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Sound generation in the searobin (*Prionotus carolinus*), a fish with alternate sonic muscle contraction

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Summary

The Northern searobin (*Prionotus carolinus*) contracts its paired sonic muscles alternately rather than simultaneously during sound production. This study describes this phenomenon and examines its effect on sound production by recording sound and EMGs during voluntary and electrically stimulated calls. Sounds produced by a single twitch resulted in a two-part sound representing contraction and relaxation sounds. The relaxation sound of one twitch coincides with the contraction sound of the next twitch of that muscle. Maximum amplitude of evoked sounds occurs between 100 Hz and 140 Hz, approximately half the fundamental frequency of a voluntarily calling fish. The muscle is capable of following electrical stimulation at frequencies of up to 360 Hz. Rapid damping and response over a wide frequency range indicate that the swimbladder is a highly damped, broadly tuned resonator. A consequence of alternate contraction is a 3.3 dB loss in acoustic pressure due to the contraction of a single sonic muscle at a time. This decrease in amplitude is offset by a doubling of fundamental frequency and a constructive interaction between the sides of the bladder, resulting in increased amplitude of each unilaterally produced sound. The alternate contraction of the bilateral sonic muscles represents a novel solution to the inherent trade-off between speed and force of contraction in rapidly contracting sonic muscles.

Key words: sound production, sonic muscle, alternate contraction, swimbladder, fundamental frequency, constructive interference, sound amplitude, *Prionotus carolinus*.

Introduction

Teleost fishes utilize a number of different mechanisms for sound production, including stridulation of bony structures and contraction of sonic muscles causing vibration of the swimbladder. Sonic muscles can be intrinsic, originating and inserting on the swimbladder wall, or extrinsic, originating elsewhere and inserting on the bladder or a structure attached to the bladder (Demski et al., 1973; Tavolga, 1971). The rate of sonic muscle contraction determines the fundamental frequency of long duration calls. Fishes producing sound in this manner generate fundamental frequencies between 100 Hz and >200 Hz, indicating that the muscles are contracting at these rates (Cohen and Winn, 1967; Skoglund, 1961; Tavolga, 1962). By comparison, white muscle contraction rates rarely get above 50 Hz (Gainer et al., 1965; Rome et al., 1996; Tavolga, 1964). In the toadfish *Opsanus tau* at 15°C, red muscle cannot generate power over 2.2 Hz and white muscle cannot generate power over 12 Hz, but sonic muscle can generate power over 100 Hz (Young and Rome, 2001). Fine et al. (2001) suggested that rapid contraction of the sonic muscles is required to move a highly damped swimbladder at velocities that can produce audible sound.

The sonic muscle of the toadfish, which has been called the fastest vertebrate striated muscle (Rome and Lindstedt, 1998; Rome et al., 1996; Tavolga, 1964), can be stimulated to contract *in situ* at rates of 500 Hz without reaching tetany (Fine et al., 2001). In order to contract at these speeds, sonic muscles express a variety of morphological and biochemical adaptations for speed. These adaptations include, but are not limited to, small diameter, radial morphology (Bass and Marchaterre, 1989; Eichelberg, 1976; Fawcett and Revel, 1961; Fine et al., 1993), multiple innervation of muscle fibres (Gainer and Klancher, 1965; Hirsch et al., 1998; Ono and Poss, 1982), abundant sarcoplasmic reticulum (Eichelberg, 1977; Franzini-Armstrong and Nunzi, 1983) and parvalbumin (Appelt et al., 1991; Hamoir et al., 1980) content, a large calcium capacity (Feher et al., 1998), the fastest calcium transient in a vertebrate muscle, and a rapid cross-bridge detachment rate (Rome et al., 1996, 1999). A result of rapid cross-bridge detachment rates is the sacrifice of much of the twitch force. The sonic muscles of toadfish produce approximately 10% of the force per cross-sectional area of white muscle myofibrils (Rome et al., 1999). However, these muscles still manage to displace the highly damped swimbladder and produce a clearly audible sound at high contraction rates (Fine et al., 2001).

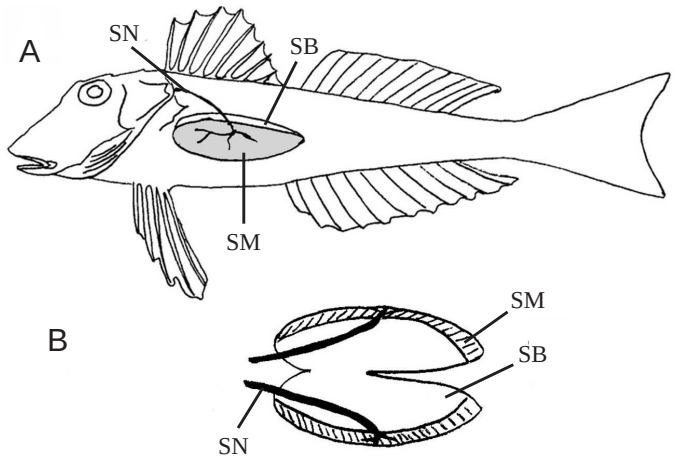

Fig. 1. (A) The position of the swimbladder (SB), sonic muscles (SM) and sonic nerve (SN) in the northern searobin, *Prionotus carolinus*. (B) A view of the dorsal surface of the swimbladder illustrating the intrinsic sonic muscles and the sonic nerve innervating them.

The Northern searobin, *Prionotus carolinus*, bears both intrinsic and extrinsic sonic muscles (Evans, 1973), and the intrinsic muscles are known to play a role in sound production (Evans, 1973; Tower, 1908). The function of the smaller, extrinsic sonic muscles is not clear and they will not be considered in this paper. Little information is available regarding the acoustic behavior of searobins. Captive (Fish, 1954) and field (Fish and Mowbray, 1970) recordings suggest that sound is used in fright and perhaps antagonistic interactions. The intrinsic muscles of the searobin are innervated ipsilaterally by fibres from the occipital nerve (Fig. 1; Bass and Baker, 1991; Evans, 1973; Finger and Kalil, 1985). Bass and Baker (1991) recorded discharges from the sonic nerve (a branch of the occipital nerve) indicating that the bilateral sonic muscles contract alternately in this species rather than synchronously, the norm among sonic fishes (Cohen and Winn, 1967; Connaughton et al., 2000; Skoglund, 1961; Tower, 1908).

The purpose of the present study was to describe sound generation in the searobin *Prionotus carolinus* using voluntary and electrically stimulated sounds. The objectives of this study were several-fold: to describe the disturbance sounds of searobins, to confirm that the sonic muscles contract alternately, to determine the optimal and maximal rates of muscle contraction producing audible sound and to determine if unilateral contraction of the sonic muscles sacrifices sound amplitude. Voluntary and evoked calls and muscle action potentials were recorded in air to avoid difficulties associated with recording in an enclosed aquatic space (Akamatsu et al., 2002; Parvulescu, 1964; Tavolga, 1962) and to allow for the determination of absolute sound pressure level at a standard distance. Carrying out the experiments in air also simplified the recording of action potentials and stimulation of the sonic nerve.

Materials and methods

Northern searobins (*Prionotus carolinus* L.) were collected in the waters off Woods Hole, MA, USA and maintained in 1500 litre flow-through salt water tanks at the Mount Desert Island Biological Laboratory, Salsbury Cove, ME, USA. Fish were held overnight prior to experiments in a 150 litre holding tank at temperatures ranging from 15°C to 17.5°C. In this study, voluntary calls refer to disturbance calls made by specimens held in-hand, out of water before and after electromyogram (EMG) electrode implantation. Frequency peaks were generated from spectra produced by fast Fourier transformation. Evoked calls refer to those induced by electrical stimulation of the sonic nerve (SN) of an anaesthetized fish.

