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Territorial vocalization in sympatric damselfish: acoustic characteristics and intruder discrimination

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ABSTRACT

Damselfishes are well known for their aggressive, territorial behaviour during which the use of vocalization behaviour has been well documented. However, agonistic acoustic signalling has been understudied in particular when the vocalizations are interspecific. In this study, we characterize and compare the previously undescribed vocalization behaviour of longfin damselfish (Stegastes diencaeus), in an agonistic context, with the closely related and sympatric dusky damselfish (Stegastes adustus). Next, we examined if these congeneric species modulate their vocalizations in a similar pattern to previously described aggressive behaviour patterns. Audio field recordings of territorial males were obtained in response to three separate stimuli: (1) conspecific male damselfish, (2) heterospecific male damselfish and (3) a common intruder, the slippery dick wrasse (Halichoeres bivittatus). The vocal repertoires of both longfin and dusky damselfish comprised the same three distinct call types: chirps, pops and pulse trains. However, temporal measures of the calls showed significant differences between species. Additionally, dusky damselfish were more vocal overall, producing more calls and spending more time calling than longfin damselfish. These responses were stimulus and species dependent, as the two species modulated acoustic response by modulating pulse number based on intruder species. These results suggest that these closely related species of damselfish use vocalization behaviours that are both unique and context dependent.

Introduction

It is widely known that many reef fish species have the capacity to produce acoustic signals (Lobel et al. 2010). However, the vocalizations of many species remain without description and their role in behavioural interactions is poorly understood. The behavioural contexts most commonly reported to include vocalization are courtship and aggression. Pomacentrids (damselfish) are a particularly well-studied family because they produce loud, broadband, pulsed sounds that are paired with easily observable behaviours (Kasumyan 2009). Male
coral reef damselfish are well known for being soniferous when they court females (Myrberg & Spires 1972; Spanier 1979; Parmentier et al. 2009) and defend their territories (Takemura 1983; Amorim 1996; Parmentier et al. 2005). Damselfish courtship behaviour consists of visual displays that include erratic swimming and changes in colouration that may advertise the quality of the male (Knapp & Kovach 1991; Myrberg et al. 1993). Because courtship has been the most heavily studied behaviour with respect to damselfish vocalization, much of our understanding of the cues contained in acoustic signals derives from this context. Females have been shown to use certain cues in courtship vocalizations when assessing males. Female bicolor damselfish (Stegastes partitus) use the spectral properties of these vocalizations to distinguish between conspecific males (Myrberg et al. 1986). Studies have indicated that body size and peak frequency may correlate with larger fish having lower peak frequencies (Myrberg et al. 1993; Colleye et al. 2009). However, a study by Lobel and Mann (1995) demonstrated that while such a correlation exists, it was highly variable suggesting that other factors were influencing individual peak frequency values. It was further demonstrated in the cichlid, Oreochromis mossambicus, that social status influenced spectral properties of male vocalizations, including peak frequency (Amorim & Almada 2005). Vocalizations can vary among species in measurements of pulse number, pulse duration and interpulse interval (Maruska et al. 2007). Pulse number has been shown to encode species identity (Myrberg et al. 1986) while the role of the other measures remains unclear.

Similar to courtship vocalizations that attract females, males produce a ‘keep out’ signal, which has been shown to repel other males (Myrberg & Spires 1972) and is necessary for maintaining ownership of the territory (Myrberg & Riggio 1985; Myrberg 1997). Characteristics of these vocalizations allow for individual recognition of neighbouring males (Myrberg & Riggio 1985; Myrberg et al. 1993) suggesting that they contain cues that complement visual information obtained from an intruder. Stout (1975) studied the structure of agonistic vocalizations in the Cyprinid, Notropis analostanis, and found during playback experiments that ‘single knock sounds’ did not affect male behaviour whereas a ‘rapid series’ did affect male behaviour. This implies that signal repetition or duration may be one of the acoustical characteristics available as a cue for males to judge their opponents. Further evidence that aggressive vocalizations may contain cues that signal male quality or status was demonstrated by Remage-Healey and Bass (2004) when they found that steroid hormones rapidly increased the duration of fictive vocalizations in the midshipman, Porichthys notatus.

