

SHORT COMMUNICATION

Sound-production mechanism in *Pomatoschistus pictus*

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ABSTRACT

Fish acoustic signals play a major role during agonistic and reproductive interactions. Among the sound-generating fish, Gobiidae, a large fish family with 1866 valid species, is one of the most studied groups of acoustic fishes, with sound production being documented in a number of species. Paradoxically, the sound-producing mechanism remains poorly studied in this group. The painted goby, *Pomatoschistus pictus*, produces two distinct sounds called drums and thumps. A combination of morphological and experimental analyses involving high-speed videos synchronized with sound recordings supports that drums are produced during lateral head movements involving at least the alternate contractions of the levator pectoralis muscles originating on the skull and inserting on the pectoral girdle. These movements are reported in many Gobiidae species, suggesting the pectoral-girdle-based mechanism is common in the family and could have evolved from locomotory movements.

KEY WORDS: Acoustic, Communication, Gobiidae, Gobionellinae

INTRODUCTION

Sound production in Gobiidae is documented in 23 species (Horvatić et al., 2016; Lugli and Torricelli, 1999; Lugli et al., 1996; Malavasi et al., 2008) belonging to 10 different genera. Four different kinds of calls have been described: (1) pulsed sounds or drums consisting of pulse trains repeated at a slow to fast rate (around 5–100 pulses s⁻¹), which can be amplitude modulated (Bass and McKibben, 2003; Malavasi et al., 2008; Zeyl et al., 2016); (2) tonal sounds showing a sinusoidal-like waveform with no inter-pulse intervals (Bass and McKibben, 2003; Malavasi et al., 2008); (3) complex sounds involving a combination of both aforementioned sounds (Bass and McKibben, 2003); and (4) thumps, which are short (74–89 ms) non-pulsed sounds of very low frequency (below 100 Hz) and with sound energy under 1 kHz (Amorim and Neves, 2007). Tonal, pulsatile and complex sounds exemplify the continuum from trains of pulsed sounds to tonal sounds (Lugli et al., 1995; Zeyl et al., 2016) and can be considered as members of the same sound category or group (G1). Thumps are non-pulsed sounds and form a second group (G2).

Different sound-production mechanisms have been proposed in gobies: muscles acting on the swimbladder (Lugli et al., 1995), hydrodynamic mechanisms with ejection of water through the gill

openings (Stadler, 2002) and contraction of muscles inserting on the pectoral girdles (Lugli et al., 1996). Only the latter hypothesis has been experimentally tested in *Gobius paganellus*, whose sounds are clearly related to movements of the pectoral girdle owing to contractions of the levator pectoralis (Parmentier et al., 2013). This muscle exhibits many characteristics found in specialized sonic muscles: ribbon-like myofibril structure, well-developed sarcoplasmic reticulum tubules, a mitochondria-dense core and numerous peripheral mitochondria (Parmentier et al., 2013). However, additional studies on sound-production mechanisms are required to seek a common mechanism within the family.

Atlantic–Mediterranean gobies can be subdivided into the gobiine-like gobiids (Gobiinae; e.g. *Gobius* and *Padogobius*) and the gobionelline-like gobiids (Gobionellinae; e.g. *Pomatoschistus* and *Knipowitschia*) (Agorreta et al., 2013; Horvatić et al., 2016). Both subfamilies show a clear distinction in sounds, suggesting a correlation between acoustic structures and phylogenetic relationships. Tonal, pulsatile and complex sounds from G1 were reported within Gobiinae (Malavasi et al., 2008) but only pulsatile calls from this group were described within Gobionellinae (Amorim et al., 2013). Thumps (G2) have currently only been described in the Gobionellinae species *Pomatoschistus pictus* and *P. canstrinii* (Amorim and Neves, 2007; Malavasi et al., 2009).

Painted goby [*Pomatoschistus pictus* (Malm 1865)] produce two types of calls (Vicente et al., 2015). Drums (in G1) and thumps (in G2) are made to attract and court females, but only drums are used during agonistic interactions (Amorim and Neves, 2007, 2008; Bolgan et al., 2013). Simultaneously with drum emission, males erect the pelvic fins, elevating the body, and exhibit lateral and frontal displays such as extending the fins, darkening the chin and quivering the body (Amorim and Neves, 2007).

The description of the sound-producing mechanism in *P. pictus* and its comparison with *Gobius paganellus* should highlight whether the sonic mechanism is the same in these two species belonging to distinct phylogenetic groups.

MATERIALS AND METHODS

All experimental procedures followed a protocol that was approved by the local ethics committee of the University of Liège (protocol no. 1226). *Pomatoschistus pictus* is not an endangered or protected species, and specimens were not caught in protected areas.

Fish were collected in February and March 2016 at Parede (38° 41' N, 9° 21' W) and Arrábida (38° 26' N, 9° 06' W) in Portugal. Fish were maintained in tanks (32×25×25 cm) containing sand substrate and shelters. The artificial filtered seawater was kept at ca. 16°C, fish were fed daily with shrimp and mussels, and the natural photoperiod was 12 h:12 h light:dark.

