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RESEARCH ARTICLE

Diversity and evolution of sound production in the social behavior of *Chaetodon* butterflyfishes

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ABSTRACT

Fish produce context-specific sounds during social communication, but it is not known how acoustic behaviors have evolved in relation to specializations of the auditory system. Butterflyfishes (family Chaetodontidae) have a well-defined phylogeny and produce pulsed communication sounds during social interactions on coral reefs. Recent work indicates that two sound production mechanisms exist in the bannerfish clade and that other mechanisms are used in the *Chaetodon* clade, which is distinguished by an auditory specialization, the laterophysic connection (LC). Here, we determine the kinematic action patterns associated with sound production during social interactions in four *Chaetodon* subgenera and the non-laterophysic fish *Forcipiger flavissimus*. Some *Chaetodon* species share the head bob acoustic behavior with *F. flavissimus*, which along with other sounds in the 100–1000 Hz spectrum, are probably adequate to stimulate the ear, swim bladder or LC of a receiver fish. In contrast, only *Chaetodon* species produced the tail slap sound, which involves a 1–30 Hz hydrodynamic pulse that is likely to stimulate the receiver's ear and lateral line at close distances, but not the swim bladder or LC. Reconstructions of ancestral character states appear equivocal for the head bob and divergent for the tail slap acoustic behaviors. Independent contrast analysis shows a correlation between sound duration and stimulus intensity characters. The intensities of the tail slap and body pulse sounds in *Chaeotodon* species are correlated with body size and can provide honest communication signals. Future studies on fish acoustic communication should investigate low-frequency and infrasound acoustic fields to understand the integrated function of the ear and lateral line, and their evolutionary patterns.

KEY WORDS: Acoustic, Auditory, Behavior, Coral reef, Fish, Hearing, Infrasound, Sound

INTRODUCTION

Many fish produce context-specific sounds during social interactions that are used in recognition of individuals, sex or species, during the defense of food resources or mates, and courtship or spawning (Ladich and Myrberg, 2006; Myrberg and Lugli, 2006). Fish sounds are produced by a diversity of motor behaviors (Ladich and Fine, 2006) but all produce an acoustic field that includes both a localized hydrodynamic flow that may stimulate the lateral line and inner ear at close distances, and a sound pressure wave that may independently stimulate the inner ear at larger distances from the source (Kalmijn, 1988; Coombs and Montgomery, 1999). Studies on the evolution of fish sounds used for intraspecific communication are based largely on meta-analyses of sound production mechanisms compared in broad taxa (e.g. Ladich and Bass, 2003; Ladich and Fine, 2006; Parmentier and Diogo, 2006) and the characteristics of sounds produced by different genera within fish families (Amorim, 2006). A few studies on sympatric congener species show evidence for conservation or divergence in temporal and spectral features of sounds: African cichlids (Amorim et al., 2004, 2008; Lobel, 1998), sunfishes (Gerald, 1971), gobies (Lugli et al., 1997), mormyrids (Crawford et al., 1997) and marine damselfishes (Kenyon, 1994; Lobel and Mann, 1995; Myrberg and Riggio, 1985; Myrberg et al., 1993; Spanier, 1979). However, the diversity of natural sounds remains to be assessed in relation to social behavior and hearing capabilities in a phylogenetic evolutionary context.

Butterflyfishes of the family Chaetodontidae include approximately 130 species and 10 genera that are abundant members of coral reef fish communities and well known for their diverse color patterns, social behaviors and food habits (Hourigan, 1989). Most are highly social species, which form long-term monogamous pairs or harems that defend food and other resources from competitors, or larger associations for planktivorous feeding or movements across the reef. Phylogenetic studies (Blum, 1988; Smith et al., 2003; Fessler and Westneat, 2007; Bellwood et al., 2010) show a well-defined phylogeny that consists of the bannerfish clade and the *Prognathodes–Chaetodon* clade (Fig. 1). The genus *Chaetodon* includes approximately 90 species distributed within four clades with 10 subgenera (Bellwood et al., 2010) all of which have a diagnostic laterophysic connection (LC) – a novel morphological association of the swim bladder, anterior swim bladder horns and the cranial lateral line canal system (Webb, 1998). Several *Chaetodon* subgenera show distinct differences in the length, width and proximity of the paired anterior swim bladder horns to a medial opening in the lateral line canals in the supracleithral bone of the head and in the morphology of the swim bladder (Webb and Smith, 2000; Webb et al., 2006, 2010). The LC system was proposed to enhance hearing capabilities during social interactions via the transduction of sound pressure waves by the anterior swim bladder horns that may stimulate fluid motion in the lateral line canal and particle motion at the inner ear (Webb, 1998; Webb et al., 2006; Tricas et al., 2006). However, the acoustic behaviors and sound characteristics used in communication by *Chaetodon* remain unknown for all but one species, and are needed to interpret the putative function and evolution of the LC within the family.

Recent studies show that sound production during social interactions occurs widely among butterflyfish genera (Fig. 1) but details on the mechanics of sound production for *Chaetodon* species come only from field observations. Acoustic behaviors are known but not quantified for *C. multicinctus*, *C. ornatissimus*, *C. kleinii*

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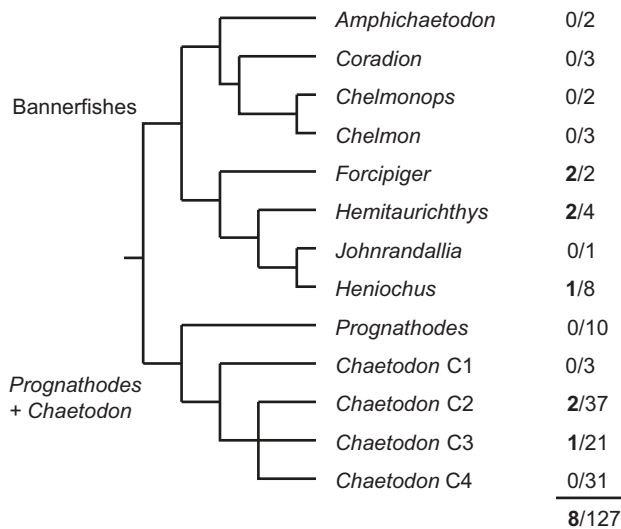
Fig. 1. Simplified diagrammatic phylogeny of the 10 butterflyfish genera in the family Chaetodontidae. Butterflyfishes include 8 genera and approximately 25 species of in the bannerfish clade and approximately 102 species in the *Prognathodes+Chaetodon* clade. The genus *Chaetodon* includes 92 species distributed across four *Chaetodon* clades (C1–C4). Numbers (bold) show the fraction of total species in each taxon for which sound production is known (but not necessarily quantified). Note the low number of *Chaetodon* species studied, all of which possess a laterophysic connection proposed to enhance hearing of social acoustic stimuli. The phylogeny and species numbers are taken from Bellwood et al. (2010). Branch lengths are not scaled.

(Tricas and Boyle, 2014) and *C. ulietensis* (Lobel et al., 2010) with kinematic data available for only *C. multicinctus* (Tricas et al., 2006). Lab and field studies on bannerfishes (all of which lack the LC) reveal two primary acoustic behaviors. The head bob sound is produced by *Forcipiger* species and is driven by the epaxial muscles (Boyle, 2011; Boyle and Tricas, 2011; Tricas and Boyle, 2014). The swim bladder buckling sound is produced by *Hemitaurichthys* species, which is driven by contraction of anterior hypaxial muscles (Boyle and Tricas, 2010; Boyle et al., 2013; Tricas and Boyle, 2014). A similar internal sound production mechanism occurs in *Heniochus chrysostomus* that is associated with contraction of obliquus superioris hypaxial muscles (Parmentier et al., 2011). These studies show that sound production involves distinct acoustic behaviors, includes different muscle systems and likely preceded the evolution of the swim bladder horns and LC character in the *Chaetodon* genus. However, sound production behaviors and associated sound characteristics must be identified for *Chaetodon* to estimate their ancestral character states and evolution. In addition, the potential adaptive value of acoustic signal communication in *Chaetodon*, such as providing honest signals about the size, condition or health of the individual as shown for *Forcipiger flavissimus* (Boyle and Tricas, 2011) remains unexplored.

In this study, we determine the acoustic behavior of different *Chaetodon* subgenera and identify basic evolutionary patterns of sound production within the family. We compare sound production in five species with a range of LC morphologies to that of the related forcepsfish, *F. flavissimus*, which lacks the LC. Herein, we (1) assess the external motor patterns that are associated with sound production; (2) quantify the sound characteristics among species; (3) test the hypothesis of honest acoustic signals related to sex and body size in *Chaetodon* acoustic communication; and (4) assess the ancestral character states and independent contrasts of sound characteristics within the family based on their published molecular phylogeny. These findings are further interpreted in relation to the auditory sensitivity of these species in a companion paper (Tricas and Boyle, 2015).

RESULTS

Acoustic behavior and sound characteristics

A total of 41 butterflyfish were tested for sound production after the introduction of a conspecific individual to the test aquarium. Sound production was recorded for 30 subjects: adult *Chaetodon multicinctus* (*n*=10, 56% of tested fish, SL=71.2±14.2 mm, mean± s.d.), *Chaetodon auriga* (*n*=5, 100%, SL=111.0±7.0 s.d. mm) including one immature individual, *Chaetodon kleinii* (*n*=4, 100%, SL=78.0±4.6 mm), *Chaetodon ornatissimus* (*n*=3, 60%, SL=78.7+ 5.9 s.d. mm), *Chaetodon unimaculatus* (*n*=4, 100%, SL=122.0+29.2 mm) and *F. flavissimus* (*n*=4, 80%, SL=99.7+12.1 mm). In total, 59% of resident fish and 16% of introduced fish produced sound. The majority (79%) of total sounds were produced by the resident fish as it approached in close proximity to the conspecific usually within 1–2 body lengths. Some body movements associated with sound production were not visible because of the orientation of the fish, the camera angle or blocked view by the body of the second fish.

Two sound types were recorded for *F. flavissimus* during social interactions with conspecifics. The head bob pulse sound occurred during a very brief dorsal–ventral motion of the head that was sometimes only slightly visible in the video and produced most frequently by the introduced non-resident fish (Table 1). This sound was of short average duration (29 ms) with an average peak frequency of 254 Hz (Table 2, Fig. 2). The head bob behavior sometimes included a slight movement of the jaw that was neither prominent nor frequent in the video. Thus, we assume jaw movement in this species was a passive action associated with the upward motion of the head and not involved in sound production. All resident fish produced the anal fin retraction pulse sound during aggressive interactions with the introduced individual. This sound occurred after extension of the anal fin spine but was coincident with the retraction motion. It was of longer average duration (54 ms) and much lower average peak frequency (27 Hz) than the head bob pulse.

