Introduction

The adaptive gap between aquatic and terrestrial acoustic communication is mirrored by a lack of understanding of the eco-evolutionary mechanisms which allowed the vertebrates to cross the water-to-land ecological barrier.

Some insights come from recent paleontological studies of Devonian prototetrapods, the tetrapods’ most recent common ancestors, e.g. [1,2]. The fossil record indicated that several adaptive radiation occurred in shallow aquatic intertidal habitats of tropical deltas and flooding plains, and that key adaptations to the terrestrial environment such as limbs were apparently exaptations selected in aquatic conditions. In particular, it might be expected that during the first phases of this transition, exaptations also facilitated both the exploration of the terrestrial acoustic world, and terrestrial acoustic communication. In this respect, the Devonian tetrapod Ichthyostega was discovered to have a uniquely modified ear region, interpreted as an underwater communicative value. A correlative analysis amongst acoustical properties and video-acoustical recordings in slow-motion supported first hypotheses on the emission mechanism. Acoustic transmission through the wet exposed substrate was also discussed. These observations were used to support an “exaptation hypothesis”, i.e. the maintenance of key adaptations during the first stages of water-to-land vertebrate eco-evolutionary transitions (based on eco-evolutionary and palaeontological considerations), through a comparative bioacoustic analysis of aquatic and semiterrestrial gobidi taxa. In fact, a remarkable similarity was found between mudskipper vocalisations and those emitted by gobiioids and other soniferous benthonic fishes.

Abstract

Coupled behavioural observations and acoustical recordings of aggressive dyadic contests showed that the mudskipper Periophthalmodon septemradiatus communicates acoustically while out of water. An analysis of intraspecific variability showed that specific acoustic components may act as tags for individual recognition, further supporting the sounds’ communicative value. A correlative analysis amongst acoustical properties and video-acoustical recordings in slow-motion supported first hypotheses on the emission mechanism. Acoustic transmission through the wet exposed substrate was also discussed. These observations were used to support an “exaptation hypothesis”, i.e. the maintenance of key adaptations during the first stages of water-to-land vertebrate eco-evolutionary transitions (based on eco-evolutionary and palaeontological considerations), through a comparative bioacoustic analysis of aquatic and semiterrestrial gobidi taxa. In fact, a remarkable similarity was found between mudskipper vocalisations and those emitted by gobiioids and other soniferous benthonic fishes.
most gobioid groups, phylogenetic relationships below the genus level are presently unresolved. Nonetheless, the close relationship of oxudercines with other aquatic gobioids is supported both by morphological [9,22] and molecular data [5,21,23,24], allowing evolutionary comparative analyses at suprageneric level.

Several studies on the social behaviour and communication of *Periophthalmus* and *Boleophthalmus* spp. showed that these species are highly territorial, and make use of intense visual displays both during agonistic and reproductive intraspecific interactions, e.g. [25–34]. Nursall [35,36] also investigated interspecific interactions during agonistic and reproductive intraspecific interactions, e.g. highly territorial, and make use of intense visual displays both while the majority contained both (Fig. 3a, c), and repeated at a relatively low rate; most pulse energy was concentrated below 100 Hz (grand mean of the peak fundamental frequency, Table 1, Fig. 3d). Tonal segments were continuous sine waves made of rapidly repeated pulses (Fig. 3a, c), and composed by a stronger first harmonic band (grand mean of the fundamental frequency: 168 Hz, Table 1) and 1–3 much weaker ones (Fig. 2). They were both amplitude (Fig. 2, top panel) and frequency modulated (Fig. 3c, 4b). Within bouts, units were spaced by highly variable time intervals, although pulse-tonal intervals were much shorter than tonal-pulse intervals (Table 1).

The presence of acoustic communication was documented in several basal and derived aquatic gobioids, both during reproductive and aggressive encounters, e.g. [37–39]. Nonetheless, except for few anecdotal accounts of audible sounds produced during feeding, e.g. [40], and few behavioural and physiological reports of their hearing capacities, e.g. [41,42], terrestrial acoustic communication has not been previously demonstrated in mudskippers.

