Hearing ability in three clownfish species

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SUMMARY

Clownfish live in social groups in which there is a size-based dominance hierarchy. In such a context, sonic cues could play a role in social organisation because dominant frequency and pulse length of sounds are strongly correlated with fish size. Data on the hearing ability of these fish are, however, needed to show that they have the sensory ability to detect the frequencies in their sounds. The present study determines the hearing sensitivity in three different anemonefish species (Amphiprion frenatus, Amphiprion ocellaris and Amphiprion clarkii), and compares it with the frequencies in their calls. The frequency range over which the three species can detect sounds was between 75 and 1800 Hz, and they were most sensitive to frequencies below 200 Hz. During sound production, dominant frequency is clearly related (R²=0.95) to the fish size, whatever the species. Dominant frequency extends from 370 to 900 Hz for specimens having a size between 55 and 130 mm. The best hearing sensitivity of small specimens were found to be lower than the dominant frequency of their own calls. However, they were found to be close to the dominant frequency of larger fish calls. The interest of juveniles lies in localising the adults and thus their location on the reef.

Key words: AEP, hearing sensitivity, frequency, clownfish, Amphiprion.

INTRODUCTION

Numerous species of Pomacentridae (damselﬁsh) are well-known sound producers (Allen, 1975). The most comprehensive studies of damselfish sound production have been performed with the signal jumps of four members of the Stegastes genus. These fish are sympatric, they establish nearby territories and they share the same reproductive season (Myrberg, 1972; Myrberg and Spire, 1972; Spanier, 1979; Myrberg et al., 1986). These pomacentrids have been shown to be able to discriminate species based upon the number of pulses and the pulse rate of a call (Myrberg and Spire, 1972; Spanier, 1979). The sounds associated with courtship behaviour have also been described in different Dascyllus species (Lobel and Mann, 1995; Mann and Lobel, 1995; Mann and Lobel, 1997; Mann and Lobel, 1998; Parmentier et al., 2009), in Abudefduf species (Lobel and Kerr, 1999; Maruska et al., 2007) and in Amphiprion species (Takeamura, 1983). Although sound production ability has been examined and highlighted in several pomacentrid species, hearing thresholds have been only determined in adults of the Stegastes and Abudefduf species (Myrberg and Spires, 1980; Kenyon, 1996; Egner and Mann, 2005) and in the larvae of Pomacentrus nagasakiiensis (Wright et al., 2005).

Audiograms for P. nagasakiiensis larvae have shown the highest sensitivity at 100 Hz (Wright et al., 2005). In Abudefduf saxatilis, fish are most sensitive to the lower frequencies (100–400 Hz). However, the frequency range over which fish are able to detect sounds has been shown to be dependent upon the size of the fish; larger fish were found to be more likely to respond to higher frequencies (Egner and Mann, 2005). A matching in the frequency domain between sound production and hearing has been shown in Abudefduf abdominalis, demonstrating that sounds are useful for courtship. Frequency peaks of sounds were found to be between 90 and 380 Hz whereas the auditory evoked potential (AEP) threshold indicated best sensitivity at low frequencies, between 95 and 240 Hz (Maruska et al., 2007).

Among pomacentrids, clownfish live in social groups in which there is a size-based dominance hierarchy (Fricke, 1979; Pautou and Allen, 1997; Buston, 2003). Anemonefish are protandrous hermaphrodites that change sex from male to female. Thus, in one group the largest anemonefish is typically female and the other anemonefishes are males. When the female is removed, the next largest fish changes sex to become the dominant female. In Amphiprion akallopisos, dominant frequency and pulse length are strongly correlated (R²=0.97) with fish size (Colleye et al., 2009). In such a context, sonic cues could convey information on the identity of the emitter and, consequently, play a previously unexpected role in social organisation. Data on the hearing ability of clownfish are, however, scarce. According to Simpson and colleagues, embryos of the clownfish Amphiprion ephippium and Amphiprion rubrocinctus are capable of responding to sound (Simpson et al., 2005). However, these authors studied fish from three to nine days post-fertilisation, and it difficult to rely on these data for these fish at the time they were able to communicate. The aim of the present study is to determine the hearing ability in three different anemonefish species (Amphiprion frenatus, Amphiprion ocellaris and Amphiprion clarkii) and to compare their hearing ability with their sound frequencies that comprise their calls.

