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SHORT COMMUNICATION

Sound production mechanism in triggerfish (Balistidae): a synapomorphy

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ABSTRACT

The ability to produce sounds for acoustic communication is known in different Balistidae species but the eventual synapomorphic aspect of the mechanism remains to be shown. In *Rhinecanthus aculeatus*, sounds result from alternate sweeping movements of the right and left pectoral fins, which push a system of three scutes against the swim bladder wall. In this study, we made a comparison between the sounds produced by this species and two additional ones (*Balistapus undulatus* and *Rhinecanthus rectangulus*) using hand-held specimens to provide a description of the sound mechanism. The results highlighted that the sound production mechanism is similar in the three species. According to recent phylogenetic data and shared morphological features, this mechanism could be common to the majority of Balistidae family members and all species could be capable of sound production using pectoral fins.

KEY WORDS: Acoustic repertoire, Exaptation, Vocal communication, Swimbladder

INTRODUCTION

Triggerfishes (Balistidae) are commonly found in shallow tropical waters (Berry and Baldwin, 1966; Chen et al., 2001; Fish, 1948; Lobel and Johanne, 1980). They are known to produce sounds during agonistic interactions or when a fish is chased into a narrow hole (Salmon et al., 1968).

Sounds were also recently described in *Rhinecanthus aculeatus* in a distress situation when the fish were held in the hand. This study allowed the description of the associated mechanism of sound production (Parmentier et al., 2017b). The acoustic signals in adult specimens sound like a short drum roll, with an average duration of 85 ms and a dominant frequency of 193 Hz (Parmentier et al., 2017b). They result from alternate sweeping movements of the right and left pectoral fins, the spines of which push a system of three scutes against the swimbladder wall. The scutes are first bent inward, pushing the wall of the swim bladder, before passive recoil restores the scute resting position. These back-and-forth movements cause a single cycle within the call. Together, the alternating work pattern of the two pectoral fins produces cycles of acoustic signals (Parmentier et al., 2017b).

In the present study, we investigated sound production in three species of triggerfishes in French Polynesia that are similar in size, occur at the same depth and share the same diet: orange-lined triggerfish *Balistapus undulatus* (Park 1797), Picasso triggerfish *Rhinecanthus aculeatus* (Linnaeus 1758) and wedge-tail triggerfish *Rhinecanthus rectangulus* (Bloch & Schneider 1801). *Balistapus undulatus* is widely distributed both in the lagoon and on the external slope (Bean et al., 2002). *Rhinecanthus aculeatus* lives in all the sandy parts of the reef but it is more abundant in the shallow parts of the lagoon. *Rhinecanthus rectangulus* lives mainly on the superior part of the external slope, but it can also live on large sandy areas. The objectives of this study were to compare the acoustic characteristics of sounds in *B. undulatus*, *R. aculeatus* and *R. rectangulus* adults, and to determine the potential unity of their sonic mechanisms.

MATERIALS AND METHODS

Study site and sampling

All the individuals used in the sound analysis were collected along the north coast of Moorea (French Polynesia) between February and April 2015. Ten *B. undulatus* [standard length (SL) 10.59–15.35 cm] were collected by hand while snorkelling in the lagoon, from the crevices where they take refuge, at a depth of 0.5–1.5 m. Eleven *R. aculeatus* (SL: 12.7–17.4 cm) were collected with a bottom gillnet (length: 25 m, mesh: 2.5 cm) on a sandbank at a maximum depth of 3 m in the lagoon. Ten specimens of *R. rectangulus* (SL: 8.1–15.3 cm) were collected by hand while scuba-diving in the shallowest part of the external slope close to the pass of Taotoi. An additional five *B. undulatus* were collected in the atoll of Fakarava in June 2017 for analysis of their sound mechanism.

Recordings and sound analyses

After 10 days of acclimatization in tanks (137 cm×68 cm×60 cm) with running seawater on a natural light cycle (12 h light:12 h dark), the specimens were recorded individually in a glass aquarium (83 cm×53 cm×23 cm) with water temperature maintained at 26.5 ±0.5°C and equipped with a hydrophone HTI-96-MIN (sensitivity: −163.9dB V μPa^{-1}; High Tech Inc., Long Beach, MS, USA) connected to a TASCAM DR-07 recorder (TEAC, Wiesbaden, Germany). Individuals were held in the hand underwater and the distance between the fish mouth and the hydrophone was 3 cm. The sounds were digitized at 44.1 kHz (16-bit resolution) and analysed with AvisoftSAS LabPro 5.2 software (Avisoft Bioacoustics, Glienicke/Nordbahn, Germany). A low-pass filter at 3.5 kHz was applied to all recordings to delete the frequencies above the lowest resonant frequency of the aquarium calculated from the equation presented in Akamatsu et al. (2002). In Fakarava, recordings of *B. undulatus* were additionally made after immobilization of one (or both) pectoral fin(s). All the experiments complied with the ethical rules of the University of Liège and the CRIOBE.