Appropriate laboratory conditions and equipment allowed to record and analyse vocalizations of the mudskipper *Periophthalmus septemradiatus* (Hamilton) (Fig. 1) during agonistic interactions, demonstrating that sounds are effectively transmitted at short distances through the wet exposed substrate. Our study aimed at: (i) description of the structure of the mudskipper call, assessing the main acoustical properties of the vocalisations transmitted through the prevalent transmitting medium; (ii) assessment of intraspecific variability in mudskipper call structure, testing also for correlation amongst acoustical properties and describing their association with visible movements (iii) exploring affinities of mudskipper acoustical signals with respect to other soniferous fishes, with further discussion of the possible evolutionary insights concerning the eco-evolutionary transition from aquatic to terrestrial habitats (exaptation hypothesis).

**Results**

Vocalizations were analysed and organised in bouts, each containing different combinations of pulses and tonal segments (Figs. 2, 3, 4a; Tables S1, S2, Fig. S1). A small proportion of bouts contained either only trains of pulses or only tonal segments, while the majority contained both (Fig. 4a). Each pulse was broad band, composed by 1–3 rapidly damped cycles (Fig. 3b), and repeated at a relatively low rate; most pulse energy was

![Figure 1. One of the males of *Periophthalmus septemradiatus* (photo by G. Polgar). doi:10.1371/journal.pone.0021434.g001](image)

**Figure 2. Oscillogram (top panel), spectrogram (bottom panel) and power spectrum (bottom left panel) of a representative mudskipper acoustical bout, composed of a tonal segment and nine pulsatile units; amplitude on a linear scale of 100 mV per division (arbitrary units).**

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| Table 1 |
|---|
| Table 2 |
| Table S3 |

MANOVA showed that bouts' mean acoustic properties were significantly different as a whole in different individuals (Wilks test, effect $\text{df} = 135$, $F = 2.0$, $p < 0.05$). Nonetheless, a significant variation was found only in tonal rate, tonal fundamental frequency, and tonal fundamental frequency measured at the final (F) portion of each tonal segment (univariate one-way ANOVAs: $\text{df} = 9$; $p < 0.05$ after Holm-Bonferroni correction, Table 2). Both within-individual and between-individual variability of all acoustic properties were relatively high ($\text{C}V_w = 1.6–300$; $\text{C}V_b = 8.3–118$, respectively; Table 1, Fig. S2b). The fundamental frequency of tonal segments was both the least variable acoustic property ($\text{C}V_b$, $\text{C}V_w$ and $\text{C}V_b$/ $\text{C}V_w$, Table 1, Fig. S2), and the only one with a $\text{C}V_b$/ $\text{C}V_w$ ratio $> 1.0$ (Table 1).

The correlative analysis among the bouts' acoustical properties (individual means of bouts' mean properties) revealed statistically significant constraints and trade-offs between rate or frequency and duration, between frequency and rate of different types of units, and between frequency and time intervals (alpha level 0.05, Table S3). The observed significant constraints between rate or frequency and duration, or between frequency and rate of different types of units, included the negative correlations between bout duration and tonal rate; between bout duration and the fundamental frequency of both the final and initial portions of tonal segments; between tonal duration and both tonal rates and the fundamental frequencies of the final portions of tonal segments; and between tonal frequency modulation (C-I and F-I) and both pulse duration and pulse rate. The observed significant constraints between frequency and intervals included the positive correlation between tonal frequency modulation (F-I) and the
tonal–pulse interval. No significant correlation was found between size and any of the studied acoustic properties (Tables S2, S3). 10 behavioural acts were recognised as simultaneous or contiguous to acoustical emissions (Table 3; video S1). In particular, D1,2 and GAP were typical mudskipper aggressive visual displays. Aggressive sequences were therefore characterised by the combined use of acoustic and visual displays.

Video-acoustical recordings were also examined in slow-motion. Immediately before the emission of each pulse, the head was slightly lifted, and during pulse emission the fish made a short, rapid and downwardly directed vertical movement of the mandible (during gaping), or of the whole head (closed mouth). No movements were ever observed during tonal sounds. The head was never in contact with the substrate during vocalizations. Two specimens (one male and one female), which were euthanised and dissected, lacked a gas bladder.

