



# Are there individual acoustic signatures in the damselfish *Dascyllus albisella*?

Salomé Laboury,<sup>1,a)</sup> D Eric Parmentier,<sup>1</sup> D and Phillip S. Lobel<sup>2</sup>

<sup>1</sup>Laboratory of Functional and Evolutionary Morphology, FOCUS, University of Liège, Liège, 4000, Belgium <sup>2</sup>Department of Biology, Boston University, Boston, Massachusetts 02115, USA

# **ABSTRACT:**

Many animal species are known to show individuality in their acoustic communication. This variation in individual male signatures can be decisive for female choice. Within the damselfishes, *Dascyllus* species are known for prolific sound production during the realization of movements associated with courtship (i.e., the signal jump) and spawning (mating sounds). However, the ability to distinguish the individuality of male *Dascyllus* sound signatures is unknown. We investigated the variability in the courtship and mating sounds of 17 males of *Dascyllus albisella* at Johnston Atoll, Central Pacific Ocean, to determine whether it was possible to distinguish them from one another and thus have information on their ability to convey individual information. Acoustic analyses confirmed that courtship sounds differed from mating sounds. Comparative analyses suggest that acoustic signals cannot serve as distinctive traits unless the individuals are of different sizes. Males of *D. albisella* do not use individual signatures in a reproductive behavioral context. However, it cannot be ruled out that variations in the sound production rate may serve as a discriminative feature. © 2025 Acoustical Society of America. https://doi.org/10.1121/10.0034790

(Received 1 July 2024; revised 4 December 2024; accepted 6 December 2024; published online 8 January 2025) [Editor: Shane Guan] Pages: 48–56

#### I. INTRODUCTION

The ability to discriminate between individuals based on their vocalizations has been demonstrated in several taxa (Narins et al., 2006; Quam et al., 2017; Suthers et al., 2016). When individuals differ in the presence or absence of certain signal attributes or when there is a greater betweenindividual variability than within-individual variability in one or more signal properties, it results in distinctiveness (Beecher, 1982, 1989; Bee et al., 2001). This distinctiveness ensures that the signals are more recognizable from those of other conspecifics, enhancing communication effectiveness among those exhibiting these distinctive traits. Caller recognition provides many benefits in parental care or territorial ownership. For birds and mammals that breed in colonies, it is crucial to distinguish between their offspring and those of others to provide proper parental care to their progeny (Blank and Yang, 2017; Tan et al., 2022). Acoustic individual discrimination has also been demonstrated among territorial animals, such as songbirds and anurans (Bee et al., 2001; Ceugniet et al., 1999; Cornec et al., 2014). This ability allows territory residents to minimize unnecessary conflicts with neighbors (Gerhardt and Bee, 2006), a phenomenon known as the neighbor-stranger or dear-enemy effect (Fisher, 1954). Residents typically respond less aggressively to intrusions by neighboring individuals than non-neighbors, conserving energy and preventing escalated confrontations (Bee et al., 2001; Fisher, 1954; Ydenberg et al., 1988). Species defending long-term or multi-purpose

territories, like songbirds, are particularly likely to exhibit this form of social recognition (Temeles, 1994). In some cases, highly distinctive acoustic parameters constitute a uniquely recognizable "vocal signature" (Casey *et al.*, 2013; Janik *et al.*, 2006). For example, bottlenose dolphins have an identification system based on the tremendous amount of interindividual variability in their signature whistle, similar to the use of names in humans (Janik *et al.*, 2006).

In species whose females invest in mate choice and males invest in courtship, females may choose a mate that provides direct benefits, such as parental care or territories, to increase their fitness and enhance offspring survival and development (Amorim, 2023; Andersson, 1994). Females could also gain indirect benefits, such as "good genes" that will improve the genetic fitness of the offspring (Amorim, 2023; Andersson, 1994). To assess those benefits in fishes, females might use important information in male acoustic signals, such as the size and potential fitness of the emitter (Amorim, 2023; Parmentier and Fine, 2016; Oliver and Lobel, 2013). For example, it is well known that dominant frequency is a reliable indicator of size for many species (Amorim, 2023; Bertucci et al., 2012; Colleye et al., 2011; Fine and Parmentier, 2015; Lobel and Mann, 1995; Myrberg et al., 1993; Parmentier and Fine, 2016). Larger fishes produce lower frequency as the sound-producing apparatus usually scales with the body size (Amorim, 2023; Fine and Parmentier, 2015; Parmentier and Fine, 2016). Therefore, differences in frequency spectra could allow individuals of some species to identify conspecifics (Bertucci et al., 2012; Colleye et al., 2009; Crawford et al., 1997; Ladich et al., 1992; Myrberg et al., 1993).

