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"YOU TALKING TO ME!?": THE ROLE OF VISUAL-ACOUSTIC CUES IN ANEMONEFISH SOCIAL BEHAVIOUR

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ABSTRACT

Acoustic communication is integral to the social dynamics of many animal species, yet fish remain underrepresented in bioacoustics research despite their ecological diversity and environmental importance. This study investigates the vocalisations and associated behaviours of anemonefish, focusing on the orange clownfish (*Amphiprion percula*). Through in situ audio-video recordings of wild populations in Papua New Guinea, we documented a diverse repertoire of sounds and their behavioural contexts, highlighting their critical role in social hierarchy maintenance, group cohesion, and territorial interactions. To understand how anemonefish integrate sensory information, we conducted a visual-acoustic cue manipulation experiment, showing the reliance on multisensory cues for social behaviours. Our findings reveal the diversity and functional significance of fish vocalisations while highlighting potential vulnerabilities to underwater noise pollution. By combining behavioural ecology and experimental manipulation this work provides new perspectives on sensory ecology in marine systems and emphasizes the need for conservation strategies in increasingly noisy underwater environments.

Keywords: *visual-acoustic cues, size hierarchy, vocalisations, size assessment, behaviour*

1. INTRODUCTION

Despite fish representing 35% of the total chordate biomass and half of vertebrate species [1], knowledge about acoustic communication in fishes is limited compared to other taxa. The main reason for this bias is fishes' apparent limitation of acoustic signalling which is restricted to certain families and species [2]. Nevertheless, several fish taxa use acoustic signals for a variety of functions.

Anemonefishes (genus *Amphiprion*) are ideal to study acoustic communication in wild fish populations since. They display a complex array of behaviours both interacting with other species and within their social groups with strict size hierarchies and mutualistic relationship with their sea anemone host, which makes them extremely site attached [3]. Anemonefish groups are formed by unrelated individuals and have a strict size-based social hierarchy where individuals queue for the breeding position [4]. The largest anemonefish is the dominant female and the second largest is the sub-dominant male, the breeding pair, which can also have non-reproductive subordinates [5]. Acoustic cues may be key to maintain their strict hierarchical social structure within groups [6], by signalling dominance/submission and allowing group members to be differentiated.

Anemonefishes can hear sounds between 75 and 1800 Hz [7] and produce sounds using their jaw teeth and vibrations from their rib cage, which generates variation in their vocalisations related to their body size [8]. Consequently, these acoustic features may convey information on the social rank of the emitter within the group [9]. In addition, individuals might use their side vision or mechanosensors to assess conspecifics size through lateralisation or side-by-side swimming [11-12]. Thus, anemonefish can obtain information form visual and acoustic cues to assess a conspecifics' size and visual-

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acoustic cues might be essential to maintain hierarchy stability within the group. However, the relative importance of these cues and their role in social interactions has not been studied.

This study examines the acoustic communication of orange clown anemonefish (*Amphiprion percula*) in natural reef habitats in Papua New Guinea, with the goal of advancing our understanding of acoustic communication in wild fish populations. The specific aims of this study are to: (1) characterize the vocal repertoire and associated behavioural contexts of *A. percula*; and (2) evaluate the integration of visual and acoustic cues in social interactions through sensory manipulation experiments.

2. METHODS

2.1 Baseline data collection

The study was conducted Kimbe Bay, Papua New Guinea. Anemonefish groups were recorded using GoPro Hero 9 action cameras and AudioMoths version 1.7.1 which were placed in front of groups on stationary tripods set close to the anemone to obtain video and audio data. A total of 142 vocalisation trains (594 individual vocalisations) were recorded *in situ* from *A. percula* groups. All individuals of the group were caught and measured underwater to obtain their standard length (SL) in mm.

2.2 Visual-acoustic cues experiment

For this experiment, three treatments (with three trials each, Tab. 1) manipulating different sensory cues were conducted: I) congruent visual-acoustic cues (A, E, I), II) constant visual cues (B, E, H) and III) constant acoustic cues (D, E, F). To simulate different size conspecifics (difference of less than 1cm, 25% larger, and 25% smaller) we used mirrors with different lenses and UW30 underwater speakers to playback sounds from different size individuals, previously recorded from the same population. The trials were recorded using GoPro cameras. For the experimental trials, only the subordinates (rank 3) of 18 groups were used. The dominant individuals, rank 1 and 2 were removed to remove effects of gender and social status as studies have shown that individual behaviour is influence by social rank [12].

Table 1. Table showing the combination of visual-acoustic cues used in the experiment.

