

# Diving into dual functionality: Swim bladder muscles in lionfish for buoyancy and sonic capabilities

Eric Parmentier<sup>1</sup>  | Anthony Herrel<sup>2</sup>  | Marine Banse<sup>1</sup>  | Heidie Hornstra<sup>3</sup>  |  
Frédéric Bertucci<sup>4</sup>  | David Lecchini<sup>5,6</sup> 

<sup>1</sup>Laboratory of Functional and Evolutionary Morphology, FOCUS, University of Liège, Liège, Belgium

<sup>2</sup>Département Adaptations du Vivant, Bâtiment, UMR 7179 MECADEV C.N.R. S/M.N.H.N., d'Anatomie Comparée, Paris, France

<sup>3</sup>Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona, USA

<sup>4</sup>UMR MARBEC, IRD-CNRS-IFREMER-INRAE-University of Montpellier, Sète, France

<sup>5</sup>PSL University, EPHE-UPVD-CNRS, USR 3278 CRIOBE, Moorea, French Polynesia

<sup>6</sup>Laboratoire d'Excellence "CORAIL", Perpignan, France

## Correspondence

Eric Parmentier, Laboratory of Functional and Evolutionary Morphology, FOCUS, University of Liège, Liège, Belgium.  
Email: [e.parmontier@uliege.be](mailto:e.parmontier@uliege.be)

## Funding information

Agence Nationale de la Recherche, Grant/Award Number: ANR-19-CE34-0006-Manini; Fondation de France, Grant/Award Number: 2019-08602; Fonds De La Recherche Scientifique—FNRS, Grant/Award Number: T.0192.20 and U.N054.23; LabEx CORAIL, Grant/Award Number: project 2018 Emul

## Abstract

Although the primary function of the swim bladder is buoyancy, it is also involved in hearing, and it can be associated with sonic muscles for voluntary sound production. The use of the swim bladder and associated muscles in sound production could be an exaptation since this is not its first function. We however lack models showing that the same muscles can be used in both movement and sound production. In this study, we investigate the functions of the muscles associated with the swim bladder in different Pteroinae (lionfish) species. Our results indicate that *Pterois volitans*, *P. radiata* and *Dendrochirus zebra* are able to produce long low-frequency hums when disturbed. The deliberate movements of the fin spines during sound production suggest that these sounds may serve as aposematic signals. In *P. volitans* and *P. radiata*, hums can be punctuated by intermittent louder pulses called knocks. Analysis of sonic features, morphology, electromyography and histology strongly suggest that these sounds are most likely produced by muscles closely associated with the swim bladder. These muscles originate from the neurocranium and insert on the posterior part of the swim bladder. Additionally, cineradiography supports the hypothesis that these same muscles are involved in altering the swim bladder's length and angle, thereby influencing the pitch of the fish body and participating in manoeuvring and locomotion movements. Fast contraction of the muscle should be related to sound production whereas sustained contractions allows modifications in swim bladder shape and body pitch.

## KEYWORDS

acoustic communication, aposematism, *Pterois*, sonic muscles, swim bladder

## 1 | INTRODUCTION

In actinopterygians, the swimbladder is a gas-filled chamber situated between the digestive tract and the vertebral column. The primary function of the swim bladder is hydrostatic. By regulating gas volume in the chamber, fishes adjust the swim bladder to compensate for downdrift caused by the high density of the skeleton and to obtain adequate buoyancy in water (Fänge, 1953). In all fishes, the swimbladder is also involved in hearing abilities since its walls can be set into motion by the pressure component of sound

and produce a particle motion component that can be detected by the inner ear (Popper, 2023; Popper & Fay, 2011; Yan et al., 2000). Some phylogenetically unrelated species are also able to use the swimbladder to produce sounds for communication. Mechanisms that rely on the swimbladder for sound production result from evolutionary convergence and are based on the same basic principle: fish provoke the vibration of the gas-filled structure (Parmentier & Fine, 2016). In most cases, the swim bladder needs the action of fast contracting muscles to produce sounds since slow bladder movements are silent (Connaughton et al., 2000; Fine et al., 2001;

Millot et al., 2011) although slow muscles have been found in carapid fishes (Parmentier et al., 2006). Depending on the species, these sonic muscles have evolved from occipital, epaxial, hypaxial or pectoral girdle muscles primarily involved in locomotion (Parmentier & Fine, 2016). The use of the swim bladder and associated muscles in sound production could be an exaptation since this is not the first function of the swim bladder (Parmentier et al., 2017). Although some ontogenetic data can support this assertion (Ladich & Bass, 1998; Millot & Parmentier, 2014; Rauther, 1945), we lack models showing that the same muscles can be used in both locomotion and sound production.

Within scorpaenids, lionfish (*Pterois* sp.) appear to be of particular interest for two specific components of their behaviour. When stalking their prey, lionfish show a high manoeuvrability, being able to position their body forwards or backwards and to straighten or tilt it without apparent pectoral fin movements. Before striking prey, lionfish also pitch their body (head above horizontal or head below horizontal) to take better aim at their prey. These behaviours suggest that lionfish may use a mechanism other than fins to control orientation (Peterson & McHenry, 2022). Due in part to its function as a hydrostatic organ and to its position relative to the centre of buoyancy, the swimbladder is a candidate for such control.

Scorpaenidae also contain species that produce sounds using their swimbladders and associated muscles (Miyagawa & Takemura, 1986). In *Pterois* species, some muscles are associated with the swimbladder and therefore constitute good a priori candidates to be involved in sound production. According to Yabe (Yabe, 1985), lionfish possess extrinsic muscles originating on the occipital region of the skull and that insert on the swimbladder. Recently, sounds were recorded in unidentified species of *Pterois*, *P. volitans* and *P. miles* (Beattie et al., 2017; Schärer-Umpierre et al., 2019). *Pterois* spp. produced two distinct call types when agitated. The most common call, knocks, consisted of one to eight pulses. The hum contained continuous energy at relatively low frequencies (Beattie et al., 2017). A third sound, corresponding to an intermittent purr of a constant tone ending in a slight down sweep has been associated with courtship (Schärer-Umpierre et al., 2019).

In this study, we first record sounds of different species from the taxa *Pterois/Dendrochirus* expecting the same kind of sounds should be produced using the same mechanism. We then investigate the functions of the muscles associated with the swimbladder in *Pterois* species. We describe the swimbladder and associated muscle anatomy, record sounds to infer the sound production mechanism and study the movements of the swimbladder in relation to fish manoeuvrability.

## 2 | MATERIALS AND METHODS

All procedures were approved by the ethical commission of the University of Liège (ethics case 1759).

### 2.1 | Sound production

#### 2.1.1 | *Pterois volitans*

Seven individuals (95–127 mm total length) were captured using hand nets during snorkelling sessions performed in May 2019 in 'Grand Cul-de-Sac Marin' in the North of the Guadeloupe archipelago (16°21' N; 61°34' W) at a depth of 5–10 m. The sex of individuals is unknown. They were housed together in a tank (70 × 52 × 45 cm, 170 L) with seawater (31 mg/L) maintained at 26 ± 2°C, and with a 2.5 cm sandy bottom. The same kind of tank was used for the recordings.

#### 2.1.2 | *Pterois radiata*

A field campaign was conducted at the CRIOBE research station in Moorea Island (French Polynesia) in April 2022. Six *P. radiata* (from 15 to 20 cm total length) were captured in the North Lagoon of Moorea with hand nets. Fish were stocked in a circular 850-L tank (diameter: 1.5 m; height: 0.7 m) with running seawater (28–29°C) on a natural light cycle (12 h light:12 h dark). It was equipped with aerators for oxygenation and cinder blocks as shelters.