The majority of soniferous species produce sounds in intraspecific encounters. However, many species also produce sounds in confrontations with interspecifics. Given the high occurrence of territorial male damselfish attacking many species of intruders (Myrberg & Thresher 1974), it is not surprising the vocalizations also occur during attacks against heterospecifics. The Pomacentrid, Abudefduf luridis, vigorously attacks any intruder, including wrasse species and vocalizes to both conspecifics and heterospecifics with no change in acoustic characteristics (Santiago & Castro 1997). Alternately, the Hawaiian damselfish, Dascyllus albisella, has aggressive repertoire that is made up of two distinct vocalizations that differ in duration. Dascyllus albisella differentially uses them based on intruding species preferring the longer vocalization when confronted with conspecifics and the shorter when vocalizing to interspecific species (Mann & Lobel 1998). Here our intent is to examine vocalizations against heterospecifics and conspecifics, recorded in situ on reef territories, to determine if territorial defenders vocally differentiate between intruding species in terms of the acoustic characteristics and rate of vocalizations.
We focused on two closely related and sympatric species, longfin damselfish (*Stegastes diencaeus*) and the dusky damselfish (*Stegastes adustus*). The vocalizations of the dusky damselfish during conspecific interactions have been described previously (Burke & Bright 1972; Spanier 1979; Albrecht 1981) and here, we provide detailed acoustic analysis. We also present the first description, to our knowledge, of the longfin damselfish's vocalization. These species were selected because they are: (1) phylogenetically closely related (Mullen et al. 2012), (2) are similar in adult morphology (although the longfin damselfish has a larger adult size), (3) share the territorial polygynous territorial social system found among all Caribbean *Stegastes* species and (4) show considerable habitat overlap (see Section Methods). Robertson (1995) demonstrated that both species often compete for the same territorial space. Based on their close phylogenetic relationship, we anticipated that their vocalizations would have a highly similar structure. However, because both species have been demonstrated to behaviourally differentiate between males of their own species and the other species (Little et al. 2013; Black, Draud et al. 2014) we expected a similar discrimination in their vocal behaviour. We expected that the same forces that selected for a divergence in visual behaviour would also select for a divergence in their vocalizations.

We also examined the vocalizations that dusky and longfin damselfish produced to an unrelated species, the slippery dick wrasse (*Halichoeres bivittatus*). The slippery dick is an egg predator and is attacked vigorously by both damselfish species. Slippery dick wrasse have little resemblance to damselfish because they are not territorial and have a shape and colouration that bears no similarities to any damselfish in the Caribbean (see Bohlke & Chaplin 1968). Previous studies have demonstrated that the longfin damselfish attacks the slippery dick more intensely than the dusky damselfish (Black, Imhoff, et al., 2014), and both species attack the slippery dick wrasse with a lower intensity than conspecific individuals (Little et al. 2013; Black, Imhoff, et al. 2014). Given that the longfin and dusky damselfish respond differently to the slippery dick wrasse, we anticipated that this would be reflected in their vocalizations. Specifically, we expected that both species would vocalize with a lower call rate towards the slippery dick wrasse than towards their own conspecifics.

**Methods**

**Study site**

Wild male dusky and longfin damselfish were observed in a shallow coral reef located offshore of the Bellairs Research Institute (McGill University), in Holetown, Barbados, West Indies (13°10′N, 59°30′W) during a ten-day period between June and July 2011. At the onset of the study, 10 ‘focal’ males of each species were identified and their territories marked with numbered tags to allow for repeated observations. Marking territories is less stressful to the fish than tagging individuals directly, and damselfish territories are highly stable and rarely usurped by intruders. Previous studies in a related species (*S. leucostictus*) using elastomer tags showed males remained on tagged sites for at least 60 days (Itzkowitz et al. 1995) and similar patterns are seen in dusky and longfin damselfish where even brief territorial absences can result in usurpation (Bartels 1984; McDougall & Kramer 2007; Turgeon et al. 2010). Dusky damselfish territories were located approximately 15–20 m from shore at depths of 1–2 m and longfin damselfish territories were slightly farther from shore.
The reef at this site is severely degraded and covered in turf algae on which the damselfish graze (for more detailed description, see Turgeon et al. 2010).

