	n = 250
Call duration (ms)	93 \pm 63	71 \pm 42
Dominant frequency (Hz)	140 \pm 24	248 \pm 126
Sound level (dB SPL)	152 \pm 3	139 \pm 14
Peak number	10.5 \pm 6	11.6 \pm 5.5

Figures

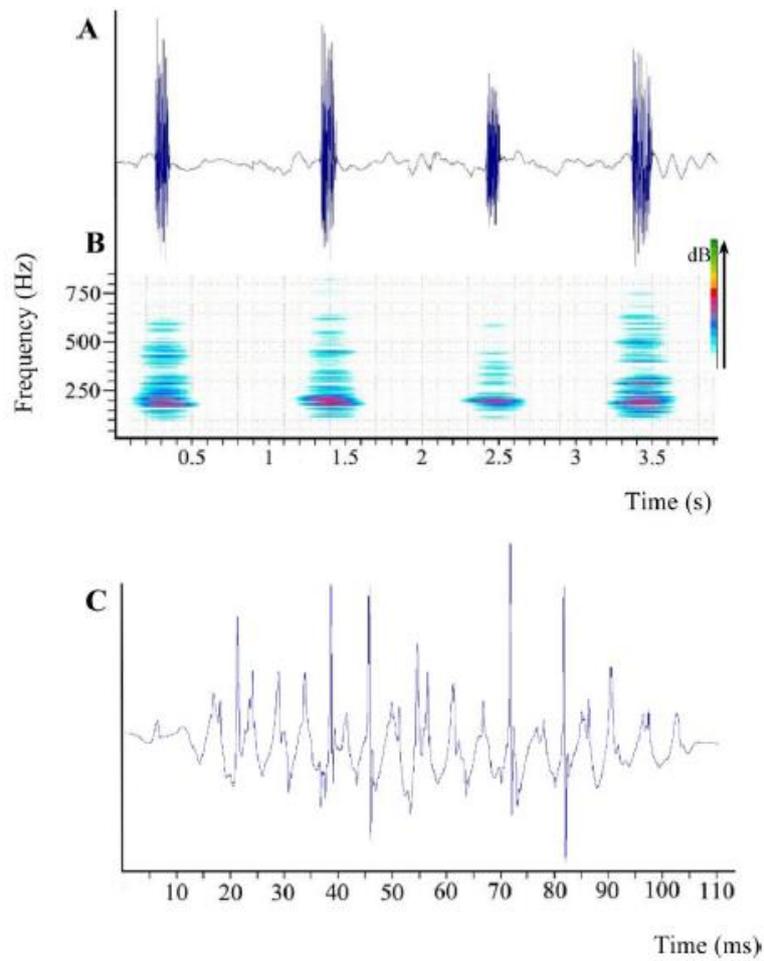


Fig. 1. Oscillogram (A) and corresponding spectrogram (B) of four sounds in *Rhinecanthus aculeatus*. (C) An enlargement of A showing the different peaks of one sound. The colour scale in B corresponds to the relative intensity of the sound.

