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Pulse sound generation, anterior swim bladder buckling and associated muscle activity in the pyramid butterflyfish, *Hemitaurichthys polylepis*

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SUMMARY

Acoustic behaviors are widespread among diverse fish taxa but mechanisms of sound production are known from relatively few species, vary widely and convergent mechanisms are poorly known. We examined the sound production mechanism in the pyramid butterflyfish, *Hemitaurichthys polylepis*, a member of the socially and ecologically diverse reef fish family Chaetodontidae. In the field, fish produce pulse trains at dusk during social interactions that are probably related to mate attraction and courtship. In laboratory experiments, sound production was synchronized to high-speed video to determine body movement associated with sound generation. In addition, electromyography (EMG) recordings tested the activity of six candidate muscles. Fish produced individual pulses with a mean peak frequency of 97 Hz in rapid succession. EMG experiments show that anterior hypaxial muscles contract at high bilaterally synchronous rates (up to 120 Hz) in near perfect association with rapid inward buckling visible outside the body over the anterior swim bladder. Muscle activity often showed EMG doublets that occurred within the time of a single sound pulse but was not sustained. Buckling and sound pulse rates correlated strongly ($R^2 \approx 1.00$) and sound pulse rate measured over two successive pulses (maximum of 38 pulses s^{-1}) was lower than muscle firing rate. These results show that the extrinsic swim bladder muscles of pyramid butterflyfish involve single contractions that produce pulses in a manner similar to distantly related teleosts, but involve a novel doublet motor-neuron firing pattern. Thus, the sound production mechanism in pyramid butterflyfish is likely convergent with several percomorph taxa and divergent from the related chaetodontid genus *Forcipiger*.

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Key words: Chaetodontidae, sound production, sonic muscle, electromyography, EMG, sonic mechanism, hypaxial muscle.

INTRODUCTION

Teleost fish have evolved a wide array of mechanisms for the production of sound in acoustic communication. It is probable that these diverse mechanisms evolved independently several times (Ladich, 2001; Ladich and Bass, 2003; Ladich and Bass, 2005). Some evidence exists for a conserved developmental pattern of vocal musculature and innervation among ray-finned fish and tetrapods (Bass et al., 2008); however, data on developmental morphology of actinopterygian fish are limited to few taxa. Although sound production is not described for most fish, acoustic communication is widespread and occurs among phylogenetically diverse lineages (Ladich, 2001). Despite the independent origins of fish sound-production structures, many species utilize muscle-driven contractions of the compressible, gas-filled swim bladder as a sound source (Zelick et al., 1999).

Swim bladder muscles for sound production are classified as either intrinsic or extrinsic on the basis of their association with the swim bladder (Tavolga, 1971). Intrinsic swim bladder muscles insert entirely on the swim bladder. However, extrinsic swim bladder muscles originate elsewhere on the body, such as the occipital region of the neurocranium, on trunk musculature and associated bones or both, and insert on or are positioned adjacent to the swim bladder. The antagonistic mechanism for these sonic muscles is the swim bladder tunic (Demski et al., 1973). Sound-production muscles in teleost fish have evolved independently and their homologies with generalized teleost musculature are not known entirely; however, in many cases they appear to be derived from hypaxial or epaxial trunk musculature (Winterbottom, 1974).

Fish with intrinsic sonic muscles, such as the oyster toadfish *Opsanus tau* (Skoglund, 1961; Fine et al., 2001) and plainfin midshipman *Porichthys notatus* (Cohen and Winn, 1967; Bass and Baker, 1991), often are characterized by high, synchronous contraction rates that correspond to the frequency of the sound produced. In the northern sea robin *Prionotus carolinus*, however, antiphasic bilateral firing produces sounds with fundamental frequencies at twice the contraction rate (Bass and Baker, 1991; Connaughton, 2004). In the few species examined with extrinsic swim bladder muscles, such as the southern pigfish *Congiopodus leucopaecilus* (Packard, 1960), the longspine squirrelfish *Holocentrus rufus* and squirrelfish *H. adscensionis*) (Winn and Marshall, 1963; Gainer et al., 1965), the bigscale soldierfish *Myripristis berndti* (Salmon, 1967) and the weakfish *Cynoscion regalis* (Connaughton et al., 2000), pulse emission rates correspond to the firing frequency of the muscles. The fundamental frequency of these single muscle-twitch pulses is hypothesized to be related more to the duration of muscle contraction than to the resonance of the swim bladder, which is highly damped by the surrounding fish tissues (Connaughton et al., 2000) and can be modeled as an impedance-matching device between the sonic musculature and the

surrounding water medium (Sprague, 2000). Thus, the firing rate of weakfish muscles sustained over a fish call is much lower (20 Hz) than those of toadfish (200 Hz) over the course of a toadfish boatwhistle sound (Connaughton et al., 2000).

The butterflyfish family (Chaetodontidae) includes 11 genera and ~122 species that occur on reefs in tropical and temperate seas (Nelson, 2006). Several members of this family have been shown recently to produce social sounds (Tricas et al., 2006; Boyle and Tricas, 2009). In the pebbled (multiband) butterflyfish, *Chaetodon multicinctus*, agonistic sound production includes hydrodynamic stimuli produced in part from strong, caudal flexion, pulsatile sounds similar to sounds that involve the swim bladder, and broadband click-like signals that are consistent with a stridulatory mechanism (Tricas et al., 2006), but the proximate mechanisms remain unclear. Within the butterflyfish bannerfish clade (*sensu* Fessler and Westneat, 2007), members of the genus *Forcipiger* produce short, pulsatile sounds that are associated with rapid dorsal elevation of the head, anterodorsal motion of the ventral pectoral girdle and dorsal elevation of the caudal skeleton that elongate the body cavity and likely stimulate sound emission from the swim bladder (Boyle and Tricas, 2009) (K.S.B. and T.C.T., unpublished data).

The presence of extrinsic or intrinsic swim bladder musculature has not yet been reported for any butterflyfish. Several morphological studies exist on the swim bladder and associated musculoskeletal morphology of the chaetodontid genera (*Chelmon*, *Forcipiger*, *Hemitaurichthys* and *Johnrandallia*), in the context of putative lateral line and auditory function of the laterophysic connection that is unique to *Chaetodon* (Webb, 1998; Webb and Smith, 2000; Smith et al., 2003; Webb et al., 2006). Despite the morphological diversity in features of potential importance for sound reception, descriptions of structures around the swim bladder associated with sound production are unknown.

In this study, we describe and compare the gross anatomy, muscle activity and sound production of the extrinsic swim bladder sonic mechanism for the pyramid butterflyfish, *Hemitaurichthys polylepis*. We examined the spectral and temporal patterns of sound emission in recordings taken in the field on Hawaiian coral reefs and the laboratory. In the laboratory, we recorded sounds synchronized with high-speed video to determine the pattern of movement of the body and underlying swim bladder. Electromyography (EMG) was conducted on free-swimming fish to identify: (1) the muscle activity associated with sound emission, (2) bilateral synchrony of activity and (3) the relationship between muscle firing, sound emission and body movement. These experiments provide evidence for an extrinsic swim bladder sonic mechanism that is divergent from that in the related genus *Forcipiger* but similar to mechanisms reported in distantly related teleost fish.

MATERIALS AND METHODS

Field observations of sound production behavior

Field and laboratory experiments were conducted on the pyramid butterflyfish, *Hemitaurichthys polylepis* (Bleeker), a zooplanktivorous species with an Indo-Pacific distribution (Randall, 2007). Fish were observed during summer months (June–August) in 2008 and 2009 along a reef drop-off at ~20 m depth on the west coast of the island of Hawaii (Puako, 19°58′6″N, 155°51′11″W). Fish were observed during periods of intense courtship activity from 15.00 to 19.00 h by divers using closed-circuit rebreathers to mitigate bubble exhaust noise associated with scuba. Field temperatures ranged between 24 and 28°C. Fish behavior and sounds were recorded on digital video tape with either a Sony TRV-950 camera (manual audio gain control; Tokyo, Japan) in an Amphibico housing (Montreal, QC, Canada) connected to an external hydrophone (HTI min96; High-Tech, Gulfport, MS, USA) extended from the camera on a 1 m PVC tube or with a Canon Optura camera (automatic audio gain control; Tokyo, Japan) in an Amphibico housing connected to a hydrophone. Video and audio recordings were imported into a PC computer and audio recordings were extracted using Cool Edit Pro 2.0 (Syntrillium, Phoenix, AZ, USA). Tonal camera hum noise from the Sony TRV-950 was digitally filtered with a notch filter at 149.8 Hz, 100 dB attenuation, with the super-narrow notch-width setting in Cool Edit Pro 2.0. Sound files were analyzed in the same manner as laboratory sound data (see below).