Jaw protrusion was the only sound type recorded for *C. kleinii*. This acoustic behavior was shown by both resident and introduced fish and most commonly included a clear extension and retraction of the oral jaws and occasionally a brief shake (lateral motion) of the head (Table 1). This sound was of very short average duration (17 ms) and showed an average peak frequency (516 Hz) (Table 2, Fig. 2). We observed no vertical motion of the head, although it may be detected at higher video frame rates.

Two sound types were produced by *C. unimaculatus*. Both resident and introduced fish produced the head bob–jaw protrusion pulse sound that was a very fast motion and visible in only 1–2 frames of video. This behavior was always associated with a clear extension and retraction movement of the jaws, a frequent vertical head bob motion and an infrequent lateral head shake as for *C. kleinii* above (Table 1). It appeared to be of longer average duration (52 ms) and peak frequency (1031 Hz) than that for the jaw protrusion sound of *C. kleinii*, although our sample size was too small for statistical comparisons (Table 2, Fig. 2). The tail slap sound was recorded for one resident *C. unimaculatus* and involved motion of the whole body directed at the nearby conspecific. This sound waveform was of long duration (424 ms) and very low peak frequency (<1 Hz) as reported for tail slaps by other *Chaetodon* species described below (Table 2).

Table 1. Proportion of motor patterns (expressed as a percentage) that are associated with sound production in *F. flavissimus* and *Chaetodon* butterflyfishes during social interactions with a conspecific individual

	Sound type	N, n	By resident (%)	Motor patterns (%)							
				Jaw protrusion	Head bob	Head shake	Body shake	Pelvic fin extend	Anal fin retract	Caudal fin motion	None
F. flavissimus	Head bob	3, 77	33±52**	–	100	–	–	–	–	–	–
	Anal fin retract	2, 24	100	–	–	–	–	–	100	–	–
C. kleinii	Jaw protrusion	3, 6	50±50[n.s.]	83±29	–	17±29	–	–	–	–	–
C. unimaculatus	Head bob–jaw protrusion	3, 12	43±39[n.s.]	100	67±58	17±29	17±29	–	–	–	–
	Tail slap	1, 1	100	–	–	–	–	–	–	100	–
C. multicinctus	Body motion	9, 93	67±42**	–	–	3±9	25±21	13±21	2±6	57±38	7±10
	Body shake	1, 2	100	–	–	–	100	–	–	–	–
	Tail slap	7, 45	86±38**	–	–	–	5±8	6±10	–	100	–
	Tail click	2, 2	100	–	–	–	–	–	–	100	–
C. ornatissimus	Body motion	1, 4	100					67±58	–	67±58	–
	Tail slap	1, 7	100	–	–	7±12	7±12	13±23	–	100	–
C. auriga	Head bob–jaw protrusion	5, 17	80±45**	67±47	73±43	–	–	–	2±5	–	–

Data show percentage (mean and s.d.) of acoustic behaviors that were associated with movement averaged among individuals. *N*, number of fish; *n*, total number of sounds recorded. Percentage of acoustic behaviors produced by resident fish are averages for each resident fish among trials, Chi-square tests for differences in sound production by residents and introduced fish were computed for all sounds produced by all test fish (*n*); **$P<0.001$; n.s., not significant.

Four pulse sounds were produced by *C. multicinctus* during social interactions with conspecifics. The body pulse sound was produced most frequently by the resident fish in association with lateral motions of the caudal peduncle, lateral body shake, flick of the pelvic fin or rarely without any observed body motion (Table 1). This acoustic behavior was often preceded by erection of the dorsal and anal fins, which did not appear to be associated with sound production. The body sound occurred in trains or single pulses that had a short average duration (25 ms), covered a wide range of peak frequencies (range, <1465 Hz; mean, 137 Hz; median, 231 Hz), and showed a wide average bandwidth of 350 Hz

(Table 2, Fig. 2). A single resident fish produced a novel body shake sound with a peak frequency of 29 Hz that involved brief lateral oscillations of the body in close juxtaposition to the introduced fish. The tail slap sound was produced primarily by resident fish, of relative long duration (418 ms), and very low average peak frequency (9 Hz) and bandwidth. Although movement was not quantified, the displacement motion of the tail slap ranged from weak to strong and sometimes displaced the body of the receiver fish by the generated hydrodynamic flow. We also recorded the previously reported 'click' sound pulse that occurs after the tail slap (Tricas et al., 2006), but these were

Table 2. Acoustic features of sounds produced by *Forcipiger flavissimus* and five species of *Chaetodon* butterflyfishes during social interactions with a conspecific

Species (clade)	Sound	Trains (%)	Duration (ms)	Peak frequency (Hz)	Median frequency (Hz)	6 dB Bandwidth (Hz)	Pk–Pk SPL (dB re. 1 μPa)
F. flavissimus (BF)	Head bob	7±9	29±14 (19–49)	254±41 (221–306)	283+42 (273–374)	287±75 (284–351)	123±8 (115–133)
	Anal fin retract	87±10	54±19 (40–68)	27±2 (25–28)	39±2 (38–41)	64±7 (59–69)	108±2 (107–110)
C. kleinii (C2)	Jaw protrusion	0	17±13 (8–33)	516±712 (45–1335)	645±677 (50–1382)	358±262 (58–542)	127±13 (115–140)
C. unimaculatus (C2)	Head bob–jaw protrusion	0	52±38 (9–79)	1031±1704 (20–2999)	1019±1568 (23–2826)	227±169 (34–347)	127±4 (124–132)
	Tail slap	0	424	<1	7±4 (3–10)	6	126
C. multicinctus (C2)	Body motion	36±44	25±25 (4–67)	137±191 (<1–465)	231±188 (62–530)	350±360 (117–947)	116±4 (112–120)
	Body shake	0	55	29	39	62	133
	Tail slap	40±44	418±381 (118–1191)	9±5 (3–17)	10±5 (3–18)	15±9 (4–29)	131±7 (120–139)
	Tail click	0	16	2752±349 (2505–2999)	2489±243 (2317–2661)	708±45 (677–740)	134±2 (133–135)
C. ornatissimus (C2)	Body motion	0	134±47 (94–186)	10±7 (3–17)	13±9 (4–23)	19±11 (10–32)	123±6 (116–127)
	Tail slap	27±46	366±390 (138–817)	6±3 (3–8)	7±4 (3–10)	14±9 (4–19)	131±13 (123–147)
C. auriga (C4)	Head bob–jaw protrusion	13±30	93±43 (43–143)	23±32 (< 1–78)	31±36 (10–95)	42±35 (23–106)	123±8 (112–133)

Data are presented as means±s.d. for averages among individuals test fish. Maximum and minimum values for each variate are indicated in brackets. Sample sizes for each sound are provided in Table 1. Clades: BF, bannerfish; C, *Chaetodon*. SPL, sound pressure level.
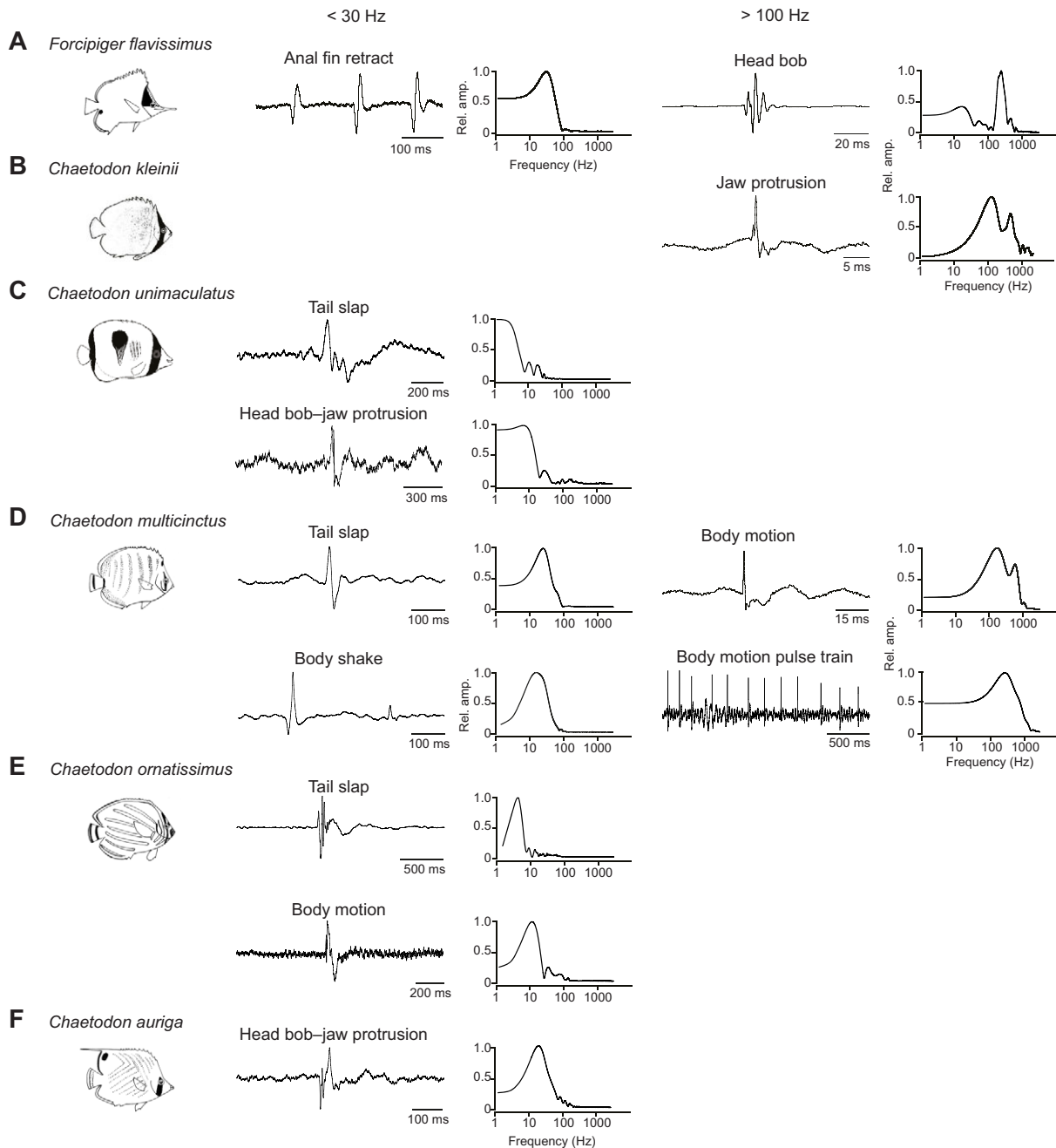
Fig. 2. Representative waveforms, spectra and motor patterns associated with sounds produced by *Forcipiger flavissimus* and five species of *Chaetodon* butterflyfishes during social interactions with conspecifics. Sounds are categorized as those with average peak frequencies near infrasound (<30 Hz) and with peak frequencies >100 Hz. (A) The forcepsfish *F. flavissimus* produced a low-frequency pulse sound associated with erection of the anal fin and a higher frequency pulse sound associated with the head bob–jaw protrusion behavior. (B) The blacklip butterflyfish *C. kleinii* produced a short pulse sound during protrusion of the jaw with average peak frequency near 500 Hz. (C) The teardrop butterflyfish *C. unimaculatus* produced pulse sounds with peak frequency <10 Hz during slap behavior or protrusion of the jaw. (D) The multiband butterflyfish *C. multicinctu*s produced low-frequency pulse sounds from 10–30 Hz during tail slap and body shake acoustic behaviors. Single and trains of pulse sounds were produced during the body motion sound which had a higher average peak frequency of 137 Hz. (E) The ornate butterflyfish *C. ornatissimu*s produced low-frequency pulse sounds near 10 Hz during both tail slap and body motion acoustic behaviors. (F) The threadfin butterflyfish *C. auriga* produced the head bob–jaw protrusion sound which had a low average peak frequency near 20 Hz. Fast-Fourier transforms of sound waveforms show relative amplitude (Rel. Amp.) of example peak frequencies.

relatively infrequent in the lab setting (Tables 1 and 2, Fig. 3). This distinct broadband sound was of very short average duration (16 ms) and much higher peak frequency (2752 Hz) than any other sound recorded.