		Visual cues		
		Smaller size	*Similar size*	*Larger size*
Auditory cues	*Smaller size*	A	B	
	Similar size	D	E	F
	Larger size		H	I

2.3 Behavioural and acoustic data

Video recordings from the baseline and experimental data were used to score individuals behaviour using BORIS (v. 7.12) (Tab. 2). Unwanted noise interferences present in the acoustic data were removed using a low-frequency filter in Audacity. Acoustic data in format *.wav* was cut in smaller clips and imported to Raven Pro v 1.6 to extract the acoustic parameters of all vocalisations, which were exported in selection tables. Vocalisations were labelled in relation to the behaviours that were displayed by the vocalising fish and the social rank of the fish.

Table 2. Ethogram used in the study showing the behavioural categories and descriptions of each behaviour.

Behaviour	**Description**
Aggression	Fish rapidly swims towards the mirror and bumps into it
	Fish rapidly darts and turns prior to or during aggressive contact.
Submission	Fish shakes body side to side in jolty fashion.
Social Interactions	Fish simultaneously approach, stay or follow each other within 1 body length
Territorial displays	Fish with stiffened body posture while vocalising
Lateralisation	Fish turns on their side within a body size of distance to the mirror
Time outside anemone	Fish swims outside the perimeter of the anemone
Out of sight	Fish cannot be seen because it enters anemone, moves under or behind anemone, or swims beyond the video frame.

2.4 Statistical analysis

All statistical analyses were performed in R v. 4.4.2 [13]. We used package *seewave* [14] to visualize the acoustic data, creating spectrograms and oscillograms. Using the packages *warbleR* and *Rraven* [15-16], we imported the selection tables created in Raven to analyse the parameters of the spectrograms. We used the *glmmTMB* package to fit generalised linear mixed models (GLMM) that accounted for zero-inflation in the data [17], using treatment, cues size and focal individual size as fixed effects. Individual ID as random effect to account for non-independence of individuals' behaviour. We used Akaike's Information Criterion (AIC) [18] for model selection. The *emmeans* package [19] was used to perform pairwise comparisons and the *performance* package was used to calculate the marginal R^2 (provides the variance of the model explained by the fixed effect) and conditional R^2 (provides the variance explained by both fixed and random effects) using Nakagawa's R^2 [20].

3. RESULTS

3.1 Vocalisations and behavioural context

We found that *A. percula* vocalisations are composed by pulses of sounds with a frequency range between 0.2 and 1.08 kHz and a duration between 0.007 and 0.361 ms. The pulses of sound could be produced individually or in a train of consecutive pulses that could range from 2 to 20 pulses, with an average of 0.036 ms of pause between pulses.

We identified five behaviours that were associated with vocalisations of *A. percula*: Interspecific aggression (aggressive displays to other fish species), intragroup aggression (aggressive displays to other fish within the social group), social interactions (non-agonistic interactions between members of the social group), submission (submissive displays to other members of the social group) and territorial displays, in which individuals would get close to the edge of the anemone and produce vocalisations without a direct interaction with another fish.

The pulse number analysis revealed several significant differences between behavioural categories. Submissive vocalisations were significantly different from aggressive (estimate = -1.899, $p < 0.01$, Fig. 1), competitive (estimate = -1.094, $p < 0.05$), territorial (estimate = 1.240, $p < 0.01$) and social vocalisations (estimate = -1.269, $p < 0.01$). However, no significant differences were found between vocalisations associated with other behaviours. These results suggest that the submissive sounds differed significantly from vocalisations associated with other behaviours.

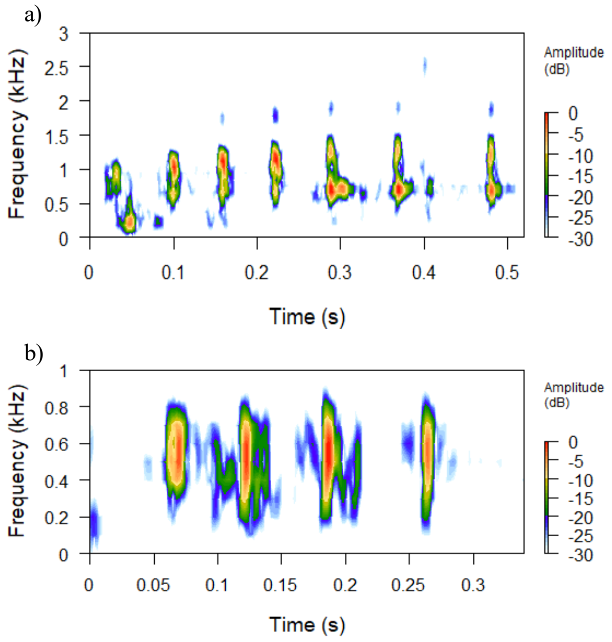

Figure 1. Spectrograms of *A. percula* vocalisation trains showing the frequency in kHz and the duration of the call in seconds (s). The spectrogram (a) correspondence with a submissive vocalisation while the spectrogram (b) corresponds with an aggressive vocalisation.