MATERIALS AND METHODS

Hearing

Fish acquisition and maintenance

Five Amphiprion frenatus Brevort 1856 [31–52 mm total length (TL); 0.62–3.02 g], seven Amphiprion ocellaris Cuvier 1830 (42–55 mm TL; 1.58–3.21 g) and five Amphiprion clarkii Bennett 1830 (38–46 mm TL; 0.94–1.26 g) were purchased from a pet store and were kept in the same community tank (60x30x30 cm, salinity=35; temperature=26°C) with an aerator at the University of South Florida (USF, FL, USA). All fish were held for 1–3 days before testing. They were fed a few pinches of ‘Tropical Fish Flakes’
were calculated using an 8192-point FFT (fast Fourier transform). Hearing thresholds were determined using power spectra, which were analyzed using AVISOFT-SAS LAB PRO 4.33 software. The most sensitive frequency was 150 Hz for all AEP waveforms and were analysed for the presence of significant peaks (peaks at twice the frequency of the stimulus that were at least 3 dB above background levels). AEP thresholds were defined as the lowest sound level at which significant FFT peaks for the dominant frequency were apparent.

**Experimental setup**

Hearing thresholds were measured using the AEP technique. AEP is an electrophysiological technique for measuring hearing thresholds in fish and other vertebrates (Kenyon et al., 1998). Electrodes inserted subdermally in proximity to the brainstem, directly measure bulk neural responses generated in the VIIIth nerve and brain in response to sounds (Corwin et al., 1982). Signal-averaging is used to filter the evoked potential signal from background noise.

The experimental setup in the present study was similar to the one used for the study of hearing in *A. saxatilis* (Egner and Mann, 2005). Each fish was immobilised in a custom-made harness, restricting body and tail movements while allowing normal respiration. Subdermal stainless steel needle electrodes (Rochester Electro-Medical, Tampa, FL, USA) were used to record the AEP signal. An electrode was inserted approximately 1 mm into the head, over the otic region. The reference electrode was placed within the fish’s epaxial musculature, and a ground electrode was placed directly in the water in close proximity to the fish. Fish were suspended 10 cm below the surface in a steel tube (1.2 m high, 20.3 cm diameter, 0.95 cm thickness), closed at the bottom with a square steel plate (60.6 x 60.96 cm), and oriented vertically. Four anti-vibration floor mounts (Tech Products Corp., 51700 Series, Philadelphia, PA, USA) were placed under each corner of the base of the steel tube. The tube was filled with saltwater of approximately 26°C up to a height of 1.12 m and a loudspeaker was placed at the bottom of the steel pipe.

**Sound generation and AEP acquisition**

A Tucker-Davis Technologies (TDT, Alachua, FL, USA) AEP workstation was used to generate sound stimuli and record AEP waveforms (see Egner and Mann, 2005). TDT SigGen and BioSig software were used to generate sound stimuli with an RP2.1 enhanced real-time processor, a PAS programmable attenuator to control sound level and a power amplifier (Hafler Trans.Ana P1000, 110 W professional power amplifier, Tempe, AZ, USA) before being sent to a UW-30 underwater speaker (Lubell Labs, Columbus, OH, USA) where sound was emitted. Stimuli consisted of 50 ms pulsed tones gated with a Hanning window. The phase of the tone was alternated between presentations to minimise electrical artefacts from the recordings. Acoustic stimuli were calibrated with a Reson enhanced real-time processor, the hydrophone was positioned near to the fish in the experimental setup, and the sound levels were measured with BioSig, without phase alternation. During each trial, nine different frequencies were presented: 75, 150, 300, 450, 600, 900, 1200, 1500 and 1800 Hz. Sound levels at each frequency were presented up to 164 dB re 1 μPa 1/2. During calibration, the hydrophone was positioned near to the fish in the experimental setup, and the sound levels were measured with BioSig, without phase alternation. During each trial, nine different frequencies were presented: 75, 150, 300, 450, 600, 900, 1200, 1500 and 1800 Hz. Sound levels at each frequency were presented up to 164 dB re 1 μPa and were decreased in 6 dB steps until a threshold level was determined. Evoked potentials recorded by the electrode were fed through a TDT HS4-DB4 amplifier (10,000 x gain) connected to an RP2.1, routed into the computer and averaged by BioSig software. A total of 2000 signal presentations were averaged to measure the evoked response at each level of each frequency.