Laboratory sound production experiments

Fish were collected for laboratory experiments from the main Hawaiian Islands by commercial suppliers. Experiments were conducted in a 110 l aquarium (76 cm wide, 30 cm deep, 46 cm high) with flow-through seawater, which was turned off during experiments, at a temperature of 28°C. Water level was kept low (~20 cm deep, 43% of aquarium capacity). Fish sounds were elicited from solitary fish in the aquarium either when an observer approached the aquarium in a well-lit room or by introducing a conspecific into the aquarium. These sounds were pooled for analysis because no differences were observed between their acoustic features (one individual produced multiple sounds in both behavioral contexts; Mann–Whitney *U*-tests for differences in duration, peak frequency, median frequency and sound pressure level $P>0.05$). Sounds from fish in the aquarium were detected with a calibrated Brüel and Kjær 8103 hydrophone (–211 dB re. 1 V µPa^{-1}; Nærum, Denmark; connected to a Nexus conditioning amplifier with gain set to 31.6 mV Pa^{-1}; Nærum, Denmark) positioned ~3 cm from the end wall of the aquarium. Sounds were recorded digitally with a CED Micro 1401 data acquisition unit and Spike2 software (Cambridge Electronic Design, Cambridge, UK) sampled initially at 40 k sample s^{-1}. Sound files were then low-pass filtered in Cool Edit Pro 2.0 and downsampled at 4 kHz using the high-quality setting. This spectrum is well below the minimum resonance frequency of 4574 Hz calculated for the aquarium at the 20 cm water depth (Akamatsu et al., 2002).

Body kinematics associated with sound production was recorded on high-speed video. Subjects were illuminated in the aquarium with four 500 W quartz halogen lights. During experiments, sound production events were pre-trigger-recorded at 300, 600 and 1200 frames s^{-1} at resolutions of 512×384, 432×192 and 336×96 pixels, respectively, using a Casio Ex-F1 Exilim camera (Tokyo, Japan). Sound and EMG data (see below) were synchronized to video with a flasher circuit in which LEDs were recorded visually by the camera while square pulses were digitized and recorded simultaneously on the hydrophone channel in Spike2.

EMG recording experiments

Contraction activity was determined for candidate sonic muscles using EMG recordings in free-swimming subjects. Bipolar recording electrodes were made from pairs of 0.05 mm insulated tungsten wire (California Fine Wire, Grover Beach, CA, USA) in which the insulation at the tip (1 mm) of each wire was removed, the wire was inserted into a 28 gauge hypodermic needle and the exposed tips were bent back into hooks. Fish were anesthetized with 100 mg l^{-1} of tricaine methanesulfonate (MS-222; Argent Labs, Redmond, WA, USA) and ventilated with seawater and anesthetic solution while the electrodes were implanted and the hypodermic needle tips were removed. A loop of surgical silk suture thread was inserted in the

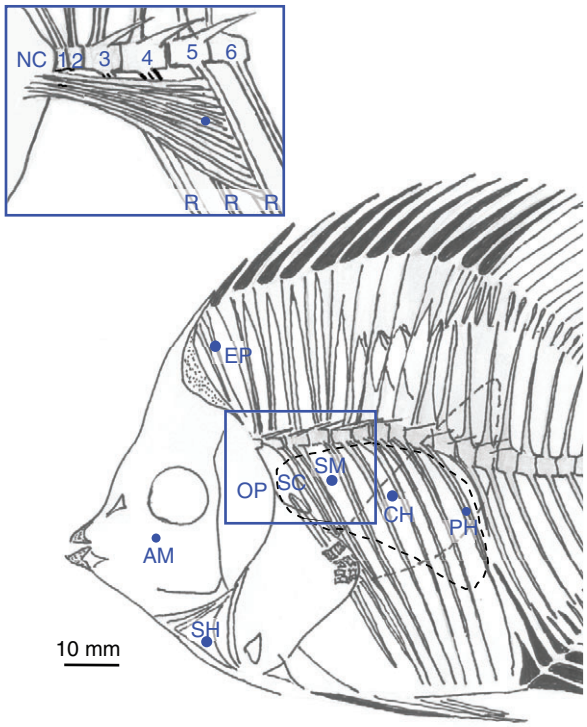

Fig. 1. Location of sonic muscle used during sound production by the pyramid butterflyfish *Hemitaurichthys polylepis*. Note that the sonic muscle is attached to the caudal neurocranium and rib of the fifth vertebra. Blue circles indicate the approximate location of bipolar electromyography recording electrodes relative to the skeleton and body of experimental fish. Short dashed line (black) indicates the location of the swim bladder; long dashed line (gray) indicates the location of the pectoral fin rays. Inset shows expanded view of the sonic musculature with the opercle, supracleithrum and cleithrum bones removed. AM, A1 of adductor mandibulae; C, cleithrum; CH, central hypaxialis; EP, anterior epaxialis; NC, neurocranium; OP, opercle; PH, posterior hypaxialis; R, rib; SC, supracleithrum; SH, sternohyoideus; SM, sonic muscle. Numbers indicate vertebra number.

dorsal trunk musculature, tied and glued with cyanoacrylate around both electrodes for strain relief to prevent dislodgement of the electrodes. EMG recordings were amplified in a four-channel differential amplifier (AM Systems, Sequim, MA, USA) with 10,000× gain and band-pass filtered between 100 and 5000 Hz with a 60 Hz notch filter. Up to four concurrent EMG recordings were digitized at 10 kHz with the CED Micro1401 using Spike2.

Based on observations of a related butterflyfish, *Forcipiger flavissimus* (K.S.B. and T.C.T., unpublished), EMG electrodes were placed (Fig. 1) in the anterior epaxial musculature (fish 1 and 2) ~0.5 cm caudal to the supraoccipital bone; in the sternohyoideus (fish 1 and 2) at the level of the caudal portion of the urohyal; in the A1 of the adductor mandibulae (fish 1); in the hypaxial musculature (fish 2) at the level of the swim bladder and approximate middle of the body cavity (i.e. central hypaxial musculature); and in the hypaxial musculature (fish 1 and 2) at the caudal end of the body cavity over the swim bladder (i.e. posterior hypaxial musculature). After observing the kinematic activity over the anterior body cavity at the rostral end of the swim bladder (see Results) and recording EMG waveforms from the above muscles, EMG electrodes in fish 2 were placed shallowly (1–2 mm below the dermis) and bilaterally in the putative sonic muscle (see Results) in the hypaxial musculature between the pleural ribs of the fourth and fifth vertebrae immediately caudal to and behind the pectoral girdle (Fig. 1). Putative sonic muscle EMG electrodes were implanted in the left and right sides of fish 3 and in the left side only of fish 4. After recovery from anesthesia, fish were placed in the aquarium setup as described above.

Sound and EMG analyses

Sound waveforms were visually inspected in Cool Edit Pro 2.0 software to determine duration (relative to background noise). Pulses in trains with silent periods between them were considered as separate sounds. Sound spectrograms, power spectra, and intensity measurements were estimated using custom Matlab 7.0 scripts (MathWorks, Natak, MA, USA). Sound power spectra were determined from 1024-point fast Fourier transforms (FFT) with a Hanning window of zero-padded sounds. From power spectra, peak frequency (frequency with the highest intensity) and median 10 dB frequency (the median frequency value of all frequencies of the power spectrum that were within 10 dB intensity of the peak frequency) were determined. Sound pressure level (SPL) from sounds in laboratory experiments was estimated from the root-mean-square pressure level of sound waveforms. Sounds were recorded from free-swimming fish, thus distance to the hydrophone was variable and could not be determined from our video. SPL in a shallow aquarium is likely to decrease with distance between the theoretical extremes of cylindrical and spherical spreading (~3 and 6 dB per doubling of distance, respectively) (Mann, 2006), thus SPL values should be considered as estimates. Most sound events probably occurred within 20 cm of the hydrophone and low intra-individual variability was found (interquartile variability for each individual ranged from 4 to 7 dB).

Sound events sometimes included pulses in close succession, with no silent interpulse intervals (see Results). To determine the timing and duration of these individual pulses and the timing relative to EMG events, full sample sounds were high-pass filtered at 20 Hz to remove the low-frequency noise that occurred on some events, rectified and smoothed with a timing constant of 0.02 s using Spike2 software. EMG data were rectified and smoothed with a time constant of 0.002 s. The timing onset and offset of individual pulses and EMG firings were then identified using the time at which the rectified waveform was 50% of the maximum rectified amplitude. Timing of body musculature movement (inward buckling) over the anterior swim bladder (see Results) relative to hydrophone and EMG data was determined from frame-by-frame examination of video in QuickTime 7.5 (Apple, Cupertino, CA, USA).