Both species were recorded with a hydrophone HTI-96-Min (sensitivity: -163.9 dB re 1 V μPa<sup>-1</sup>; flat frequency response range 2 Hz–30 kHz; High Tech, Inc. Long Beach, MS, USA) that was connected to a TASCAM DR-05 portable audio recorder (sampling frequency: 44 kHz, 16-bit resolution; TEAC, Wiesbaden, Germany). During recordings in tanks, the filters, water flow, bubblers and the light of the aquarium were turned off to reduce non-biological noises. At sea and in the tank, sound production was elicited in the same way. A plastic rod was used to disturb the fish. This methodology aimed to elicit the type of sounds produced by the fish during distressing events. It is interesting to note that the fish erected its fins in the direction of the plastic rod simultaneously to the production of sounds. These recordings were also made at sea (within 1.2 m depth) to avoid any issues with sound deformation that can occur due to reverberation in an aquarium setting (Banse et al., 2023). Fish were positioned at approximately 3 cm from the hydrophone until sounds were obtained. Recordings were conducted for a maximum of 3 min and were ceased when at least 30 sounds/specimens were recorded.

#### 2.1.3 | *Dendrochirus zebra*

A field campaign was conducted at Negros Island, Philippines, (9°3'3" N; 123°7'15" E) during June 2022. A single specimen of *D. zebra* was recorded using a GoPro7 (GoPro, San Mateo, CA, USA), placed in a housing (Spy-fish, Liège, Belgium) and coupled with an external hydrophone HTI-96-Min (sensitivity: -164 dBV μPa<sup>-1</sup>, flat frequency response range: 20 Hz–20 kHz, sampling rate of 44.1 kHz, 16-bit resolution; High Tech Inc, Long Beach, MS, USA).

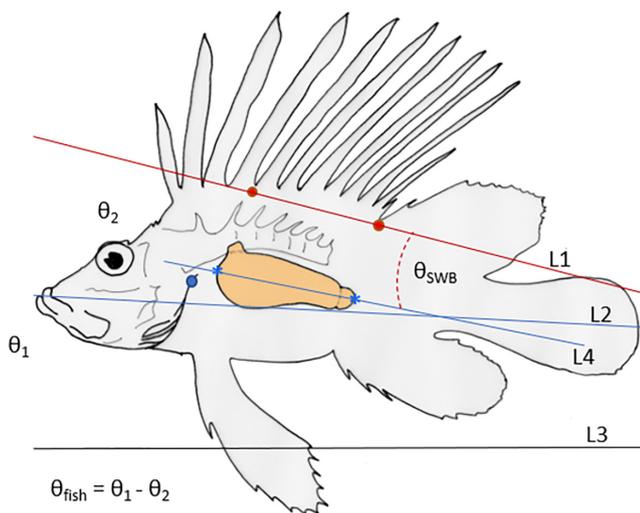
## 2.2 | Acoustic data processing

All recordings were digitized at 44.1kHz (16-bit resolution) and analysed with Avisoft SASLab Pro version 5.2.13 software (Avisoft Bioacoustics, Glienicke, Germany). The following acoustic features were measured: the sound duration (from the beginning of the first sound to the end of the last sound), the number of sounds in a series, the pulse period (peak-to-peak interval between two consecutive pulses in a sound) and the dominant frequency. Temporal features were measured from oscillograms whereas frequencies were obtained from logarithmic power spectra (Fast Fourier Transform FFT, 128 points, Hamming window, 75% overlap).

## 2.3 | Cineradiography

Three *P. volitans* from commercial suppliers were housed together in a tank (70×52×45 cm, 170L) with seawater (31 mg/L), and with a 2.5 cm sandy bottom. The tank was maintained at  $26 \pm 2^\circ\text{C}$ . Lionfish were acclimated for 24 h before experiments. Each specimen was anaesthetized with MS-222 (150 Mg/L) (Sigma Chemical Company, St Louis, MO, USA) to implant radio-opaque markers used as reference points for cineradiographic imaging and movement analysis. Two markers were inserted below the insertion of the dorsal fin spines and one at the level of the opercle (Figure 1).

X-ray videos and photographs were recorded using a Philips Optimus M200 X-ray generator (Royal Philips Electronics NV, Eindhoven, The Netherlands) coupled to a 14-inch image intensifier and a Redlake (San Diego, CA, USA) Motion Pro camera. Videos were



**FIGURE 1** Calculation of swimbladder angle ( $\theta_{SWB}$ ) and fish pitch ( $\theta_{fish}$ ).  $\theta_{SWB}$  corresponds to the angle between L1 and L4.  $\theta_{fish} = \theta_1$  (angle between L1 and L3)  $- \theta_2$  (angle between L1 and L2). Negative values indicated the head was below horizontal (pitched down) and positive values indicated the head was above the horizontal (pitched up). SWB, swim bladder; \*, tips of the swim bladder; ●, markers at the level of the dorsal fin; ●, marker at the level of the opercle.

recorded in lateral view and were recorded at 250 frames per second. Five sequences were obtained for each individual fish ( $n=3$ ) for a total of 13 usable pitching sequences. To induce pitching, we offered prey items (small, live shrimp or fish) on the end of 30 cm long forceps. This allowed to move the prey up and down within the filming chamber, causing the fish to pitch head-up or head-down relative to horizontal. After the recording sessions, the animals were euthanized by an overdose of MS-222 and frozen to determine the location of their centre of mass according to the method of Webb and Weihs (Webb & Weihs, 1994).

Five landmarks (the three radio-opaque markers and both the anterior and posterior tips of the swimbladder) were digitized frame-by-frame from the high-speed X-ray videos using either Midas 2.0 (Xcitex, Inc.) or the custom-designed routine QuickImage for NIHImage (Walker, 1998). The two markers at the level of the dorsal fin determine a dorsal line (L1). Three additional lines were used for calculation: the first intersected the tip of the snout and the most posterior, central point of the caudal peduncle (L2), the second used two points along the bottom of the filming chamber to serve as a horizontal reference line (L3), the third crossed the two landmarks associated with the swimbladder (L4, Figure 1).

## 2.4 | Analyses

Coordinates from images were used to determine whether modifications in the swimbladder can alter the body posture. Fish pitch ( $\theta_{fish}$ ) was calculated by subtracting the angle between L1 and L3 ( $\theta_1$ ) from the angle between L1 and L2 ( $\theta_2$ ) (Figure 1). Negative values indicated the head was below the horizontal (pitched down) and positive values indicated the head was above the horizontal (pitched up). The swimbladder angle ( $\theta_{SWB}$ ) was the angle between L1 and L4 (Figure 1). The swimbladder length is the distance between the anterior and posterior tips of the swimbladder (Figure 1). As pitch can be affected by yaw and roll, we also estimated them for the cineradiographic sequences. On video sequences, yaw should correspond to a decrease in the length between both dorsal markers and roll should correspond to a decrease in the distance between the anterior dorsal marker and the opercle marker. Comparison of these two values with  $\theta_{SWB}$  allows determining whether changes in  $\theta_{SWB}$  were related to yaw and roll. KaleidaGraph v4.0 (Synergy Software) was then used to follow measurements through time.

## 2.5 | Anatomy

Four *Pterois volitans* and three *Pterois radiata* specimens were euthanized using an overdose of tricaine methanesulfonate solution. Specimens were fixed in a 5% formalin solution for 2 days and then transferred to 70% ethyl alcohol. A specimen of *P. volitans* has been frozen. Two specimens of each species were dissected and examined using a Wild M10 binocular microscope (Leica Microsystems GmbH, Germany) equipped with a camera lucida. After formalin fixation,

the left muscle associated with the swimbladder in *Pterois volitans* was dehydrated in butanol, decalcified, embedded in paraffin, sectioned serially at 10  $\mu\text{m}$  (Reichert microtome) and stained using Gill III haematoxylin. Histological sections were observed with a Digital microscope VHX-7000 (Keyence, Osaka, Japan) that allowed measurements on the cross sections.

