

## Sound production in four damselfish (*Dascyllus*) species: phyletic relationships?

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Most studies of fish sounds show that the sounds are species-specific, with unique spectral and timing characteristics. This raises the question as to whether these sounds can be used to understand phyletic relationships between species and which acoustic parameters are subject to variation between species. In the present study, 597 sounds (and 2540 pulses) related to signal jumps of four *Dascyllus* species (*Dascyllus aruanus*, *Dascyllus trimaculatus*, *Dascyllus albisella*, and *Dascyllus flavicaudus*) from different geographic regions (Madagascar, Moorea, Rangiroa, and Hawaii) were analysed. It was possible to discern species-specific sounds, but also variation in sounds between populations. Large variations in sound length were found between *Dascyllus* species, whereas differences in interpulse duration were found to be variable between populations. In the regions where species live in sympatry, it appears that they restrict the variability in their sounds. This could comprise evidence of adaptation with character displacement of sonic characteristics where different species co-occur. However, sonic characteristics still overlapped substantially between species, suggesting that females would need to sample more than one sound and potentially use other cues to discriminate between species. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, 97, 928–940.

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### INTRODUCTION

A key problem in evolution is understanding how diverging species might avoid mating with each other, thereby escaping the mixing of genes that results from sexual reproduction. This has led to the suggestion that, in most cases, reproductive isolation and speciation must result from populations becoming physically separated, such as through the separation of ocean basins.

In sympatric speciation, variations in sounds are considered pre-zygotic isolating mechanisms leading

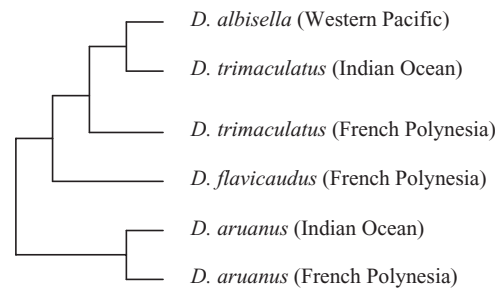
to speciation (Cocroft & Ryan, 1995; Grant & Grant, 1996; Slabbekoorn & Smith, 2002); the receivers are the selective force on the evolution of the signal (Higgins & Waugaman, 2004). Sexual selection can lead to the divergence of traits involved in mate recognition and thus generates pre-zygotic reproductive isolation between lineages. Several examples demonstrate divergence in acoustic signals between closely-related species (Mendelson & Shaw, 2005). Acoustic studies in the Hemiptera have shown how rapidly pre-zygotic reproductive barriers involving acoustic signals may become established (Claridge & de Vrijer, 1993). In mammals, horseshoe bats from Sulawesi comprise three separate size forms, each

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calling at a different frequency of the same harmonic series, and DNA analysis has confirmed that these bats are on their way to becoming distinct species (Kingston & Rossiter, 2004). In Mediterranean gobies, Malavasi, Collatuzzo & Torricelli (2008) suggested congruence between acoustic characters and morphological/genetic data. Their study demonstrated that some acoustic properties of sounds could be used to differentiate species, or groups of closely-related species. These findings are reinforced by the geographical variation in acoustic signals in numerous vertebrate taxa including frogs (Cocroft & Ryan, 1995), birds (Grant & Grant, 1996; Slabbekoorn & Smith, 2002), and mammals (Peters & Tonkin-Leyhausen, 1999; Bazua-Duran & Au, 2004). In the fish *Opsanus tau*, a variation of dialect was also recorded in the field, along the Atlantic coast of North America (Fine, 1978a), and from sounds evoked by electrical stimulation of the brain (Fine, 1978b). Parmentier *et al.* (2005) found that acoustic parameters differed between two distant (6500 km) populations of the clownfish, *Amphiprion akallopisos*. More recently, allopatric differentiation was also found in the acoustic communication of weakly electric fish, *Marcusenius macrolepidotus* (Lamml & Kramer, 2007).

The damselfish genus, *Dascyllus* (Pomacentridae), comprises ten species of both large- and small-bodied fishes distributed over the entire Indo-West Pacific (Randall & Allen, 1977; Godwin, 1995; Bernardi & Crane, 1999). According to Godwin (1995) and McCafferty *et al.* (2002), the genus is divided into two groups, one basal consisting of two species of small-bodied fishes (*Dascyllus aruanus* and *Dascyllus melanurus*) and the other consisting of larger species. All *Dascyllus* species are demersal spawners. Courting males prepare nests by removing debris from an area of substratum, usually near the base of the coral colony, and spend a great deal of time chasing other fishes away from the site. These males perform courtship dips, also called signal jumps or gambolling, to attract females. The courtship dip consists of a male rising in the water column and then rapidly swimming downwards at the same time as making a pulsed sound (Lobel & Mann, 1995). Females travel to a male-guarded nest to lay eggs, which are subsequently tended by the male until hatching (Thresher, 1984).

The most comprehensive studies of damselfish sound production have been performed with the signal jumps of four members of the *Stegastes* genus, which are sympatric, establish nearby territories, and share the same reproductive season (Myrberg, 1972; Myrberg & Spires, 1972; Spanier, 1979; Myrberg, Mohler & Catala, 1986). Species-specific recognition within these pomacentrids has been experimentally demonstrated to be based upon the number of pulses



**Figure 1.** *Dascyllus* phylogenetic relationships based on the studies of Bernardi & Crane (1999), and McCafferty *et al.* (2002). The tree concerns only the populations used in the present study. *Dascyllus flavicaudus* appears as a single population because the mitochondrial DNA analysis did not allow discrimination between the populations of Moorea and Rangiroa (McCafferty *et al.*, 2002).

and the pulse interval of a call (Myrberg & Spires, 1972; Ha, 1973; Spanier, 1979). On the other hand, frequency spectrum and amplitude do not appear to be critical for species recognition (Ha, 1973). Within each species, however, the frequency appears also to be a predictor of body size (Myrberg, Ha & Shambloot, 1993; Lobel & Mann, 1995). The repetition rate of courtship dips may act as an indicator of male vigour (Knapp & Kovach, 1991; Karino, 1995; Mann & Lobel, 1995). The sounds associated with courtship behaviour have also been described in detail in *Dascyllus albisella* (Lobel & Mann, 1995; Mann & Lobel, 1995, 1997, 1998), in *Abudefduf sordidus* (Lobel & Kerr, 1999), and in *Abudefduf abdominalis* (Maruska *et al.*, 2007).

The present study compared the sounds associated with signal jumps in four *Dascyllus* species: the whitetail damselfish *D. aruanus*, the threespot damselfish *Dascyllus trimaculatus*, the Hawaiian damselfish *D. albisella*, and the yellowtail damselfish *Dascyllus flavicaudus*. Moreover, different populations of *Dascyllus* of the same species were recorded in distant geographic regions. The study aimed to determine whether the sounds produced by these different taxa (Fig. 1) vary in a way that corresponds to the existing phylogeny (McCafferty *et al.*, 2002).