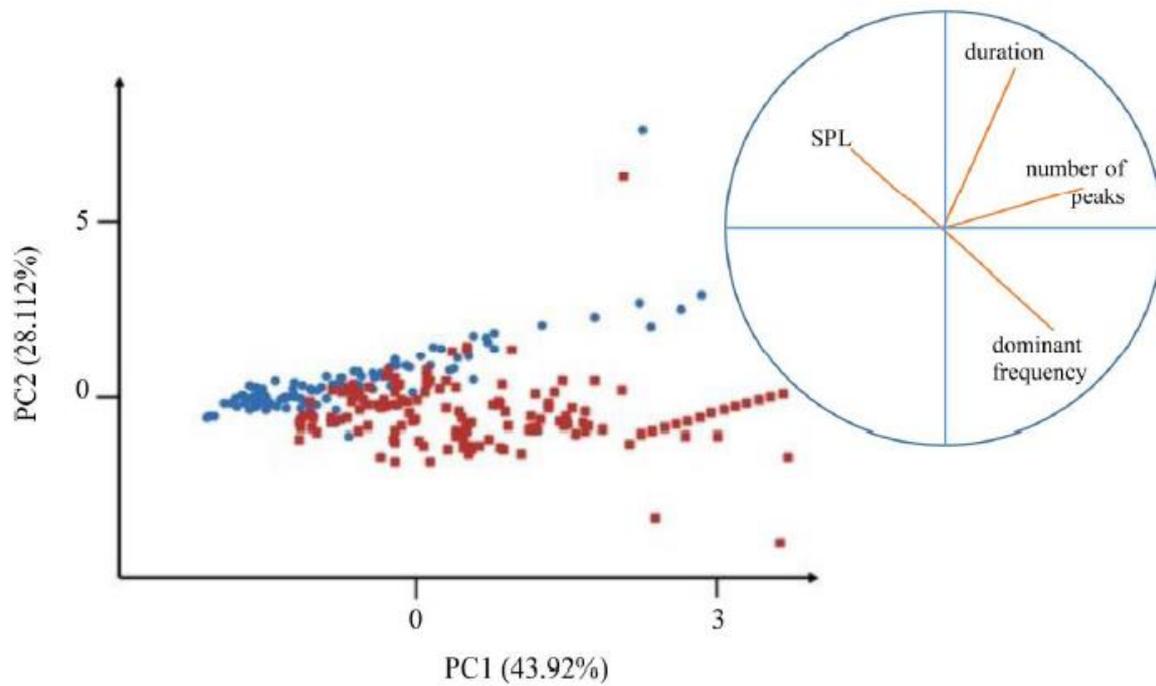


Fig. 2 Scatterplot of principal component PC1 versus PC2 from a principal component analysis on the four acoustic parameters (A) whose loadings are shown in the projection of the variables on the factorial plan (circle). Each point corresponds to a sound: *Rhinecanthus aculeatus* adults are in blue and juveniles are in red.

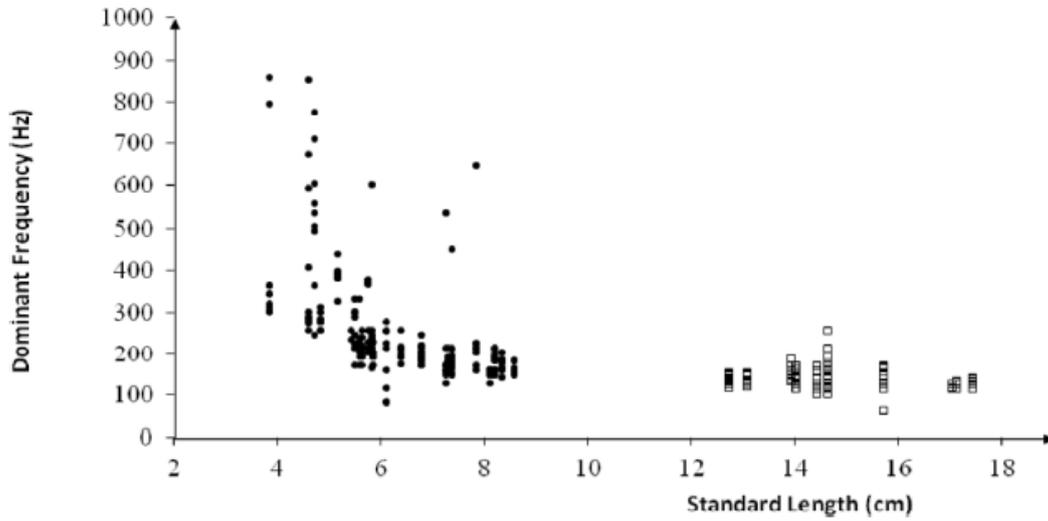


Fig. 3 Relationship between *Rhinecanthus aculeatus* size and dominant frequency of its sound. Black dots: juveniles (n = 25), white squares: adults (n = 11). Ten sounds/specimen are used.