Three-dimensional reconstructions of the skeleton and swimbladder of *Pterois volitans* specimens was performed at the Royal Belgian Institute of Natural Sciences (Belgium) using the specimen BE-RBINS-VZ-Pisces-15,415. Scanning was completed using a RX EasyTom (RX Solutions, Chavanod, France; <http://www.rxsolutions.fr>), with an aluminium filter. Images were generated at a voltage of 110 kV and a current of 272  $\mu\text{A}$  and power of 30 W, with a set frame rate of 12.5 images/s and 4 average frames per image. This generated 4709 images and a voxel size of 43.1  $\mu\text{m}$ . Three-dimensional (3D) images were produced in 16-bit and subsequently converted into 8-bit voxels using ImageJ (Abramoff et al., 2014). Three-dimensional processing and rendering, performed according to the protocols described by Zanette et al. (2014), were obtained after semi-automatic segmentation of the skeleton and swimbladder using a 'generated surface'. Direct volume renderings (iso-surface reconstructions) were used to visualize a subset of selected voxels of body, brain and inner ear in AMIRA 2019.2.

## 2.6 | Electromyography

An individual of *Pterois volitans* was anaesthetized by bubbling  $\text{CO}_2$  into its tank until it lost control of buoyancy and its ability to right itself. Next, the fish was placed on a grate over a tank of synthetic seawater. A hose attached to a small, submerged pump was inserted into its mouth, allowing water from the tank to circulate over its gills during the experiment. The swimbladder and associated muscle was then exposed before stimulation using a silver bipolar electrode connected to a Grass S6 stimulator. Stimulations consisted of a square wave of 0.1 ms duration and were delivered to the anterior and posterior bellies of the left swimbladder muscle at various frequencies (1–80 Hz). This experiment was recorded with a JVC digital video camera so that muscle movements could be correlated with voltages and frequencies of stimulation.

## 3 | RESULTS

### 3.1 | Sounds

#### 3.1.1 | *Pterois volitans*

*Pterois volitans* produced hums (Figure 2) and simultaneously erected its fins in the direction of the plastic rod. Hums were produced both in the tank and at sea. These sounds consist of long series of regular low-amplitude pulses that are highly discernible on the oscillograms. Hums were produced when the plastic rod approached the body.

In this case, the fish pointed its fins at the plastic rod and produced the hum. In the tank, sound durations are quite variable, mainly depending on the disturbance with the plastic rod. On average, hums lasted ( $x \pm \text{SD}$ )  $1231 \pm 1247$  ms ( $n=11$ ). They possess a peak frequency around  $65 \pm 4$  Hz ( $n=11$ ). Pulses are produced at a period of  $13.5 \pm 2.6$  ms ( $n=84$ ). Although the acoustic data are all statistically different using the same specimens in both the tank and the field, (Mann Whitney,  $p < 0.01$ ), they are still within the same magnitude order. In the field, hums last  $2220 \pm 1740$  ms ( $n=14$ ) and possess a peak frequency around  $53 \pm 15$  Hz ( $n=14$ ). Pulses are produced at a period of  $19.5 \pm 3.2$  ms ( $n=50$ ). In both the field and the tank, pulses can be punctuated by intermittent louder pulses, knocks, lasting  $83 \pm 47$  ms ( $n=14$ ) and having a peak frequency of  $115 \pm 43$  Hz ( $n=14$ ).

#### 3.1.2 | *Pterois radiata*

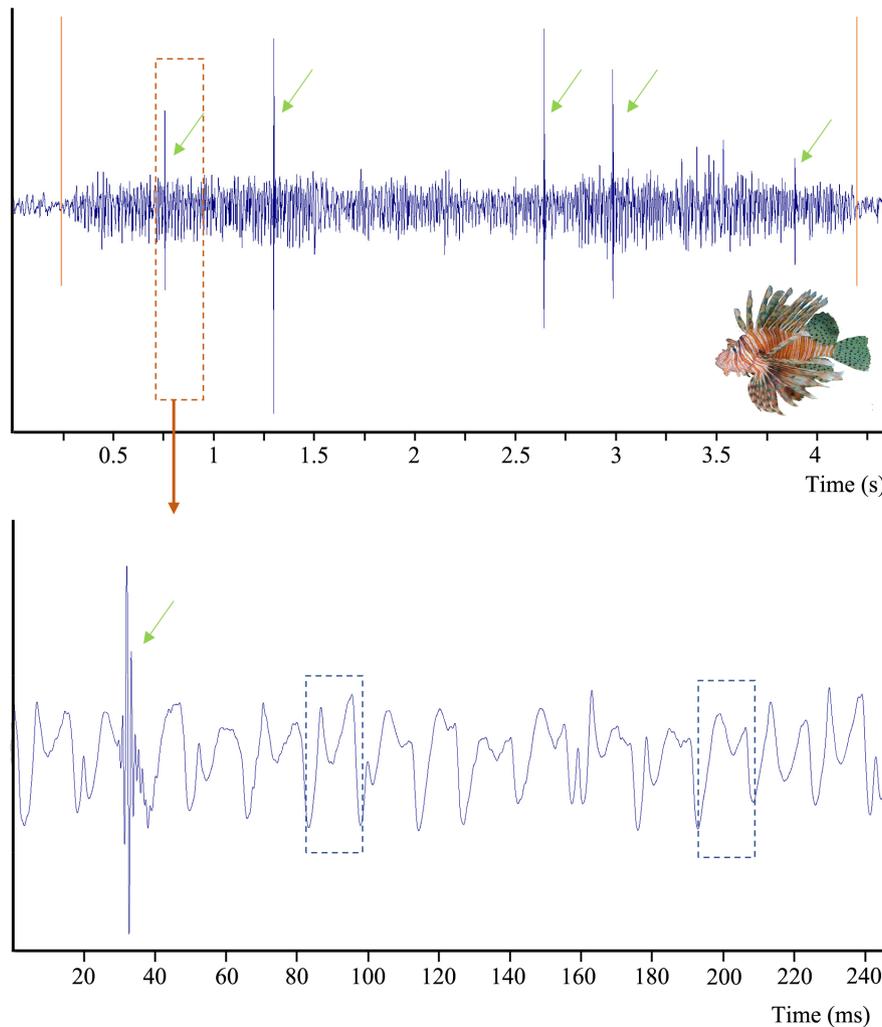
As in *P. volitans*, hums were produced both in the tank and at sea (Figure 3). Each fish erected its fins in the direction of the plastic rod while simultaneously producing sounds. These sounds consist of long series of regular low-amplitude pulses that can be punctuated by intermittent louder knocks. These hums lasted as long as the fish was disturbed (Suppl. Mat. 1), up to 98 sec in this study. The period between pulses in hums was  $54 \pm 6$  ms ( $n=66$ ) and the dominant frequency was  $57 \pm 15$  Hz ( $n=44$ ). In *P. radiata*, knocks were produced every  $10 \pm 2$  ms ( $n=27$ ) hum pulses corresponding to a period of  $667 \pm 284$  ms ( $n=235$ ). The dominant frequency in knocks varies from 50 to 180 Hz. It is worth noting that knocks were recorded at sea at a period of  $526 \pm 304$  ms ( $n=52$ ), but we were not able to distinguish the hums which could be masked by the background noise.

