

RESEARCH ARTICLE

Sound pressure enhances the hearing sensitivity of *Chaetodon* butterflyfishes on noisy coral reefs

Timothy C. Tricas^{1,2,‡} and Kelly S. Boyle^{1,2,*}

ABSTRACT

Butterflyfishes are conspicuous members of coral reefs that communicate with acoustic signals during social interactions with mates and other conspecifics. Members of the genus *Chaetodon* have a laterophysic connection (LC) – a unique association of anterior swim bladder horns and the cranial lateral line – but the action of the LC system on auditory sensitivity is unexplored. Here, we show in baseline auditory evoked potential threshold experiments that *Forcipiger flavissimus* (which lacks swim bladder horns and LC) is sensitive to sound tones from 100 Hz up to 1000 Hz, and that thresholds for three species of *Chaetodon* are 10–15 dB lower, with extended hearing ranges up to 1700–2000 Hz. The relatively high thresholds to sound pressure and low pass response near 500 Hz for all four species are consistent with a primary sensitivity to hydrodynamic particle acceleration rather than sound pressure. Deflation of the swim bladder in *F. flavissimus* had no measurable effect on auditory sensitivity. In contrast, displacement of gas from the swim bladder horns in *Chaetodon multicinctus* and *Chaetodon auriga* increased thresholds (decreased sensitivity) by 5–20 dB, with the greatest effect at 600 Hz. The evolution of swim bladder horns associated with the LC system in *Chaetodon* species has increased hearing sensitivity through sound pressure transduction in the frequency bands used for social acoustic communication. The close affiliative behaviors that are common in *Chaetodon* species and other butterflyfish facilitate sound perception and acoustic communication at close distances relative to the high background noise levels found in their natural reef environment.

KEY WORDS: Behavior, Bioacoustic, Communication, Fish, Hydrodynamic, Infrasonic, Social behavior

INTRODUCTION

The perception of communication signals in fishes can involve transduction mechanisms in both the inner ear and lateral line systems. The acoustic field of underwater sound includes a hydrodynamic flow field in close proximity to the source that can accelerate the body of a nearby receiver and directly stimulate the inner ear (Kalmijn, 1988; Braun and Grande, 2008). Within distances of a few body lengths from the source, the local flow field gradient across the skin may also stimulate the mechanosensory lateral line system and provide additional information such as direction of the source (Braun and Coombs, 2010). In addition, sound pressure waves

propagate farther distances, penetrate the body of a receiving fish and can induce pulsations of a gas-filled swim bladder, anterior swim bladder horns or bullae, if present. These secondary particle motions may also stimulate the inner ear (Schellart and Popper, 1992; Braun and Grande, 2008). Thus, the perception of biologically relevant sounds by a receiver fish is complex and dependent upon many factors related to the distance and direction of the receiver from the sound producer, the frequency spectrum and intensity of the acoustic field, the configuration of the lateral line system and the presence of morphological associations between the inner ear and swim bladder.

Several fish groups have independently evolved adaptations that reduce the distance between the gas-filled swim bladder and the inner ear to enhance hearing capabilities. Bilaterally-paired swim bladder horns that approach or contact the otic capsule have evolved independently in several families of acanthopterygian fishes (reviewed by Schellart and Popper, 1992; Braun and Grande, 2008). Behavior and physiology experiments show a general trend for enhanced sensitivity or extended high frequency range of hearing that is associated with the presence of rostral extensions of the swim bladder among taxa that include holocentrid squirrelfishes (Tavolga and Wodinsky, 1963; Coombs and Popper, 1979), the Atlantic cod *Gadus morhua* (Chapman and Hawkins, 1973), several distant and sister cichlid genera (Schulz-Mirbach et al., 2012), mormyrids (Yan and Curtsinger, 2000; Fletcher and Crawford, 2001), sciaenid drums and croakers (Ramcharitar et al., 2006; Horodysky et al., 2008), the geriid mojarra (Parmentier et al., 2011b) and others (reviewed in Braun and Grande, 2008). Although this diversity of swim bladder and inner ear associations is widespread among teleost fishes, a detailed assessment of the relationship between swim bladder horn morphology among congener species is lacking, especially in relation to the frequency response of the ear and contexts of sounds used in social communication.

Butterflyfishes (family Chaetodontidae) include approximately 130 species in 10 genera that are common inhabitants of coral reefs. The genus *Chaetodon* (~90 species) is distinguished by a laterophysic connection (LC), which is a unique morphological association between rostral projections of the swim bladder and the lateral line at the posterior margin of the skull (Blum, 1988; Webb, 1998). *Chaetodon* subgenera differ with respect to the dimensions of the horns (length and width) and proximity to the lateral line in the supracleithrum and inner ear. These features are proposed to translate sound pressure stimuli into fluid motion in the lateral line canal and particle motion in the inner ear (Webb and Smith, 2000; Webb et al., 2006, 2010), and to favor the efficient perception of auditory information among fish pairs (Tricas et al., 2006). Recent work shows that *Chaetodon*, *Forcipiger*, *Hemitaenichthys* and *Heniochus* species produce several forms of pulsed sounds with peak frequencies and bandwidths from <1 Hz to >1000 Hz during social interactions in the field and lab (Tricas et al., 2006; Boyle and Tricas, 2011; Parmentier et al., 2011a; Tricas and Boyle, 2014, 2015). In *Chaetodon*, this includes very low frequency hydrodynamic stimuli (<1–30 Hz)

¹Department of Biology, University of Hawaii, 2538 The Mall, Honolulu, HI 96822, USA. ²Hawaii Institute of Marine Biology, 46-007 Liliupuna Road, Kaneohe, HI 96744, USA.

*Present address: Département d'Ecologie et de Gestion de la Biodiversité, Muséum National d'Histoire Naturelle, 57 rue Cuvier, Paris 75231, Cedex 5, France.

[‡]Author for correspondence (tricas@hawaii.edu)

Received 19 September 2014; Accepted 20 February 2015

List of symbols and abbreviations

a	estimated inverse polynomial regression parameter
AEP	auditory evoked potential
f_l	lower cutoff frequency of the octave band
f_u	upper cutoff frequency of the octave band
LC	laterophysic connection
PAL	particle acceleration level
SNR	signal to noise ratio
SPL	sound pressure level
SPL_{int}	intensity spectrum level
Y_0	estimated inverse polynomial regression parameter

(Tricas et al., 2006; Hanke et al., 2008; Tricas and Boyle, 2015) that can likely stimulate the inner ear by whole body accelerations and the mechanosensory lateral line of the receiver fish. Pulsed acoustic sounds from >100–1000 Hz are produced by all genera by a diversity of motor mechanisms (Boyle and Tricas, 2010, 2011; Parmentier et al., 2011a; Tricas and Boyle, 2015). Of relevance to perception of these acoustic stimuli by *Chaetodon* is the strong pairing of monogamous species that defend territories from conspecific competitors (Reese, 1975; Hourigan, 1989; Tricas, 1989a; Roberts and Ormond, 1992). Acoustic stimuli are produced during social interactions and are directed towards both mates and conspecific competitors within distances of a few body lengths. However, the effect of the swim bladder horns on perception of the sounds used in natural behaviors by *Chaetodon* and sister taxa is untested.

Butterflyfishes live in a noisy coral reef environment with high levels of background noise from abiotic, vertebrate and invertebrate sources (e.g. Wenz, 1962; Tricas and Boyle, 2014; Lammers et al., 2008) that overlap with the spectrum of their communication sounds (Tricas and Boyle, 2015). Ambient background noise can have several effects on sound detection and communication in fishes and it is necessary to understand the sound characteristics of their natural habitats to determine their auditory efficiency and behavior (Ladich, 2013, 2014).

In this study, we test the hypothesis that the LC system enhances the auditory sensitivity of *Chaetodon*. We compare butterflyfish hearing sensitivity to tone stimuli in three *Chaetodon* species with different LC morphologies with that of *Forcipiger flavissimus*, which lacks swim bladder horns and the LC. We use the auditory evoked potential (AEP) technique to test the effects of the rostral horns and swim bladder on hearing sensitivity in the 100–2000 Hz range. We interpret their audiograms with respect to the frequency spectrum of sounds produced during social communication, and the presence of high ambient noise levels recorded in butterflyfish territories on coral reefs. Our results support the hypothesis that the rostral extensions of the *Chaetodon* swim bladder associated with the LC has increased both auditory sensitivity and hearing range, which may promote perception of conspecific acoustic signals in their noisy coral reef environment. We also interpret these findings in relation to the likely co-stimulation of the lateral line.