## MATERIAL AND METHODS

### SOUND RECORDINGS

Field recordings of sound production by *D. aruanus* and *D. trimaculatus* were made in the same coral patch area, in the lagoon in front of the coast of Tulear (Mozambique Channel, West coast of Madagascar, 23°22'S, 43°40'E; 26 °C). Recording sessions, each lasting approximately 2 h, were made during

November 2005. Field recordings of signal jumps by *D. trimaculatus*, *D. aruanus*, and *D. flavicaudus* were made in French Polynesia during July 2007. Specimens of each species were recorded in front of Opanohu Bay, Moorea (17°29'S, 149°51'W; 28 °C). A second population of *D. flavicaudus* was recorded in Rangiroa Lagoon (14°57'S, 147°39'E; 28 °C). The duration of recording sessions was 1–4 h. Sounds were recorded using an Orca hydrophone (sensitivity: –186 dB re 1V/μPa) connected via an Orca-made amplifier (ORCA Instrumentation) to a Sony TCD-D8 digital audio tape-recorder (recording band width: 20–22 000 Hz ± 1.0 dB). The hydrophone was attached to a 2-m boom so that it floated 0.5 m above the coral patch on which the *Dascyllus* lived; the water depth was in the range 1–3 m on a flat bottom. All recordings were made with fish approximately 0.5–2 m from the hydrophone. In each case, short video sequences were made with a Digital Olympus video camera placed in an underwater housing. These videos were used to confirm that the recorded sounds corresponded to the signal jump.

Field recordings of sound production by *D. albisella* were made during scuba dives made at Johnston Atoll, Central Pacific Ocean (16°44'N, 169°31'W) in April 1994 and at Kaneohe Bay, Oahu, Hawaii (21°27'N, 157°47'W) in May 1992. Average sea water temperature in the lagoon was 25.8 °C in April and 26.4 °C in May. Sounds were recorded using a hydrophone (flat response 10–3000 Hz; nominal calibration of –162 dB re 1V/μPa) (BioAcoustics) coupled to a Sony V-9 8-mm Handycam in an underwater housing. All recordings were made with fish approximately 1–2 m from the hydrophone in an approximately 4-m depth of water on a flat bottom. All the dip sounds used in the study were recorded by Mann & Lobel (1998). To make the comparisons between species, these sounds were digitized with Adobe Audition 2.0 and analysed in the same manner as the other recordings.

Sounds were digitized at 44.1 kHz (16-bit resolution) and analysed using AvisSoft-SAS Lab Pro 4.33 software. Only the sounds with a high signal to noise ratio were used in the analysis. Temporal features were measured from oscillograms, and frequency parameters were obtained from power spectra (FFT size 256 points, time overlap 96.87%, and a rectangular window). The sound parameters measured were: sound duration; number of pulses in a sound; pulse period (measured as the average peak-to-peak interval between consecutive pulses in the entire sound); interpulse interval (IPI) (measured as the time from the end of one pulse to the beginning of the next); pulse length (measured as the time from the beginning of one pulse and its end); dominant frequency.

#### STATISTICAL ANALYSIS

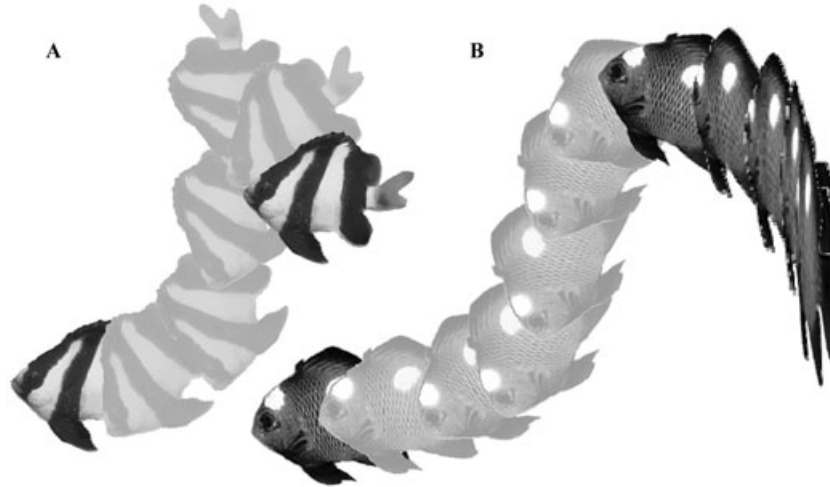
The analysis assumes that the pulses are independent. Because of field constraints, the analysis was performed by grouping calls together from multiple individuals. For analysis of acoustic data, each sonic variable (pulse length, pulse number, IPI, main frequency, pulse period) was first correlated against each other. This showed that the pulse period was highly correlated with the IPI in each species ( $r > 0.7$ ,  $P < 0.01$ ). Consequently, pulse period was removed from statistical analysis involving principal component analysis (PCA) and canonical variate analysis (CVA). Sound duration was removed from all comparisons because it depended on the number of pulses in a call.

#### INTER-SPECIFIC VARIATION

To test sound variations among each studied *Dascyllus* species, species was used as grouping factor. First, for a preliminary exploration of the sound dataset, a PCA was used to identify the variables explaining the most variance among all recorded fishes. Second, a multivariate analysis of variance (MANOVA) was performed on all call parameters to testing for differences among species. Wilk's lambda was then used as a statistical test for the presence of overall differences among species and acoustic properties. To assess the variations among species, we used canonical discriminant analysis. Also referred to as CVA, this is a widespread technique for assessing and displaying variation among groups relative to the variation within the groups. The method successively extracts axes (canonical variates, CVs) from multidimensional space that have the greatest ratio of among-group to within-group variance. Therefore, plots of the first few CVs are optimal displays of differences among groups. In addition to these analyses, a cluster analysis was performed to illustrate the similarities in call parameters between species. This procedure was applied to the matrix of Mahalanobis distances produced by the CVA. A phenogram was calculated employing the unweighted pair group method with arithmetic mean (UPGMA) algorithm using these Mahalanobis distances.

#### INTER-POPULATION VARIATION

*Dascyllus aruanus* and *D. trimaculatus* were recorded from populations in Tulear (Madagascar) and in Moorea (French Polynesia), which are separated by a distance of 15 000 km. *Dascyllus flavicaudus* were recorded from populations in Moorea and Rangiroa (French Polynesia), which are separated by 375 km. In each *Dascyllus* species, differences in call parameters between the two populations were compared



**Figure 2.** Comparison of the signal jump movements in *Dascyllus aruanus* (A) and *Dascyllus trimaculatus* (B).

and tested using *t*-tests. The comparison with respect to *D. albisella* concerned two populations separated by 1000 km, and is provided elsewhere (Mann & Lobel, 1998).