Discussion
A comparative analysis between the main call properties of mudskippers and 19 other soniferous gobioid species (Table 4) immediately recovers clear affinities in terms of acoustic patterns. The general acoustic structure of the mudskippers’ calls, that is a combination of pulsatile and tonal elements characterised by low dominant frequencies (approximately 100 Hz), closely corresponds to the typical pattern found in known soniferous gobioids, that is either a pulsatile or a mixed (tonal plus pulsatile elements) pattern, and peak/dominant frequencies comprised between 80 and 200 Hz (e.g. Padogobius martensis, junior syn. of P. bonelli (Bonaparte, 1846), [43]; Table 4). In a parsimonious scenario, the occurrence of similar patterns of acoustic emissions in several gobioid genera, including a member of the basal family Odontobutidae ([38,44]; Table 4), and the wide geographic distribution of these species in different aquatic habitats (Table 4) both suggest (1) a fundamental similarity of the unknown sound production mechanism and (2) that aquatic acoustic communication is a plesiomorphic trait in gobioids.

These observations support the guiding “exaptation hypothesis” in mudskippers. The gobioid structure of P. septemradiatus vocalizations both suggests that during their eco-evolutionary transition to an amphibious lifestyle mudskippers retained ancestral acoustic traits, and that other oxudercine gobies are soniferous. In an adaptive perspective, our results also suggest that in amphibious gobies eco-ethological adaptations likely preceded rather than followed new adaptations to terrestrial conditions.

In fact, mudskippers may communicate acoustically inside their water-filled burrows, involving both aggressive and reproductive behaviours; nonetheless, no underwater interactions were obtained in the laboratory during this study. In this respect, the acoustic sensitivity of the closely related species Periophthalmus barbarus (Linnaeus, 1766), measured under water (100–900 Hz [42]) and inferred in behavioural studies made out of water in Periophthalmus
koelreuteri (Pallas, 1770) = jen. syn. of P. barbarus (258–651 Hz [41]) reveals a good overall correspondence with the observed peak frequencies of sound emissions.

In a wider perspective, gobioids are typically benthonic fishes, and the structure of mudskippers’ sounds is also similar to other non-gobioid benthonic teleosts, such as toadfishes (Batrachoididae; [45–47]), blennies (Blennidae; [48]) and sculpins (Cottidae; [49,50]), some of which have well-known sonic mechanisms associated with extremely specialised anatomical structures [47]. This suggests (1) that this peculiar sound structure might have evolved multiple times in aquatic benthonic habitats, and (2) that a benthonic lifestyle might have facilitated water-to-land transitions in mudskippers.

The mean fundamental frequency of tonal segments (1) was significantly different among individuals; (2) exhibited a relatively stereotyped nature; and (3) had a CVb/CTW ratio >1.0, therefore showing a potential for acoustic communication, and suggesting that tonal segments act as acoustical taggers [51] and neighbour-stranger discriminants (see enemy effect; [43]). In the aquatic goby P. bonelli, the wider scope for frequency and amplitude modulation of tonal sounds apparently improved both propagation and signal recognition [46,52]. Acoustical tagging and dear enemy effects would be advantageous for territorial mudskipper species. Future playback and discrimination experiments could verify these hypotheses.

In general, all the examined acoustic properties of P. septemradiatus would be classified as dynamic [53], due to their high level of within-individual variation, a feature also observed in other gobies [54,55], which nonetheless does not imply low repeatability [53]. The contiguous or simultaneous emission of sounds with visual aggressive displays that were previously described in oxudercine species (Periophthalmas sp.; [25]), such as gaping and dorsal fin erection, suggests the use of multimodal visual-acoustic communication, as hypothesised in other gobies [56].

Sonar organs are unknown in gobiods. In P. septemradiatus, the temporal association of pulsatile and tonal units, which never overlapped, suggests either a unique or two synchronised sonic mechanisms. In the first, most parsimonious hypothesis, the adjustable frequency of a sonic muscle would produce both pulses and tonal segments [57]. Significant correlations between size and acoustic properties were observed in many soniferous fishes (e.g. Triglidae [58]; Mormyridae [59]; Osphronemidae [60]; Mocho- kidae [61]; Pomacentridae [62]; Batrachoididae [63]; and Gobiidae [64]). Nonetheless, P. septemradiatus is not an isolated exception: no correlation was found between body size and acoustic properties in either aggressive and courtship sounds also in the freshwater goby Podabrius bonelli [65], whose structural acoustic properties closely resemble those of P. septemradiatus. Therefore, unless the mechanisms of sound emission are clarified in gobiods, no general conclusion on the relationship between body size and acoustic properties can be drawn.

