Sounds produced by the longsnout seahorse: a study of their structure and functions

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Abstract

Seahorses are known to produce sounds in different behavioural contexts, but information on the sound production in this fish group is scarce. Here we examined the acoustic behaviour of the longsnout seahorse *Hippocampus reidi* by analysing sound production when fish were introduced to a new environment and during feeding, handling and courtship. We show that males and females produce two distinct sound types: 'clicks' (main energy between 50 and 800 Hz) during feeding and courtship, and previously undescribed 'growls' (main energy concentrated below 200 Hz). The latter consists of series of sound pulses uttered in stress situations when the animals were handheld. Growls were accompanied by body vibrations, and may constitute an additional escape mechanism in seahorses, which might startle predators. During reproductive behaviour, clicks were most abundant on the third (last) day of courtship; they were particularly associated with the males' pouch-pumping behaviour, suggesting synchronization between sound production and courtship behaviour. This is consistent with the biology of *Hippocampus* species, which are mostly monogamous and form pair bonds. Thus, a courtship call may be used to signal readiness to mate.

Introduction

Fishes produce sounds in a wide range of contexts, such as during territorial defence, in disturbance situations, during feeding, territory advertisement, mate attraction, courtship and spawning (for a review, see Ladich & Myrberg, 2006; Myrberg & Lugli, 2006; Kasumyan, 2009; Luczkovich, Sprague & Krahforst, 2011). Bony fishes possess the largest diversity of sound-producing mechanisms of all vertebrate classes (Ladich & Fine, 2006). The majority of vocal species studied so far produce low-frequency sounds by vibrating their swim bladders via intrinsic or extrinsic drumming muscles. Some taxa such as catfish generate broadband stridulatory sounds by rubbing pectoral spines in grooves of the shoulder girdle (Fine & Ladich, 2003; Ladich & Fine, 2006; Parmentier *et al.*, 2010; Ladich & Bass, 2011).

Sound production in seahorses (*Hippocampus* spp.) has been mentioned in several ecological and behavioural studies, mainly during feeding events (e.g. Bergert & Wainwright, 1997; Felício *et al.*, 2006; Anderson, 2009). The most conspicuous sounds emitted by those fish are broad-band clicking sounds, which are generated by a skull stridulatory mechanism (Colson *et al.*, 1998). Additionally, seahorses reportedly vocalize when introduced to new environments, in stress situations (i.e. when handheld) and during courtship (Dufossé, 1874; Fish, 1953; Fish & Mowbray, 1970; Colson *et al.*, 1998; Anderson, 2009; Anderson *et al.*, 2011).

The first probable record of sound production by seahorses dates from the nineteenth century (Dufossé, 1874). Nonetheless, until recently, specific studies have been rare and limited to a few species (*H. hippocampus*: Dufossé, 1874; *H. erectus*: Fish, 1953; Fish & Mowbray, 1970; Colson *et al.*, 1998; Anderson, 2009; Anderson *et al.*, 2011; *H. zosterae*: Colson *et al.*, 1998; *H. kuda*: Chakraborty *et al.*, 2014).

Besides sound production, seahorses exhibit complex behaviours and life histories, such as low mobility, small home ranges, mate fidelity (in most species studied), a complex courtship behaviour and male 'pregnancy' (Foster & Vincent, 2004). Therefore, seahorses provide an opportunity to assess fish acoustic communication from a unique perspective. The present study investigated the sound repertoire and sound

characteristics of *H. reidi* Ginsburg, 1933 produced in different behavioural contexts.

