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CORRECTION

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

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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.

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RESEARCH ARTICLE

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

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ABSTRACT

The ability to produce sound 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 the buccal teeth and movements of the fins. The best-supported hypothesis involves movements of the pectoral fin against the lateral part 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*, which are like short drum rolls with an average duration of 85 ms, 193 Hz dominant frequency and 136 dB SPL level at 3 cm distance. The 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 of 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 probably found in many members of Balistidae because these peculiar scutes occur 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.

KEY WORDS: Acoustic, Communication, Swimbladder

INTRODUCTION

In the field of fish acoustic communication, triggerfishes (Balistidae) are particularly well known because their ability to produce sounds has been reported for over a century (Moseley, 1879; Cunningham, 1910; Fish, 1954). 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 these have not been clearly described. One example is sounds produced during feeding activities. These result from collision of the buccal teeth, e.g. in *Balistes capriscus* (Vincent, 1963), or occur during grating of pharyngeal teeth in *B. capriscus*, *Balistes 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 and Boyle, 2014), it is unclear whether they are used for communication. In some triggerfishes (*O. niger*, *B. capriscus*), sounds could also result from movement of the spine of the first dorsal fin, which can be locked into an erect position (Fish et al., 1952; Schneider, 1961). However, this sound-producing mechanism requires greater description and an associated behavior has not been discovered. 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*, *Melichthys vidua*, *Sufflamen bursa* and *Sufflamen fraenatum* (Salmon et al., 1968). The swimbladder is large and is in close contact with 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 sound (Fine and Parmentier, 2015). As a result, the updated hypothesis was that 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; Sörensen, 1895). Two observations contradict this assumption. First, it has been experimentally shown in distantly related species that 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 instead 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 these two species at least,

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Received 26 July 2016; Accepted 17 October 2016

sounds are dictated by contraction dynamics of superfast sonic muscles (Fine et al., 2001, 2009; Millot et al., 2011). Studies in these fishes suggest that long sounds recorded in different members of the Balistidae are unlikely to be the result of a single strike (Salmon et al., 1968). Second, in their 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.

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 and Johannes, 1980). The male is polygynous and actively defends a territory housing two to five females (Künzli and Tachihara, 2012; Kuwamura, 1997). Calls of the species were reported 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 (1889) believed drumming sounds resulted from stridulation between the post-cleithrum and the inner surface of the cleithrum. 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 movement of structures.

The aim of this study was to provide a first quantitative description of the sound produced by the triggerfish *R. aculeatus* and to unravel its sound-producing mechanism. Moreover, sounds were recorded in specimens of different lengths to determine the effect of size on acoustic characteristics.

MATERIALS AND METHODS

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.

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″W) and Pihaena beach (17°29′06″S, 149°49′50″W). 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 (28–29°C) on a natural light cycle (12 h light:12 h dark). Nine subadult 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 saltwater aquaria (with sand and hiding places) at 26–28°C 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 μPa^{-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 held by hand in the tank approximately 3 cm from the hydrophone, with the dorsal fin blocked. Ten sounds were recorded for each fish.

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; the recording tank was 99 cm×39 cm×25 cm in Liège and 83 cm×53 cm×23 cm in French Polynesia, with estimated minimum resonance frequencies of 3640 and 3670 Hz, respectively (Akamatsu et al., 2002). A band-pass filter (0.05–3.5 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 (time from the beginning to the end of the sound, ms), pulse period (time between the onset of two consecutive pulses, ms), pulse duration (time 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, fast Fourier transform (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).

Morphological study

Six specimens (TL: 4.4–18.4 cm) were killed 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 standard length, 233 mm TL) was scanned at the AST-RX technical platform at the Muséum National d'Histoire Naturelle with a microCT 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 µm. Segmentation and surface rendering of the cleithrum, post-cleithrum, radials, scutes and swimbladder were done in Amira 5.4.0 (FEI, Hillsboro, OR, USA). Automatic thresholding was used to identify bones, whereas the swimbladder membrane was outlined by hand.