Statistical analyses

Means and s.e.m. were determined from averages of each individual for sound and EMG features. Differences between acoustic features from field and laboratory recordings were tested with a two-sample *t*-test or with a Mann–Whitney *U*-test when assumptions of normality and homogeneity of variance were not met. Inter-individual differences in acoustic parameters (sound duration, pulse duration, peak frequency, median 10 dB frequency and SPL) from laboratory experiments failed assumptions of equal variance and thus were tested with Kruskal–Wallis one-way ANOVAs followed by Dunn's *post hoc* tests. EMG timing onset relative to pulse onset, buckling onset and left sonic muscle *vs* right sonic muscle onsets were tested by multiple regression, in which a subject factor (individual fish) and interaction term (fish × onset) were included. Sonic muscles often fired multiply (usually twice, see Results) and thus the first and second firings associated with the nearest sound were tested separately. Differences between putative sonic muscles

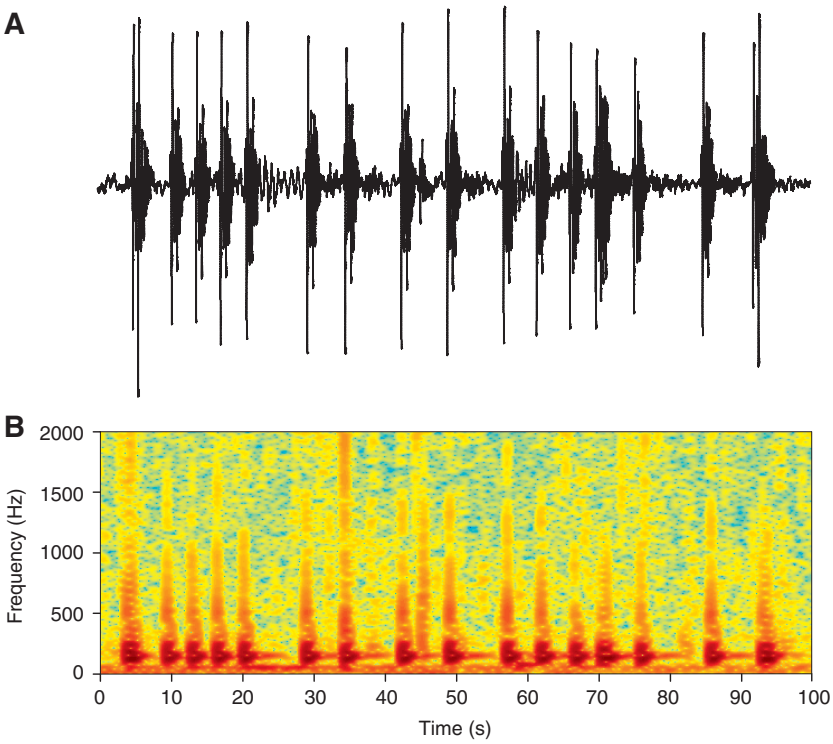

Fig. 2. Representative pulse train sound produced by a free-swimming pyramid butterflyfish that approached a heterospecific under a coral ledge. (A) Oscillogram and (B) spectrogram show the repeated pulses (16) emitted over a 2.1-s period. The sound spectrum includes frequency components near 1000 Hz, with the strongest intensity near 100 Hz. Spectrogram settings: 1024 point fast Fourier transform, 2.5% window length, 95% window overlap.

and non-sonic muscles in the absolute firing onset (|EMG onset – pulse onset|) were tested among 10 groups (sonic muscles from three fish, epaxial muscle from two fish, sternohyoideus from two fish, anterior hypaxial muscle from one fish and posterior hypaxial muscle from two fish). The absolute firing onsets did not meet assumptions of equal variance and thus Kruskal–Wallis test followed by Dunn's *post hoc* test was used to assess differences. All statistical tests were conducted in Minitab v. 13.0. Multiple comparisons from the 20 conducted tests were corrected with a sequential Bonferroni procedure to an adjusted family-wide α of 0.05 (Rice, 1989). *P*-

Table 1. Acoustic properties of pyramid butterflyfish sounds recorded in the field and during laboratory experiments

	N	*n*	*n* Range	Mean ± s.e.m.
Sound duration (s)				
Field (total)	6	114	8–40	0.083±0.025
Field (agonistic courtship)	2	115	7–8	0.091±0.074
Field (agonistic to heterospecifics)	2	33	9–24	0.113±0.084
Field (reacting to diver presence)	4	66	9–27	0.106±0.019
Lab	4	822	87–307	0.194±0.031
Pulse duration (s)				
Field (total)	6	142	8–50	0.038±0.004
Field (agonistic courtship)	2	17	8–9	0.045±0.010
Field (agonistic to heterospecifics)	2	41	9–32	0.034±<0.001
Field (reacting to diver presence)	4	84	9–9	0.035±0.002
Lab	4	1027	104–339	0.058±0.006
Peak frequency (Hz)				
Field (total)	6	114	8–40	116±21.1
Field (agonistic courtship)	2	115	7–8	155±17.0
Field (agonistic to heterospecifics)	2	33	9–24	143±13.4
Field (reacting to diver presence)	4	66	9–27	94±24.2
Lab	4	822	8–307	97±32.6
Median 10 dB frequency (Hz)				
Field (total)	6	114	8–40	182±32.2
Field (agonistic courtship)	2	115	7–8	232±94.9
Field (agonistic to heterospecifics)	2	33	9–24	176±45.1
Field (reacting to diver presence)	4	66	9–27	138±2.4
Lab	4	822	87–307	116±26.2
SPL (dB re. 1 μPa)				
Lab	4	822	87–307	123±3.7

N, number of individual fish observed for each category; *n*, number of events per category; *n* Range, range of *n* observed per individual fish.
Data are means from individual fish averages.
Categories: Field, field observations (behavioral context); Field (total), average of all field sounds pooled per individual; Lab, laboratory experiments.
SPL, sound pressure level.

values for most of the 16 statistical tests used in this study were very low and a type I family-wide alpha level of 0.0125 was calculated after the sequential Bonferroni procedure.

Pulse-emission-frequency distributions in bins of 2.5 pulses s^{-1} were determined by calculating the instantaneous pulse emission rate determined from two, three, four and five consecutive pulses [1/(duration of pulses + inter-pulse intervals); in s]. A minimum estimate was calculated for the time each pulse rate was sustained (no. successive pulses/emission rate, where emission rate is in pulses s^{-1}). Similarly, distributions of sonic muscle EMG firing rate in bins of 10 events s^{-1} were determined by calculating the instantaneous EMG firing rate for two, three, four, five, and six successive EMG events.

RESULTS

Acoustic behavior

Pyramid butterflyfish were observed in the field producing pulse trains during late-afternoon hours (15.00–19.00 h), at a period when most individuals were closer to the reef (i.e. not high in the water column feeding on plankton) and engaged in courtship behavior. Some individuals, which lacked a swollen abdomen, occupied areas close to the substrate below reef ledges and vigorously chased other non-swollen conspecifics, as well as nearby *Chaetodon miliaris* Quoy & Gaimard and *Acanthurus nigrofuscus* (Forsskål). These pyramid butterflyfish were assumed to be males based on previous observations of synchronized dusk spawning and coincident enlargement of the female abdomen by egg hydration in other Hawaiian chaetodontids (Lobel, 1978; Tricas and Hiramoto, 1989). A total of 114 sounds from six separate individuals were analyzed from field recordings. Pulse train sounds (Fig. 2, Table 1) were emitted during courtship interactions between putative male and female fish, in which the pair would carousel and engage in short chases; during agonistic interactions between adjacent putative males; during agonistic interactions between heterospecifics that entered areas below reef ledges; and when diver observers approached fish beneath ledges (supplementary material Movie 1). Sounds from each behavioral context were acoustically similar in terms of overall sound duration, pulse duration, peak frequency and median 10 dB frequency (Table 1), but the small sample sizes of several contexts (2–4 individuals) precluded any statistical comparisons between different contexts or between contexts and sounds recorded in the laboratory.

Fish held in the aquarium readily produced pulse sounds in the presence of conspecifics and solitary fish produced sounds when approached by observers. A total of 822 sounds were recorded and analyzed from four fish. Pulse sounds were produced singly or in trains (Fig. 3). Sounds recorded in the laboratory were similar to

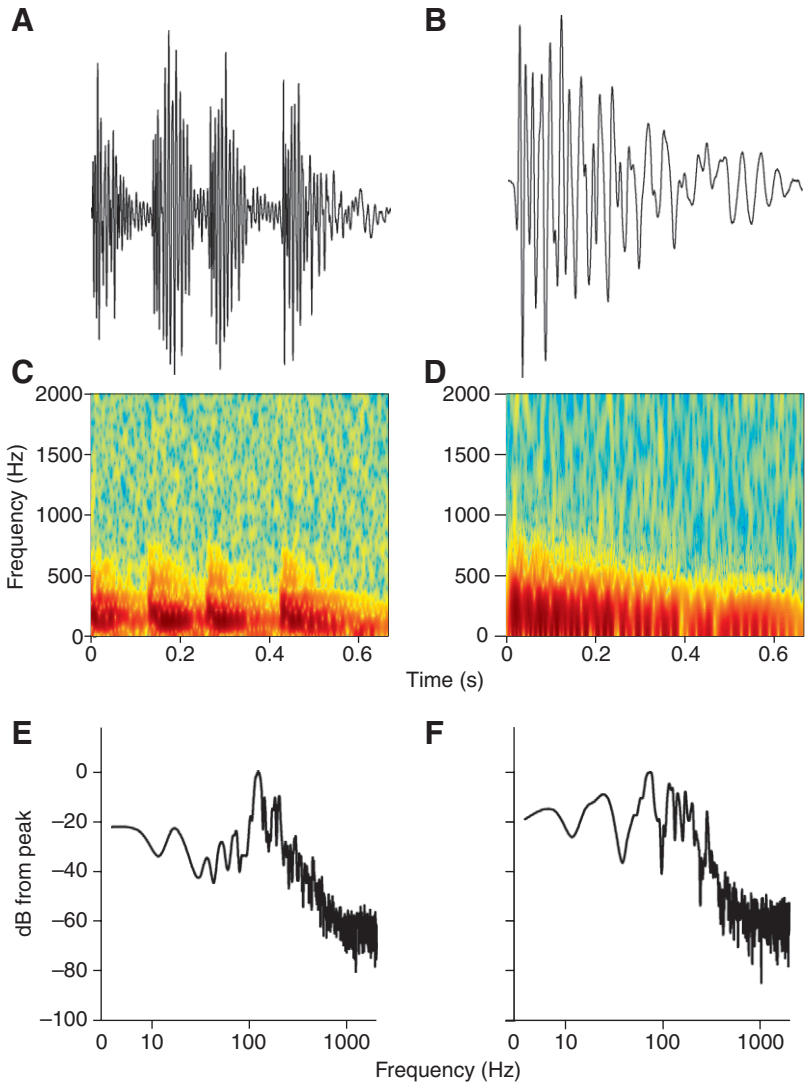

Fig. 3. Acoustic features of individual sound pulses produced in the laboratory by the pyramid butterflyfish. (A,B) Oscillograms, (C,D) spectrograms and (E,F) power spectra from a quadruple pulse train (A,C,E) and single pulse (B,D,F) sound event. Power spectra show peak frequency near 100 Hz [1024 point, zero-padded, Hanning window, fast Fourier transform (FFT)]. Spectrogram settings: 1024 point FFT, 2.5% window length, 95% window overlap.