Methods

Animals

Our study focuses on the longsnout seahorse *H. reidi*, which is distributed from Cape Hatteras, United States, to Brazil and the Gulf of Mexico (Lourie, Vincent & Hall, 1999). Captive bred animals were supplied by the Haus des Meeres – Aqua Terra Zoo, a public aquarium in Vienna, Austria. Males ($n = 10$; body height: 10.9–17.3 cm) and females ($n = 11$; 11.6–17.0 cm) used in this study were kept separately in two bare bottom tanks ($100 \times 50 \times 50$ cm) filled with artificial sea water (salinity 35; Reef Crystals, Aquarium Systems Inc., Sarrebourg, France) and kept under a 12:12 h L : D cycle at 25 ± 1°C. Biological filtration was accomplished with a sintered glass medium (Siporax, Schott Inc., Mainz, Germany) in an external canister filter and open-celled polyurethane (PU) foam. Several artificial holdfasts (plants and corals) were provided. Animals were fed *ad libitum* twice daily with frozen mysid shrimps (Ruto Inc., Zevenhuizen, the Netherlands). Thawed food was supplemented with ascorbic acid to prevent spoilage. Specimens were individually identified by natural colour patterns and went through an acclimation period of at least 1 week before being tested.

Sound recordings

Experimental set-up

Sound recordings were performed in an experimental tank ($60 \times 30 \times 30$ cm) placed on a vibration-isolated table in a soundproof room. The tank bottom was covered with sand or open-celled PU foam (2 cm thick). Tank walls (except front) were lined inside with acoustically absorbent material (air-filled packing wrap) to reduce resonances and reflexions (for the effect, see fig. 1 in Wysocki & Ladich, 2002). Temperature was kept at 25 ± 1°C, and a 20% water change was performed in the end of every trial. An artificial plant was provided as a holdfast in all trials; in the courtship trials, an artificial coral was also provided.

Sounds and acoustic behaviour were recorded using a hydrophone (Brüel & Kjaer 8101, Brüel & Kjaer Sound & Vibration Measurement A/S, Naerum, Denmark) connected to a power supply (Brüel & Kjaer 2804) and by a video camera (Sony CCD-VX1E, Sony Corporation, Tokyo, Japan) positioned behind a curtain. Both hydrophone and camera were connected to an S-VHS HiFi VCR (JVC HRD 4700 EG, JVC Kenwood Corporation, Yokohama, Japan), so that behaviours and sounds were recorded simultaneously. The hydrophone was positioned in the centre of the tank (16 cm away from the bottom) in all trials, except during courtship; in this case, it was placed closer to bottom (7 cm away), where the seahorses spent most of their time.

Behavioural contexts

Introduction to a new environment

In each trial ($n = 16$), one specimen was transferred to the test tank and recorded for 1 h. As none of the individuals tested produced sounds, recordings in that context were not further considered.

Feeding

In each trial ($n = 13$, following the aforementioned 1-h period), mysid shrimps were offered to the seahorse and the sounds produced were recorded. Recordings lasted until the animal ceased feeding (15–36 min). The position each animal assumed in the tank for every feeding strike was recorded. Only sounds associated to the effective capture of food, that is, when the mysid shrimp was completely ingested, were considered for analysis, following Anderson (2009).

Stress situation

Each seahorse ($n = 16$) was held dorsally by the trunk and positioned laterally at a distance of 2 cm from the hydrophone. Recordings lasted 1–4.3 min. Although handling has a level of artificiality, it does provoke fish to produce sounds as if they were captured by a predator. Seahorses are frequently grabbed and held by predators such as frogfish before being swallowed (Lourie *et al.*, 1999; Kuiter, 2009).

Courtship

Five pairs of *H. reidi* were tested. As most seahorse species studied so far exhibit size-assortative mating (Foster & Vincent, 2004), males and females were selected for similar body height (±0.5 cm; measurements followed Lourie *et al.*, 1999). As *H. reidi* is a diurnal species (Felício *et al.*, 2006), each pair was observed *ad libitum* (Lehner, 1996) in the morning, as follows: from 08:00 to 11:00 h on the first and second days, and from 08:00 until copulation on the third day (when all copulations took place). The resulting specific ethogram encompassed only the behaviours associated with sounds produced during courtship. The frequency of sound production was assessed according to those behaviours, as well as to courtship day and to the sex of seahorses. Sound production by all animals occurred simultaneously to a clear upward movement of the animals' head, enabling the individual producing the sounds to be recognized with confidence. Males and females were considered as being 'together' when they were ≤15 cm apart and at least one of the individuals presented any interaction behaviour (such as brightening); they were considered 'apart' when the distances exceeded 15 cm (Anderson, 2009). The frontal glass of the test tank was divided into 50 sectors (6×5 cm each) so that distances could be visually determined. Animals were not fed during the courtship trials; thus, no feeding clicks were recorded.