Functional study

Pectoral fin (spine and rays) movements of five fish were recorded at 500 frames s^{-1} with a high-speed camera (model NX4-S1, IDT Vision, Tallahassee, FL, USA; 640×456 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, IDT Vision) allowing synchronization with an 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, Shaw Island, WA, USA) on the pectoral fin. Movements were observed with MotionStudio software (IDT Vision).

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; and (3) after deflation of the swimbladder, by puncturing the swimbladder wall of anesthetized specimens and removing gas using a 1 ml syringe. Sounds of treated specimens were compared with controls.

Statistics

Juvenile and adult 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. Juvenile and adult sounds were compared with Wilcoxon signed-rank tests. The correlation between body size and acoustic features was examined with a Spearman correlation matrix associated with a *P*-value (Holm–Bonferroni adjusted method) matrix. The sounds produced by one or both pectoral fins were compared with the non-parametric 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.

RESULTS

Sounds

Sounds were recorded in both juveniles (4.8–9.8 cm) and adults (15.9–20.6 cm). The calls were clearly similar in the two groups (Table 1) and sounded like short drum rolls (Fig. 1). These sounds were composed of 13.7±3.5 peaks (mean±s.d.; 9 specimens, 90 sounds, TL: 6.4–10.1 cm). They lasted 85±22 ms, and had a dominant frequency of 193±38 Hz and an amplitude of ca. 136±16 dB re. 1 µPa.

A PCA was conducted to determine whether variation in mean sound characteristics among individual fish is related to sexual maturity. The PCA incorporated the spectral and duration features of individual sound events, and indicated broad acoustic similarity between juveniles and adults (Fig. 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 in the number of peaks ($P=0.014$).

Size was positively correlated with SPL ($r_s=0.49$, $P<0.01$) 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 was not linear (Fig. 3); dominant frequency decreased with fish size before reaching a plateau (between 6 and 85 mm TL).

Table 1. Acoustic characteristics of sounds recorded in *Rhinecanthus aculeatus*

Calls	Adults (*N*=110)	Juveniles (*N*=250)
Call duration (ms)	93±63	71±42
Dominant frequency (Hz)	140±24	248±126
Sound level (dB SPL)	152±3	139±14
No. of peaks	10.5±6	11.6±5.5

Eleven adults (total length, TL: 15.9–20.6 cm) and 25 juveniles (TL: 4.8–10.1 cm) were used. Data are means±s.d. SPL, sound pressure level.

Morphology

Previous dissections of 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. The portion of the swimbladder that does not have a tunica externa is usually called the swimbladder fenestra (Mok et al., 2011; Parmentier et al., 2003, 2016). The swimbladder is conical, enlarged at the anterior end and tapering 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 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. The 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 following bones: the post-temporal, supracleithrum, cleithrum, scapula, coracoid and post-cleithrum. Three osseous protrusions can de distinguished. The first is found on the cleithrum (Fig. 4A), between the bases 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

