The Journal of Experimental Biology 214, 3829-3842
© 2011. Published by The Company of Biologists Ltd
doi:10.1242/jeb.062554

RESEARCH ARTICLE

Sound production in the longnose butterflyfishes (genus *Forcipiger*): cranial kinematics, muscle activity and honest signals

Kelly S. Boyle[1,2,*] and Timothy C. Tricas[1,2]

[1]Department of Zoology, University of Hawai'i at Mānoa, Edmondson 152, 2538 McCarthy Mall, Honolulu, HI 96822, USA and
[2]Hawai'i Institute of Marine Biology, 46-007 Lilipuna Road, Kane'ohe, HI 96744, USA

*Author for correspondence (kboyle@hawaii.edu)

Accepted 22 August 2011

SUMMARY

Many teleost fishes produce sounds for social communication with mechanisms that do not involve swim bladder musculature. Such sounds may reflect physical attributes of the sound-production mechanism, be constrained by body size and therefore control signal reliability during agonistic behaviors. We examined kinematics of the cranium, median fins and caudal peduncle during sound production in two territorial chaetodontid butterflyfish sister species: forcepsfish (*Forcipiger flavissimus*) and longnose butterflyfish (*F. longirostris*). During intraspecific agonistic encounters, both species emit a single pulse sound that precedes rapid cranial rotation at velocities and accelerations that exceed those of prey strikes by many ram- and suction-feeding fishes. Electromyography showed that onsets of activity for anterior epaxialis, sternohyoideus, A1 and A2 adductor mandibulae muscles and sound emission are coincident but precede cranial elevation. Observations indicate that sound production is driven by epaxial muscle contraction whereas a ventral linkage between the head and pectoral girdle is maintained by simultaneous activity from the adductor mandibulae and sternohyoideus. Thus, the girdle, ribs and rostral swim bladder are pulled anteriorly before the head is released and rotated dorsally. Predictions of the hypothesis that acoustic signals are indicators of body size and kinematic performance were confirmed. Variation in forcepsfish sound duration and sound pressure level is explained partly by cranial elevation velocity and epaxial electromyogram duration. Body size, however, explains most variation in duration and sound pressure level. These observed associations indicate that forcepsfish sounds may be accurate indicators of size and condition that are related to resource holding potential during social encounters.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/214/22/3829/DC1

Key words: Chaetodontidae, sound production, *F. flavissimus*, *F. longirostris*, forcepsfish, electromyography, EMG, anterior epaxialis, cranial rotation.

INTRODUCTION

Acoustic behaviors provide important cues and signals used during social communication by fishes. These include functions for advertisement of territory boundaries (Myrberg, 1997), mate attraction, courtship and spawning (Myrberg et al., 1986; Lobel and Mann, 1995; Lobel, 1996; Maruska et al., 2007). The relationships between the kinematics of body parts involved in sound production are largely undescribed but may have important consequences for signal costs and honesty (Fitch and Hauser, 2002; Mitchell et al., 2008). Honest signals reflect properties of the signaler (e.g. body size or health) that may be of importance to both signalers and receivers (e.g. mates or territorial rivals). Variation in the ability to generate movements required for signal production (i.e. kinematic performance) may be reflected in relevant sound features. Features such as pulse repetition rate of underwater sounds can carry information for species identification (Myrberg and Spires, 1972) and spectral content related to swim bladder size for recognition of individuals (Myrberg and Riggio, 1985). Other characteristics of fish sounds, such as duration of sound or intensity, may be reliable indicators of body size and be of value to both sender and receiver in assessments of potential mates or rivals.

Teleost fishes have independently evolved a variety of mechanisms for social sound production that include musculoskeletal elements beyond the most familiar extrinsic and intrinsic swim bladder muscle mechanisms (Tavolga, 1971; Ladich, 2004; Ladich and Bass, 2003). Examples include pectoral fin tendon plucking in a gourami (Osphronemidae) (Kratochvil, 1978), pectoral girdle vibration of sculpins (Cottidae) (Barber and Mowbray, 1956; Bass and Baker, 1991), drumming of the pectoral fins against the body in triggerfishes (Balistidae) (Moulton, 1958; Salmon et al., 1968) and stridulation of pharyngeal tooth plates in grunts (Haemulidae) (Burkenroad, 1930; Moulton, 1958). Recently, a functional morphology study documented the production of pulse sounds in the yellowtail clownfish (*Amphiprion clarkii*), which involves elevation and coincident lowering of the hyoid bar, and a unique ligament that closes the jaw following high-amplitude cranial elevation (Parmentier et al., 2007). The mechanism may involve exaptations of kinematics and morphological features that evolved for feeding. In other fishes with sound-production mechanisms co-opted from structures typically involved in other activities such as feeding, the interaction between anatomical features and the properties of the emitted sound are often unknown.

Sounds emitted during agonistic interactions, such as territorial defense, are predicted to reflect physical attributes related to the resource holding potential of the signaler. These features include body size, condition and performance ability, which are associated with the action patterns involved in sound signal generation. Relationships between sound features (e.g. duration, amplitude and frequency) and body size are known from a variety of fishes (reviewed by Amorim, 2006; Ladich and Fine, 2006). Evidence of physiological-condition-related constraints for fish sound production is known from fishes with intrinsic sonic muscles that produce long, potentially costly signals (Mitchell et al., 2008; Amorim et al., 2010). The relationship between kinematic performance and sound features in fishes without sonic swim bladder muscles, however, is not well understood but is potentially important for the evolution of sound-production mechanisms.

Social sound production was recently described for three species of the coral-reef-dwelling butterflyfish family (Chaetodontidae). The pebbled butterflyfish (*Chaetodon multicinctus*) is a socially monogamous species, and produces several classes of sounds during agonistic interactions with conspecifics in defense of coral feeding territories and towards mates in a putative distress call context (Tricas et al., 2006). The direct mechanism for sound production by pebbled butterflyfish, however, remains unknown. In two closely related butterflyfish species, pyramid butterflyfish (*Hemitaurichthys polylepis*) and pennant bannerfish (*Heniochus chrysostomus*), pulse train sounds are produced that involve activity of hypaxial musculature in close association with the anterior swim bladder and ribs (Boyle and Tricas, 2010; Parmentier et al., 2011). These recent findings indicate that diversity in sound production mechanisms and behavioral contexts likely exists within the family Chaetodontidae and leads to the question of whether communicative sounds provide honest signals during interactions with conspecifics.

The genus *Forcipiger* includes two Indo-Pacific butterflyfish species: the forcepsfish (*F. flavissimus*) and the longnose butterflyfish (*F. longirostris*) (Allen et al., 1998). These species are sympatric on the Kona coast of Hawai'i, where the forcepsfish is socially haremic and the longnose butterflyfish is socially monogamous. Both species defend feeding territories against conspecifics, which involves acoustic behaviors (Boyle, 2011). In aquaria, agonistic sounds are emitted readily towards conspecifics and sound emission involves a rapid, stereotyped body motion with cranial elevation. The relationships between head kinematics and sound production were previously unknown but may have important consequences for signal costs and honesty in these highly social species. In this study, we describe the kinematics of the cranium, body and median fins that are involved in sound emission for this genus. Additionally, we test predictions of the hypothesis that acoustic signals are indicators of kinematic performance and examine relationships between: (1) the sound features of frequency, duration and sound pressure level; (2) body size and kinematics; and (3) the activity of several muscles associated with movement of the head, the oral jaws and the hyoid arch. Results from this study support the existence of a relationship between acoustic signal features, body size and cranial elevation velocity in this sound-production mechanism, which was previously unknown among teleost fishes.

MATERIALS AND METHODS

Sound production kinematic experiments

Experiments were conducted in the laboratory on 14 forcepsfish, *Forcipiger flavissimus* Jordan and McGregor 1898, and four longnose butterflyfish, *F. longirostris* (Broussonet 1782), two species with broad Indo-Pacific distributions (Randall, 2007) obtained from Hawaiian waters by commercial suppliers. Experimental procedures were approved by the University of Hawai'i Institutional Animal Care and Use Committee. Experiments were conducted in a flow-through 110 l aquarium (76×30×46 cm, width×depth×height). During experiments, water flow was shut off to minimize background noise, and the water level was lowered to approximately 20 cm (43% of aquarium capacity). Individual test fish were placed within the aquarium and allowed to acclimate for 30 min. Sounds were evoked by direct introduction of a second fish into the test tank or separated by a thin piece of acrylic when aggressive interactions were extreme. All experiments took place at a water temperature between 25 and 28°C.

The aquarium was illuminated with four 500 W quartz halogen lights and an acrylic sheet with a 1 cm grid was placed behind the fish. Videos of body kinematics during sound production events were pre-trigger recorded at 600 frames s^{-1}, 432×192 pixels with a Casio Ex-F1 Exilim camera (Tokyo, Japan) and stored as Quicktime 7.5 files (Apple, Cupertino, CA, USA). All image sequences used in analyses had a resolution of at least 7.1 pixels cm^{-1} and 75% of all sequences had a resolution >10 pixels cm^{-1}. Fish were represented in all image sequences at a minimal resolution of 75 pixels per body length (standard length, SL) with 75% of all observations >120 pixels SL^{-1}. This resolution allowed for cranial elevation rotation estimates (see Results) at a resolution of at least 1.7 deg $pixel^{-1}$, with 75% of all observations with resolutions of 0.71 deg $pixel^{-1}$ or greater. Video, sound and electromyography (EMG) data (see below) were synchronized with a digital pulse circuit that illuminated LEDs in the video and were recorded simultaneously on the hydrophone data channel after a sound event (see below).

Sounds were recorded with a Brüel and Kjaer 8103 hydrophone (–211 dB re. 1 V μPa^{-1}; Nærum, Denmark) connected to a Nexus conditioning amplifier with 60 dB gain (Nærum, Denmark) positioned approximately 3 cm from the aquarium end. A CED micro 1401 data acquisition system and Spike2 software (Cambridge Electronic Design, Cambridge, UK) were used to digitize and store sounds on a computer (raw sample rate at 40 kHz). Sound files were low-pass filtered and downsampled at 4 kHz with Cool Edit Pro 2.1 software (Syntrillium, Phoenix, AZ, USA) set at the 'high-quality' setting. The resulting bandwidth (0–2 kHz) was well below the minimum resonance frequency of 4574 Hz estimated for the 20 cm water depth in the aquarium (Akamatsu et al., 2002), thus sounds recordings were not significantly biased in this experimental setup.

