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THE EFFECTS OF SEASONAL HYPERTROPHY AND ATROPHY ON FIBER MORPHOLOGY, METABOLIC SUBSTRATE CONCENTRATION AND SOUND CHARACTERISTICS OF THE WEAKFISH SONIC MUSCLE

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Summary

Male weakfish *Cynoscion regalis* possess highly specialized, bilateral, striated sonic muscles used in sound production associated with courtship. Androgen-driven hypertrophy of the muscles during the late spring spawning period results in a tripling of sonic muscle mass followed by post-spawning atrophy. This study examined the morphological and biochemical changes underlying seasonal changes in sonic muscle mass and the functional effects of these on contraction as measured by sound production. Sonic muscle fiber cross-sectional area (CSA) increased significantly during the period of hypertrophy and then decreased by nearly 60 %. Both the CSA of the contractile cylinder and that of the peripheral sarcoplasm decreased significantly by late summer, with the peripheral ring of sarcoplasm virtually disappearing. Muscle protein content followed a similar trend, suggesting a major loss of structural elements during atrophy. Muscle glycogen and lipid content decreased precipitously in early June during the period of maximal sound production. Sound pressure level increased and sound pulse duration decreased with increasing sonic muscle mass, indicating that sonic muscle fibers contract with greater force and shorter duration during the spawning season. Neither the pulse repetition rate nor the number of pulses varied seasonally or with muscle mass, suggesting that the effects of steroids on the acoustic variables are more pronounced peripherally than in the central nervous system. Seasonal sonic muscle hypertrophy, therefore, functions as a secondary sexual characteristic that maximizes vocalization amplitude during the spawning period.

Key words: sound production, bioacoustics, muscle fiber, glycogen, lipid, protein, weakfish, *Cynoscion regalis*.

Introduction

Like most sciaenid species, male weakfish *Cynoscion regalis* possess sexually dimorphic, extrinsic sonic muscles (Smith, 1905; Tower, 1908; Tavolga, 1964), which first develop during sexual maturation (Hill *et al.* 1987). The bilaterally paired muscles lie on the body wall of the male, surrounding the swimbladder (Fig. 1A,B). They extend nearly the entire length of the body cavity and attach to the lateral body wall musculature by connective tissue (Ono and Poss, 1982). The sonic muscle fibers run dorsoventrally, originating on an aponeurotic sheet over the dorsal surface of the swimbladder (Fig. 1B) and inserting on the abdominal hypaxial muscles (Ono and Poss, 1982; Hill *et al.* 1987). Muscle contraction produces sound (Fig. 1C), which is amplified by vibration of the swimbladder (Tower, 1908; Harris, 1964; Demski *et al.* 1973).

Male weakfish sound production is seasonal and correlated with late spring and early summer spawning activity in the field (Connaughton and Taylor, 1995*b*) and with courtship behavior observed prior to spawning in captivity (Connaughton and Taylor, 1996). Merriner (1976) noted that the size of the muscle changed seasonally and that its color deepened to a dark red during the spawning season. In fact, the muscle triples in mass and doubles in thickness during the spawning season in response to seasonally increasing plasma androgen titers (Connaughton and Taylor, 1994). Sonic muscle hypertrophy has been elicited and maintained in the laboratory using testosterone implants (Connaughton and Taylor, 1995*a*).

Morphological, biochemical and functional correlates of this seasonal, steroid-induced change in muscle size and use are entirely unexamined. The objectives of this study were threefold. First, we described the changes in sonic muscle fiber morphology and protein concentration associated with muscle hypertrophy. Second, we examined the metabolic stores of the muscle across the course of the spawning period. Finally, we determined the effects of changing sonic muscle mass on sound, hypothesizing that a more massive muscle will contract with greater force and generate a sound of greater amplitude.

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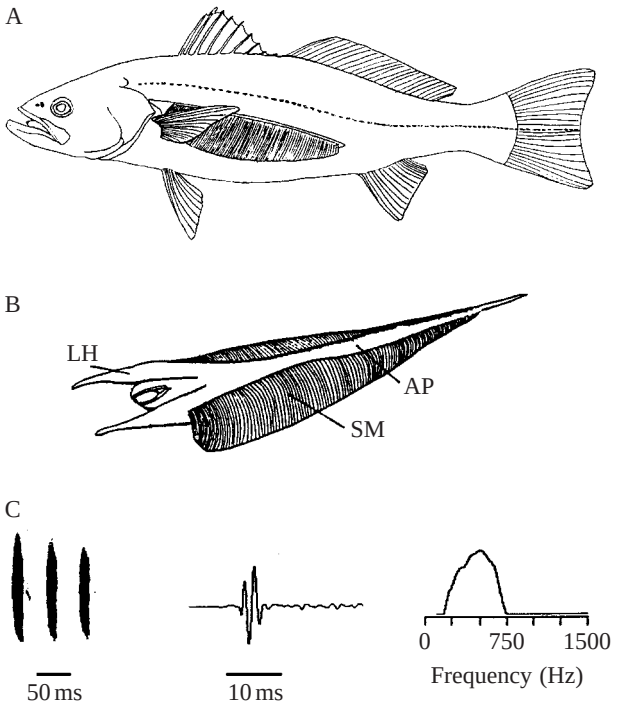