Determination of sound pressure levels

Instantaneous sound pressure levels (SPL; L_{LFP}, linear frequency weighting, 5–20 kHz, root mean square fast time

Table 1 Characteristics of sounds produced by *Hippocampus reidi* in different behavioural contexts

Sound characteristics	Sound type	Total	Males	Females	*t*-test
Feeding	Click	*n* = 13	*n* = 7	*n* = 6	
Pulse duration (ms)		16.1 ± 1.8 (7.2–27.1)	15.6 ± 2.7 (7.2–27.1)	16.7 ± 2.4 (8.7–25.3)	*t* = −0.28
Dominant frequency (Hz)		323.5 ± 40.3 (122.3–576.6)	362.1 ± 50.2 (188.1–559.8)	278.4 ± 64.7 (122.3–576.6)	*t* = 1.04
SPL (dB re 1 μPa)		119.8 ± 1.8 (112.3–133.8)	118.9 ± 2.7 (112.3–133.8)	120.9 ± 2.2 (115.2–131.0)	*t* = −0.57
Courtship	Click	*n* = 10	*n* = 5	*n* = 5	
Pulse duration (ms)		19.4 ± 3.8 (8.0–44.6)	20.0 ± 6.8 (8.0–44.6)	18.8 ± 4.3 (10.5–35.2)	*t* = 0.15
Dominant frequency (Hz)		368.9 ± 41.2 (211.0–648.0)	358.9 ± 82.6 (211.9–648.0)	378.9 ± 27.9 (275.7–440.0)	*t* = −0.23
SPL (dB re 1 μPa)		105.0 ± 2.4 (93.6–115.5)	111.8 ± 1.9 (104.4–115.5)	99.1 ± 1.6 (93.6–102.4)	*t* = 5.10[a]
Stress situation	Growls	*n* = 16	*n* = 8	*n* = 8	
Pulse duration (ms)		35.6 ± 0.8 (30.0–41.1)	36.3 ± 1.3 (31.9–41.1)	34.9 ± 1.0 (30.0–39.2)	*t* = −0.84
Pulse period (ms)		111.2 ± 6.8 (77.1–164.3)	113.6 ± 8.7 (90.3–164.3)	108.9 ± 11.0 (77.1–154.4)	*t* = −0.34
Pulse repetition rate (s^{-1})		8.8 ± 0.6 (4.2–13.4)	8.3 ± 0.7 (4.2–10.3)	9.2 ± 1.0 (5.6–13.4)	*t* = 0.72
Dominant frequency (Hz)		115.3 ± 1.7 (103.0–125.0)	113.5 ± 2.5 (105.0–123.0)	117.1 ± 2.4 (103.0–125.0)	*t* = 1.06
SPL (dB re 1 μPa)		115.0 ± 0.5 (112.0–119.0)	114.4 ± 0.9 (112.0–118.0)	115.6 ± 0.6 (114.0–119.0)	*t* = 1.15

Data presented as mean ± standard error of the mean and range in brackets.