EMG experiments

EMGs were recorded from several candidate muscles of forcepsfish in order to determine associations with sound production. Two to four muscles were tested for activity within each free-swimming subject. Candidate muscles were chosen based on preliminary analysis of high-speed video during sound emission that revealed a rapid cranial elevation component (see Results) and based on observations of a related chaetodontid, the pyramid butterflyfish (Boyle & Tricas, 2010). EMG recording electrodes (Fig. 1) were placed in the anterior epaxial musculature (EP, N=7) approximately 0.5 cm posterior to the supraoccipital bone and at a dorso-ventral level approximately 50% of the dorso-ventral axis of the supraoccipital. Activity of the sternohyoideus (SH, N=5) was monitored near the caudal portion of the urohyal, approximately 1–2 mm off the midline. Electrodes were placed in both the A1 (AM1) and A2 (AM2) subdivisions of the adductor mandibulae (N=2). In one individual, an EMG electrode was inserted into tail epaxial musculature (TEP) midway between the caudal end of the body cavity and caudal peduncle, approximately 1 cm above the midline. Based on observations of muscle activity during sound production in pyramid butterflyfish (Boyle & Tricas, 2010), we monitored the anterior hypaxial (HP) musculature caudal to the

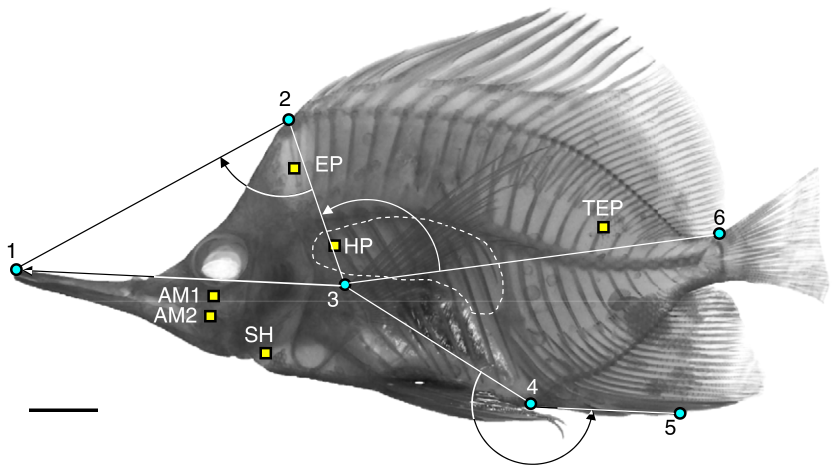

Fig. 1. Cleared and stained forcepsfish specimen indicates the skeletal elements, location of swim bladder (outlined by dotted line), points digitized for kinematic analyses (blue circles), angles and displacements calculated from digitized points (lines and arrows) and electromyography (EMG) recording electrode locations (yellow squares). Cranial elevation measured change in angle 1–2–3, anal fin erection measured change in angle 3–4–5, caudal peduncle elevation measured change in angle 2–3–6 and premaxilla protrusion measured change in displacement from 1 to 3. AM1, A1 subdivision of the adductor mandibulae; AM2, A2 subdivision of the adductor mandibulae; EP, anterior epaxial musculature; HP, anterior hypaxial musculature; SH, sternohyoideus; TEP, tail epaxial musculature. Scale bar, 1 cm.

pectoral girdle and rib of the fourth vertebra, at a level approximately 25% along the ventral-dorsal axis of the supracleithrum (*N*=1).

Bipolar EMG recording electrodes were fabricated with 0.05 mm insulated tungsten wire (California Fine Wire, Grover Beach, CA, USA), inserted into a 28 gauge hypodermic needle with tips (1 mm) exposed and bent back to form opposing hooks. Prior to electrode implantation, fish were anesthetized in 100 mg l^{-1} of tricaine methanesulfonate (MS-222; Argent Labs, Redmond, WA, USA) and then ventilated with anesthetic while the electrodes were implanted and the hypodermic needle tips were removed. Wire leads were tied and glued with cyanoacrylate to a loop of surgical thread placed in the dorsal trunk musculature to provide strain relief and prevent dislodgement of the electrodes. Fish were revived by ventilation with seawater and placed back in the aquarium after a recovery period of approximately 30 min.

Muscle function experiments

Several manipulative experiments were conducted to determine the role of muscle activity in sound emission. Experiments were conducted after recording several sounds from the same individual fish prior to manipulation. In order to examine the role of the SH firing in muscles to pulse sound emission during headbob sounds (see Results), fish were anesthetized with MS-222 (as above) and the SH was inactivated with an injection of 30 μl of 2.0% lidocaine in the muscle. An initial injection of 30 μl of 0.2% lidocaine (Crawford and Huang, 1999) did not appear to cause any inactivation to the SH. To test efficacy, 30 μl of 0.2% lidocaine was injected into the abductor superficialis of the pectoral fin. No reduction in pectoral fin activity was observed, so a 30 μl injection of 2.0% lidocaine was tested, which did result in a temporary (~20 min) cessation of activity. A 2.0% 30 μl injection was then administered to both right and left SH muscles in the same individual. The experiment was repeated with the same volume and full lidocaine dosage in a second test fish. After apparent recovery (100 min later), a 30 μl injection was administered in the right and left anterior epaxialis musculature of these subjects.

Additionally, sound production was recorded from three individuals prior to and after SH transection. Fish were deeply anesthetized with MS-222 and a cut was made across SH fibers, perpendicular to and deep to the lateral edge of the caudal portion of the urohyal, below the opercle. Fibers of the SH insert on the broad, lateral face of the urohyal and thus the perpendicular cut was expected to weaken the linkage between the pectoral girdle and hyoid bar, but not sever all fibers of the muscle.

Kinematic analyses

Kinematic movie files were converted to tiff image stacks and analyzed with ImageJ software (National Institutes of Health, Bethesda, MD, USA). *X–Y* position coordinates in the lateral view were calculated for six skeletal features based on external landmarks (Fig. 1): (1) the tip of the premaxilla, (2) the dorsal portion of the neurocranium (estimated from external morphology), (3) the dorsal margin of the pectoral fin base (reference point), (4) the origin of the anal fin, (5) the distal tip of anal fin spine II and (6) the dorsal margin of the caudal peduncle. Initial frames of reference were set at 10 frames (0.0167 s) before onset of the sound waveform. These initial frames were used to record: (1) cranial elevation angle estimated as the angle between landmarks 1, 2 and 3; (2) premaxillary protrusion estimated as the distance from landmark 1 to landmark 2; (3) anal fin erection angle estimated as the change in angle 3–4–5; and (4) caudal peduncle elevation angle estimated as the angle 2–3–6. Digitization noise from kinematic data was reduced with a fourth-order Butterworth zero phase-shift low-pass filter (e.g. Van Wassenbergh et al., 2007) of 100 Hz, and velocity and acceleration were calculated.

Several variables were calculated for cranial elevation, premaxillary protrusion, anal fin erection and caudal peduncle elevation kinematic data: (1) the maximum cranial elevation angle, maximum premaxillary protrusion, minimum anal fin erection angle and minimum caudal peduncle elevation angle; (2) the time of position at extreme relative to sound onset; (3) the greatest linear velocity for premaxillary protrusion and greatest angular velocity for all other features; and (4) the greatest linear acceleration for premaxillary protrusion and greatest angular velocity for all other features. Values for 3 and 4 were calculated for the negative phase of anal fin erection and caudal peduncle elevation.

Sound and EMG analyses

Sound waveforms were examined aurally and visually. Sound duration was determined by visual inspection of sounds relative to background noise. Custom MATLAB 7.0 (MathWorks, Natick, MA, USA) programs were used to measure peak-to-peak sound pressure level (SPL; dB re. 1 μPa) and to estimate power spectra with a zero-padded 1024 point fast Fourier transforms (FFTs) with a Hanning window. The following spectral features were determined from 512 frequency bins and relative amplitudes values obtained from generation of the FFTs: (1) peak frequency, =frequency bin with greatest amplitude; (2) proportion of bandwidth within –10 dB of peak (0.316×peak amplitude), =number of frequency bins

>(0.316×peak amplitude)/512; (3) minimum frequency within –10 dB from peak, 10 dB min=lowest frequency bin >(0.316×peak amplitude); (4) maximum frequency within –10 dB from peak, 10 dB max=greatest frequency bin >(0.316×maximum peak amplitude); and (5) median frequency within –10 dB of peak, median frequency bin with amplitude >(0.316×peak amplitude).

EMG oscillograms were rectified with Spike2 software. Muscle activity was estimated by calculating time periods for which the rectified waveform was three times the average background noise level.

Statistical analyses

Means and standard errors were determined from averages of each individual for sound, kinematic and EMG features. Properties of sounds between species were compared using a two-sample *t*-test with data averaged for each individual fish. In order to test for a relationship between acoustic features and body size, simple linear regressions were performed with means of acoustic features (median frequency, duration and SPL) *versus* the SL of the fish (to the nearest mm) for each species. These analyses provided evidence for a relationship between body size and some acoustic features (see Results), so size was included as a covariate in analyses used to examine relationships between acoustic features, kinematics and muscle activity. Relationships between acoustic features (dependent variables) and cranial kinematics (independent variables) were assessed with multiple regression models that included body size (SL) as a covariate. Regression models initially included categorical variables for individual subjects and subject–kinematic interactions. Models were simplified in a stepwise fashion, first by removing insignificant interaction terms ($P>0.05$), and then by removing individual variables if $P>0.05$ and the variable is not associated with an significant interaction term ($P<0.05$). Separate analyses were conducted for each acoustic feature (median frequency, sound duration and SPL) and cranial kinematic variables: displacement peak (maximum cranial elevation angle), displacement latency (time of displacement peak relative to onset of sound emission), velocity (maximum angular velocity) and acceleration (maximum angular acceleration). The same multiple regression procedures were used to test for relationships between acoustic features (dependent variables) and muscle activity (independent variables: EP, SH, AM1 and AM2). Multiple regressions without a size covariate were used to test for a relationship between cranial kinematic features (independent variables: displacement peak, displacement latency, velocity and acceleration) and EP muscle activity (independent variable). Initial models, as above, initially included all individual subject terms and interactions but were removed from the model iteratively when $P>0.05$. Multiple comparisons from the separate regression models were corrected with a sequential Bonferroni procedure (Rice, 1989). Data that failed to meet assumptions for normality and homogeneity of variance were $\log_{10}$ or rank transformed. SH muscle function experiments were analyzed with a general linear model (GLM) with a random subject factor and a two-sample *t*-test was used to test the effect of lidocaine on anterior epaxial musculature in one individual. All statistical tests were conducted with Minitab v.13.31 software (Minitab, Inc., State College, PA, USA).

RESULTS

A total of 218 forcepsfish and 14 longnose butterflyfish pulse sounds were obtained during agonistic interactions in which conspecifics were in close proximity. Both species produced similar sounds. However, individual longnose butterflyfish were less aggressive in the aquarium and less likely to produce sound in our experimental conditions, so sample sizes are lower for the latter species.