Only sounds with a high signal to noise ratio (at least 3 dB above the background noise) were analysed with AviSoft-SAS Lab Pro 5.2 software. The call duration (time from the beginning to the end of the sound) was measured on the oscillogram while the dominant

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frequency (frequency component with the most energy, Hz) was measured on power spectra [Hamming window, fast Fourier transform (FFT): 512 points].

Morphology and mechanism analysis

Three specimens of each species were killed with an overdose of MS222, fixed in 5% formaldehyde for 2 days and transferred to 70% alcohol for conservation. The scutes and the pectoral fin of each species were examined and photographed under a stereoscopic microscope coupled with a camera (Leica Mc170 Hd with LAS EZ). In Fakarava, pectoral fin movements of two *B. undulatus* were recorded at 120 frames s^{-1} with a GoPro (model HERO 4) and observed with GoPro Studio software.

Statistical analysis

Ten sounds were recorded from 10 specimens of each species. Data concerning *R. aculeatus* were taken from a prior study (Parmentier et al., 2017b). Statistical analyses were done with R software 3.0.2. The normality of the data was checked with the Shapiro–Wilk test. The homoscedasticity of the variances was checked with the Bartlett test. As the conditions for parametric tests were not met, the acoustic characteristics of the different species were compared with univariate Kruskal–Wallis (K–W) tests followed by Siegel and Castellan *post hoc* tests for species comparison. No individual means were calculated. According to normal distribution, the correlation between body size and acoustic features was examined with a Spearman or a Pearson test. The level of uncertainty was 0.05 for all the tests.

RESULTS AND DISCUSSION

Sounds were produced in the same behavioural context. Recording these sounds when the fish were held in the hand shows they can be emitted in a stressful situation. It is interesting to note that the same kinds of sounds were produced when the fish were caught in a net or when a diver was chasing them or removing them from the cavities where they take refuge (X.R., unpublished observation). Sound production under these circumstances is probably a behavioural adaptation which, in addition to the bony plates and trigger mechanism, serves to increase survival of these relatively slow-moving fish (Salmon et al., 1968).

In the three species, sounds were similar to short drum rolls and consisted in a series of consecutives cycles (Fig. 1). The sound duration was 70±26 ms (mean±s.d.) in *B. undulatus* (n_{fish}=10, n_{sounds}=100) and 93±37 ms in *R. rectangulus* (n_{fish}=10, n_{sounds}=100). The dominant frequency was 140±23 and 154±28 Hz in *R. rectangulus* and *B. undulatus*, respectively. In comparison, adults and juveniles of *R. aculeatus* produce sounds with a duration of 93±63 ms (n_{fish}=11, n_{sounds}=110) and a dominant frequency of 140±24 Hz (Parmentier et al., 2016).

The sounds of the three species showed significant differences at the level of both the total duration (P<0.01, χ^2=26.85, d.f.=2; K–W test) and the dominant frequency (P<0.01, χ^2=22.23, d.f.=2; K–W test). Sounds of *B. undulatus* were different from those of *R. aculeatus* and *R. rectangulus* in terms of total duration and dominant frequency (all P<0.05; Table 1). The sounds of *B. undulatus* were shorter and had a higher frequency than those of the *Rhinecanthus* species. The total duration and dominant frequency of *R. aculeatus* and *R. rectangulus* were not differently significant (P>0.05 for both; Table 1).

The configuration of the sound-producing mechanism that was described by Parmentier et al. (2017b), i.e. a large rostral scute, a large caudal scute and a small ventral scute facing the drumming membrane of the swimbladder, was found in the three species (Fig. 2). However, the rostral and the caudal scutes were smaller in *B. undulatus* and they had an additional large transition scale that we can call the dorsal scute (Fig. 2C).