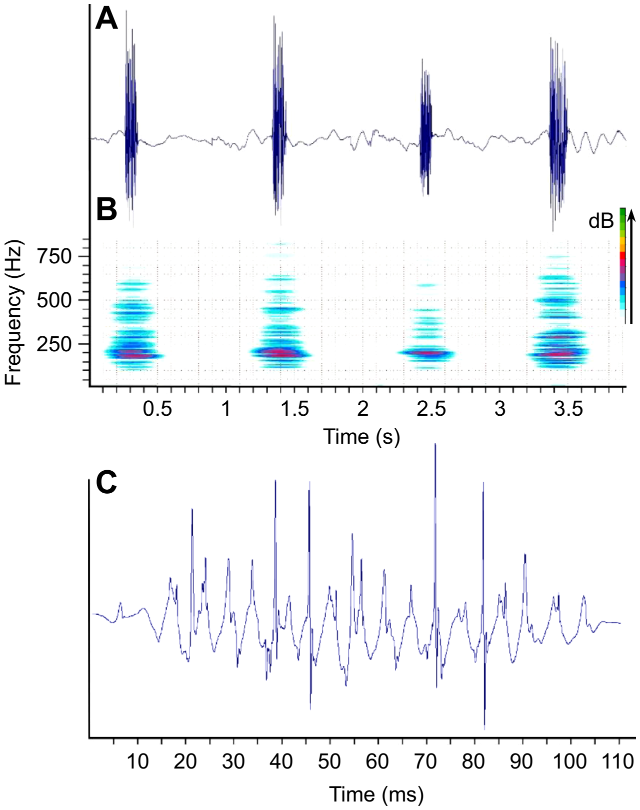

Fig. 1. Illustration of sounds produced by *Rhinecanthus aculeatus*. (A) Oscillogram and (B) corresponding spectrogram of four sounds. (C) An enlargement of A showing the different peaks of one sound. The color scale in B corresponds to the relative intensity of the sound.

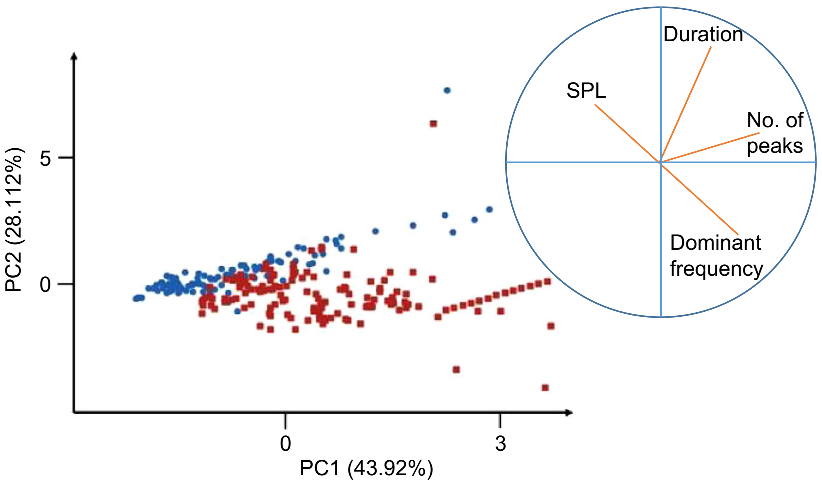

Fig. 2. Principal component analysis of acoustic parameters. The scatterplot is of principal component 1 (PC1) versus PC2; loadings are shown in the projection of the variables on the factorial plan (circle). Each point in the scatterplot corresponds to a sound: *R. aculeatus* adults are in blue and juveniles are in red. SPL, sound pressure level.

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).

Sound production mechanism

No drumming sounds were detected when both pectoral fins were held immobile against the side of the body. However, immobilization of one pectoral fin revealed sounds consisting of a suite of pulses produced by the alternating movements of the left and right pectoral fins (Fig. 5). Holding one fin did not change the sound duration but reduced the number of peaks by half (Fig. 5; Table 2), highlighting that a pulse is made of two consecutive cycles (Fig. 5). In this case, the pulse period was 23±10 ms (Table 2), which indicates it was highly variable, from 14 to 40 ms (Fig. 6). The period usually increased 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 revealed they were not statistically different (Fig. 6; Oja, *P*=0.08), meaning the mechanism was the same for both fins.

Removing the dorsal pectoral spine (Fig. 4) decreased sound amplitude by 33 dB but did not change sound duration or dominant frequency (*P*=0.55 and 0.45, respectively; Friedman test). Moreover, the number of peaks was similar in the two cases (*P*=0.86, paired Student *t*-test).

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

High-speed video showed 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 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 (Fig. 6, blue boxes). Comparison of high-speed movies with the waveform in oscillograms showed the first cycle corresponds to the first contact of the scutes with the swimbladder and the second cycle corresponds to the second contact. During each contact, the negative peak of the cycle (Fig. 6, A_1 or B_1) corresponds to the buckling (i.e. inward displacement of the scutes) of the body wall and the positive part of the cycle (Fig. 6, A_2 or B_2) 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 two cycles again when touching the scutes. In summary, the pulse is made of two consecutive cycles and corresponds to one fin movement.