Sounds produced by both *Forcipiger* species consisted of prominent single pulses of short duration with most energy below 750 Hz (Fig. 2, Table 1). Pulses of both species were characterized

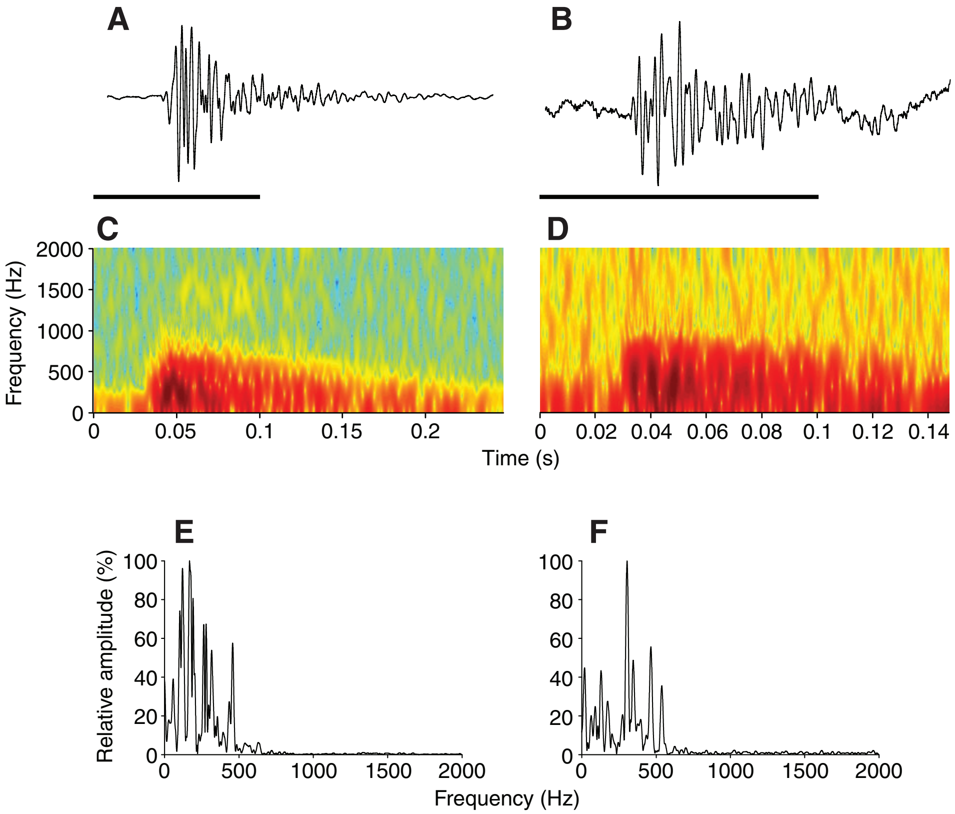

Fig. 2. Acoustic features of representative pulse sounds from the (A,C,E) forcepsfish and (B,D,F) longnose butterflyfish. (A,B) Oscillogram, (C,D) spectrogram and (E,F) power spectrum. Note the similarity between sounds from both species and the concentration of acoustic intensity from 100 to 500 Hz. Spectrogram settings: 1024 point FFT, 2.5% window length, 95% window overlap. Scale bar in A and B, 0.1 s.

Table 1. Acoustic properties of sounds produced by the forcepsfish and the longnose butterflyfish

Species	*N*	*n*	*n* range	Duration (s)	Proportion of bandwidth	Peak frequency (Hz)	10 dB min (Hz)	10 dB max (Hz)	Median frequency (Hz)	SPL (dB)
Longnose butterflyfish	14	218	3–29	0.098±0.010	0.06±0.01	100±21	16±6	333±42	145±20	132±2
Forcepsfish	4	14	2–7	0.074±0.007	0.03±0.01	62±33	3±2	156±62	74±32	127±2
Between-species comparisons (*P*-value)				0.100	0.010	0.315	0.188	0.047	0.105	0.131

N, number of individual fish observed; *n*, number of sounds; *n* range=range of *n* observed per individual fish; SPL, sound pressure level.
Data are presented as means of individual fish means ± s.e.m.
Between-species comparisons were made using a two-sample *t*-test (d.f.=7) of individual sound characteristic means.
Note that all *P*-values were >0.0031, the sequential Bonferroni-adjusted alpha for experiment-wide multiple comparisons.

by an initial onset of several half cycles rising in amplitude over a period of approximately 25 ms or less, followed by a longer period of exponential decay (Fig. 2). Sounds of both species tended to be less than 100 ms duration, had narrow bandwidth (less than 10% of the 2 kHz bandwidth within 10 dB of peak), were characterized by a 10 dB low-pass well below 500 Hz (333 Hz for forcepsfish and 156 Hz for longnose butterflyfish), and had similar peak-to-peak SPLs (Table 1). No measurable differences between sound characteristics from each species were observed; however, statistical power was low because of the small number of longnose butterflyfish observed (*N*=4) and the sequential Bonferroni multiple comparison corrections (Table 1).

High-speed video of both species revealed stereotyped kinematic action patterns that were associated with sound emission onset. During sound emission, both species were found to elevate the cranium rapidly (Table 2, Fig. 3, supplementary material Movie 1). Cranial elevation from these ‘headbob’ sounds always included moderate cranial elevation angles relative to the pectoral girdle and body, and also high angular velocities and accelerations (Table 2). In all observations, cranial elevation occurred after the initial onset of sound emission and the latency to peak displacement relative to sound onset was highly variable (Fig. 4). Sound emission kinematics of forcepsfish usually co-occurred with erection of the anal fin spines and rays and elevation of the caudal peduncle (Table 2, Figs 3 and 4). Longnose butterflyfish individuals, however, did not erect the anal fin during sound emission and showed more moderate and inconsistent elevation of the caudal peduncle (Table 2, Figs 3 and 4). Additionally, longnose butterflyfish usually protruded the upper jaw (premaxilla) and dentary (observed but not quantified here) during headbob sounds in contrast to forcepsfish, which showed relatively minor jaw movement during cranial elevation (Table 2, Figs 3 and 4).

EMG recordings revealed burst activity of several muscles with close association to sound emission and before visible cranial elevation (Fig. 5). Sound emission included brief activity of EP, SH, AM1 and AM2, and was closely associated with the initial onset of sound emission before cranial elevation (Fig. 6). Additional EMGs in one individual showed activity towards the tail by TEP for some but not all (12 of 18) sound events. The onset of TEP was closely associated with the onset of sound emission, but the mean (±s.d.) onset of EP lagged relative to sound onset (–0.08±3.68 ms) compared with TEP (–0.71±3.22 ms, paired *t*-test, *N*=12, *T*=2.01, *P*=0.069). In the same individual, HP musculature, a region shown to be active during sound production in *H. polylepis* (Boyle & Tricas, 2010), was found to fire for only six out of 18 events, and to lag behind the onset of EP (mean EP onset=–1.33±1.51 ms *versus* HP mean=6.32±5.17 s, paired *t*-test, *N*=6, *t*=–4.61, *P*=0.006).

Variation in forcepsfish acoustic signals may be explained in part by body size differences (Table 3). An analysis of individual means of sound characteristics revealed that both duration and SPL were positively correlated with body size (Table 3, Fig. 7). A statistical association between frequency and body size in forcepsfish was not observed. No size associations were observed for these sound features in longnose butterflyfish; however, the small sample size

Table 2. Summary of kinematic features of sound production for the forcepsfish and longnose butterflyfish

	N	*n*	*n* range	Displacement		Velocity (deg s^{-1})	Acceleration (deg s^{-2})
				Peak (deg)	Latency (s)		
Cranial elevation							
Forcepsfish	14	146	3–29	6.3±0.3	0.057±0.003	932±52	191,521±12,269
Longnose butterflyfish	4	14	2–7	6.2±1.1	0.070±0.006	588±108	116,894±22,141
Anal fin elevation							
Forcepsfish	14	129	3–27	–8.3±0.8	0.059±0.003	–1609±340	–390,294±83,113
Longnose butterflyfish	4	13	1–7	–6.0±1.8	0.100±0.018	–497±44	–147,562±5565
Caudal peduncle elevation							
Forcepsfish	14	130	2–27	–3.3±0.3	0.062±0.002	–396±33	–90,653±6508
Longnose butterflyfish	4	14	2–7	–1.4±0.7	0.058±0.017	–194±35	–53,033±5742
	N	*n*	*n* range	Peak (cm)	Latency (s)	Velocity (cm s^{-1})	Acceleration (cm s^{-2})
Premaxillary protrusion							
Forcepsfish	14	146	3–29	0.11±0.01	0.056±0.004	24±1	7809±332
Longnose butterflyfish	4	14	2–7	0.76±0.13	0.075±0.010	79±10	13,973±1818

N, number of individual fish observed; *n*, number of sounds; *n* range, range of *n* observed per individual fish.
Latency is the time to peak displacement since the onset of sound emission; velocity and acceleration are both peak values. See Materials and methods for details.
Data are presented as means of individual fish means ± s.e.m.

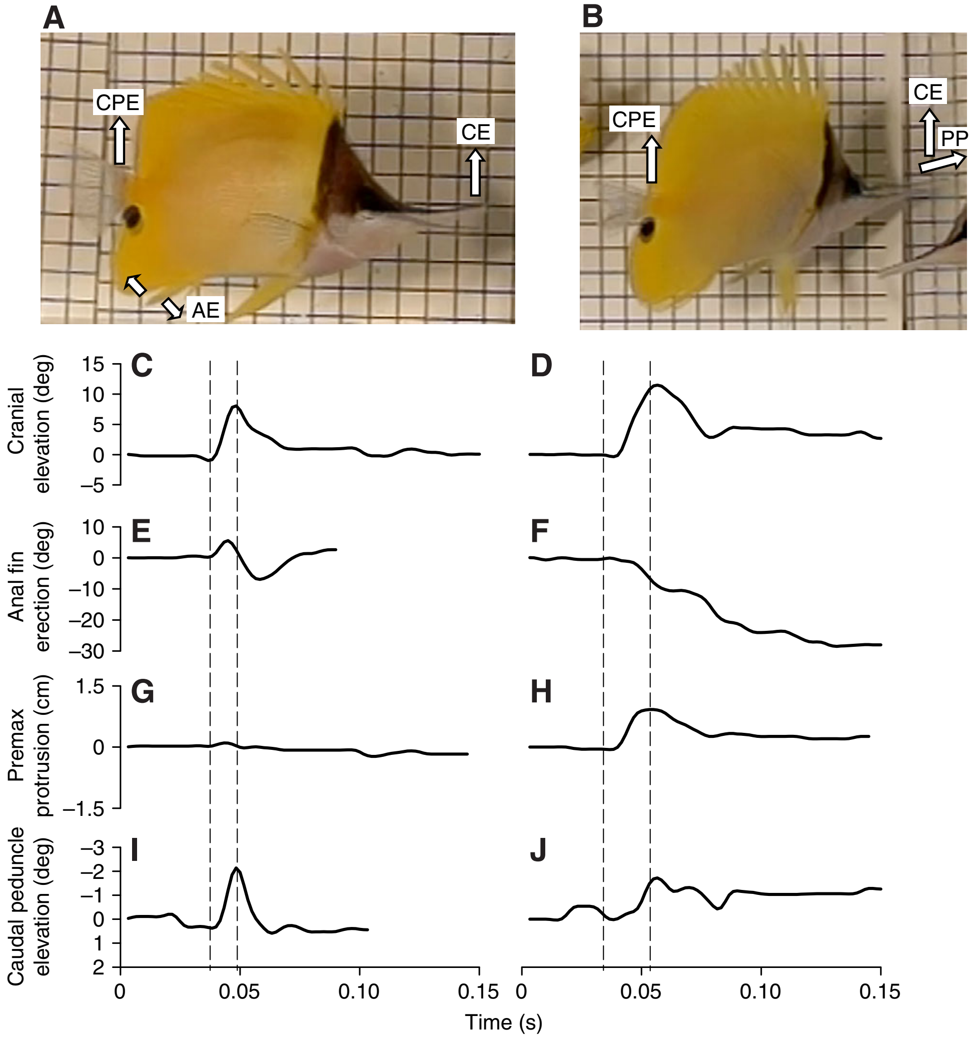

Fig. 3. Kinematics associated with pulse sound generation in the forcepsfish and longnose butterflyfish. Superimposed images from two separate frames from video (600 frames s^{-1}) recorded during sound emission with arrows to indicate the main direction of motion from (A) forcepsfish and (B) longnose butterflyfish. Grid, 1 cm. Motion profiles (see Materials and methods for details) of (C,D) cranial elevation, (E,F) anal fin erection, (G,H) premaxillary protrusion and (I,J) caudal peduncle elevation for forcepsfish (left) and longnose butterflyfish (right). Dotted lines indicate time periods of the two superimposed images. Note the rapid cranial elevation that occurs for both species, the anal fin erection and caudal peduncle elevation that occurred mainly for forcepsfish and the premaxillary protrusion that occurred only for longnose butterflyfish. AE, anal fin erection; CE, cranial elevation; CPE, caudal peduncle elevation; PP, premaxilla protrusion.