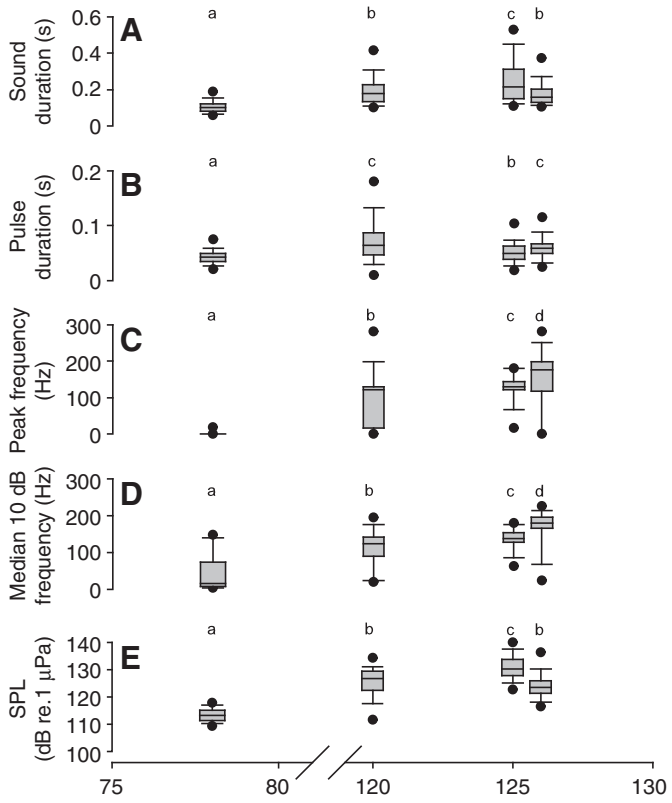

Fig. 4. The relationship between features of sound production and body size in the pyramid butterflyfish. Boxplots (box bounds quartiles and median line, lines extend to 10th and 90th percentiles and points indicate 5th and 95th percentiles) show differences between individual fish in (A) overall sound duration, (B) rectified pulse duration, (C) peak frequency, (D) median 10 dB frequency and (E) sound pressure level (SPL). Kruskal–Wallis tests revealed overall differences between individuals for sound duration (H=362.4, d.f.=3, P<0.0001), pulse duration (H=138.7, d.f.=3, P<0.0001), peak frequency (H=484.9, d.f.=3 P<0.0001), median 10 dB frequency (H=429.2, d.f.=3 P<0.0001) and SPL (H=610.3, d.f.=3 P<0.0001). Letter groups indicate statistically different groups after Dunn's *post hoc* test and sequential Bonferroni correction.

those recorded in the field. Sound waveforms of the pyramid butterflyfish involved an initial low-amplitude deflection of positive or negative polarity, followed by a series of larger-amplitude cycles that decayed exponentially and resulted in pulse durations that often lasted 40 ms. When pulses were emitted in rapid succession, the resulting sound blended together with no silent period. Power spectra indicated that most energy in an overall sound occurred below 500 Hz, with peak frequencies typically below 150 Hz and median 10 dB frequencies below 200 Hz. Spectrograms indicated a general downwards shift in frequency energy as the pulse proceeded and the waveform decayed.

Sounds in the laboratory tended to be longer in duration, as would be expected owing to the quieter recording environment than the field, hydrophone placement closer towards the sound source and, potentially, from reflections on tank walls, but neither overall sound duration nor rectified pulse duration were statistically different after sequential Bonferroni correction (two-sample t-tests, d.f.=4, P=0.053 and d.f.=6, P=0.031, respectively, Bonferroni-adjusted α=0.0125). Both were of low frequency (peak frequency two-sample t-test, d.f.=5, P=0.629; median 10 dB frequency Mann–Whitney U-test, N=6 and 4, P>0.05) with most energy concentrated below 500 Hz (Figs 2, 3, Table 1). Sound pressure-level approximations from the laboratory were high for small reef fish, with a mean of 123 dB re. 1 µPa, a maximum value of 148 dB re. 1 µPa and a minimum value of 104 dB re. 1 µPa. Train sounds were produced in rapid succession and in some cases successive pulses occurred without any period of silence between pulses (Fig. 3A). Thus, individual sounds were composed of varying numbers (from one to seven) of repeating, sometimes blended, low-frequency pulses, with single pulses produced most commonly (mean ± s.e.m., field 91±5%, laboratory 84±6%).

There were several acoustic differences between individual fish (Fig. 4). Most individuals produced statistically different sound and pulse durations, peak frequency, median 10 dB frequency and SPL (Fig. 4). Fish 4 was smaller than the other fish and produced substantially shorter sounds of weaker intensity (Fig. 4). Spectral features (peak frequency and median 10 dB frequency) varied between individuals, with larger fish producing higher-frequency sounds (Fig. 4). These individual differences in sound features, however, did not correspond to differences in timing of muscle activity or body movements (see below).

Muscle and motor buckling activity

High-speed video revealed a unique buckling mechanism that involved a small area of dermal tissue and body musculature (~0.5 cm diameter, 0.2 cm^2) located lateral to the anterior swim bladder and immediately caudal to the dorsal pectoral girdle (Fig. 5; supplementary material Movie 2). Mean visible displacement during inward buckling occurred close to the start of sound emission (mean ± s.e.m., 0.026 ± 0.003 s after sound onset). The region of buckling included the obliquus superioris hypaxial musculature below the dorsal midline, caudal to the supracleithrum and the dorsal cleithrum. Muscle fibers in this area were packed loosely in gross dissection and were less stiff than caudal and ventral hypaxial musculature in fresh and preserved specimens. On the basis of these observations, EMG electrodes were placed in the center of this putative sonic musculature in the area of loose fibers between the pleural ribs of the fourth and fifth vertebrae in order to examine muscle activity in association with sound emission and buckling (Fig. 1). The sonic muscle lies ventral to the midlateral horizontal septum, caudal to the supracleithrum, and ventral to the third visible epaxialis myocomma behind the skull. Fibers of this musculature originate on the pterotic process of the neurocranium, on the posteromedial surface of the cleithrum, on the medial surface of the supracleithrum and on Baudelot's ligament. Fibers insert on the lateral faces of large laminae of the anterior ribs of vertebrae three to five (v3–v5), on a posterolateral myocomma at the level of the rib of v5, and some on the tunica externa of the swim bladder between the enlarged space between the ribs of v4 and 45. Anteriorly, the swim bladder extends to the rib of v3, which is located in a narrow space between the ribs of v3 and v4. Manipulation of muscle fibers of fish specimens lends support to the hypothesis that the large rib of v5 and the attached underlying swim bladder may be pulled anteriorly by contractions of this muscle, which allows for a buckling between the space between the ribs of v4 and v5.

Sonic muscle firing estimated by EMG was characterized by strong amplitude, short duration (Table 2) and occurrence before the onset of sounds (Fig. 6). EMG waveforms recorded from local motor units resembled a single muscle action potential, similar to patterns shown in fish with sonic muscle composed of a single fiber type and with fibers innervated by multiple axons, although this has not been confirmed for this species. Each sound pulse usually occurred with one or two firings (singlets and doublets) (Table 3)

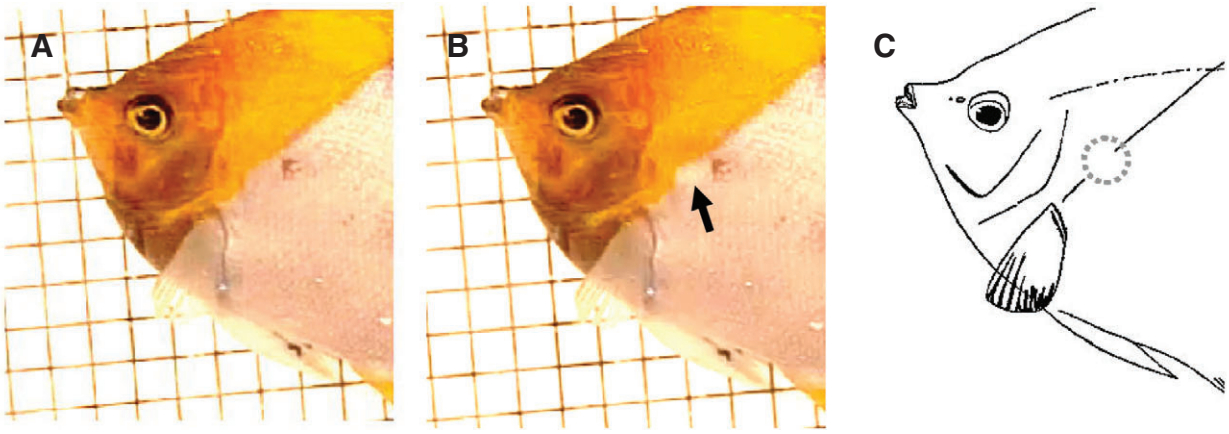

Fig. 5. Kinematic buckling during sound production in the pyramid butterflyfish. (A) Pre-acoustic condition of sonic buckling area from a video image. (B) The same fish 3.3 ms later shows buckling over the region behind the pectoral girdle (arrow). (C) Diagrammatic representation of buckling location highlighted by dashed circle. Data taken from video at 300 frames s^{-1}.

and, for train sounds, often occurred as doublets followed by singlets (Fig. 6). Rectification and smoothing of EMG and sonic waveforms (Fig. 6) allowed for subsequent timing comparisons between EMG, buckling and individual pulses, the latter of which often appeared as a single complex sound with amplitude modulation prior to smoothing.