#### 3.1.3 | *Dendrochirus zebra*

The single specimen was recorded on the coral reef. Sounds were produced when we tried to touch the fish with the plastic rod (Figure 4). The specimen produced six sounds made up of a train of 4 to 25 pulses. The sounds lasted  $61 \pm 33$  ms ( $n=6$ ) and produced pulses at a period averaging  $5.8 \pm 0.5$  ms ( $n=45$ ). The peak frequency of this sound was  $173 \pm 8$  Hz ( $n=6$ ).

### 3.2 | Cineradiography

Nine out of the 13 pitching sequences were affected by yaw and/or roll. In each of the four remaining sequences, changes in  $\theta_{\text{SWB}}$  appeared before changes in fish pitch, suggesting a time lag in response of the fish body to changes in swimbladder angle (Figure 5). There was a direct relationship between change in  $\theta_{\text{SWB}}$  and fish pitch. An increase in  $\theta_{\text{SWB}}$  corresponded to an increase in the pitch of the fish (the head of the fish rises) and a decrease in  $\theta_{\text{SWB}}$  corresponded to a decrease in the fish pitch (the head of



**FIGURE 2** Oscillograms at different time scales showing hums and knocks that were recorded in *Pterois volitans* when disturbed. Recordings at sea. Blue dotted line rectangles delineate the low-amplitude pulses of the hum; Green arrows show the knocks; red lines correspond delineate the sound duration.

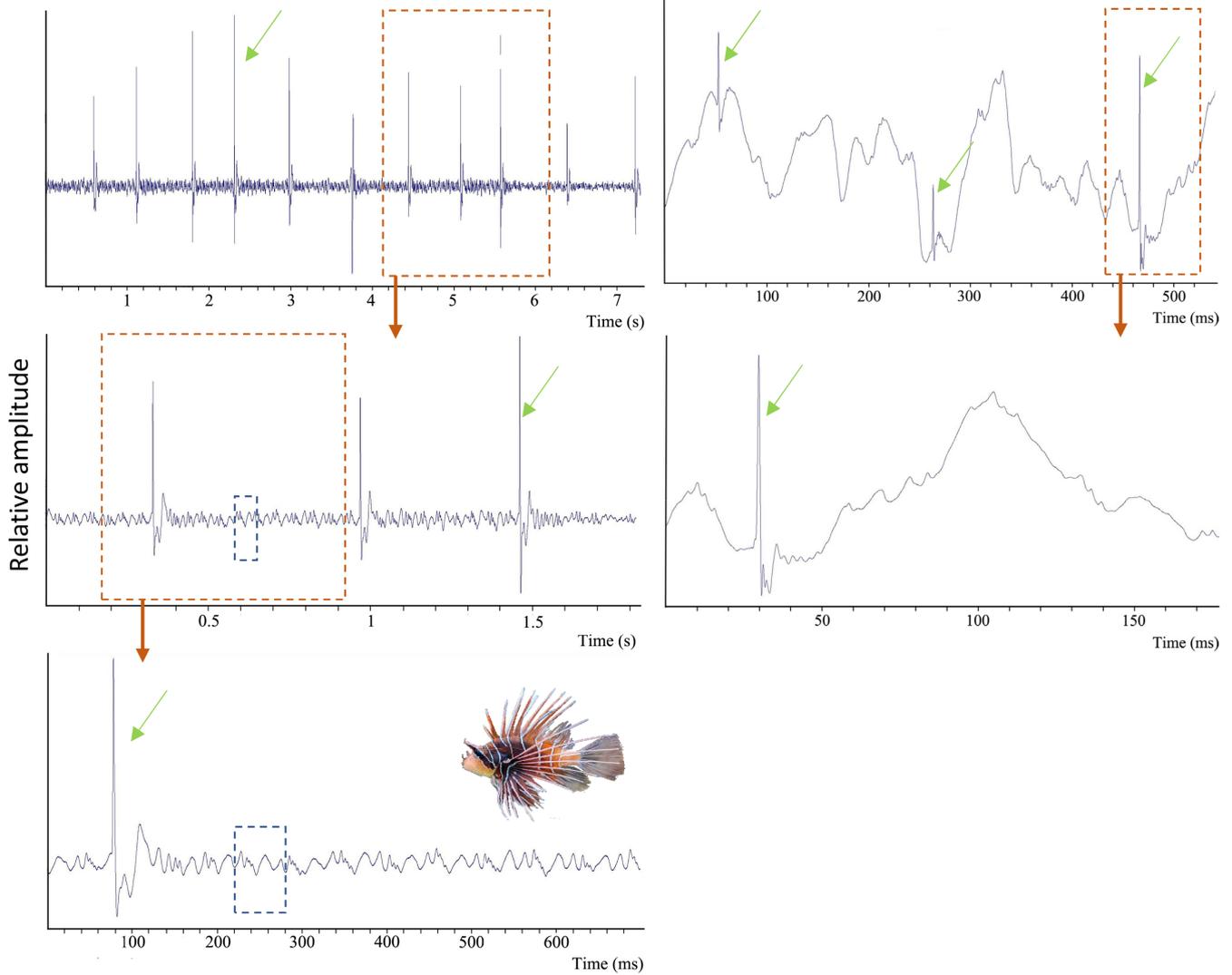
the fish falls). Changes in  $\theta_{\text{SWB}}$  (Table 1) ranged from  $2.9^\circ$  to  $11.3^\circ$  (with a mean of  $6.9^\circ$ ) and resulted in a change in  $\theta_{\text{fish}}$  from  $21^\circ$  to  $42.2^\circ$  (with a mean of  $29.3^\circ$ ). Further observation of the cineradiographic sequences suggested that modifications in  $\theta_{\text{SWB}}$  were associated with a visible shortening of the gas bladder with the posterior end of the bladder moving rostrally.

### 3.3 | Anatomy

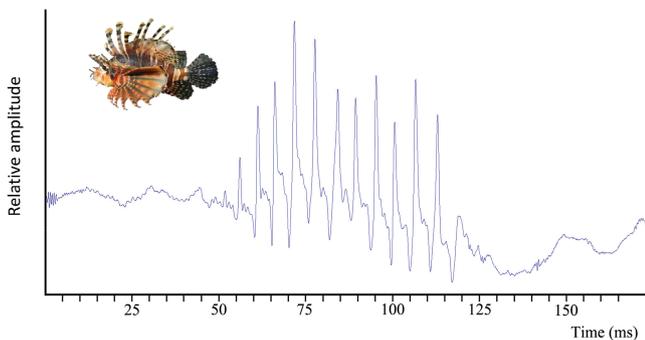
In *Pterois volitans*, the physoclistous swimbladder is oval but the anterior portion of the bladder appears to be slightly lobed and heart-shaped. On its anterior lateral part, three bands of connective tissue originating from the *tunica externa* of the swimbladder firmly attach the swimbladder to the anterior vertebral centra, the remaining part of the swimbladder being free from the vertebral column (Figure 6). The posterior end of the gas bladder is rounded and bulbous. This bulb, which looks like a cap encapsulating the terminal part of the swim bladder results from a circular fold of the *tunica externa*

(Figure 6). The inner membrane of the swim bladder (the *tunica interna*) compartmentalizes the posterior part of the bladder from the anterior part via a vascularized septum that shows a circular hole in its centrum. The septum lies approximately at the level of the bulb, close to the posterior end (Figures 6 and 7). In *P. radiata*, the swimbladder possesses only two bands connecting the swimbladder to the vertebral column and the contact zone between the swimbladder and the vertebral column is longer than in *P. volitans*.