(*N*=4) provides little statistical power (Table 3). Thus, the duration and SPL may be influenced by the size for at least the forcepsfish.

Several kinematic features of cranial elevation in forcepsfish were associated with acoustic features after the influence of size and subject differences were explained (Table 4). Body size was a contributor to the variation in all models (Table 4). Individual terms were kept in all models because of strong individual variation and interaction terms were present in seven models. Median frequency was strongly influenced by body size and individual differences, but no strong kinematic main effects were observed. Sound duration trended positively with peak cranial displacement in all but two individuals, had a negative relationship with latency to peak displacement for nine of 14 individuals, and trended positively with cranial velocity for all individuals (Table 4, Fig. 8). SPL was positively correlated with peak displacement in all individuals, positively correlated with peak velocity in all but two individuals and positively correlated with peak acceleration in all but one individual (Table 4, Fig. 8). For longnose butterflyfish, variation in median frequency was driven by body size, individual differences and interaction terms; however, no main effects with kinematic features were observed (Table 4). No other relationships between kinematics, acoustic features or individuals were observed for longnose butterflyfish; however, the power of analysis was likely low for this species because of the low sample size of individuals and replicates. Thus results from this analysis indicate that for at least forcepsfish, cranial elevation motion may influence the duration and intensity of sounds.

Anterior epaxial activity was the only EMG variable associated with sound features. Activity of EP trended positively with sound duration in all individuals and was positively correlated with SPL in all but one individual (Table 5, Fig. 9). Activity of EP musculature was positively correlated with maximum cranial displacement in all but one individual (Table 6, Fig. 9), but not did not contribute substantially to the variation observed in other cranial kinematic variables.

Evidence of a ventral cranial–pectoral girdle linkage for pulse sound emission was provided from lidocaine and muscle transection experiments. Application of 2% lidocaine to left and right SH musculature in two individuals reduced the sound duration (GLM on sound duration ranks with random subjects factor: lidocaine effect $F_{1,20}$=361.00, *P*=0.033, individual $F_{1,20}$=11,000, *P*=0.006, individual–lidocaine interaction $F_{1,20}$=0.00, *P*=0.950; back-transformed mean rank, pre-lidocaine 0.119 s, post-lidocaine 0.108 s). Sound intensity, however, was not affected by lidocaine administration (GLM on SPL: lidocaine effect $F_{1,20}$=0.11, *P*=0.748). Similarly, transection of the right and left SH in three individuals reduced sound duration (GLM on sound duration ranks with random subjects factor: muscle transection effect $F_{1,70}$=349.70, *P*=0.022, individual $F_{1,70}$=436.54, *P*=0.002, individual–transection interaction $F_{1,70}$=0.10, *P*=0.905; back-transformed mean rank, pre-transection

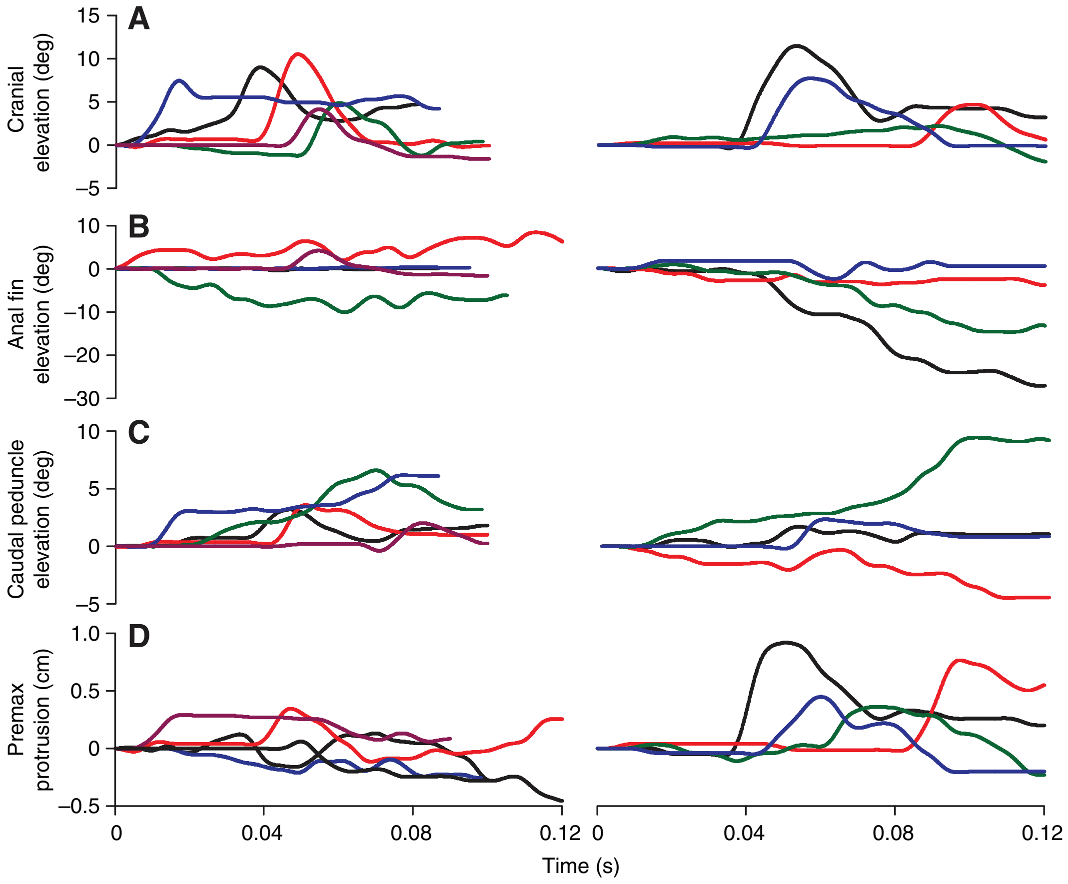

Fig. 4. Kinematic profiles from sound production events in the forcepsfish (left) and longnose butterflyfish (right). (A) Cranial elevation, (B) anal fin elevation, (C) caudal peduncle elevation and (D) premaxilla protrusion. Each trace shows the corresponding kinematic variable produced during the loudest sound of different individual fish, indicated with different shades for each fish (N=5 shown for forcepsfish, N=4 for longnose butterflyfish). Events are aligned by the start of sound emission, which occurs at 0.0167 s. Note that both species produce a strong cranial elevation component in which the time of maximum elevation occurs well after the onset of sound production and that the latency until maximum elevation is highly variable in relation to the onset of sound emission.

0.075 s, post-lidocaine 0.066 s), but an overall drop in intensity was not observed with GLM on sound intensity (muscle transection effect $F_{1,70}$=1.32, P=0.345). These experiments provide partial support for the hypothesis that the SH muscle may act antagonistically with anterior EP musculature at the initial onset of sound emission in order to delay cranial elevation and to pull the pectoral girdle and anterior swim bladder anteriorly.

Direct evidence of the contribution of EP musculature to sound emission was identified after application of 2% lidocaine to the right and left EP musculature of one individual after recovery from a previous SH injection. After application of lidocaine to the anterior EP, sound duration was reduced (two-sample t-test, t=3.18, d.f.=13, P=0.007, pre-epaxial injection mean ± s.d. 0.189±0.051 s, post-injection 0.115±0.040 s). Intensity also was reduced following injection (two-sample t-test, t=2.27, d.f.=13, P=0.041, pre-epaxial injection mean ± s.d. SPL 135.70±3.72 dB, post-injection 130±5.27 dB). There was evidence for recovery over time after epaxial injection, as cranial elevation velocity was strongly and positively associated with time past injection (simple linear regression, F_7=15.75, P=0.007, R^2=0.724) and no sounds were emitted until 30 min after injection. The range of cranial elevation was low during the first 48 min following injection (max. 3.3 deg, mean 1.9 deg). This experiment indicates that EP musculature is directly involved in sound emission and cranial elevation.

DISCUSSION

Acoustic behaviors provide important cues and signals for social communication in fishes, but the nature and modulation of relevant signals are poorly known for species without intrinsic or extrinsic swim bladder muscles. This study found relationships between quantitative features of sound, cranial rotation and body size in the highly social longnose butterflyfishes of the genus *Forcipiger*, which lack swim bladder muscles. Sound production in this ditypic genus involves a rapid elevation of the head, for which the sound duration and SPL are related to aspects of maximum angular displacement, velocity and acceleration. Both forcepsfish and longnose butterflyfish produce sounds with a similar, delayed rapid cranial rotation, and we demonstrate a relationship between signal features, kinematics and body size for the forcepsfish. Differences in actions among species include anal fin erection and caudal elevation in the forcepsfish and protrusion of the oral jaws during cranial rotation by longnose butterflyfish. Results also indicate that agonistic sounds from the haremic forcepsfish may convey information about the signaling animal such as body size and condition.

The positive correlations of body size, pulse duration and SPL found in this study are consistent with results observed from other studies for distantly related fishes with different sonic mechanisms. Similar associations between body size and pulse durations were observed in sciaenids (Connaughton et al., 2000; Tellechea et al., 2011) and a gobiid (Amorim and Neves, 2008), and between body size and call duration in a pomacentrid (Lobel and Mann, 1995). SPL was also shown to correlate positively with body size for a variety of fishes, including a batrachoidid (Fine et al., 2001), a sciaenid (Connaughton et al., 2000), gobiids (Takemura, 1984; Lindström and Lugli, 2000) and an osphronemid (Wysocki and Ladich, 2001). A hypothesis that explains this consistent relationship is that the swim bladder is a sound radiator, which increases in surface area with body size, and if swim bladder oscillation velocities remain constant with body size, volume velocity and SPL would increase with an increase in surface area (Fine et al., 2001).