<sup>&</sup>lt;sup>a)</sup>Current address: Allée du six Août, 11, 4000 Liège, Belgium. Email: S.Laboury@uliege.be

JASA

Adults and juveniles Dascyllus albisella are found on coral heads in aggregations of a few to more than 100 individuals (Fishelson, 1998; Stevenson, 1963). Males guard territories where they have established their nests (i.e., a cleaned area of coral) (Oliver and Lobel, 2013; Stevenson, 1963). During courtship, males perform a display (called the signal jump) to attract females (Lobel and Mann, 1995; Oliver and Lobel, 2013). This courtship dip involves the male rising into the water column and then swimming rapidly downward while emitting a pulsed sound (Lobel and Mann, 1995; Myrberg and Spires, 1972). Females travel between males until they spawn in a male's nest (Oliver and Lobel, 2013; Stevenson, 1963). During incubation, males fan and guard the eggs from intruders (Oliver and Lobel, 2013; Stevenson, 1963). Males produce pulsed sounds during courtship and mating (Lobel and Mann, 1995; Mann and Lobel, 1998). The mating sound is produced as the male quivers during spawning (Lobel and Mann, 1995; Mann and Lobel, 1998). Courtship sounds differ from mating sounds by having more pulses and a longer duration (Lobel and Mann, 1995; Mann and Lobel, 1998). There is a negative relationship between the body size of males and the dominant frequency of their courtship sounds, with larger males producing lower-frequency sounds (Lobel and Mann, 1995). The overall mating success of D. albisella was correlated with the rate of courtship call repetition and no other male traits (Oliver and Lobel, 2013).

As males *D. albisella* defend their territory for reproduction purposes and form great aggregations on coral heads, we hypothesize that those fish could use individual distinctive sounds to attract females. The goal of our study is to examine the variability in the reproductive sounds of males *D. albisella*, investigating possible individual discrimination.

#### **II. MATERIAL AND METHODS**

#### A. Study site

The data used in this study were collected in Johnston Atoll ( $16^{\circ} 44' \text{ N}$ ,  $169^{\circ} 31' \text{ W}$ ) in the Central Pacific Ocean.

Recordings were made of free-living fish in the lagoon from May 31 to June 4, 1991, between 4 and 6 m depth. The average water temperature in the lagoon was  $26.4 \,^{\circ}$ C. Seventeen colonies of *D. albisella* were recorded. They comprised two to nine individuals associated with isolated coral heads or bared rocks.

#### **B. Recordings**

Behaviors associated with sound production were recorded with a SONY model V-9 8 mm video camera (SONY, Tokyo, Japan) coupled to a hydrophone (sensitivity:  $-162 \text{ dBV } \mu \text{Pa} - 1$ , flat frequency response range: 10 Hz to 3 kHz, a sampling rate of 48 kHz, 16 bit resolution) (BioAcoustics, Falmouth, MA) (Fig. 1). Audio-video recordings of courting and mating were made while scubadiving with controlled breathing to avoid excessive regulator and bubble noise (Fig. 1). The hydrophone was generally placed between 0.5 and 1.0 m of the fish. The diver was positioned 3-5 m away, operating the video camera. The hydrophone was attached to the video camera by a 5 m-long cable and manipulated on a sound boom (a 2.5 m pole) that rested on the bottom. The hydrophone was located at the end of the boom with 40 cm of free cable and buoyed so it floated freely in the water column. Each sound was classified according to the behavior of the male producing the sound, as seen in the video (Supplementary Material 1). Sounds were detected on oscillograms and spectrograms. Only individual sounds distinguishable from the background noise were used (Supplementary Material 1). Sounds associated with reproductive behaviors were analyzed with Raven Lite 2.0.1 (Cornell Lab of Ornithology, Ithaca, NY). Sounds were not down-sampled. The following acoustic parameters were measured: sound duration (in milliseconds; Fig. 2), number of pulses in a sound (Fig. 2), pulse period (measured as the time from the beginning of one pulse to the beginning of the next one, in milliseconds; Fig. 2), pulse duration (measured as the time from the beginning of one pulse and its end, in milliseconds; Fig. 2), interpulse interval



FIG. 1. (Color online) The audio–video recording instrumentation with the hydrophone extended on a pole from the camera. Modified from Lobel, Mar. Technol. Soc. J. **35**, 19–28 (2001). Copyright Marine Technology Society 2001.





FIG. 2. (Color online) Oscillogram of a sound produced by male number 1 *Dascyllus albisella* composed of three pulses and depicting sound duration, pulse period, pulse duration, and interpulse interval.

(measured as the time from the end of one pulse to the beginning of the next one, in milliseconds; Fig. 2), and dominant frequency (frequency component with the most energy, in Hertz). Temporal parameters were measured from oscillograms, while the dominant frequency was obtained from power spectra.

### C. Statistics

All the statistics were carried out with *R* 4.1.2 (GNU General Public License, Free Software Foundation, Boston, MA), and the significance level was  $\alpha = 0.05$ . The Shapiro–Wilk normality test was used to test the normality of the parameters measured on the sounds. The homoscedasticity of the variances was checked with the *F*-test of equality of variances or the Bartlett test. A principal component analysis (PCA) was run on the six acoustic parameters from the correlation matrix to give an overview of the potential acoustic differences between the two reproductive behaviors and between the individuals. Spearman's rank correlation coefficients (rS) were used to quantify the relation between principal components and acoustic parameters (and adjusted *p*-values with Holm's method). The Bonferroni correction was applied when multiple statistical tests were carried out.