Focal and stimulus fish

Ideally, we would have preferred to control for size in both the focal and the stimulus species (Table 1), yet due to sampling and time constraints, this was not possible. Therefore, in order to eliminate the confound of size within stimulus species, we presented size-matched dusky and longfin damselfish intruders. In doing so, the stimulus longfin was typically smaller than the focal territorial longfin while the stimulus dusky was similar in size to the focal territorial dusky. Because of the scarcity of small territorial longfins, it was not possible to compare size-matched focal longfin and dusky damselfish and maintain a sufficient sample size. As a practical necessity, the slippery dick wrasse was longer than all focal damselfish due to its ‘pencil-like’ morphology, which made it difficult to capture smaller specimens.

Stimulus presentations

Here, we control for some parameters including stimulus location that are difficult or often not controlled in previous field studies by placing stimulus species within clear plastic bottles within the territories of focal damselfish species (i.e. ‘model bottles’ after Myrberg & Thresher 1974).

Territorial males were presented with each stimulus placed in clear plastic cylindrical bottles (165 mm diameter × 240 mm height). During the presentation of each stimulus, two-minute audio recordings were taken and all acoustic measures were derived from these focal subject recordings. Each territorial male was tested three times (for a total of nine stimulus presentations) with each stimulus species. Stimuli were captured away from the study area and released near the same location immediately after testing to ensure that they were unfamiliar fish. The same stimulus individual was presented to all focal subjects in a given day to control for individual variation among stimuli. Presentations occurred over the course of several days and were completed alternating focal fish order to avoid any effect from time of day or presentation sequence (i.e. day one: dusky males #1–10 followed by longfin males #1–10; Day 2: longfin males #10–1 followed by dusky males #10–1). On days where focal males were presented with more than one stimulus, several hours were allowed to elapse between testing, which has been shown to be sufficient time to prevent habituation (Itzkowitz et al. 1995). Observations of dusky males were taken while snorkelling while longfin male observations required the use of SCUBA, since their territories tended to occur in somewhat deeper water. At the onset of each presentation trial, the

Table 1. Focal and stimulus fish mean standard length (SL), mean total length (TL), and standard deviation (SD).

<table>
<thead>
<tr>
<th></th>
<th>Focal</th>
<th>SL (mm) ± SD</th>
<th>TL (mm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal</td>
<td>Stegastes adustus</td>
<td>63.7 ± 6.3</td>
<td>83.1 ± 7.7</td>
</tr>
<tr>
<td></td>
<td>Stegastes diencaeus</td>
<td>84.9 ± 15.3</td>
<td>110.5 ± 18.6</td>
</tr>
<tr>
<td>Stimulus</td>
<td>Stegastes adustus</td>
<td>72.3 ± 1.2</td>
<td>92.7 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Stegastes diencaeus</td>
<td>67.7 ± 2.1</td>
<td>90.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Halichoeres bivittatus</td>
<td>94.3 ± 3.5</td>
<td>110.3 ± 2.9</td>
</tr>
</tbody>
</table>
bottle was placed in the centre of the territory and a hydrophone (Aquarian H2a-0) (Range: 10 Hz–100 kHz; Sensitivity: –180 dB re: 1 V/μPa) coupled to a SONY digital audio recorder (linear PCM1M10) with a sampling rate of 44.1 kHz was positioned within 10 cm directly in front of the bottle. Observers moved roughly 4 m away to reduce background noise and to minimize the influence of the observer on behaviour.