Two sounds were produced infrequently by resident *C. ornatissimus*, which was the most timid species in the laboratory setting. The body pulse sound occurred primarily during movements of the caudal peduncle and/or extension of the pelvic fins (Table 1). This was similar to the body sound motion of *C. multicinctus*, but lacked movements of several additional body parts. It had an average duration of 134 ms and a very low average peak frequency of 10 Hz (Table 2, Fig. 2). Resident fish produced both weak and strong tail slap behaviors (as described above) which were of long average duration (366 ms) and low average peak frequency (6 Hz).
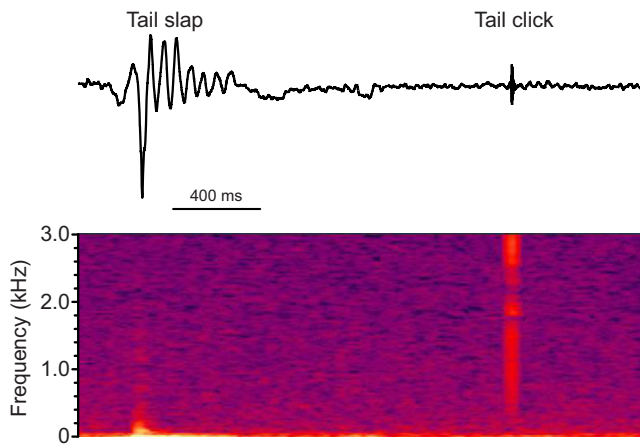
Fig. 3. Low- and high-frequency sounds associated with the agonistic tail slap behavior in *Chaetodon multicinctus* during social interactions with conspecifics. A vigorous tail slap behavior directed at a conspecific individual produces a strong infrasonic hydrodynamic acceleration at peak frequencies of <10 Hz. Subsequent motion of the tail is associated with the production of a second and brief, broadband tail click pulse sound with peak frequency >2 kHz.

Only the head bob–jaw protrusion pulse was produced by *C. auriga*. This sound was associated primarily with a clear vertical elevation of the head and a strong protrusion of the jaws. The head bob motion was very similar to that observed for *F. flavissimus* (which did not show active jaw protrusion) and the jaw motion was very similar to that observed in *C. kleinii* (which did not show evidence of a vertical 'head bob' motion). It was produced frequently by the resident fish (Table 1) and occurred in single pulses of relatively long duration (93 ms) and low peak frequency (23 Hz) (Table 2, Fig. 2), and less frequently in a two-pulse train (Table 3).

Comparison of sound characteristics

The acoustic stimuli produced by *Chaetodon* species span at least four frequency decades (from <1 to >1000 Hz) and show a strong and non-linear inverse relationship between average sound duration and peak frequency (Fig. 4). Extreme low-frequency sounds with an average peak frequency of <10 Hz and long duration (400 ms) were associated with the tail slap behavior in *C. ornatissimus*, *C. multicinctus* and *C. unimaculatus*. The tail slap sound did not differ among *C. multicinctus* and *C. ornatissimus* in either median duration (Mann–Whitney U-test, $U=0.8$, $n=7$ and 3, $P=0.67$), average peak frequency (t-test, d.f.=8, $t=0.834$, $P=0.43$), average 6 dB median frequency (t-test, d.f.=8, $t=0.467$, $P=0.467$) or average

6 dB bandwidth (t-test, d.f.=8, $t=0.171$, $P=0.87$). Other pulsed low-frequency sounds that had peak frequencies from ~10 to 30 Hz and durations near or <100 ms were produced by *C. multicinctus*, *C. ornatissimus*, *C. auriga* and *F. flavissimus*. Sounds with peak frequencies from 100–1000 Hz were readily produced in social interactions in the lab by *C. kleinii*, *C. unimaculatus*, *C. multicinctus* and *F. flavissimus*, with pulse durations ≤50 ms. Analysis of variance identified no differences in peak or median frequency, or bandwidth among these sounds. The unique click sound of *C. multicinctus* showed a much higher average peak frequency of 2732 Hz. These data show that the acoustic fields produced by fish at close distances span a wide frequency range and that longer pulses carry information at lower frequencies.

There were several similarities and differences in the bandwidth of sounds produced among species (Fig. 5). With the exception of *C. kleinii*, all *Chaetodon* species produced at least one sound type that had a peak frequency or bandwidth that fell within the <1–30 Hz and the 10–100 Hz ranges. The lowest frequency sounds included the tail slap behaviors produced by *C. unimaculatus*, *C. ornatissimus* and *C. multicinctus*. The 10–100 Hz band included several sound types produced among species, and often extended to higher frequencies. Sounds within the >100 Hz band included different sound types produced by *F. flavissimus* (head bob), *C. kleinii* (jaw protrusion), *C. unimaculatus* (head bob–jaw protrusion) and *C. multicinctus* (body motion and click), but sounds in this band were not recorded for *C. auriga* or *C. ornatissimus* (which is known to produce sounds in this band in the wild, see Discussion). These comparisons show that the acoustic repertoire for some species covers a wide bandwidth through the production of several sound types (as in *C. unimaculatus* and *C. multicinctus*) or may have a more limited vocalization bandwidth.

Several pulsed sounds were produced by *F. flavissimus* and *Chaetodon* species in trains that included 2–12 discrete pulses separated by <1 s (Fig. 6, Table 3). The most common pulsed trains occurred in the body and tail slap sounds of *C. multicinctus*. The average rate of pulse train production in *Chaetodon* ranged from a low of 2 Hz for the tail slap sound of *C. ornatissimus* to a high average of 6 Hz for the body pulse by *C. multicinctus*, but both trains were highly variable in pulse number (Table 3). The sequential production of multiple head bobs was shown by both *F. flavissimus* and *C. auriga* and the sequential production of multiple tail slap sounds by both *C. multicinctus* and *C. ornatissimus*.

The average pressure levels of sounds produced by individual subjects (as measured within a few cm of the source) ranged from a low of 107 dB re. 1 μPa for the anal fin retract pulse of *F. flavissimus* to a maximum of 134 dB re. 1 μPa for the tail click pulse by *C. multicinctus* (Table 2). Pressure levels for the tail slap sound were of the strongest

Table 3. Characteristics of pulse train sounds produced by *Forcipiger flavissimus* and three species of *Chaetodon* butterflyfishes

	Sound type	*N, n*	No. of trains per fish	Train duration (ms)	Pulses per train	Pulse interval (ms)	Pulse period (ms)	Rate (Hz)
F. flavissimus	Head bob	2, 4	2±0 (2)	590±290 (143–931)	2±0 (2)	554±289 (350–758)	573±291 (125–609)	5.6±4.2 (2.1–14.0)
	Anal fin retract	2, 4	2.0 (1–3)	852±286 (305–1864)	5.3±1.89 (3–10)	134±1 (62–332)	185±15 (114–363)	6.2±6.8 (5.4–9.8)
C. multicinctus	Body motion	4, 17	4.3±1.7 (2–6)	680±630 (174–2227)	3.6±0.59 (2–12)	265±113 (1–896)	289±107 (4–904)	6.2±1.3 (2.2-11.5)
	Tail slap	4, 9	2.3±1.5 (1–4)	1030±656 (341–3001)	2.8±0.96 (2–7)	272±96 (55–593)	444±121 (228–771)	3.5±1.5 (1.8–5.9)
C. ornatissimus	Tail slap	1, 2	2	1085 (913–1257)	2 (2)	810 (741–879)	948 (834–1062)	1.9 (1.6–2.2)
C. auriga	Head bob–jaw protrusion	1, 1	1	677	2	608	645	3

Data are means±s.d.; ranges are given in brackets. *N*, number of fish; *n*, number of total trains.
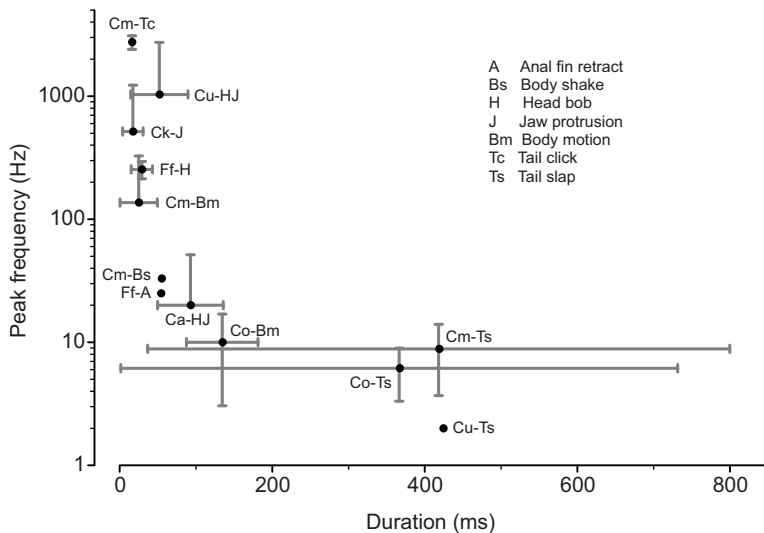
Fig. 4. The relationship between average sound duration and average peak frequency for sounds produced by *Forcipiger flavissimus* and five species of *Chaetodon* butterflyfishes. Error bars represent s.d. for averages among individual fish. Species: Ca, *C. auriga*; Ck, *C. kleinii*; Cm, *C. multicinctus*; Co, *C ornatissimus*; Cu, *C. unimaculatus*; Ff, *F. flavissimus*.

magnitude recorded and did not differ among *C. ornatissimus* (131 dB re. 1 µPa) and *C. multicinctus* (131 dB re. 1 µPa, *t*-test, *t*=−0.13, d.f.=8, *P*=0.90,). However, within species we confirm that the intensity of the tail slap sound (131 dB re. 1 µPa) was greater than the body motion pulse sound (120 dB re. 1 µPa) produced by individual *C. multicinctus* subjects (paired *t*-test, *t*=−4.15, d.f.=5, *P*<0.01), which collectively span a broad frequency spectrum (Fig. 7A).