[a]Significant difference between sexes: *P* < 0.01.

n, number of seahorses sampled; SPL, sound pressure level.

weighting) were determined in parallel to sound recordings using a sound level meter (Brüel & Kjaer Mediator 2238) connected to the hydrophone power supply. As seahorses produced sounds at different distances to the hydrophone (during feeding and courtship trials), the sectors placed on the frontal glass of the test tank were used to better record fish position during sound emission. A feeding click was selected (one whose sound characteristics were similar to the mean values according to Table 1) and continuously played back at a constant SPL by an underwater speaker (Fuji 7G06, 8 ohm, 0.8 W; 33 mm in diameter), in each sector of the tank in the front and back half of the aquarium. To determine SPL values independently of the animals' distance to the hydrophone, the relative difference between the SPL measured 2 cm away from the hydrophone and at the sections where the seahorses produced sounds was calculated; this value was added to the SPL values measured during recordings (following Wysocki & Ladich, 2001). This correction of SPL values was applied to the two hydrophone positions. In order to increase accuracy during SPL measurements, holdfasts were placed halfway between the hydrophone and the tank walls. The seahorses could grasp these holdfasts, which reduced their movement in the tank. All trials started only when the animal was holding the holdfast. Moreover, in feeding trials, mysid shrimps were offered in the central longitudinal line of the tank, which minimized seahorse movement towards the front and the back walls of the tank. Finally, determination of SPL during playback was simultaneously video recorded, and positions of the camera and the observer were the same in both playback and recording trials.

Sound analysis

Sound recordings were analysed using Cool Edit 2000 (Syntrillium Software Corporation, Phoenix, AZ, USA) and S_TOOLS-STx 3.7.8 (Acoustics Research Institute, Austrian Academy of Sciences, Vienna, Austria). We analysed 3–10 feeding clicks, 1–12 courtship clicks and 30 sound pulses of growls per animal recorded (sampling rate 44.1 kHz). Pulse duration was analysed in all behavioural contexts. In distress growls, we measured pulse period (time between maximum peaks of consecutive pulses within a growl) and repetition rate (number of pulses s^{-1}). The dominant frequency of the sounds was determined through cepstrum-smoothed power spectra (Noll, 1967). In order to attenuate the effect of tank resonances, all recordings were low pass filtered (3000 Hz) (Akamatsu *et al.*, 2002).

Data analysis

All data were verified for normal distribution and homogeneity of variances using Shapiro–Wilk's and Levene's tests, respectively. When these assumptions were not met, nonparametric tests were performed. Means of sound characteristics were calculated for each fish and each behavioural context, and used for further analyses.

Differences between sexes in dominant frequency, pulse duration, pulse period and pulse repetition rate (when applicable), and SPL of sounds produced, were calculated using *t*-test. Relationships between seahorse height and sound characteristics were determined using Pearson's (log-transformed growls' pulse period values) and Spearman rank (for feeding clicks' SPL values) correlation coefficients.

In order to calculate differences in sound characteristics recorded in different behavioural contexts (feeding, stress, courtship), a Kruskal–Wallis test was performed followed by a Dunn's post hoc test. The difference in the number of sounds emitted during courtship was compared using the Mann–Whitney *U*-test (between males and females) and the Friedman test (among courtship days), followed by a Dunn's post hoc test.

Results

Hippocampus reidi produced two distinct sounds (Table 1) in different behavioural contexts: *click sounds* – single pulses, recorded during feeding and courtship, frequently audible to the observer during trials; and *growling sounds* – a series of sound pulses emitted only when handheld and never during intraspecific interactions.

Feeding clicks

Feeding clicks were produced during prey capture and consisted of short broadband sounds that were typically uttered singly (mean duration: 16.1 ms), with the main energy ranging from 50 to 800 Hz. The mean SPL (L_{LFP} re: 2 cm) of feeding clicks was 119.8 dB re 1 μPa (see Table 1). They were produced in all feeding events recorded (Fig. 1a).

Click duration [$r = -0.297$, $n = 13$, not significant (NS)], dominant frequency ($r = 0.279$, $n = 13$, NS) and SPL ($r_S = -0.022$, $n = 13$, NS) were not significantly correlated to seahorse height. Moreover, no differences were found between males and females in these sound characteristics (*t*-test; $P > 0.05$ in all cases; see Table 1).