**Signal analysis**

To compare the fine-scale structure of vocalizations between dusky and longfin damselfish, analysis of the acoustic signal was initially performed using Raven Pro 1.3 bioacoustics software (The Cornell Lab of Ornithology, Ithaca, NY). The signal was low-pass filtered at 1500 Hz and individual calls identified. Vocalizations were categorized into a descriptive repertoire: chirp, pop and pulse train. Only calls with high signal-to-noise ratios were further analysed for temporal and frequency measures. Peak frequency values were calculated using the power spectra in Raven Pro for each pulse within a call. Raven Pro calculates peak frequency as the frequency with maximum power. Frequency values for each pulse were then averaged within each vocalization.

Examination of acoustic temporal measures was performed in MATLAB Student Version R2012a. Following the method of Mann and Lobel (1998), the signal was divided by its rms amplitude, rectified and an envelope function was calculated using a 3 ms decay, then the signal was smoothed using a moving 3 ms window. Individual pulses were detected by gating the envelope record, which outputs the ‘on’ and ‘off’ times of individual pulses from envelope threshold crossings. The threshold was dynamically defined for each signal as the maximum background noise level in a 20 ms period before the vocalization plus 20% of the maximum signal amplitude (see Mann & Lobel 1998 and Figure 1 for definitions). The following temporal measurements were obtained from the detected signals: Pulse number (number of pulses in each call), pulse duration (length in ms of individual pulses) and interpulse interval [(IPI) length in ms between the end of one pulse and the start of the next. Because ‘pops’ were most often composed of one pulse, IPIs were not analysed for this call type. The analysis of the fine-scale measures for the dusky damselfish included 125 chirps and 102 pops and for the longfin 89 chirps and 123 pops were analysed. Because of the high rate of noise disturbance in the natural setting and because all values were being averaged within individual fish (for a maximum sample of 10) there was not a high enough sample size for each individual to compare each fine-scale measurement based on stimulus species. Additionally, the longfin damselfish were less vocal overall than dusky damselfish vocalizing least of all to themselves. Therefore, vocalizations to conspecifics from territorial longfins were limited. We sampled from each stimulus species as evenly as possible to control for any inherent variation in measure based on stimulus species.

Next, the number of pulses in the ‘chirp’ call were tallied for both dusky and longfin damselfish by stimulus species to see if the focal species differentially modulated this measure. This was not performed on pops because of the calls’ inherent low variability in pulse number, nor on pulse trains because of their rarity. We were able to obtain data from a larger sample of chirps (dusky \( n = 308 \); longfin \( n = 247 \)) because minor background noise disturbance did not prevent us from accurately counting the number of pulses in an individual chirp.
Lastly, we examined broader vocalization behaviour towards each stimulus species. We did this by quantifying their calling behaviour in a series of measures that captured the calling rate and the distribution of repertoire use. ‘Call number’ (total number of all vocalizations and repertoire of vocalizations produced to each stimulus), and ‘time calling’ (total time calling to each stimulus measured by adding the length of each call produced) were tallied.

At the conclusion of the study, each focal fish was captured using a cast net (2 m diameter) and several size measurements were obtained [standard length (SL), total length (TL), height (H) and width (W)] before they were released back to their territories (Table 1).

**Statistical analysis**

All measures recorded from focal fish were averaged for each individual (focal dusky; \( n = 10 \) and focal longfin; \( n = 10 \)) and mean values were entered into each model and analysed in
SPSS. For each analysis, alpha was set at 0.01. All graphs were generated using MATLAB and Excel.

For the call type ‘pops’, we were only able to obtain a complete data-set on three acoustic measures (pulse number, pulse duration and peak frequency) due to missing data resulting from the pop being often comprised of a single pulse. For both focal species, pulse trains were excluded from the statistical analysis because they were rare causing the sample size to be extremely low. Two separate MANOVAs were performed, one for each call type (chirps and pops) to test for differences between focal species. When the MANOVA produced significant results, subsequent univariate ANOVAs were then preformed on each measure.

In response to three different stimuli (dusky, longfin and wrasse), there were five behavioural variables (time calling, call number, number of pops, number of chirps and number of pulse trains) recorded from both focal species. Because the behavioural variables are interdependent, we analysed the five variables together with three separate MANOVAs (one for each stimuli species). Again, when the one-way MANOVA yielded significant results, they were followed by univariate ANOVAs (focal species x stimulus species) for each acoustic variable.