RESULTS

Hearing sensitivity of *Forcipiger flavissimus* and *Chaetodon* species

Auditory thresholds between 100–2000 Hz were obtained for the four study species. Representative AEP waveforms are shown for a 200 Hz stimulus at different intensities for *F. flavissimus* and *Chaetodon ornatissimus* (Fig. 1). The AEP waveform is visible above background noise at a stimulus level of 125 dB re. 1 μ Pa for *F. flavissimus*, and at a lower intensity of 111 dB re. 1 μ Pa for *C. ornatissimus*. Comparisons of the baseline threshold audiograms show several similarities and differences in the frequency response characteristics among species

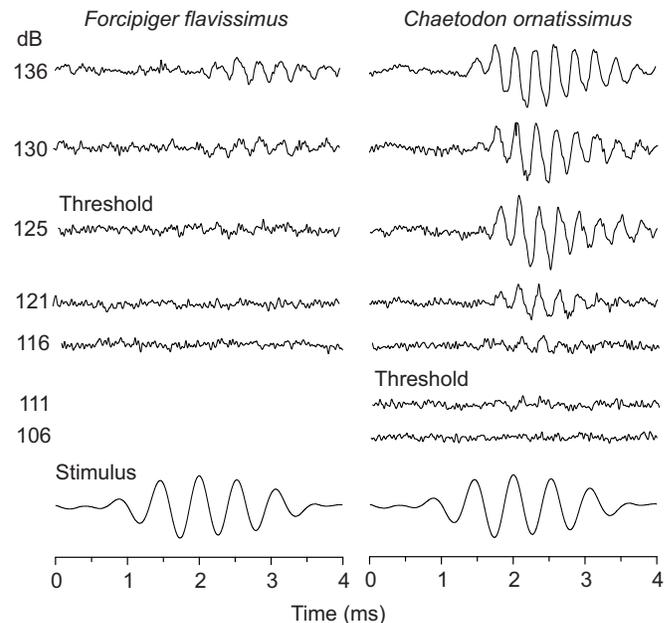


Fig. 1. Representative auditory evoked potential (AEP) waveforms used to determine intensity thresholds for butterflyfish species at 200 Hz stimulation. (A) AEP waveforms for the forcepsfish *Forcipiger flavissimus* show a stimulus threshold at 125 dB re. 1 μ Pa (left, third trace from top). The ornate butterflyfish *Chaetodon ornatissimus* shows a lower threshold level at 111 dB re. 1 μ Pa (right, sixth trace from top). Bottom traces show the stimulus waveform recorded by the hydrophone at the position of the fish head.

(Fig. 2A). All *F. flavissimus* individuals responded to stimuli from 100 to 800 Hz: a higher frequency response at 1000 Hz recorded for two individuals (33%) and no responses at higher frequencies. In contrast, all individuals of all three *Chaetodon* species responded to stimuli up to 1000 Hz. An extended frequency response range was apparent for both *Chaetodon multicinctus* and *Chaetodon auriga* with the maximum recorded frequency response at 1700 Hz for only a few individuals. This frequency response was exceeded by *C. ornatissimus*, with hearing thresholds observed for all individuals at 1300 Hz and a response at 2000 Hz for one individual. Both sound pressure and particle acceleration audiograms (Fig. 2B) further indicate a low-pass response (flat sensitivity) to frequencies ≤ 400 Hz in *F. flavissimus* and below about 500–600 Hz in *Chaetodon* species. Audiograms for responses to particle acceleration show a decreasing threshold response with frequency in *F. flavissimus*, with a minimum threshold of 77 dB re. 1 μ m s⁻² at 100 Hz compared with a relatively flat low-pass band of 65–75 dB re. 1 μ m s⁻² below 500–600 Hz for all *Chaetodon* species. However, the average audiogram threshold curve for *F. flavissimus* is 5–15 dB higher than those for *Chaetodon* species in the 200–1000 Hz stimulus range. A two-way repeated-measures ANOVA (GLM) tested for differences in frequency sensitivity and species across the 100–800 Hz band and confirms differences between species and stimulus frequencies, with no interaction of factors (Table 1). A *post hoc* Student Newman–Keuls test shows that *C. multicinctus* has an overall lower threshold than *F. flavissimus* ($P < 0.05$) but the test did not detect sensitivity differences among *Chaetodon* species in this range. The maximum frequency at which auditory thresholds differed between *F. flavissimus* and *C. multicinctus* was 600 Hz for both total sound pressure level (25 dB difference: 134.8 vs 109.6 dB re. 1 μ Pa, respectively) and particle acceleration level (29 dB difference: 93.8 vs 64.9 dB re. 1 μ m s⁻², respectively) (Fig. 2). Because *F. flavissimus* is not uniformly sensitive to auditory stimuli >800 Hz, we tested for differences in AEP responses at 1000 and 1300 Hz among the three

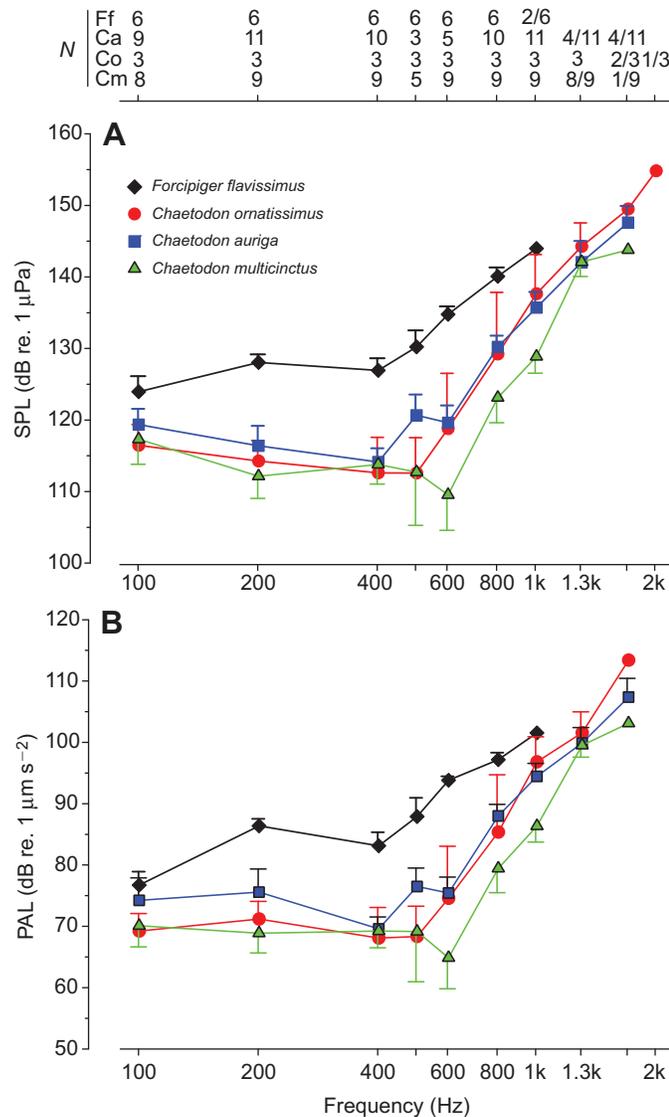


Fig. 2. Hearing threshold audiograms for *Forcipiger flavissimus* and three *Chaetodon* species as measured by the AEP technique.

(A) Audiograms measured as total sound pressure level (SPL) are similar among *Chaetodon* species with lower thresholds and an extended response range to 1700 Hz for all species and to 2000 Hz for *C. ornatissimus*.

(B) Audiograms measured as particle acceleration level (PAL) show relative patterns that are similar to SPL curves for *Chaetodon* and *F. flavissimus* across most tested frequencies. We did not calibrate the accelerometer at 2000 Hz; thus, that data point is lacking for *C. ornatissimus*. *N*=sample size of fish tested at each frequency. Fractions indicate the proportion of tested fish that showed a response. Data are means and s.e. Ff, *Forcipiger flavissimus*; Ca, *Chaetodon auriga*; Co, *Chaetodon ornatissimus*; Cm, *Chaetodon multinctus*.

Chaetodon species. Repeated-measures ANOVA (GLM) show that responses differ among frequencies but not among species (Table 1) and was confirmed by the *post hoc* Student Newman–Keuls test ($P < 0.05$). While we were able to run the above ANOVA tests on reciprocal transformed normalized data in terms of sound pressure, we were not able to normalize particle acceleration data for these species and frequency comparisons.

Action of the swim bladder and horns on hearing sensitivity

Analysis of horn and swim bladder experiments confirms differences between species and stimulus frequencies. Deflation of the swim

Table 1. Statistical comparisons of audiograms by two-way ANOVA with repeated measures (general linear model)

	Sound pressure level			
	d.f.	MS	<i>F</i>	<i>P</i>
All species 100–800 Hz				
Species	3	0.0000068	4.86	0.009
Individual	23	0.0000015		
Frequency	5	0.0000022	12.94	<0.001
Frequency×species	15	0.00000028	1.64	0.077
Error	97	0.00000017		
All <i>Chaetodon</i> 1 and 1.3 kHz				
Species	2	99.39	2.57	0.111
Individual	14	40.01		
Frequency	1	299.85	19.86	0.001
Frequency×species	2	47.58	3.15	0.087
Error	10	151.00	15.10	
<i>F. flavissimus</i> swim bladder deflation				
Individual	5	775.30		
Treatment	1	32.33	2.10	0.207
Treatment×individual	5	15.40		
Frequency	3	92.50	4.65	0.017
Frequency×individual	15	19.90		
Treatment×frequency	3	4.03	0.62	0.616
Error	15	6.55		
<i>C. multinctus</i> horn fill and swim bladder deflation				
Individual	3	188.06		
Treatment	2	454.24	31.39	<0.001
Treatment×individual	6	14.47		
Frequency	2	5.07	0.22	0.808
Frequency×individual	6	22.94		
Treatment×frequency	4	81.41	4.77	0.015
Error	12	17.05		
<i>C. auriga</i> horn fill and swim bladder deflation				
Individual	3	0.00000061		
Treatment	2	0.0000018	22.92	0.002
Treatment×individual	6	7.9×10^{-8}		
Frequency	3	0.00000013	0.67	0.59
Frequency×individual	9	0.00000002		
Treatment×frequency	6	0.00000013	2.75	0.045
Error	18	4.7×10^{-8}		