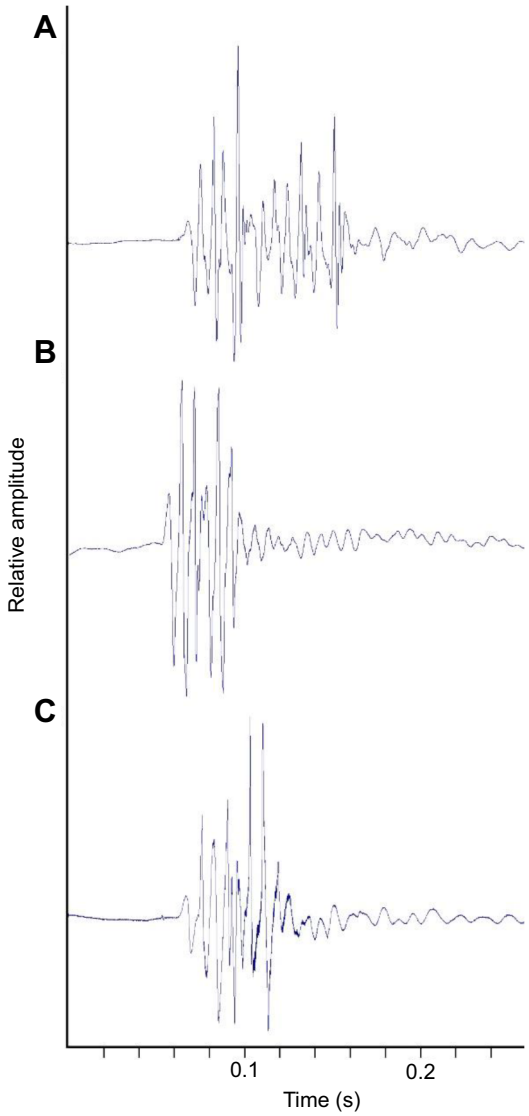


Fig. 1. Oscillograms of pectoral sounds. (A) *Rhinecanthus aculeatus*, (B) *Rhinecanthus rectangulus* and (C) *Balistapus undulatus*.

No sound was detected when both pectoral fins were immobilized against the body. When both pectoral fins were free, in-depth analysis of call waveform was difficult, probably because the sound peaks resulting from the movement of one pectoral fin could alter the sound resulting from movement of the other fin. After the immobilization of one pectoral fin, the waveform showed more

Table 1. Pairwise comparisons (Siegel and Castellan *post hoc* test) of sounds in Balistidae

	Frequency		Duration	
	Test	*P*	Test	*P*
R. rectangulus versus *B. undulatus*	2.90	0.011	5.03	<0.05
R. rectangulus versus *R. aculeatus*	1.71	0.26	1.52	0.38
B. undulatus versus *R. aculeatus*	4.68	<0.05	3.63	<0.05

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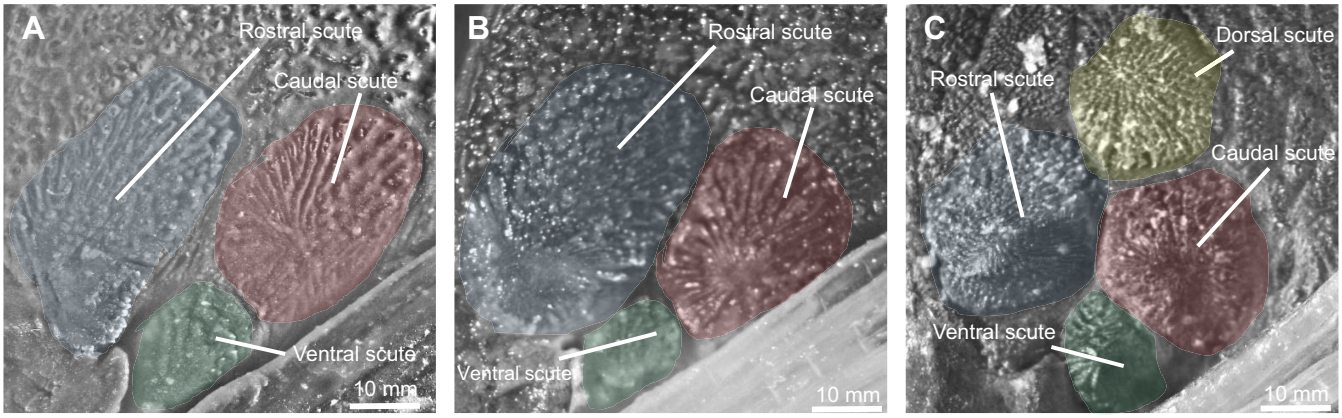


Fig. 2. Balistidae scutes. (A) *Rhinecanthus rectangulus*, (B) *Rhinecanthus aculeatus* and (C) *Balistapus undulatus*. The rostral scute is in blue, the caudal scute is in red, the ventral scute is in green and the dorsal scute is in yellow.

distinct units and a repetitive schema where each pulse was mainly made of two cycles. The pulse duration was 14±3 ms (n=19) and the pulse period was 19±3 ms (n=9). This short pulse period suggests there is an overlap between pulses when both pectoral fins are free to move and most probably explains why it is not possible to discriminate correctly groups of cycles in fish with free pectoral fins (Fig. 3). The videos did not allow an accurate description of the movement of the scutes as previously done in *R. aculeatus* (Parmentier et al., 2017b) because movements were only recorded at 120 frames s^{-1}. However, the videos clearly show that the sounds were produced during sweeping movements of the pectoral fins against the scutes. Typically, the pectoral fin was first aligned against the lateral wall, rostral to the scutes. This was followed by a rapid backward movement of the fin (the pectoral spine being against the body) and the production of the first cycle before the fin returned to its initial position, initiating the second main cycle.