The ‘number of pulses’ in chirps measure was the only structural measure that we could examine with a sufficient sample size for each stimulus species. So, we examined this structural measure separately with a two-way mixed ANOVA. The between-subjects factor was focal species, the within-subjects factor was stimulus species, the dependant variable was pulse number and the random factor was fish. Once again, when the ANOVA yielded a significant interaction, follow up univariate ANOVAs were performed.

**Results**

**Repertoire characterization**

We first examined the vocalizations of each species and categorized the repertoire according to empirical parameters. While the dusky has received some descriptions (Burke & Bright 1972; Spanier 1979; Albrecht 1981), the longfin has not been previously reported as a soniferous species. Figure 2 illustrates that both longfin and dusky damselfish displayed similar repertoires of calls. The ‘chirp’ was the most commonly produced call for both species. The dusky damselfish repertoire was composed of 62.8% chirps, 34.6% pops and 2.6% pulse trains. The longfin repertoire was made up of 50.6% chirps, 47.9% pops and 1.4% pulse trains.

**Signal analysis**

We next examined the fine-scale temporal properties and peak frequency of the repertoire call types and compared them between species. Dusky and longfin damselfish significantly differed in ‘chirp’ measures (MANOVA; Pillai’s Trace = 0.997, $F_{4,15} = 36.04, p < 0.001$). The dusky damselfish had a higher ‘pulse number’ (ANOVA; $F_{1,18} = 33.64, p < 0.001$) and longer ‘pulse duration’ (ANOVA; $F_{1,18} = 14.04, p < 0.001$), while longfin damselfish had higher ‘IPIs’ (ANOVA; $F_{1,18} = 57.90, p < 0.001$; Table 2). ‘Peak frequency’ was not significantly different between species (ANOVA; $F_{1,18} = 4.92, p = 0.040$)
Dusky and longfin damselfish also differed significantly in measures of the call type 'pop', (MANOVA; Pillai's Trace = 0.693, $F_{3,16} = 12.06, p < 0.001$) with dusky damselfish having a longer 'pulse duration' (ANOVA; $F_{1,18} = 34.17, p < 0.001$). 'Pulse number', in pops was insignificant between focal species (ANOVA; $F_{1,18} = 7.45, p = 0.014$). There was also no significant difference between the two focal species in ‘peak frequency’ for this call type (ANOVA; $F_{1,18} = 0.61, p = 0.44$; Table 3).
**Evoked vocal behaviour**

To determine if there were species-specific patterns of repertoire use (chirps, pops and pulse trains) or general vocalization behaviour (‘call number’ and ‘time calling’), we evoked acoustic behaviour by presenting commonly encountered intruders: conspecific (dusky or longfin damselfish), congeneric (dusky or longfin damselfish) and a heterospecific egg predator (wrasses). Analysis revealed significant differences in evoked acoustical behaviour between the two focal species for all three stimuli groups (MANOVA: Wrasse, Pillai’s Trace = 0.908, $F_{5, 14} = 24.77$, $p < 0.001$; Longfin, Pillai’s Trace = 0.944, $F_{5, 14} = 41.45$, $p < 0.001$; Dusky, Pillai’s Trace = 0.933, $F_{5, 14} = 38.53$, $p < 0.001$).

For the five-evoked vocal behaviour measures (time calling, number of calls produced, number of chirps, number of pops and number of pulse trains produced in the presence of the stimulus fish), there was a main effect of focal species with $F_{1, 18}$ ranging from 23.91 to 414.33, all $p < 0.001$. Clearly, dusky damselfish scored higher on all vocal measures than did longfin damselfish indicative of their tendency towards more vigorous vocalization behaviour overall. However, there were also effects of the stimulus species for some of these vocal behaviour measures.