#### VARIATIONS AMONG SYMPATRIC POPULATIONS

In Moorea and Tulear, we recorded three (*D. aruanus*, *D. trimaculatus*, and *D. flavicaudus*) and two (*D. aruanus* and *D. trimaculatus*) populations of species living in sympatry respectively. For each geographical area, MANOVA was performed on sound variables for testing differences among the species. Then CVA were also used to assess variates explaining the most variation among groups.

#### RESULTS

The courtship dip consists of a rapid, almost vertical dive movement. The video allowed only a qualitative description of the movements. We found differences in the dip movement between *D. aruanus* and the other three species (Fig. 2). In *D. aruanus*, the male stops swimming, makes a forward rotating movement by raising the caudal fin, and then swims rapidly downward. In successive signal jumps, the fish stops swimming between each dip, and rises in the water column at the same time as raising its tail before the next jump. The movement resembles a sinusoidal curve. In the other three species, the swimming fish turns abruptly at a right angle and begins the dive to the side and not forwards, as occurs in *D. aruanus*. In all four species, sounds are made not only during the descent, but also during the rise.

**Table 1.** Percentage and cumulative percentage of three first axis of principal component analysis (PCA) with PCA loadings for the three axes extracted from the four acoustic properties

	PC1	PC2	PC3
Percentage	45.18	27.72	17.32
Cumulative percentage	45.18	72.79	90.22
Pulse number	0.617	-0.467	0.624
Pulse length	-0.712	-0.562	0.133
IPI	-0.436	0.722	0.530
Frequency	0.854	0.237	-0.069

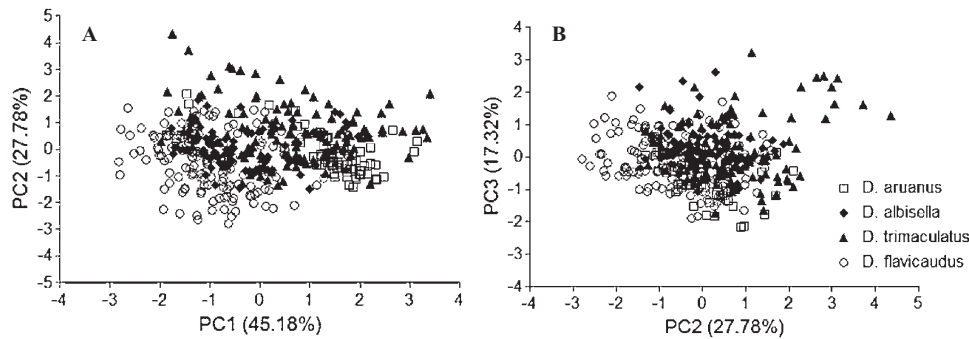
IPI, interpulse interval.

#### INTER-SPECIFIC VARIATION

In general, the sounds were strikingly similar between the four species. The plot of the species along the first three principal components (PC1, 45.18%; PC2, 27.72%; and PC3, 17.32% of the sound variance, respectively) showed largely overlapping samples (Fig. 3). PCA suggested that none of the different acoustic properties can be used as a strong distinctive character (Table 1). The first axis (PC1) is principally loaded by variables describing the frequency and the pulse length, the second axis (PC2) by the IPI, and the third axis (PC3) by the pulse number. In spite of high overlaps in sound parameters among species, the four *Dascyllus* species differed significantly in their average acoustic signals (Wilk's lambda = 0.346,  $F_{12,1122} = 46$ ,  $P < 0.0001$ ).

Each sound associated with the signal jump consisted of a series of three to 11 pulses (Fig. 4). *Dascyllus flavicaudus* generally produces a lower number





**Figure 3.** Scatterplot of principal component (PC) 1 versus PC2 (A) and PC2 versus PC3 (B) from principal component analysis on the four acoustic parameters. PC1 is loaded by variables describing the frequency and the pulse length, PC2 by the interpulse interval and PC3 by the pulse number.

**Table 2.** Comparison (mean  $\pm$  SE) of signal jumps in four *Dascyllus* species

	<i>Dascyllus aruanus</i>	<i>N</i>	<i>Dascyllus trimaculatus</i>	<i>N</i>	<i>Dascyllus albisella</i>	<i>N</i>	<i>Dascyllus flavicaudus</i>	<i>N</i>
Number of fishes	7		11		6		9	
IPI (ms)	17 $\pm$ 0.3	376	29 $\pm$ 0.4	640	28 $\pm$ 0.3	408	26 $\pm$ 0.4	614
Pulse period (ms)	30 $\pm$ 0.3	374	49 $\pm$ 1	185	46.6 $\pm$ 0.4	258	48 $\pm$ 0.4	738
Pulse length (ms)	13 $\pm$ 0.2	452	15.3 $\pm$ 0.1	779	20 $\pm$ 0.2	500	26 $\pm$ 0.3	571
Frequency (Hz)	466 $\pm$ 4	452	465 $\pm$ 5	679	401 $\pm$ 4	500	348 $\pm$ 2	792

IPI, interpulse interval; *N*, pulse number.