Patterns of sound production across the butterflyfish phylogeny

The phylogenetic tree map of butterflyfish acoustic behaviors from this and previous studies (Boyle and Tricas, 2010, 2011; Parmentier

et al., 2011) indicates the possibility for both unique and shared sound production traits among the bannerfishes and *Chaetodon* species examined thus far (Fig. 8). The head bob occurs in *Chaetodon* clades 2 and 4 (but not clade 3) and is similar to that for *F. flavissimus* in which the epaxial muscles elevate the cranium. In contrast, the sound production mechanisms in *Heniochus* and *Hemitaurichthys* species are driven by mechanisms that involve the hypaxial muscles. Preliminary EMG recordings during production of the similar body pulse sound in *C. multicinctus* (our unpublished data) tentatively show activity in the ventral anterior epaxial musculature lateral to the anterior swim bladder (note that the bilateral swim bladder horn extensions project dorsal to the
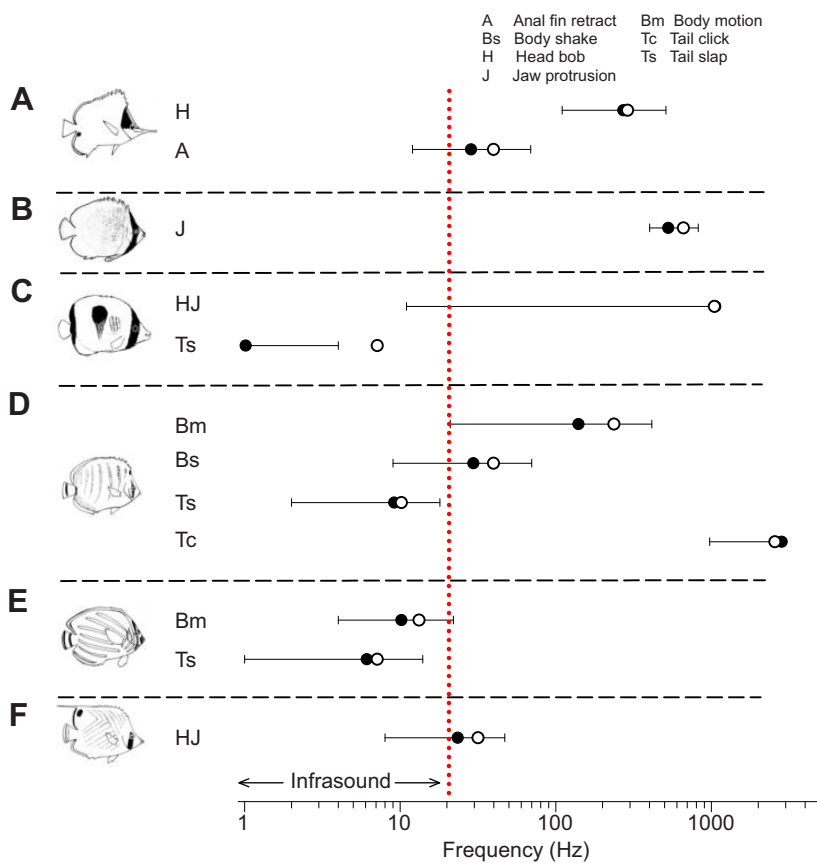
Fig. 5. The 6 dB bandwidth of butterflyfish social sounds recorded for *Foricipiger flavissimus* and *Chaetodon* species in laboratory experiments. (A) *F. flavissimus* produced the head bob and anal fin retract sounds that collectively span the 10–1000 band. (B) *C. kleinii*, produced only the jaw protrusion sound which was limited to near 500–1000 Hz. (C) *C. unimaculatus* produced an infrasonic tail slap and the head bob–jaw protrusion sound that spans higher frequencies to 1000 Hz. (D) *C. multicinctus* produced the body motion, body shake, tail slap and tail click sounds that span the <1 to >1000 Hz band. (E) *C. ornatissimus* produced the body motion and tail slap sounds which span the infrasound range. (F) *C. auriga* produced a single low-frequency head bob–jaw protrusion sound type. Both peak (black circles) and median (open circles) frequencies are shown for each sound type. Vertical dotted line indicates upper limit of the <20 Hz infrasound range.
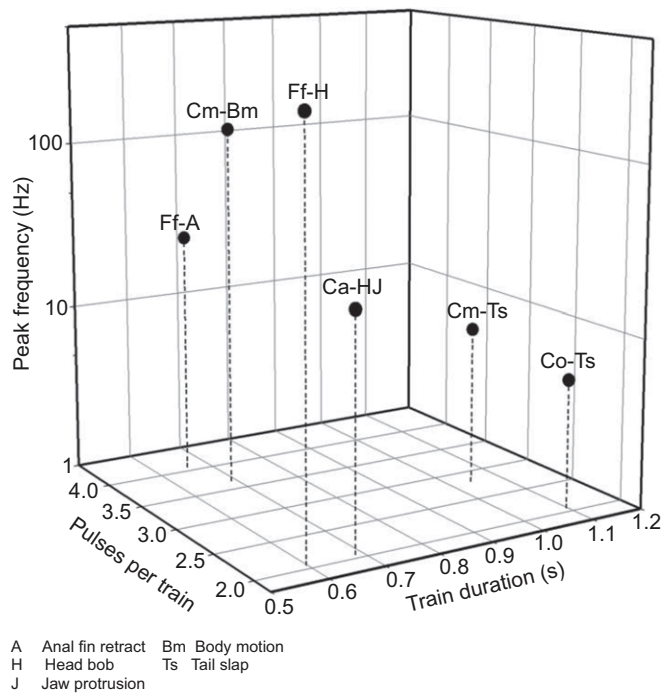
Fig. 6. Temporal and spectral characteristics of pulsed train sounds produced by *Forcipiger flavissimus* and three species of *Chaetodon* butterflyfishes during social interactions with conspecifics. Pulse trains were defined as the production of sequential pulses that were separated by an inter pulse interval of <0.5 s. Data points indicate averages for individual fish. Sound types were also produced as single pulse events. Species names: Ff, *F. flavissimus*; Ca, *Chaetodon auriga*; Cm, *C. multicinctus*; Co, *C. ornatissimus*.

horizontal septum). In comparison, the tail slap behavior occurs in 4 of 5 *Chaetodon* species, but not in the bannerfishes studied so far.

We estimated the ancestral character states for the head bob and tail slap behavior using the maximum-likelihood and parsimony methods (Fig. 9). The anal fin and body motion behaviors, and jaw protrusion in *C. kleinii* currently appear to be autapomorphies. The jaw protrusion in other species appears to be a by-product of the head bob. Maximum-likelihood analysis shows that the ancestral state for the head bob is undetermined, with equivocal likelihoods at all ancestral nodes, whereas the maximum parsimony method indicates independent evolution of this trait among species (Fig. 9A). The observation of the tail slap behavior only in *Chaeotodon* showed similar ancestral state patterns for both the likelihood and parsimony methods with high probability at all clade nodes (Fig. 9B). The lack of the observed tail slap behavior in *C. kleinii* indicates the potential loss of this character. In addition, it is equivocal as to whether the common ancestor of the bannerfish and *Chaetodon* clades showed tail slap behavior.

Independent contrast analyses of the continuous variable sound characteristics (duration, peak frequency, median frequency, bandwidth and intensity) for the tail slap or most prominent sound type observed for each species show evidence for correlated changes in sound characteristics (Table 4). We found no significant relationship in the slope of the independent contrasts and corrected branch length for any character. Further analysis showed a correlated change between sound duration and sound pressure characteristics ($R=0.86$, $P=0.03$) and the expected strong correlation between peak and median frequency ($R=0.98$, $P<0.001$) characters.
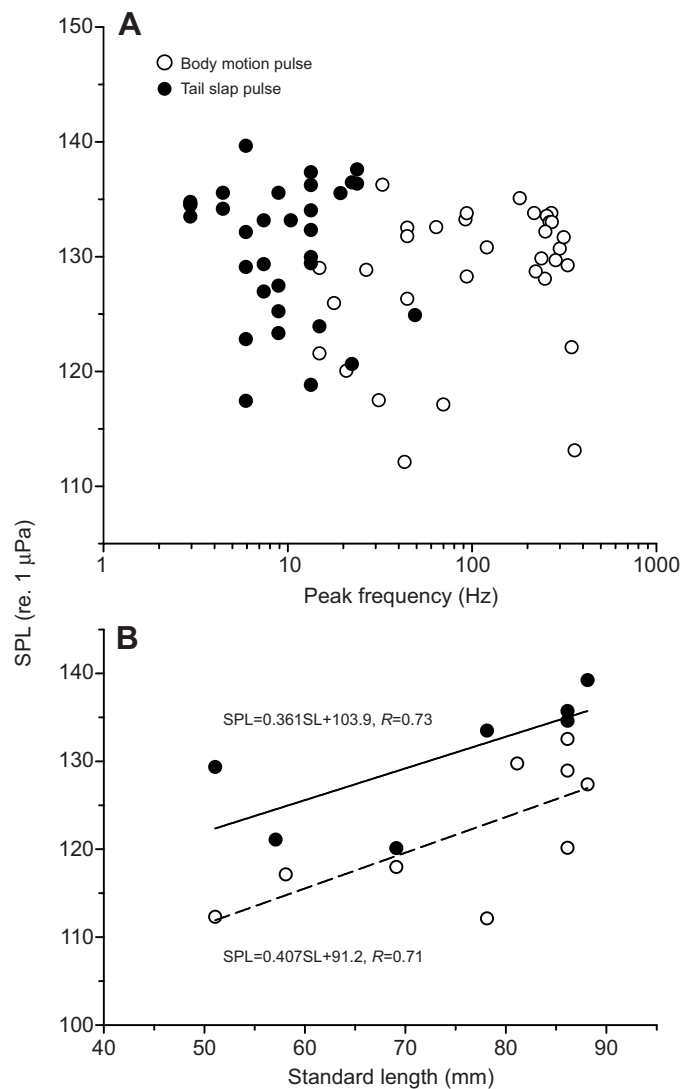

Fig. 7. Intensity of the two most common sounds produced by the multiband butterflyfish *Chaetodon multicinctus*. (A) The intensities for both sounds showed great overlap in sound pressure level (SPL) but are separated in peak frequency near 10–20 Hz. Data are shown for all sounds recorded in the study. (B) Intensity increases as a function of body size in both the tail slap (black circles) and the body motion sound types (open circles) when averaged for individual fish.

We did not include continuous sound characteristic data available for other butterflyfish species beyond this study because of differences in the quantitative measures that may be recorded in different test aquaria.