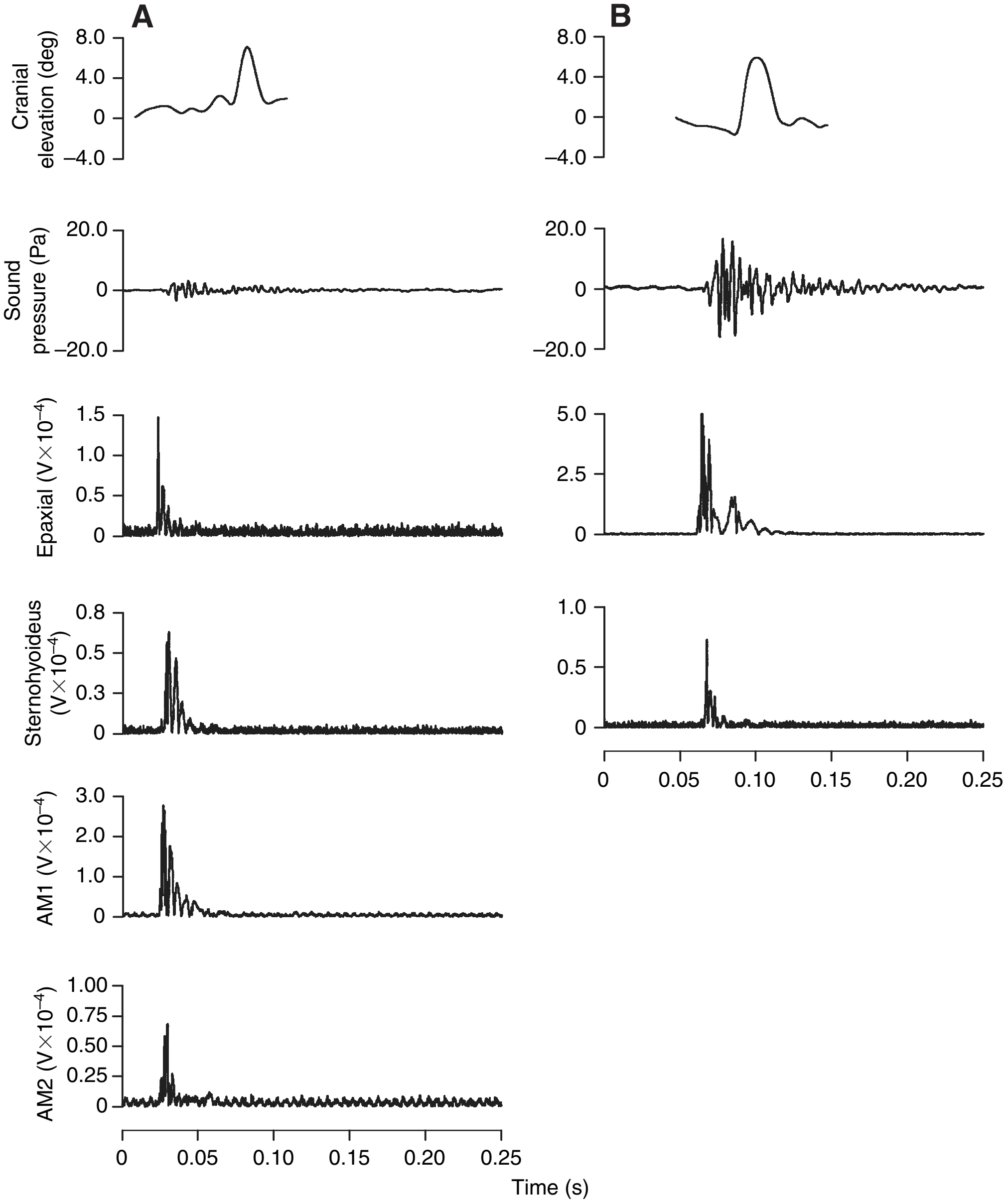

Fig. 5. Representative kinematic profiles, sound waveforms and rectified EMGs from the forcepsfish, which show timing of muscle activity, sound emission and cranial elevation. (A) Example from a 117 mm (standard length, SL) individual in which EMG recording electrodes were placed in the anterior epaxialis (epaxial), sternohyoideus, A1 subdivision of the adductor mandibulae (AM1) and A2 subdivision of the adductor mandibulae (AM2). (B) Example from a 148 mm SL individual in which the epaxial and sternohyoideus EMG electrodes were implanted. Note the onset of activity from these muscles near the occurrence of sound emission and the variable delay until cranial elevation. Sound pressure and durations for A and B are shown on the same scale. Note the greater sound pressure level (150 *versus* 136 $\mathrm{dB_{peak-peak}}$ re. 1 μPa) and duration (0.137 *versus* 0.174 s) from the larger fish shown in B.

In this study, frequency components did not correlate with body size. Many studies have reported negative correlations with body size in a variety of vocal fishes with different sonic mechanisms. Negative frequency–body size relationships were found for fishes with stridulatory mechanisms (Takemura, 1984; Pruzsinszky and Ladich, 1998), a tendon-plucking sonic mechanism (Henglmüeller and Ladich, 1999), extrinsic sonic muscles (Crawford et al., 1997; Connaughton et al., 2000; Gannon, 2007; Tellechea et al., 2011) and unknown sonic mechanisms (Myrberg et al., 1993; Lobel and Mann, 1995; De Jong et al., 2004). The batrachoidid and triglid fishes possess intrinsic sonic muscles and frequency is related to the rate of muscle contraction rather than body size (Skoglund, 1961; Cohen and Winn, 1967; Bass and Baker, 1991; Fine et al., 2001; Connaughton, 2004). A hypothesis proposed to explain the contrast between size-dependent and -independent relationships with sound frequency is that musculature that scales proportionally will take longer to contract, which results in a lower-frequency sound (Connaughton et al., 2002). The lack of a size–frequency relationship in forcepsfish may be explained by the high variability of cranial elevation displacement, velocity and acceleration among all sizes as evidenced by the presence of individual effects and interaction terms in the regression models. Nevertheless, most of the spectral energy of all forcepsfish sounds emitted in this study fell within a relatively narrow range of frequencies and within the bandwidth (≤500 Hz) of best hearing as measured by auditory evoked potentials (T.C.T. and K.S.B., unpublished data).

Cranial rotation velocity and acceleration also explained the variation recorded for forcepsfish sound duration and amplitude. Rapid cranial elevation that immediately follows sound emission was previously undescribed in teleosts but involves motion used for fast prey capture. Cranial elevation is a major component of stereotyped prey capture in teleost fish feeding, occurs in taxa that span the ram–suction feeding continuum and involves a series of movement of cranial elements in order to lower the hyoid and expand the buccal cavity (Gibb and Ferry-Graham, 2005). The rapid cranial elevation velocities and accelerations seen during sound production rival and exceed (>2×) those reported from feeding studies of voracious predators such as largemouth bass

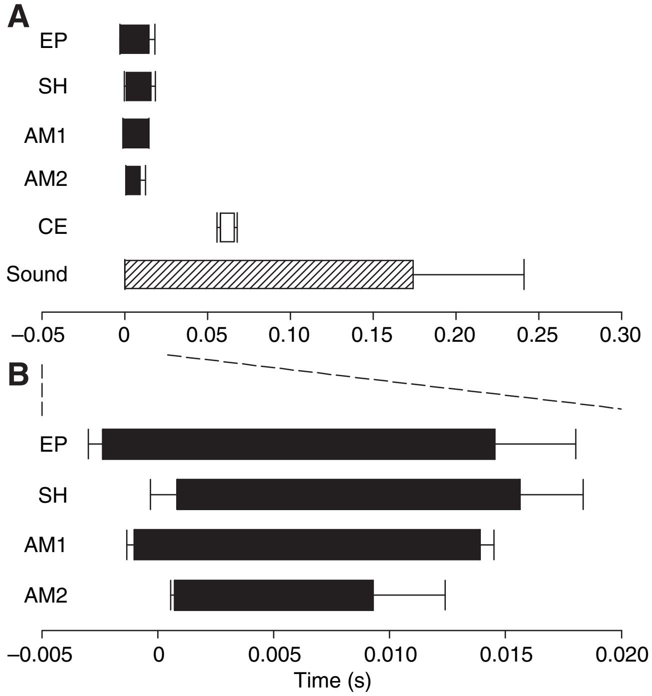

Fig. 6. (A) Timing of muscle activity (solid bars) and cranial elevation (open bar) relative to sound emission (hatched bar) in the forcepsfish. Sound emission onset occurs at time 0 and bars represent mean ± s.e.m. onset and offset times. (B) Dashed line shows magnified portion on the time axis showing muscle activity times in detail. Cranial elevation onset is represented by the time of 25% of maximum elevation. AM1, A1 subdivision of the adductor mandibulae; AM2, A2 subdivision of the adductor mandibulae; CE, cranial elevation; EP, anterior epaxialis; SH, sternohyoideus. Note that muscle activity is associated closely with the onset of sound emission and occurs well before cranial elevation.

(*Micropterus salmoides*) (Svanbäck et al., 2002). Based on maximum cranial elevation displacement and time to maximum cranial elevation data from several fish families (Gibb and Ferry-Graham, 2005), cranial elevation during forcepsfish sound production is faster than most feeding strikes from all but zebrafish (*Danio rerio*) and bay pipefish (*Syngnathus leptorhynchus*). Cranial rotation observed in this study during sound production by both *Forcipiger* species also involved more rapid cranial elevation than was shown for the same species during feeding in a separate study in which mean peak elevations were less than 4 deg and occurred over approximately 30 ms (Ferry-Graham et al., 2001). Members of the seahorse and pipefish family Syngnathidae, however, produce cranial elevations during feeding that far exceed those measured during sound production in *Forcipiger* (Van Wassenbergh et al., 2008; Flammang et al., 2009; Roos et al., 2009). The relationship between suction feeding in fishes such as largemouth bass may be explained by both cranial kinematics (Svanbäck et al., 2002) and motor patterns of associated muscles (Grubrich and Wainwright, 1997). Thus the muscle activity patterns associated with kinematics observed during sound production may predict variation in acoustic signals in some fishes.