EMG initiation of sonic muscles, onset time of individual sound pulses measured from rectified and smoothed waveforms, and buckling were all highly correlated (Fig. 7). Several multiple regression models were used to test the relationship between sonic muscle firing onset (dependent variable) and onset of sound emission, second sonic firing and visible buckling (independent variables); these included an individual fish subject factor and an interaction term. In all models, interactions between individual fish and individual firing time did not contribute substantial variation to the models (P>0.05). The onset of first firing of sonic muscles as determined by EMG was highly correlated with the onset of sound emission (R^2≈1.00, F=326,835, d.f.=3, 337, P<0.0001; Fig. 7, x-axis *vs* y-axis). A similar result was found for the second sonic firing, from instances when the sonic muscle fired two or more times per sound (R^2≈1.00, F=1,081,000, d.f.=3, 329, P<0.0001). Similarly, onset of first sonic EMG firing was highly correlated with the onset of visible buckling (R^2≈1.00, F=195,484, d.f.=3, 293, P<0.0001; Fig. 7, z-axis *vs* y-axis). Second sonic muscle firings in instances of doublets or more firings were also highly correlated with buckling (R^2≈1.00, F=203,376, d.f.=3, 226, P<0.0001). EMG recordings from sonic muscles were synchronized and bilateral. Sonic muscle firing onset from left muscles was strongly correlated with buckling (R^2≈0.996, F=21,058, d.f.=3, 230, P<0.0001). In all experiments (EMG and non-EMG), the start of visible buckling was strongly correlated with the start of rectified, smoothed sound waveforms (Fig. 7, z-axis *vs* x-axis). A multiple regression of buckling onset (dependent variable) *versus* sound pulse onset (independent variable) showed that the onset of visible buckling was highly correlated with the onset of sound emission (R^2≈1.00, F=958,412, d.f.=3, 568, P<0.0001).

Sound pulses produced in close succession by a stationary fish showed the same initial phase (positive or negative direction of zero-crossing) as would be expected from an acoustic swim bladder source that involves a consistent first deflection. However, the phase of onset of pulsed sound waveforms differed with location of the recording hydrophone relative to the fish body (Fig. 8). An examination of 37 cases of successive pulses with high signal-to-noise ratios and silence between pulses provided clear zero-crossing estimates and revealed that 86% of positive phase pulses occurred while the head of the fish was oriented towards the hydrophone and 100% of the negative phase pulses occurred while the head of the fish was oriented away from the hydrophone (Fisher's exact test, P<0.001, N=15 negative, 22 positive). This observation indicates that the sound source is more complex than a simple monopole, and is consistent with the hypothesis of rostral expansion of the anterior end of the swim bladder during lateral buckling.

EMG waveforms were recorded from the following additional muscles of the body and head from two individuals during sound production: the anterior epaxialis, the A1 adductor mandibulae, the posterior hypaxial musculature over the swim bladder, the central hypaxial musculature (between the sonic musculature and the posterior recording location) and the sternohyoideus. Unlike the putative sonic muscles, these muscles did not fire consistently during sound emission events and recordings from the A1 did not occur near sound emission. In the instances that the muscles did show activity near sound emission, a comparison of the muscle firing time relative to sound emission onset (|muscle onset time–sound onset|)

Table 2. Sonic muscle electromyography (EMG) firing durations of first and second EMG events in pyramid butterflyfish

Sonic firing	*N*	*n*	*n* Range	Mean ± s.e.m. duration (s)
First	3	442	41–237	0.007±0.002
Second	3	332	31–187	0.005±0.001

First and second sonic firings are the first and second EMG events, respectively, within a single sound pulse emission. *N*, number of individual fish; *n*, number of EMG firings; *n* Range, range of *n* recorded per individual fish.

Table 3. Occurrence of single and multiple electromyography firing types within pulse sound events of pyramid butterflyfish

		Occurrence (%)				
N	*n*	Singlets	Doublets	Triplets	Quadruplets	≥5
3	442	27±3.6	60±8.4	6±1.5	5±3.3	2±1.1

N, number of individual fish; *n*, number of EMG firings.
Values are means ± s.e.m.

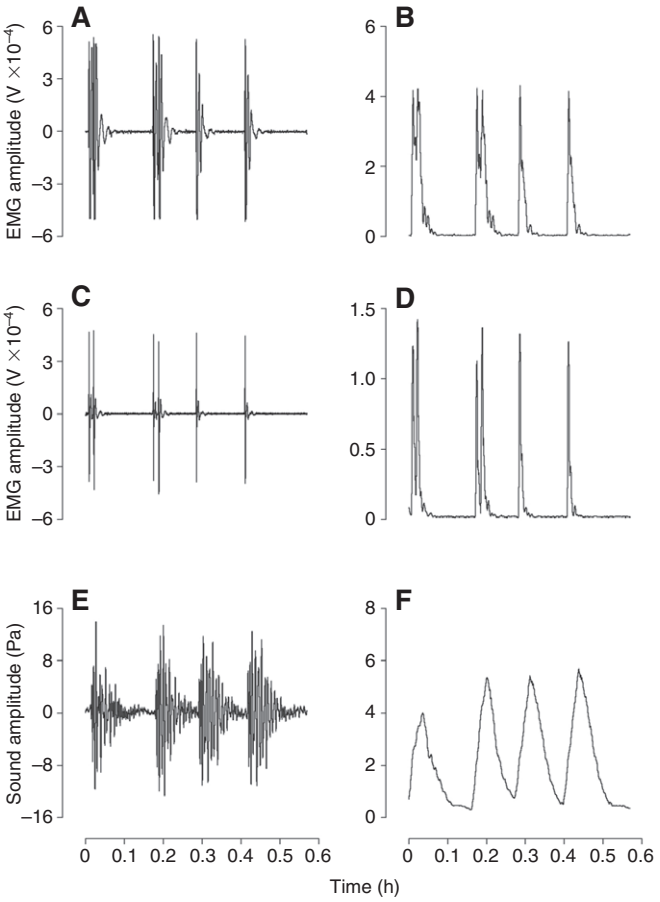

Fig. 6. Muscle activity during sound production by the pyramid butterflyfish. Electromyography recording from the fish's (A) right and (C) left sides. (E) Associated sound waveform. Sound wave form consists of a single pulse sound followed by a triple pulse (single sound consisting of three pulses in rapid succession). Sonic muscles on both sides fired twice for the first sound and the first pulse of the second sound and once for the remaining two pulses. (B,D,F) The same waveforms after rectification and smoothing (see Materials and methods). Onset and offset times of muscle firing and individual pulses were estimated from the time at which the smoothed waveform was half of the maximum intensity for that event.

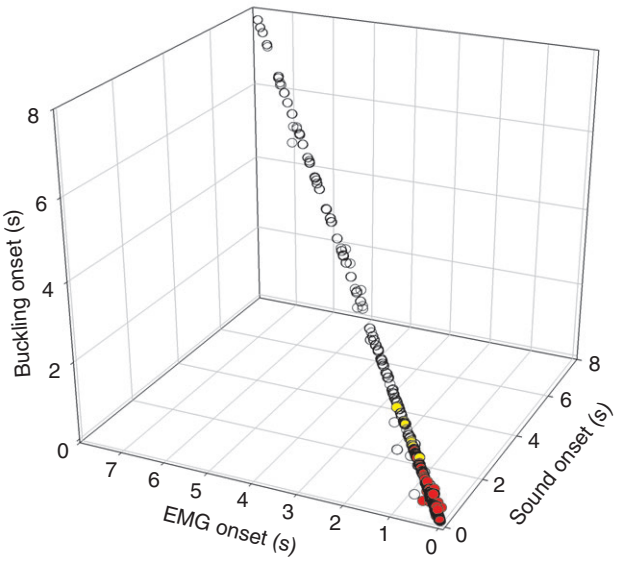

Fig. 7. Relative onset timing of sonic muscle activity, pulse sound onset and visible buckling over the anterior swim bladder during sound production in the pyramid butterflyfish. *x*-axis, sound onset; *y*-axis, left sonic muscle electromyography (EMG) recording start time; *z*-axis, start of visible buckling. Data points from individual fish (*N*=3) are presented in different colors (yellow, red and open circles). Note the strong correlation between sound onset, EMG activity and visible swim bladder buckling.

was conducted which revealed that firing of putative sonic muscles occurred much closer to muscle onset time than these additional muscles. Sonic muscle recordings from all three fish had EMG onset times much closer to sound emission onset time than EMG onset times from the anterior epaxialis, sternohyoideus and posterior hypaxialis muscles (Table 4). These values were statistically different (Kruskal–Wallis, H=652.2, d.f.=9, P<0.001) for all non-sonic muscles tested (Dunn's *post hoc* test, P<0.001 for each fish–muscle combination, N=3 sonic, 6 non-sonic) except central hypaxial muscle (P>0.05), which had a low absolute onset difference, but for which there was a small sample size of 14 EMG waveforms as this musculature only fired 5% of the time during sound emission.