Simultaneous EMG and acoustic recordings were made from voluntarily calling searobins in air. In-air and in-water recordings of weakfish (*Cynoscion regalis*; Connaughton et al., 2000) and Atlantic croaker (*Micropogonias undulatus*; J. Drummond and M. A. Connaughton, unpublished data) indicate that sounds produced under these conditions are identical except for a greater number of pulses in calls recorded in air. In-air recording trials were kept to less than 1 min in duration, after which the fish was anaesthetized for electrical stimulation of the SN (see below). EMGs were recorded differentially (World Precision Instruments, DAM 50 amplifier, Sarasota, FL, USA) using Teflon-coated wire electrodes placed no more than 2 mm apart in either the left or right sonic muscle through a 1 cm incision in the lateral body wall. In four experiments, recordings were made simultaneously from the left and right sonic muscles using a single amplifier, with only one electrode placed in each of the muscles. Acoustic recordings were made with a pressure zone microphone (Realistic, Tandy Corp., Fort Worth, TX, USA; frequency response flat from 20 Hz to 18 kHz) held 10 cm from the fish. The signal was amplified (Radio Shack Karaoke Mate), digitized (MacLab; AD Instruments, Castle Hill, NSW, Australia) and recorded at 11.1 kHz on a Macintosh computer. A 500 Hz, 70 dB (re: 20 μPa) calibration tone, measured with a sound level meter (Realistic) adjacent to the microphone, was recorded to permit measurement of absolute sound pressure level (SPL). Recordings were analyzed with Scope oscilloscope software (v. 3.3; AD Instruments) and Canary bioacoustic workstation software (v. 1.2; Cornell Laboratory of Ornithology, Ithaca, NY, USA). For comparison with action potential repetition rate, acoustic repetition rate (= fundamental frequency) was calculated from the period of the waveform.

Specimens were anaesthetized with 100 mg l^{-1} MS-222 (tricaine methanesulfonate; Sigma Chemical Co., St Louis, MO, USA) buffered to a neutral pH. Fish were placed in a recording chamber with water and anaesthetic recirculating over the gills. The SN was exposed by dissection of a 1 cm×2 cm window in the hypaxial musculature and stimulated (MacLab) *via* hook electrodes with 0.5 ms, 1 V, square wave pulses in 90 ms sweeps (the approximate duration of a disturbance call) at frequencies ranging from 50 Hz to 500 Hz. Simultaneous unilateral EMG

and acoustic recordings were made with the microphone at 6 cm from the fish during SN stimulation. In four stimulation experiments, one, then both SNs were stimulated at frequencies between 100 Hz and 150 Hz for comparison of unilateral and bilateral SPL within a fish. SPL was determined as the average sound intensity across a 90 ms stimulation sweep. Fish were then sacrificed *via* an overdose of anaesthetic at low temperatures. Total length, total mass, total sonic muscle mass and average sonic muscle thickness were measured for each fish (13 male and 12 female). All experimental protocols were approved by the Mount Desert Island Biological Laboratory Institutional Animal Care and Use Committee.

A pooled *t*-test, or Wilcoxon signed rank test in the case of non-parametric data, was used to compare male and female data. Acoustic parameters were regressed across total length and holding tank temperature. A pooled *t*-test was used to compare the duration of acoustic waveforms from evoked and voluntary single twitches. Bonferroni multiple comparison tests (summed α=0.05) were used to compare among various parts of single twitch acoustic waveforms and among peak-to-peak negative pressure intervals. A paired *t*-test was used to compare unilateral and bilateral SPL for each fish at each stimulus rate.

The sharpness of tuning (Q) of the swimbladder was calculated as:

$$Q_{3dB}=f_r/bw$$

where f_r is the stimulation frequency that produced the maximal amplitude response and bw is the bandwidth, or frequency range, across which the amplitude of sounds produced by the resonator (the swimbladder) was within 3 dB of the maximal amplitude (Bradbury and Vehrenkamp, 1998).

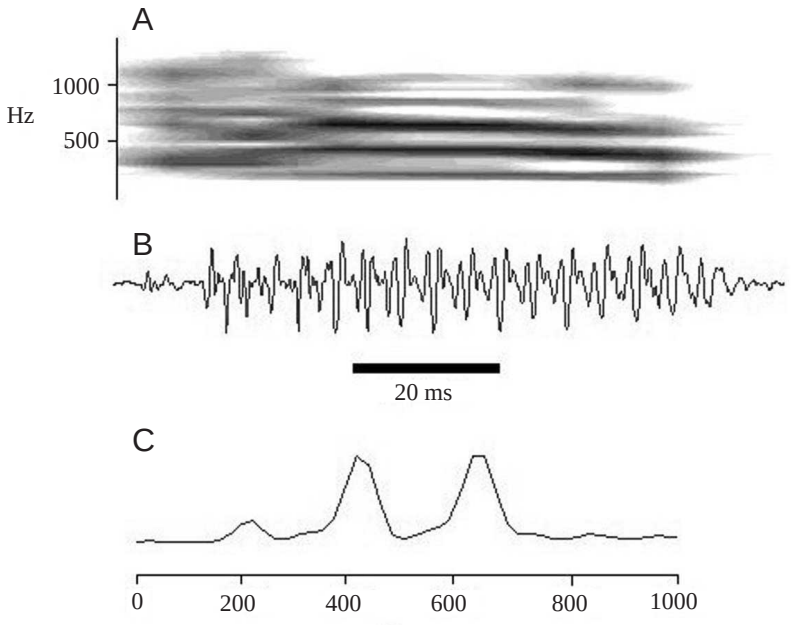

Fig. 2. The sonagram (A), waveform (B) and power spectrum (C) of a *Prionotus carolinus* voluntary disturbance call recorded in air.

Results

Sonic muscles

Total length ranged from 21.5 cm to 27.5 cm and did not vary with sex. Sonic muscle mass ranged from 1.3% to 2.4% of total mass and expressed a mean of 1.8±0.3%. Relative sonic muscle mass was significantly greater (P=0.0037) in males (mean ± S.D., 2.0±0.2%) than in females (1.7±0.3%). Similarly, sonic muscle thickness ranged from 0.9% to 2.4% of total length, expressed a mean of 1.60±0.33% and was also greater (P=0.0231) in males (1.8±0.3%) than in females (1.5±0.3%).

Disturbance calls

Prior to electrode implantation, duration, SPL and peak frequencies were collected for six disturbance calls from each fish (10 male, 7 female) and none of these call characters varied significantly with sex. Calls had a mean duration of 62.8±16.1 ms, a SPL of 75.4±2.0 dB (re: 20 μPa at 10 cm), a fundamental frequency of approximately 200 Hz (204.1±15.5 Hz) with harmonics at approximately 400 Hz and 600 Hz (Fig. 2). However, some compound calls (ranging in duration from 100.6 ms to 132.2 ms) were recorded from one fish, and some longer sounds (240.4–933 ms) were recorded from two other fish. These compound and longer calls are not included in the mean data.

Call duration did not vary significantly with total length or temperature. SPL increased significantly with total length (y=0.50x+63.1, r^2=0.238, P=0.047) but did not vary over a temperature range of 2.5°C.

The power spectra of voluntary calls exhibited three or more clear frequency peaks. The frequency with the greatest amplitude often varied among these peaks even within the calls of a single individual. For example, a typical fish would produce a fundamental frequency of 217 Hz and two harmonic peaks at 435 Hz and 652 Hz. The range of fundamental frequencies observed was 174–217 Hz. The ranges for the two harmonic peaks were more variable, 348–481 Hz and 522–674 Hz, respectively. Peak frequencies did not vary significantly with total length. Fundamental frequency increased significantly with temperature, rising 43 Hz over a modest 2.5°C change in temperature (Fig. 3A).

Alternate muscle contraction during disturbance calls

Trains of EMGs in unilateral recordings of disturbance calls (N=12) expressed a mean duration of 89.5±15.6 ms and consisted of 4–8 action potentials (mean action potential duration= 6.2±1.0 ms) followed by an extended return to baseline (30.4±10.8 ms; Fig. 4A). The number of sound pulses per call ranged from 6 to 15 (11.2±2.2). The interval between action potentials for one muscle averaged 9.5±0.8 ms while the interval between sound pulses was 4.8±0.4 ms. Action potential repetition rate was half the sound