Fig. 1. (A) An illustration of the sonic muscles in the weakfish *Cynoscion regalis* (modified from an illustration by H. L. Todd in Goode, 1884). (B) An oblique view of the swimbladder and associated sonic muscles; SM, sonic muscle; LH, lateral horn of the swimbladder; AP, aponeurosis overlying the dorsal surface of the swimbladder (modified from Ono and Poss, 1982). (C) An example of weakfish sound production. From left to right: a sonogram of a three-pulse train (frequency against time); an oscillogram of a single pulse with two cycles of acoustic energy (amplitude against time); a power spectrum of a single pulse (amplitude against frequency).

Materials and methods

Specimen and sample collection

Weakfish *Cynoscion regalis* (Bloch and Schneider, 1801) were collected by gill net or hook-and-line from the southwest portion of Delaware Bay near Lewes, DE, USA, from May to August 1992. Immediately after capture, the fish were killed by exposure to MS-222 (tricaine methane sulfonate, Sigma Chemical Co., St Louis, MO, USA) and low temperature, and the left sonic muscle was removed, frozen in liquid nitrogen and stored at –80 °C for later biochemical analysis. The right sonic muscle was removed, weighed and preserved in 10 % buffered formalin for histological analysis.

To standardize for variations in specimen size, sonic muscle-somatic index (SMSI) was calculated as (total sonic muscle mass/total body mass)×100. For field-collected specimens, total sonic muscle mass was calculated as twice the right sonic muscle mass, as previous studies indicated that the sonic muscles were bilaterally symmetrical (Connaughton and Taylor, 1994). On one of the collecting trips (11 June), epaxial musculature was also sampled.

Live specimens of nearly identical size (26–29 cm total length) were acquired for bioacoustic analyses by haul-seine along the southwest coast of the Delaware Bay in May and June 1993. Specimens were maintained at 18 °C and ambient photoperiod throughout the summer and early autumn in 1500 l tanks and were fed *ad libitum* three times a week.

Morphological analyses

Formalin-fixed sonic muscle samples were embedded in paraffin wax, sectioned at 5 μm and stained with hematoxylin and eosin. Sonic muscle tissue was not pinned prior to fixation as the curved path of the muscle around the swimbladder complicated determination of muscle resting length. As a result, CSA values may be slightly high due to contraction of the fibers during fixation. The total cross-sectional area (tCSA) of the fiber, the cylinder cross-sectional area (cCSA) and the total diameter were measured using a computer-assisted image-analysis system (C-2 Olympus Image Analyzer, Morphometry software, ver. 2.2; N=12 fibers from 4–10 specimens per date). Measurements of tCSA included the peripheral ring of sarcoplasm, the myofibrillar region and the fragmented central core noted in many fibers, whereas cCSA measurements included only the myofibrillar and central core regions. The area of the ring of peripheral sarcoplasm was calculated as a percentage of tCSA as [(tCSA–cCSA)/tCSA]×100. Epaxial muscle fibers had neither a noticeable ring of sarcoplasm nor a central core; consequently, only a single diameter and CSA measurement were taken from these fibers.

Biochemical analyses

Protein concentration was determined by the Bio-Rad colorimetric protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Samples were homogenized in 0.07 mol l^{-1} sodium phosphate buffer (pH 7.4) and run against a standard curve established with bovine serum albumin. Absorbance was measured at 595 nm.

Tissue samples were dried to constant mass at 50 °C for 10–12 days to determine water content. For determination of soluble lipid content, dried samples were extracted in petroleum ether at room temperature for 48 h, and then dried at 50 °C for 24 h. The extraction was repeated until the lipid-free dry mass was constant (Given, 1988).

Glycogen concentration was measured following the protocol of Marsh and Dawson (1982). Briefly, muscle tissue was first homogenized in 0.6 mol l^{-1} perchloric acid, and the amount of free glucose was determined using the glucose oxidase procedure (Sigma glucose diagnostic kit no. 510A, Sigma Diagnostics, St Louis, MO, USA). Glycogen was then hydrolyzed to glucose with amyloglucosidase (Sigma Chemical Co.), and total glucose concentration was measured. Glycogen concentration was calculated as the difference between total and free glucose.

Ash content was calculated by subtracting from 100 % the sum of the percentage wet mass represented by protein, glycogen, lipid and water.

Acoustic analyses

The effect of seasonally decreasing sonic muscle mass on

sound characteristics was studied by recording from and then killing (for the calculation of SMSI) three groups of fish over a period of 3.5 months. The first group was recorded on 14 June during the peak of the spawning period, the second on 19 July closely following the spawning period, and the third on 24 September well after spawning had finished. These dates encompassed the period of peak sonic muscle mass (maximal SMSI) and the subsequent atrophy of the muscle.