DISCUSSION

The present study provides the first physical description of the drumming sounds in *R. aculeatus*. Although some data are lacking

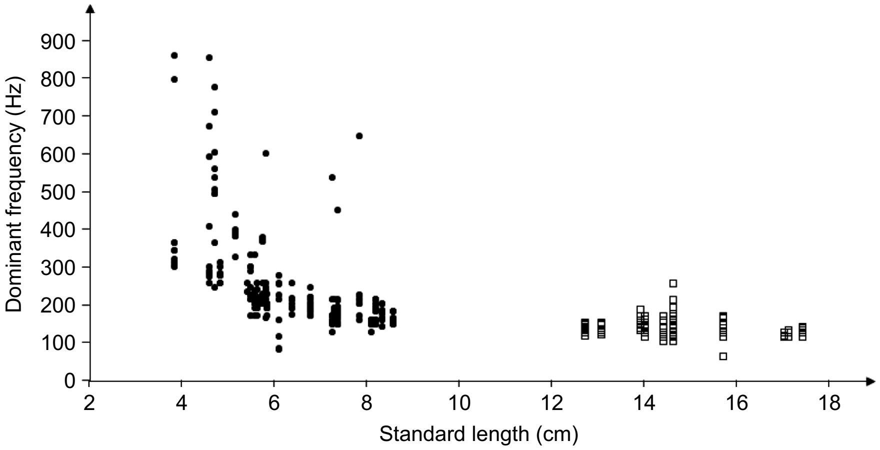

Fig. 3. Relationship between *R. aculeatus* size and the dominant frequency of its sound. Black circles, juveniles (*N*=25); white squares, adults (*N*=11). Ten sounds per specimen were used.