Probabilities of a type 1 error < 0.05 are indicated in bold. MS, mean square. See text for additional details.

bladder in *F. flavissimus* had no effect on thresholds across frequencies (Fig. 3A). A two-way ANOVA (frequency and swim bladder as factors, individuals as random subjects) shows no effect on thresholds following deflation of the swim bladder, no change in frequency response or statistical interactions of these factors (Table 1). Deflation of the swim bladder in *C. ornatissimus*, which has short horns that could not be independently evacuated, appeared to show a 5 dB increase in thresholds at 200 and 400 Hz, but we could not test for statistical differences because of low sample size (Fig. 3B). In comparison, displacement of gas from the swim bladder horns and subsequent deflation of the swim bladder increased hearing thresholds in *C. multinctus* and *C. auriga*. Fig. 4 shows the AEP responses for an individual *C. multinctus* with a baseline threshold of 107 dB re. 1 μ Pa and sequential 10 dB increases following gas displacement in the horns and deflation of the swim bladder. Differences in threshold responses at 200, 400 and 600 Hz were found for *C. multinctus* across treatments (Two-way repeated-measures ANOVA for subjects with all three treatments at these test frequencies; Table 1, Fig. 3) but there were no differences in thresholds at 100 Hz among treatments (One-way repeated-measures ANOVA for subjects with all three treatments at 100 Hz, d.f.=2,2,4; $F=0.40$; $P=0.694$). *Post hoc* Student Newman–Keuls test shows no differences among horn or swim

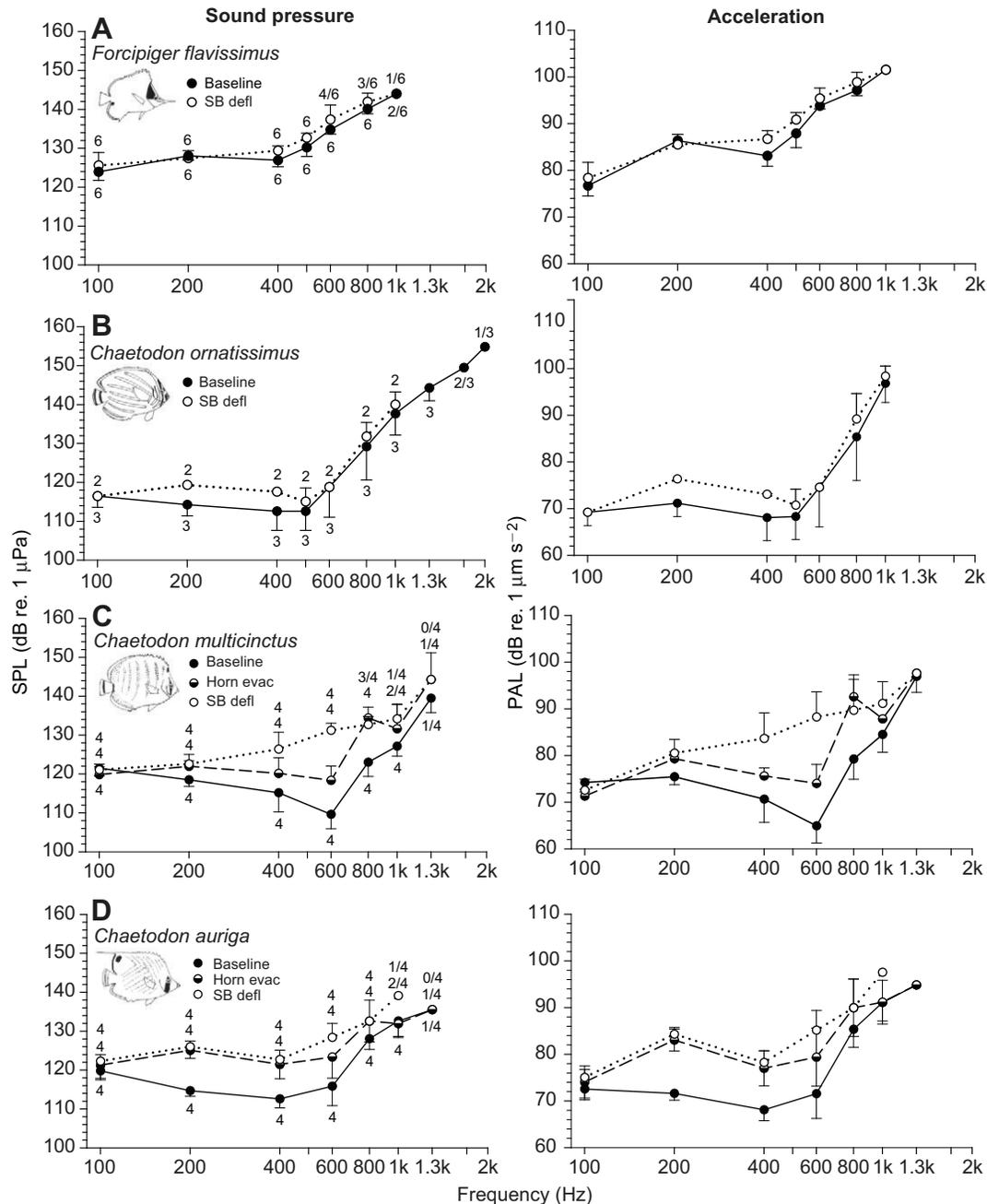


Fig. 3. Effect of the gas-filled swim bladder horns and chamber on hearing sensitivity in *Chaetodon* butterflyfish with different laterophysic morphologies as determined by the AEP technique. (A) *F. flavissimus*, which lacks an LC and swim bladder horns shows no change in normal AEP threshold (black circles) following deflation of the swim bladder (open circles). (B) *C. ornatissimus* has short swim bladder horns with an indirect connection to the LC that we were not able to manipulate. The baseline thresholds extended to 2000 Hz and appear to increase by approximately 5 dB in the 200–400 Hz band following deflation of the swim bladder. (C) *C. multicinctus* has long swim bladder horns with an indirect connection to the LC. Baseline thresholds increased in the 200–600 Hz band after gas was evacuated from the swim bladder horns (half-filled circles) with a maximum increase of 10 dB at 600 Hz. Subsequent deflation of the swim bladder demonstrated further threshold increases most notable at 600 Hz. (D) *C. auriga* has long swim bladder horns with a direct connection to the LC. Baseline thresholds increased by about 10 dB at 200–600 Hz after gas was evacuated from the swim bladder horns and swim bladder. AEP threshold data are provided in relation to sound pressure level (left column) and particle acceleration level (right column). Data are means and s.e. among individuals. Numbers at data points indicate sample size at each test frequency, or fraction of test subjects for which an AEP was recorded.

bladder treatments at 200 Hz, but an increase in threshold from baseline by both treatments (but their thresholds did not differ) and a sequential increase in threshold by swim bladder deflation following displacement of gas from the horns. A similar effect was observed for *C. auriga* at the same test frequencies (Fig. 3D). Deflation of the horns increased thresholds at 100, 200, 400 and 600 Hz, but the subsequent deflation of the swim bladder had no additional effect (Two-way

ANOVA, Table 1). The reduction in the number of fish that showed responses after manipulation of the horns and swim bladder precluded further statistical tests, but the loss of hearing sensitivity may extend to frequencies >600 Hz, as seen in post manipulation audiogram plots for *C. multicinctus* (Fig. 3C). Comparisons of the net changes in hearing sensitivity following removal of gas from the swim bladder and horns show about a 5 to 20 dB decrease among *Chaetodon* species (Fig. 5).

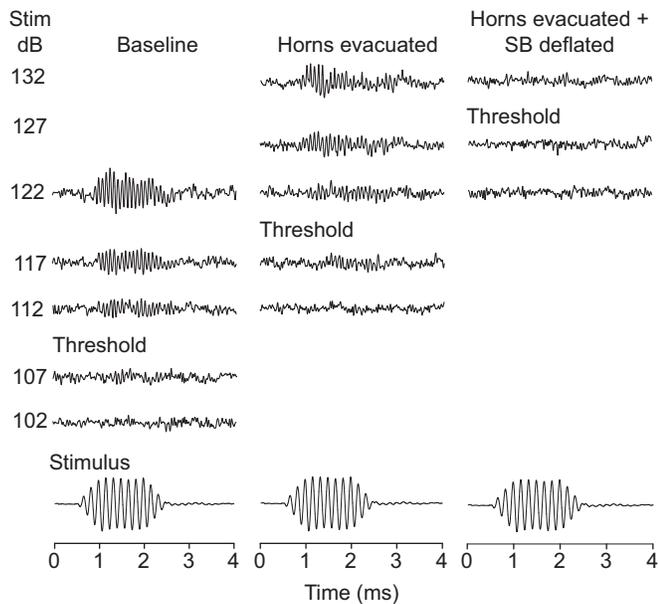


Fig. 4. Relative change in AEP thresholds after sequential displacement of gas from the swim bladder horns and then evacuation of gas from the swim bladder in an individual *Chaetodon multicinctus* at 600 Hz stimulation. Baseline threshold is at 107 dB re. 1 μ Pa. Displacement of gas from the swim bladder horn by a small injection of gel increased the threshold to 117 dB re. 1 μ Pa. Subsequent evacuation of gas from the swim bladder increased the threshold to 127 dB re. 1 μ Pa. Bottom traces show the stimulus waveform recorded by the hydrophone at the position of the fish head.