Sound recordings from the laboratory indicated that pyramid butterflyfish were capable of high sound pulse emission rates, but these events were clustered in time and composed of a low number of pulses. Calculation of instantaneous pulse emission rate from two consecutive pulses showed that pulses sometimes occurred in rapid succession (max.=39 pulses s^{-1}) but were sustained briefly (0.051 s). The mode of all pulse emission rates calculated from two successive pulses, however, indicated a lower typical pulse emission rate of 7.5 pulses s^{-1} (sustained for a longer duration of 0.27 s) (Fig. 9A). Pulse emission rates sustained for five consecutive pulses were substantially lower (Fig. 9D). Pulse emission rates recorded from the field (max.=59, mode=17.5 pulses s^{-1} for two consecutive pulses, and max.=19, mode=5.0 pulses s^{-1} for five consecutive pulses) were somewhat higher than rates recorded in the laboratory, but showed a similar pattern. Thus, pulses were emitted at a moderately high rate but were only sustained over short periods.

Sonic muscle firing rates measured across two consecutive EMG events had a maximum firing rate of 500 events s^{-1} and a bimodal distribution because of the presence of EMG doublets on many sounds. Measured across two consecutive firings, sonic muscle firing rate had a high mode of 120 events s^{-1} (sustained briefly, 0.017 s) and a low mode of 10 (sustained for 0.2 s) (Fig. 10A). Firing rates measured across three firings were unimodal, with a maximum of 375 events s^{-1} (sustained for 0.008 s) and a mode of 20 (sustained for 0.15 s) (Fig. 10B). Measured over six firings, there was a maximum firing rate of 13 events s^{-1} (sustained for 0.46 s) and a mode of 10 (sustained for 0.6 s) (Fig. 10E). Thus, sonic muscle firing occurred at high rates that were sustained very briefly during a doublet, and at moderately high rates for short duration, which correspond to multiple doublets fired during a pulse train sound. These high firing rates, however, were not sustained for long periods.

DISCUSSION

This study demonstrates that sound production in the pyramid butterflyfish involves hypaxial musculature and buckling over the anterior swim bladder in a manner that is similar to that reported in distantly related percomorph fish but different from the sonic mechanism in the closely related chaetodontid genus *Forcipiger*. Pyramid butterflyfish produce repeated, short-duration, pulsed sounds with highly localized and previously unreported inward

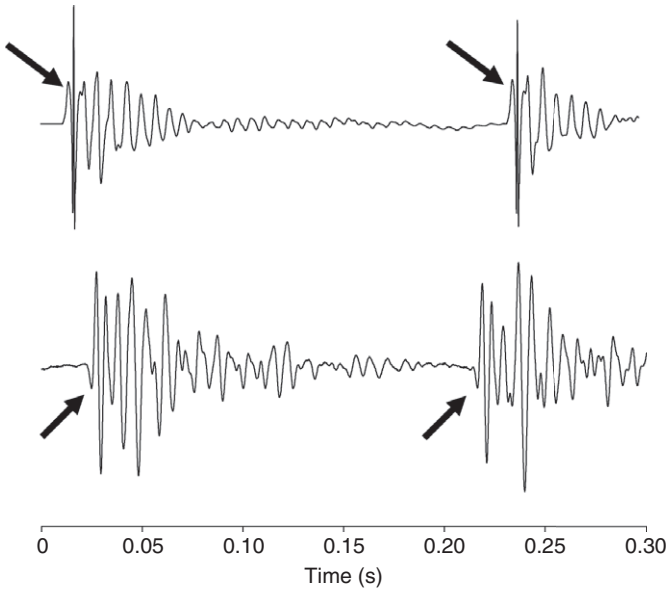

Fig. 8. Representative waveforms with different initial pulse zero-crossings from successive sound by the pyramid butterflyfish at a fixed location with orientation relative to the recording hydrophone. Initial zero-crossings (arrows) in the top trace were positive while the head of the fish was oriented towards the hydrophone. Initial zero-crossings on the bottom trace were negative while the fish was oriented away from the hydrophone.

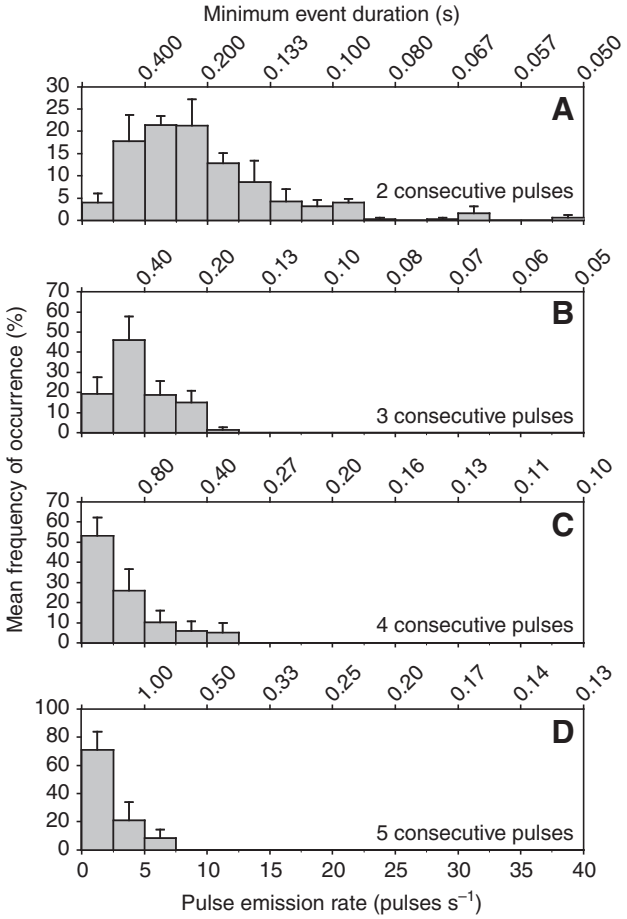

Fig. 9. Variability of sound pulse emission rates in the pyramid butterflyfish. Instantaneous pulse sound emission rate measured over (A) two, (B) three, (C) four and (D) five consecutive pulses. Mean frequency of occurrence (% of total) and error bars (±s.e.m.) of pulse emission rate (2.5 pulses s^{-1}) from four fish are shown on the bottom *x*-axis, and the minimum event duration for which this emission rate is sustained (s) is shown on the top *x*-axis (no. of pulses/duration of consecutive pulse events). Note that pulse emission rates can be moderately high for two consecutive pulses (up to 39 pulses s^{-1}) but are not sustained, indicated by lower emission rates when calculated across three or more consecutive pulses.

buckling over the rostral swim bladder. These experiments also show that sonic muscles fire at high rates, which correspond to pulse train emissions of moderately high rates. Pulse emission and rapid firing rates, however, are not sustained over long periods. These findings appear to be different from previously studied butterflyfish, but bear resemblance to some members of distantly related percomorph taxa (e.g. Congiopodidae, Holocentridae, Sciaenidae) that produce pulse train sounds with fast extrinsic sonic muscles. The pyramid butterflyfish is a member of a monophyletic clade that includes the genera *Forcipiger*, *Heniochus* and *Johnrandallia* (Fessler and Westneat, 2007). *Forcipiger*, unlike *Hemitaurichthys*, produces sounds by rapid cranial elevation and body flexion that include synchronous firing of the anterior epaxialis, sternohyoideus and adductor mandibulae (K.S.B. and T.C.T., unpublished). These muscles fired only occasionally during pyramid butterflyfish sound production experiments from the present study. When they did fire, they were not associated as closely with sound emission, and no cranial elevation was observed.

Table 4. Absolute electromyography (EMG) onset relative to sound start for sonic muscle, anterior epaxialis, sternohyoideus, central hypaxial musculature and posterior hypaxial musculature in pyramid butterflyfish

Muscle	*N*	*n*	*n* Range	Mean ± s.e.m. (s)
Sonic muscle	3	837	100–463	0.021±0.005
Epaxial onset	2	182	21–161	0.125±0.070
Sternohyoideus	2	324	131–193	0.123±0.047
Central hypaxial	1	14	131	0.019
Posterior hypaxial	2	419	62–357	0.235±0.098

N, number of individual fish in which EMG firings were recorded for that muscle; *n*, number of EMG firings per muscle; *n* Range, range of *n* recorded per individual fish.