3.2 Visual-acoustic cues manipulation experiment

Focal fish exhibited different rates of display between behaviours and a higher interaction rate when visual-acoustic cues were congruent (Fig.2). There were significant differences in behavioural category (χ^2_3 = 60.355, $P < 0.001$) and experimental treatments (χ^2_2 = 6.407, $P < 0.01$). Aggressive, lateralisation, and territorial behaviours had positive estimates (Aggression: 0.498 ± 0.283 behaviour/min; Lateralisation: 1.083 ± 0.276 behaviour/min; Territorial: 0.509 ± 0.275 behaviour/min), while submission had a negative estimate (-0.414 ± 0.343 behaviour/min). Among treatments, the congruent cues experiment had the highest behavioural rate (0.498 ± 0.283 behaviour/min), compared to constant acoustic (0.126 ± 0.117 behaviour/min) and constant visual cues (-

0.184 ± 0.123 behaviour/min). There was no significant interaction between behavioural category and treatment. The fixed effects explained 30.1% of the variance (R^2m = 0.301, R^2c = 0.395).

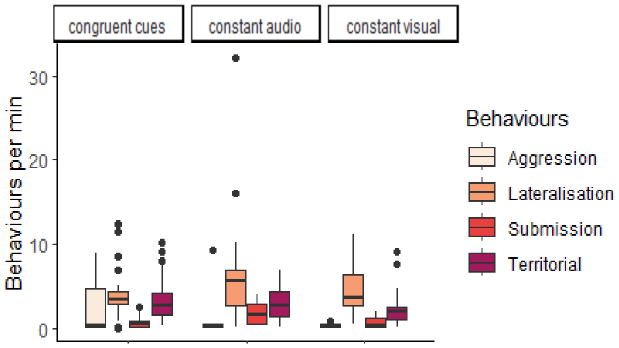

Figure 2. Distribution of behavioural rates (behaviours per minute) across treatments (congruent visual-acoustic cues, constant acoustic cues, and constant visual cues) for four behaviours: aggression, lateralization, submissions and territorial displays. Each box represents the interquartile range (IQR), with the median indicated by the horizontal line.

Lateralisation rates were significantly different between trials in the congruent ($\chi^2_2 = 10.774$, $P < 0.01$, Fig. 3) and constant acoustic cues treatments ($\chi^2_2 = 15.126$, $P < 0.001$). Individuals displayed higher lateralization rates when presented with similar-sized cues (congruent cues: 1.591 ± 0.609; constant acoustic cues: 2.535 ± 0.680), compared to cues from larger (congruent cues: -1.706 ± 0.625; constant acoustic cues: -2.126 ± 0.705) or smaller conspecifics (congruent cues: 0.634 ± 0.672; constant acoustic cues: 1.266 ± 0.692). This suggests that visual cues representing similar-sized conspecifics led to more pronounced lateralisation behaviours. In contrast, in the constant visual cues treatment, lateralization rates were the lowest when individuals were presented with similar-size cues, with the highest rates displayed when presented with small acoustic cues. However, these differences were not significant.

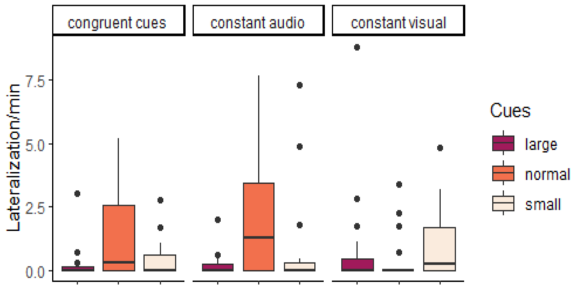

Figure 3. Lateralization events per minute across different cue sizes (larger cues, normal/similar size cues, and smaller cues) and treatments (congruent visual-acoustic cues, constant acoustic cues, and constant visual cues). Boxplots show the distribution of lateralization behaviour for each cue size within each treatment group. Each box represents the interquartile range with the median shown as a horizontal line; whiskers extend to 1.5× the IQR.