Courtship clicks

These comprised short single pulses with mean duration of 19.4 ms (Table 1; Fig. 1b), main energy between 100 and 800 Hz and SPL (re: 2 cm) of 105.0 dB. The SPLs of courtship clicks were lower than of feeding clicks but did not differ from distress growls (see next topic) (Kruskal–Wallis test: $H = 12.9$; $n = 20$, $P < 0.01$; Dunn's post hoc: $P < 0.001$). Male courtship clicks had higher SPLs than clicks of females [*t*-test: $t = 5.10$, degrees of freedom (d.f.) = 11, $P < 0.01$; Table 1], but with similar duration and dominant frequency (*t*-tests, $P > 0.05$ in both cases; Table 1). Sex differences in SPL were not due to differences in size as the sound characteristics of courtship clicks were not correlated to body height (SPL: $r = 0.490$; duration: $r = 0.276$; dominant frequency: $r = 0.468$; in all cases, $n = 10$, $P > 0.05$).

Hippocampus reidi's courtship lasted for 3 days and encompassed a sequence of behaviours accompanied by sound production (Table 2). The behaviour *pouch pumping* was the most frequently associated with clicks (63.6%), followed by *promenading* (25.0%), *brightening* (9.1%) and *raising* (2.3%), whereas no sounds were emitted during circling, quivering, pointing, attempting to copulate and copulating.

Both males and females produced click sounds during courtship, and sound production mostly (86.1%) took place when the distances between the fishes were <15 cm. The number of sounds produced by males and females during courtship did not differ significantly from one another, neither considering all days together (*U*-test: $U = 6.0$, $n = 10$, NS) nor separately (*U*-tests, $n = 10$ in all cases: day 1, $U = 10.0$, NS; day 2, $U = 9.5$, NS; day 3, $U = 7.0$, NS). A considerable increase in click production was observed on the last (third) day of courtship (Friedman test: $\chi^2 = 15.2$, d.f. = 2, $P < 0.001$, Dunn's post hoc: $P < 0.05$; Fig. 2). When considering sexes
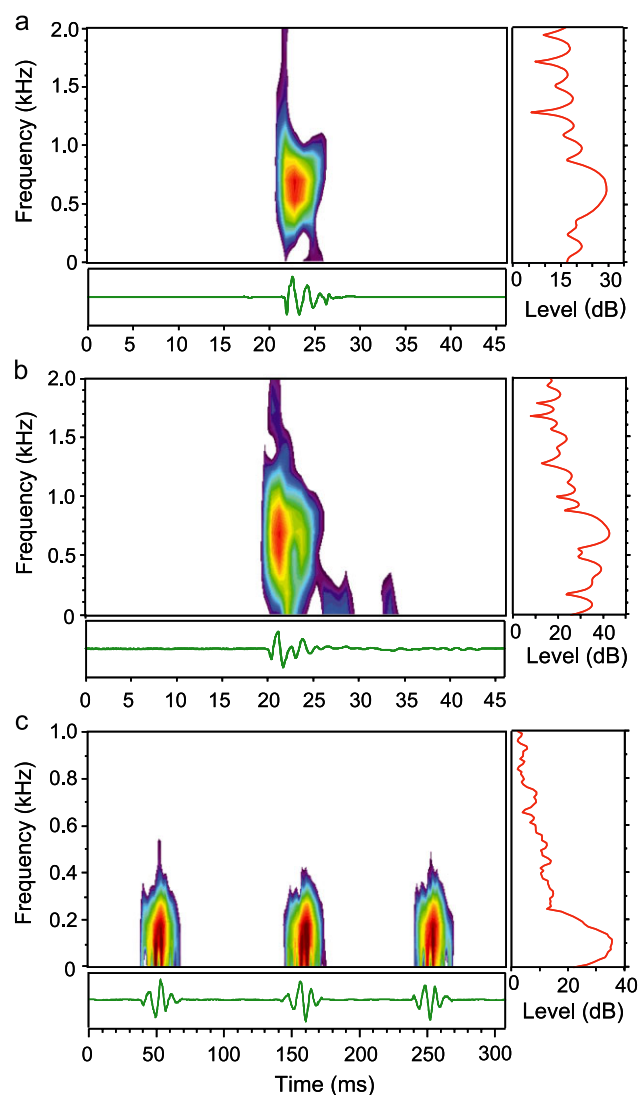
Figure 1 Sonograms, oscillograms (below) and spectra (on the right) of sounds produced by *Hippocampus reidi*. (a) Feeding click, (b) courtship click and (c) three pulses of a growling sound produced when handheld. Sampling rate: 44.1 kHz; filter bandwidth: 250 Hz (a), 300 Hz (b) and 150 Hz (c); 75% overlap, Hanning window.