We examined repertoire use by dusky and longfin damselfish based on stimulus species (Figure 3). For both ‘pops’ and ‘pulses’, there was a main effect for focal species based largely on the dusky being more vocal (ANOVA: Pops, $F_{1, 18} = 174.30$, $p < 0.001$; Pulses, $F_{1, 18} = 23.91$, $p < 0.001$) while no significant main effect was found for stimuli species (ANOVA: Pops, $F_{2, 36} = 1.469$, $p = 0.244$; Pulses, $F_{2, 36} = 1.108$, $p = 0.341$) or focal species x stimuli species interaction (ANOVA: Pops, $F_{2, 36} = 3.046$, $p = 0.060$; Pulses, $F_{2, 36} = 2.152$, $p < 0.131$). ‘Chirps’ revealed a significant main effect for stimulus species with more chirps being produced in the presence of the wrasse and less in the presence of the other two stimulus species, which were roughly equal (ANOVA; $F_{2, 36} = 19.21$, $p < 0.001$). There was also a significant main effect of focal species (ANOVA; $F_{1, 18} = 263.249$, $p < 0.001$) but no significant interaction was found (ANOVA; $F_{2, 36} = 0.271$, $p = 0.765$).

For ‘time calling’, in addition to a significant main effect for focal species (Figure 4A) (ANOVA: $F_{1, 18} = 67.582$, $p < 0.001$), there was a main effect of stimuli species (ANOVA: $F_{2, 36} = 26.52$, $p < 0.001$). However, this appears to be a result of the focal species x stimuli species interaction (ANOVA: $F_{2, 36} = 16.855$, $p < 0.001$) because focal longfin and dusky damselfish spent the least amount of time calling to their own conspecific stimuli and the most amount of time calling to the wrasse.

We examined whether focal species modulated the total number of calls produced (‘call number’) when presented with the stimuli species during the two minute trial (Figure 4B). Again, there was a significant main effect for focal species demonstrating the dusky damselfish’s tendency to vocalize more frequently (ANOVA: $F_{1, 18} = 414.335$, $p < 0.001$). There was also a significant main effect of stimuli species (ANOVA: $F_{2, 36} = 17.02$, $p < 0.001$), with more total calls in the presence of the wrasse than in the presence of the other two stimulus species. No significant focal species x stimuli species interaction was found (ANOVA: $F_{2, 36} = 0.02$, $p = 0.981$) illustrating that both focal species did not differentiate between the two stimulus damselfish.

We next examined the pulse number in chirps in response to each stimulus species (Figure 5). There was a main effect of focal species ($F_{1, 18} = 98.787$, $p < 0.001$, partial $n^2 = 0.846$). There was no significant main effect of stimulus species ($F_{2, 36} = 2.316$, $p = 0.113$, partial $n^2 = 0.114$).
There was a significant interaction between focal species and stimulus species \( F_{2,36} = 48.059, \ p < 0.001, \ \text{partial } \eta^2 = 0.728 \). This showed that dusky and longfin damselfish differentially modulate their pulse number based on stimulus species. Univariate ANOVAs were run to test between subject effects (focal species). There were statistically significant differences in number of pulses produced between focal species with dusky stimulus \( F_{1,18} = 21.014, \ p < 0.001, \ \text{partial } \eta^2 = 0.539 \); longfin stimulus \( F_{1,18} = 143.924, \ p < 0.001, \ \text{partial } \eta^2 = 0.889 \); and wrasse stimulus \( F_{1,18} = 20.471, \ p < 0.001, \ \text{partial } \eta^2 = 0.532 \). The number of pulses within chirps directed at the heterospecific damselfish was increased relative to the other stimuli (see Figure 5).

**Discussion**

In this study, we examined in natural context, the aggressive vocal behaviour of two closely related, sympatric damselfish species: the dusky damselfish and the longfin damselfish. Despite these two species having expected similarities in the structure and behavioural
Figure 4. Evoked behaviour (A) Comparison of *S. adustus* (grey bars) and *S. diencaeus* (white bars) time spent vocalizing to stimulus species (average time calling per 2 min ± s.e.). (B) Average calling rate ± s.e. directed at each stimulus species (*S. adustus*, *S. diencaeus* and *Halichoeres bivittatus*) by the *S. adustus* (grey bars) and the *S. diencaeus* (white bars).