Taken together, all of the above results indicate that both *F. flavissimus* and *Chaetodon* species are sensitive to particle acceleration stimuli. In *Chaetodon* species, sound pressure actions on the swim bladder and horns enhances frequency sensitivity from 200 to 600 Hz and increases the maximum frequency response range to at least 1300 Hz.

Ambient noise levels in coral reef territories, sound production and hearing

Data from the field recordings of ambient noise levels in reef territories of multiband butterflyfish, *C. multicinctus*, were compared with data on auditory thresholds and spectral levels of their communication sounds (Tricas and Boyle, 2015) to provide a first estimate of the potential constraints on hearing perception in their natural setting. The total background noise levels across the full 10–24 kHz band show variation in intensity of about 10–30 dB among territories and among frequencies that vary with depth (Fig. 6, background noise curves). The greatest difference in background noise was observed in territories <6 m deep, with relatively high average noise levels in the 10–100 Hz band compared with territories at deeper sites. Overall, the noise levels are near but below the AEP auditory thresholds to frequency tones. Total noise levels were also below the peak frequency levels reported for the four sounds produced by this species during communication at close distances (Tricas and Boyle, 2015) as shown by representative sound intensity spectral curve plots in Fig. 6. The tail slap pulse is a very low frequency stimulus associated with accelerations of a local hydrodynamic flow field with an average peak frequency of ~10 Hz. The body shake sound is produced infrequently and has a low peak frequency ~29 Hz. The body pulse sound is produced very frequently during social interactions and has an average peak frequency of 137 Hz (median 231 Hz) and bandwidth of 350 Hz that overlaps well with their range of highest frequency sensitivity from 100–600 Hz. A high frequency and broadband click sound is also

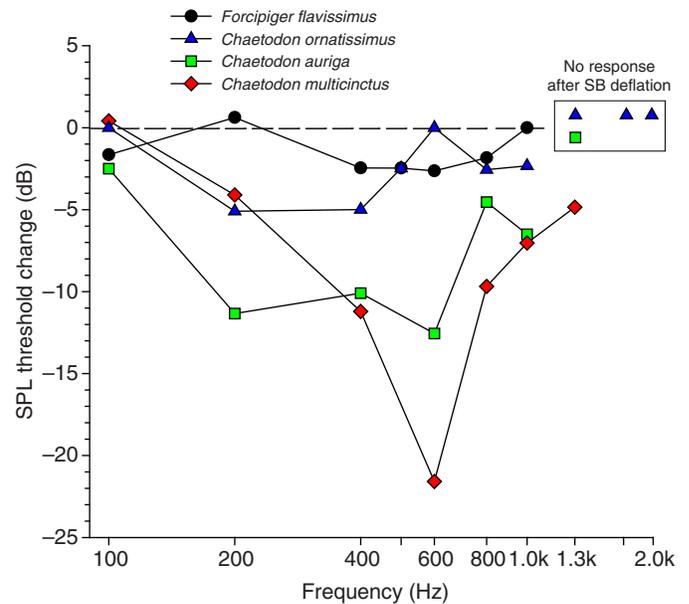


Fig. 5. Summary of relative changes in the average sensitivity thresholds to sound pressure stimuli following deflation of the swim bladder in four butterflyfish species. Averaged response changes for individual fish across stimulus frequencies showed no statistical differences following swim bladder deflation in *F. flavissimus*, which lacks a laterophysic connection (LC) and the associated swim bladder horns. Different magnitudes of decreased thresholds were observed for *C. ornatissimus* which has short swim bladder horns, and *C. auriga* and *C. multicinctus*, both of which have long and wide swim bladder horns of the LC. Note that the peak effects on swim bladder deflation (including evacuation of the swim bladder horns) for all *Chaetodon* species ranged from 200 to 600 Hz, with smaller effects at higher frequencies. Responses were lost for all test individuals in some species at higher frequencies following swim bladder deflation (box). Reference at 0 dB (dashed line) indicates relative baseline threshold level for each test subject.

often produced during the tail slap behaviour, but has an average peak frequency far above the tone hearing range of 100–1700 Hz.

The estimated intensity spectrum level (SPL_{int}), in which sound intensities are corrected for a 1 Hz band to provide a more realistic comparison of relative intensity across a range of frequencies, confirms that ambient noise levels on the reef substrate (where fish forage in pairs) decrease non-linearly with water depth [inverse polynomial function, $SPL_{int}=Y_0+(a/\text{depth})$: $SPL_{int}=73.36+(22.90/\text{depth})$, $P=0.012$] and a similar relationship is found for noise levels measured 1 m above the substrate where fish occasionally swim [$SPL_{int}=76.10+(16.82/\text{depth})$, $P=0.033$] (Fig. 7A). However the parameters for these two regression curves do not differ (ANOVA: Y_0 , d.f.=1,20,21, $F=2.10$, $P=0.163$; a , d.f.=1,20,21, $F=0.374$, $P=0.548$) and indicate that total sound levels did not differ with depth at the two elevations within a territory. When their respective sound frequency bands are included in the estimation of band noise, the relationship between ambient noise and territory depth differs for the two most common sounds. The noise level within the frequency band of the tail slap sound decreases rapidly with territory depth [$SPL=(-1.75\times\text{depth})+127.30$, $P<0.001$, $R=0.68$], whereas ambient noise in the body pulse sound band does not change with depth [$SPL=(-0.083\times\text{depth})+105.40$, $P=0.782$, $R=0.095$] (Fig. 7B). Comparison of these band-specific background noise levels with the 6 dB bandwidth levels for each sound type (from Tricas and Boyle, 2015) indicates that the signal to noise ratio of the tail slap sound increases with depth of the territory [$SNR=(1.57\times\text{depth})+3.70$, $P=0.021$, $R=0.68$], but that for the

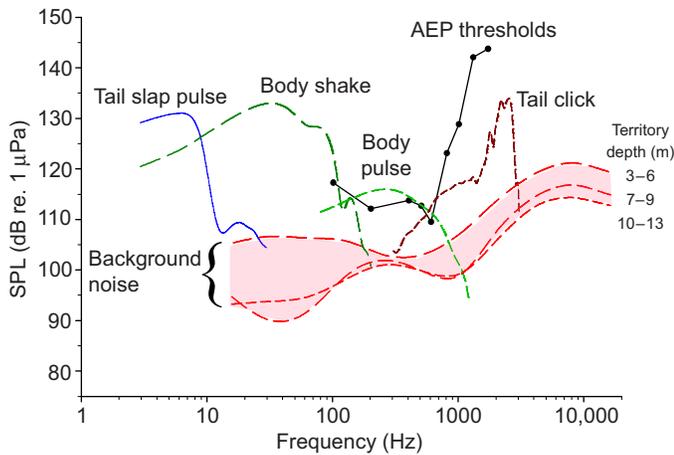


Fig. 6. Hearing thresholds and spectra of vocalizations used during social communication by the multiband butterflyfish *Chaetodon multicinctus* and the spectra of ambient noise in fish territories at Puako Reef in Hawaii. Fish AEP thresholds determined in the lab were tested from 100 to 2000 Hz and show a low-pass sensitivity below 600 Hz (black circles). Spectra of four representative sound types are shown. Best hearing sensitivity as determined by the AEP technique is nearest to the band of the body pulse sound used commonly in social interactions. The infrasound tail slap and body shake pulse are produced during vigorous agonistic interactions but sensitivity to these low-frequency particle acceleration stimuli remain to be determined. The broadband high-frequency tail click sound is probably beyond their hearing capabilities. The average ambient background octave noise bands are shown for 11 territories recorded at sites 2.5–12.8 m deep (red dashed lines at bottom; shaded area indicates range) and illustrate higher noise levels in the fish hearing range in territories in shallow habitats ≤ 6 m deep. Waveforms used for intensity estimates of the four representative fish sounds were recorded by Tricas and Boyle (2015).

body pulse sound does not change with territory depth [SNL=(0.08×depth)+10.60, $P=0.78$, $R=0.10$] (Fig. 7C).

DISCUSSION

This study demonstrates that the auditory sensitivity of *Chaetodon* butterflyfishes is enhanced by the perception of sound pressure and is mediated by the swim bladder and horns associated with the LC. As for most AEP studies we used tone stimuli to determine auditory thresholds across relevant frequencies. Below we use the audiogram data to interpret their likely sensitivity to the spectra of different sounds that are important for acoustic communication and the effects of ambient noise spectra encountered in their natural coral reef environment. We recognize the limitation that the AEP tone stimulation method shows responses to single brief tones and not more complex sounds used for communication. In addition, the AEP technique can also include direct responses of the lateral line and possibly those mediated by the LC, which we also discuss.