Descriptive statistics (mean and standard deviation) were calculated for sounds associated with both behaviors. Seventeen males produced courtship sounds, and five males produced mating sounds. Wilcoxon *t*-test was used to compare courtship and mating sounds for each acoustic parameter. Wilcoxon *t*-tests were also used to compare courtship and mating sounds for each acoustic parameter. Wilcoxon *t*-tests were also used to compare courtship and mating sounds for the first two principal components. Descriptive statistics (mean and standard deviation) were calculated for all the individuals for each behavior. Kruskal–Wallis tests were used to compare the individuals for each acoustic parameter for each behavior. They were followed by Dunn's multiple comparison tests for pairwise comparisons. Kruskal–Wallis tests were used to compare courtship and mating sounds between the individuals for the first two principal components.

The between-individual coefficient of variation (CVb)/ within-individual coefficient of variation (CVw) ratio is

usually used to define the potential for individual identity coding (Ceugniet et al., 1999; Bee et al., 2001; Terrazas et al., 2003). For each acoustic parameter and each studied individual, the ratio between the CVb and the CVw was calculated. A low ratio (CVb/ CVw) for a given signal property indicates low stereotypy since acoustic parameters exhibit a much greater variation within than between individuals. Therefore, low CVb/CVw ratios reveal sound consistency between the individuals (Ceugniet et al., 1999). A CVb/CVw ratio superior to a value of 2 could indicate that the parameter serves as an individual marker (Ceugniet et al., 1999). The coefficient of variation corresponds to [(standard deviation/mean)  $\times$  100]. Within-individual coefficients of variation were based on means and standard deviations calculated from the sounds produced by each individual. Between-individual coefficients of variation were determined on grand mean and standard deviation based on the means of all individuals (Bee et al., 2001).

A flexible discriminant analysis (FDA) was employed to assess the individual distinctiveness of reproductive sounds, specifically courtship and mating sounds. The objective of the FDA was to classify signals based on the individual males who produced them. FDA generated canonical discriminant functions, representing linear combinations of the original variables, to maximize the separation between groups (individual males) in multidimensional signal space. Classification

TABLE I. Comparison of the different acoustic parameters (mean  $\pm$  standard deviation) characterizing the reproductive sounds produced by *Dascyllus albisella* grouped according to the associated behavior. Numbers between brackets correspond to the number of analyzed sounds. Seventeen males produced courtship sounds, and five males produced mating sounds.

	Courtship sounds $(N = 398)$	Mating sounds $(N = 230)$
Duration (ms)	$274 \pm 70$	143 ± 51
Number of pulses	$6 \pm 1$	$3 \pm 1$
Pulse period (ms)	$49 \pm 6$	$50 \pm 7$
Pulse duration (ms)	$24 \pm 2$	$24 \pm 2$
Interpulse interval (ms)	$26 \pm 6$	$26 \pm 6$
Dominant frequency (Hz)	447 ± 77	$386 \pm 51$



FIG. 3. (Color online) (a) and (b) Oscillogram (top) and spectrogram (bottom) of a sound produced by male number 1 *Dascyllus albisella* during (a) courtship, (b), spawning. fast Fourier transform length: 512, window: Hann, frame: 50%. Colors on the spectrogram indicate sound intensity based on the "Jet" color scheme. The color scale ranges from blue, representing lower intensity, to red, representing higher intensity. Intermediate colors (green and yellow) indicate medium levels of intensity, with red marking the points of highest acoustic energy.



FIG. 4. (Color online) A scatterplot of principal component PC1 versus PC2 was performed with the six acoustic parameters of courtship sounds (circle) and mating/spawning sounds (triangle) produced by *Dascyllus albisella*. Ellipses correspond to 95% of the observations, and dots correspond to ellipse centers.

success, measured by the accuracy rate of classifying signals to their respective males, is a key indicator of the effectiveness of the discriminant functions (Terrazas *et al.*, 2003). The FDA analysis encompassed 398 courtship sounds from 17 males and 230 mating sounds from five males, incorporating all six variables to comprehensively capture the acoustic characteristics of the signals.

#### **III. RESULTS**

Courtship sounds were significantly longer (Wilcoxon,  $W = 86\,623$ , P < 0.001) and made of more pulses (Wilcoxon,  $W = 86\,056$ , P < 0.001) than mating sounds produced during the spawning [Table I; Figs. 3(a) and 3(b)]. However, the pulse period (Wilcoxon,  $W = 43\,818$ , P = 1), the pulse duration (Wilcoxon,  $W = 48\,374$ , P = 1), and the interpulse interval (Wilcoxon,  $W = 41\,662$ , P = 0.36) were not significantly different between courtship and mating sounds [Table I; Figs. 3(a) and 3(b)]. Finally, the dominant frequency of courtship sounds was also higher (Wilcoxon,  $W = 57\,606$ ; P < 0.001) [Table I; Figs. 3(a) and 3(b)].

The acoustic diversity associated with the reproduction was summarized by a PCA based on the six acoustic parameters (Fig. 4). The first two principal components of the PCA explained cumulatively 72.4% of the variation, with principal component 1 (PC1) and principal component 2 (PC2) explaining, respectively, 39.2% and 33.2% of the variation. The number of pulses (rS = 0.95, P < 0.001), the sound duration (rS = 0.88, P < 0.001), and the dominant frequency (rS = 0.68, P < 0.001) mostly contributed to the first principal component. The pulse period (rS = 0.93, P < 0.001) and the interpulse interval (rS = 0.88, P < 0.001) were principally associated with the second principal component. The reproductive sounds are easily distinguishable within this acoustic space along PC1 (Fig. 4). PC1 (Wilcoxon, W = 84783, P < 0.001) and PC2 (Wilcoxon, W = 57345, P < 0.001) support significant differences between courtship and mating sounds.