separately, differences in click production among courtship days were detected for both females (Friedman test: $\chi^2 = 7.176$, d.f. = 2, $P < 0.05$, Dunn's post hoc: NS) and males (Friedman test: $\chi^2 = 8.588$, d.f. = 2, $P < 0.05$, Dunn's post hoc: $P < 0.05$), both of which produced more clicks on the last day in comparison with the first day.

Growling sounds

Seahorses emitted low-frequency growling sounds while handheld. Growls consisted of series of sound pulses often lasting as long as the fish were handheld (maximum duration: 260 s) (Fig. 1c). The mean duration of sound pulses was 35.6 ms,

Table 2 Ethogram of sound-associated behaviours exhibited by *Hippocampus reidi* during courtship

Behaviour	Description
Together	Distance between seahorses ≤15 cm, and at least one of the individuals presented any interaction behaviour (such as brightening).
Apart	Distance between seahorses >15 cm.
Brightening	The seahorse brightened its body colouration, except for the head and natural marks (i.e. dorsal saddles, blotches) and the keel.
Promenading	Seahorses brightened in colour and, positioned side by side, with or without intertwined tails, moved close to the tank bottom and over/around holdfasts. Frequently, lowered the head, positioning the snout close to the trunk, in a jackknife position; concomitantly, the body quivered laterally, with rapid movements.
Pouch pumping (males only)	Male's tail moved towards the pouch; concomitantly, the seahorse inflated the pouch with water.
Raising	Seahorse(s) raised the head, pointing the snout upward and rose in the water column, towards the water surface.
Click production	Seahorse rapidly raised the head, opened the mouth, protruded the hyoid and returned to the original position. This movement was accompanied by a click sound.

Definitions based on Masonjones & Lewis (1996), Vincent (1994) and Anderson (2009), with modifications.
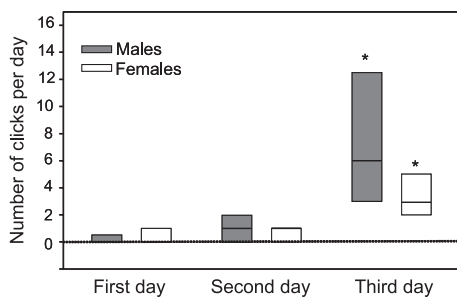
Figure 2 Seahorse sound production on different days of courtship. Median (line within boxes, lower line on first day, upper line on second day female), 25th and 75th percentile (lower and upper limit of box) of the number of clicks per day produced by both sexes during courtship within the first 3 days. Asterisks indicate significant differences between days within sexes.

with a mean pulse period of 111.2 ms. The mean SPL was 115.0 dB and the main energy was concentrated below 200 Hz (see Table 1). During production of growling sounds the seahorse's body vibrated. Growls could not be recorded during feeding or courtship.

The acoustic characteristics of growling sounds were neither related to seahorse height (Pearson's correlation, *P* > 0.05 in all cases) nor differed between sexes (*t*-test, NS in

all cases; Table 1). The dominant frequency of growling sounds was significantly lower than that of both feeding and courtship clicks (Kruskal–Wallis test: *H* = 11.9; *n* = 20, *P* < 0.01; Dunn's post hoc: *P* < 0.05).