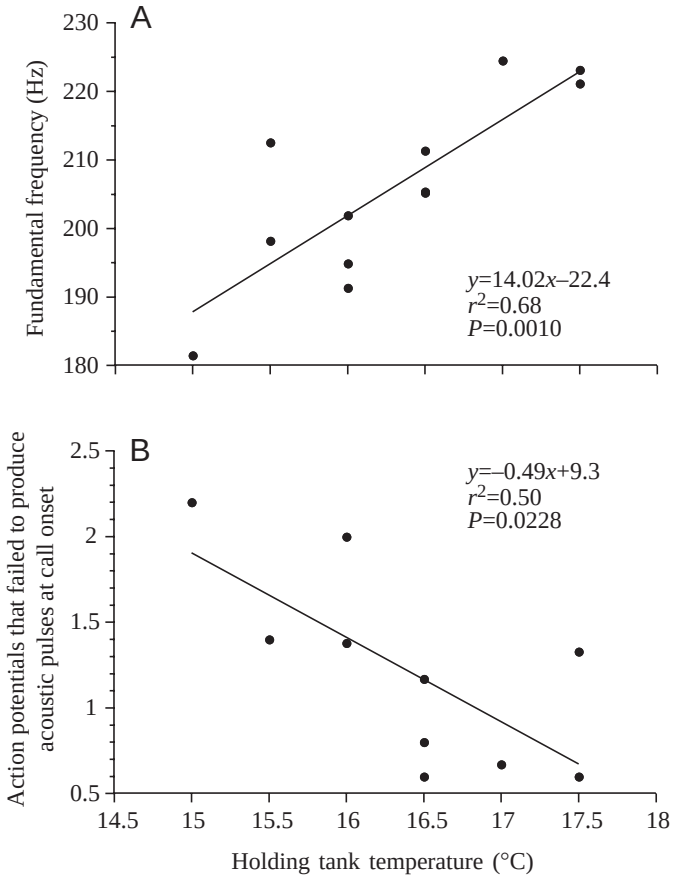

Fig. 3. (A) Fundamental frequency (N=12) and (B) number of action potentials that failed to produce an acoustic pulse from call onset (N=10) regressed across holding tank temperature. Data were taken from simultaneous acoustic and unilateral electromyogram recordings of voluntary in-air disturbance calls.

repetition rate (fundamental frequency; Fig. 5), a typical call expressing values of 107 Hz and 213 Hz, respectively. Mean action potential repetition rate (106.5±8.3) was close to half that of the sound repetition rate (206.4±16.1). In unilateral recordings, either the muscle with the electrode or the contralateral muscle might contract first. Since the first action potential did not always generate an acoustic pulse, it was not always possible to determine if the contralateral muscle contracted first. When the ipsilateral muscle contracted first, the time between onset of the action potential and the first sound pulse was 2.1±0.5 ms.

An action potential was present prior to alternate sound pulses in unilateral recordings (Fig. 4A), whereas in bilateral EMG recordings, every sound was preceded by an action potential (Fig. 4B). Between the large amplitude sound pulses, smaller single, double or more complex waveforms were visible. These smaller waveforms varied between fish. At the onset of most traces, one or more action potentials failed to produce acoustic pulses, most commonly the first, second or third action potential. The number of action potentials without resultant sounds at the onset of a call decreased with increasing temperature (Fig. 3B). Amplitude of sound pulses in a call increased to a maximum at the 5th (= mode; mean=5.7±0.5) pulse. Two additional, attenuated sound pulses followed the final action potential (Fig. 4A,B). The durations of the negative pressure peaks of these attenuated pulses (2.9±0.5 ms and 3.3±0.6 ms, respectively) were significantly greater than those of sound pulses associated with action potentials (2.0±0.4 ms; P=0.0001 for both).

Single twitch mechanics

Simulating the SN at 50 Hz provided the opportunity to examine single twitch mechanics, as the acoustic waveform of one twitch was complete before the next stimulus in the series occurred. The acoustic waveform resulting from a single twitch consisted of two distinct sound pulses (see Table 1 for durations) putatively described as the contraction and relaxation components of the sound, following Fine et al. (2001). The first, or contraction waveform, began with a negative half-cycle followed by a positive half-cycle of acoustic pressure (B in Fig. 6A). A plateau containing little or no sound energy followed this waveform (D). The second, or relaxation waveform, began with a positive half-cycle followed

Table 1. *Duration of acoustic waveforms produced by evoked and voluntary single twitches*

Waveform	Fig. 6 labels	Evoked duration (ms)	Voluntary duration (ms)
Interval between action potential and 1st waveform	A	2.05±0.4	2.29±0.1
1st waveform* duration	B	2.63±0.3‡	2.70±0.4§
2nd waveform† duration	C	3.63±0.7‡	5.59±0.4§
Interval between waveforms	D	5.47±0.8	6.38±0.4
Total dual-waveform duration	B+C+D	11.7±1.07	14.1±0.05
1st waveform* peak frequency		426.0±32.40¶	517.33
2nd waveform† peak frequency		311.72±25.60¶	438.67

Data were collected from twitches evoked by unilateral stimulation of the sonic nerve at 50 Hz or from voluntary single twitches (abortive calls). Data are presented as means ± S.D. (N=6–9 for evoked calls, N=2 for voluntary calls, N=1 for voluntary frequency). Shared symbols indicate significantly different values within evoked and voluntary twitches as determined by a paired t-test (i.e. the t-test compares 1st and 2nd waveform duration for evoked calls, etc.). *1st waveform, contraction sound; †2nd waveform, relaxation sound; ‡P=0.008; §P=0.028; ¶P=0.0001.

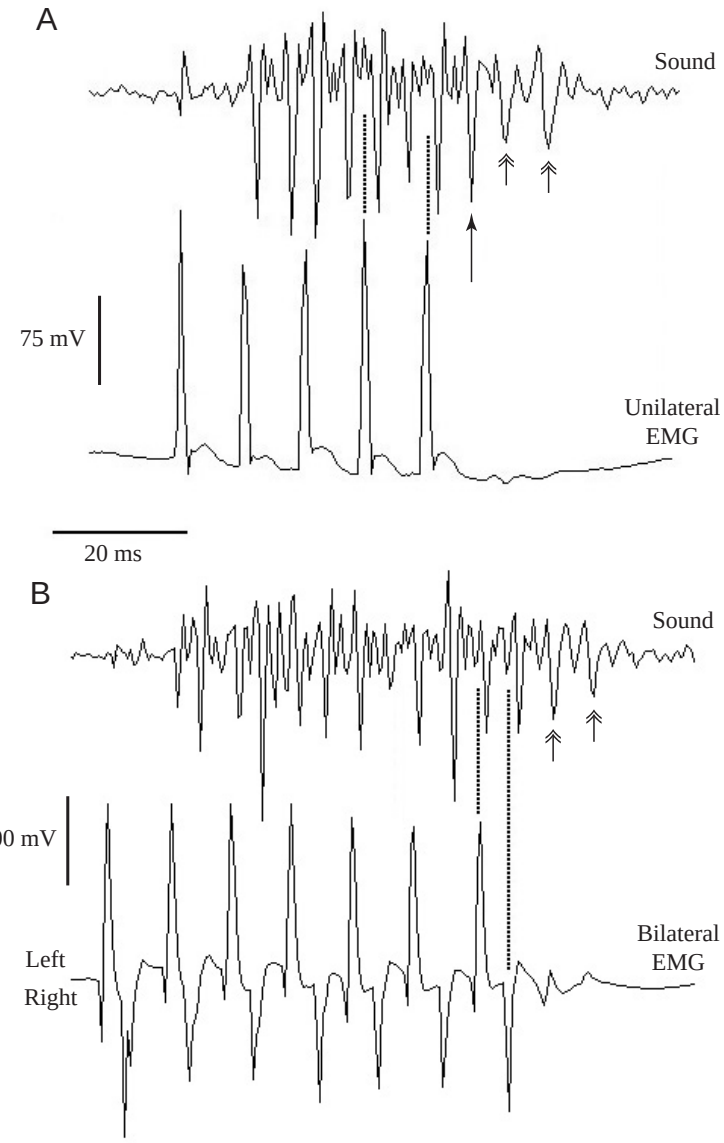

Fig. 4. Sound and (A) unilateral or (B) bilateral sonic muscle electromyogram (EMG) traces from voluntary disturbance calls recorded in air. In the bilateral trace, action potentials from the right sonic muscle deflect down, while those from the left muscle deflect up. Muscle action potentials occur before alternate sound pulses in the unilateral trace but before each sound in the bilateral trace (dotted lines). Note the action potentials failing to produce acoustic pulses early in both calls and the two attenuated sound pulses at the end of each call that are not associated with any action potentials (double arrows in both traces). Note also the sound pulse in the unilateral trace denoted with a single arrow. This sound indicates that the contralateral (off-trace) sonic muscle contracted last in this call.

by a negative half-cycle and another positive half-cycle of acoustic pressure (C). Data from evoked single twitches (*N*=9 fish, 4 twitches each) were corroborated by voluntary single twitches recorded during abortive calls (*N*=2 fish, a total of 9 twitches). The only significant differences between the dual-waveform sounds of evoked and voluntary single twitches were the duration of the relaxation waveform (*P*=0.0039) and, consequently, the total duration (*P*=0.0048; Table 1). The durations of the contraction and relaxation waveforms differed significantly within evoked and voluntary twitches, as did peak frequencies (Table 1).