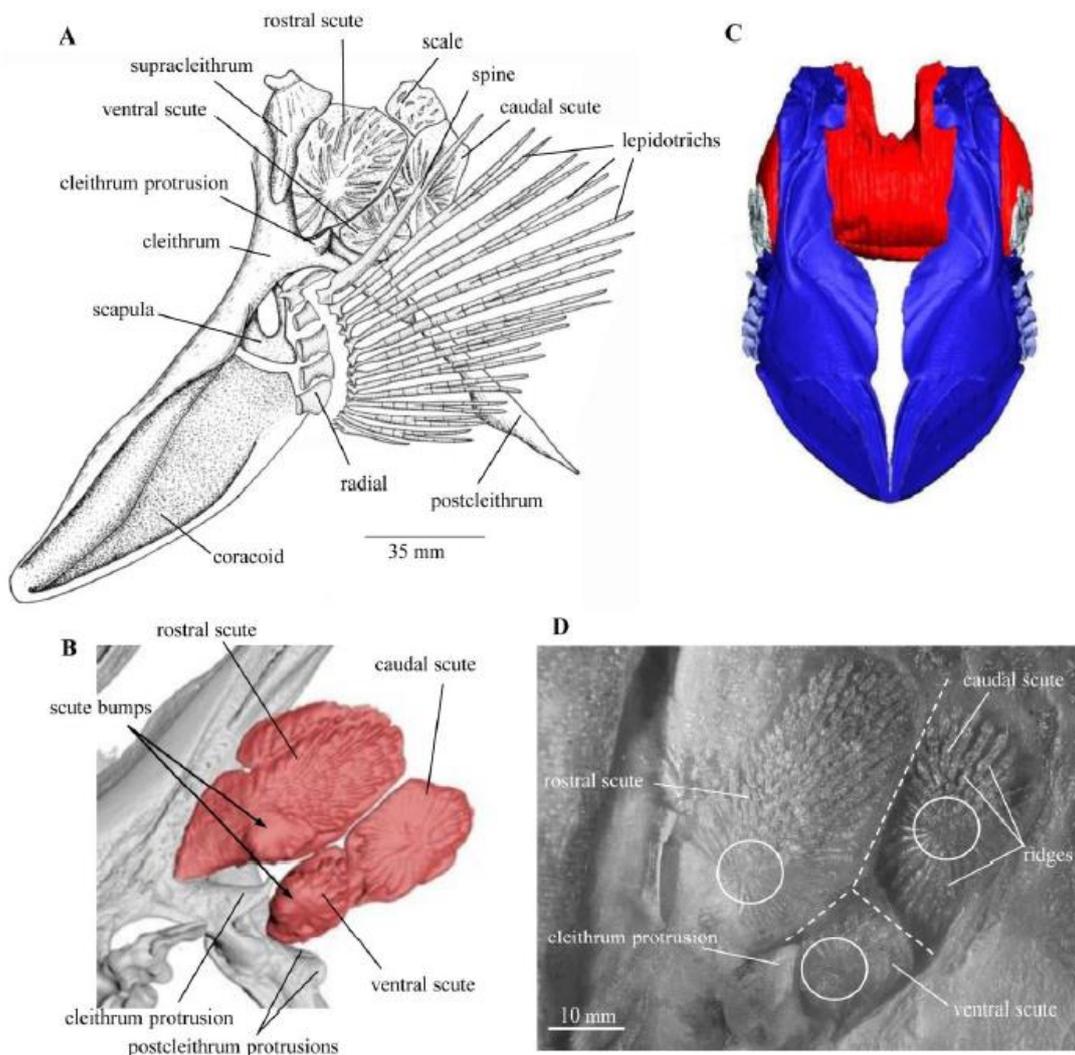


Fig. 4. *Rhinecanthus aculeatus*. Left lateral view of left scutes, pectoral girdle and fin (A), Reconstruction of the scutes based on CT scan (B). Frontal reconstruction (C) of the swimbladder (red), pectoral girdle (blue), radials (grey) and scutes (white) based on CT scan. Picture of the left lateral scutes (D). The dotted lines correspond to the inner junctions of the scutes and the zone of high flexibility of the swim bladder fenestra. Circles are placed at the level of the bumps of the scutes. The three scutes cover the swim bladder fenestra.