The acoustic behaviors and ecologies differ in several ways among the pyramid butterflyfish and those reported for the more distant confamilial pebbled butterflyfish. The pyramid butterflyfish is a diurnal planktivore that forms large social groups during the day. Use of closed-circuit rebreathers permitted us to approach closely and record these fish in midwater, but sound production was not detected among fish within these feeding groups. We identified production of the pulse sound only during dusk hours during apparent courtship activity and when approached by divers on the bottom. By comparison, the pebbled butterflyfish forms long-term monogamous pairs that defend permanent coral feeding territories from conspecifics during the day (Tricas, 1989). This species produces at least six different acoustic behaviors during interactions with conspecific territory intruders; these include clicks, pulses and pulse trains in the field (Tricas et al., 2006). Of these, the pulse grunt sound of the pebbled butterflyfish appears most similar to the pulse of the pyramid butterflyfish. This sound was proposed to function as an alert or distress call to the pair mate, has a slightly

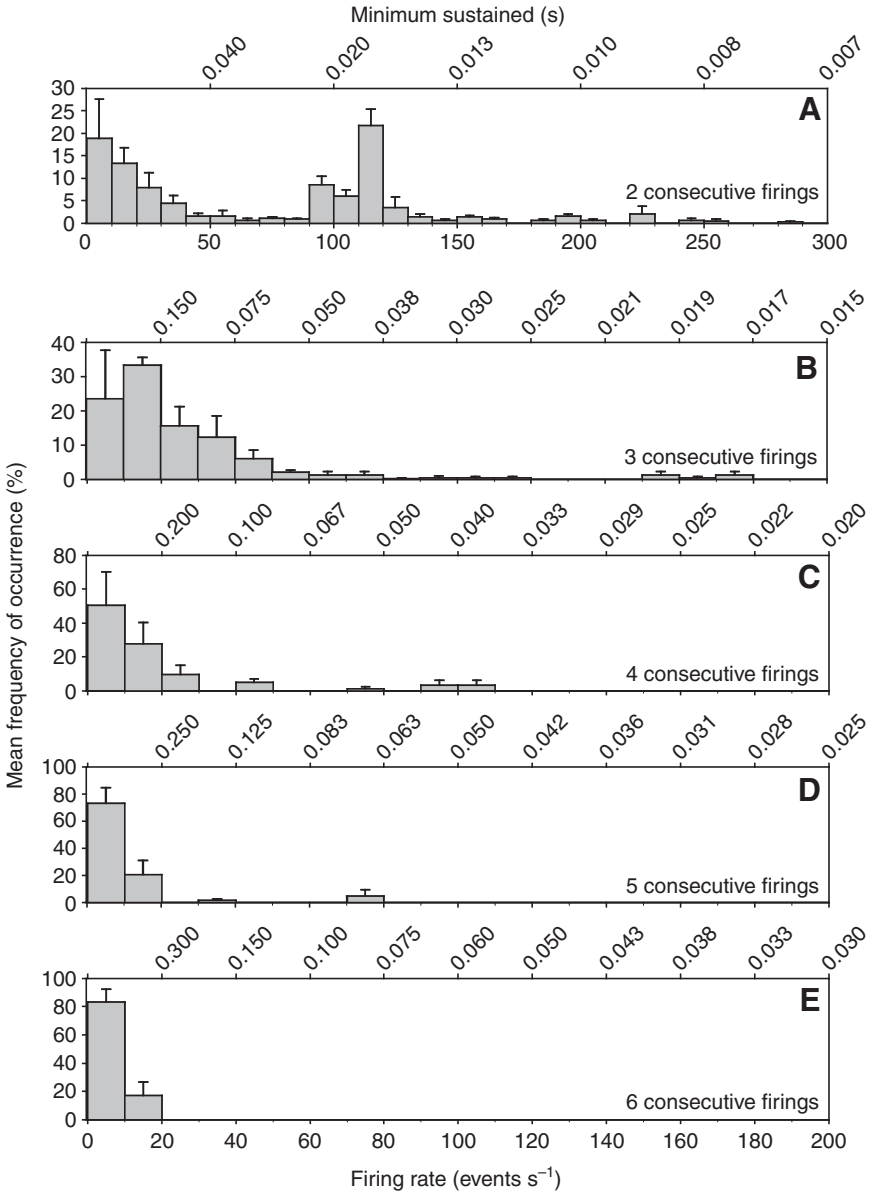

Fig. 10. Variability of sonic muscle firing emission rates in the pyramid butterflyfish. Instantaneous firing rate measured over (A) two, (B) three, (C) four, (D) five and (E) six consecutive firings. Mean frequency of occurrence (% of total) and error bars (±s.e.m.) of firing rate (bins of 10 events s^{-1}) from three fish are shown on the bottom *x*-axis, and the minimum firing duration for which this rate is sustained (no. of firings/duration of successive firing events) is shown on the top *x*-axis (s). Note the skewed and bimodal distribution for instantaneous firing rates (the higher mode at 110 events s^{-1}), which results from the presence of electromyography doublets for some sounds (top). Firing rate calculated across more firings drops to more typical skeletal muscle firing rates of 20 events s^{-1} or less (bottom).

higher peak frequency of 163 Hz, a similar pulse duration of 42 ms and a lower pulse rate (~3 pulses s^{-1}). In addition, it was the only sound reported for *Chaetodon* that was not associated with overt body movement and was proposed to result from the action of internal musculature not directly associated with locomotion (Tricas et al., 2006). More work is needed to determine whether the muscles that produce these similar sounds in *Chaetodon* and other genera are conserved or divergent from those of *Hemitaurichthys*.

Sound emission rates from pyramid butterflyfish in this study were moderately high (up to 38 Hz) for short durations. These sound emission rates are comparable to those reported for teleost fish species that produce similar pulse train sounds. Emission rates were higher than typical repetition rates of southern pigfish (Congiopodidae; 8 Hz) (Packard, 1960), haddock (Gadidae; 8 Hz) (Hawkins and Amorim, 2000) and pearlfish (Carapidae; 7 Hz) (Parmentier et al., 2003), but were comparable to pulse emission rates of weakfish (Sciaenidae; 20 Hz) (Connaughton et al., 2000) and Atlantic croaker (Sciaenidae; ~30 Hz) (Fine et al., 2004; Gannon, 2007), cusk eel sounds (Ophidiidae; up to 25 Hz) (Mann et al., 1997) and jump sounds of the pebbled butterflyfish (20 Hz) (Tricas et al., 2006). Sound emission rates from the present study, however, were not as high as those measured from holocentrids, i.e. squirrelfish (85 Hz) (Winn and Marshall, 1963) and soldierfish (~90 Hz) (Salmon, 1967), or from John dory (Zeidae; 71 Hz) (Onuki and Somiya, 2004). Pyramid butterflyfish pulse emission rates were higher than those of many distantly related ray-finned fish, but still fell within the range known for teleosts.

EMG firing rates in the present study were measured in free-swimming fish and are within the range of those reported for other sonic species. Sonic muscles usually show highly synchronous, short-duration contractions without tetany (Fine et al., 2001). Fish with tonal swim bladder sounds have the highest firing rates and are capable of sustaining activity for long durations, up to several minutes in the plainfin midshipman (Bass and McKibben, 2003). These tonal sounds show fundamental frequencies that correspond either directly to the rate of bilateral muscle contraction (e.g. toadfish and midshipman) (Skoglund, 1961; Cohen and Winn, 1967; Fine et al., 2001) or twice the firing rate of alternating individual muscles (e.g. northern sea robin) (Bass and Baker, 1991; Connaughton, 2004). The sustained sonic muscle firing rates for the pyramid

butterflyfish were lower than for tonal fish species, similar to those measured for weakfish (Connaughton et al., 2000) and southern pigfish (Packard, 1960), but less than the brief sustained levels of squirrelfish (115 firings s^{-1}) (Gainer et al., 1965) and bigscale soldierfish (~120 firings s^{-1}) (Salmon, 1967). These acoustically similar, non-tonal, pulse-train-emitting species produce individual pulse sounds by single muscle contractions. Muscle firing in this study was highly synchronous between the right and left sides of the body, as demonstrated for other fish examined (Packard, 1960; Skoglund, 1961; Cohen and Winn, 1967; Connaughton et al., 2000), except for triglids, which have antiphasic firing (Bass and Baker, 1991; Connaughton, 2004). Pyramid butterflyfish muscle firing rates appear most similar to those of fish species that produce non-tonal, percussive swim bladder sounds, although available data are limited as EMG recordings are reported from relatively few soniferous fish species.

Sonic muscle activity measured in this study most often involved two muscle action potentials in rapid succession. This firing pattern is unusual and was not previously reported for other sonic fish. The rapid sequential firings did not produce sounds that were distinguishable from those produced by single firings, and thus the function remains unclear. Some video sequences showed subtle muscular movement during the buckling period, while the overall buckling area remained inward. This observation, however, was inconsistent and was not obviously associated with single or double firings. The highest typical firing rates measured in this study (120 Hz) were associated with doublets. In toadfish, which have highly apomorphic sonic muscles, a stimulation frequency of 60 Hz causes complete tetany in skeletal muscle but still produces clear, distinct contractions on swim bladder musculature (Rome et al., 1999). Perhaps doublet firing produces a fused single contraction or allows the fish to recruit more motor units to ensure reliable pulse production, as EMG recordings reported for southern pigfish, which do not produce doublets, sometimes show unilateral contraction failure during a pulse train (Packard, 1960).