The influence of body size and sex on sound production

Analyses of the acoustic characteristics for male and female *C. multicinctus* of different sizes show several trends in their body motion and tail slap pulse sounds. The intensity of both sound pulses was correlated with body size ($R=0.73$, $P<0.003$ and $R=0.71$, $P<0.001$, respectively) (Fig. 7B). Both sexes produced these two sound types in very similar proportions during the test trial periods (body pulses: males, 68.4% vs females, 68.1% of sounds; tail slaps: males, 30.1% vs females, 26.8% of sounds) although there was considerable variation of total number of sounds produced among individual fish (males, 1–34 total sounds per trial; females, 9–20 total sounds per trial). For the body pulse sound, we found no
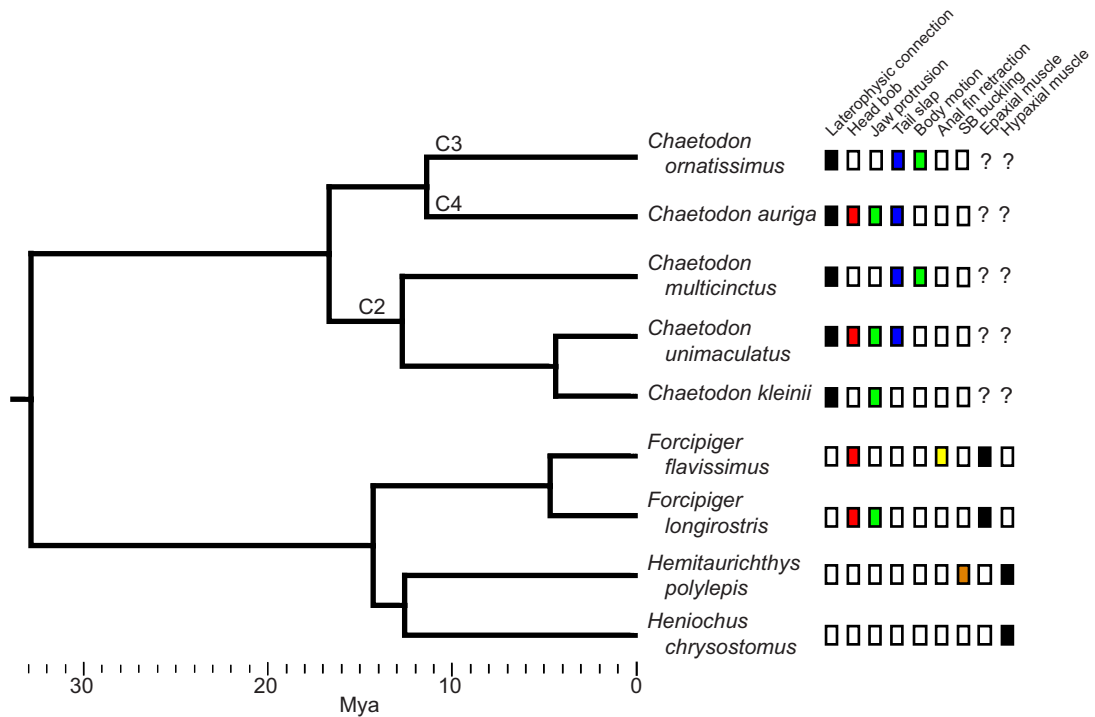
Fig. 8. Character states associated with sound production for nine species in the two major butterflyfish clades. In the bannerfish clade (bottom), the head bob sound is known so far only for *Forcipiger* species and involves epaxial muscle activity. In contrast, sound production by *Heniochus chrysostomus* and *Hemitaurichthys polylepis* does not include the apparent head bob motion and involves the hypaxial muscles. A pronounced head bob sound occurs in *Chaetodon* species (top), including clade 4, not clade 3, and was variable among species in clade 2. Note the frequent, but not consistent, linkage between the head bob and jaw protrusion movements. Of note, the tail slap behavior is found only in *Chaetodon* and is represented in clades 2, 3 and 4 (C2, C3, C4). The box matrix below the sound character traits indicate the presence (filled) or lack (open) documented so far for each species. ?, verification of muscle activity remains to be tested; Mya, million years ago. Supplemental data from other sources are included for the laterophysic connection (Smith et al., 2003), *Forcipiger* (Boyle and Tricas, 2011), *Hemitaurichthys* (Boyle and Tricas, 2010) and *Heniochus* (Parmentier et al., 2011). The phylogeny and clock estimates taken from Bellwood et al. (2010).

difference between sexes in average pulse duration (Mann–Whitney *U*-test, $U=2.0$, $n=3$ and 5, $P=0.14$), peak frequency (*t*-test, $t=0.67$, d. f.=6, $P=0.53$), median frequency (*t*-test, $t=1.02$, d.f.=6, $P=0.35$), or 6 dB bandwidth (*t*-test, $t=0.60$, d.f.=6, $P=0.57$), but sample size for tail slaps by females was too small for statistical comparison (Fig. 10).

DISCUSSION
Butterflyfish social behavior and acoustic communication
Butterflyfishes are well known for their diverse feeding ecology and social behaviors (Hourigan, 1989; Roberts and Ormond, 1992) and all species used in this study spend large amounts of time in close social affiliations in the wild. In Hawaii, the forcepsfish *F. flavissimus*
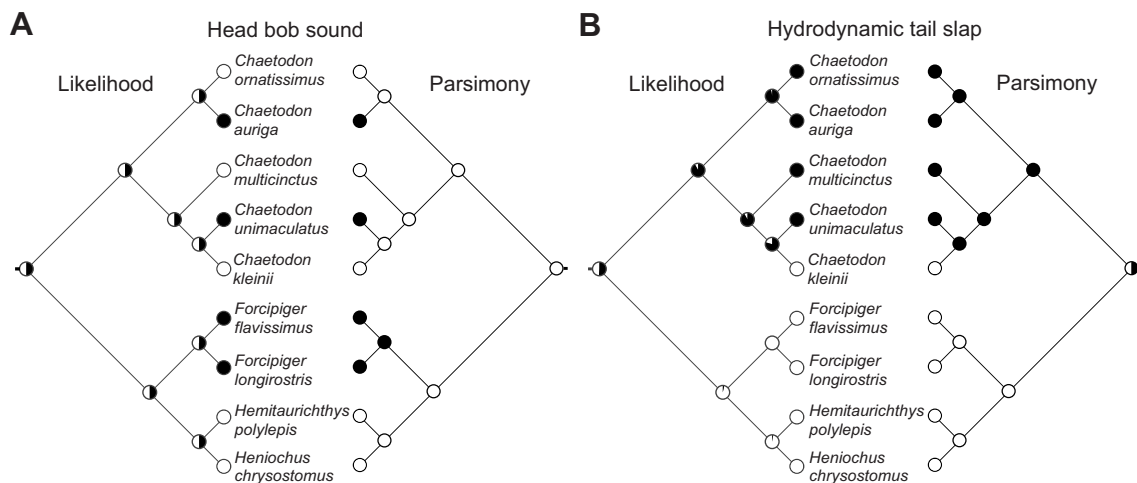
Fig. 9. Ancestral state reconstruction of two acoustic behaviors used during social interactions in butterflyfishes. (A) The head bob acoustic behavior occurs in both *Chaetodon* and *Forcipiger* with an equivocal likelihood for it as the ancestral state (left), and the possible independent evolution among clades in the parsimony model (right). (B) The tail slap behavior is so far observed during social interactions only in *Chaetodon* species and produces a very low frequency acoustic stimulus that is directed towards the receiver fish. It is not yet documented in the bannerfish clade and both the likelihood (left) and parsimony (right) models are equivocal on the presence of this trait in the common ancestor. The darkened portion of circles indicates the model probability that the trait is present at each node.

Table 4. Independent contrasts for continuous sound characteristics of the primary acoustic behaviors for six butterflyfish species in this study

	Character correlation (R, slope, P)			
	Peak frequency	Median frequency	Bandwidth	SPL
Duration	−0.44, −2.48 P=0.39	−0.54, −2.29 P=0.27	−0.74, −0.81 P=0.10	0.86, 0.02 **P=0.03**
Peak frequency	–	0.98, 0.92 **P<0.001**	0.33, 0.07 P=0.71	−0.20, 0.01 P=0.70
Median frequency	–	–	0.37, 0.36 P=0.47	−0.24, −0.01 P=0.64
Bandwidth	–	–	–	−0.37, −0.02 P=0.47

Slope statistics for character correlations are shown for the least squares regression (d.f.=4 for all character comparisons). P values in bold for character correlations are statistically significant. SPL, sound pressure level.

forms social harems (Boyle, 2011); the teardrop butterflyfish *C. unimaculatus* and threadfin butterflyfish *C. auriga* form social pairs and larger groups (Reese, 1975; Fricke, 1986; Hourigan, 1989); the multiband *C. multicinctus* and ornate *C. ornatissimus* butterflyfish are found in monogamous pairs (Reese, 1975; Tricas, 1989; Kosaki, 1999); and the blacklip butterflyfish *C. kleinii* forms plankton-feeding associations in the water column or social pairs on the reef (Hourigan, 1989). These close associations among individuals may facilitate the exchange of important acoustic information with mates, other conspecifics and heterospecifics in the noisy reef environment (Tricas and Boyle, 2015).

The perception of butterflyfish sounds

In this study, we consider sound to be the acoustic field produced by fish motor behaviors that forms both a local hydrodynamic flow field and a propagating sound pressure wave (Kalmijn, 1988). At
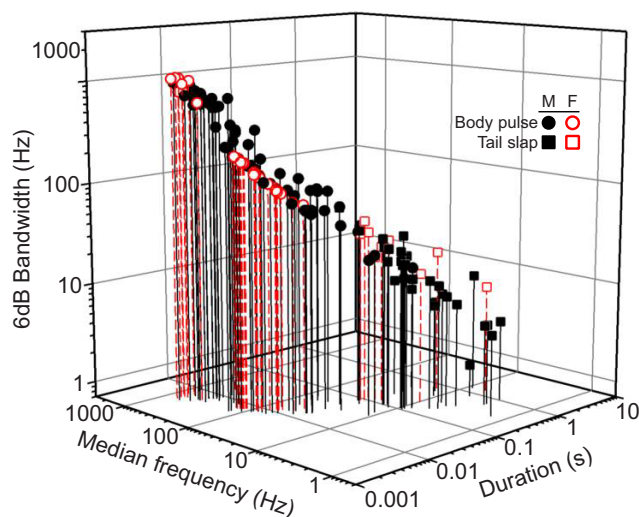
Fig. 10. Characteristics of pulsed sound production by male and female multiband butterflyfish *Chaetodon multicinctus* during social interactions with conspecifics. Both males (black symbols) and females (red symbols) produce body pulse (circles) and tail slap (squares) sounds that form a continuous gradient across median frequency, duration and bandwidth. Median frequency is the median frequency bin among all FFT power spectrum frequencies within 6 dB from peak. Data are for all sounds recorded in the study.

distances close to the source, the hydrodynamic flow field can stimulate the inner ear of a receiver by whole body accelerations and at very close distances also the mechanosensory lateral line by the pressure gradient across the surface of the skin. In contrast, the sound pressure wave passes through the body but can cause compression of a gas-filled swim bladder (or other structure), which can impart local particle motions upon an adjacent or coupled inner ear. Thus features of the acoustic field can stimulate both the inner ear via hearing and the lateral line via mechanoreception by a variety of mechanisms (Coombs and Montgomery, 1999; Braun and Grande, 2008).