In voluntary calls, the intervals between action potentials and between sound pulses did not vary from the beginning to the end of the call. Based on the period between peak negative pressures, the interval between the contraction and relaxation waveforms in single twitches (E on Fig. 6) matched the interval between alternate sound pulses within a voluntary call (F; Table 2). This suggests that in a voluntary call, the relaxation waveform from a single twitch coincides with the contraction waveform of the next twitch of that sonic muscle (Fig. 7). The interval between the contraction and relaxation waveforms of a voluntary twitch was similar to the intervals preceding the two attenuated pulses (Table 2). This suggests that the attenuated pulses, although not associated with any action potential, represent the relaxation waveform of the final muscle twitch of each alternately contracting sonic muscle (Fig. 7).

Table 2. *Intervals between acoustic waveforms from single twitches and between sound pulses from voluntary calls*

Interval	Fig. 6 labels	Duration (ms)
1st to 2nd waveform* – evoked twitch	E	9.29±0.9†,‡
1st to 2nd waveform* – voluntary twitch	(E, not shown)	10.79±0.6
Alternate (unilateral) sound pulses – both associated with action potentials	F	9.89±0.6§
2nd to last sound pulse associated with an action potential to 1st attenuated sound pulse	G	10.50±0.6†,¶
Last sound pulse associated with an action potential to final attenuated sound pulse	H	12.06±1.1‡,§,¶

Intervals were measured from peak to peak negative acoustic pressure (see Fig. 6). Data are presented as means ± S.D. (*N*=9 except voluntary single twitches in which *N*=2). Intervals were compared using a Bonferroni multiple comparison with a pooled α of 0.05. Shared symbols indicate significantly different waveform durations. *1st waveform, contraction sound; 2nd waveform, relaxation sound; $^{\dagger}P$=0.0288; $^{\ddagger}P$=0.0001; $^{\S}P$= 0.0001; $^{\P}P$=0.0018.

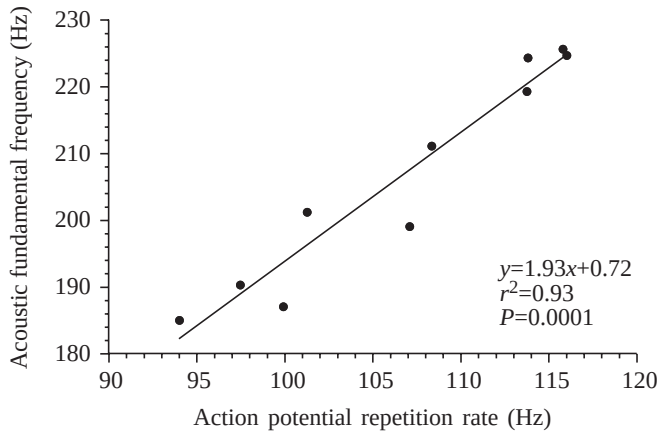

Fig. 5. Acoustic fundamental frequency (sound repetition rate) regressed across unilateral action potential repetition rate. Values were generated from the period of the respective waveforms, not from fast Fourier transformation (N=10).

Evoked trains

The sonic muscle followed the SN stimulus rate (produced sound of that frequency) from 50 Hz to 360 Hz. At 400 Hz, the peak frequency in the power spectra was half the stimulus rate, indicating that the muscle was contracting in response to alternate stimuli. Greatest sound amplitudes were recorded between 100 Hz and 140 Hz, with the maximal amplitude (98.8 dB re: 20 μPa) at 120 Hz (Fig. 8). A Q_{3dB} of 1.98 was calculated from the response of the swimbladder to this range of stimuli.

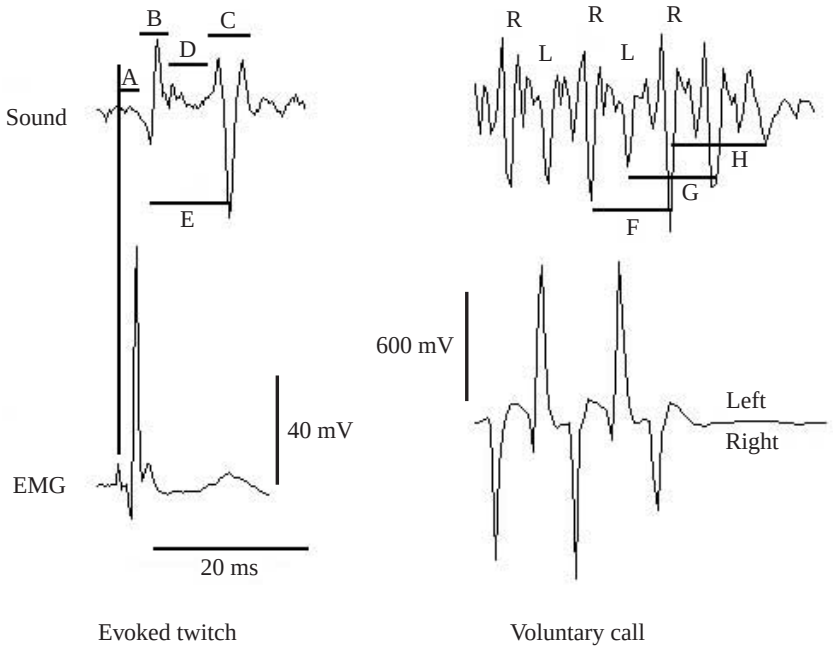

Fig. 6. Acoustic (top panels) and bilateral sonic muscle electromyogram (EMG; bottom panels) traces from a single evoked twitch and a voluntary call. The single twitch was evoked by stimulating the sonic nerve at 50 Hz and generated a two-part acoustic waveform. In the voluntary call, sound pulses from the right (R) and left (L) sonic muscle twitches are noted. Descriptions and mean durations of intervals A–H can be found in Tables 1, 2.

Action potential amplitude began to decrease by the end of any stimulus series above 120 Hz (Fig. 9). At frequencies as high as 360 Hz (data not shown) the muscle responded to each stimuli, although action potential amplitude dropped sharply after the first few stimuli. At 400 Hz, the muscle was observed to respond to alternate stimuli, while at 500 Hz the muscle either did not respond after the first stimulus or did so irregularly (Fig. 9).

Evoked trains of sound at 100–150 Hz (see Fig. 9) were similar to voluntary calls. They began with a small initial sound followed by one or more action potentials without resultant sounds. Amplitude of the sound pulses increased to a maximum and then declined. As the stimulus rate increased, the highest amplitude sound pulse came later in the series, shifting from the second pulse at a 50 Hz stimulus rate to the fifth or sixth pulse at 160–180 Hz (Table 3). In addition, as the stimulus rate increased, more initial action potentials failed to produce sounds and the muscle often failed to produce sounds through the end of the stimulus series. At stimulus rates for which sound was produced through the end of the series, the final sound pulse was not associated with any stimulus or action potential and was attenuated (see 100 Hz in Fig. 9). Note that there was only one attenuated sound pulse in evoked calls.

Bilateral vs unilateral amplitude

In evoked calls, synchronous bilateral contraction of the sonic muscles produced greater amplitude sound than unilateral contraction at all stimulus rates (P=0.005, 0.0021, 0.005 and 0.001 for four fish; see a sample distribution for one fish in Fig. 10A). The SPL difference between bilaterally and unilaterally evoked sounds (3.3±0.6 dB) ranged from 2.6 dB at 100 Hz stimulation to 3.8 dB at 140 Hz (Fig. 10B). Individual differences in unilateral and bilateral SPL ranged as high as 5.9 dB, an approximate doubling of acoustic pressure.