EMG experiments in this study demonstrate a link between EP activity, sound duration and SPL. EP activity was also associated with an increase in cranial elevation displacement. Studies on muscle activity during teleost feeding demonstrated that anterior EP musculature activity often occurs simultaneously with the SH, which serves to elevate the neurocranium and lower the hyoid, respectively, and initiate opening of the oral jaws and expansion of the buccal cavity. These actions are followed by activity of subdivisions of the adductor mandibulae in order to close the jaws (Lauder et al., 1986; Westneat and Wainwright, 1989; Grubrich and Wainwright, 1997; Alfaro and Westneat, 1999). The duration of anterior EP muscle activity in the present study (17 ms) is comparable to that reported during feeding in centrarchids (Lauder et al., 1986; Grubrich, 2000), scarids (Alfaro and Westneat, 1999) and a labrid (Westneat and Wainwright, 1989). Activation of the SH and both adductor mandibulae subdivisions in sound production by forcepsfish is more aligned with the onset of EP activity and of shorter duration compared with feeding in most other species. This observation is consistent with the hypothesis that EP-driven elevation of the head occurs while tension is maintained between the hyoid and cleithrum *via* SH activity, and also between the hyoid and neurocranium *via* activity of the adductor mandibulae. This motion results in an antero-dorsal motion of the pectoral girdle along with postcleithral bones and ribs, which are closely associated with the swim bladder. Synchronous activity of the adductor mandibulae and anterior epaixialis near the onset of sound emission is predicted by this hypothesis. Thus the brief early activity of the adductor mandibulae during sound production is expected to differ from the later onset and longer duration pattern associated with closing of the jaws during a feeding strike. Manipulation of a cleared and stained specimen (K.S.B., unpublished) shows that elevation of the head produces forward movement of the pectoral girdle and anterior pleural ribs. Further support for this hypothesis comes from experiments in which sound duration was reduced following partial lidocaine inactivation or transection of the SH muscle. The incomplete ablation of sound production in these experiments may have resulted from the residual bone and connective tissue linkages or fiber attachments at the extreme caudal end of the urohyal. In the extreme case of rapid cranial rotation seen in pipefish, the onset of EP activity occurs much earlier (from 300 ms to nearly 0.5 s) than the beginning of cranial rotation, and thus provided strong evidence for a power-

Table 3. Relationships between body size and sound features in the forcepsfish and the longnose butterflyfish

	Forcepsfish					Longnose butterflyfish			
Sound characteristic	d.f.	*F*	*P*	Relationship	r^2	d.f.	*F*	*P*	r^2
Median frequency	1, 12	2.87	0.116		19.3	1, 2	2.65	0.245	57.0
Duration	1, 12	13.42	**0.003**	+	0.528	1, 2	1.68	0.325	45.6
Sound pressure level	1, 12	16.13	**0.002**	+	0.573	1, 2	0.54	0.538	21.4

Results are from individual simple linear regression models to test the relationship between body size (standard length) and mean sound characteristics for individual fish in the study.

Bold *P*-values are lower than the sequential Bonferroni-adjusted alphas for experiment-wide multiple comparisons (0.0038 for forcepsfish and 0.0031 for longnose butterflyfish).

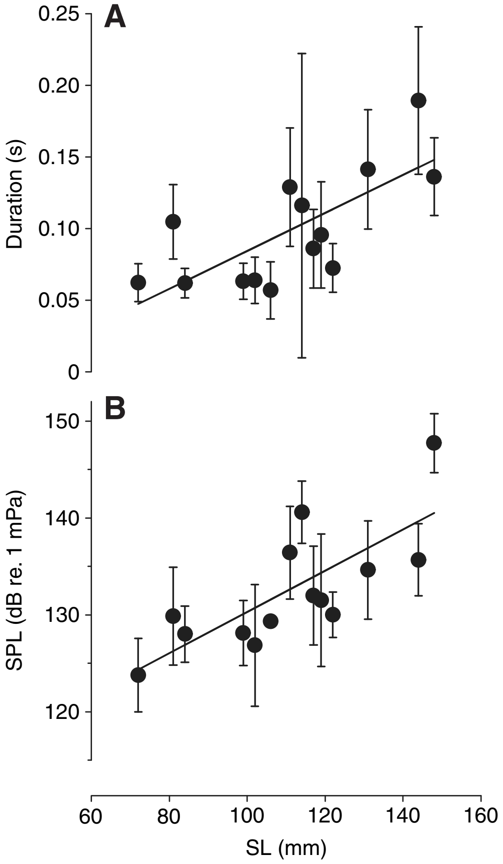

Fig. 7. Relationship between (A) mean sound duration and body size (SL) and (B) mean sound pressure level (SPL) and body size for the forcepsfish. Symbols are means ± s.d. from different individuals (N=14) and the best-fit line is from a simple linear regression of individual sound means *versus* body size. Note the strong positive scaling of both duration and SPL with body size.

amplification system (Van Wassenbergh et al., 2008) similar to that seen in other animals with ballistic-like movements, such as plethodontid salamanders (Deban et al., 2007). Cranial elevation during forcepsfish sound production occurs after EP activity but likely results from less power amplification because EP activity duration was correlated with cranial elevation velocity, unlike the independent relationship found in bay pipefish (Van Wassenbergh et al., 2008). Furthermore, EP activity indicates a tension load for much shorter periods in this study and less potential energy stored in connective tissue elements. EP activity in this study consistently occurred immediately before sound emission yet cranial elevation occurred with variable latency relative to sound onset. This action was negatively correlated with sound duration in some individuals but positively correlated in others. Further, latency to peak cranial elevation was variable with respect to EP onset and uncorrelated with EP activity duration. These observations are consistent with the hypothesis that cranial elevation occurs as a byproduct of sound production, after antagonistic activity of the SH and adductor mandibulae ceases and the head is released.

The cranial elevation pattern of sound production observed in this study is unusual both within the Chaetodontidae and broadly among teleosts. Social sound production was documented only recently in butterflyfishes. Territorial sounds of unknown mechanistic origin are described for pebbled butterflyfish (Tricas

Table 4. Relationships between individual cranial elevation kinematic features and sound features for the forcepsfish and the longnose butterflyfish

	Forcepsfish								Longnose butterflyfish							
	d.f.	F	Total test P	SL P	Kinematic P	Subj. factor	Interaction	Relationship	R^2	d.f.	F	Total test P	SL P	Kinematic P	Subj. factor	Interaction	R^2
Median frequency																	
Displacement peak	7, 138	15.94	**<0.001**	**<0.001**	0.262	s	i		0.447	5, 8	16.25	**0.001**	0.005	0.835	s	i	0.910
Displacement latency	5, 140	19.95	**<0.001**	**<0.001**	0.476	s	–		0.416	5, 8	16.64	**<0.001**	0.007	0.650	s	i	0.912
Velocity peak	6, 139	18.45	**<0.001**	**<0.001**	0.109	s	i		0.443	5, 8	16.42	**0.001**	0.006	0.733	s	i	0.911
Acceleration peak	5, 140	20.07	**<0.001**	**<0.001**	0.359	s	–		0.417	5, 8	16.71	**0.001**	0.007	0.627	s	i	0.913
Duration																	
Displacement peak	10, 135	18.89	**<0.001**	**<0.001**	0.016	s	i	+ (12/14)	0.583	2, 11	0.74	0.501	0.774	0.774	–	–	
Displacement latency	23, 122	10.72	**<0.001**	0.314	**0.001**	s	i	– (9/14)	0.669	2, 11	1.23	0.330	0.314	0.349	–	–	0.182
Velocity peak	7, 138	22.53	**<0.001**	**<0.001**	0.017	s	–	+ (all)	0.533	2, 11	0.82	0.468	0.588	0.645	–	–	0.129
Acceleration peak	7, 138	21.76	**<0.001**	**<0.001**	0.061	s	i		0.525	2, 11	0.71	0.513	0.380	0.847	–	–	0.114
SPL																	
Displacement peak	3, 142	79.86	**<0.001**	**<0.001**	**<0.001**	s	–	+ (all)	0.628	2, 11	0.55	0.593	0.326	0.792	–	–	0.910
Displacement latency	5, 140	68.78	**<0.001**	**<0.001**	0.593	s	–		0.698	2. 11	0.83	0.462	0.278	0.451	–	–	0.131
Velocity peak	10, 135	48.42	**<0.001**	**<0.001**	**<0.001**	s	i	+ (12/14)	0.782	2, 11	0.66	0.534	0.289	0.604	–	–	0.108
Acceleration peak	5, 140	64.48	**<0.001**	**<0.001**	**<0.001**	s	i	+ (13/14)	0.697	2, 11	0.80	0.474	0.234	0.481	–	–	0.127

Results are from individual multiple regression models to test the relationship between sound features (dependent variable) and cranial kinematic features (independent variable) with body size (standard length, SL) included as a covariate. Interaction terms (i) and individual subject factors (s) were kept in the model when P<0.05. Direction of relationships between kinematic variables and sound features in cases with significant main effects indicated (+ or –) are shown for N=14 forcepsfish subjects. Bold P-values are less than the sequential Bonferroni-adjusted alpha for experiment-wide multiple comparisons (0.0038 for forcepsfish and 0.0031 for longnose butterflyfish).

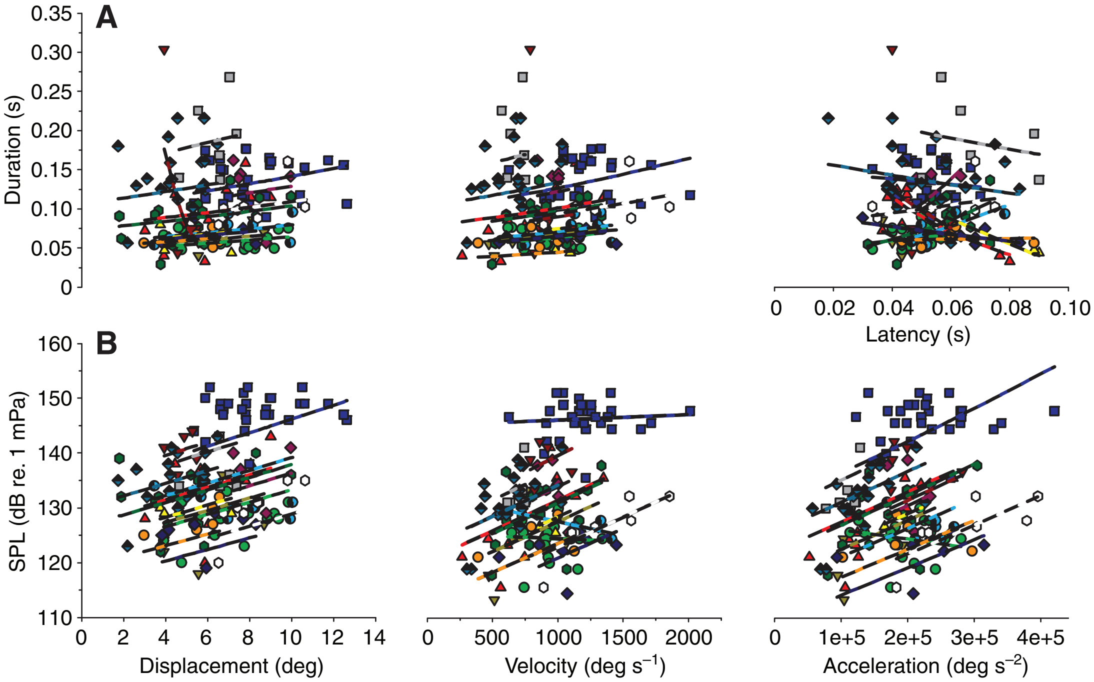

Fig. 8. Scatter plots of acoustic and kinematic features during sound production by individual forcepsfish. (A) Sound duration *versus* cranial elevation displacement (left), cranial elevation velocity (center) and latency to peak cranial elevation displacement (right). (B) SPL *versus* cranial elevation displacement (left), cranial elevation velocity (center) and cranial elevation acceleration (right). Sounds recorded from different individuals (N=14) are indicated by different symbols and colors. Fitted lines were determined from multiple regression models (see Materials and methods for details) that included body size as a covariate and, in addition, categorical variables for individuals when $P<0.05$ (all cases) and interactions when $P<0.05$ (all cases except duration *versus* velocity and SPL *versus* peak displacement). Note that duration scaled positively for 12 of 14 individuals with peak displacement, positively with velocity in all individuals and negatively with latency for 9 of 14 individuals. Sound pressure level scaled positively with peak displacement for all individuals, positively with velocity for 12 of 14 individuals and positively with acceleration for 13 of 14 individuals.