The genus *Chaetodon* is distinguished by the presence of paired anterior swim bladder horns that form the LC and project towards the inner ear (Webb and Smith, 2000; Webb et al., 2006). The LC is proposed to translate sound pressure fluctuations from the swim bladder horns to fluid motion in the lateral line canal and also secondary particle motions to the ear (Webb, 1998) that potentially enhance the perception of the acoustic field during social interactions (Tricas et al., 2006). The gas-filled swim bladder and swim bladder horns are thought to be relatively insensitive to infrasonic sound pressure and have complex resonant properties at higher frequencies between 100–2000 Hz that can vary considerably among species and with depth (Fine et al., 2009; Lewis and Rogers, 1996; Sand and Hawkins, 1973). Below, we discuss the detection of acoustic fields by the ear and lateral line in the context of butterflyfish social interactions, and the potential contributions to each by the LC.

The inner ear

Butterflyfish sound pulses cover a very broad frequency spectrum from <1 Hz to >1000 Hz that can directly stimulate the inner ear. With the exception of *C. kleinii*, each species produced at least one sound type that showed a very low peak frequency and bandwidth within the infrasound frequency range of <20 Hz (*sensu* Sand and Karlsen, 1986). The tail slap behaviors of *C. unimaculatus*, *C. multicinctus* and *C. ornatissimus* showed peak frequencies <1–9 Hz and were commonly produced as part of a visual lateral display behavior by resident fish. The visual features of their bright color patterns enhance the recognition of conspecifics (Boyle and Tricas, 2014). The lateral display is an agonistic visual stimulus commonly used during disputes by territorial *Chaetodon* species (Sutton, 1985; Yabuta, 2002; Zumpe, 1965) that precedes the tail slap behavior and is delivered at a distance of about 1–2 body lengths in the wild (Tricas et al., 2006). A tail slap at low intensity by *C. multicinctus* can impose weak hydrodynamic vortex rings to the side of the receiver's body (Hanke et al., 2008), whereas a high-intensity tail slap can displace the body of the receiver fish (Tricas et al., 2006). Low-frequency hydrodynamic motions can stimulate the otolith organs of the inner ear, which are highly sensitive to low-frequency linear accelerations of the body (Karlsen, 1992; Sand and Karlsen, 2000). The relative action of sound pressure on the swim bladder is thought to be negligible at these low frequencies (Sand and Hawkins, 1973). The potential importance of low-frequency sound during social interactions is further indicated by the body shake (*C. multicinctus*), body (*C. ornatissimus*), head-jaw (*C. auriga*) and anal fin (*F. flavissimus*) motions that are also associated with low-frequency stimuli. The detection of low-frequency hydrodynamic stimuli by the butterflyfish ear needs to be determined, but the sensitivity of the fish ear to acceleration at frequencies <100 Hz is probably equivalent to particle motion sensitivity at higher frequencies (Sand and Karlsen, 1986, 2000). In addition, the biological relevance of these low-frequency stimuli needs to be tested by manipulation and playback experiments on behaving fish.

Several pulsed sounds in the 137–1031 Hz band were produced by *F. flavissimus*, *C. kleinii*, *C. unimaculatus* and *C. multicinctus* and add to a growing body of work on their acoustic repertoires. Sound with a peak frequency >100 Hz was not recorded from *C. ornatissimus* in this study, but does occur during social interaction (346 Hz) in the field (Tricas and Boyle, 2014). Thus, with the exception of *C. auriga*, all species in this study are now known to produce pulsed sounds within the 100–1000 Hz band. Many sounds share temporal and spectral characteristics with single 'pop' and pop train sounds produced by damselfishes (Amorim, 2006; Lobel et al., 2010; Maruska et al., 2007) and other reef species (Lobel et al., 2010), and are all within the hearing range of the inner ear (Ladich and Fay, 2013). Recent manipulation experiments using the auditory evoked potential technique show that *Chaetodon* has enhanced sound pressure sensitivity in the 100–700 Hz frequency band that is mediated by the swim bladder horns associated with the LC (Tricas and Boyle, 2015). Thus, the particle acceleration component of the acoustic field may directly stimulate the ear, whereas the swim bladder horns add additional sensitivity, likely by the transduction of sound pressure that secondarily stimulates the inner ear as in cichlids (Schulz-Mirbach et al., 2012) and other species that lack an otophysic connection (Ladich and Fay, 2013).

The lateral line

Rapid lateral motions of a strong tail slap presumably creates a dipole or higher order flow field in the horizontal plane. The steep pressure gradient across the skin of the receiver will quickly dissipate beyond distances of a few body lengths (Coombs and Montgomery, 1999). Weaker *Chaetodon* tail slaps can create a fluid vortex directed towards the skin of the receiver (Hanke et al., 2008). The motion of these flow fields across the skin can provide somatotopic information about the intensity and frequency components of water velocity by stimulation of superficial neuromasts (Weeg and Bass, 2002) and water acceleration by stimulation of canal neuromasts (Kroese and Schellart, 1992). Furthermore, the acceleration detected by canal neuromasts can provide information about the direction and distance from the source as described for the localization of prey (Coombs et al., 1996). During close social interactions between only two butterflyfish, their locations are evident by visually conspicuous markings in clear coral reef waters. However, in territory border conflicts among pairs (Tricas, 1989; Tricas et al., 2006) discrimination of a sexually monomorphic rival may be facilitated by hydrodynamic information to the lateral line that is integrated with auditory (and visual) input to provide directional information (Braun et al., 2002; Coffin et al., 2014) that can be potentially used to discriminate friend from foe. In addition, the lateral line may be activated by sound pressure stimuli that are transduced by the LC. Thus both a segment of the lateral line and the inner ear may receive coincident sound pressure information for processing, but this hypothesis requires testing. Future experiments such as the pharmacological or physical ablation of neuromasts are needed to demonstrate the relative contribution of the lateral line and LC system to perception of the acoustic field.

The evolution of sound production in *Chaetodon*

Motion analyses in this study show that several motor patterns are shared among species with divergent phylogenetic histories (Fig. 8). Most notable is the head bob motion in *F. flavissimus* and species in two *Chaetodon* clades. The head bob motion in *F. flavissimus* is driven by the action of the epaxial muscles on a ventral linkage between the head and pectoral girdle, which is maintained by simultaneous activity of the adductor mandibulae and

sternohyoideus muscles (Boyle and Tricas, 2011). This results in the anterior motion of the pectoral girdle, ribs and rostral swim bladder before the head is released and rotated dorsally. Our study shows that the head bob motion for *C. unimaculatus* (clade 2) is also rapid and includes a prominent and active protrusion of the jaws that appears independent from the upward motion of the head. In contrast, only the prominent jaw protrusion and not the head bob is seen in *C. kleinii* (clade 2) and may be an autapomorphy (further studies can be done to test this), whereas jaw protrusion in the other fish appears to be associated with, or a by-product of, the head bob. The apparent diversity in head bob actions among *Chaetodon* species leaves the question of the ancestral character state across the phylogeny undetermined (Fig. 9A). The head bob behavior is apparently lacking in *C. multicinctus* (also clade 2) and *C. ornatissimus* (clade 3). Preliminary EMG experiments on *C. multicinctus* demonstrate activity of muscles in the ventral region of anterior epaxial muscles lateral to the anterior swim bladder horns during the production of the body motion sound (our unpublished data), which tentatively appears most similar to that observed for the nearby region of the hypaxial muscles in both *Hemitaurichthys* (Boyle and Tricas, 2010) and *Heniochus* species (Parmentier et al., 2011). Further kinematic, anatomical and electromyography analyses are needed to test the hypotheses that the head bob sound is a homologous trait nested deep within the butterflyfish phylogeny, was lost at least once in the bannerfishes and twice in *Chaetodon*, or has evolved in parallel. Furthermore, it remains to be determined whether these acoustic behavior characters exist for *Prognathodes*, the sister genus to *Chaetodon*, the remaining bannerfish genera and their putative outgroups such as the pomacanthid angelfishes and ephippid spadefishes (Fig. 1).

The most distinctive acoustic behavior in *Chaetodon* was the tail slap, which is not yet reported in the bannerfishes. Reconstruction of ancestral states show this character as prominent at all *Chaetodon* nodes (Fig. 9B). However, we emphasize that laboratory tests are of limited utility because the tail slap behavior was not observed for *C. auriga*, but is seen in the field (Tricas and Boyle, personal observations). Similarly, the tail slap behavior was not observed for *C. kleinii* in the lab but may occur in the wild. Thus, both field and lab studies are needed to better define the evolution of sound production.

Patterns are emerging to indicate that acoustic signals used during social interactions are adaptive in butterflyfish communication. Our preliminary independent contrast analyses show evidence for correlated changes in sound duration and sound pressure characteristics for social signals. Honest signals provide accurate information about the condition of the signaler (Fitch and Hauser, 2002) and are often linked to physical attributes, such as body size or mass. In this study, we have shown that the intensity of both the tail slap and body motion sounds produced by *C. multicinctus* increases with body size and that both sound types are shared among sexes. Feeding territory size in wild *C. multicinctus* pairs is highly correlated with body size (Tricas, 1989). Defense of feeding territories towards unfamiliar non-mate adults or juveniles is known for many *Chaetodon* species (Hourigan, 1989; Roberts and Ormond, 1992; Tricas, 1989; Tricas et al., 2006) and we expect that sound production may be used as an indicator of condition or resource holding potential by many *Chaetodon* species as shown for *F. flavissimus* (Boyle and Tricas, 2011) and other fishes (Amorim et al., 2010; Ladich, 1998). The strong visual and acoustic displays during territorial contests may provide useful information about the relative size of an opponent, reduce the likelihood of escalated combat and lower the risk of injury. The acoustic stimuli provided during close social interactions in *Chaetodon* may provide valuable

information that reinforces other visual and chemical signals or cues related to mate or non-mate recognition (Reese, 1975; Boyle and Tricas, 2014; Hanke et al., 2008) in their noisy coral reef environment (Tricas and Boyle, 2015).