For courtship sounds, a few differences were found between the individuals for the sound duration (Kruskal–Wallis, d.f. = 16,  $\chi^2 = 45.87$ , P < 0.001) and the number of pulses (Kruskal–Wallis, d.f. = 16,  $\chi^2 = 40.48$ , P < 0.001) (Table II; Supplementary Material 2, Tables V and VI). More statistical differences were found between the individuals for pulse period (Kruskal–Wallis, d.f. = 16,  $\chi^2 = 193.15$ , P < 0.001), pulse duration (Kruskal–Wallis, d.f. = 16,  $\chi^2 = 126.22$ , P < 0.001), interpulse interval (Kruskal–Wallis, d.f. = 16,  $\chi^2 = 204.6$ , P < 0.001), and dominant frequency (Kruskal–Wallis, d.f. = 16,  $\chi^2 = 134.12$ , P < 0.001), but no male could be distinguished from all the others (Table II; Supplementary Material 2, Tables VII–X).

The acoustic diversity associated with the courtship was summarized by a PCA based on the six acoustic parameters (Fig. 5). The first two principal components of the PCA explained cumulatively 69.2% of the variation, with PC1 and PC2 explaining, respectively, 36.3% and 32.9% of the variation. The sound duration (rS = 0.80,



TABLE II. Comparison to the number of analyz	n of the diffe ed sounds.	erent acoust	ic paramete	ers (mean :	+ standard	deviation) cł	naracterizin	ig the court	ship sound:	s produced	by the 17 n	nales of <i>D</i> <sub>6</sub>	iscyllus albi:	<i>iella</i> . Numb	ers betwee	n brackets	correspond
	Male 1 $(N = 65)$	Male 2 $(N = 14)$	Male 3 $(N = 25)$	Male 4 $(N = 12)$	Male 5 $(N = 9)$	Male 6 $(N = 33)$	Male 7 $(N = 57)$	Male 8 $(N = 34)$	Male 9 $(N = 9)$	Male 10 $(N = 20)$	Male 11 $(N = 7)$	Male 12 $(N = 12)$	Male 13 $(N = 11)$	Male 14 $(N = 16)$	Male 15 $(N = 10)$	Male 16 $(N = 51)$	Male 17 $(N = 13)$
Duration (ms)	$249 \pm 36$	276 ± 38	$291 \pm 84$	$293 \pm 52$	$306 \pm 34$	$283 \pm 104$	$260 \pm 55$	$251 \pm 57$	224 ± 64	$263 \pm 40$	245 ± 42	271 ± 61	$286 \pm 111$	273 ± 62	271 ± 53	317 ± 86	307 ± 82
Number of pulses	$6 \pm 1$	$7 \pm 1$	$6 \pm 1$	$6 \pm 1$	$7 \pm 1$	$6 \pm 2$	$6 \pm 1$	$6 \pm 1$	$6 \pm 1$	$6 \pm 1$	$6 \pm 1$	$6\pm1$	$6 \pm 2$	$6 \pm 1$	$7 \pm 1$	$6 \pm 2$	$7 \pm 2$
Pulse period (ms)	$48 \pm 4$	$44 \pm 2$	$54 \pm 5$	$51 \pm 4$	$46 \pm 2$	$55 \pm 8$	$46 \pm 4$	$48 \pm 2$	44 ± 3	$45 \pm 3$	$42 \pm 2$	$50 \pm 3$	$48 \pm 4$	$53 \pm 4$	$44 \pm 2$	$55 \pm 7$	$47 \pm 5$
Pulse duration (ms)	$23 \pm 2$	$25 \pm 1$	$24 \pm 1$	$23 \pm 2$	$24 \pm 1$	$25 \pm 2$	$25 \pm 1$	$23 \pm 1$	$24 \pm 2$	$24 \pm 1$	$23 \pm 1$	$23 \pm 2$	$22 \pm 2$	$25 \pm 2$	$24 \pm 1$	$24 \pm 2$	$22 \pm 2$
Interpulse interval (ms)	$25 \pm 4$	$19 \pm 2$	$30 \pm 5$	$28 \pm 5$	$22 \pm 3$	$31 \pm 8$	$21 \pm 4$	$25 \pm 3$	$20 \pm 3$	$21 \pm 3$	$20 \pm 2$	$27 \pm 4$	$27 \pm 5$	$28 \pm 3$	$20 \pm 2$	$32 \pm 7$	$25 \pm 4$
Dominant frequency (Hz)	402 ± 43	439 ± 49	443 ± 44	325 ± 58	$417 \pm 31$	451 ± 76	412 ± 60	442 ± 55	501 ± 44	504 ± 55	492 ± 56	428 ± 58	$501 \pm 93$	426 ± 65	507 ± 90	514 ± 68	515 ± 117





FIG. 5. (Color online) A scatterplot of principal component PC1 versus PC2 was performed with the six acoustic parameters of courtship sounds produced by 17 *Dascyllus albisella* males. The different forms distinguish the males. Ellipses correspond to 95% of the observations, and dots correspond to ellipse centers.