Several teleosts apparently use a gas bladder to amplify the vibrations produced by sonic muscles [46]; nonetheless, not unlike many mudskipper species [66], P. septemradiatus lacks a gas bladder. Mudskippers may also be able to use the gas bubble retained during air-gulping [67] as a resonant structure; nonetheless, during our observations sounds were also produced with apparently deflated opercular chambers. Stridulatory mechanisms are also improbable, since these sounds generally have much higher fundamental frequencies [46]. The observed condition is compatible with a sonic muscle utilising a part of the body as a sound transducer (e.g. the pectoral girdle; [46,49]). Our correlation analysis of the acoustic properties supports this hypothesis: the observed constraints and trade-offs are consistent with a more

| Acoustic property | \( CV_b \) | \( CV_w \) | Grand mean ± SD | \( CV_b \) | \( CV_b/\overline{CV_w} \) |
|-------------------|-----------|-----------|----------------|-----------|-----------------|
| Bout duration (s) | -         | 80.8 (24.0–135.8) | 3.2 ± 1.6 (1.2–5.2) | 50.1 | 0.6 |
| Number of pulses per bout | -         | 85.9 (52.2–129.9) | 4.8 ± 2.1 (1.2–7.1) | 42.6 | 0.5 |
| Number of tonal segments per bout | -         | 55.5 (35.1–100.7) | 1.6 ± 0.5 (1.2–2.5) | 29.1 | 0.5 |
| Pulse rate (1/s) | -         | 55.7 (37.7–98.6) | 1.8 ± 0.6 (1.0–2.8) | 32.0 | 0.6 |
| Tonal rate (1/s) | -         | 74.4 (31.7–112.7) | 1.1 ± 0.6 (0.3–1.9) | 54.4 | 0.7 |
| Pulse duration (ms) | 32.4 (0.0–102.2) | 33.9 (18.0–85.4) | 58. ± 16 (33–76) | 27.3 | 0.8 |
| Pulse peak frequency (Hz) | 17.8 (0.0–63.9) | 20.0 (6.9–28.2) | 60. ± 6 (51–72) | 10.1 | 0.5 |
| Tonal duration (ms) | 37.4 (6.7–88.2) | 43.5 (27.8–62.1) | 461. ± 120 (279–713) | 26.0 | 0.6 |
| Tonal fundamental frequency (Hz) | 8.3 (0.1–18.6) | 6.6 (1.6–13.9) | 168. ± 14 (147–192) | 8.4 | 1.3 |
| Tonal fundamental frequency I | 15.4 (0.3–67.4) | 14.7 (9.1–36.9) | 194. ± 23 (155–229) | 11.7 | 0.8 |
| Tonal fundamental frequency C | 13.0 (1.0–45.6) | 12.3 (1.7–23.3) | 219. ± 18 (199–254) | 8.3 | 0.7 |
| Tonal fundamental frequency F | 13.0 (0.0–47.3) | 13.6 (2.1–23.4) | 210. ± 24 (182–257) | 11.6 | 0.9 |
| Tonal frequency modulation (C-I) | 58.4 (2.1–136.0) | 60.1 (30.2–82.2) | 29 ± 16 (13–62) | 53.9 | 0.9 |
| Tonal frequency modulation (F-C) | 84.8 (20.7–149.2) | 85.5 (38.6–134.4) | 23 ± 9 (9–36) | 37.6 | 0.4 |
| Tonal frequency modulation (F-I) | 81.2 (2.7–139.5) | 77.2 (59.9–105.6) | 31 ± 19 (10–70) | 62.3 | 0.8 |
| Pulse-pulse interval (ms) | 91.3 (11.5–199.5) | 76.3 (43.2–109.9) | 608. ± 384 (184–1,341) | 63.1 | 0.8 |
| Pulse-tonal interval (ms) | 142.1 (32.6–200.0) | 178.7 (88.4–300.0) | 56 ± 66 (0–181) | 118.0 | 0.7 |
| Tonal-pulse interval (ms) | 60.7 (3.2–149.4) | 61.5 (15.9–141.4) | 406. ± 202 (147–794) | 49.9 | 0.8 |
| Tonal-tonal interval (ms) | 82.8 (12.8–137.5) | 73.8 (16.2–137.1) | 1000. ± 493 (501–2,217) | 49.3 | 0.7 |