Discussion

This study has shown that the bilateral, intrinsic sonic muscles of the searobin *Prionotus carolinus* contract alternately rather than synchronously. The highly damped, short time-course of the sound produced by a single twitch (11–14 ms) and a low Q (1.98) suggest that the swimbladder of the searobin is a broadly tuned, highly damped sound source. The response of the bladder to any frequency imparted on it supports the conclusion that the sonic muscle, not the natural frequency of the swimbladder, determines the fundamental frequency of the sounds produced. The coincidence of the two-part acoustic waveform produced by consecutive twitches of a muscle

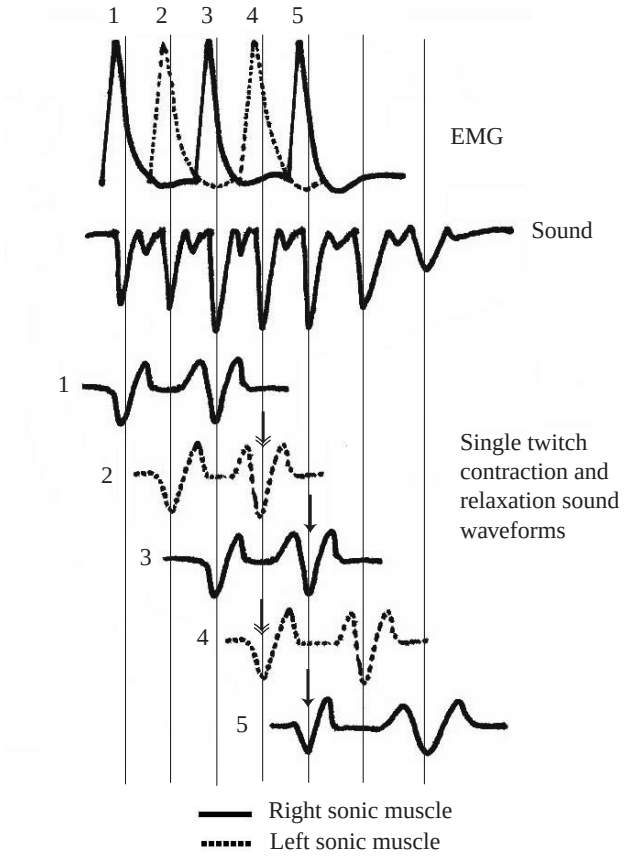

Fig. 7. A diagram of sonic muscle electromyogram (EMG; 1–5) and acoustic traces from a voluntary disturbance call with a breakdown of dual-waveform sounds produced by each muscle twitch (vertical 1–5). Right and left EMG traces are superimposed on the same trace all deflecting up and the acoustic trace has been simplified to highlight the negative pressure peaks. Note that the 2nd sound waveform (relaxation sound; see text) of a single twitch coincides with the 1st waveform (contraction sound) of the next twitch of that sonic muscle, not the contralateral muscle (compare traces 2 and 4, double arrows, and traces 3 and 5, single arrows). Note also that the first two sound pulses in the call are not reinforced in this manner. Finally, note that the relaxation sounds of the final twitch of the right and left sonic muscles account for the two attenuated sound pulses at the end of the call.

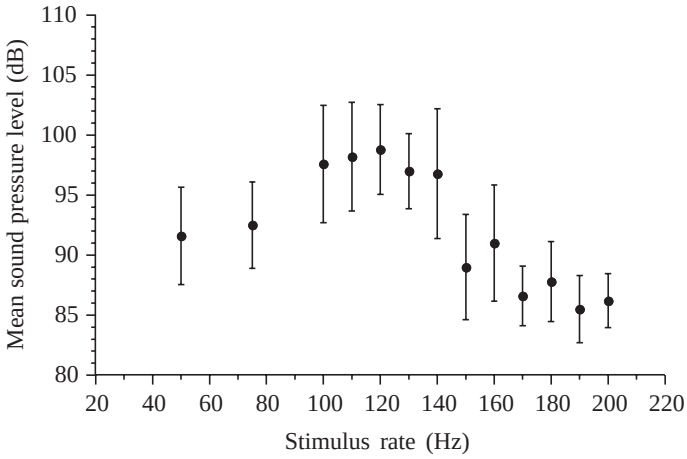

Fig. 8. Mean (± S.D.; *N*=2–5) sound pressure level of sounds evoked by stimulation of the sonic nerve at frequencies of 50–200 Hz. Sound pressure level was measured as the average pressure (re: 20 μPa) over the duration of a 90 ms stimulus sweep.

Table 3. *Mean sound pulse number expressing the greatest amplitude in an evoked train of sounds*

Stimulus rate (Hz)	Mean sound pulse number	*N*
180	5.50	2
160	5.40	5
150	4.60	5
140	4.50	10
130	3.83	6
120	3.18	11
110	2.71	7
100	2.55	11
75	1.80	5
50	2.00	5

Sounds were evoked by unilateral stimulation of the sonic nerve with a 90 ms stimulus train.

results in constructive interference that increases sound amplitude during voluntary calls.

The voluntary disturbance sounds produced by the searobin in this study were similar in duration and frequency to 'barks' recorded in captivity (Fish, 1954) and in the field (Fish and Mowbray, 1970). Fundamental frequency increased with temperature as it does in other species producing long, non-pulsatile sounds (Brantley and Bass, 1994; Fine, 1978; Schneider, 1967). The basis for this change in frequency is an increase in discharge rate of the sonic motor nucleus with temperature (Bass and Baker, 1991).

Searobin sonic muscles contract alternately, as suggested by the observation of asynchronous sonic nerve discharges (Bass and Baker, 1991). This is a unique arrangement for a teleost sonic system, as all other sonic muscles examined to date contract synchronously (Cohen and Winn, 1967; Connaughton et al., 2000; Packard, 1960; Skoglund, 1961; Tower, 1908). During voluntary disturbance calls, the searobin sonic muscles contracted alternately at a mean of 105 Hz, producing a fundamental acoustic frequency of approximately twice this value. When driven by stimulation of the sonic nerve, the sonic muscle produced maximal sound amplitudes at 120 Hz, somewhat higher than the muscle operates during voluntary calling. Midshipman (*Porichthys notatus*) produce a courtship buzz at fundamental frequencies between 98 Hz and 108 Hz by synchronously contracting their sonic muscles (Cohen and Winn, 1967; Ibara et al., 1983). Therefore muscles of the searobin and midshipman contract at approximately the same rate, although a twofold difference in acoustic fundamental frequency results. The sonic muscles of the toadfish and pigfish (*Congiopodus leucopaecilus*) produce 200 Hz calls *via*

synchronous muscle contraction (Fine, 1978; Packard, 1960; Skoglund, 1961) and thus function at twice the contraction rate of the searobin. The extrinsic sonic muscles of two catfish, *Galeichthys felis* and *Bagre marinus*, produce 150 Hz fundamentals (Tavolga, 1962), falling between the toadfish and the searobin.

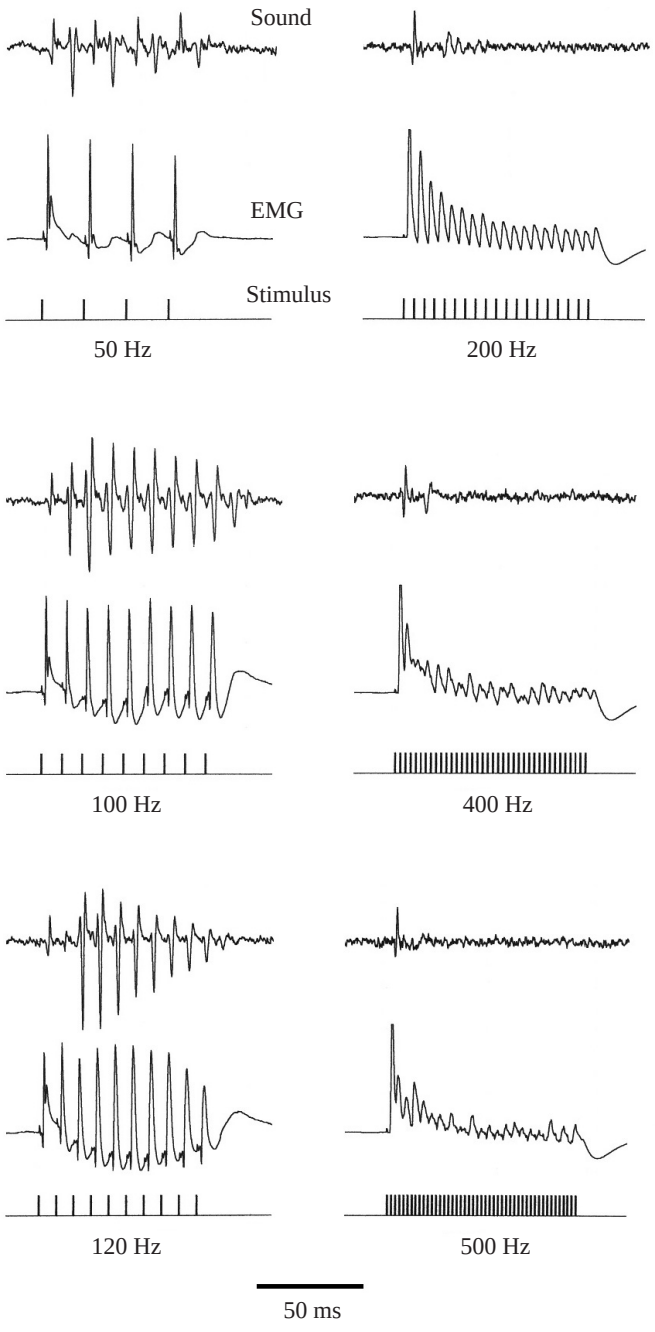

Fig. 9. Acoustic and electromyogram traces from calls evoked by 90 ms trains of stimuli applied unilaterally to the sonic nerve. The sonic muscle is fast enough that stimulation at 50 Hz resulted in a series of separate twitches in which the entire two-part waveform of the produced sound can be seen. Action potentials matched stimulation frequencies to 360 Hz (not shown) but responded to alternate stimuli at 400 Hz.