**RESULTS**

**Hearing**

AEP measurements suggest a low sensitivity to acoustic pressure (Fig. 1). Mean thresholds for all fish species show that they were most sensitive at the lower frequencies tested (75–900 Hz) and required loud sound levels (>140 dB sound pressure level (SPL)) in order to detect tones at higher frequencies (900–1800 Hz). No artefacts were detected at the high sound levels when a dead fish control was run. The most sensitive frequency was 150 Hz (mean=99.9 dB, s.e.m.=3.28) in *A. frenatus*, 75 Hz (mean=95.6 dB, s.e.m.=3.77) in *A. clarkii* and 75 Hz (mean=104 dB, s.e.m.=1.56) in *A. ocellaris*. In the three species, the sensitivity was found to be higher up to 300 Hz (from 103 to 112 dB), after which there was a significant drop in the auditory threshold to 600 Hz. This threshold was then found to be quite regular from 600 Hz to 900 Hz. All the fish were in the same size range, and there were no significant differences found between the three species for each tested frequency [analysis of variance (ANOVA) *P*>0.05].

**Sound production**

The dominant frequency varied from 370 to 800 Hz (Table 1; Fig. 2). The differences in the dominant frequency were not related to the species but to the size of each specimen (Fig. 3). The lack of a species difference in dominant frequency can be demonstrated by two specimens of the same size: the dominant frequency of the female *A. ocellaris* (79 mm) and the male *A. frenatus* (80 mm) was found to be 627 and 625 Hz, respectively (Mann–Whitney, *P*=0.84). Another example is found between *A. frenatus* (72 mm) and *A. clarkii* (73 mm), having a dominant frequency of 679 and 698, respectively (Mann–Whitney, *P*=0.41). The correlation between size and dominant frequency was *R*=0.95 for all species grouped together.
DISCUSSION

In Hawaii, the hearing ability of *A. abdominalis* has previously been measured by the AEP technique, showing that sound production and hearing in adults of *A. abdominalis* (TL: ±124 mm) are closely matched in the frequency domain (Maruska et al., 2007). Their best sensitivity was found to be at low frequencies, between 95 and 240 Hz (Maruska et al., 2007). The best hearing sensitivity was around 100–500 Hz in *Pomacentrus partitus* and *Pomacentrus variabilis*, and appears also to match the frequency of their own sounds (Kenyon, 1996). These species were found to undergo an ontogenetic change in their auditory sensitivity, in which the threshold decreased with increasing size. By contrast, various studies have also revealed that dominant frequency and pulse length of fish sounds are highly correlated with fish size in several species (Myrberg et al., 1993; Lobel and Mann, 1995; Connaughton et al., 2000), including clownfish (*Colleye et al., 2009*). In *A. akallopisos*, fish size has been shown to be highly related to the dominant frequency of the calls, suggesting that these fish are able to identify the size of the (conspecific) emitter (Colleye et al., 2009). Teleost fishes such as *Gobius niger* (Gobiidae), *Corvina nigra* (Sciaenidae) and *Sargus annularis* (Sparidae) are able to discriminate tonal sounds differing in frequency of approximately 10%; the frequency discrimination ability at 400 Hz is approximately 40 Hz (Fay, 1988). Such a capacity to discriminate frequency differences has already been shown in damselfish; females of *Stegastes partitus* preferentially respond to lower frequency chirps of a larger male than those from a smaller fish (Myrberg et al., 1986). In *A. saxatilis*, fish size has a significant effect on the auditory sensitivity (Egner and Mann, 2005); all fish are most sensitive to the lower frequencies (100–400 Hz) but the larger fish are more likely to respond also to higher frequency (800–1600 Hz). In *Amphiprion*, this observation is of significant importance due to the size-based dominance hierarchy displayed by these fish (Buston and Cant, 2006).