When fresh, the muscles associated with the swimbladder in *P. volitans* appear clear to opaque and pinkish in colour (Figure 7). The swimbladder muscles on average are  $\sim 120\%$  of the length of the gas bladder and scale linearly with fish standard length ( $r^2=0.972$ ,  $n=5$ ). The extrinsic sound-producing muscles are unusual since they are composed of two distinct bellies that are separated to a fascia (Figures 6 and 7). This fascia is not inserted on the swimbladder. The fascia is short in *P. radiata*. Rostrally, the anterior bundle has a membranous insertion that originates on the dorsal part of the occipital and is connected to the intermediate tendon at the level of swimbladder connecting bands. The second bundle is inserted into



**FIGURE 3** Oscillograms at different time scales showing hums and knocks that were recorded in *Pterois radiata* when disturbed. Recordings in plastic tank (left) and at sea (right). Blue dotted line rectangles delineate the low-amplitude pulses of the hum. Green arrow: knocks.



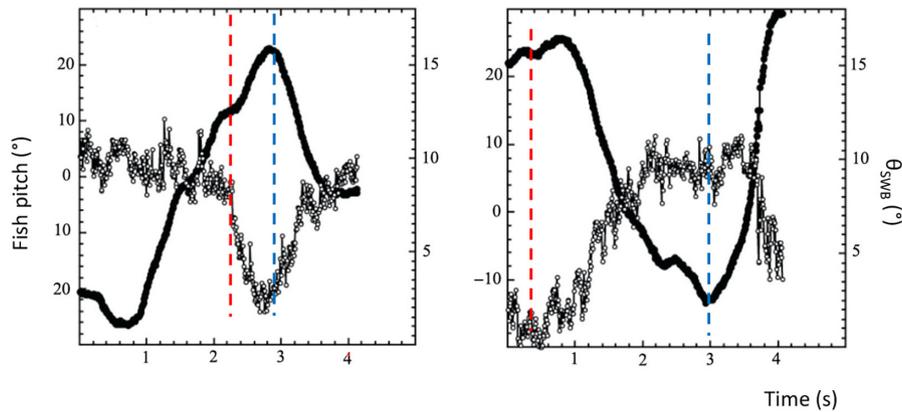
**FIGURE 4** Oscillogram showing sounds that were recorded in *Dendrochirus zebra* when disturbed. Recordings were made at sea.

the fold of the *tunica externa* that delineates the anterior part of the swimbladder (Figure 6). The gas bladder muscles in *P. volitans* are striated and fibres in the anterior muscle belly that are normally

distributed, with nerves and blood vessels scattered throughout (Figure 7). Fibre diameters range in size from 35 to 154  $\mu\text{m}$  in the anterior bundle with an average fibre diameter of  $72 \pm 30 \mu\text{m}$  ( $n=228$ ).

### 3.4 | Electromyography

No visible response was detected when the swimbladder muscle was stimulated with  $<10$  volts at  $1 \text{ pulse}\cdot\text{s}^{-1}$ . At 10 volts, there was some twitching of the swimbladder due to muscle contraction. When the posterior belly of the swimbladder muscle (Figure 2) was stimulated directly, a tetanic contraction was produced with 10V at 80Hz, with time to full contraction averaging 100ms. The result of contraction of posterior belly was movement of the posterior end of the bladder towards the contracting muscle. Stimulation of the anterior belly did not induce movements at the level of the posterior end of the gas bladder.



**FIGURE 5** Plots of two sequences where yaw and roll did not affect  $\theta_{\text{SWB}}$ . Fish pitch (solid circles) and  $\theta_{\text{SWB}}$  (open circles) were plotted through time (s). In each plot, changes in  $\theta_{\text{SWB}}$  (red dotted line) occur before changes in fish pitch (blue dotted line) and there is a direct relationship: an increase in  $\theta_{\text{SWB}}$  is followed by an increase in fish pitch, and vice versa.

**TABLE 1** Relationships between modification in the swimbladder angle ( $\theta_{\text{SWB}}$ ) and fish pitch ( $\theta_{\text{fish}}$ ).

	Change in $\theta_{\text{SWB}}$ (°)	Change in $\theta_{\text{fish}}$ (°)	Lag time (s)
Head-up pitching			
<i>Pterois</i> 1A	+10.1	+20.9	1.1
<i>Pterois</i> 1B	+2.9	+36.3	0.4
<i>Pterois</i> 2A	+11.8	+42.2	2.5
Head-down pitching			
<i>Pterois</i> 1C	+3.5	-22.3	1.0
<i>Pterois</i> 2B	+7.0	-25.1	0.6

Note: Lag time corresponds to the duration between the onset of modification in  $\theta_{\text{SWB}}$  and  $\theta_{\text{fish}}$ .

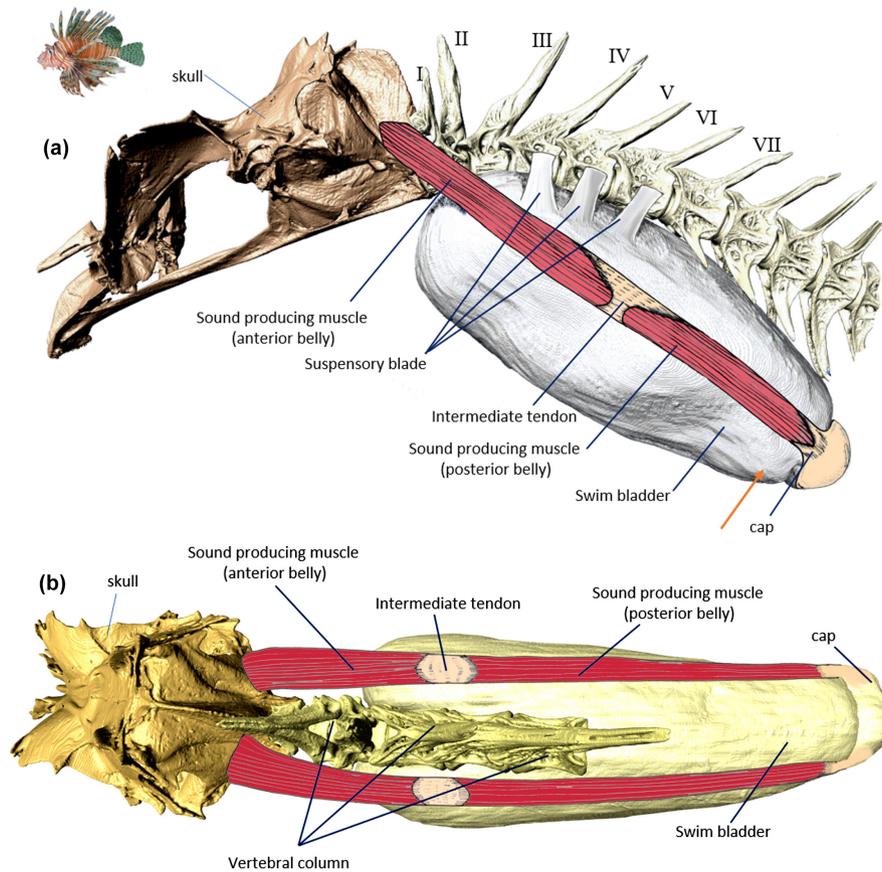
## 4 | DISCUSSION

In this study, *P. volitans* and *P. radiata* share a common sound, the hum, that was produced during recordings in both the tanks and at sea. Hums appear to be unusual since they can be punctuated by intermittent pulses having much higher amplitude. These sounds most probably correspond to the sounds previously recorded in *Pterois* spp. (Beattie et al., 2017). In all cases, sounds were recorded when the fish was agitated and oriented its fins towards the plastic rod, suggesting that it may be an aposematic sound (Raick et al., 2022) since spine movements may be used for defence, protection and intimidation in lionfish (Galloway & Porter, 2019). Similar types of sound, hums with punctuated pulses (clicks), were recorded in different boxfish species: *Ostracion meleagris* and *Ostracion cubicus* when hand held (Parmentier et al., 2019). In both these species, the positioning of two pairs of sound-producing muscles, being extrinsic and intrinsic sonic muscles, suggest that each pair could be responsible for the production of a part of the hum. In a third boxfish species, *Lactoria cornuta*, that is also able to produce hums and clicks, this

hypothesis is supported by ultra-structural differences between both sonic muscles (Parmentier et al., 2020).