The sonic muscles of the toadfish have been reported to tetanize at 250–300 Hz (at 25°C) in isolated bundles (Rome et al., 1996) but are able to produce trains of sound *in situ* at 400 Hz and can respond to alternate stimuli without tetany at 500 Hz (Fine et al., 2001). The sonic muscles of *Bagre* also did not fully tetanize at 500 Hz, while those of *Galeichthys* reached tetany at 300–400 Hz (Tavolga, 1962). The sonic muscles of another searobin, *Prionotus scitiulus*, have been observed to tetanize in half a second at stimulus rates of 340–380 Hz (Tavolga, 1964). Finally, the slower sonic muscles of the squirrelfish (*Holocentrus* sp.) and the red hind (*Epinephalus guttatus*) have been observed to tetanize between 150 Hz and 200 Hz (Gainer et al., 1965; Tavolga, 1964).

Determination of tetany requires a constant muscle force at a known stimulation frequency. This study did not measure force, nor can it be determined with any certainty that all of the muscle fibres in the stimulated muscles were activated by each stimulus during evoked trains. Having said this, sound production might be used to make some simple suggestions about the capabilities of the searobin sonic muscle. Based on sound amplitude, it appears that the searobin sonic muscle

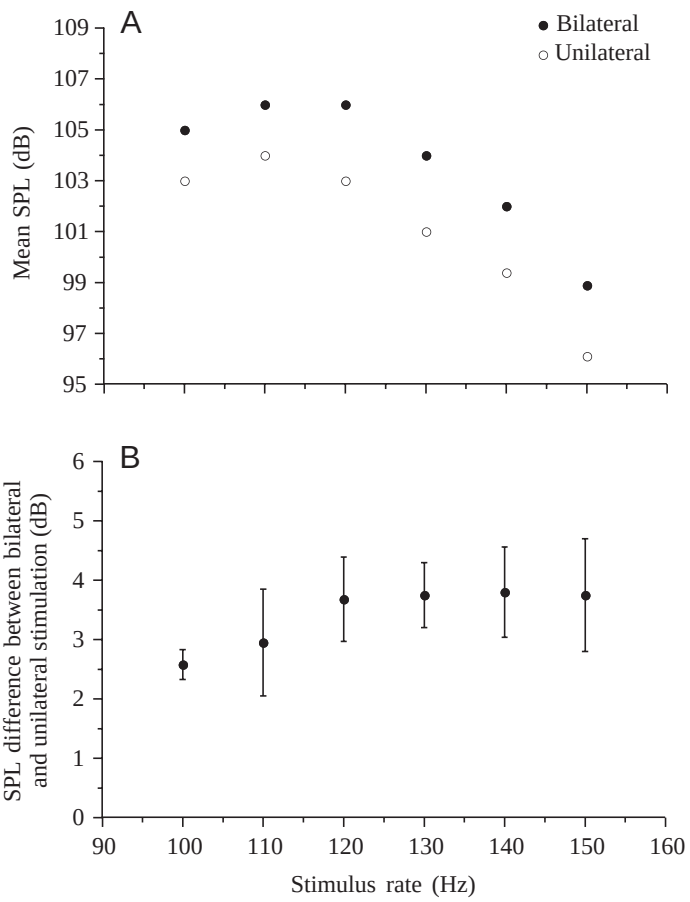

Fig. 10. (A) Sample distribution of sound pressure level (SPL) across stimulus rate for sounds evoked bilaterally and unilaterally from the same fish (90 ms sweeps at 100–150 Hz). (B) SPL difference between bilaterally and unilaterally evoked calls (mean ± S.D.; N=4). SPL was measured as the average pressure (re: 20 μPa) over the duration of a 90 ms stimulus sweep.

(more accurately the sonic muscle–swimbladder system) was operating optimally when stimulated between 100 Hz and 140 Hz. The muscle was capable of producing sound of matching frequencies at stimulus rates of up to 360 Hz but, at a stimulus rate of 400 Hz, the searobin sonic muscle contracted during alternate stimuli and, at 500 Hz, the muscle ceased to respond after the initial stimulus. Thus, using sound production as a tentative indicator of muscle mechanics, the sonic muscle of the searobin appears to be capable of contraction rates in the mid-range for sonic muscles and at rates similar to those reported for *P. scitiulus* (Tavolga, 1964).

A number of characteristic acoustic traits identified in voluntary calls are also present in evoked calls. The commonality of these traits in voluntary and evoked calls suggests that they may reflect important features of the mechanics of the sonic motor system in searobins. The first few sound pulses are of low amplitude or are even missing, i.e. an action potential is present but no obvious sound is recorded. Action potentials that failed to produce acoustic pulses were more common at lower temperatures in voluntary calls and at stimulation rates above 150 Hz in evoked calls. Also, a gradual increase in amplitude of the sound pulses within a call is evident in both voluntary and evoked calls. Action potentials without resultant sounds and delayed amplitude maxima are probably due to an interaction of calcium release and reuptake, sonic muscle twitch characteristics and swimbladder displacement resulting in constructive interference.

Low initial sound amplitude and a gradual increase to maximum amplitude can be see in the traces of evoked calls in toadfish (Fine et al., 2001) and voluntary calls in midshipman (Cohen and Winn, 1967). When the toadfish sonic nerve is stimulated at 200 Hz, peak amplitude was reached at the sixth muscle twitch (Fine et al., 2001). In the voluntary grunts (175–200 Hz) produced by the midshipman, *Porichthys notatus*, the fourth muscle twitch produced maximal sound amplitude (Cohen and Winn, 1967). Fine et al. (2001) used a laser vibrometer to measure swimbladder displacement during sound production evoked at 200 Hz. They observed that the swimbladder did not reach full oscillations until the fifth or sixth action potential, coincident with peak sound amplitude. Rome et al. (1996) observed a similar delay in the development of maximal force from bundles of toadfish sonic fibres. Fine et al. (2001) have suggested that the initial summation of bladder displacement and resultant low acoustic amplitude may be the result of the initial timing of calcium release and reuptake by the sarcoplasmic reticulum. This hypothesis may explain the low initial amplitude and, in conjunction with the concept of constructive interference (see below), the gradual build up to maximal amplitude in voluntary and evoked searobin calls. It may also explain the action potentials lacking acoustic pulses observed at call onset in this study. In voluntary calls, the number of action potentials without resultant sounds decreased with increasing temperature, and a warmer sarcoplasmic reticulum can cycle (release and reuptake) calcium more rapidly (Feher et al., 1998). More rapid cycling of calcium would allow the muscle to relax and twitch again, producing a sound that might otherwise have been missing due to sonic muscle twitch and bladder displacement summation early in the call.

Recordings of evoked and voluntary single twitches revealed a sound consisting of two distinct waveforms of acoustic energy separated by 5–6 ms of relative quiet. One possibility is that these two pulses might represent twitches of the two sonic muscles; i.e. a twitch of the contralateral muscle might be responsible for the second waveform in unilateral EMG recordings. However, this two-part waveform was confirmed in bilateral recordings. In addition, the same double sound was produced by unilateral twitches evoked by stimulation of one SN. As the sonic motor nuclei of the searobin only branch ipsilaterally (Bass and Baker, 1991; Finger and Kalil, 1985), a feedback mechanism from the stimulated SN to the contralateral nerve is unlikely. Furthermore, duration, dominant frequency (Table 1) and waveform shape (Fig. 7) of the two waveforms were distinct, suggesting that these sounds were not the result of identical, alternate twitches of the two sonic muscles.