Comparison of audiograms indicates that *Chaetodon* species are more sensitive than *Forcipiger* to sound stimuli at frequencies between 100–800 Hz, and also have an extended frequency range of hearing. In addition, hearing audiograms for butterflyfishes are similar in several respects to species that are primarily sensitive to particle acceleration rather than sound pressure. Sound pressure thresholds at best frequency were within the low-frequency band of 100–600 Hz for all species and ranged from 124 dB re. 1 μ Pa for *F. flavissimus* to 110 dB for *C. multicinctus*. These minimum SPL thresholds are similar to those reported for species that lack anterior projections of the swim bladder such as the pumpkinseed sunfish (Wysocki and Ladich, 2003), oyster toadfish (Yan et al., 2000), gobies (Lugli et al., 2003) and larval snappers and jacks (Wright et al., 2010).

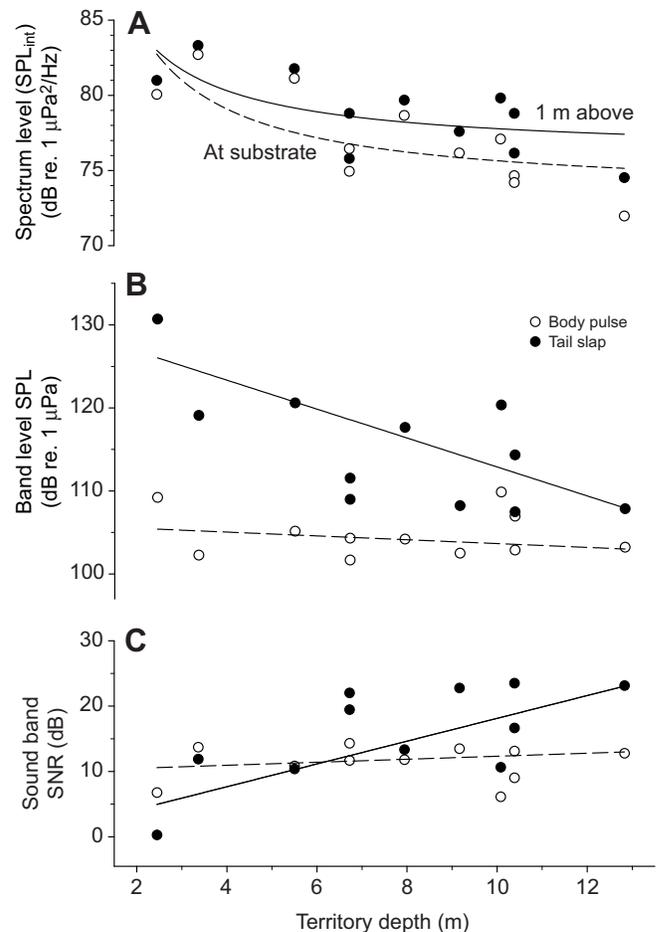


Fig. 7. The potential action of sound-band-specific ambient noise on the transmission of communication sounds in coral reef territories of *Chaetodon multicinctus* at Puako Reef in Hawaii. (A) Estimated intensity spectrum levels (SPL_{int}) from ambient sound recordings made within territories where fish pairs forage over the reef surface (open circles) are similar to levels measured 1 m up into the water column where fish also swim less frequently (black circles). (B) Background noise band pressure within the predominate frequency range (top 6 dB of the power spectrum) of the body pulse (21–414 Hz) and tail slap sounds (2–18 Hz) indicate that the background noise levels change with territory depth for the tail slap but not for the body pulse sound. (C) Estimated ratios of signal to noise (SNR) of sound amplitudes near the source to ambient noise levels for the frequency bands of the body pulse and tail slap sounds at different territory depths. Perception of the tail slap but not the body pulse sound should improve at deeper territory locations.

These SPL thresholds are far greater than those reported by many AEP studies on species with peripheral auditory structures known to enhance sensitivity to sound pressure such as the Weberian apparatus of some cyprinids and catfish (Kenyon et al., 1998; Ladich, 1999; Amoser and Ladich, 2005; Lechner and Ladich, 2008), the otic gasbladder of mormyrids (Yan and Curtsinger, 2000) and the suprabranchial organ of gouramis (Ladich and Yan, 1998), although AEP audiograms are not considered to be directly comparable between studies because of differences in techniques (Ladich and Fay, 2013). Primary sensitivity to particle acceleration in butterflyfish is further indicated by the descending low-pass characteristics and relatively flat low-pass curves below 500 Hz. The band of highest frequency sensitivity was from 100 to 400 Hz for *F. flavissimus* and 100 to 600 Hz for *Chaetodon* species. This coincides with the narrower 200–300 Hz band of best sensitivity recorded for larval and juvenile spotfin butterflyfish, *Chaetodon ocellatus* (Webb et al.,

2012). These bands of best sensitivity are well below the 1–3 kHz range seen in many sound pressure sensitive species with otophysical connections (Ladich and Fay, 2013). The maximum frequency response for some *F. flavissimus* individuals extended to 1000 Hz whereas *Chaetodon* species showed sensitivities to 1700 and 2000 Hz. Thus, while all butterflyfishes in this study appear sensitive to particle acceleration stimuli, *Chaetodon* species showed higher frequency sensitivity than *F. flavissimus*.

The differences in response characteristics observed for *F. flavissimus* and *Chaetodon* species support the hypothesis that the anterior swim bladder horns in *Chaetodon* enhance both the hearing frequency range and thresholds via an added sensitivity to sound pressure stimuli. Deflation of the swim bladder in *F. flavissimus* had no effect on auditory thresholds and supports the conclusion for an auditory sensitivity only to particle acceleration. However, displacement of gas from the relatively long swim bladder horns in both *C. multinctus* and *C. auriga* increased the auditory thresholds between 200 and 600 Hz and therefore decreased hearing sensitivity to sound pressure in this range (Fig. 4C,D). Scanning electron microscopy and computed tomographic imaging analyses of the maculae, hair cell populations, otoliths and swim bladder of several LC variants reveal that the butterflyfish inner ear has an unremarkable morphology and lacks a physical otophysical connection (Webb et al., 2010). However, the swim bladder horns of both species are long and have equivalent relative lengths when corrected for body size (Woods, 2006). In addition, the swim bladder horns of *C. multinctus* approach the lagena of the inner ear at close distances (1 mm) in both the transverse and horizontal planes, whereas the horns of *C. auriga* approach the lagena at twice that distance (~2 mm) and in only the transverse plane. This closer proximity of the horns to the inner ear and the presence of a blunter rostral horn apex found in LC indirect variants indicate a potentially stronger effect of the horns on auditory sensitivity in *C. multinctus* (and other LC indirect variants) than *C. auriga* (and other LC direct variants) (Woods, 2006). Despite these morphological differences in horn morphology and proximity to the inner ear, our results indicate that the relative effect of the horns on sound pressure sensitivity may be more prominent in *C. auriga*. Displacement of gas from the swim bladder horns in *C. auriga* reduced sensitivity at 200–600 Hz, but there was no additional statistical effect on sensitivity after subsequent swim bladder deflation as observed for *C. multinctus*. Recent work on the hearing abilities of representatives of four cichlid genera with different swim bladder and horn morphologies (Schulz-Mirbach et al., 2012) shows that species with anterior swim bladder horns have improved auditory sensitivities of 20–40 dB (SPLs) between 0.5 and 1 kHz. That work also indicated that swim bladder size was involved in extending the upper frequency range to 3 kHz. Of note in this study is that the highest hearing sensitivity was observed at 2 kHz for *C. ornaticornis*, a species with very short swim bladder horns (LC variant Ind2), but also approach the lagena at a short 1 mm in the transverse plane (Woods, 2006). The physical responses to sound pressure stimuli by these morphological variants of the LC, swim bladder horns, and swim bladder need to be quantified in order to confirm their contribution to butterflyfish hearing.

The relative action of acoustic stimuli on the head and trunk lateral line system was not addressed in this study. Recent work shows that there can be a significant contribution to the AEP from hydrodynamic motions across the lateral line canals especially at lower stimulus frequencies (Higgs and Radford, 2013). Thus, AEP responses at low frequencies should be considered as multimodal rather than only auditory evoked potentials derived from the inner

ear. While our AEP responses probably also include a contribution from the lateral line system, the observed changes in threshold following evacuation of gas from the swim bladder and horns is more likely due to changes in excitation of the inner ear. This is because the LC portion of the lateral line adjacent to the swim bladder horns includes only a few associated neuromasts of the supracleithrum and lateral line canal scales (Webb et al., 2006), and presumably represents only a very small portion of the lateral line input. Nonetheless, the physical and neurophysiological actions of the LC system on the butterflyfish ear and lateral line need to be clarified as there is likely an integrative role in the detection and processing of information encoded from both systems during butterflyfish acoustic communication.