Territorial display rates were significantly influenced by focal individual standard length (Fig. 4). Territorial rates decreased with standard length increase in both the congruent treatment ($\chi^2_1 = 4.959$, $P < 0.05$, -0.091 ± 0.040). This trend was also observed in the constant acoustic and constant visual treatments, however, there were not significant effect found.

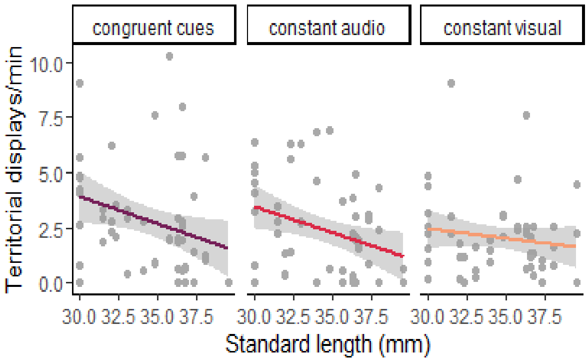

Figure 4. Relationship between standard length (mm) and frequency of territorial displays (displays per minute) across experimental treatments. Grey points indicate individual observations, while coloured lines represent linear regression with 95% confidence intervals.

Aggressive and submissive displays were primarily observed when individuals were presented with similar-size cues across all treatments. However, only the submission rate in the congruent cues treatment showed significant differences between trials ($\chi^2_2 = 10.774$, $P < 0.01$).

4. DISCUSSION

Our findings highlight the complexity of social communication in *A. percula*, emphasizing the critical role of multimodal signals—particularly the integration of visual and acoustic cues—in conspecific size assessment and social interactions. Across experimental treatments, individuals displayed distinct behavioural responses depending on the type and congruency of the sensory stimuli, with congruent visual-acoustic cues eliciting the strongest reactions. These included increased rates of interaction, heightened body shakes and lateralisation, and more time spent outside the anemone, suggesting that multimodal input enhances signal reliability and informs decision-making during social and agonistic encounters [22-23].

We demonstrated that vocalisations are used frequently in natural settings and across diverse social contexts—not only during aggression, but also in neutral interactions and interspecific encounters. These results expand upon previous research by showing that vocal signals are not limited to conflict scenarios but also serve broader social functions [24]. Notably, we observed vocalisations occurring independently of direct interaction, such as at the edge of the anemone or during interspecific interactions—an observation not previously reported in anemonefish bioacoustics literature. This broader range of social contexts highlights the limitations of laboratory settings, where social complexity is often reduced [25].

Lateralisation also emerged as a key behaviour, especially in response to similar-sized visual cues. This supports the hypothesis that lateralisation allows individuals to monitor conspecific size and evaluate their social status [10-11], potentially influencing their decision to engage in conflict [29]. This is consistent with findings in anurans, where vocal frequency is used to assess body size [30-31]. Interestingly, lateralisation was also increased in response to smaller acoustic cues under constant visual treatment, suggesting that individuals rely more on acoustic cues when visual differences are subtle.

Territorial display rates were inversely related to focal fish size, with smaller individuals showing higher rates, particularly in response to larger conspecific cues. This suggests that smaller individuals may use indirect strategies, such as territorial displays, to signal presence or dominance without engaging in direct conflict [32]. These displays may also serve as alert signals to other group members, though further research is needed to clarify their role in group-living contexts.

Behaviourally, body shakes and aggression were more frequent when individuals were exposed to similar-sized conspecific cues, particularly under congruent visual-acoustic treatments. While only body shakes showed statistically significant increases, this trend aligns with predictions that individuals engage in either aggressive or appeasing behaviours when size differences are minimal [26-27]. These behaviours may appear contradictory, but reflect strategic decisions based on social context and perceived dominance, as body size is a strong predictor of conflict outcomes [28].

Overall, our study reinforces the importance of multimodal sensory integration in social interactions and hierarchy maintenance among *A. percula*. Congruent visual-acoustic cues prompted stronger behavioural responses, supporting their role in enhancing recognition, reducing uncertainty, and guiding social decisions. While visual cues often dominated in conflict scenarios, acoustic cues provided critical size information when visual assessment was ambiguous. These findings contribute to a broader understanding of how sensory systems mediate social behaviour in complex group-living animals and underscore the need for future research that integrates ecological realism, individual variation, and dominance structure. Importantly, the reliance on acoustic signals in diverse social contexts highlights the potential vulnerability of these animals to anthropogenic noise, emphasizing the need to consider acoustic pollution as a disruptive force in reef fishes social dynamics.

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