The two acoustic waveforms produced by a single twitch were nearly identical to the ‘contraction’ and ‘relaxation’ sounds that Fine et al. (2001) recorded from the toadfish sonic muscle. Based on simultaneous bladder displacement and sound measurements, they have modelled the movement of the bladder relative to the positive and negative pressure peaks of the resultant sounds. Their model suggests quadrupole movement of the swimbladder, rather than the monopole movement associated with an oscillating sphere (Harris, 1964). They found that the initial negative peak of the contraction sound represents inward displacement of the side of the swimbladder by shortening of the sonic muscle. The following positive pressure peak represents the outward displacement of the bottom of the bladder caused by increasing internal pressure. The relaxation sound begins with a positive pressure peak as the muscle relaxes and the sides of the bladder move back out. The bottom then falls, producing a negative peak, and as the bottom stops moving the sides complete their outward movement, producing the final positive peak.

While this model of bladder movement (Fine et al., 2001) accurately describes the two waveforms produced by a single searobin sonic muscle twitch, it cannot explain the extended interval of relative quiet between these two waveforms. This 5–6 ms plateau between the contraction and relaxation sounds represents an interval of relative stillness of the swimbladder and would result from sonic muscle mechanics and/or the morphology of the swimbladder. The interval between the toadfish contraction and relaxation sounds coincides with minimal velocity of the bladder surface, indicative of complete muscle contraction (Fine et al., 2001). It is possible that a delayed relaxation of the muscle might produce the longer interval between the contraction and relaxation sounds in the searobin. Alternatively, this interval might be the result of swimbladder morphology. The searobin swimbladder is bi-lobed and distinct from that of the heart-shaped toadfish bladder (Evans, 1973; Tower, 1908). The tapered oval lobes

are separated by anterior and posterior clefts and are connected only by a constricted passage towards the anterior end of the bladder. Perhaps inertia resulting from the constriction between the bi-lobed halves of the bladder might delay the displacement waveform long enough to produce the interval.

This interval is crucial in allowing constructive interference and a resultant amplification of sound to occur during the searobin call. The peak negative pressure of the relaxation sound produced by one twitch coincides with that of the contraction sound of the next twitch of that sonic muscle (not the contralateral muscle; see Fig. 7). This process can be observed in evoked trains of sound at 50, 100 and 120 Hz (Fig. 9). At 50 Hz, the entire dual sound waveform can be seen between action potentials. At 100 Hz, the small negative acoustic pressure peak that appears just prior to the next action potential (and resultant contraction sound) is the relaxation sound of the preceding twitch. At 120 Hz, these two negative peaks superimpose. Each muscle twitch reinforces the next twitch of that muscle by compounding the displacement of the bladder, resulting in increased sound amplitude. Note that the first two acoustic pulses will not be reinforced in this manner. In addition to the cycling of calcium, this may help explain why some action potentials at call onset fail to produce an audible sound.

The same phenomenon has been observed in sounds evoked from toadfish sonic muscles, but the reinforcement takes place at higher muscle contraction frequencies. In the toadfish, the interval of ~5.3 ms between contraction and relaxation sounds should produce reinforcement of the next sound at 188 Hz, and such reinforcement can be observed at 200 Hz (Fine et al., 2001). Similarly, the 9.3 ms interval in evoked calls in the searobin produces maximal reinforcement at 107.6 Hz for one muscle. The longer interval between the contraction and relaxation sounds in voluntary single twitches, 10.8 ms, would produce maximal reinforcement at 92.7 Hz. Both of these values are close to the 105.6 Hz mean frequency of contraction in a voluntary call.

Constructive interference and sonic fibre characteristics are both likely to play a role in determining the range of stimulation frequencies at which the searobin can produce maximum sound amplitudes (100–140 Hz; see Fig. 10A). Constructive interference of negative pressure peaks might also explain the dominance of negative acoustic pressure in the waveforms of searobin and toadfish calls when both positive and negative acoustic pressure is evident in the sounds produced by single twitches in both species (Fine et al., 2001).

This constructive interference may be an inherent part of the mechanism of sound production in searobins, toadfish and other species producing sound *via* trains of muscle contractions rather than single twitches. Although the sonic motor systems of toadfish and searobins are considered homologous, they differ in three of nine compared vocal control traits (Bass and Baker, 1991), in sonic muscle function (synchronous *vs* alternate contraction) and in swimbladder morphology (Evans, 1973; Tower, 1908). The appearance of an essentially identical mode of constructive interference in both species suggests the importance of this mechanism in amplifying sounds produced by a highly damped and inefficient sound source such as a swimbladder (Connaughton et al., 2002; Fine et al., 2001). The timing of the contraction and relaxation sounds produced by a single twitch plays an essential role in this constructive interference and should be studied further.

A final characteristic of both voluntary calls and evoked trains is rapid damping, expressed by the greater duration and lower amplitude of the final two sound pulses. Bilateral EMG recordings confirm that these attenuated sound pulses were not produced by twitches of the contralateral sonic muscle. These attenuated sounds do not represent resonance of the swimbladder, rather they represent the relaxation sound of the final twitch of each alternately contracting muscle (see Fig. 7; Table 2). In evoked calls there is only one attenuated sound because only one muscle is being stimulated. Similarly, single attenuated sound pulses are observed in trains of evoked sounds in toadfish (Fine et al., 2001) and in voluntary calls of midshipman (Cohen and Winn, 1967). In one case, a single muscle is being stimulated and in the other the sonic muscles are contracting synchronously.

The rapid damping of the searobin sonic system supports the proposal that swimbladders are low Q, broadly tuned sound sources and are unlikely to resonate (Connaughton et al., 2002; Fine et al., 2001). The swimbladder of the searobin produced sound at any frequency imparted upon it, from 50 Hz to 360 Hz, and a Q_{3dB} of 1.98 was calculated based on these data. A Q_{3dB} of 1.45 has been reported for the toadfish swimbladder (Fine et al., 2001), and frequency response curves generated by sounds evoked from *Galeichthys* and *Bagre* (Tavolga, 1962) produce very low Q_{3dB} values of 0.89 and 0.33, respectively. The time-course of sound produced by single muscle twitches in the searobin is brief, and both evoked (11.7 ms) and voluntarily (14.1 ms) produced sounds ended abruptly after the cessation of the action potential. Similar short-duration, highly damped waveforms have been observed in toadfish (Fine et al., 2001) and weakfish (Connaughton et al., 2002), supporting the hypothesis that swimbladders are inefficient sound sources and need to be driven rapidly in order to produce sound (Fine et al., 2001).

The searobin can produce a call with a fundamental frequency of 200 Hz while each muscle twitches at 100 Hz. A trade-off that accompanies this mode of function is that only half of the total sonic muscle mass is displacing the swimbladder during any given contraction, resulting in an average 3.3 dB decrease in SPL when comparing bilateral and unilateral sounds. Decreased SPL has been noted in other species when comparing unilateral and bilateral sound production (Tavolga, 1962; Winn and Marshall, 1963), but these species do not naturally contract their sonic muscles alternately.

A possible adaptation to the decrease in sound amplitude resulting from contraction of only one sonic muscle at a time is a bigger or thicker sonic muscle. In the present study, the more massive sonic muscles of the males expressed a mass of 2.0% of total mass and a thickness of 1.8% of total length. By

comparison, the seasonal sonic muscles of the male weakfish *Cynoscion regalis* express a mass of 3.2% of total mass and a thickness of 1.6% of total length at the peak of the spawning season (Connaughton and Taylor, 1994). The sonic muscle of male Atlantic croaker (*Micropogonias undulatus*), also seasonal, peaks at a mass of 1.8% and a thickness of 0.8% (S. Modla, M. L. Lunn and M. A. Connaughton, unpublished data). The sonic muscle of the calling, nest-building, type I male midshipman peaks at 1.5% of body mass (Brantley et al., 1993) and that of male toadfish at ~1.3% (calculated from a regression of sonic muscle mass *vs* total mass in Fine et al., 1990). Although these data are limited, it appears that searobin sonic muscle expresses the greatest relative thickness and second greatest relative mass of those species examined thus far, perhaps as an adaptation to compensate for decreased amplitude due to alternate contraction of the sonic muscles.