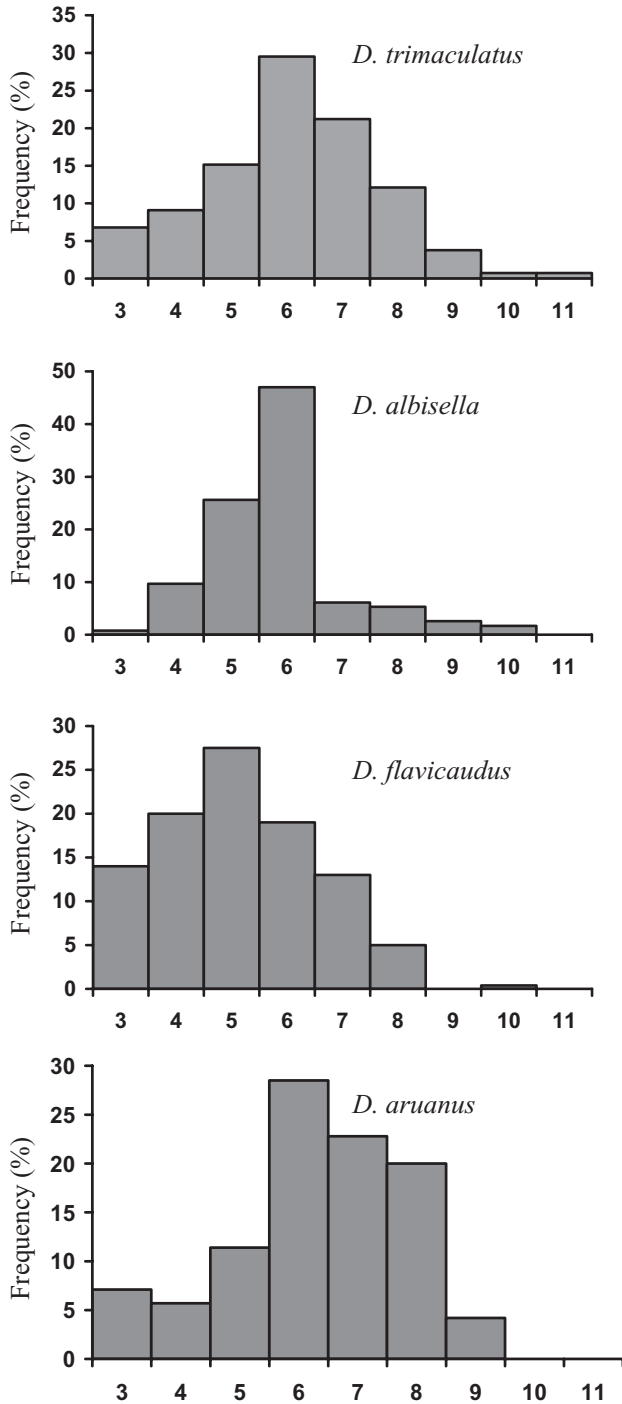
of pulses per sound than the other three species (Tukey's test,  $P < 0.001$ ). The mode number of pulses was six in *D. trimaculatus*, *D. albisella*, and *D. aruanus*, whereas *D. flavicaudus* was the sole species to have a mode of five pulses per sound. However, *D. flavicaudus* and *D. albisella* had mostly four to six pulse sounds (66.5% and 82.3% of the cases, respectively), *D. trimaculatus* had five to seven pulse sounds (65.8%), and *D. aruanus* had six to eight pulse sounds (71.3%).

In each case, the number of pulses was correlated with the sound duration, indicating that the calls had a constant pulse period (Fig. 5). The pulse period was the shortest in *D. aruanus* (30 ms) and significantly different from all other three species (Tukey's test,  $P < 0.001$ ). The pulse periods in the three other species were similar (i.e. in the range 46–49 ms) and not significantly different between them (Tukey's test,  $P > 0.001$ ) (Table 2). However, the pulse period depends on both the pulse duration and the IPI. Both the mean pulse duration and mean IPI were significantly different between these species, except in the IPI between *D. albisella* and *D. trimaculatus* (Tukey's test,  $P > 0.05$ ). The mean pulse duration was the shortest in *D. aruanus* (13 ms; Tukey's test,  $P < 0.001$ ), which is closer to the mean pulse duration

in *D. trimaculatus* (15.3 ms) than in *D. flavicaudus* (20 ms) and *D. albisella* (24 ms). All of these mean pulse durations were statistically significantly different from each other (Tukey's test,  $P < 0.001$ ).

In each species, the pulse duration tended to increase from the first to the last pulse; although the increase was not linear in all the cases. The first pulse was found to be shorter than the last pulse in 82% of cases in *D. trimaculatus*, in 89% of cases in *D. albisella*, in 90% of cases in *D. flavicaudus*, and in 92% of cases in *D. aruanus*. The difference in pulse length between the first and the last pulse was 5.4 ms in *D. trimaculatus*, 5.3 ms in *D. albisella*, 5.7 ms in *D. aruanus*, and 8.1 ms in *D. flavicaudus* (Fig. 6). The durations of the first and last pulse were not significantly different in *D. trimaculatus* and in *D. aruanus*. Because *D. aruanus* had the shortest pulse length, IPI, and pulse period, the sound length was logically the shortest, regardless of the number of pulses. For sounds with the same number of pulses, the sound length was similar only in *D. albisella* and *D. flavicaudus*.

The results obtained regarding peak frequency have to be treated carefully because many studies have shown that this characteristic could depend on the fish size (Myrberg *et al.*, 1993; Lobel & Mann,



**Figure 4.** Frequency distribution of pulse numbers in dip sounds in four *Dascyllus* species.

1995). The fish that we recorded in the field were not collected and so it was not possible to know their size. It is, however, interesting to note that the peak frequency was not significantly different between *D. aruanus* (465 Hz) and *D. trimaculatus* (466 Hz), which comprise two species with large differences in

**Table 3.** Percentage and cumulative percentage of three first axis of canonical variate analysis (CVA) with CV loadings for the three axes extracted from the four acoustic properties

	CV1	CV2	CV3
Percentage	78.04	21.42	0.54
Cumulative percentage	78.04	99.46	100
Pulse number	-0.184	-0.278	1.020
Pulse length	0.938	-0.125	-0.072
IPI	0.151	-1.062	-0.010
Frequency	-0.096	-0.394	0.299

IPI, interpulse interval.

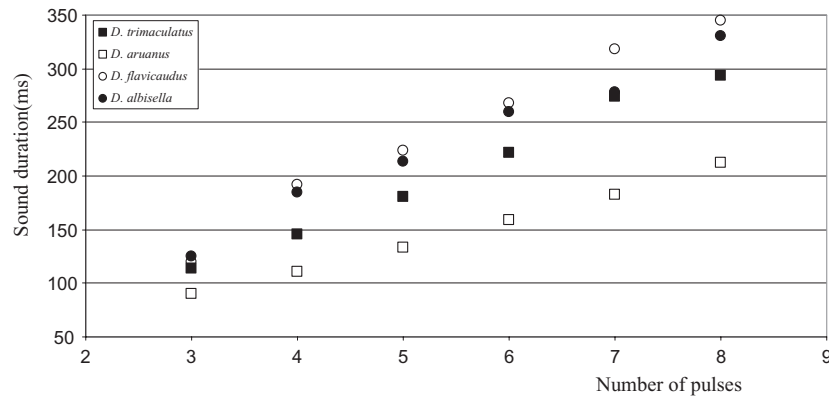
size at the adult stage (a maximum of 60 and 100 mm, respectively; Randall & Allen, 1977). All other pairwise comparisons indicated significant differences between the groups (Tukey's test,  $P < 0.001$ ). Figure 7 does not show a species-specific distribution of the pulses in a call. Each spectrogram shape could apply to any *Dascyllus* species in the present study. For example, the main frequency was found to be higher, equal or lower in the first pulse than in the last pulse in 40%, 18%, and 42% of the cases ( $N = 35$ ) in *D. trimaculatus*.