These morphological data together with the functional data from *B. undulatus* and *R. aculeatus* clearly support the use by these three species of the same sound-producing mechanism. Salmon et al. (1968) described the same kinds of movements of the stiff pectoral fin spine across the scutes in *R. aculeatus*, *Melichthys niger* and *Balistes vetula*. Deprived of a high-speed camera and knowledge on the swimbladder wall properties, they erroneously suggested a 'drumming membrane mechanism'. The more detailed description of the mechanism we provided in our previous study (Parmentier et al., 2017b) has to be considered as a better explanation. These morphological features related to sound production – pectoral fins, enlarged scales (scutes) forming a flexible tympanum above the pectoral fin base and associated with the swim bladder – were observed in all the 12 Balistidae genera except *Canthidermis* and *Xanthichthys* (Matsuura, 1979, 2001, 2015).

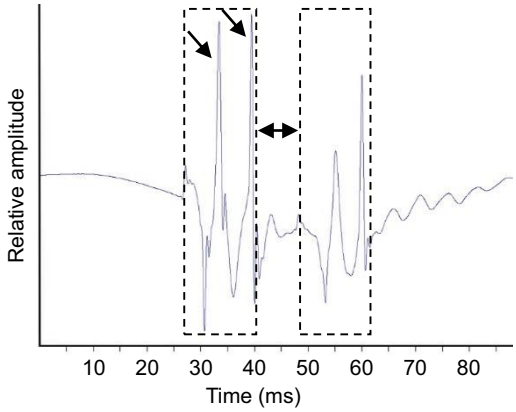


Fig. 3. Waveforms of the sounds produced by *B. undulatus* with one pectoral fin immobilized. Each pulse is made of two main cycles (single arrows within the box). It is not possible to distinguish the silent duration (double-headed arrows) when left and right pectoral fins are free (compare with Fig. 1C) because they are both involved in sound production.

McCord and Westneat (2016) recently divided Balistidae (42 species) into three clades, with *Rhinecanthus* in Clade 3 and *Balistapus* in Clade 2. Interestingly, Clade 2 also includes *Melichthys* and Clade 3 includes *Sufflamen*, which are both able to produce sound with their pectoral fins (Salmon et al., 1968). All together, these data are in support of the sound-producing mechanism being common to the two clades. However, *Balistes vetula* Linnaeus 1758, which belongs to Clade 1, also uses its pectoral fins to make sounds (Salmon et al., 1968), suggesting that the mechanism is common to all the family. In another phylogeny (Santini et al., 2013), Balistidae are divided into two clades: *Balistes vetula* and *Rhinecanthus* sp. are in clade A and *Balistapus undulatus* is in clade B, leading to the same conclusion regarding the sound-production mechanism. However, the situation is less clear in *Canthidermis* and *Xanthichthys* genera. The current phylogenies indicate that both genera are distributed among calling species (Faircloth et al., 2013; McCord and Westneat, 2016) but they seem to be deprived of enlarged scales above the pectoral fin base (Matsuura, 2001) meaning they are not able to make sounds or that the mechanism has been modified in these genera.

In the sister family (Monacanthidae), similar morphological features have not been described and sounds resulting from pectoral fin movements have not been reported. Therefore, we can reasonably argue that the detailed sonic mechanism using pectoral fins, scutes and the swim bladder could be a strong Balistidae feature. It could have evolved from locomotory movement and be a new example of exaptation (Parmentier et al., 2017a). Despite large similarities, sounds of *B. undulatus* are different from the sounds of *R. aculeatus* and *R. rectangulus* in terms of total duration and dominant frequency. More in-depth studies on the mechanism are needed to determine how these acoustic features are modulated.

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Competing interests
The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.P.; Methodology: X.R., E.P.; Validation: E.P.; Formal analysis: X.R., L.K., O.C., F.B., E.P.; Investigation: X.R., L.K., O.C., F.B.; Resources: D.L.; Data curation: D.L.; Writing - original draft: E.P.; Writing - review & editing: L.K., O.C., F.B., E.P.; Visualization: D.L., E.P.; Supervision: E.P.; Funding acquisition: D.L., E.P.

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