It is worth noting that the basic part of the hum having continuous energy could not be detected at sea in *Pterois radiata*. This could be attributed to the background noise masking it, making that part of the sound detectable only at short distances. Although recordings at sea allow for correct identifications without tank artefacts (Banse et al., 2023), recordings in tanks can be useful in some cases to describe the different sonic components. The hum observed in both *Pterois* sp. was not detected in *Dendrochirus zebra* when disturbed. This species produced trains of pulses having the same amplitude range. Our results show that this species, phylogenetically close to *Pterois* taxa (Kochzius et al., 2003), is also able to produce sounds. Additional experiments should be carried on this taxon because some parts of the sounds could have been masked in *D. zebra* that was recorded only at sea. In combination with results from previous studies (Beattie et al., 2017; Schärer-Umpierre et al., 2019), sounds have now been recorded in *D. zebra*, *P. volitans*, *P. radiata* and *P. miles*, suggesting all Pteroinae should be able to produce sounds. This feature should be taken into account in future studies dealing with the biology of these species.

Sound production using muscles associated with the swimbladder appears supported by different features in *Pterois* species. The different *Pterois* sounds that were recorded are characterized by their low frequency. Low frequency supports the fact that sounds are not stridulatory but are based on sound-producing muscles acting on the swimbladder (Fine & Parmentier, 2015; Fine & Parmentier, 2022). *Pterois* and the closely related false kelpfish *Sebasticus marmoratus* (Smith) possess many features in common. Both species have a long and slender muscle originating on the skull and inserting on the posterior part of the swimbladder; the swimbladder is divided into two parts by a septum having a hole in its centrum. In *S. marmoratus*, electromyographic data support the idea that sonic muscles are responsible for the sound production (Miyagawa & Takemura, 1986). Their sound-producing muscles receive an occipital nerve with multiterminal innervation allowing rapid stimulation of the entire sonic

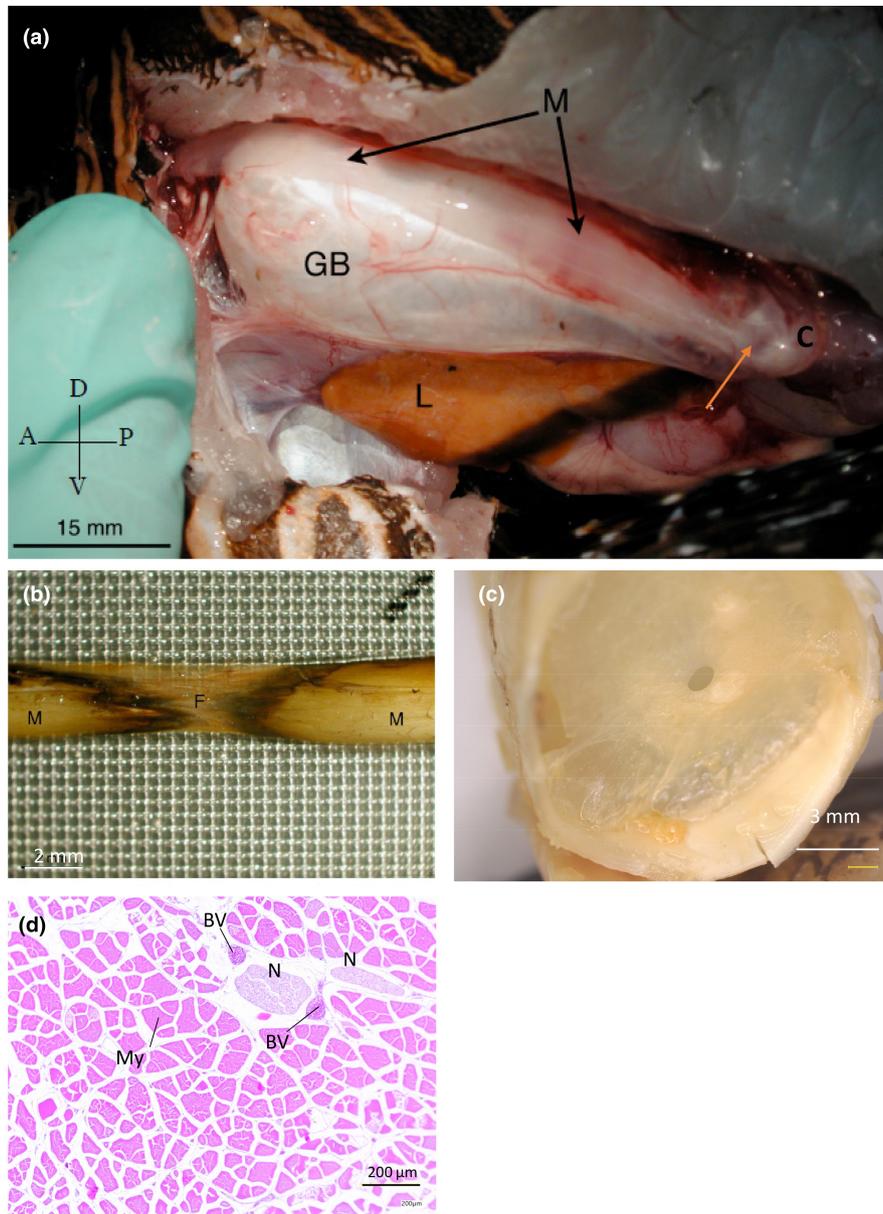


**FIGURE 6** Left lateral (a) and dorsal (b) view of the sound-producing mechanism in *Pterois volitans*. Vertebrae VI to X were removed in B to see the posterior part of the swimbladder. Arrow corresponds to the positioning of the septum (see [Figure 7](#)).

muscle (Onuki & Somiya, 2007; Yoshimoto et al., 1999). In *S. marmoratus*, fast contraction is related to the phasic character of the sound-producing muscle (Kobayashi et al., 1990). Contraction of sound-producing muscles shortens the posterior end of the swim bladder and forces the gas in the posterior chamber to flow through the small hole in the centre of the internal septum and into the larger anterior chamber. In *S. marmoratus*, the flow of gas through this hole has been suggested to cause the internal septum to vibrate and produce sound, which is resonated by the swimbladder (Dotu, 1951). This hypothesis related to septum vibration is, however, doubtful because it has never been experimentally tested. The well-known vocal Batrachoididae (Fänge & Wittenberg, 1958; Rice & Bass, 2009), the gurnard *Trigla lineata* (Rauther, 1945), and the cichlid *Oerochromis niloticus* (Longrie et al., 2009), for example also all possess a septum containing a sphincter that separates anterior and posterior parts of the bladder but a mechanism based on air flow has never been supported in these species. In *Pterois*, the similarity in the mechanism described for *S. marmoratus* suggests that swimbladder-associated muscles are involved in sound production but the air flow mechanism is not demonstrated in this study. Moreover, the tetanisation at 80 Hz supports the idea that the muscle is able to produce the fast contractions that are required for swimbladder-based sounds (Parmentier et al., 2003). It corresponds at least to the frequency of sounds found at the level of the hums. In the framework

of this study, we cannot, however, explain the alternance of pulses having low and high amplitude. As *Dendrochirus* and *Pterois* species are closely related, the complex '*Pteropterus-Dendrochirus*' clade is not yet resolved (Kochzius et al., 2003), it strongly suggests the sound-producing mechanism is similar in *D. zebra*.