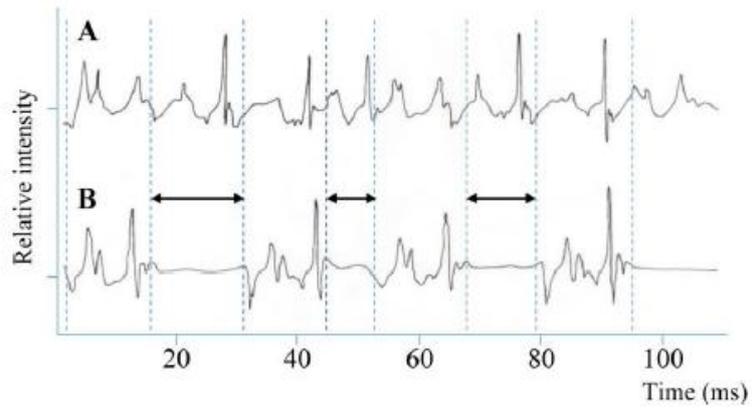


Fig. 5. Comparison of the waveforms of the sounds produced by hand-held *Rhinecanthus aculeatus* in two situations: both pectoral fins are free to move (A) and one pectoral fin is immobilized (B). Dotted lines allow the distinction between both situations with silent periods (double arrow) in B.

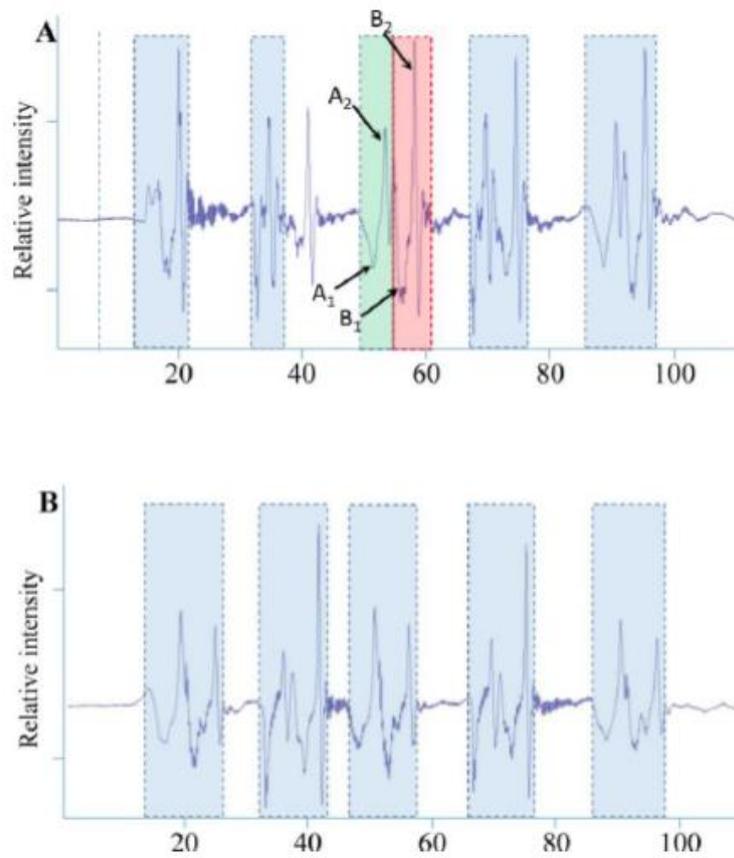


Fig. 6. Oscillogram comparing the sounds produced by hand-held *Rhinecanthus aculeatus* in two situations: left (A) or right (B) pectoral fin is immobilized. Blue boxes in dotted lines distinguish the different pulses. Each pulse is made of two main cycles. The green box corresponds to the first cycle and red box to the second cycle. The negative peak of the first (A1) and second cycles (B1) corresponds to the buckling (= the inward movement of the scutes) of the scutes and the positive peaks of the first (A2) and second (B2) cycles correspond to the recoil movements (= the outward movement of the scutes).