Discussion

Hippocampus reidi produced two types of sounds: clicks and growling sounds. Although click sound production is commonly known from other members of the family Syngnathidae (e.g. Fish, 1953; Colson *et al.*, 1998; Ripley & Foran, 2007; Anderson, 2009), a 'tambour' (=drum) sound was only described by Dufossé (1874) in *H. hippocampus*.

Contexts and functional significance

Similar to other fish, seahorses utter sounds in several behavioural contexts. The best investigated acoustic behaviour in seahorses and pipefishes is the production of clicking sounds during feeding (e.g. Colson *et al.*, 1998; Ripley & Foran, 2007; Anderson, 2009). Moreover, seahorses produce sounds during courtship, in stress situations (Dufossé, 1874; Anderson, 2009; present study), during male–male competition (Colson *et al.*, 1998) and when introduced in a new environment (Fish, 1953).

Feeding

The functional significance of feeding clicks is unknown. Feeding clicks are not related to the success in capturing prey (Anderson, 2009), nor are they restricted to food intake events because they were also recorded in unsuccessful attempts (T. P. R. Oliveira, pers. obs.). It is unlikely that they are merely a by-product of prey capture because that may increase predation risk. Anderson (2009) suggested that clicks produced by *H. erectus* during feeding signal a food source to a mate or may help in locating potential mates in a population where individuals are sparsely distributed.

The duration of the feeding clicks produced by *H. reidi* was similar to that of *H. zosterae* (5–20 ms: Colson *et al.*, 1998) and two pipefish species (5–22 ms: Ripley & Foran, 2007), but differed from those produced by the sympatric and morphologically similar *H. erectus* (110 ms: Anderson, 2009). Interspecific differences in temporal patterns of sounds might be used in species discrimination, especially in sympatric and closely related species (e.g. Myrberg, Spanier & Ha, 1978; Malavasi, Collatuzzo & Torricelli, 2008; Colleye *et al.*, 2011).

Courtship

Sound production during reproductive behaviour is well known in at least 20 fish families, and typically, sounds are produced by males when advertising their territories, while attracting females to their nest sites or during courtship and spawning (for a review, see Myrberg & Lugli, 2006). So far female courtship and spawning sounds are known from the croaking gourami *Trichopsis vittata* (perciform family Osphronemidae) (Ladich, 2007) and from seahorses. In

H. reidi (this study) and *H. erectus* (Anderson, 2009) both sexes vocalize during courtship, in particular on the last day, before copulation.

Seahorses are known for their unique life-history and courtship behaviour: most species of *Hippocampus* studied are monogamous, form pair bonds and exhibit complex courtship behaviour, which is performed by both sexes and culminates in the female transferring the oocytes into the males' pouch (Foster & Vincent, 2004). Moreover, several syngnathid fish (i.e. pipefish) show sex role reversal and females are the more active sex (see Rosenqvist & Berglund, 2011 for a review), and is usually the courting sex that produces sound. Courtship sounds in fishes may help to advertise and increase spawning readiness or bring individuals together (Fish, 1953; see Myrberg & Lugli, 2006 for a review). It is assumed that female pre-spawning sounds stimulate and coordinate spawning behaviour in *T. vittata* (Ladich, 2007).

In *H. reidi*, the predominance of the click sounds on the last day of courtship, and mostly associated with the male pouch pumping, corroborates previous studies showing that the frequency of courtship displays in other seahorse species escalates on the last day (*H. erectus*: Anderson, 2009; *H. zosterae*: Masonjones & Lewis, 1996; *H. fuscus*: Vincent, 1990). Vincent (1990, 1994) states that pouch pumping may allow females to assess males prior to mating. Accordingly, an increase in male click production may communicate to females a readiness to mate. Anderson (2009) concluded based on muting experiments in *H. erectus* that the acoustic signalling may help to maintain pair bonding and identify sexually mature partners. This suggests that absence of the acoustic signals affects the courtship behaviour in seahorses, which is based on mutual signalling (Vincent, 1990, 1994; Masonjones & Lewis, 1996). The production of clicks potentially transmits information in the sexual context of seahorses, as a component of a multimodal signalling system.