Experiments were conducted in 35 liter tanks set on vibration-absorbing material. Following Amorim and colleagues (2013), a male was placed in the tank provided with a PVC shelter, and females were then added to the tank.

Seventeen video recordings (26 frames s⁻¹) from 17 males were obtained with a video camera (Sony DCR-SR15) synchronized with

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the Bruel & Kjaer 8104 hydrophone positioned in the nest chimney. In addition, lateral and dorsal views were filmed with a Redlake MotionPro high-speed camera ($500 \text{ frames s}^{-1}$) also coupled with the Bruel & Kjaer 8104 hydrophone. The male was illuminated with an LED torch situated ca. 1 m behind the camera. On the basis of four fish, four lateral and one dorsal views allowed the detailed description of movements during drums.

Ten males were then euthanized with an excess of MS222 (tricaine methane sulphonate; Pharmaq, Norway) and conserved in ethanol (70%). Two of these males were alizarin stained (Taylor and Van Dyke, 1985) and the others were dissected to identify the muscles involved in the sound-production mechanism.

Sounds of 17 males ($4.28 \pm 0.25 \text{ cm TL}$) were analyzed with Raven 1.5 for Windows (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA). The acoustic pulse is considered the fundamental unit of the drum sound (Lindström and Lugli, 2000). Drums were analyzed for sound duration (ms), total number of pulses in a drum sound and pulse period (ms).

RESULTS AND DISCUSSION

The mechanism dealing with sound production in gobiids was originally experimentally studied in *G. paganellus* (Parmentier et al., 2013). In this fish, it appears that pectoral girdles are involved because (1) electromyography revealed the contraction of the levator pectoralis during the sound production and (2) these shoulder girdle muscles possess characteristics of high-speed muscles usually found in calling fishes (Parmentier and Fine, 2016). Electromyography was not used in the framework of this study because of the small size of the fish. However, dissections revealed the strong similarities between the musculo-skeletal system of *G. paganellus* and *P. pictus*. A complete description of the head skeleton and muscles is provided for *Pomatoschistus lozaoni* (Adriaens et al., 1993) and is similar to that of *P. pictus* (Fig. 1). The relevant parts of the pectoral girdle are the post-temporal, the supracleithrum and the cleithrum. The coracoid and scapulae are associated with the cleithrum. The post-temporal consists of a basal plate with two rostrally directed processes that form a fork with a dorsal and a lateral attachment to the skull. The rostral tip of the dorsal process is flattened and is firmly connected to the epiotic bone,

whereas the ventral process extends rostrally into a ligament that is attached to the neurocranium. The supracleithrum articulates with the post-temporal and the cleithrum. The levator pectoralis muscle is divided into two bundles: the pars lateralis and the pars medialis. The pars lateralis originates on the caudal margin of the pterotic bone from the neurocranium and is attached to the rostral margin of the cleithral bone. The pars medialis originates on the exoccipital bone and is inserted on the medial side of the supracleithrum.

High-speed videos clearly show a strong relationship between lateral head movements and the production of pulses. Movements observed during drum production concerned mainly the mouth, the opercles, the lateral head and body movements, and the back-and-forth sweeping of pectoral fins. However, only pectoral fins and lateral movements were correlated with drum pulses. A complete cycle of lateral head movements was related to the production of four pulses (Fig. 2). The first pulse occurred when the head was on the left side. The second pulse took place when the head was in the axis of the fish body. No pulse was detected when the head was on the right side. The third pulse was produced when the head came back in the body axis and the fourth pulse was produced at the end of the cycle, meaning when the head was again on the left. The total cycle was performed in approximately 84 ms at $200 \text{ frames s}^{-1}$. The pulse period was $23 \pm 4 \text{ ms}$ ($n=210$), a cycle corresponding to four pulses. The mean number of pulses of 21 ± 9 ($n=211$) corresponded to ca. four to six complete head cycles during drum production. The lateral head movements were probably amplified by the sweepings of pectoral fins: posterior displacement of the right fin helped to turn left and vice versa. These lateral displacements of the head were also exhibited when there was no call, but they were slower in this case.

In the Gobiinae species *G. paganellus* (Parmentier et al., 2013), nodding was associated with drum production whereas lateral head movements were observed in the Gobionellinae species *P. pictus*. Although movements are different in both species, kinematic analysis indicated a relationship between the head movement and the pulse construction. These head movements are observed during the production of drums in many other gobiid and cottid species (Colley et al., 2013; Lugli et al., 1997; Polgar et al., 2011; Zeyl et al., 2016). Although they are related to sound production, they could be mainly a visual display that reinforces the acoustic signal.