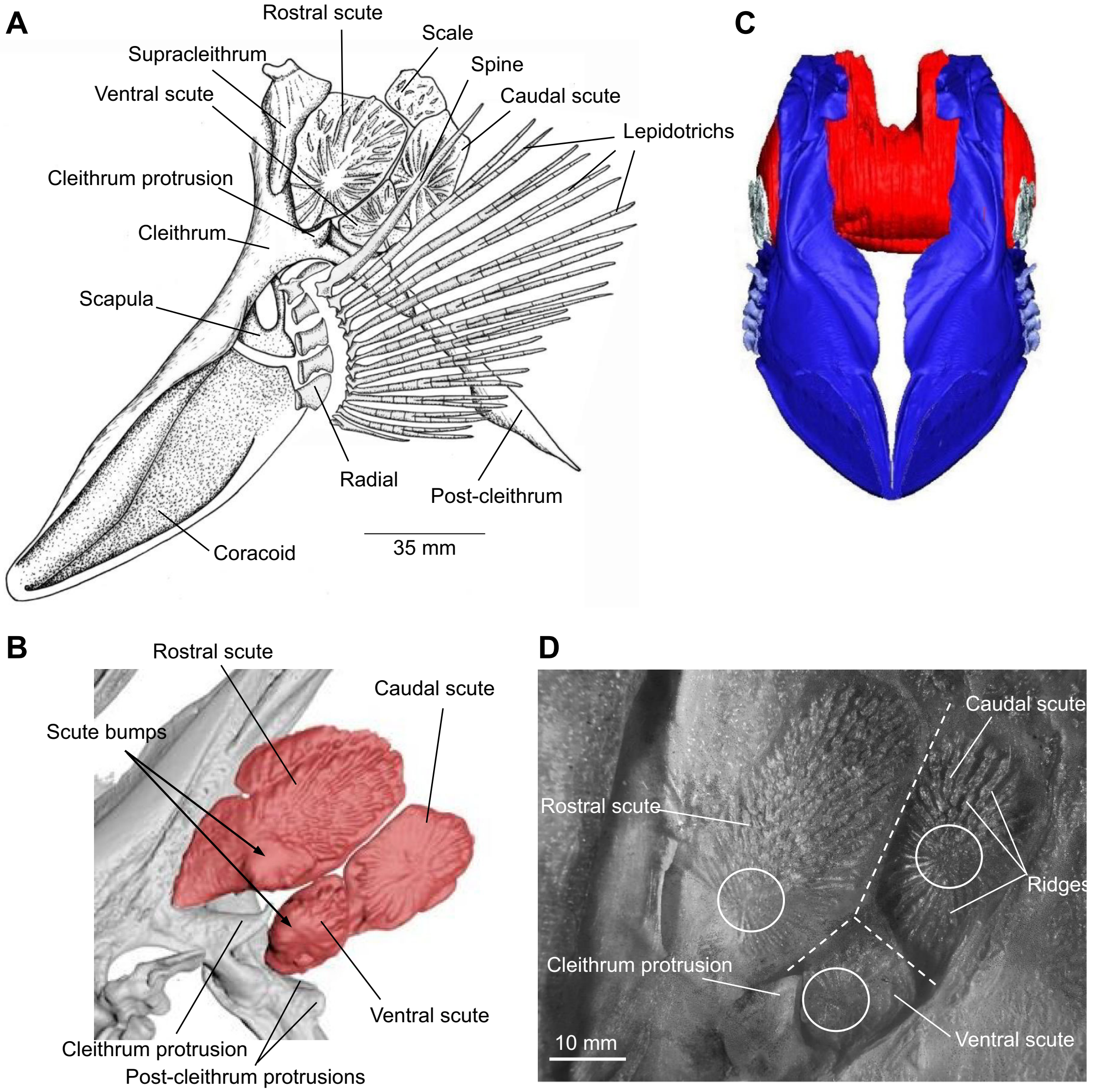

Fig. 4. ***Rhinecanthus aculeatus.*** (A) Left lateral view of left scutes, pectoral girdle and fin. (B) Reconstruction of the scutes based on the CT scan. (C) Frontal reconstruction of the swimbladder (red), pectoral girdle (blue), radials (gray) and scutes (white) based on the CT scan. (D) Picture of the left lateral scutes. 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.

from other balistids (*B. capriscus*, *B. undulatus*, *R. rectangulus*, *M. niger*) that appear to use a similar pectoral fin–swimbladder mechanism to produce sounds (Salmon et al., 1968; Vincent, 1963), the sounds produced by *R. rectangulus* appear to be 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, 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. More-detailed 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 that of *R. rectangulus*.

In most of the fishes that use 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, 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).

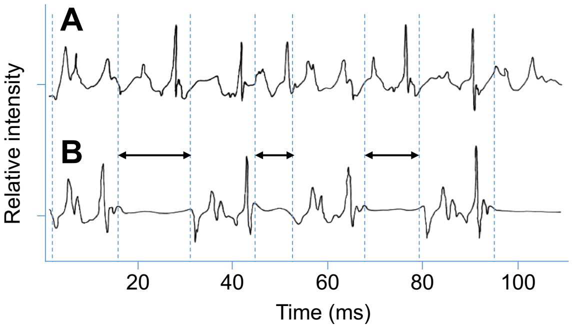

Fig. 5. Comparison of the waveforms of the sounds produced by *R. aculeatus* with and without pectoral fin immobilization. Fish were held by hand and (A) both pectoral fins were free to move or (B) one pectoral fin was immobilized. Dotted lines allow comparison between the two situations, with silent periods indicated by the double-headed arrows in B.