P < 0.001) and the interpulse interval (rS = 0.66, P < 0.001) mostly contributed to the first principal component. The pulse period (rS = -0.76, P < 0.001), the number of pulses (rS = 0.73, P < 0.001), and the interpulse interval (rS = -0.69, P < 0.001) were principally associated with the second principal component. The individuals are not easily distinguished within this acoustic space along PC1 and PC2 (Fig. 5). None of the first two components allows us to significantly distinguish a male from all the others (Kruskal–Wallis, d.f. = 16, PC1  $\chi^2 = 120.66, P < 0.001$  and PC2  $\chi^2 = 110.31, P < 0.001;$ Supplementary Material 2, Tables XI and XII).

For mating sounds produced during spawning, a few differences were found between the five individuals for all the acoustic parameters, but no male could be distinguished from all the others (Kruskal–Wallis, d.f. = 4, sound duration  $\chi^2 = 31.6$ , P < 0.001, number of pulses  $\chi^2 = 33.99$ , P < 0.001, pulse period  $\chi^2 = 12.87$ , P = 0.012, pulse duration  $\chi^2 = 36.26$ , P < 0.001, interpulse interval  $\chi^2 = 30.59$ , P < 0.001 and dominant frequency  $\chi^2 = 37.88$ , P < 0.001; Table III; Supplementary Material 2, Tables XIII–XVIII).

The acoustic diversity associated with the mating was summarized by a PCA based on the six acoustic parameters (Fig. 6). The first two principal components of the PCA explained cumulatively 74.3% of the variation, with PC1 and PC2 explaining, respectively, 42.2% and 32.1% of the variation. The number of pulses (rS = 0.88, P < 0.001), the sound duration (rS = 0.65, P < 0.001), and the dominant frequency (rS = 0.62, P < 0.001) mostly contributed to the first principal component. The pulse period (rS = 0.71, P < 0.001) and the sound duration (rS = 0.64, P < 0.001) were principally associated with the second principal component. The individuals are not easily distinguished within this acoustic space along PC1 and PC2 (Fig. 6). None of the first two components allows us to significantly distinguish a male from all the others (Kruskal–Wallis, d.f. = 4, PC1  $\chi^2 = 40.33$ , P < 0.001 and PC2  $\chi^2 = 26.5$ , P < 0.001; Supplementary Material 2, Tables XIX and XX).

The within and between-individual coefficients of variation (CVw and CVb, respectively) were calculated for all the acoustic parameters for courtship and mating sounds (Table IV; Supplementary Material 2, Tables XXI and XXII). For courtship sounds, the between-individual variation appeared slightly higher than the within-individual variation, but CVb/CVw was lower than 1.5 for the sound duration, number of pulses, pulse duration, and dominant frequency (Table IV; Supplementary Material 2, Table XXI). These low ratios indicate some consistency among males for these parameters. For pulse period and interpulse interval, the ratios are higher (CVb/CVw > 1.5), meaning more variability between individuals (Table IV; Supplementary Material 2, Table XXI). For the mating sounds, all the ratios were slightly greater than 1 (CVb/CVw < 1.5) (Table IV; Supplementary Material 2, Table XXII).

In the analysis of reproductive sounds, flexible discriminant function analysis (FDA) was conducted using the

TABLE III. Comparison of the different acoustic parameters (mean  $\pm$  standard deviation) characterizing the mating sounds produced by the five males of *Dascyllus albisella*. Numbers between brackets correspond to the number of analyzed sounds.

	Male 1 ( $N = 152$ )	Male 3 ( $N = 21$ )	Male 6 ( $N = 31$ )	Male 8 ( $N = 5$ )	Male 14 ( $N = 21$ )
Duration (ms)	130 ± 37	$161 \pm 59$	$169 \pm 70$	$260 \pm 58$	$152 \pm 38$
Number of pulses	$3 \pm 1$	$4 \pm 1$	$4 \pm 1$	$6 \pm 1$	$4 \pm 1$
Pulse period (ms)	$50 \pm 6$	54 ± 12	$50 \pm 6$	$46 \pm 2$	$47 \pm 4$
Pulse duration (ms)	$23 \pm 2$	$24 \pm 2$	$25 \pm 1$	$23 \pm 1$	$25 \pm 1$
Interpulse interval (ms)	$27 \pm 5$	$30 \pm 11$	$26 \pm 6$	$23 \pm 2$	$23 \pm 4$
Dominant frequency (Hz)	$370 \pm 44$	$402 \pm 42$	430 ± 53	476 ± 35	$402 \pm 32$





FIG. 6. (Color online) A scatterplot of the principal component PC1 versus PC2 was performed with the six acoustic parameters of mating sounds produced by five *Dascyllus albisella* males. The different forms distinguish the males. Ellipses correspond to 95% of the observations, and dots correspond to ellipse centers.

sounds of 17 males and including six acoustic parameters. The results showed that 90.6% of the sounds (568 of 628) were classified correctly according to the associated behavior. For courtship sounds, FDA was performed using the sounds of 17 males and including the same six acoustic parameters. Only 38.9% of the courtship sounds (154 of 398) were classified correctly according to the identity of the male. For mating sounds, FDA was performed using the sounds of five males and including the same six acoustic parameters. A higher classification accuracy was observed, with 73.9% of the mating sounds (169 of 230) correctly classified based on the male's identity.