Recent work shows that our four test species produce several sound types with bandwidths that span four frequency decades from <1 Hz to >1000 Hz. The body pulse and other sounds produced by *C. multinctus* in the field and lab have peak frequency content from 137 to 466 Hz (Tricas et al., 2006; Tricas and Boyle, 2014, 2015) and the pulsed sounds at 346 Hz produced by *C. ornaticornis* (Tricas and Boyle, 2014). Thus sounds produced by both species overlap with their best frequency sensitivities demonstrated in this study. We would expect these to be the most effective sound types to stimulate the swim bladder and LC at close and greater distances from the source. In contrast, the prominent tail slap and other sounds produced during social interactions with conspecifics by *C. multinctus*, *C. ornaticornis*, *C. auriga* and *F. flavissimus* contain hydrodynamic and sound pressure components in the infrasound range of <1–30 Hz, which should not affect horns or swim bladder transduction (Sand and Karlens, 2000). The response of the butterflyfish inner ear to such low-frequency linear accelerations needs to be determined to understand the full range of acoustic signals used by these fish in their social interactions. In addition, the discrimination of sound stimuli needs to be determined beyond the small laboratory tank environment so that the particle motion, sound pressure and spatial configuration of communication sounds can be presented to stimulate the ear in more-natural ways.

The rostral extensions of the swim bladder may serve other functions in addition to the facilitation of acoustic communication. The connection between the modified swim bladder and ear in the mojarra *Eucinostomus argenteus* imparts a high hearing sensitivity but that species apparently does not produce sound for communication (Parmentier et al., 2011b). In this case, the swim bladder morphology was argued to facilitate body orientation during feeding on small benthic prey and a similar hydrostatic function may occur in coral feeding butterflyfish which must orient with great precision to selectively feed on small coral polyps (Tricas, 1989b). Enhanced hearing sensitivity and frequency range may also function for the detection of predators or prey, as suggested for the highly sensitive otophysans in quiet freshwater habitats (Ladich, 2000). Such potential non-communication functions of the modified associations between the swim bladder, ear and LC need further investigation in relation to the detection of prey, predators, ambient background noise levels and body orientation behaviors.

Butterflyfish sound communication on coral reefs

The best sensitivities for both *F. flavissimus* (~125 dB SPL) and *Chaetodon* species (~115 dB SPL) are similar to those reported for some other coral reef-dwelling damselfish (e.g. Egner and Mann, 2005; Maruska et al., 2007) and are higher than that of many non-reef-dwelling species (Ladich and Fay, 2013). Although differences in recording techniques preclude direct comparisons, it is possible that the noisy coral reef environment constrains adaptations for

higher hearing sensitivity, as seen in aquatic environments with less background noise (Ladich, 2014). The difference in hearing sensitivity between *F. flavissimus* and *Chaetodon* species is not likely to be explained by differences in habitat selection because the two species overlap extensively in their distribution over the reef, although this remains to be tested.

The efficiency of acoustic communication by butterflyfish on noisy coral reefs may be facilitated by the selection of optimal acoustic sub-habitats. Ambient sound recordings taken within territories of the coral-feeding multiband butterflyfish, *C. multicinctus*, show that total noise levels are higher in shallow areas of the reef (<6 m depth), especially within the lower frequency band of 10–100 Hz (Fig. 6). Such low-frequency background noise in shallow reef waters comes from several abiotic sources, such as wind, waves, tidal flow and also biological sources (Wenz, 1962; Urlick, 1983). This noise spectrum includes the frequency range for the tail slap and body shake sounds, which are used during social interactions with mates and other conspecifics (Tricas and Boyle, 2014, 2015). The intensity of ambient noise near the reef substrate (where butterflyfish pairs swim and feed together on corals) in these shallow territories was approximately 10–15 dB greater than the intensity within territory sites 10–13 m deeper on the reef, and this relationship was maintained when noise levels were adjusted for the frequency band of the tail slap sound (Fig. 7). In comparison, the intensity of ambient noise within the frequency band of the body pulse sound type did not change with territory depth. These differences indicate the possibility for different actions of ambient reef noise levels on the perception of different sound types. Are there fitness advantages related to acoustic communication efficiency (information transfer, useful distance of acoustic communication etc.) among mates and rivals that establish territories in deeper areas of the reef with lower ambient noise levels? In addition, other studies on ambient reef noise reveal different spectral signatures among reefs and habitats (Simpson et al., 2005; Kennedy et al., 2010; Radford et al., 2014) but have not yet distinguished between the hydrodynamic surge/water turbulence and sound pressure components of sounds that also differ respectively with habitats and depth. Future work should address the acoustic characteristics of reef environments associated with the reef fish distributions and their potential impact on sound perception during acoustic communication.

The perception of a sound stimulus in a reef habitat with high background noise would be enhanced by a closer association of two fish (small distance of separation) compared with fish in a habitat with low background noise. Previous studies indicate that signal to noise level ratios are 10–20 dB at threshold and are dependent upon the relative directions of a sound and the ambient noise source (Chapman, 1973; Chapman and Sand, 1974; Hawkins and Sand, 1977; Fay, 1988). The signal to noise levels estimated in the present study were determined from independent measurements of AEP hearing thresholds in the relatively quiet lab environment and compared with empirical field measurements of sound band-specific noise levels (corrected for the frequency range of a specific sound type) in the field. By this method we estimated sound signal to ambient noise level ratios to be 10–20 dB near the source (Fig. 7C). Unfortunately, there is not a rigorous association for all species between hearing thresholds measured by the AEP and behavior assay techniques (Ladich and Fay, 2013); thus, the behavioral thresholds to tone stimuli and also to their complex sound waveforms remain unknown. Behavioral thresholds are needed to determine the maximum distance of separation for the perception of conspecific sounds in the presence of noise in the coral reef environment. The signal jump sound used to attract

females to nest sites by male Hawaiian Dascyllus damselfish, *Dascyllus albisella*, was estimated to be detectable by a receiver fish at a distance of 11–12 m from the source where the signal to noise ratio was 5–10 dB (Mann and Lobel, 1997). Unlike damselfishes, butterflyfishes are broadcast spawners that do not form nesting sites. Many *Chaetodon* butterflyfish form monogamous pairs that spend a large portion of their day foraging together separated by only a few body lengths, and are not known to produce acoustic advertisements for mates at great distances. This leads to the conclusion that pairing and other close social behaviors of butterflyfish promote efficient acoustic communication in a noisy reef environment. Future studies on hearing capabilities in the coral reef environment should investigate the reception sounds produced by conspecifics (rather than tones) to determine the extent that the intense ambient noise may reduce sound perception. In addition, the potential contribution of sound pressure transduction by the LC to acoustic communication at near and greater distances needs to be assessed to better understand the potential adaptive values for their close affiliative behaviors.

METHODS

Study species

Spectral sensitivity was determined for three *Chaetodon* species with different LC morphologies (including long and short rostral swim bladder extensions) that belong to different subgenera and *Forcipiger flavissimus* (sensu Webb et al., 2006). The threadfin butterflyfish, *C. auriga* ($N=11$, $SL=116.0\pm 9.7$ mm; mean \pm s.d.) has a direct LC with mucoid connective tissue between the medial opening in the supracleithral lateral line canal, and has long and wide swim bladder horns (LC variant Dir1). The multiband butterflyfish *C. multicinctus* ($N=9$, $SL=76.0\pm 6.7$ mm) has an indirect LC with a layer of epaxial muscle (and no mucoid connective tissue) between the medial opening of the supracleithrum, and long and wide swim bladder horns (LC variant Ind2). The ornate butterflyfish *C. ornatissimus* ($N=3$, $SL=89.3\pm 7.3$ mm) has an indirect LC with short horns that do not closely approach the anterior lateral line or inner ear (LC variant Ind3). The forcepsfish *F. flavissimus* ($N=6$, $SL=106.2\pm 15.6$ mm) lacks swim bladder horns and an LC (no medial opening in the supracleithrum). Adult fish were acquired from local fish collectors, transported to the lab and acclimated in 40 litre tanks with coral/sessile invertebrate food and fresh flow through seawater (25–28°C) for ≥ 24 h before experiments were performed.

Stimulus generation and AEP recordings

The auditory responses of butterflyfishes to sound stimulation were determined using the AEP physiology technique which detects compound neural responses to estimate the hearing sensitivity of fish and used to determine the effect of gas-filled anterior swim bladder and horns on auditory sensitivity (Kenyon et al., 1998; Yan et al., 2000). We followed recording procedures on reef fish used previously in our lab (Maruska et al., 2007). This non-invasive technique was preferred because frequency thresholds could be rapidly determined and allowed sequential manipulation of the gas-filled swim bladder horns and swim bladder chamber of individual fish.

Experimental fish were immobilized with an injection of pancuronium bromide into the dorsal body musculature, lightly restrained in a mesh harness with a clamp suspended from a PVC frame, positioned slightly below the water surface of a 23 litre bucket (29 cm diam \times 35 cm high) and ventilated through the mouth with fresh seawater. Stainless steel electrodes (Rochester Electro-Medical, Inc., Tampa, FL, USA; 6–12 k Ω impedance) were sealed on the ends with glue and nail polish so that only ~ 1 mm of metal was exposed at the tip. The recording electrode tip was inserted 3–5 mm deep into the cranial musculature along the dorsal midline above the brainstem. The reference electrode was inserted into the cranial musculature between the eyes, and a ground wire placed in the water near the fish.