Figure 5. Average number of pulses in all chirps produced ± s.e. by *S. adustus* (black bars) and *S. diencaeus* (white bars) towards stimulus species: *S. adustus*, *S. diencaeus* and *H. bivittatus*.
use of their vocalizations, we found striking differences. The dusky damselfish produced vocalizations at a much higher rate than the longfin even while using the same repertoire of call types. Upon examining the fine-scale structure of the vocalizations, we found that the dusky and the longfin had significant differences on many measures. Lastly, we found that in response to different stimulus intruders, the dusky and the longfin damselfish modulated the pulse number in the chirp differentially based on intruder species.

**Repertoire characterization**

We described the agonistic vocalizations of the longfin damselfish for the first time and described the dusky damselfish to provide a framework from which to make comparisons of these closely related, sympatric species. As we anticipated, both species produced identical vocal repertoires. Most damselfish, even those that are phylogenetically distantly related and found in the Pacific, produce broadband signals similar to the Caribbean dusky and the longfin (Lobel & Mann 1995; Amorim 1996; Santiago & Castro 1997; Maruska et al. 2007; Parmentier et al. 2009); thus it is not surprising that the basic call structure of both the closely related longfin and dusky damselfish is highly similar.

**Signal analysis**

The chirp was the most commonly produced vocalization by both damselfish species. However, the number of pulses in the chirp differed between species with the dusky having more pulses than the longfin. The duration of each pulse was also significantly different between dusky and longfin damselfish. However, despite this difference, in biological terms it was small (< 5 ms). Previous work has shown that pulse duration can vary as much as 5 ms over a 2 m range of recording distances (Mann & Lobel 1998). Focal fish in this study remained close to the bottle and the hydrophone throughout the recording (never straying more than half a metre from the hydrophone). However, even small variation in location from the recording device can alter the signal to noise ratio and could have an effect on the measured pulse duration. This suggests that pulse duration would be a less reliable element to communicate information, such as species identity. The pulse duration for the chirp in longfin damselfish had smaller variable than the dusky which may indicate tighter control of vocalization character.

The IPI has been demonstrated to be a key feature for species identification (Myrberg et al. 1978; Crawford et al. 1997; Kihlslinger & Klimley 2002; Amorim et al. 2008). The IPI of the chirp was also statistically different between longfin and dusky damselfish with the dusky having a smaller average IPI than the longfin. The longfin also displayed smaller variation in this measure.

Finally, the spectral measurement of peak frequency did not vary between the two species. The longfin damselfish produced a lower average peak frequency, which is expected with their slightly larger body size. However, a large variation occurred in both species. Other studies have suggested that females use peak frequency to assess males (Myrberg et al. 1993). Playback recordings combined with behavioural studies would be needed to determine if any of these cues hold biologically meaningful differences.

The pop results did not have a significant difference in pulse number which is to be expected given the low variability in that measure for this call type. For the pulse duration
measure, both species had differences similar to the chirp with the dusky damselfish having significantly longer pulse duration. IPI was not analysed because of the frequency of single-pulse vocalizations. The longfin again trended towards overall lower peak frequency, however, differences were not significant due to high variation among individuals.

The pulse train was a distinct but rarely produced call. For this reason, we had insufficient recordings with a high signal to noise ratio and were unable to analyse the vocalizations for temporal and spectral measures. However, the trends observed suggest that in this call type, the longfins maintained their lower peak frequency and shorter pulse duration but also with the addition of a potentially much shorter IPI. A larger sample size would be needed to statistically validate these differences.

In comparing the vocalizations of dusky and longfin damselfish for fine-scale measures, we used three different stimulus fish. Because we could only use calls with little to no background noise, the sample size of acceptable calls was relatively low per individual focal fish. The longfin damselfish in particular was less vocal than the dusky restricting our sample size further still. So, we were not able to statistically compare these focal species based on stimulus species for these measures. However, the dusky being more vocal than the longfin provided a higher sample size and we performed an analysis of the dusky measures for pulse duration, IPI and peak frequency based on stimulus species to consider whether these measures might be variable. The dusky data did not yield any significant differences in these measures based on stimulus species (data not shown). We are unable to statistically test the longfin vocalizations for any potential species-specific variation. However, the longfin measurements had smaller variation than did the dusky damselfish.