## **IV. DISCUSSION**

*Dascyllus albisella* produced sounds associated with courtship and spawning behaviors that are statistically different. Mating sounds were shorter than courtship sounds since they were composed of fewer pulses. These results are congruent with those of Lobel and Mann (1995) on male *D. albisella* from Johnston Atoll in 1991 and 1994.

TABLE IV. Comparison of the ratios between the between-individual coefficient of variation (CVb) and the within-individual coefficient of variation (CVw) calculated for different acoustic parameters in *Dascyllus albisella* for courtship and mating sounds.

	CVb/CVw for courtship sounds	CVb/CVw for mating sounds
Duration	1.1	1.2
Number of pulses	1.1	1.1
Pulse period	1.6	1.1
Pulse duration	1.1	1.3
Interpulse interval	1.6	1.1
Dominant frequency	1.3	1.3

In this study of 17 specimens, none of the six acoustic parameters, either individually or combined, can isolate males from each other based on courtship or mating sounds. The relatively low CVb/CVw ratios for both behaviors indicate low stereotypy, as the temporal and frequency parameters showed a slightly greater variation between individuals than within them. These low ratios reflect vocal similarities among individuals of supposed similar size (College et al., 2009), which may limit the acoustic properties' ability to identify individuals. Additionally, the results of the discriminant analysis support the absence of distinctiveness. It is important to note that the FDA conducted on mating sounds yielded higher classification accuracy than the one performed on courtship sounds. However, the difference can be attributed to sampling: only five males produced mating sounds, with one male accounting for about 65% of the sample. In contrast, 17 males produced courtship sounds more evenly, reducing the likelihood of misclassifying mating sounds compared to courtship sounds in this study.

Our findings suggest that it is challenging to distinguish between *D. albisella* individuals based solely on their vocalizations. The absence of distinct acoustic signatures in male vocalizations diminishes the likelihood of female recognition based only on this sensory channel. However, it remains plausible that alternative communication channels, such as olfactory or visual, could play a significant role, such as in Cichlidae and Gasterosteidae (Jordan *et al.*, 2003; Kidd *et al.*, 2006; Knight and Turner, 1999; Plenderleith *et al.*, 2005; Rafferty and Boughman, 2006). Therefore, while our study does not rule out the possibility of individual recognition, it indicates that if such recognition occurs, it is unlikely to rely solely on vocal means. Playback experiments are the next step in testing these results.

While individual recognition through acoustic signals plays a role in mate selection (Myrberg *et al.*, 1986) and male-male interactions in some species, such as *Stegastes* 



partitus, where males distinguish between familiar and unfamiliar sounds (Myrberg and Riggio, 1985), our study did not detect such distinctiveness in D. albisella. Acoustic individuality in fish often relies on spectral characteristics, particularly dominant frequency, which did not distinguish individuals in our study. Although we could not measure the size of the individuals, their presumed similarity may explain the lack of detectable variation in dominant frequency. In other species, such as Mormyridae (Pollimyrus adspersus) and cichlids (Metriaclima zebra), individuality arises from complex combinations of acoustic features, including spectral and temporal dimensions (Bertucci et al., 2012; Crawford et al., 1997). While D. albisella vocalizations appear less distinctive, we cannot rule out the possibility that individual identification through size-related spectral characteristics occurs (Colleye et al., 2009; Lobel and Mann, 1995; Myrberg et al., 1993).

While our study suggests individual recognition through vocalizations is unlikely in D. albisella, the potential role of acoustic cues in male reproductive success cannot be dismissed (Amorim et al., 2015). In other fish species, vocal parameters, such as calling rate, calling effort, and dominant frequency, have been shown to influence mating outcomes, as seen in the Lusitanian toadfish (Halobatrachus didactylus) and the painted goby (Pomatoschistus pictus) (Amorim et al., 2013; Vasconcelos et al., 2012). These parameters likely serve as honest signals of male condition or reproductive motivation (Vasconcelos et al., 2012), and a similar mechanism might be present in D. albisella. For instance, Oliver and Lobel (2013) observed a correlation between male courtship rates and mating success in D. albisella, suggesting that acoustic signaling plays a role in female mate choice, even without individual recognition.

#### **V. CONCLUSION**

The different analyses performed in this study suggest that *D. albisella* males do not possess any individual acoustic signatures. No male could be distinguished from all the others based on our parameters. Either alternative communication channels are involved, or individual recognition is not requisite for this species' biology. However, communication in this species is multimodal, combining acoustic signaling with color changes and a dance behavior (the signal jump) performed by the vocalizing individual. A combination of these elements could define individual characteristics. It would be interesting to extend the study with experimentations, using playback experiments to assess whether the males of *D. albisella* can or cannot differentiate the sounds of other males.

#### SUPPLEMENTARY MATERIAL

See the supplementary material for video and audio recordings (Supplementary Material 1), supplementary results (Supplementary Material 2), and codes and data (Supplementary Material 3). This work was supported through S.L. by the Fonds de la Recherche Scientifique (40016018), the University of Liège (2022/MOB/05895), and the LEAR foundation. Field studies at Johnston Atoll were supported through P.S.L. by the Army Research Office (DAAAG55-98-1–0304 and DAAD19-02-1–0218) and the Office of Naval Research (N00014-19-J1519 and N00014-92-J-196). S.L. is a scholarship holder from the Fonds de la Recherche Scientifique - FNRS (40011238). We also thank Arnout Van Messem for his help in the statistical analysis. We thank Arthur Van Damme for his reasoning during the study. We also thank the reviewers for their insightful remarks and advice, which improved this manuscript.