Our results concerning fish positioning clearly show that swimbladder can be involved in other functions than sound production. The lionfish swimbladder system may function to change body pitch by shifting the animal's centre of buoyancy through changes in bladder shape or orientation. The seahorse, *Hippocampus brevisrostris*, uses its gas bladder to change its body angle while swimming (Peters, 1951). This is achieved by shortening or lengthening the gas bladder through contractions of muscles that are associated with the anterior chamber. In *Pterois*, the shortening of the gas bladder seen during the muscle stimulation experiment should provide modification in the swimbladder position, which in turn alters the posture of the body as observed in the X-ray videos. In this case, the simultaneous contraction of the right and left muscles shortens the posterior part of the swimbladder while the anterior part does not move since it is firmly attached to the vertebral column ([Figure 2](#)). The air in the posterior chamber flows through to the hole in the vertical septum altering the buoyancy and changing the pitch of the animal. Previous studies by Peters (1951), Litvinenko (Litvinenko, 1980), and the current study indicate that not all muscles associated with the swim



**FIGURE 7** (a) Left lateral view of the swim bladder (GB) and associated muscle (M) in *Pterois volitans*. (b) Sonic muscle after being stained with a cholinesterase motor end plate stain showing that the swim bladder muscles is made of two bellies (M) that are connect by a fascia (F) or intermediate tendon. (c) View of the posterior septum of the swimbladder. (d) Cross section in the anterior belly of the sonic muscle. BV, blood vessel; C, swimbladder cap; L, liver; N, nerve and D, V, A, and P, dorsal, ventral, anterior, and posterior directions, respectively. Arrow corresponds to the positioning of the septum.

bladder are solely dedicated to sound production. Instead, these muscles may serve as a mechanism for enhancing manoeuvrability in certain fish species, either independently or in conjunction with the use of fins. In *Pterois*, this mechanism allowing for a change in body pitch and fine-tuning of position can be important during prey capture. It can help the fish to adjust their heading towards the prey's position and potentially eliminates the use of the pectoral fins in locomotion or control of body orientation during stalking (Peterson & McHenry, 2022). Interestingly, stimulation of the posterior belly of the muscle induced swimbladder shortening which is not the case with the stimulation of the anterior muscle. The fascia between both bellies could cushion the effect of the anterior belly shortening. We

hypothesize sound production should be due to synchronize contraction of left and right muscles that provokes back and forth movements of the swimbladder cap and, consequently, modifications of the air volume in both compartments of the swimbladder. Additional experiments are required since the emg study was not accompanied by sound recording. In this case, sounds should result from fast contraction of the swimbladder muscle. Modification of the body pitch is possible if the muscle contraction is sustained for a longer period allowing modification in the swimbladder configuration. It suggests the anterior belly would be predominantly responsible of sound production but Other scorpaenid genera (*Scorpaenodes*, *Hoplosebastes*, *Ebosia*, *Brachypterois*, *Parapterois* and *Dendrochirus*) that have a

fascia dividing their gas bladder muscles into two pieces (Ishida 1994; Matsubara 1934; Yabe, 1985) should be used to study both sound production and manoeuvring abilities.

In different species, it has been suggested that the mechanism of sound production is the result of an exaptation process. Existing anatomical structures are modified into complex effectors for sound production without major changes to their overall 'Bauplan' (Parmentier et al., 2017).

In lionfish, we suggest that the associated swim bladder muscles originated from the swimming body musculature and subsequently evolved to serve two functions: buoyancy and sound production. Nevertheless, within the scope of the present study, it remains challenging to discern which of these functions was first derived function associated with this pair of muscles.

## AUTHOR CONTRIBUTIONS

Eric Parmentier, Marine Banse, David Lecchini and Frédéric Bertucci collected fish sounds. Anthony Herrel and Heidie Hornstra performed cineradiography and electromyography. Eric Parmentier analysed sounds. Heidie Hornstra and Eric Parmentier performed anatomical studies. Eric Parmentier and Marine Banse realised 3D reconstruction. Eric Parmentier wrote the first draft of the MS. Marine Banse, Heidie Hornstra and Anthony Herrel edited and revised the manuscript.

## ACKNOWLEDGEMENTS

Mrs S. Smeets kindly helped in the microscopic study. Jonathan Brecko and O. Pauwels (IRSNB) helped for the specimen loan and scanning.

## FUNDING INFORMATION

Funding provided to M. B. and E. P. by the Fonds De La Recherche Scientifique—FNRS, (Grant/Award Number: T.0192.20 and U.N054.23) and to D. L. by Fondation de France (2019-08602), LabEx CORAIL (project 2018 Emul) and ANR-19-CE34-0006-Manini.

## DATA AVAILABILITY STATEMENT

All data collected and analysed for this study are included in this published article in adherence with disclosure policy of the journal. Sounds are available upon reasonable request.