The CVA analysis was performed to assess the occurrence of acoustic differences among species. The analysis emphasized the predominant role of the pulse length on the CV1 and IPI on the CV2 to distinguish the four species (Table 3). Discriminant analysis of sonic parameters provided an overall percent-correct classification, with 64.9% accuracy for all species. However, the scores vary according to the species. Sound parameters allow correct categorization for the sounds of *D. aruanus* (83.5%), *D. trimaculatus* (70.8%), and *D. flavicaudus* (79.3%). However, the accuracy of categorization is only 16.2% in *D. albisella*. This indicates that the sound parameters of *D. albisella* are highly variable and overlap the other three species.

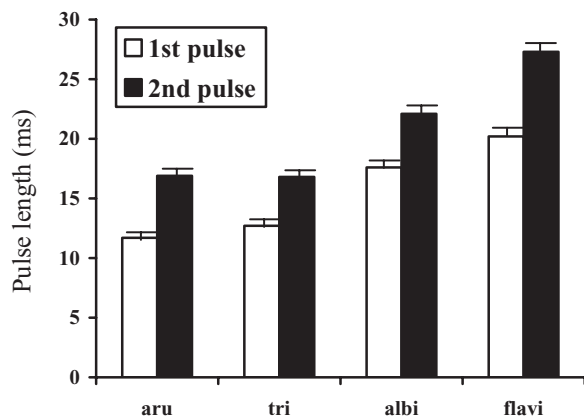
The UPGMA phenogram based on the matrix of Mahalanobis distances among the mean sound of each species allows the determination of the degree of acoustic signal similarity between species (Fig. 8). It revealed that *D. aruanus* had the greatest differentiation with respect to the other species, which branch off into two main clusters: one formed by *D. flavicaudus* and the other formed by *D. trimaculatus* and *D. albisella*.

#### INTER-POPULATION VARIATION

The *D. trimaculatus* populations in Madagascar and French Polynesia had significantly different pulse



**Figure 5.** Relationship of sound duration to the mean number of pulses in a call in *Dascyllus trimaculatus*, *Dascyllus aruanus*, *Dascyllus flavicaudus*, and *Dascyllus albisella*. The curve was fitted by:  $y = 37.8x - 3.1$  ( $r = 0.99$ ) in *D. trimaculatus*,  $y = 24.3x + 14.4$  ( $r = 0.99$ ) in *D. aruanus*,  $y = 44.2x + 1.3$  ( $r = 0.98$ ) in *D. flavicaudus*, and  $y = 38.6x + 19.3$  ( $r = 0.98$ ) in *D. albisella*.



**Figure 6.** Mean  $\pm$  SE pulse length in the first pulses (white bar) and last pulses (black bar) of dip sounds in *Dascyllus aruanus* (aru), *D. trimaculatus* (tri), *D. albisella* (albi), and *D. flavicaudus* (flavi).

periods (Fig. 9, Table 4). The pulse durations of the two populations were not significantly different. The 14 ms difference in the IPI between the *D. trimaculatus* of Madagascar and French Polynesia was responsible for the difference in the pulse period. The peak frequency was also almost 150 Hz lower in Moorea compared to Tulear (Fig. 9, Table 4).

*Dascyllus aruanus* pulse periods and IPIs were also significantly different between the populations in Moorea and Tulear. As in *D. trimaculatus*, the IPI was shorter in the Tulear population, and the pulse duration was similar (Fig. 9, Table 4).

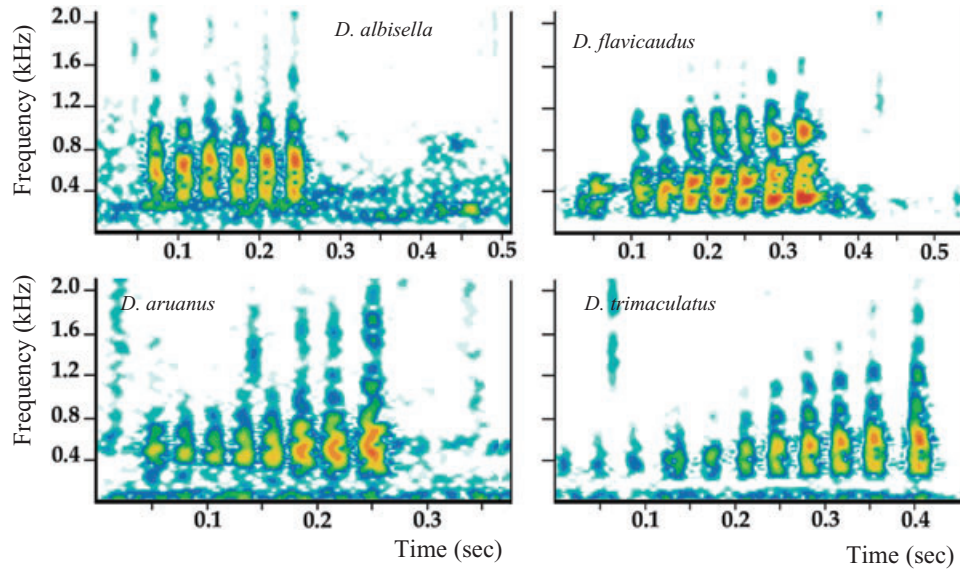
The yellowtail damselfish *D. flavicaudus* differed significantly between Rangiroa and Moorea in the IPI, pulse duration, and pulse period. On the other hand, the peak frequency was not found to be significantly different between populations (Fig. 9, Table 4).

In the Hawaiian *Dascyllus*, Mann & Lobel (1998) noted that populations at Johnston Atoll and Hawaii differed by only a small difference in the pulse length ( $P = 0.011$ ). The study did not provide any indication concerning the dominant frequency of both populations. Mann & Lobel (1998) measured an IPI of 38 ms and a pulse duration of 13 ms using a different technique than the one used in the present study. Our reanalysis produced an IPI of 26 ms and a mean pulse duration of 24 ms.

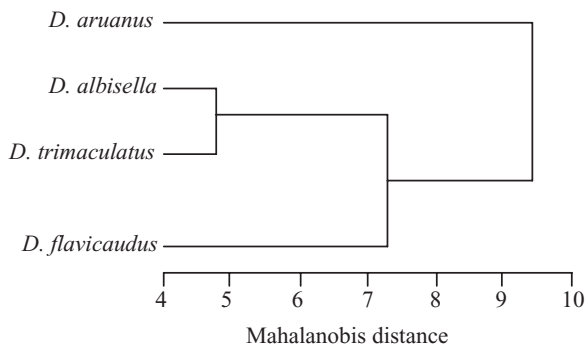
On the basis of mitochondrial DNA sequence data, populations of *D. trimaculatus* from the Indo-Pacific were more closely related to *D. albisella* than they were to *D. trimaculatus* from Moorea (McCafferty *et al.*, 2002). This assumption was sufficient for us to carry out a comparison of the sound characteristics between *D. trimaculatus* from both Tulear and Moorea and *D. albisella*. Pulse lengths were not found to be significantly different between the *D. trimaculatus* of Moorea and Tulear, but they did differ from those of *D. albisella*. Peak frequency and period were different for all three groups ( $P < 0.001$ ). The difference in the IPI from Tulear *D. trimaculatus* and *D. albisella* was only 1.6 ms (Tables 1, 2).