MATERIALS AND METHODS

Study species

Lab experiments were performed to characterize sound production in five Hawaii butterflyfish species that are members of three of the four *Chaetodon* clades based on their molecular phylogeny (Fessler and Westneat, 2007; Bellwood et al., 2010) and previously placed in four subgenera each of which has different LC variants (Smith et al., 2003; Webb et al., 2006). Species in *Chaetodon* clade 1 (*Chaetodon hoeferi*, *Chaetodon robustus* and *Chaetodon marleyi*) were not tested. Three test species from clade 2 are the blacklip butterflyfish *C. kleinii* and teardrop butterflyfish *C. unimaculatus* [subgenus *Lepidochaetodon*; indirect LC with mucoid connective tissue, long horns (LC variant Ind1)] and the multiband butterflyfish, *C. multicinctus* [subgenus *Exornator*; indirect LC without mucoid connective tissue, long horns (LC variant Ind2)]. The ornate butterflyfish *C. ornatissimus* is a member of clade 3 [subgenus *Citharoedus*; indirect LC without mucoid connective tissue, short stubby horns (LC variant Ind3)]. The threadfin butterflyfish *C. auriga* is a member of *Chaetodon* clade 4 [subgenus *Rabdophorous*; direct LC with mucoid connective tissue, long horns (LC variant Dir1)]. We also tested for the acoustic behavior in the forcepsfish *F. flavissimus,* which is a member of the bannerfish group that possesses no swim bladder horns or LC. Details of sound production by *F. flavissimus* and *Forcipiger longirostris* were reported in a previous study (Boyle and Tricas, 2011).

Experimental fish were obtained from commercial collectors, transported to the lab and held for 1–3 days in holding tanks or aquaria (80–125 liter) equipped with fresh flow-through sea water from Kaneohe Bay (annual range, 25–28°C). Fish were supplied with colonies of live corals or sessile invertebrates for food. Adult fish were used in most trials (see Results, Table 4). Most subjects were not killed to determine sex, with the exception of *C. multicinctus,* which was tested for the effects of body size and sex on the acoustic features of different sounds. Following these experiments, individuals were measured for standard length and sexed by either gonad catheterization or after euthanization. All experimental procedures were approved by the University of Hawaii Institutional Animal Care and Use Committee.

Sound and kinematic recordings

Acoustic behavior experiments were conducted in a flow-through sea water 125 liter aquarium with interior dimensions of 77.5×44.5×35.5 cm, L×W×H (water depth). Water temperature across the duration of the study ranged from 25 to 28°C. A resident test fish was acclimated for 3–24 h in the aquarium that contained at least one live coral head. Sound production was evoked by the introduction of a second conspecific stimulus fish enclosed in a 20×20×20 cm wire frame covered with fine 2.5 cm mesh monofilament netting that allowed visual contact between fish and sound transparency. Video of body movements during sound production events was recorded on a Sony video camera (TRV-950) at 30 f s^{-1} through a glass window on the tank side. Sounds were recorded with Brüel and Kjaer 8103 (−211.8 dB re. 1 V µPa^{-1}; Naerum, Denmark) and 8104 (−205.4 dB re. 1 V µPa^{-1}) hydrophones connected to a Nexus 2692 conditioning amplifier at 60 dB gain (Naerum, Denmark) and positioned approximately 3 cm from the face of the cage with the stimulus fish. The 0.1–20 kHz frequency response of the hydrophones includes part of the infrasound spectrum (<1–20 Hz). Hydrophone signals were digitized at 48,000 samples s^{-1} on one audio track of the camera for reference on the video (16 bit) and on one channel of a Sony DAT recorder (PCMM-1, 16 bit, 20 Hz–22 kHz playback response) for waveform analysis. Strong frequency components <20 Hz occurred in several sound waveforms, thus are likely underrepresented in our subsequent analyses because of the low-frequency filter of the DAT recorder. Resident fish would most commonly approach the exposed side of the cage to produce sound, thus intensities for resident and stimulus fish were recorded at approximately 1–10 cm of the source. Water flow was

stopped during acoustic recordings to minimize background noise. Trials were typically of 15 min duration but sometimes were extended up to 30 min to obtain representative sounds. Two trials were run for each resident fish although up to eight trials were required to evoke sound production from some individuals.

Digital video and sound recordings were transferred to a PC and stored as uncompressed AVI and WAV files, respectively. Sound files were filtered (60 Hz notch) in Cool Edit Pro 2.1 software with a 100 dB attenuation and 'super narrow' setting. Most sound files were low-pass filtered at 2 kHz and downsampled at 4 kHz with Cool Edit Pro 2.1 software set at the 'high-quality' setting. The resulting bandwidth (0–2 kHz) was below the minimum resonance frequency of 2871 Hz estimated for the 35.5 cm water depth in the aquarium (Akamatsu et al., 2002), thus sounds recordings of most sound types were not significantly biased in this experimental setup. However, we did record high-frequency clicks from several species as reported by Tricas et al. (2006), which had peak frequencies from 2 to 3 kHz. Thus, we analyzed these sounds both at full sampling frequency and after low-pass filtering at 3 kHz.

Sounds were produced as single pulse events or in trains. We measured the duration (ms) of all pulsed sounds. Sequential sound events separated by pulse intervals <1 s were considered to be part of a pulse train. For pulse trains, we measured the total time of the train, pulse period (time between successive pulse onsets) and interpulse interval (time between pulse events). We counted the number of pulses in the train to calculate the average pulse rate of each sound train (pulses s^{-1} or Hz). Spectral features of individual pulse events were analyzed using custom MatLab (v7.0) scripts. Power spectra of 3 kHz sampled files were generated from 4096 point fast-Fourier transforms of Hanning windowed data. From these power spectra, we determined the peak frequency, median 6 dB frequency (the median frequency bin of all frequencies ≤6 dB from peak), the minimum 6 dB frequency (the lowest frequency bin ≤6 dB from peak), the maximum 6 dB frequency (the highest frequency bin ≤6 dB from peak) and the 6 dB bandwidth proportion (the percent of frequency bins ≤6 dB from the frequency peak bin).

Motor patterns during sound production

Body movements associated with sound production were qualitatively described and categorized from frame-by-frame analysis of the video recordings with QuickTime 7. The body part (e.g. jaw, head, body, caudal region; dorsal, anal, pelvic or caudal fin), direction (dorsal, ventral, lateral) and form (e.g. extension, retraction, protraction, shake, slap) of motion by the body part(s) were determined. Subsequent review of the video data sometimes resulted in assignment of more descriptive action patterns such as the 'head bob' to indicate motion in the dorsal–ventral plane and 'head shake' as oscillation of the head in the horizontal plane. In this analysis, each movement pattern we report to be associated with the acoustic behavior always overlapped movements associated with production of a sound, but was not necessarily coincident with the sound event. Our normal speed video recordings were of relatively low temporal resolution (30 frames s^{-1}=33 ms) and we may not have reliably captured more rapid motions. In addition, we recognize that many of these movements do not define causal mechanisms, rather we assume they are associated with the internal sound production mechanism.

Data analyses

Sound characteristics

Measures of the acoustic characteristics of individual sounds were averaged for each individual and then averaged among individuals to estimate the population statistic for each species. For comparisons among species, data were tested for the assumptions of normality and homogeneity of variance and, when violated and could not be transformed, non-parametric tests were used. Sound characteristics of low-frequency sound types were compared with a series of one-way ANOVA tests and Student–Newman–Keuls *post hoc* tests or Kruskal–Wallis tests with Dunn's *post hoc* tests. *t*-tests or non-parametric Mann–Whitney *U*-tests were used to compare characteristics of sounds shared between two species. Characteristics of two sound types produced by individuals of a species were tested for differences with the paired *t*-test.

Phylogenetic analyses

We used Mesquite software (v3.02; Maddison and Maddison, 2015) to explore the diversity and evolution of the acoustic behavior characters that are associated with sound types and characteristics across the recent molecular phylogeny of Bellwood et al. (2010). We reconstructed the ancestral character states of the head bob and tail slap behaviors using the Mk1 model that reports the proportional likelihood of the ancestral characters across the phylogeny, and also a parsimony reconstruction (unordered, one step). We used the PDAP:PDTREE module (v1.16; Midford et al., 2005) to conduct a preliminary analysis on the continuous characters of sounds for our six species (e.g. duration, mean frequency, bandwidth, intensity) to get insight on possible correlated sound characteristics across our test phylogeny. We recognize that this is a very small sample size of species (∼5%) within the family and that these analyses will undoubtedly change as more taxa are added. Nonetheless, we view these analysis as providing insight for the development of testable hypotheses in future research on the evolution of sound production.

References

Akamatsu, T., Okumura, T., Novarini, N. and Yan, H. Y. (2002). Empirical refinements applicable to the recording of fish sounds in small tanks. *J. Acoust. Soc. Am.* **112**, 3073-3082.

Amorim, M. C. P. (2006). Diversity of sound production in fish. In *Communication in Fishes*, Vol. I (ed. F. Ladich, S. P. Collin, P. Moller and B. G. Kapoor), pp. 71-104. Enfield, NH: Science Publishers.

Amorim, M. C. P., Knight, M. E., Stratoudakis, Y. and Turner, G. F. (2004). Differences in sounds made by courting males of three closely related Lake Malawi cichlid species. *J. Fish Biol.* **65**, 1358-1371.

Amorim, M. C. P., Simões, J. M., Fonseca, P. J. and Turner, G. F. (2008). Species differences in courtship acoustic signals among five Lake Malawi cichlid species (*Pseudotropheus* spp.). *J. Fish Biol.* **72**, 1355-1368.

Amorim, M. C. P., Simões, J. M., Mendonca, N., Bandarra, N. M., Almada, V. C. and Fonseca, P. J. (2010). Lusitanian toadfish song reflects male quality. *J. Exp. Biol.* **213**, 2997-3004.

Bellwood, D. R., Klanten, S., Cowman, P. F., Pratchett, M. S., Konow, N. and van Herwerden, L. (2010). Evolutionary history of the butterflyfishes (f: Chaetodontidae) and the rise of coral feeding fishes. *J. Evol. Biol.* **23**, 335-349.

Blum, S. D. (1988). Osteology and phylogeny of the Chaetodontidae (Pisces: Perciformes). PhD thesis, Zoology, University of Hawaii, Honolulu, HI, USA.

Boyle, K. S. (2011). Proximate and ultimate aspects of acoustic and multimodal communication in butterflyfishes. PhD thesis, Zoology, University of Hawaii, Honolulu, HI, USA.

Boyle, K. S. and Tricas, T. C. (2010). Pulse sound generation, anterior swim bladder buckling and associated muscle activity in the pyramid butterflyfish, *Hemitaurichthys polylepis*. *J. Exp. Biol.* **213**, 3881-3893.

Boyle, K. S. and Tricas, T. C. (2011). Sound production in the longnose butterflyfishes (genus *Forcipiger*): cranial kinematics, muscle activity and honest signals. *J. Exp. Biol.* **214**, 3829-3842.

Boyle, K. S. and Tricas, T. C. (2014). Discrimination of mates and intruders: visual and olfactory cues for a monogamous territorial coral reef butterflyfish. *Anim. Behav.* **92**, 33-43.

Boyle, K. S., Dewan, A. D. and Tricas, T. C. (2013). Fast drum strokes: novel and convergent features of sonic muscle ultrastructure, innervation, and motor neuron

organization in the pyramid butterflyfish (*Hemitaurichthys polylepis*). *J. Exp. Biol.* **274**, 377-394.