Sound stimuli were generated (and AEPs recorded) with a Cambridge Electronics Design (CED, Cambridge, UK) Micro 1401 controlled by Spike

2 software and a CED 3505 attenuator. Conditioned signals were differentially amplified (UMA 352; Peavey Electronics, Meridian, MS, USA) and sent to an underwater speaker (UW-30; frequency response 100–10 kHz) placed at the bottom of the test bucket and approximately 28–30 cm below the fish head. A total of eight primary stimulus frequencies from 100–800 Hz were tested for each subject and 1–2 kHz when possible. Acoustic pips at test frequencies of ≥ 200 Hz consisted of 2000 pure tone 20 ms pulses (10 ms plateau with rise and fall times of 5 ms), and at a 100 Hz pulse plateau, rise and fall times of 10 ms. Stimulus artifacts in the AEP recordings were minimized by sequential alternation of the pip phase. Each trial began at a suprathreshold intensity (136–156 dB rms re. 1 μPa) and was decreased in 5 dB steps to a sound level below the presumed threshold (90–125 dB rms re. 1 μPa). Threshold was determined for each frequency (described below) before moving to the next test frequency. Sound pressure levels produced by the loudspeaker were calibrated with a Brüel and Kjær (Nærum, Denmark) hydrophone (model #8103; sensitivity=211 dB re. 1 V Pa⁻¹; frequency response 0.1 Hz–180 kHz) placed in the experimental tank at the position that the fish head normally occupies. We confirmed that SPL was uniform across the position of the fish body. For calibration, pips were presented without phase alternation, and voltage levels of sounds at all frequencies and intensity levels were measured with the hydrophone, Brüel and Kjær Nexus amplifier (Model 2692, sensitivity 10 mV Pa⁻¹ or 31.6 mV Pa⁻¹) and then signal averaged with our Spike 2 routine to determine actual sound pressure levels in dB rms re. 1 μPa . We also calibrated the tank for particle acceleration with a EDO Corp. P/N 51815-4, sensitivity 14.1 mV/(m s²) accelerometer kindly provided by Joe Sisneros (University of Washington) and Michael Gray (Georgia Tech).

AEP waveforms were differentially amplified (10,000 \times) and band-pass filtered (1–10,000 Hz) with a differential amplifier (DP-301, Warner Instruments, LLC, Hamden, CT, USA), digitized on the CED Micro 1401 (described above) running Spike 2 software, and stored on the computer hard drive. Responses to a total of 2000 repetitions were averaged for each sound intensity and test frequency. Power spectra (FFT, 512 or 1024-points) of the averaged waveforms were calculated and examined for peaks at twice the stimulus frequency that results from the opposed orientation of ear hair cells and non-linearities in the auditory system. Thresholds were determined by both the averaged AEP trace and power spectrum and were defined as the lowest sound level to show a repeatable AEP trace above background noise, and an FFT peak at twice the stimulus frequency. In cases where the threshold appeared between adjacent 5 dB intensity steps, the threshold level was verified by a repeated test of the averaged AEP.

Experimental manipulation of the swim bladder horns and swim bladder

Each fish was tested for changes in baseline hearing thresholds after manipulation of the swim bladder horns (*C. multincinctus* and *C. auriga*) and/or swim bladder (all four species). After baseline control responses were recorded, gas in the rostral swim bladder horns was displaced (evacuated) by injection of 100 μl of Super Poligrip™ or Vaseline (petroleum jelly) into the swim bladder horns and hearing thresholds re-tested. The swim bladder was then deflated with a hypodermic needle and syringe and the fish tested one final time. Only pre-manipulation controls and swim bladder deflation thresholds were determined for *C. ornatissimus*, which has short inaccessible horns at the rostral end of the swim bladder and *F. flavissimus*, which lacks rostral swim bladder horns. Fish were euthanized after experiments by immersion in MS-222, and filling of the rostral horns and deflation of the swim bladder were confirmed by dissection. We also performed AEP experiments on freshly euthanized specimens to confirm that the AEP responses were abolished and not a recording artifact.

Estimates of average hearing thresholds and standard error were calculated for each species from data on individual fish. Normal hearing sensitivity was determined by pooling thresholds from individuals not used for manipulation experiments and also baseline control data for fish in which the swim bladder or horns were subsequently manipulated. Repeated-measures general linear models (rmGLM) were used in order to test for species-level differences in threshold at each frequency. Because multiple frequencies were tested for each individual, thresholds at each frequency are not statistically independent. Thus we used rmGLM in which individual fish

were used as a random subject factor, frequency and species were used as fixed factors, and threshold as the response: individual fish were nested within species, and species and frequency were crossed. Because the hearing range varied among species, two separate tests were also conducted. One rmGLM tested for differences in thresholds among all species and at frequencies between 100 and 800 Hz. A second test examined thresholds among all three *Chaetodon* species (but not *F. flavissimus*), between 1000 and 1300 Hz. Experiments that tested the effect of the swim bladder and horns on hearing thresholds were also analyzed with rmGLM separately for each species in which individual fish was a random subject factor, frequency and experimental phase [pre-manipulation, horn injection (where applicable), swim bladder deflation] were fixed factors, and threshold was the response. In these experiments individual fish are crossed with treatment and frequency, and the factors treatment and frequency are crossed. *Post hoc* pairwise multiple comparisons were performed with the Student Newman–Keuls method. All statistical analyses were performed using SigmaPlot (v11.0 and 12.5).

Reef noise and communication sound band estimates

In order to determine the variation in ambient noise levels encountered by butterflyfishes in their normal coral reef habitats, we first recorded background sound within 11 *C. multincinctus* territories using scuba at Puako Reef, Hawaii. Prior to recordings, focal butterflyfish pairs and territory boundaries were determined from direct observation over a 4–6 week period. A stereo portable digital audio tape recorder (Sony DAT PCM-M1) in a custom housing (Sexton Corporation) with two ATI MIN 96 hydrophones was placed within an open area near a central location of each territory. A constant gain was used for recordings and compared to a calibrated tone of known voltage amplitude. One hydrophone was placed ≤ 10 cm above the substrate and the second hydrophone suspended directly 1 m above in the water column to provide simultaneous recordings at two depths. Depth of fish territories (at the location of the substrate hydrophone) ranged from 2.4 to 12.8 m with various mixtures of dead coral, rock and live coral substrates. Recordings of approximately 5 min duration were made on 24 August 2004 between 1455 and 1615 h. After deployment of the recording apparatus, scuba divers left the area to eliminate bubble noise in the recordings. Background sound recordings were downloaded to a computer and reviewed on Cool Edit Pro software. Approximately 1 min of continuous recording was identified for analysis that represented the frequency spectrum and intensity for each territory location, except for one territory, for which only 16 s of recording could be analyzed because of anomalous hydrophone interference. Noise estimates were calculated from a 16,384 point FFT using the ‘scan’ button in Cool Edit Pro. Estimates of spectrum level (dB re. 1 $\mu\text{Pa}^2 \text{Hz}^{-1}$), which provides a standardized estimate of frequency-specific noise, were obtained by subtracting 4.67 dB (10 log BW) to adjust for the 2.93 Hz bandwidth of the FFT. Estimates of octave band sound pressure levels (center frequencies 15.6, 31.3, 62.5, 125 etc.) for each territory were obtained by taking the average among linear values for the FFT bins that correspond to the upper (f_u) and lower (f_l) cutoff frequency of the octave band. The averaged value was then converted to a dB scale and the amplitude was adjusted based on the bandwidth of the octave band and original FFT bins (2.9 Hz) in order to determine SPL dB re. 1 μPa for each octave band: $10 \log(f_u - f_l) - 10 \log(2.9)$. In addition, we calculated the background noise of frequency bands that correspond to the majority of spectral energy of the two most common sounds of *C. multincinctus*: the tail slap and body pulse (Tricas and Boyle, 2015). The average minimum and maximum frequencies within 6 dB of peak of sounds from those recorded in a laboratory study (Tricas and Boyle, 2015) were used to represent the bandwidth of the tail slap and body pulse, 2–18 Hz and 21–414 Hz, respectively. For each territory at the substrate and 1 m above, estimates of noise levels obtained for these two bands were determined from the average linear noise values from noise measurements described above for the corresponding frequencies. The average was then converted to a dB value and adjusted by adding $[10 \log(\text{sound bandwidth}) - 10 \log(2.9)]$. We then estimated the total pressure level across the respective bandwidth for each sound type signal to noise ratio (SNR dB) for each of these two sound types at each territory and recording location (substrate and 1 m) were estimated by taking the average sound

pressure level recorded in the laboratory (Tricas and Boyle, 2015) for the tail slap (131 dB) and body pulse (116 dB) and subtracting the corresponding estimated sound type band noise level.

Acknowledgements

We thank Adam Dewan, Karen Maruska and Lisa Privitera for their assistance with the lab experiments and analyses. We thank Jacqueline Webb (who read an earlier version of this manuscript) and two anonymous reviewers; thanks also to Whitlow Au and John Allen for many discussions on butterflyfish sounds, bioacoustics and fish hearing.

Competing interests

The authors declare no competing or financial interests.

Author contributions

This study was conceived and designed by T.C.T., and enhanced by K.S.B. Lab and field data were collected, analyzed and interpreted by both K.S.B. and T.C.T. The manuscript was written, revised and edited by T.C.T. with help from K.S.B.

Funding

This work was supported, in part, by the National Science Foundation [IBN01-37883 to T.C.T.].