**Evoked vocal behaviour**

We further examined repertoire based on allocation of call types to each stimulus species and found that while the dusky was more vocal overall, there was no change in trends in how they divided their repertoire. The chirp was the most commonly employed call, followed by the pop, while the pulse train was least commonly produced. This pattern held across both species and in the presence all three stimuli. It is unclear why the males produce this variety of call types in agonistic interactions; or whether they convey meaningful information in other social interactions. Interestingly, neither focal species differentiated between conspecific and congeneric species with regard to number of calls produced. Both species chirped more towards the wrasse compared to the conspecific and heterospecific damselfish suggesting that the wrasse represents a distinct threat and that they employ alternate strategies to deal with this type of intruder. While wrasses are known to be egg predators, male damselfish compete amongst themselves for resources and territory. Because dusky and longfin damselfish have overlapping territory ranges, this competition also overlaps species. Itzkowitz (1991) observed that the closely related Caribbean beaugregory damselfish (S. leucostictus) was more likely to threaten the egg-eating bluehead wrasse (Thalassoma bifasciatum) because direct attacks would be more likely to displace the defending beaugregory male away from its eggs but allow other bluehead wrasse easy access. Quite possibly the chirp vocalization is directed towards a threat and thus the dusky and the longfin damselfish may strategically choose to vocalize in order to minimize the need to directly attack and pursue the slippery dick wrasse.
Pops were also more commonly produced by dusky damselfish, but unlike chirps, there were no differences for stimulus species. The pulse trains also showed little difference in use rate between the three stimulus species but were not commonly used by either focal species. Based on the uniform rates of pops and pulse trains, their signal function is likely unrelated to the differences in threat presented by the three stimulus species.

Despite some behavioural similarities such as repertoire content and use, we supported the hypothesis that differences in the vocalizations between these two species match their differential behaviour response towards stimuli species. A significant difference between the two focal species was the larger number of calls generated by the dusky for all types of vocalizations and in all circumstances. This difference is likely related to their divergent social behaviour. Under natural conditions, the dusky is clearly more active than the long-fin and spends considerably more time engaging aggressively with neighbours and other intruders (Black, Imhoff, et al. 2014, pers obs). Our results demonstrate that the dusky is inherently more soniferous than the longfin when presented with single standardized intruders suggesting a link between heightened levels of other aggressive behaviours and aggressive vocalizations.

We observed that both focal damselfish species modulated the time calling when presented with heterospecific damselfish as compared to conspecifics without modulating the number of calls produced. This suggests that they were either altering one or all of the measured acoustic values (pulse number, IPI, pulse duration) to lengthen their calls when signalling to heterospecifics or altering the repertoire of calls produced. While the difference in overall call numbers is clear, neither focal species differentiated call patterns between heterospecific and conspecific damselfish. However, the focal dusky increased the average pulse number within chirp calls which contributed to an increased time calling without increasing the overall number of chirp calls. Both focal species were similar in vocalizing to the slippery dick wrasse by increasing the number of calls and the calling time without changing the pulse number. Thus, both species demonstrate that they do indeed differentiate between conspecific and heterospecific damselfish and that they appear to use the same mechanism to modulate their call length. Since we were unable to examine the fine-scale measures of these calls based on stimulus species, we do not know if those properties are similarly modulated.

In summary, both the dusky and the longfin damselfish respond similar to each other and to the slippery dick wrasse. However, there are distinct differences illustrating that both differentiate between conspecifics and heterospecific damselfish, and in this regard, supports the hypothesis. Our data clearly show that these two closely related species differentiate between conspecifics and heterospecifics within their habitat and that they use vocalizations differentially in their interactions with them. Both damselfish species vocalized more frequently to the slippery dick wrasse suggesting it represents a unique threat unlike either damselfish species. Future playback experiments may help resolve the salience of these calls across species to convey information in the context of territorial defence.

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