#### INTRA-REGION VARIATION

The purpose of this analysis was to determine whether the sonic characters of the sympatric populations of different species also overlap. Accordingly, we conducted an analysis of call variations in Moorea where *D. aruanus*, *D. flavicaudus*, and *D. trimaculatus* co-occur. MANOVA revealed that differences among species were statistically significantly different in Moorea (Willk's lambda = 0.402,  $F_{8,304} = 21.9$ ,  $P < 0.0001$ ). The CVA also illustrated differences among species (Fig. 10). Although the species aver-



**Figure 7.** Sonogram of dip sounds in four *Dascyllus* species.



**Figure 8.** Phenogram resulting from Mahalanobis distance; unweighted pair group method with arithmetic mean method based on the means of the acoustic properties.

ages were clearly distinct, some overlap was present in the scatter of data (Fig. 10). The CV1 set *D. trimaculatus* apart from the other two species, and was mainly associated with the IPI (Table 3). The CV2 was primarily a contrast of *D. aruanus* versus *D. flavicaudus*, corresponding to differences in the pulse length (Table 3). The discriminant analysis of sonic parameters of species from Moorea correctly classified a great proportion of the specimens (81% of total correct classification).

In Tulear, *D. aruanus* and *D. trimaculatus* significantly differed (t-test,  $P < 0.001$ ) for all acoustic parameters.

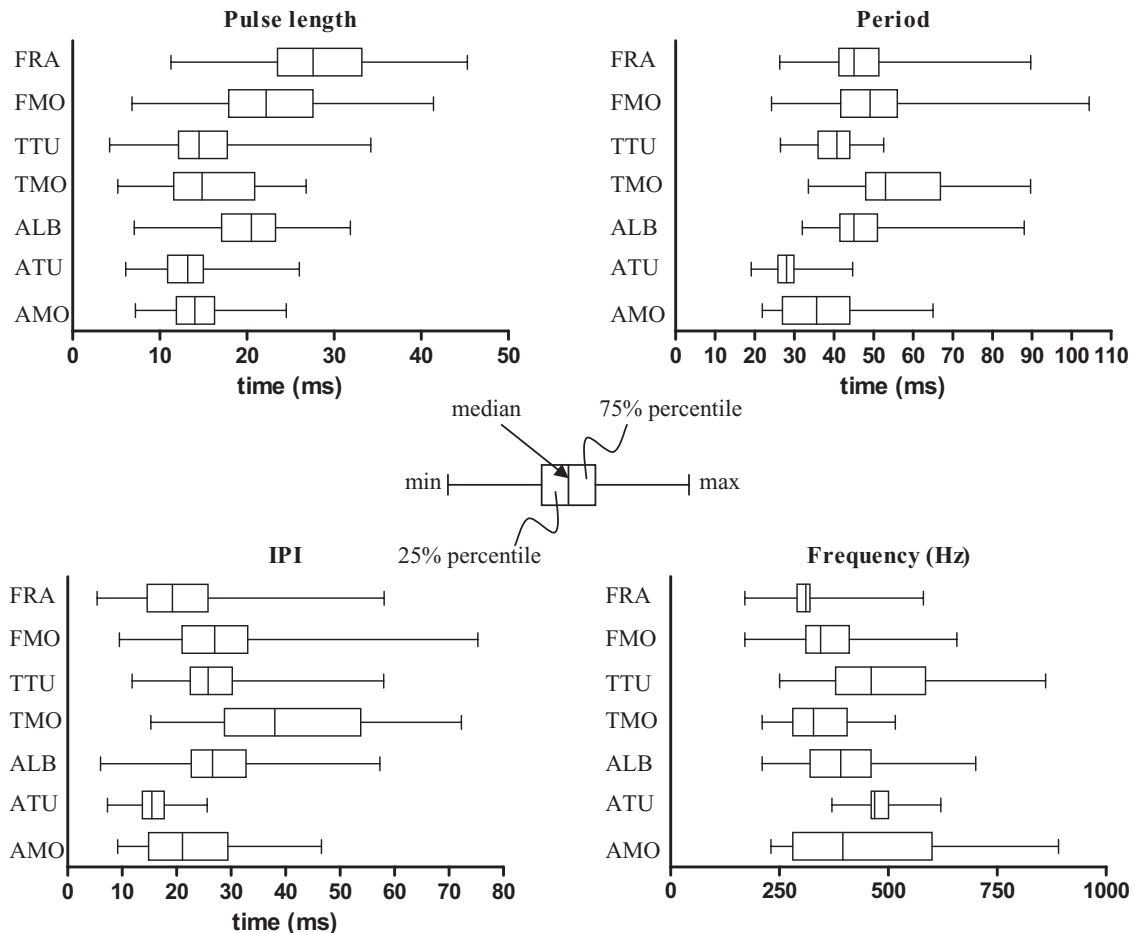
## DISCUSSION

All species of *Dascyllus* produce acoustic pulse trains for communication. There was a great deal of overlap

in the acoustic parameters analysed in the present study between species. However, it was possible to discriminate species-specific differences in the means of the parameters that were analysed. Mean differences were also detectable between populations for *D. trimaculatus*, *D. aruanus*, and *D. flavicaudus*. There was little variation in temporal properties between *D. trimaculatus*, *D. albisella*, and *D. flavicaudus*: differences in mean IPI between these species were in the range 1–3 ms. *Dascyllus aruanus* had the shortest IPI, which was 9–12 ms shorter than the other species. The dominant frequency of the sounds was also similar between species (< 150 Hz variation), despite there being a large difference in the sizes of the different species. Within the framework of the present study, the comparison of the different pulses within each dip sound did not give additional information, except for the fact that the shortest duration was found in the first pulse for each species.