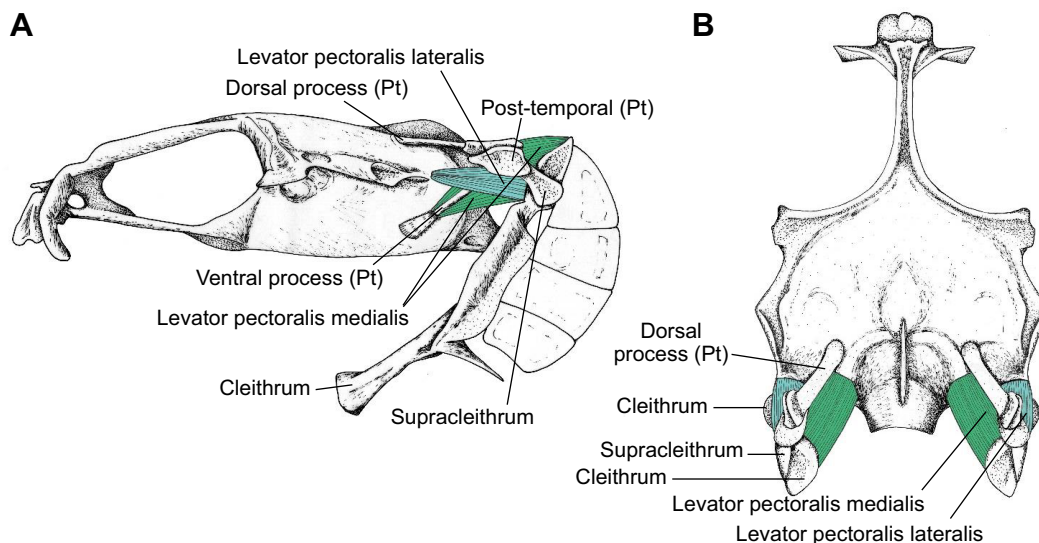


Fig. 1. Sound producing mechanism in *Pomatoschistus pictus*. (A) Left lateral view of the neurocranium, pectoral girdle and sonic muscles. (B) Dorsal view of the neurocranium and pectoral girdle (left) and with sonic muscles indicated (right). Pt, post-temporal.

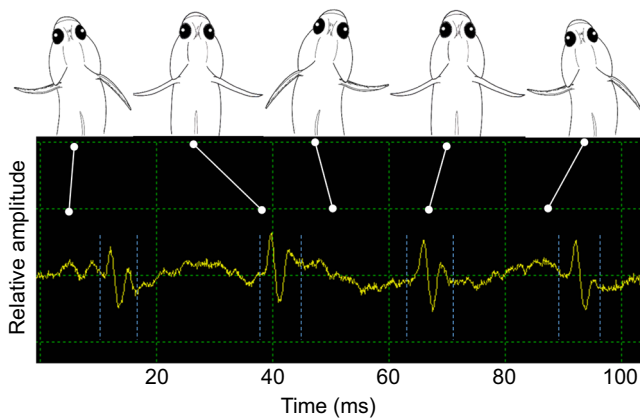


Fig. 2. Oscillogram of drumming sound in relation to a dorsal view of associated movements of the head and opposed motions of pectoral fins in *P. pictus*. The white lines identify the movement of the fish and the corresponding position on the oscillogram. Pulses are delimited by the blue dashed lines.

In addition to the common ability to produce drums, *G. paganellus* and *P. pictus* share many similar characteristics: pectoral girdle, levator pectoralis and head movements during the calls. Moreover, these characteristics are also found in Cottidae (Barber and Mowbray, 1956; Bass and Baker, 1991). We thus suggest that the pectoral girdle is involved in drum production in *P. pictus* and that the mechanism is actuated by the contractions of the levator pectoralis. As *G. paganellus* and *P. pictus* are found in two sister clades (Gobiinae and Gobionellinae) within the Gobiidae, the mechanism should be common to the family. However, it remains to be determined how the sound is radiated. The pectoral girdle could be the sound transducer (Barber and Mowbray, 1956; Whang and Janssen, 1994) or their vibrating movements could be amplified by the radials at the level of the fins (Parmentier et al., 2013). Lateral movements of the head (and in less extent of the body) suggest hydrodynamic-based sounds. However, strong similarities between mudskipper vocalizations that are produced out of water and underwater sounds of other gobies (Polgar et al., 2011) do not support this hypothesis. Moreover, the movements would probably need to occur much faster to be able to generate the short drum pulses.

Gobiidae do not show obvious sonic mechanical structures but have the classical Bauplan of Perciformes (Lugli et al., 1997; Malavasi et al., 2008; Stadler, 2002). In different taxa, sound production results from the independent modification of existing structures originally having other functions (Parmentier et al., 2017). We suggest that in Gobiidae, sounds could have evolved from locomotory movements related to the pectoral girdle.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.P., M.C.P.A.; Methodology: E.P., M.C.P.A., P.J.F.; Software: M.P.; Validation: E.P., M.C.P.A., P.J.F.; Formal analysis: M.P.; Investigation: E.P., M.P., P.J.F.; Resources: M.C.P.A.; Data curation: P.J.F.; Writing - original draft: E.P., M.P., M.C.P.A.; Writing - review & editing: M.C.P.A., P.J.F.; Supervision: E.P., M.C.P.A.; Funding acquisition: E.P., M.A., P.J.F.

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