\( CV_b \): mean within-bouts coefficient of variation (= mean of the 78 bouts’ CVs; Fig. S5a); \( CV_w \): mean within-individual coefficient of variation (= mean of the 10 individual CVs; Fig. S5b); Grand mean: mean of the 10 individual means, each obtained as the mean of the bouts’ means (Table S5); CVb: between-individual CV; \( CV_w \): within-individual CV ratio; ranges in parentheses. doi:10.1371/journal.pone.0021434.t001
Table 2. Univariate one-way ANOVAs of the mean bouts’ values amongst individuals, for each acoustic properties.

| Acoustic property                  | n. bouts | N  | F (df 9) | p-value |
|-----------------------------------|----------|----|----------|---------|
| Bout duration (s)                 | 7.8±5.0  | 78 | 2.8      | 0.007   |
| Number of pulses per bout         | 7.8±5.0  | 78 | 3.1      | 0.004   |
| Number of tonal segments per bout | 7.8±5.0  | 78 | 1.3      | 0.240   |
| Pulse rate (1/s)                  | 7.7±4.8  | 77 | 2.5      | 0.014   |
| Tonal rate (1/s)                  | 7.7±4.8  | 77 | 3.3      | 0.001*  |
| Pulse duration (ms)               | 7.4±5.2  | 74 | 2.4      | 0.019   |
| Pulse peak frequency (Hz)         | 7.4±5.2  | 74 | 1.3      | 0.246   |
| Tonal duration (ms)               | 7.6±5.0  | 76 | 1.7      | 0.115   |
| Tonal fundamental frequency (Hz)  | 7.6±5.0  | 76 | 8.8      | 0.000*  |
| Tonal fundamental frequency I     | 7.6±5.0  | 76 | 2.2      | 0.034   |
| Tonal fundamental frequency F     | 7.5±5.0  | 75 | 4.9      | 0.000*  |
| Tonal frequency modulation (C-I)  | 7.6±5.0  | 76 | 2.2      | 0.035   |
| Tonal frequency modulation (F-C)  | 7.5±5.0  | 75 | 0.5      | 0.834   |
| Tonal frequency modulation (F-I)  | 7.5±5.0  | 75 | 2.7      | 0.009   |
| Pulse-pulse interval (ms)         | 5.7±5.0  | 57 | 1.5      | 0.193   |
| Pulse-tonal interval (ms)         | 6.4±5.5  | 64 | 1.1      | 0.382   |
| Tonal-pulse interval (ms)         | 5.1±3.4  | 51 | 1.7      | 0.124   |
| Tonal-tonal interval (ms)         | 2.7±1.5  | 27 | 0.3      | 0.958   |

*p statistically significant p-values (α = 0.05), after Holm-Bonferroni correction for multiple comparisons; n. bouts: mean and SD of the number of bouts contributed by the 10 individuals to the measured variate (all individuals differently contributed to each measured variate); N: number of bouts recorded for the measured variate amongst all individuals.

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The fish turns around the cage where the intruder is confined, apparently trying to reach the intruder

Table 3. Descriptions of the aggressive behaviours examined in the territorial owner during the dyadic contests, that were simultaneous or contiguous to acoustic emissions.

D1,2  Aggressive display of the first and second dorsal fins [25]

APP  Directed and rapid movement (‘tripod’ locomotion; [78]) towards the cage containing the intruder

ENT  The fish presses the snout against the cage, in an apparent attempt to reach the intruder

GAP  Head slightly lifted, mouth wide open, hyoid depressed and extension of branchiostegal membranes (gaping; [25]), darkening of body colouration

SLT  The fish draws away from the cage, heading towards the defended shelter

TUR  The fish turns around the cage where the intruder is confined, apparently trying to reach the intruder
males’ competitive feeding. Sounds could be recorded with a hydrophone (B&K 8103 Naerum, Denmark; sensitivity $-210 \text{dB re} \ 1 \mu \text{Pa}$) inserted into the wet mud and connected to a conditioning amplifier (B&K 2626 Naerum, Denmark) and to a portable digital audio tape recorder (DAT: Sony D7 Park Ridge, NJ, USA).

Therefore, standardised protocols were designed to observe dyadic male-male encounters and record the associated sound production. Single males were isolated in experimental tanks (same size and equipment of the communal tanks), but only a single shelter made of slate pieces or terracotta was provided, which was rapidly filled with mud. A single hydrophone was inserted into the mud at a depth of 2–5 cm, in front of the opening of the resident’s shelter and within an acceptable range of the attenuation distance from the source of possible vocalizations [76], being connected both to the DAT and a conditioning amplifier (B&K 2626 Naerum, Denmark) and to a portable digital audio tape recorder (DAT: Sony D7 Park Ridge, NJ, USA).