Braun, C. B. and Grande, T. (2008). Evolution of peripheral mechanisms for the enhancement of sound reception. In *Fish Bioacoustics* (ed. J. F. Webb, R. R. Fay and A. N. Popper), pp. 99-144. New York: Springer.

Braun, C. B., Coombs, S. and Fay, R. R. (2002). What is the nature of multisensory interaction between octavolateralis sub-systems? *Brain Behav. Evol.* **59**, 162-176.

Coffin, A. B., Zeddies, D. G., Fay, R. R., Brown, A. D., Alderks, P. W., Bhandiwad, A. A., Mohr, R. A., Gray, M. D., Rogers, P. H. and Sisneros, J. A. (2014). Use of the swim bladder and lateral line in near-field sound source localization by fish. *J. Exp. Biol.* **217**, 2078-2088.

Coombs, S. and Montgomery, J. C. (1999). The enigmatic lateral line system. In *Comparative Hearing: Fish and Amphibians* (ed. R. R. Fay and A. N. Popper), pp. 319-362. New York: Springer.

Coombs, S., Hasting, M. and Finneran, J. (1996). Modeling and measuring lateral line excitation patterns to changing dipole source locations. *J. Comp. Physiol. A* **178**, 359-371.

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.

Fessler, J. L. and Westneat, M. W. (2007). Molecular phylogenetics of the butterflyfishes (Chaetodontidae): taxonomy and biogeography of a global coral reef fish family. *Mol. Phylogenet. Evol.* **45**, 50-68.

Fine, M. L., King, C. B. and Cameron, T. M. (2009). Acoustical properties of the swimbladder in the oyster toadfish *Opsanus tau*. *J. Exp. Biol.* **212**, 3542-3552.

Fitch, W. T. and Hauser, M. D. (2002). Unpacking 'honesty': vertebrate vocal production and the evolution of acoustic signals. In *Acoustic Communication* (ed. A. M. Simmons, R. R. Fay and A. N. Popper), pp. 65-137. New York: Springer.

Fricke, H. W. (1986). Pair swimming and mutual partner guarding in monogamous butterflyfish (Pisces, Chaetodontidae): a joint advertisement for territory. *Ethology* **73**, 307-333.

Gerald, J. W. (1971). Sound production during courtship in six species of sunfish (Centrarchidae). *Evolution* **25**, 75-87.

Hanke, W., Boyle, K. and Tricas, T. C. (2008). Flow measurements during the multimodal communication in Hawaiian butterflyfish. In *Lasermethoden in der Strömungsmesstechnik* (ed. B. Ruck, A. Leder and D. Dophedide), pp. 53.1-53.6. Karlsruhe: German Association for Laser Anemometry.

Hourigan, T. F. (1989). Environmental determinants of butterflyfish social systems. *Environ. Biol. Fish.* **25**, 61-78.

Kalmijn, A. J. (1988). Hydrodynamic and acoustic field detection. In *Sensory Biology of Aquatic Animals* (ed. J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga), pp. 83-130. New York: Springer.

Karlsen, H. E. (1992). The inner ear is responsible for detection of infrasound in the perch (*Perca fluviatilis*). *J. Exp. Biol.* **171**, 163-172.

Kenyon, T. N. (1994). The significance of sound interception to males of the bicolor damselfish, *Pomacentrus partitus*, during courtship. *Environ. Biol. Fish.* **40**, 391-405.

Kosaki, R. K. (1999). Behavioral mechanisms of coexistence among coral-feeding butterflyfishes. PhD thesis, Zoology, University of Hawaii, Honolulu, HI, USA.

Kroese, A. B. A. and Schellart, N. A. M. (1992). Velocity- and acceleration-sensitive units in the trunk lateral line of the trout. *J. Neurophysiol.* **68**, 2212-2221.

Ladich, F. (1998). Sound characteristics and outcome of contests in male croaking gouramis (Teleostei). *Ethology* **104**, 517-529.

Ladich, F. and Bass, A. H. (2003). Underwater sound generation and acoustic reception in fishes with some notes on frogs. In *Sensory Processing in Aquatic Environments* (ed. S. P. Collin and N. J. Marshall), pp. 173-193. New York: Springer.

Ladich, F. and Fay, R. R. (2013). Auditory evoked potential audiometry in fish. *Rev. Fish. Biol. Fisheries* **23**, 317-364.

Ladich, F. and Fine, M. L. (2006). Sound-generating mechanisms in fishes: a unique diversity in vertebrates. In *Communication in Fishes*, Vol. I (ed. F. Ladich, S. P. Colin, P. Moller and B. G. Kapoor), pp. 3-43. Enfield: Science Publishers.

Ladich, F. and Myrberg, A. A.Jr. (2006). Agonistic behavior and acoustic communication. In *Communication in Fishes*, Vol. I (ed. F. Ladich, S. P. Colin, P. Moller and B. G. Kapoor), pp. 121-148. Enfield: Science Publishers.

Lewis, T. N. and Rogers, P. H. (1996). The vibrational response of single-chambered fish swimbladders to low-frequency sound. *J. Mar. Sci.* **53**, 285-287.

Lobel, P. S. (1998). Possible species specific courtship sounds by two sympatric cichlid fishes in Lake Malawi, Africa. *Environ. Biol. Fish.* **52**, 443-452.

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.

Lobel, P. S., Kaatz, I. M. and Rice, A. N. (2010). Acoustical behavior of coral reef fishes. In *Reproduction and Sexuality in Marine Fishes* (ed. K. S. Cole), pp. 307-348. Berkeley: University of California Press.

Lugli, M., Torricelli, P., Pavan, G. and Mainardi, D. (1997). Sound production during courtship and spawning among freshwater gobiids (Pisces, Gobiidae). *Mar. Fresh. Behav. Physiol.* **29**, 1-4.

Maddison, W. P. and Maddison, D. R. (2015). Mesquite: a modular system for evolutionary analysis. Version 3.02 http://mesquiteproject.org.

Maruska, K. P., Boyle, K. S., Dewan, L. R. and Tricas, T. C. (2007). Sound production and spectral hearing sensitivity in the Hawaiian sergeant damselfish, *Abudefduf abdominalis*. *J. Exp. Biol.* **210**, 3990-4004.

Midford, P. E., Garland, T.Jr and Maddison, W. P. (2005). PDAP Package of Mesquite. Version 1.07.

Myrberg, A. A.Jr and Lugli, M. (2006). Reproductive behavior and acoustical interactions. In *Communication in Fishes*, Vol. I (ed. F. Ladich, S. P. Colin, P. Moller and B. G. Kapoor), pp. 149-1176. Enfield: Science Publishers.

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., 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.

Parmentier, E. and Diogo, R. (2006). Evolutionary trends of swimbladder sound mechanisms in some teleost fishes. In *Communication in Fishes*, Vol. I (ed. F. Ladich, S. P. Colin, P. Moller and B. G. Kapoor), pp. 45-70. Enfield: Science Publishers.

Parmentier, E., Boyle, K. S., Berten, L., Brié, C. and Lecchini, D. (2011). Sound production and mechanism in *Heniochus chrysostomus* (Chaetodontidae). *J. Exp. Biol.* **214**, 2702-2708.

Reese, E. S. (1975). A comparative field study of the social behavior and related ecology of reef fishes of the family Chaetodontidae. *Z. Tierpsychol.* **37**, 37-61.

Roberts, C. M. and Ormond, R. F. G. (1992). Butterflyfish social behaviour, with special reference to the incidence of territoriality: a review. *Environ. Biol. Fish.* **34**, 79-93.

Sand, O. and Hawkins, A. D. (1973). Acoustic properties of the cod swim bladder. *J. Exp. Biol.* **58**, 797-820.

Sand, O. and Karlsen, H. E. (1986). Detection of infrasound by the Atlantic cod. *J. Exp. Biol.* **125**, 197-204.

Sand, O. and Karlsen, H. E. (2000). Detection of infrasound and linear acceleration in fishes. *Philos. Trans. R. Soc. B Biol. Sci.* **355**, 1295-1298.

Schulz-Mirbach, T., Metscher, B. and Ladich, F. (2012). Relationship between swim bladder morphology and hearing abilities – a case study on Asian and African cichlids. *PLoS ONE* **7**, e42292.

Smith, W. L., Webb, J. F. and Blum, S. D. (2003). The evolution of the laterophysic connection with a revised phylogeny and taxonomy of butterflyfishes (Teleostei: Chaetodontidae). *Cladistics* **19**, 287-306.

Spanier, E. (1979). Aspects of species recognition by sound in four species of damselfishes, genus *Eupomacentrus* (Pisces: Pomacentridae). *Z. Tierpsychol.* **51**, 301-316.

Sutton, M. (1985). Patterns of spacing in a coral reef fish in two habitats on the Great Barrier Reef. *Anim. Behav.* **33**, 1332-1337.

Tricas, T. C. (1989). Determinants of feeding territory size in the corallivorous butterflyfish, *Chaetodon multicinctus*. *Anim. Behav.* **37**, 830-841.

Tricas, T. C. and Boyle, K. S. (2014). Acoustic behaviors in Hawaiian coral reef fish communities. *Mar. Ecol. Prog. Ser.* **511**, 1-16.

Tricas, T. C. and Boyle, K. S. (2015). Sound pressure enhances the hearing sensitivity of *Chaetodon* butterflyfishes on noisy coral reefs. *J. Exp. Biol.* **218**, 1585-1595.

Tricas, T. C., Kajiura, S. M. and Kosaki, R. K. (2006). Acoustic communication in territorial butterflyfish: test of the sound production hypothesis. *J. Exp. Biol.* **209**, 4994-5004.

Webb, J. F. (1998). Laterophysic connection: a unique link between the swimbladder and the lateral line system in *Chaetodon* (Perciformes: Chaetodontidae). *Copeia* **1998**, 1032-1036.

Webb, J. F. and Smith, W. L. (2000). The laterophysic connection in chaetodontid butterflyfish: morphological variation and speculations on sensory function. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**, 1125-1129.

Webb, J. F., Smith, W. L. and Ketten, D. R. (2006). The laterophysic connection and swim bladder of butterflyfishes in the genus *Chaetodon* (Perciformes: Chaetodontidae). *J. Morphol.* **267**, 1338-1355.

Webb, J. F., Herman, J. L., Woods, C. F. and Ketten, D. R. (2010). The ears of butterflyfishes (Chaetodontidae): 'hearing generalists' on noisy coral reefs? *J. Fish Biol.* **77**, 1406-1423.

Weeg, M. S. and Bass, A. H. (2002). Frequency response properties of lateral line superficial neuromasts in a vocal fish, with evidence for acoustic sensitivity. *J. Neurophysiol.* **88**, 1252-1262.

Yabuta, S. (2002). Uncertainty in partner recognition and the tail-up display in a monogamous butterflyfish. *Anim. Behav.* **63**, 165-173.

Zumpe, D. (1965). Laboratory observations on the aggressive behaviour of some butterfly fishes (Chaetodontidae). *Z. Tierpsychol.* **22**, 226-236.