Clicks emitted during courtship were lower in level than those emitted during feeding. This may indicate that feeding and courtship clicks are addressed to different receivers or to receivers at different distances. This, however, remains to be confirmed in field studies. Kenyon (1994) mentioned that male bicolour damselfish *Stegastes* (formerly *Pomacentrus*) *partitus* produce high-level chirp sounds to advertise their nest site and repel neighbouring males, and attract females over long distances, and that they emit low-level grunts when a female enters the nest site. Lowering the sound level during courtship reduces spawning intrusion by neighbouring males. Similarly, Ladich (2007) showed that female pre-spawning sounds in *T. vittata* are of a lower level than female aggressive sounds, again indicating that they are not intended for other mates and that they also reduce spawning intrusions or predation. The assumption is therefore that the courtship clicks produced by *H. reidi* are important during mating.

Our results also revealed that male *H. reidi* produced courtship clicks of higher SPL than females. Louder sounds may indicate higher fitness and higher success rates during competitive interactions. Louder sounds may influence the outcome of agonistic interactions between males, as in other species (Ladich, 1998). Indeed, the production of click sounds

during male–male competition has been observed in *H. zosterae* (Colson *et al.*, 1998) and in *H. reidi* in captivity (T. P. R. Oliveira, pers. obs.).

Stress

In addition to clicking sounds, *H. reidi* produces low-frequency sounds in stress situations when handheld. This is the first study to characterize this sound type. Previous studies mentioned vibration of the seahorse's body, for example, when taken out of the water, in *H. erectus* (Anderson, 2009) and in *H. hippocampus* (Dufossé, 1874). Dufossé (1874) wrote that vibrations were accompanied by 'drum'-like sounds (tambour) and that they were more frequent and more intense during the breeding season. Based on the overall lack of data, we can only suggest that some seahorse species produce this sound type in stress situations and perhaps also during courtship.

What is the possible role of growling sounds in *H. reidi*? The functional significance of distress or disturbance sounds has been frequently discussed (Fish & Mowbray, 1970; Ladich, 1997; Bosher, Newton & Fine, 2005; Ladich & Myrberg, 2006) but, due to a lack of appropriate experiments, remains unknown in fish. The assumption is that they serve, similar to other animal taxa, in warning and deterring predators, in attracting secondary predators (which would then attack the first predator) or in alarming conspecifics (Ladich, 1997; Ladich & Myrberg, 2006). Bosher *et al.* (2005), however, have shown that stridulatory sounds are ineffective in thwarting predation and have not reduced further attacks by largemouth bass. The low level of *H. reidi's* growling sounds probably makes them detectable at only very short distances, thus rendering them unsuitable to function as an alarm call unless individuals are in very close proximity. Alternatively, growls may constitute an additional escape mechanism because sound production is accompanied by body vibrations, which might startle predators (catfish: Ladich, 1997; weeping lizards: Labra *et al.*, 2013; birds: Conover, 1994).

Based on the differences in sound characteristics and on behavioural observations during sound production, clicks and growls are suggested to be produced by two different sound-generating mechanisms. Broadband clicks in seahorses are stridulatory in origin and are produced when a supraoccipital ridge of the neurocranium snaps over the grooved anterior margin of the coronet (Colson *et al.*, 1998). Growls, in contrast, are low-frequency sounds similar to drumming sounds. However, as *H. reidi* does not possess swim bladder muscles (T. P. R. Oliveira, pers. obs.), we suggest that growl emission results from rapid contraction of other muscles (e.g. lateral trunk muscles). These make the swim bladder and the body vibrate, as also mentioned by Dufossé (1874). Two different sound-generating mechanisms are uncommon in fishes and known only in representatives of the catfish families Doradidae, Pimelodidae, Mochokidae and Ariidae (Ladich & Fine, 2006). These catfishes produce stridulatory sounds by their pectoral spines and low-frequency sounds by vibrating their swim bladders (Fine & Ladich, 2003; Ladich & Fine, 2006).

Acknowledgements

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