Whatever the species, the three different species of the present study could be situated on the same slope (Fig. 2), showing the negative relationship between fish size and the dominant frequency of *Amphiprion* sounds.

### Table 1. Dominant frequency in the three clownfish species

<table>
<thead>
<tr>
<th>Amphiprion species</th>
<th>TL (mm)</th>
<th>Dominant frequency (Hz)</th>
<th>Means ± s.d.</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. ocellaris</em></td>
<td>55</td>
<td>898.226±110.93</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td><em>A. ocellaris</em></td>
<td>63</td>
<td>770.441±78.47</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td><em>A. frenatus</em></td>
<td>72</td>
<td>697.749±103.49</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td><em>A. clarkii</em></td>
<td>75</td>
<td>520.715±76.89</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td><em>A. ocellaris</em></td>
<td>75</td>
<td>668.505±55.80</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td><em>A. ocellaris</em></td>
<td>79</td>
<td>617.477±53.47</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td><em>A. frenatus</em></td>
<td>80</td>
<td>625.552±47.17</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td><em>A. frenatus</em></td>
<td>95</td>
<td>536.889±46.52</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td><em>A. frenatus</em></td>
<td>99</td>
<td>506.142±49.87</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td><em>A. frenatus</em></td>
<td>105</td>
<td>484.992±64.07</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td><em>A. clarkii</em></td>
<td>112</td>
<td>443.393±44.93</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td><em>A. frenatus</em></td>
<td>114</td>
<td>433.272±47.43</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td><em>A. clarkii</em></td>
<td>129</td>
<td>415.508±43.51</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td><em>A. clarkii</em></td>
<td>130</td>
<td>381.575±30.98</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td><em>A. clarkii</em></td>
<td>131</td>
<td>376.800±51.48</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

TL, total length; N, number of pulses.
It is interesting to note that the best hearing sensitivity of a given fish does not correspond exactly to the dominant frequency of the sound it can produce. The regression equation indicates (Fig. 3) that the smaller clownfish used in these AEP experiments would make sounds with a dominant frequency from 780 Hz for the longer (52 mm) to 900 Hz for the smaller (31 mm) fish whereas their best hearing sensitivity is at around 100 Hz. By contrast, adult clownfish would need to detect the presence of smaller fish, which constitute potential new mates or competitors for sex change. Study is supported by FNRS and ULG Research Mobility Grants. E.P. is a research associate of the Fonds National de la Recherche Scientifique de Belgium (FRS-FNRS).

REFERENCES


Fig. 4. Mean audiogram (closed circles) of Amphiprion frenatus is plotted together with power spectra of pulses of Amphiprion frenatus having different sizes. Blue: 80 mm total length (TL); purple: 99 mm TL; green: 114 mm TL; and red: 130 mm TL. The greatest energy of sound is concentrated from about 300 to 800 Hz but the dominant frequency (arrows) is lowering with increasing size. Threshold data are plotted as means of sound pressure level (SPL) (left axis) for all A. frenatus pooled. Power spectra of individual sounds are plotted as relative amplitude in dB (right axis). The scale of this axis is not given because the different power spectra were adjusted to the level of the dominant frequency to allow comparisons in the distribution of the frequencies.