Table 4. Comparative overview of sound production in gobioid fishes.

| Species | Call structure (mean peak or dominant frequency) | Context | Geographic distribution | Habitat type | References |
|---------|-----------------------------------------------|---------|------------------------|--------------|------------|
| Pomatoschistus canestrini (Ninni, 1883) | Pulsatile (130 Hz) | A, P | Med | B | [57] |
| P. minutus (Pallas, 1770) | Pulsatile (100 Hz) | P | EA, Med, BS | B, M | [55] |
| P. marmoratus (Risso, 1810) | Pulsatile (120 Hz) | P | Med, BS | B | [79] |
| P. pictus (Malm, 1865) | Pulsatile (80–200 Hz) | A, C, P | Med | M | [39,54] |
| Knipowitschia panazae (Verga, 1841) | Pulsatile (190 Hz) | P | Med | B | [79] |
| K. punctatissima (Canestrini, 1864) | Pulsatile (130 Hz) | P | Med | F | [80] |
| Podagogus bonelli (Bonaparte, 1846) | Mixed* (180 Hz) | A, P, C | Med | F | [80] |
| P. nigricans (Canestrini, 1867) | Tonal (110 Hz) | C | Med | F | [81] |
| Gobius paganellus Linnaeus, 1758 | Tonal (100 Hz) | A, C | EA, Med, BS | B, M | [57] |
| G. cobitis Pallas, 1814 | Pulsatile (90 Hz) | A, C | EA, Med, BS | B, M | [57] |
| G. niger Linnaeus, 1758 | Pulsatile (100 Hz) | A, C | EA, Med, BS | B, M | [57] |
| Zostera depressa ophioccephalus (Pallas, 1814) | Pulsatile (220 Hz) | A, C | Med, BS | B, M | [57] |
| Proterorhinus marmoratus (Pallas, 1814) | Tonal (70–130 Hz) | C | Med, BS, CS | F | [82] |
| Bathygobius soporator (Valenciennes, 1837) | Pulsatile (145 Hz) | C | Med, EA, WA | B, M | [83] |
| B. fuscus (Ruppell, 1830) | Pulsatile (120 Hz) | C | IWP | B, M | [84] |
| B. curacao (Metzelaar, 1919) | Pulsatile (100–200 Hz) | C | WA | B | [85] |
| Gobiosoma bosc (Lacepède, 1800) | Clicks (1–5 kHz) | C | WA | B, M | [86] |
| Odontobutis obscura (Temminck & Schlegel, 1845) | Pulsatile (300 Hz) | C | China, Japan, Korea | F | [38] |
| Neogobius melanostomus (Pallas, 1814) | Pulsatile (180 Hz) | C | BS, CS | F | [87] |

*the call is composed by tonal and pulsatile elements; A = aggressive; B = brackish; BS = Black Sea; C = courtship; CS = Caspian Sea; EA = Eastern Atlantic; F= freshwater; IWP = Indo-West Pacific region; M = marine; Med = Mediterranean; P = prespawning; WA = Western Atlantic.

The aggressive responses of the focal animal (resident) prevalently took place within a restricted area comprised between the cage and the shelter, at a few cm from the hydrophone. Sounds were analysed in real time (SASLab Pro® Avisoft Bioacoustics Berlin, Germany; window type: hamming, FFT: 256, frame: 100, bandwidth: 20 Hz, resolution: 16 Hz, overlap: 87.5%; Fig. S1). Analagical signals were digitalised (1,500 Hz sampling) and acoustic components which were not present in the recorded fish sounds (band: 30–500 Hz) were band-pass filtered, in order to eliminate sources of disturbance and distortion for the mudskippers’ waveforms. Only signals with higher signal to noise ratios were analysed.

The recorded calls of 10 individuals were resolved into 78 “complex bouts” by defining a minimum time gap between two subsequent sound units (5 s). Bouts were then broken down into pulsatile and tonal units [77], and several acoustic properties were defined, measured and analysed (STATISTICA v 7.0© StatSoft Tulsa, OK, USA; Table S1, S2). In particular, tonal frequency modulation was quantified as the differences between the frequency of initial (I) and central (C); initial and final (F); and central and final portions of tonal segments, each portion corresponding to 4 cycles, randomly taken from each portion of sound [57].