Pyramid butterflyfish sound waveforms recorded in this study have an unusual shape, in which the initial component of the pulse waveform is a low-amplitude deflection (half cycle), followed by several full cycles of large and variable amplitude before an exponential decay. Swim bladder sounds are highly damped because of the tissues that surround the swim bladder (Fine et al., 2001) and thus the decay observed in the pyramid butterflyfish waveform may be explained by damping of the swim bladder. EMG data from this study indicate that muscles over the swim bladder produce, at most, two complete twitches (perhaps one if tetanic fusion occurs), yet the higher-amplitude portion of the sound wave is sustained over several cycles. A single twitch sound that results in multiple cycles has been attributed to a sound production mechanism that involves excitation of the swim bladder via bones or tendons (Parmentier et al., 2006; Parmentier et al., 2010). The ribs of v4 and v5, with wide laminae in close association with the tunica externa of the swim bladder, are potential candidates for such a system in the pyramid butterflyfish.

Both single and doublet firing produced single pulse sounds and were also associated with a single buckling event. Connaughton et al. have proposed a single-twitch sonic mechanism for sciaenids based on EMG measurements of weakfish calls (Connaughton et al., 2000). Weakfish single-twitch mechanisms were modeled by Sprague as an impedance-matching device between the gas in the swim bladder and the surrounding water environment, and the fundamental frequency of the sound produced was found to be influenced both by the duration of muscle contraction and the resonance properties of the highly damped swim bladder (Sprague, 2000). An observation in weakfish that is consistent with this model is that larger fish, which likely have longer contraction duration cycles because of their longer muscle fibers, produce lower-frequency sounds (Connaughton et al., 2002). Our data indicate a trend towards sounds of higher frequency, in terms of both peak and median 10 dB frequency, with larger body size, perhaps because larger fish are able to vibrate the swim bladder with more energy. The three larger fish did produce louder sounds than the smallest fish, however, as would be expected from fish with larger swim bladders, which increase the volume velocity of the sound source (Bradbury and Vehrencamp, 1998; Connaughton et al., 2000). Perhaps larger pyramid butterflyfish, with more extrinsic musculature mass, are able to deflect the swim bladder tunic at a higher velocity and thus a higher frequency. Larger sample sizes are needed to confirm this body size–frequency relationship. In addition, further experiments are necessary to determine the relationship between body size and swim bladder motion.

The use of high-speed video to visualize movement of the musculature over the anterior swim bladder in this study demonstrated the strong association between inward buckling and sound emission. Recent studies (Parmentier et al., 2007; Longrie et al., 2009) have used a functional morphological approach to examine kinematic patterns in order to determine how anatomical structures and motor patterns are related to sound production. The cichlid obliquus inferioris hypaxial musculature adjacent to the swim bladder is involved in sound generation (Longrie et al., 2009). In our study, the sonic muscle of the pyramid butterflyfish consists of anterior hypaxial musculature behind the pectoral girdle, which contracts to produce an inward buckling. This musculature involved is consistent with the obliquus superioris hypaxial musculature (*sensu* Winterbottom, 1974) and has fibers that insert on the back of the skull, run medial to the supracleithrum and have attachments on the dorsal cleithrum, posterior myocomma and ribs, which are medial to the muscle and lateral to the swim bladder. The inward buckling of the dermis and underlying musculature occurs at the location between the second and third ribs (of v4 and v5). Our images from high-speed video do not show how the rest of the swim bladder responds during the buckling, but we expect that the internal bladder pressure increases during muscle contraction in buckling, which would cause the swim bladder to expand outwardly at other surface locations. Our data indicate that positive phase sounds tend to occur when the fish is oriented towards the hydrophone and negative phase sounds occur when the fish is oriented away from the hydrophone, which is consistent with the occurrence of initial displacement primarily at the rostral end of the swim bladder. Experiments that directly measure the displacement of the swim bladder, however, are necessary to confirm this prediction. Fine et al. examined toadfish swim bladder displacement with a laser vibrometer during sound production and found that contraction of the intrinsic swim bladder muscles caused the swim bladder, which was exposed to air in their study, to move inward from the sides and expand ventrally at the beginning of sound emission (Fine et al., 2001). Future comparative studies are needed on the motion of the swim bladder and surrounding tissues during sound production to determine whether different spatial patterns are correlated with acoustic features of sounds.

Preliminary examination of the hypaxial sound production musculature in pyramid butterflyfish indicates the presence of features that are similar (but require confirmation) to fast-twitch oxidative fibers described for other sonic fish muscles (Fine and

Pennypacker, 1988), such as lighter appearance of musculature and small muscle fibers. Additionally, the shape of the EMG waveforms observed is consistent with a strong, synchronous action potential of local motor units that would be expected from musculature composed of a single fiber type. Sonic swim bladder muscles in other taxa have probably evolved independently in different lineages, but appear often to be derived from epaxialis and obliquus superioris trunk musculature (Winterbottom, 1974). The highly apomorphic, intrinsic musculature of batrachoidid fish originally develops from anterior hypaxial musculature in the occipital region of the head, which migrates caudally in development (Tracy, 1961). Among fish with known sonic mechanisms of the swim bladder muscle, several adult patterns of muscle attachment and associated innervation patterns exist (reviewed in Onuki and Somiya, 2007). A common pattern seen among species with extrinsic sonic musculature is an insertion on the occipital region of the skull; this occurs in the Pempheridae, Terapontidae, Monocentridae, Holocentridae and Scorpaenidae (Salmon, 1967; Onuki and Somiya, 2007), as well as in the pyramid butterflyfish (this study). Cusk eels (Ophidiidae) have multiple (3–4) pairs of extrinsic sonic muscles, which originate on the back of the skull and are unusual among sound producing fish in that they appear to operate antagonistically, as opposed to using internal swim bladder pressure as the antagonist (Parmentier et al., 2006; Fine et al., 2007). Both the extrinsic sonic muscles of these fish as well as the intrinsic sonic muscles of batrachoidids and triglids are innervated by motor nerves that exit occipital foraminae (reviewed in Onuki and Somiya, 2007). Conversely, the intrinsic sonic muscles of John dory (Zeidae) and walleye pollack (Gadidae) and the extrinsic sonic muscles of piranhas (Characidae: Serrasalminae) and drums and croakers (Sciaenidae) are innervated entirely by spinal nerves (reviewed in Onuki and Somiya 2007). Based on developmental patterns of sonic motor neuron innervation in batrachoidid fish compared with anurans, birds and mammals, Bass et al. proposed a homologous region of premotor–motor vocal circuitry in the hindbrain (rhomobomere 8) that is conserved among ray-finned fish and tetrapods (Bass et al., 2008). Given the diversity of sonic muscle arrangements and innervation within derived teleosts, and a lack of information of sound production behavior and anatomy among sarcopterygian and basal actinopterygian fish, this hypothesis is worthy of exploration in more fish taxa, including the pyramid butterflyfish, but may be difficult in species that develop as small planktonic larvae. Nonetheless, further comparisons of skeletal-muscular anatomy, neuroanatomical innervation patterns and muscle ultrastructure in adults are needed to further understand homologies and other evolutionary relationships among species.

The location of sonic buckling and associated swim bladder musculature in the body of the pyramid butterflyfish is in a similar location to the laterophysic connection that is present in *Chaetodon* but absent in other butterflyfish genera including *Hemitaurichthys*. Members of the genus *Chaetodon* possess a laterophysic connection between paired anteriorly directed bullae of the swim bladder and a medial opening of the lateral line canal in the supracleithrum (Webb, 1998; Webb and Smith, 2000; Smith et al., 2003; Webb et al., 2006). This morphology is variable at the subgeneric level and has been hypothesized to impart sound-pressure sensitivity to the mechanosensory lateral line (Webb et al., 2006). The pyramid butterflyfish does not possess anterior swim bladder bullae (Webb et al., 2006), but the swim bladder extends anteriorly to the posteroventral edge of the supracleithrum and sonic muscle fibers run rostrocaudally and medial to the supracleithrum (K.S.B. and T.C.T., unpublished). It is not clear whether the possible presence of sonic musculature near the dorsal, posterior girdle influences any putative function of the laterophysic connection or whether the anterior swim bladder and bullae are involved in the similar short-pulse sounds described by Tricas et al. for the pebbled butterflyfish (Tricas et al., 2006).

The propensity and motivation of the pyramid butterflyfish to produce loud pulse train disturbance calls in the presence of human observers is distinct from that of several other chaetodontids that we have observed in the laboratory and field (*Forcipiger flavissimus*, *F. longirostris*, *Chaetodon auriga*, *C. kleinii*, *C. multicinctus*, *C. ornatissimus* and *C. unimaculatus*). As discussed above, this species usually occurs in large shoals over the reef when feeding, but during courtship individuals are more solitary and often engage in short chases of conspecifics and sometimes heterospecifics. Intense pulse trains were also produced when divers approached fish in a manner similar to that reported for longspine squirrelfish (Holocentridae) (Winn et al., 1964). The sounds from these relatively small animals are of high intensity and may be important for mate selection and defense of mating territories, thus they warrant further behavioral study.

Results from this study and ongoing work on chaetodontids indicate that sound production mechanisms may be quite variable within the butterflyfish family. Further studies on skeletal and muscle morphology, muscle ultrastructure, motor firing patterns, innervation of musculature and sonic motor neuron location in these fish will allow for comparisons in the broader context of the evolution of sonic mechanisms within butterflyfish and among teleosts.

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