References

- Amoser, S. and Ladich, F. (2005). Are hearing sensitivities of freshwater fish adapted to the ambient noise in their habitats? *J. Exp. Biol.* **208**, 3533–3542.
- Blum, S. D. (1988). Osteology and phylogeny of the Chaetodontidae (Pisces: Perciformes). 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, *Hemitaenichthys 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.
- Braun, C. B. and Coombs, S. (2010). Vibratory sources as compound stimuli for the octavolateralis systems: dissection of specific stimulation channels using multiple behavioral approaches. *J. Exp. Psychol. Anim. Behav. Processes* **36**, 243–257.
- 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-Verlag.
- Chapman, C. J. (1973). Field studies of hearing in teleost fish. *Helgol. Wiss. Meer.* **24**, 371–390.
- Chapman, C. J. and Hawkins, A. D. (1973). A field study of hearing in the cod, *Gadus morhua* L. *J. Comp. Physiol. A* **85**, 147–167.
- Chapman, C. J. and Sand, O. (1974). Field studies of hearing in two species of flatfish *Pleuronectes platessa* (L.) and *Limanda limanda* (L.) (Family Pleuronectidae). *Comp. Biochem. Physiol. A Physiol.* **47**, 371–385.
- Coombs, S. and Popper, A. N. (1979). Hearing differences among Hawaiian squirrelfish (family Holocentridae) related to differences in the peripheral auditory system. *J. Comp. Physiol. A* **132**, 203–207.
- Egner, S. A. and Mann, D. A. (2005). Auditory sensitivity of sergeant major damselfish *Abudefduf saxatilis* from post-settlement juvenile to adult. *Mar. Ecol. Progr. Ser.* **285**, 213–222.
- Fay, R. R. (1988). *Hearing in Vertebrates: A Psychophysics Databook*. Winnetka, IL: Hill-Fay Associates.
- Fletcher, L. B. and Crawford, J. D. (2001). Acoustic detection by sound producing fishes (Mormyridae): the role of gas-filled tympanic bladders. *J. Exp. Biol.* **204**, 175–183.
- 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.
- Hawkins, A. D. and Sand, O. (1977). Directional hearing in the median vertical plane by the cod. *J. Comp. Physiol. A* **122**, 1–8.
- Higgs, D. M. and Radford, C. A. (2013). The contribution of the lateral line to 'hearing' in fish. *J. Exp. Biol.* **216**, 1484–1490.
- Horodysky, A. Z., Brill, R. W., Fine, M. L., Musick, J. A. and Latour, R. J. (2008). Acoustic pressure and particle motion thresholds in six sciaenid fishes. *J. Exp. Biol.* **211**, 1504–1511.
- 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-Verlag.
- Kennedy, E. V., Holderied, M. W., Mair, J. M., Guzman, H. M. and Simpson, S. D. (2010). Spatial patterns in reef-generated noise relate to habitats and communities: evidence from a Panamanian case study. *J. Exp. Mar. Biol. Ecol.* **395**, 85–92.
- Kenyon, T. N., Ladich, F. and Yan, H. Y. (1998). A comparative study of hearing ability in fishes: the auditory brainstem response approach. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **182**, 307–318.
- Ladich, F. (1999). Did auditory sensitivity and vocalization evolve independently in otophysan fishes? *Brain Behav. Evol.* **53**, 288–304.
- Ladich, F. (2000). Acoustic communication and the evolution of hearing in fishes. *Philos. Trans. R. Soc. B Biol. Sci.* **355**, 1285–1288.
- Ladich, F. (2013). Effects of noise on sound detection and acoustic communication in fishes. In *Animal Communication and Noise* (ed. H. Brumm), pp. 65–90. Berlin Heidelberg: Springer.
- Ladich, F. (2014). Diversity in hearing in fishes: ecoacoustical, communicative, and developmental constraints. In *Insights from Comparative Hearing Research. Springer Handbook of Auditory Research*, Vol. 49 (ed. C. Köppl, G.A. Manley, A. N. Popper and R.R. Fay), pp. 289–321. 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 Yan, H. Y. (1998). Correlation between auditory sensitivity and vocalization in anabantoid fishes. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **182**, 737–746.
- Lammers, M. O., Brainard, R. E., Au, W. W. L., Mooney, T. A. and Wong, K. B. (2008). An ecological acoustic recorder (EAR) for long-term monitoring of biological and anthropogenic sounds on coral reefs and other marine habitats. *J. Acoust. Soc. Am.* **123**, 1720–1728.
- Lechner, W. and Ladich, F. (2008). Size matters: diversity in swim bladders and Weberian ossicles affects hearing in catfishes. *J. Exp. Biol.* **211**, 1681–1689.
- Lugli, M., Yan, H. Y. and Fine, M. L. (2003). Acoustic communication in two freshwater gobies: the relationship between ambient noise, hearing thresholds and sound spectrum. *J. Comp. Physiol. A* **189**, 309–320.
- Mann, D. A. and Lobel, P. S. (1997). Propagation of damselfish (Pomacentridae) courtship sounds. *J. Acoust. Soc. Am.* **101**, 3783–3791.
- 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.
- Parmentier, E., Boyle, K. S., Berten, L., Brié, C. and Lecchini, D. (2011a). Sound production and mechanism in *Heniochus chrysostomus* (Chaetodontidae). *J. Exp. Biol.* **214**, 2702–2708.
- Parmentier, E., Mann, K. and Mann, D. (2011b). Hearing and morphological specializations of the mojarra (*Euclinostomus argenteus*). *J. Exp. Biol.* **214**, 2697–2701.
- Radford, C. A., Stanley, J. A. and Jeffs, A. G. (2014). Adjacent coral reef habitats produce different underwater sound signatures. *Mar. Ecol. Progr. Ser.* **505**, 19–28.
- Ramcharitar, J. U., Higgs, D. M. and Popper, A. N. (2006). Audition in sciaenid fishes with different swim bladder-inner ear configurations. *J. Acoust. Soc. Am.* **119**, 439–443.
- 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 Karlsen, H. E. (2000). Detection of infrasound and linear acceleration in fishes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**, 1295–1298.
- Schellart, N. A. M. and Popper, A. N. (1992). Functional aspects of the evolution of the auditory system of actinopterygian fish. In *The Evolutionary Biology of Hearing* (ed. D. Webster, A. N. Popper and R. R. Fay), pp. 295–322. New York: Springer-Verlag.
- Schulz-Mirbach, T., Metscher, B. and Ladich, F. (2012). Hearing abilities—a case study on Asian and African cichlids. *PLoS ONE* **7**, e42292.
- Simpson, S. D., Meekan, M., Montgomery, J., McCauley, R. and Jeffs, A. (2005). Homeward sound. *Science* **308**, 221.
- Tavolga, W. N. and Wodinsky, J. (1963). Auditory capacities in fishes. Pure tone thresholds in nine species of marine teleosts. *Bull. Am. Mus. Nat. Hist.* **126**, 177–240.
- Tricas, T. C. (1989a). Determinants of feeding territory size in the coralivorous butterflyfish, *Chaetodon multicinctus*. *Anim. Behav.* **37**, 830–841.
- Tricas, T. C. (1989b). Prey selection by coral-feeding butterfly-fishes: strategies to maximize the profit. *Environ. Biol. Fish.* **25**, 171–185.
- Tricas, T. C. and Boyle, K. S. (2014). Acoustic behaviors in Hawaiian coral reef fish communities. *Mar. Ecol. Progr. Ser.* **511**, 1–16.
- Tricas, T. C. and Boyle, K. S. (2015). Sound production and spectral diversity in the social behavior of *Chaetodon* butterflyfishes. *J. Exp. Biol.* **218**, 1572–1584.
- 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.
- Urick, R. J. (1983). *Principles of Underwater Sound*. New York: McGraw-Hill.
- 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.
- Webb, J. F., Walsh, R. M., Casper, B. M., Mann, D. A., Kelly, N. and Cicchino, N.** (2012). Development of the ear, hearing capabilities and laterophysic connection in the spotfin butterflyfish (*Chaetodon ocellatus*). *Environ. Biol. Fish.* **95**, 275-290.
- Wenz, G. M.** (1962). Acoustic ambient noise in the ocean: spectra and sources. *J. Acoust. Soc. Am.* **34**, 1936-1956.
- Woods, C. F.** (2006). Swim bladder morphology in chaetodontid butterflyfishes with a discussion of its bioacoustic significance. MS Thesis, Biology, Villanova University, Villanova, PA, USA.
- Wright, K. J., Higgs, D. M., Cato, D. H. and Leis, J. M.** (2010). Auditory sensitivity in settlement-stage larvae of coral reef fishes. *Coral Reefs* **29**, 235-243.
- Wysocki, L. E. and Ladich, F.** (2003). The representation of conspecific sounds in the auditory brainstem of teleost fishes. *J. Exp. Biol.* **206**, 2229-2240.
- Yan, H. Y. and Curtsinger, W. S.** (2000). The otic gasbladder as an ancillary auditory structure in a mormyrid fish. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **186**, 595-602.
- Yan, H. Y., Fine, M. L., Horn, N. S. and Colon, W. E.** (2000). Variability in the role of the gas bladder in fish audition. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **186**, 435-445.