To estimate whether the observed acoustic properties varied significantly amongst individuals, the means of the bouts’ values of each individual were logarithmically transformed to conduct a one-way MANOVA to test for multivariate difference amongst individuals; and 19 one-way ANOVAs with Holm-Bonferroni corrections, to specifically test for each acoustic property (Table S4). Mean within-bout ($CV_{sb}$), mean within-individual ($CV_{w}$), and between-individual ($CV$) coefficients of variation ($CV = (SD/X) \times 100$; untransformed
The species studied and housing conditions. Tonal and pulsed artificial sounds through the substrate, recorded as particle displacements and pressure waves. Supplementary references.

**Video S1** A complex bout emitted by a male of *P. septemradiatus*. Left panel: video recording; the fish, which is a resident territorial owner, is oriented towards the cage containing the intruder (on the right, not visible). The oscillogram (top right panel) and spectrogram (bottom right panel) of the emitted bout show a train of pulses followed by a tonal segment. Acoustical and video recordings are synchronised. Note the rapid downward movements of the head made during the pulse emissions, and the behaviours preceding and following the vocalisation (dorsal fins' display and jump, respectively). During the sequence, the mudskipper is also retracting its eyes into the dermal cups positioned below the orbits (“blinking”), to clean and moisten the eye surface while out of water.

**Table S1** Descriptions of the acoustical properties of bouts and sound units.

**Table S2** Size, number of acoustic bouts and sound units of the recorded resident individuals.

**Table S3** Pearson correlation coefficients (* = p < 0.05) of the relationships amongst the individual means of each acoustic property (n = 10 specimens).

**Table S4** Mean acoustic properties of bouts per individual.

**Table S5** Individual means.

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**Author Contributions**

Conceived and designed the experiments: GP SM. Performed the experiments: VG GC. Analyzed the data: GP SM VG GC. Contributed reagents/materials/analysis tools: GP SM VG GC. Wrote the paper: GP SM JAC PT.

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**Supporting Information**

**Figure S1** Temporal organisation of a mudskipper call and some acoustic properties. (TIF)

**Figure S2** Boxplots of the within-bout (a: CV<sub>b</sub>) and within-individual (b: CV<sub>w</sub>) coefficients of variation. CV<sub>b</sub> are the coefficients of variation of acoustic properties of the sound elements measured in each of the 78 bouts (mean values = CV<sub>b</sub>). CV<sub>w</sub> are the coefficients of variation of the mean acoustic properties of the bouts of each of the 10 individuals (mean values = CV<sub>w</sub>). Boxes indicate the middle 50% of the distribution (interquartile range); whiskers indicate minimum and maximum values; horizontal lines are median values. BD bout duration; NP number of pulses; NTS number of tonal segments; PD pulse duration; PPF pulse peak frequency; PPI pulse-pulse interval; PR pulse rate; PPT pulse-tonal interval; TD tonal duration; TFF tonal fundamental frequency; TPI tonal-pulse interval; TR tonal rate; TTI tonal-tonal interval (see also Table 1).

**Figure S3** Layout of the housing terraria (community tank). FP: polystyrene foam panel; h/T: hygrometer’s and thermostat’s probes; IR: thermostated heating lamps; Md: mud; P: pool (non toxic plastic bowl); T: thermometer; z: three parallel zones separated by wooden logs and flat slate pieces to reduce aggressive interactions.

**Figure S4** Spectrograms, power spectrum and waveforms of tonal artificial sounds acoustically similar to the tonal segments of the calls of *P. septemradiatus*, synchronously recorded in terms of pressure (a, b and c) and particle velocity (d, e and f); sounds were produced as a descending scale from 300 Hz at third octave steps (hamming FFT: 512, frame: 100, bandwith: 10 Hz, resolution: 8 Hz, overlap: 93.75%); for the power spectra, amplitude on a linear scale of 100 mV per division (arbitrary units).

**Figure S5** Spectrograms, power spectrum and waveforms of pulsed artificial sounds acoustically similar to the pulsatile elements of the calls of *P. septemradiatus*, synchronously recorded in terms of pressure (a) and particle velocity (b); see Figs. S4 for more details.

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