## ORCID

Eric Parmentier  <https://orcid.org/0000-0002-0391-7530>

Anthony Herrel  <https://orcid.org/0000-0003-0991-4434>

Marine Banse  <https://orcid.org/0000-0001-9689-1079>

Frédéric Bertucci  <https://orcid.org/0000-0002-2425-9362>

David Lecchini  <https://orcid.org/0000-0002-6347-1112>

## REFERENCES

- Abramoff, M.D., Magalhaes, P.J. & Ram, S.J. (2014) Image processing with ImageJ. *Biophotonics International*, 11, 36–42.
- Banse, M., Lecchini, D., Bertucci, F. & Parmentier, E. (2023) Reliable characterization of sound features begins at sea. *The Journal of the Acoustical Society of America*, 154, 270–278.
- Beattie, M., Nowacek, D.P., Bogdanoff, A.K., Akins, L. & Morris, J.A., Jr. (2017) The roar of the lionfishes *Pterois volitans* and *Pterois miles*. *Journal of Fish Biology*, 90, 2488–2495.
- Connaughton, M.A., Taylor, M.H. & Fine, M.L. (2000) Effects of fish size and temperature on weakfish disturbance calls: implications for the mechanism of sound generation. *The Journal of Experimental Biology*, 203, 1503–1512.
- Dotu, Y. (1951) On the sound producing mechanism of a scorpaenoid fish, *Sebastiscus marmoratus*. *Science bulletin of the Faculty of Agriculture, Kyushu University*, 13, 286–288.
- Fange, R. (1953) The mechanisms of gas transport in the euphysoclit swimbladder. *Acta Physiologica Scandinavica*, 30, 1–133.
- Fange, R. & Wittenberg, J.B. (1958) The swimbladder of the toadfish (*Opsanus tau* L.). *The Biological Bulletin*, 115, 172–179.
- Fine, M.L., Malloy, K.L., King, C., Mitchell, S.L. & Cameron, T.M. (2001) Movement and sound generation by the toadfish swimbladder. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology*, 187, 371–379.
- Fine, M.L. & Parmentier, E. (2015) Mechanisms of sound production. In: Ladich, F. (Ed.) *Sound communication in fishes*. Wien: Springer, pp. 77–126.
- Fine, M.L. & Parmentier, E. (2022) Fish sound production: the swimbladder. *Acoustics Today*, 18, 13–21.
- Galloway, K.A. & Porter, M.E. (2019) Mechanical properties of the venomous spines of *Pterois volitans* and morphology among lionfish species. *The Journal of Experimental Biology*, 222, jeb197905.
- Ishida, M. (1994) Phylogeny of the suborder Scorpaenoidei (Pisces:Scorpaeniformes). *Bulletin of Nansai National Fisheries Research Institute*, 27, 1–112.
- Kobayashi, T., Kondo, D., Goto, A. & Sugi, H. (1990) Mechanisms of propagation of action potential and the electrical constants in the swimbladder muscle of a teleost *Sebastiscus marmoratus*. *Zoological Science*, 7, 1040.
- Kochzius, M., Söller, R., Khalaf, M.A. & Blohm, D. (2003) Molecular phylogeny of the lionfish genera *Dendrochirus* and *Pterois* (Scorpaenidae, Pteroinae) based on mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 28, 396–403.
- Ladich, F. & Bass, A.H. (1998) Sonic/vocal motor pathways in catfishes: comparisons with other teleosts. *Brain, Behavior and Evolution*, 51, 315–330.
- Litvinenko, N. (1980) The structure, function and origin of the drumming muscles in the North Atlantic Ocean perches of the genus *Sebastes* (Scorpaenidae). *Journal of Ichthyology*, 20, 89–98.
- Longrie, N., Van Wassenbergh, S., Vandewalle, P., Manguit, Q. & Parmentier, E. (2009) Potential mechanism of sound production in *Oreochromis niloticus* (Cichlidae). *The Journal of Experimental Biology*, 212, 3395–3402.
- Matsubara, K. (1934) Studies on the scorpaenoid fishes of Japan, I. Descriptions of one new genus and five species. *Journal of the Imperial Fisheries Institute*, 30, 199–21.
- Millot, S. & Parmentier, E. (2014) Development of the ultrastructure of sonic muscles: a kind of neoteny? *BMC Evolutionary Biology*, 14, 1–9.
- Millot, S., Vandewalle, P. & Parmentier, E. (2011) Sound production in red-bellied piranhas (*Pygocentrus nattereri*, Kner): an acoustical, behavioural and morphofunctional study. *The Journal of Experimental Biology*, 214, 3613–3618.
- Miyagawa, M. & Takemura, A. (1986) Acoustical behavior of scorpaenoid fish *Sebastiscus marmoratus*. *Bulletin of the Japanese Society of Scientific Fisheries*, 52, 411–415.
- Onuki, A. & Somiya, H. (2007) Innervation of sonic muscles in teleosts: occipital vs. spinal nerves. *Brain, Behavior and Evolution*, 69, 132–141.
- Parmentier, E., Diogo, R. & Fine, M.L. (2017) Multiple exaptations leading to fish sound production. *Fish and Fisheries*, 18, 958–966.
- Parmentier, E. & Fine, M.L. (2016) Fish sound production: insights. In: Suthers, R., Tecumseh, F., Popper, A.N. & Fay, R.R. (Eds.) *Vertebrate*

- Sound Production and Acoustic Communication*. New York, USA: Springer, pp. 19–49.
- Parmentier, E., Lagardère, J.-P., Braquegnier, J.-B., Vandewalle, P. & Fine, M.L. (2006) Sound production mechanism in carapid fish: first example with a slow sonic muscle. *The Journal of Experimental Biology*, 209, 2952–2960.
- Parmentier, E., Marucco Fuentes, E., Millot, M., Raick, X. & Thiry, M. (2020) Sound production, hearing sensitivity, and in-depth study of the sound-producing muscles in the cowfish (*Lactoria cornuta*). *Journal of Anatomy*, 238, 956–969.
- Parmentier, E., Solagna, L., Bertucci, F., Fine, M.L., Nakae, M., Compère, P. et al. (2019) Simultaneous production of two kinds of sounds in relation with sonic mechanism in the boxfish *Ostracion meleagrís* and *O. Cubicus*. *Scientific Reports*, 9, 4962.
- Parmentier, E., Vandewalle, P. & Lagardère, J.P. (2003) Sound-producing mechanisms and recordings in Carapini species (Teleostei, Pisces). *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology*, 189, 283–292.
- Peters, H.M. (1951) Contributions for the ecological physiology of the seahorse (*Hippocampus brevis*). *Zeitschrift für Vergleichende Physiologie*, 33, 207–265.
- Peterson, A.N. & McHenry, M.J. (2022) The persistent-predation strategy of the red lionfish (*Pterois volitans*). *Proceedings of the Royal Society B: Biological Sciences*, 289, 20221085.
- Popper, A.N. (2023) Hearing diversity in 34000 fish species: a personal perspective. *The Journal of the Acoustical Society of America*, 154, 1351–1361.
- Popper, A.N. & Fay, R.R. (2011) Rethinking sound detection by fishes. *Hearing Research*, 273, 25–36.
- Raick, X., Koussa, A., Zawadzki, C.H., Kurchevski, G., Godinho, A.L. & Parmentier, É. (2022) Sounds and associated morphology of *Hypostomus* species from South-East Brazil. *Journal of Zoology*, 317, 77–91.
- Rauther, M. (1945) Über die Schwimmblase und die zu ihr in Beziehung tretenden somatischen Muskeln bei den Trigliden und anderen Scleroparei. *Zoologische Jahrbücher. Abteilung für Anatomie und Ontogenie der Tiere*, 69, 159–250.
- Rice, A.N. & Bass, A.H. (2009) Novel vocal repertoire and paired swimbladders of the three-spined toadfish, *Batrachomoeus trispinosus*: insights into the diversity of the Batrachoididae. *The Journal of Experimental Biology*, 212, 1377–1391.
- Schärer-Umpierre, M.T., Zayas, C., Appeldoorn, R.S., Tuohy, E., Olson, J.C., Keller, J.A. et al. (2019) The purr of the lionfish: sound and behavioral context of wild lionfish in the Greater Caribbean. *Gulf and Caribbean Research*, 30, 15–19.
- Walker, J.A. (1998) Estimating velocities and accelerations of animal locomotion: a simulation experiment comparing numerically different algorithms. *The Journal of Experimental Biology*, 201, 981–995.
- Webb, P.W. & Weihs, D. (1994) Hydrostatic stability of fish with swim bladders: not all fish are unstable. *Canadian Journal of Zoology*, 72, 1149–1154.
- Yabe, M. (1985) Comparative osteology and myology of the superfamily Cottoidea (Pisces: Scorpaeniformes), and its phylogenetic classification. *Memoirs of the Faculty of Fisheries, Hokkaido University*, 32, 1–130.
- Yan, H.Y., Fine, M.L., Horn, N.S. & Colón, W.E. (2000) Variability in the role of the gasbladder in fish audition. *Journal of Comparative Physiology. A*, 186, 435–445.
- Yoshimoto, M., Kikuchi, K., Yamamoto, N., Somiya, H. & Ito, H. (1999) Sonic motor nucleus and its connections with octaval and lateral line nuclei of the medulla in a rockfish, *Sebastes marmoratus*. *Brain, Behavior and Evolution*, 54, 183–204.
- Zanette, I., Daghfous, G., Weitkamp, T., Gillet, B., Adriaens, D., Langer, M. et al. (2014) Looking inside marine organisms with magnetic resonance and x-ray imaging. In: Reynaud, E.G. (Ed.) *Imaging Marine Life: Macrophotography and Microscopy Approaches for Marine Biology*, pp. 122–184. Weinheim: Wiley-Blackwell.

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**How to cite this article:** Parmentier, E., Herrel, A., Banse, M., Hornstra, H., Bertucci, F. & Lecchini, D. (2023) Diving into dual functionality: Swim bladder muscles in lionfish for buoyancy and sonic capabilities. *Journal of Anatomy*, 00, 1–11. Available from: <https://doi.org/10.1111/joa.13963>