# AUTHOR DECLARATIONS Conflict of Interest

The authors have no conflicts to disclose.

#### **Ethics Approval**

This is an observational study. The Animal Ethics Commission of the University of Liège has confirmed that no ethical approval is required.

#### DATA AVAILABILITY

The data supporting this study's findings are available from the corresponding author upon reasonable request. The datasets generated and analyzed during the current study are available within the article and its supplementary material.

- Amorim, M. C. P. (2023). "The role of acoustic signals in fish reproduction," J. Acoust. Soc. Am. 154, 2959–2973.
- Amorim, M. C. P., Pedroso, S. S., Bolgan, M., Jordão, J. M., Caiano, M., and Fonseca, P. J. (2013). "Painted gobies sing their quality out loud: Acoustic rather than visual signals advertise male quality and contribute to mating success," Funct. Ecol. 27, 289–298.
- Amorim, M. C. P., Vasconcelos, R. O., and Fonseca, P. J. (2015). "Fish sounds and mate choice," in *Sound Communication in Fishes: Animal Signals and Communication*, edited by F. Ladich (Springer, Vienna), Vol. 4, pp. 1–33.
- Andersson, M. B. (1994). Sexual Selection (Princeton University Press, Princeton, NJ), pp. 3–31, available at http://archive.org/details/sexual selection0000ande (Last viewed June 20, 2024).
- Bee, M. A., Kozich, C. E., Blackwell, K. J., and Gerhardt, H. C. (2001). "Individual variation in advertisement calls of territorial male green frogs, *Rana clamitans*: Implications for individual discrimination," Ethology 107, 65–84.
- Beecher, M. D. (1982). "Signature systems and kin recognition," Am. Zool. 22, 477–490.
- Beecher, M. D. (**1989**). "Signalling systems for individual recognition: An information theory approach," Anim. Behav. **38**, 248–261.
- Bertucci, F., Attia, J., Beauchaud, M., and Mathevon, N. (2012). "Sounds produced by the cichlid fish *Metriaclima zebra* allow reliable estimation of size and provide information on individual identity," J. Fish Biol. 80, 752–766.
- Blank, D. A., and Yang, W. (2017). "Mother-young recognition in goitered gazelle during hiding period," Behav. Process. 142, 21–28.
- Casey, C., Reichmuth, C., Fregosi, S., Charrier, I., and Mathevon, N. (2013). "The acoustic signature of the male northern elephant seal: Individual variation supports recognition during competitive interactions," J. Acoust. Soc. Am. 134, 3988.

https://doi.org/10.1121/10.0034790



- Ceugniet, M., Aubin, T., Bernard-Laurent, A., and Soyez, D. (1999). "Vocal signatures of the rally call of red-legged and rock partridges and of their hybrids," C. R. Acad. Sci. III 322, 887–895.
- Colleye, O., Frederich, B., Vandewalle, P., Casadevall, M., and Parmentier, E. (2009). "Agonistic sounds in the skunk clownfish *Amphiprion akallopisos*: Size-related variation in acoustic features," J. Fish. Biol. 75, 908–916.
- Colleye, O., Vandewalle, P., Lanterbecq, D., Lecchini, D., and Parmentier, E. (2011). "Interspecific variation of calls in clownfishes: Degree of similarity in closely related species," BMC Evol. Biol. 11, 365.
- Cornec, C., Hingrat, Y., and Rybak, F. (2014). "Individual signature in a lekking species: Visual and acoustic courtship parameters may help discriminating conspecifics in the Houbara bustard," Ethology 120, 726–737.
- Crawford, J. D., Cook, A. P., and Heberlein, A. S. (1997). "Bioacoustic behavior of African fishes (Mormyridae): Potential cues for species and individual recognition in *Pollimyrus*," J. Acoust. Soc. Am. 102, 1200–1212.
- Fine, M., and Parmentier, E. (2015). "Mechanisms of fish sound production," in *Sound Communication in Fishes*, edited by F. Ladich (Springer, Vienna, Austria), Vol. 4, pp. 77–126.
- Fishelson, L. (1998). "Behaviour, socio-ecology and sexuality in damselfishes (Pomacentridae)," Ital. J. Zool. 65, 387–398.
- Fisher, J. (1954). "Evolution and bird sociality," in *Evolution as a Process* (Allen & Unwin, London), pp. 71–83.
- Gerhardt, H. C., and Bee, M. A. (2006). "Recognition and localization of acoustic signals," in *Hearing and Sound Communication in Amphibians*, Springer Handbook of Auditory Research, edited by P. M. Narins, A. S. Feng, R. R. Fay, and A. N. Popper (Springer, New York), pp. 113–146.
- Janik, V. M., Sayigh, L. S., and Wells, R. S. (2006). "Signature whistle shape conveys identity information to bottlenose dolphins," Proc. Natl. Acad. Sci. U.S.A. 103, 8293–8297.
- Jordan, R., Kellogg, K., Juanes, F., and Stauffer, J. (2003). "Evaluation of female mate choice cues in a group of Lake Malawi *Mbuna* (Cichlidae)," Copeia 2003, 181–186.
- Kidd, M. R., Danley, P. D., and Kocher, T. D. (2006). "A direct assay of female choice in cichlids: All the eggs in one basket," J. Fish Biol. 68, 373–384.
- Knight, M. E., and Turner, G. F. (1999). "Reproductive isolation among closely related Lake Malawi cichlids: Can males recognize conspecific females by visual cues?," Anim. Behav. 58, 761–768.
- Ladich, F., Bischof, C., Schleinzer, G., and Fuchs, A. (**1992**). "Intra- and interspecific differences in agonistic vocalization in croaking gouramis (genus: *Trichopsis*, Anabantoidei, Teleostei)," Bioacoustics **4**, 131–141.
- Lobel, P. S. (2001). "Fish bioacoustics and behavior: Passive acoustic detection and the application of a closed-circuit rebreather for field study," Mar. Technol. Soc. J. 35, 19–28.
- Lobel, P. S., and Mann, D. A. (1995). "Spawning sounds of the Damselfish, *Dascyllus albisella* (Pomacentridae), and relationship to male size," Bioacoustics 6, 187–198.
- Mann, D. A., and Lobel, P. S. (1998). "Acoustic behavior of the damselfish Dascyllus albisella: Behavioral and geographic variation," Environ. Biol. Fishes 51, 421–428.
- Myrberg, A. A., Jr., Ha, S. J., and Shamblott, M. J. (1993). "The sounds of bicolor damselfish (*Pomacentrus partitus*): Predictors of body size and a