The central and peripheral components of the searobin sonic motor system have evolved to produce sound *via* a novel mode of swimbladder-generated sound production. Central components, including ipsilateral branching and alternate firing of the SN (Bass and Baker, 1991), generate alternate contractions of the sonic muscles. Peripheral components are likewise specialized. Alternate contraction of the sonic muscles fulfils the need for rapid movement of the swimbladder without the need for super-fast mechanics (by the standards of sonic muscles), and optimal sound output for these muscles occurs at relatively low frequencies (100–140 Hz). Muscle mechanics and/or swimbladder morphology result in constructive interference of sequential relaxation and contraction sounds, maximizing signal amplitude at typical muscle contraction frequencies. It is hypothesized that this reinforcement may partially compensate for the loss in amplitude inherent in the use of only half the total sonic muscle mass to displace the swimbladder during unilateral muscle contraction. The relatively great mass and thickness of the searobin sonic muscle may also play a role in compensating for loss in amplitude due to alternate contraction. Although the impact of muscle twitch force on sound amplitude has never been directly examined, it seems that searobin sonic muscle function is a novel solution to the trade-off between speed and force characterized in sonic muscles by the extremely fast, low force toadfish sonic muscle (Rome et al., 1999).

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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.

Appelt, D., Shen, V. and Franzini-Armstrong, C. (1991). Quantitation of Ca ATPase, feet and mitochondria in superfast muscle fibers from the toadfish, *Opsanus tau*. *J. Muscle Res. Cell Motil.* **12**, 543-552.

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

Bass, A. H. and Marchaterre, M. A. (1989). Sound-generating (sonic) motor system in a teleost fish (*Porichthys notatus*): sexual polymorphism in the ultrastructure of myofibrils. *J. Comp. Neurol.* **286**, 141-153.

Bradbury, J. W. and Vehrenkamp, S. L. (1998). *Principles of Animal Communication*. Sunderland, MA: Sinauer Associates.

Brantley, R. K. and Bass, A. H. (1994). Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish *Porichthys notatus* Girard (Teleostei, Batrachoididae). *Ethology* **96**, 213-232.

Brantley, R. K., Marchaterre, M. A. and Bass, A. H. (1993). Androgen effects on vocal muscle structure in a teleost fish with inter- and intra-sexual dimorphism. *J. Morph.* **216**, 305-318.

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.

Connaughton, M. A. and Taylor, M. H. (1994). Seasonal cycles in the sonic muscles of the weakfish, *Cynoscion regalis*. *U.S. Fish. Bull.* **92**, 697-703.

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.

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.

Demski, L. S., Gerald, J. W. and Popper, A. N. (1973). Central and peripheral mechanisms of teleost sound production. *Am. Zool.* **13**, 1141-1167.

Eichelberg, H. (1976). The fine structure of the drum muscles of the tigerfish, *Therapon jarbua*, as compared with the trunk musculature. *Cell Tissue Res.* **174**, 453-463.

Eichelberg, H. (1977). Fine structure of the drum muscles of the piranha (Serrasalminae, Characidae). *Cell Tissue Res.* **185**, 547-555.

Evans, R. R. (1973). The swimbladder and associated structures in western Atlantic sea robins (Triglidae). *Copeia* **1973**, 315-321.

Fawcett, D. W. and Revel, J. P. (1961). The sarcoplasmic reticulum of a fast acting fish muscle. *J. Biophys. Biochem. Cytol.* **10**, 89-109.

Feher, J. J., Waybright, T. D. and Fine, M. L. (1998). Comparison of sarcoplasmic reticulum capabilities in toadfish (*Opsanus tau*) sonic muscle and rat fast twitch muscle. *J. Muscle Res.* **19**, 661-674.

Fine, M. L. (1978). Seasonal and geographical variation of the mating call of the oyster toadfish *Opsanus tau*. *Oecologia* **36**, 45-57.

Fine, M. L., Bernard, B. and Harris, T. M. (1993). Functional morphology of toadfish sonic muscle fibers: relationship to possible fiber division. *Can. J. Zool.* **71**, 2262-2274.

Fine, M. L., Burns, N. M. and Harris, T. M. (1990). Ontogeny and sexual dimorphism of the sonic muscle in the oyster toadfish. *Can. J. Zool.* **68**, 1374-1381.

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.

Finger, T. E. and Kalil, K. (1985). Organization of motoneuronal pools in the rostral spinal cord of the sea robin, *Prionotus carolinus*. *J. Comp. Neurol.* **239**, 384-390.

Fish, M. P. (1954). The character and significance of sound production among fishes of the western North Atlantic. *Bull. Bingham Ocean. Coll.* **14**, 1-109.

Fish, M. P. and Mowbray, W. H. (1970). Sounds of western North Atlantic fishes. Baltimore: The Johns Hopkins Press.

Franzini-Armstrong, C. and Nunzi, G. (1983). Junctional feet and particles in the triads of a fast-twitch muscle fiber. *J. Muscle Res. Cell Motil.* **4**, 233-252.

Gainer, H. and Klancher, J. E. (1965). Neuromuscular junctions in a fast-contracting fish muscle. *Comp. Biochem. Physiol.* **15**, 159-165.

Gainer, H., Kusano, K. and Mathewson, R. F. (1965). Electrophysiological and mechanical properties of squirrelfish sound-producing muscle. *Comp. Biochem. Physiol.* **14**, 661-671.

Hamoir, G., Gerardin-Otthiers, N. and Focant, B. (1980). Protein differentiation of the superfast swimbladder muscle of the toadfish, *Opsanus tau*. *J. Mol. Biol.* **143**, 155-160.

Harris, G. G. (1964). Considerations on the physics of sound production by fishes. In *Marine Bio-Acoustics*, vol. 1 (ed. W. N. Tavolga), pp. 233-247. New York: Pergamon Press.

Hirsch, J. E., Bigbee, J. W. and Fine, M. L. (1998). Continuous adult development of multiple innervation in toadfish sonic muscle. *J. Neurosci.* **36**, 348-356.

Ibara, R. M., Penny, L. T., Ebeling, A. W., van Dykhuizen, G. and Cailliet, G. (1983). The mating call of the plainfin midshipman fish, *Porichthys notatus*. In *Predators and Prey in Fishes* (ed. D. L. G. Noakes, D. G. Lindquist, G. S. Helfman and J. A. Ward), pp. 205-212. The Hague: Junk Publishers.

Ono, R. D. and Poss, S. G. (1982). Structure and innervation of the swim bladder musculature in the weakfish, *Cynoscion regalis* (Teleostei: Sciaenidae). *Can. J. Zool.* **60**, 1955-1967.

Packard, A. (1960). Electrophysiological observations on a sound-producing fish. *Nature* **187**, 63-34.

Parvulescu, A. (1964). The acoustics of small tanks. In *Marine Bioacoustics*, vol. 2 (ed. W. N. Tavolga), pp. 7-13. New York: Pergamon Press.

Rome, L. C., Cook, C., Syme, D. A., Connaughton, M. A., Ashley-Ross, M., Klimov, A., Tikunov, B. and Goldman, Y. E. (1999). Trading force for speed: why superfast crossbridge kinetics leads to superlow forces. *Proc. Natl. Acad. Sci. USA* **96**, 5826-5831.

Rome, L. C. and Lindstedt, S. (1998). The quest for speed: muscles built for high-frequency contractions. *News Physiol. Sci.* **13**, 261-268.

Rome, L. C., Syme, D. A., Hollingworth, S., Lindstedt, S. L. and Baylor, S. M. (1996). The whistle and the rattle: the design of sound producing muscles. *Proc. Natl. Acad. Sci. USA* **93**, 8095-8100.

Schneider, H. (1967). Morphology and physiology of sound-producing mechanisms in teleost fishes. In *Marine Bioacoustics*, vol. 2 (ed. W. N. Tavolga), pp. 135-158. New York: Pergamon Press.

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

Tavolga, W. N. (1962). Mechanisms of sound production in the Ariid catfishes *Galeichthys* and *Bagre*. *Bull. Am. Mus. Nat. Hist.* **124**, 1-30.

Tavolga, W. N. (1964). Sonic characteristics and mechanisms in marine fishes. In *Marine Bioacoustics*, vol. 1 (ed. W. N. Tavolga), pp. 195-211. New York: Pergamon Press.

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

Tower, R. W. (1908). The production of sound in the drumfishes, the sea-robin and the toadfish. *Ann. N.Y. Acad. Sci.* **18**, 149-180.

Winn, H. E. and Marshall, J. A. (1963). Sound-producing organ of the squirrelfish, *Holocentrus rufus*. *Physiol. Zool.* **36**, 34-44.

Young, I. and Rome, L. (2001). Mutually exclusive designs: the power output of the locomotory and sonic muscles of the oyster toadfish (*Opsanus tau*). *Proc. R. Soc. Lond. B. Biol. Sci.* **269**, 1965-1970.