et al., 2006). Sound production in the pyramid butterflyfish (*Hemitaurichthys polylepis*) and pennant bannerfish (*Heniochus chrysostomus*) involves activity of anterior hypaxial musculature associated with anterior ribs and swimbladder (Boyle and Tricas, 2010; Parmentier et al., 2011). Recent phylogenetic hypotheses place the genus *Forcipiger* as basal within a clade that includes *Hemitaurichthys*, *Heniochus* and *Johnrandallia* and is estimated to have separated between 9.9 and 24.2 million years ago (Fessler and Westneat, 2007; Bellwood et al., 2009). The outgroup to this clade includes four genera (Fessler and Westneat, 2007; Bellwood et al., 2009), thus more analyses of sound production functional morphology within these additional taxa are needed to determine

Table 5. Relationships between muscle activity and sound features for forcepsfish

	d.f.	F	Total test P	SL P	Muscle activity P	Subj. factor	Interaction	Relationship	R^2
Median frequency									
EP	5, 70	7.42	**<0.001**	0.013	0.483	s			0.346
SH	5, 47	9.11	**<0.001**	**0.003**	0.507	s			0.492
AM1	2, 18	15.41	**<0.001**	**<0.001**	0.223	–			0.590
AM2	2, 18	14.70	**<0.001**	**<0.001**	0.309	–			0.622
Sound duration									
EP	3, 72	15.91	**<0.001**	**0.001**	0.031	s		+	0.399
SH	6, 46	7.89	**<0.001**	0.024	0.116	s			0.507
AM1	2, 18	1.38	0.277	0.116	0.942	–			0.133
AM2	2, 18	1.62	0.225	0.432	0.524	–			0.153
SPL									
EP	6, 69	46.46	**<0.001**	**<0.001**	**0.001**	s	i	+ (5/6)	0.802
SH	2, 50	77.54	**<0.001**	**<0.001**	0.999	–			0.756
AM1	2, 18	1.38	0.276	0.235	0.368	–			0.133
AM2	2, 18	1.28	0.302	0.645	0.425	–			0.125

Results are from individual multiple regression models to test the relationship between sound features (dependent variable) and muscle activity (independent variable) with body size (standard length, SL) included as a covariate. Interaction terms (i) and individual subject factors (s) were kept in the model when $P<0.05$. The direction of relationships between kinematic variables and sound features in cases with significant main effects is indicated (+ or –) for N=6 subjects. Bold P-values are ≤0.0038, the sequential Bonferroni-adjusted alpha for experiment-wide multiple comparisons.

AM1 and AM2, A1 and A2 subdivisions of the adductor mandibulae, respectively; EP, anterior epaxial musculature; SH, sternohyoideus.

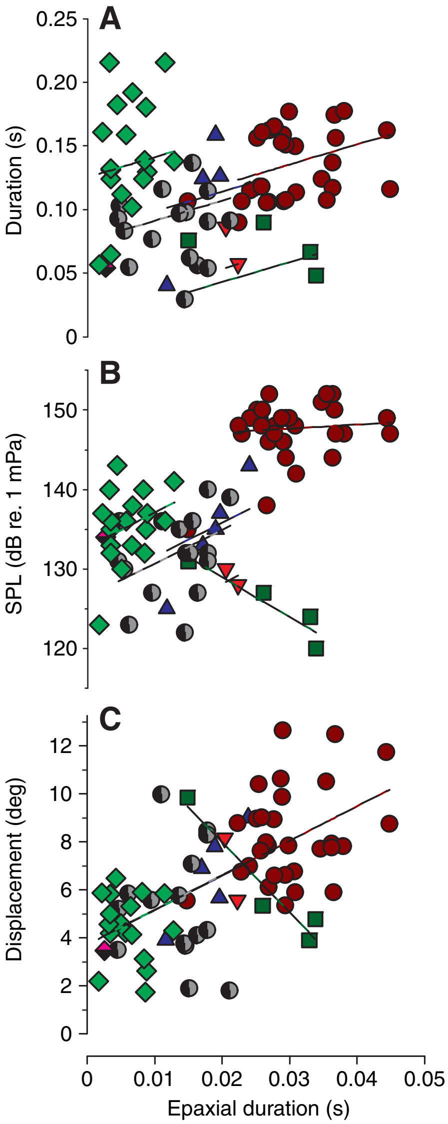

Fig. 9. Scatter plots of forcepsfish acoustic and kinematic features *versus* anterior epaxialis muscle activity. (A) Sound duration *versus* anterior epaxialis EMG duration (epaxial duration), (B) SPL *versus* epaxial duration and (C) maximum cranial elevation velocity *versus* epaxial duration. Sounds recorded from different individuals are represented by different symbols and colors. Fitted lines were determined from multiple regression models (see Materials and methods for details) that included body size as a covariate for A and B, and in addition, categorical variables for individuals when $P<0.05$ (all cases) and interactions when $P<0.05$ (all cases except duration *versus* epaxial duaration). Note that sound duration scaled positively with epaxial duration for all individuals, SPL scaled positively with epaxial duration in five of six individuals and displacement scaled positively with epaxial duration in five of six individuals.

whether the cranial elevation sound production behavior is a derived condition within *Forcipiger*. The headbob motion of sound emission is conserved in both species of *Forcipiger*, though kinematic variation was not correlated with sound emission features in longnose butterflyfish in this study, perhaps because of a small sample size. The main difference observed between these two species during sound emission was the large degree of jaw protrusion present only in longnose butterflyfish. This jaw protrusion could simply be a byproduct of an apomorphic feeding mechanism, such as the highly mobile suspensorium that allows for protrusion of the oral jaws (Ferry-Graham et al., 2001).

In yellowtail clownfish, cranial elevation is also a major component of sound production. However, sound emission occurs near peak cranial elevation, rotation of the neurocranium occurs over a longer period, sound emission results from closing of the oral jaws and sounds consist of pulse trains (Parmentier et al., 2007). Sound emission also occurs during the rapid cranial elevation of seahorse feeding (Bergert and Wainwright, 1997; Colson et al., 1998). It is not known, however, whether sounds have an agonistic or courtship social function and the mechanism of seahorses involves stridulation of the supraoccipital and coronet (Colson et al., 1998). Sound production in *Forcipiger* does not likely involve stridulation, as sounds tend to be of lower frequency and are more similar to swim-bladder-driven sounds (Ladich and Bass, 2003).

The relationships observed between fish size and cranial kinematics of forcepsfish sound production may ultimately affect signal honesty during acoustic communication. Honest signals provide accurate information about the condition of the signaler (Fitch and Hauser, 2002). For example, certain aspects of a signal may be constrained by physical attributes such as body size, and such signal features may be expected to be performed closer to their physical limits during periods of greater conflict, as was found in studies of bird song (Price et al., 2005; DuBois et al., 2009). Some observations of acoustic signaling in fishes show features of sound production consist with honesty. In a study on croaking gourami (*Trichopsis vittata*), SPL and frequency, features associated with body size, were predictors of winners between fights among males (Ladich, 1998). In the Lusitanian toadfish (*Halobatrachus didactylus*), a species that produces long, potentially costly sounds, call rate and effort during the breeding season predicted the condition of males (Amorim et al., 2010). Results from the present study demonstrate that SPL and duration are reliable indicators of forcepsfish body size. Additionally, these sound features are correlated directly with cranial elevation velocity and acceleration. The effects of body size on sound intensity are likely linked with swim bladder size and not subject to deceptive signaling strategies because swim bladder size is constrained by its hydrostatic function. Sounds of forcepsfish contain information on the signaler's size and thus may provide warning of risk associated with fights during territorial disputes as members of this species possess long jaws and 11–12 long dorsal spines (Randall, 2007) that are formidable in large adults. Many species of butterflyfishes defend feeding territories that are contiguous with conspecific pairs or groups and involve little overt aggression (Hourigan, 1989; Roberts and Ormond, 1992). Further, a positive correlation was found for male body size and territory area in a related chaetodontid fish (Tricas, 1989) and it is possible that size may be related to resource holding potential in forcepsfish as well. In addition to visual cues, size-related variation in duration and SPL of forcepsfish sounds may potentially aid receivers in recognition of familiar individuals (e.g. mates and territorial neighbors).

Our observations on cranial rotation kinematics, however, lead us to hypothesize that fish may be able to modulate SPL and duration in part with increased cranial elevation performance. Experiments in oyster toadfish (*Opsanus tau*) indicate that the apomorphic mate calling sound produced by intrinsic swim bladder muscles incurs little overall energetic costs (Amorim et al., 2002), but is fatigue limited as a result of local glycogen depletion (Mitchell et al., 2008). The metabolic costs associated with the much shorter duration cranial rotation sounds produced by forcepsfish are unknown, but are expected to be far lower than those of toadfish. However, cranial

Table 6. Relationships between epaxial muscle activity and cranial elevation variables for forcepsfish

	d.f.	*F*	Total test *P*	Epaxial activity *P*	Subj. factor	Interaction	Relationship	R^2
Displacement peak	2, 71	19.29	**<0.001**	**<0.001**	s	i	+ (5/6)	0.35
Displacement latency	2, 71	0.20	0.658	0.658	–			0.003
Velocity peak	2, 71	25.71	**<0.001**	0.084	s	i		0.420
Acceleration peak	2, 71	16.26	**<0.001**	0.230	s			0.314

Results are from individual multiple regression models to test the relationship between cranial kinematic measurements (dependent variable) and epaxial muscle activity (independent variable). Interaction terms (i) and individual subject factors (s) were kept in the model when $P<0.05$. The direction of relationships between kinematic variables and sound features in cases with significant main effects is indicated (+ or –) for $N=6$ subjects. Bold *P*-values are ≤ 0.0038, the sequential Bonferroni-adjusted alpha for experiment-wide multiple comparisons.

kinematic performance may be a reliable indicator of fish condition. Future experiments that evaluate the behavior of *Forcipiger* receivers in the presence of different acoustic signals are needed to test the ultimate functions of sound production and signal honesty in the context of territory defense and associated haremic and monogamous mating strategies.

ACKNOWLEDGEMENTS

We thank J. Allen III, W. Au, B. Bowen and K. Cole for comments on an early version of the manuscript. This is contribution #1471 from the Hawaii Institute of Marine Biology.

FUNDING

This study was supported by the National Science Foundation [grant number IBN 0137883 to T.C.T.].

REFERENCES

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

Alfaro, M. and Westneat, M. W. (1999). Motor patterns of herbivorous feeding: electromyographic analysis of biting in the parrotfishes *Cetoscarus bicolor* and *Scarus iseri*. *Brain. Behav. Evol.* **54**, 205-222.

Allen, G. R., Steene, R. and Allen, M. (1998). *A Guide to Angelfishes and Butterflyfishes*. Perth: Odyssey Publishing/Tropical Reef Research.