spectral basis for individual recognition and assessment," J. Acoust. Soc. Am. 94, 3067–3070.

- Myrberg, A. A., Jr., Mohler, M., and Catala, J. D. (1986). "Sound production by males of a coral reef fish (*Pomacentrus partitus*): Its significance to females," Anim. Behav. 34, 913–923.
- Myrberg, A. A., and Riggio, R. J. (**1985**). "Acoustically mediated individual recognition by a coral reef fish (*Pomacentrus partitus*)," Anim. Behav. **33**, 411–416.
- Myrberg, A. A., Jr., and Spires, J. Y. (1972). "Sound discrimination by the bicolor Damselfish, *Eupomacentrus partitus*," J. Exp. Biol. 57, 727–735.
- Narins, P. M., Feng, A. S., Fay, R. R., and Popper, A. N. (Eds.) (2006). *Hearing and Sound Communication in Amphibians*, Springer Handbook of Auditory Research (Springer, New York), Vol. 28.
- Oliver, S. J., and Lobel, P. S. (2013). "Direct mate choice for simultaneous acoustic and visual courtship displays in the damselfish, *Dascyllus albisella* (Pomacentridae)," Environ. Biol. Fish. 96, 447–457.
- Parmentier, E., and Fine, M. L. (2016). "Fish sound production: Insights," in *Vertebrate Sound Production and Acoustic Communication*, Springer Handbook of Auditory Research, edited by R. A. Suthers, W. T. Fitch, R. R. Fay, and A. N. Popper (Springer International Publishing, Cham, Switzerland), Vol. 53, pp. 19–49.
- Plenderleith, M., van Oosterhout, C., Robinson, R. L., and Turner, G. F. (2005). "Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish," Biol. Lett. 1, 411–414.
- Quam, R. M., Ramsier, M. A., Fay, R. R., and Popper, A. N. (Eds.) (2017). *Primate Hearing and Communication*, Springer Handbook of Auditory Research (Springer International Publishing, Cham, Switzerland), Vol. 63.
- Rafferty, N. E., and Boughman, J. W. (2006). "Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks," Behav. Ecol. 17, 965–970.
- Stevenson, R. A. (1963). "Life history and behavior of Dascyllus albisella Gill, a pomacentrid reef fish," Ph.D. thesis, University of Hawaii, Honolulu, HI, available at http://hdl.handle.net/10125/12020 (Last viewed June 15, 2024).
- Suthers, R. A., Fitch, W. T., Fay, R. R., and Popper, A. N. (Eds.) (2016). Vertebrate Sound Production and Acoustic Communication, Springer Handbook of Auditory Research (Springer International Publishing, Cham, Switzerland), Vol. 53.
- Tan, X., Li, Y., Sun, K., Jin, L., and Feng, J. (2022). "Mutual mother-pup acoustic identification in Asian particolored bats," Ecol. Evol. 12, e9554.
- Temeles, E. J. (1994). "The role of neighbours in territorial systems: When are they 'dear enemies'?," Anim. Behav. 47, 339–350.
- Terrazas, A., Serafin, N., Hernández, H., Nowak, R., and Poindron, P. (2003). "Early recognition of newborn goat kids by their mother: II. Auditory recognition and evidence of an individual acoustic signature in the neonate," Dev. Psychobiol. 43, 311–320.
- Vasconcelos, R. O., Carriço, R., Ramos, A., Modesto, T., Fonseca, P. J., and Amorim, M. C. P. (2012). "Vocal behavior predicts reproductive success in a teleost fish," Behav. Ecol. 23, 375–383.
- Ydenberg, R. C., Giraldeau, L. A., and Falls, J. B. (**1988**). "Neighbours, strangers, and the asymmetric war of attrition," Anim. Behav. **36**, 343–347.