In gobies, two acoustic temporal properties (i.e. pulse rate and sound duration) allow individuals to be clustered according to the species-specific nature of their sounds (Malavasi *et al.*, 2008). The temporal characteristics of *D. aruanus* sounds differed widely from those of the other three species, and allowed the formation of two groups, corresponding to the first deep phylogenetic branching *sensu* McCafferty *et al.* (2002) and Bernardi & Crane (1999) (Figs 1, 8). This grouping is reinforced by the courtship dip mating display associated with sound production (Fig. 2). Based on an analysis of the sound parameters, *D. trimaculatus* groups with *D. albisella* (McCafferty *et al.*, 2002), rather than one or both these fishes with *D. flavicaudus*. This grouping of acoustic characteris-





**Figure 9.** Sonic parameters in different populations of the damselfish *Dascyllus*. FRA, *Dascyllus flavicaudus* from Rangiroa; FMO, *D. flavicaudus* from Moorea; TTU, *Dascyllus trimaculatus* from Tulear, TMO, *D. trimaculatus* from Moorea; ALB, *Dascyllus albisella* from Johnston Atoll; ATU, *Dascyllus aruanus* from Tulear; AMO, *D. aruanus* from Moorea. IPI, interpulse interval.

tics matches the phylogenetic relationships among these species. Interestingly, the pulse length, but not IPI and the pulse period, varies greatly between these three large-bodied species. Comparison with outgroups would help to understand the orientation and evolution of certain sonic characters.

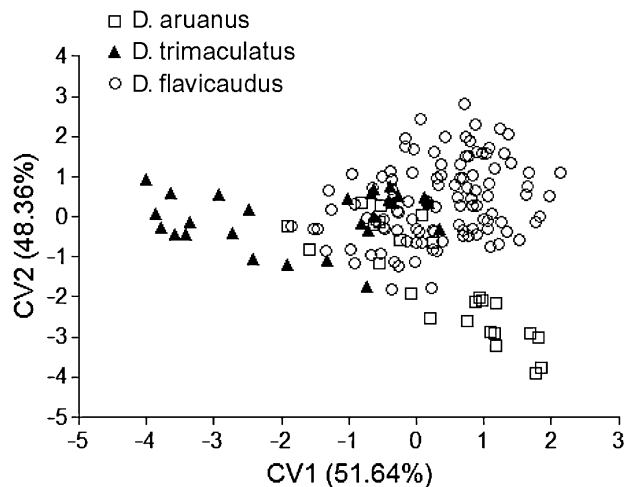
The situation is not the same at the population level: the main differences are found in the IPI and not in the pulse length. In this case, the period varies because the IPI varies. Indeed, the intra-specific comparison between populations indicates that the pulse length appears to be the same between populations in *D. albisella* (Mann & Lobel, 1998), *D. trimaculatus*, and *D. aruanus*. The observation that the signal parameters differentiating species in this subgroup are distinct from those differentiating populations within species is intriguing. It appears that acoustic signals have evolved differently in the geographical regions, most likely as a response to different abiotic

or biotic factors. For example, Tulear possess two small species (*D. aruanus* and *D. carneus*) and one large species (*D. trimaculatus*), whereas there are two large species (*D. trimaculatus* and *D. flavicaudus*) and one small species (*D. aruanus*) in Moorea (McCafferty *et al.*, 2002). Generally speaking, the two species (*D. aruanus* and *D. trimaculatus*), with the most widespread distribution, possess the greatest variability in the IPI. In the regions where they live in sympatry, it appears that these species have modulated their sounds. Curiously, IPI, pulse period and pulse length of *D. trimaculatus* and *D. aruanus* were longer in Moorea than in Tulear. On the other hand, *D. albisella* does not co-occur with other *Dascyllus* species. *Dascyllus albisella* acoustic parameters overlap all other *Dascyllus* and can not be clearly distinguished. Taken together, these results suggest that there could be character displacement of sonic characteristics when different species co-occur.

**Table 4.** Comparison of signal jumps in different populations in three *Dascyllus* species

		<i>N</i>		<i>N</i>	Difference	<i>P</i>
<i>Dascyllus trimaculatus</i>						
Number of fishes	Tulear: 6		Moorea: 5			
IPI (ms)	26.7 ± 0.3	531	41 ± 1.4	109	14.3	< 0.001
Pulse period (ms)	39.8 ± 0.6	77	57 ± 1.2	109	17.2	< 0.001
Pulse length (ms)	15.2 ± 0.2	645	15.9 ± 0.4	134	0.7	0.06
Frequency (Hz)	491 ± 5.4	645	342 ± 7	134	149	0.001
<i>Dascyllus aruanus</i>						
Number of fishes	Tulear: 3		Moorea: 4			
IPI (ms)	15.7 ± 0.2	290	23.1 ± 1	86	7.4	< 0.001
Pulse period (ms)	28.3 ± 0.4	49	36.8 ± 1	87	8.5	< 0.001
Pulse length (ms)	13 ± 0.2	339	14 ± 0.3	113	1	0.007
Frequency (Hz)	474 ± 2.4	339	442 ± 17	113	32	0.004
<i>Dascyllus flavicaudus</i>						
Number of fishes	Rangiroa: 5		Moorea: 4			
IPI (ms)	21 ± 0.6	193	26 ± 0.5	298	5	< 0.001
Pulse period (ms)	47.2 ± 0.5	277	50 ± 0.5	314	2.8	< 0.001
Pulse length (ms)	28.4 ± 0.4	234	25.6 ± 0.3	384	2.8	< 0.001
Frequency (Hz)	317 ± 3	236	351 ± 3	384	34	0.002

IPI, interpulse interval. *P*-values are the result of a *t*-test.



**Figure 10.** Scatterplot of canonical variate (CV) 1 versus CV2 from canonical variate analysis on the four acoustic parameters. The three populations live in sympatry in Moorea (French Polynesia). Each data point corresponds to a call.

In the sonic communication of four *Stegastes* species, fishes were shown to be fully capable of distinguishing their species-specific sounds from similar sounds of the three closely-related species (Myrberg & Spire, 1972; Myrberg, Spanier & Ha, 1978). Myrberg *et al.* (1978) played back dip sounds to test the responsiveness of the *Stegastes*. Interestingly, dip sounds of each species were able to elicit signal