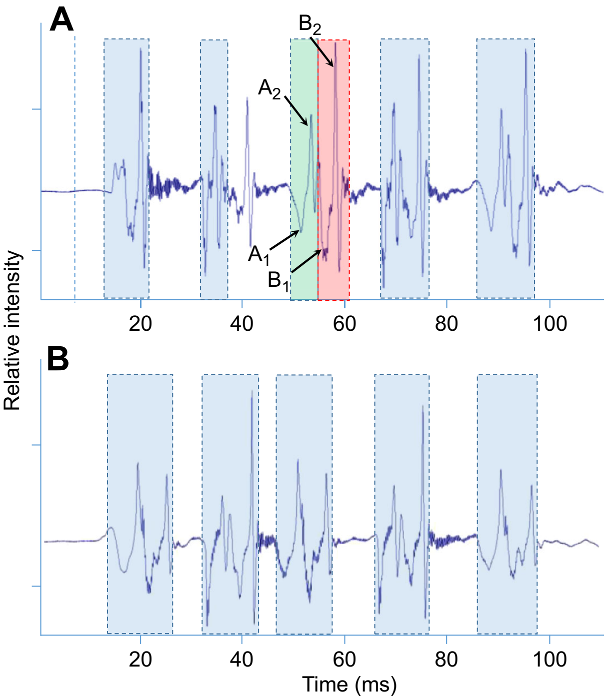

Fig. 6. Oscillogram comparing the sounds produced by *R. aculeatus* with and without pectoral fin immobilization. Fish were held by hand and (A) the left or (B) the right pectoral fin was immobilized. Blue boxes distinguish the different pulses. Each pulse is made of two main cycles: the green box corresponds to the first cycle and the red box to the second cycle. The negative peak of the first (A_1) and second cycles (B_1) corresponds to the buckling (inward movement) of the scutes and the positives peaks of the first (A_2) and second (B_2) cycles correspond to the recoil (outward movement) of the scutes.

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 (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 movements of the scutes. The pectoral fin actively deforms the scutes, which rapidly bend inward, pushing the wall of the swimbladder, increasing its internal pressure and creating the first negative peak (Fig. 6, A_1). The first positive peak (Fig. 6, A_2) is produced by passive recoil of the fenestra. This 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 sweep of the spine results in a pulse made of two cycles. However, it is not possible to distinguish the pulses when right and left pectoral fins participate together to produce sounds. During sound production, the pectoral fins complete a cycle between 15 and 40 ms, corresponding to about 25–60 beats s^{-1}. This is higher than typical rates observed for locomotion because pectoral fin beat frequencies can be from 20 to 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 have also 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 is required to determine whether acoustic related movements of the fins correspond to a new motor pattern or are co-opted from locomotion movements. Balistiform locomotion is the swimming mode in triggerfishes, which 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.

Table 2. Comparison of the sounds made by *R. aculeatus* when both pectorals are free to move and when one of the two pectoral fins is immobilized

	Free pectoral fins (*N*=9)	One pectoral fin immobilized (*N*=9)	*P*-value (Wilcoxon)
Duration (ms)	85±22	88±36	0.78
Dominant frequency (Hz)	193±38	252±121	<0.01
Sound intensity (dB SPL)	136±15	143±5	0.2
No. of peaks	13.7±3	6.9±2.5	<0.01
Pulse duration (ms)	–	12±7	–
Pulse period (ms)	–	23±10	–

Nine fish were used in each experiment. Fins were immobilized by being held by hand. Data are means±s.d.

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, which 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. 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 as its ablation corresponds to a lowering of the sound level by 26 dB. The weaker intensity due to the cutting of the pectoral spine has previously been 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 a 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 that also involves 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 socket 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 as electromyography experiments showed 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 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.

Acknowledgements

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.

Competing interests

The authors declare no competing or financial interests.

Author contributions

E.P., L.K. and D.L. designed the study; X.R., K.B., S.V. and L.K. carried out the experiments; K.B. performed µCT scans; X.R., S.V. and L.K. performed high-speed camera experiments; X.R. and E.P. analyzed the data; E.P., L.K. and X.R. wrote the manuscript. All authors gave final approval for publication.

Funding

This research was funded by a grant from the Agence Nationale de la Recherche [ANR-16-ACHN-0006-01 to S.V.W. and ANR-11-JSV7-012-01 to X.R. and D.L.] and by a grant from the Fonds De La Recherche Scientifique - FNRS [T.0056.13 to E.P.].

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.146514.supplemental

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