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

Amorim, M. C. P. and Neves, A. S. M. (2008). Male painted gobies (*Pomatoschistus pictus*) vocalise to defend territories. *Behaviour* **145**, 1065-1083.

Amorim, M. C. P., McCracken, M. L. and Fine, M. L. (2002). Metabolic costs of sound production in the oyster toadfish, *Opsanus tau*. *Can. J. Zool.* **80**, 830-838.

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

Barber, S. B. and Mowbray, W. H. (1956). Mechanism of sound production in the sculpin. *Science* **124**, 219-220.

Bass, A. H. and Baker, R. (1991). Evolution of homologous vocal control traits. *Brain Behav. Evol.* **38**, 240-254.

Bellwood, D. R., Klanten, S., Cowman, P. F., Pratchett, M. S., Konow, N. and van Herwerden, L. V. (2009). Evolutionary history of the butterflyfishes (f: Chaetodontidae) and the rise of coral feeding fishes. *J. Evol. Biol.* **23**, 335-349.

Bergert, B. A. and Wainwright, P. C. (1997). Morphology and kinematics of prey capture in the syngnathid fishes *Hippocampus erectus* and *Syngnathus floridae*. *Mar. Biol.* **127**, 563-570.

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

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

Burkenroad, M. D. (1930). Sound production in the Haemulidae. *Copeia* **1930**, 17-18.

Cohen, M. J. and Winn, H. E. (1967). Electrophysiological observations on hearing and sound production in the fish, *Porichthys notatus*. *J. Exp. Zool.* **165**, 355-370.

Colson, D. J., Patek, S. N., Brainerd, E. L. and Lewis, S. M. (1998). Sound production during feeding in *Hippocampus* seahorses (Syngnathidae). *Env. Biol. Fish.* **51**, 221-229.

Connaughton, M. A. (2004). Sound generation in the searobin (*Prionotus carolinus*), a fish with alternate muscle contraction. *J. Exp. Biol.* **207**, 1643-1654.

Connaughton, M. A., Taylor, M. H. and Fine, M. L. (2000). Effects of fish size and temperature on weakfish disturbance calls: implications for the mechanism of sound generation. *J. Exp. Biol.* **203**, 1503-1512.

Connaughton, M. A., Fine, M. L. and Taylor, M. H. (2002). Weakfish sonic muscle: influence of size, temperature and season. *J. Exp. Biol.* **205**, 2183-2188.

Crawford, J. D. and Huang, X. (1999). Communication signals and sound production mechanisms of mormyrid electric fish. *J. Exp. Biol.* **202**, 1417-1426.

Crawford, J. D., Cook, A. P. and Heberlein, A. S. (1997). Bioacoustic behavior of African fishes (Mormyridae): Potential cues for species and individual recognition in *Pollimyrus*. *J. Acoust. Soc. Am.* **102**, 1200-1212.

De Jong, K. D., Bouton, N. and Slabbekoorn, H. (2004). Azorean rock-pool blennies produce size-dependent calls in a courtship context. *Anim. Behav.* **74**, 1285-1292.

Deban, S. M., O'Reilly, J. C. O., Dicke, U. and van Leeuwen, J. L. (2007). Extremely high-power tongue projection in plethodontid salamanders. *J. Exp. Biol.* **210**, 655-667.

DuBois, A. L., Nowicki, S. and Searcy, W. A. (2009). Swamp sparrows modulate vocal performance in an aggressive context. *Biol. Lett.* **5**, 163-165.

Ferry-Graham, L. A., Wainwright, P. C., Hulsey, C. D. and Bellwood, D. R. (2001). Evolution and mechanics of long jaws in butterflyfishes (Family Chaetodontidae). *J. Morphol.* **248**, 120-143.

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

Fine, M. L., Malloy, K. L., King, C. B., Mitchell, S. L. and Cameron, T. M. (2001). Movement and sound generation by the toadfish swimbladder. *J. Comp. Physiol. A* **187**, 371-379.

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

Flammang, B. E., Ferry-Graham, L. A., Rinewalt, C., Ardizzone, D., Davis, C. and Trejo, T. (2009). Prey capture kinematics and four-bar linkages in the bay pipefish, *Syngnathus leptorhynchus*. *Zoology* **112**, 86-96.

Gannon, D. P. (2007). Acoustic behavior of Atlantic croaker, *Micropogonias undulatus* (Sciaenidae). *Copeia* **2007**, 193-204.

Gibb, A. C. and Ferry-Graham, L. A. (2005). Cranial movements during suction feeding in teleost fishes: Are they modified to enhance suction production? *Zoology* **108**, 141-153.

Grubrich, J. R. (2000). Crushing motor patterns in drum (Teleostei: Scianidae): Functional novelties associated with molluscivory. *J. Exp. Biol.* **203**, 3161-3176.

Grubrich, J. R. and Wainwright, P. C. (1997). Motor basis of suction feeding performance in largemouth bass, *Micropterus salmoides*. *J. Exp. Zool.* **277**, 1-13.

Henglmüeller, S. M. and Ladich, F. (1999). Development of agonistic behaviour and vocalization in croaking gouramis. *J. Fish Biol.* **54**, 380-395.

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

Kratochvil, H. (1978). Der bau des lautapparates vom knurrenden gurami (*Trichopsis vittatus* Cuvier & Valenciennes) (Anabantidae, Belontiidae). *Zoomorphologie* **91**, 91-99.

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

Ladich, F. (2004). Sound production and acoustic communication. In *Senses of Fishes* (ed. G. von der Emede and J. Mogdans), pp. 210-230. New Delhi: Narosa Publishing House.

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

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

Lauder, G. V., Wainwright, P. C. and Findeis, E. (1986). Physiological mechanisms of aquatic prey capture in sunfishes: functional determinants of buccal pressure changes. *Comp. Biochem. Physiol.* **84A**, 729-734.

Lindström, K. and Lugli, M. (2000). A quantitative analysis of the courtship acoustic behaviour and sound patterning in male sand goby, *Pomatoschistus minutus*. *Environ. Biol. Fish.* **58**, 411-424.

Lobel, P. S. (1996). Spawning sound of the trunkfish, *Ostracion meleagris* (Ostraciidae). *Biol. Bull.* **191**, 308-309.

Lobel, P. S. and Mann, D. A. (1995). Spawning sounds of the damselfish, *Dascyllus albisella* (Pomacentridae), and the relationship to male size. *Bioacoustics* **6**, 187-198.

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.

Mitchell, S. L., Poland, J. and Fine, M. L. (2008). Does muscle fatigue limit advertisement calling in the oyster toadfish *Opsanus tau*? *Anim. Behav.* **76**, 1011-1016.

Moulton, J. M. (1958). The acoustical behavior of some fishes in the Bimini area. *Biol. Bull.* **114**, 357-374.

Myrberg, A. A., Jr (1997). Sound production by a coral reef fish (*Pomacentrus partitus*): evidence for a vocal, territorial 'keep-out' signal. *Bull. Mar. Sci.* **60**, 1017-1025.

Myrberg, A. A., Jr and Riggio, R. J. (1985). Acoustically mediated individual recognition by a coral reef fish (*Pomacentrus partitus*). *Anim. Behav.* **33**, 411-416.

Myrberg, A. A., Jr and Spires, J. Y. (1972). Sound discrimination by the bicolor damselfish, *Eupomacentrus partitus. J. Exp. Biol.* **57**, 727-735.

Myrberg, A. A., Jr, Mohler, M. and Catala, J. (1986). Sound production by males of a coral reef fish (*Pomacentrus partitus*) its significance to females. *Anim. Behav.* **34**, 913-923.

Myrberg, A. A., Jr, Ha, S. J. and Shamblott, M. J. (1993). The sounds of bicolor damselfish (*Pomacentrus partitus*): predictors of body size and a spectral basis for individual recognition and assessment. *J. Acoust. Soc. Am.* **94**, 3067-3070.

Parmentier, E., Colleye, O., Fine, M. L., Frédérich, B., Vandewalle, P. and Herrel, A. (2007). Sound production in the clownfish *Amphiprion clarkii. Science* **316**, 1006.

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

Price, J. J., Earnshaw, S. M. and Webster, M. S. (2005). Montezuma oropendolas modify a component of song constrained by body size during vocal contests. *Anim. Behav.* **71**, 799-807.

Pruzsinszky, I. and Ladich, F. (1998). Sound production and reproductive behaviour of the armoured catfish *Corydoras paleatus* (Callichthyidae). *Environ. Biol. Fish.* **53**, 183-191.

Randall, J. E. (2007). *Reef and Shore Fishes of the Hawaiian Islands*. Honolulu, HI: University of Hawai'i Sea Grant College Program.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223-225.

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.

Roos, G., Leysen, H., Van Wassenbergh, S., Herrel, A., Jacobs, P., Dierick, M., Aerts, P. and Adriaens, D. (2009). Linking morphology and motion: a test of a four-bar mechanism in seahorses. *Physiol. Biochem. Zool.* **82**, 7-19.

Salmon, M., Winn, H. E. and Sorgente, N. (1968). Sound production and associated behavior in triggerfishes. *Pac. Sci.* **22**, 11-20.

Skoglund, C. R. (1961). Functional analysis of swim-bladder muscles engaged in sound production of the toadfish. *J. Biophys. Biochem. Cytol.* **10**, 187-200.

Svanbäck, R., Wainwright, P. C. and Ferry-Graham, L. A. (2002). Linking cranial kinematics, buccal pressure, and suction feeding performance in largemouth bass. *Physiol. Biochem. Zool.* **75**, 532-543.

Takemura, A. (1984). Acoustical behavior of the freshwater goby *Odontobutis obscura*. *Bull. Japan Soc. Sci. Fish.* **50**, 561-564.

Tavolga, W. N. (1971). Sound production and detection. In *Fish Physiology*, Vol. 5 (ed. W. S. Hoar and D. J. Randall), pp. 135-205. New York: Academic Press.

Tellechea, J. S., Norbis, W., Olsson, D. and Fine, M. L. (2011). Calls of the black drum (*Pogonias cromis*: Sciaenidae): geographical differences in sound production between northern and southern hemisphere populations. *J. Exp. Zool.* **315**, 48-55.

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

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.

Van Wassenbergh, S., Herrel, A., Adriaens, D. and Aerts, P. (2007). Interspecific variation in sternohyoideus muscle morphology in clariid catfishes: functional implications for suction feeding. *J. Morphol.* **268**, 232-242.

Van Wassenbergh, S., Strother, J. A., Flammang, B. E., Ferry-Graham, L. A. and Aerts, P. (2008). Extremely fast prey capture in pipefish is powered by elastic recoil. *J. R. Soc. Interface* **5**, 585-296.

Westneat, M. W. and Wainwright, P. C. (1989). Feeding mechanism of *Epibulus insidiator* (Labridae; Teleostei): evolution of a novel functional system. *J. Morphol.* **202**, 129-150.

Wysocki, L. E. and Ladich, F. (2001). The ontogenetic development of auditory sensitivity, vocalization and acoustic communication in the labyrinth fish *Trichopsis vittata*. *J. Comp. Physiol. A* **187**, 177-187.