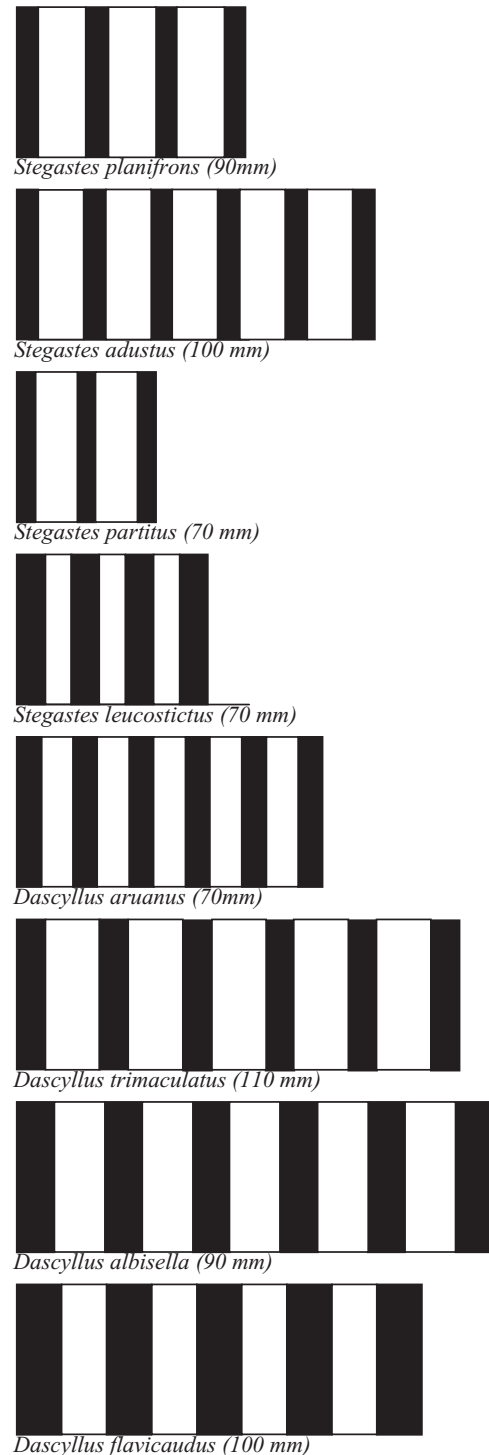
jumps in the test males of all the other species, demonstrating that the sound characteristics did not isolate the species completely. However, significantly more responsiveness was shown by males to sounds of their own species than to sounds from congeners (Myrberg *et al.*, 1978). The experiments with *Stegastes* species clearly showed that sounds can elicit responses, even if all the criteria are not present, and even if the sounds are made by another species. Myrberg *et al.* (1978) postulated that inconsequential courtship based on misidentification operated in damselfish. The reason for misidentification appears to be linked to the broad and overlapping ranges in frequency spectra and temporal data. The same overlaps are found for the *Dascyllus* genus. As a whole, the four *Dascyllus* species of the present study possess overlapping temporal and spectral characteristics. The four *Stegastes* species can be distinguished on the basis of the number of pulses per call, and this is more difficult to distinguish between the four *Dascyllus* species.

In teleost fishes, temporal features within a call, including pulse duration, rate and number, can all be important to the communicative value of a call (Bass & McKibben, 2003; Malavasi *et al.*, 2008). Temporal sound features have been effectively and reliably encoded in auditory neurones in some fishes (Suzuki, Kozloski & Crawford, 2002; Wysocki, 2006). In the mormyrid *Pollimyrus adspersus* for example, Crawford (1997) showed that these neurones are sensitive to periodicities in the same range used by these fish

for auditory communication. Myrberg *et al.* (1978) reported that damselfish could be capable of distinguishing temporal differences of sounds at 5–10 ms. By analysing auditory evoked potentials in response to sounds with varying periods in different species, Wysocki & Ladich (2002, 2003) showed that temporal resolution ability was below 1.5 ms. Differences in temporal parameters appear to be small in the *Dascyllus* species: the IPI is in the range 16–28 ms (up to 41 ms in *D. trimaculatus* from Moorea only) and the pulse length is in the range 13–28 ms). Further research is needed to determine the abilities of *Dascyllus* species to discriminate different call parameters. However, the wide overlap between species suggests that sonic recognition would require sampling of multiple calls to perform reliable species discrimination.

Because these fish sounds are percussive and the temporal properties appear to be the most important in species recognition, a graph analogous to a bar code was created, aiming to visualize the differences between the sound species (Fig. 11). This graph was made on the basis of a succession of six pulses and five intervals. It includes the data of four *Stegastes* species (Myrberg *et al.*, 1978) and the four *Dascyllus* species of the present study. Each bar code was based on the calculation of the means and not on the range of the data. The graph shows, as a minimum, *D. aruanus* is clearly separated from the other three *Dascyllus*. The same observation can be made for *Stegastes leucostictus* and the other three *Stegastes* species. Unfortunately, the phylogenetic relationships between the four *Stegastes* species are not known. In *Dascyllus*, it was shown in the present study that *D. trimaculatus*, *D. albisella*, and *D. flavicaudus* show important variations, mainly in pulse length. This is not the case in *Stegastes*, where the more important variations between *Stegastes planifrons*, *Stegastes adustus* and *Stegastes partitus* are mainly found at the level of the IPI (Spanier, 1979). This shows that the calling signal may not have the same kind of evolution in the different taxa. As a result, fishes belonging to different taxa (e.g. *D. aruanus* and *S. leucostictus*) can use a similar code resulting from a different evolutionary path. However, other characters, such as colour and swimming movements, also occur in courtship behaviour and may provide additional information regarding species recognition and partner selection.

On the basis of the bar code graph and of knowledge concerning the auditory temporal resolution, we postulate that the fish brain may have a type of punch card image corresponding to the bar code. The closer the temporal code to the punch card, the greater the responsiveness will be. At present, this model is speculative. However, it can in part explain



**Figure 11.** Bar codes corresponding to the typical dip sounds in different species of damselfish of the *Stegastes* and *Dascyllus* genera. The black bars correspond to a pulse and its width to the pulse length; and the white bars correspond to the pulse interval (IPI) and its width to the IPI duration. The size in parenthesis refers to the biggest size for each species.

how the *Stegastes* can respond to nonspecific sounds (Myrberg & Spires, 1972; Myrberg *et al.*, 1978) and why females differ in how they select a spawning partner. Damselfish repeat these sounds hundreds of times a day (Mann & Lobel, 1995), so it is possible that a female could sample many calls before making a decision to spawn with a given male.

In Cichlidae, the radiation in Lake Malawi has been characterized in three historical stages (Kocher, 2004; Genner & Turner, 2005). The first stage corresponds to habitat divergence. The second stage is the elaboration of morphologically distinct taxa. In the third stage, species differ primarily in colour pattern, which indicates the action of sexual selection (Kocher, 2004; Schliewen *et al.*, 2006). Each stage allows the taxa to diverge. This succession of events can also explain, for example, how fishes with the same colour pattern can be phylogenetically distant. These three first stages (habitat, morphology, and communication) are not restricted to cichlids, and are found in different vertebrate radiations (Streelman & Danley, 2003). It is also the case in Pomacentridae (Frédérich *et al.*, 2008, 2009). The third stage, communication, involves male secondary sexual characteristics and has been usually associated with visual displays. Sounds are also responsible for mating of fishes, and could correspond to the fourth stage of speciation or have an active role in the third stage. Inter-specific mate recognition could be mediated by multimodal signals (Amorim *et al.*, 2008).

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