Given the difficulties of recording in an enclosed aquatic space (Tavolga, 1962; Schneider, 1967), acoustic conditions were improved by recording sound production in air. Recordings were made with a Realistic pressure zone microphone (Radio Shack, Tandy Corp., Fort Worth, TX, USA; frequency response flat from 20 Hz to 18 kHz) and an Aiwa HS-J470 cassette recorder (Aiwa Corp., Mahwah, NJ, USA) on standard magnetic audio tape (Sony, type II High Bias LX, Sony Electronics, Park Ridge, NJ, USA). Each specimen was held 10 cm from the microphone during recording. The process of removing the fish from the water was enough to elicit sonic behavior. Sonograms and power spectra of sounds recorded in the field and during spawning in captivity (M. A. Connaughton, M. L. Fine and M. H. Taylor, in preparation) are nearly identical with those recorded in air in the present study. A 500 Hz, 80 dB (re: 20 μPa) calibration tone, measured with a Realistic sound level meter (Radio Shack) adjacent to the microphone, was recorded on the tape to permit measurement of absolute signal amplitude, or sound pressure level (SPL).

Sounds were digitized at an 11 kHz sampling rate and analyzed with a Macintosh personal computer using Canary bioacoustic workstation software (ver. 1.2, Cornell Laboratory of Ornithology, Ithaca, NY, USA). A pulse refers to a single sound consisting of two cycles of acoustic energy, and a train describes several pulses in a continuous series. Sounds were analyzed for SPL, the number of pulses in a train, the repetition rate within a train (number of pulses per second), the pulse duration and the dominant frequency. SPL, pulse duration and dominant frequency were measured for the ten pulses of greatest amplitude within each recording (as determined from the oscillogram). The number of pulses per train and the repetition rate were determined for all trains (two or more pulses) in each recording.

Statistical analyses

Morphological data

Contractile cylinder and total CSA values were compared for each specimen on each collecting date using a paired *t*-test. As diameter and CSA varied with the total length of the fish and the total length varied seasonally (data not shown), the diameter and CSA data were examined across date using analysis of covariance (ANCOVA), with total length of the specimen evaluated as a covariate (Zar, 1984). In addition, the CSA data in Fig. 3 are presented after normalization for specimen size, calculated as fiber CSA/total length of the fish. SMSI and the percentage of tCSA reflected in the peripheral ring of sarcoplasm were evaluated across date using one-way analysis of variance (ANOVA) after arcsine-transformation of the data. In addition, cCSA and tCSA were correlated with sonic muscle mass using the Spearman rank correlation.

Biochemical data

Protein, glycogen, water, lipid and ash data, presented as percentages of sonic muscle tissue wet mass, were arcsine-transformed prior to one-way ANOVA across date. Protein concentration was correlated with SMSI and glycogen content with water content using the Spearman rank correlation.

Acoustic data

Variations in SMSI, SPL and pulse duration across date were analyzed *via* one-way ANOVA. SPL, pulse duration, dominant frequency, number of pulses in a train and repetition rate were regressed across SMSI. SMSI data were arcsine-transformed prior to analyses.

An α level of 0.05 was used to determine significance in all analyses. Values are presented as means ± S.D.

Results

Morphological analyses

SMSI varied significantly during the collecting period ($P \leqslant 0.0001$), peaking at 3.6±0.41 % body mass (mean ± S.D.) in early June and decreasing to 1.2±0.30 % (see Fig. 4B) by August. Cross sections of sonic muscle tissue revealed that the fibers were small and variable in shape. Peripheral rings of sarcoplasm and fragmented central cores were obvious in hypertrophied fibers, and were reduced or missing in the atrophied fibers (Fig. 2). Mean fiber diameter decreased from a peak value of 39.3±4.45 μm during the spawning period to 23.1±5.30 μm (N=8–9, $P \leqslant 0.0001$) in August. Both cCSA and tCSA varied significantly with date (P=0.038 and P=0.0002, respectively; Fig. 3), reaching maximal values in early June (554.8±142.66 μm^2 for cCSA and 781.8±133.40 μm^2 for tCSA, non-normalized data) when SMSI was maximal, before declining to minimum values in August (295.9±121.25 μm^2 for cCSA and 316.4±127.22 μm^2 for tCSA). These changes represent a decrease in cCSA of 46.7 % and a decrease in tCSA of 59.5 % across the collecting period. Both cCSA and tCSA measurements were significantly correlated with sonic muscle mass (r^2=0.25 for cCSA, r^2=0.36 for cCSA), and tCSA was significantly greater than cCSA on all sampling dates ($P \leqslant 0.0001$ for all dates; Fig. 3). The percentage of tCSA represented by the peripheral ring of sarcoplasm decreased from a peak of 38.15±8.23 % during the spawning period to 5.37±3.41 % in August ($P \leqslant 0.0001$).

By comparison, the epaxial muscle fibers were much larger in size, averaging 109.7±8.95 μm in diameter, more irregular in shape and did not express an outer ring of sarcoplasm. Mean epaxial CSA (7138.9±906.914 μm^2; N=9) was nine times greater than the largest and 23 times greater than the smallest mean tCSA of the sonic muscle fibers.

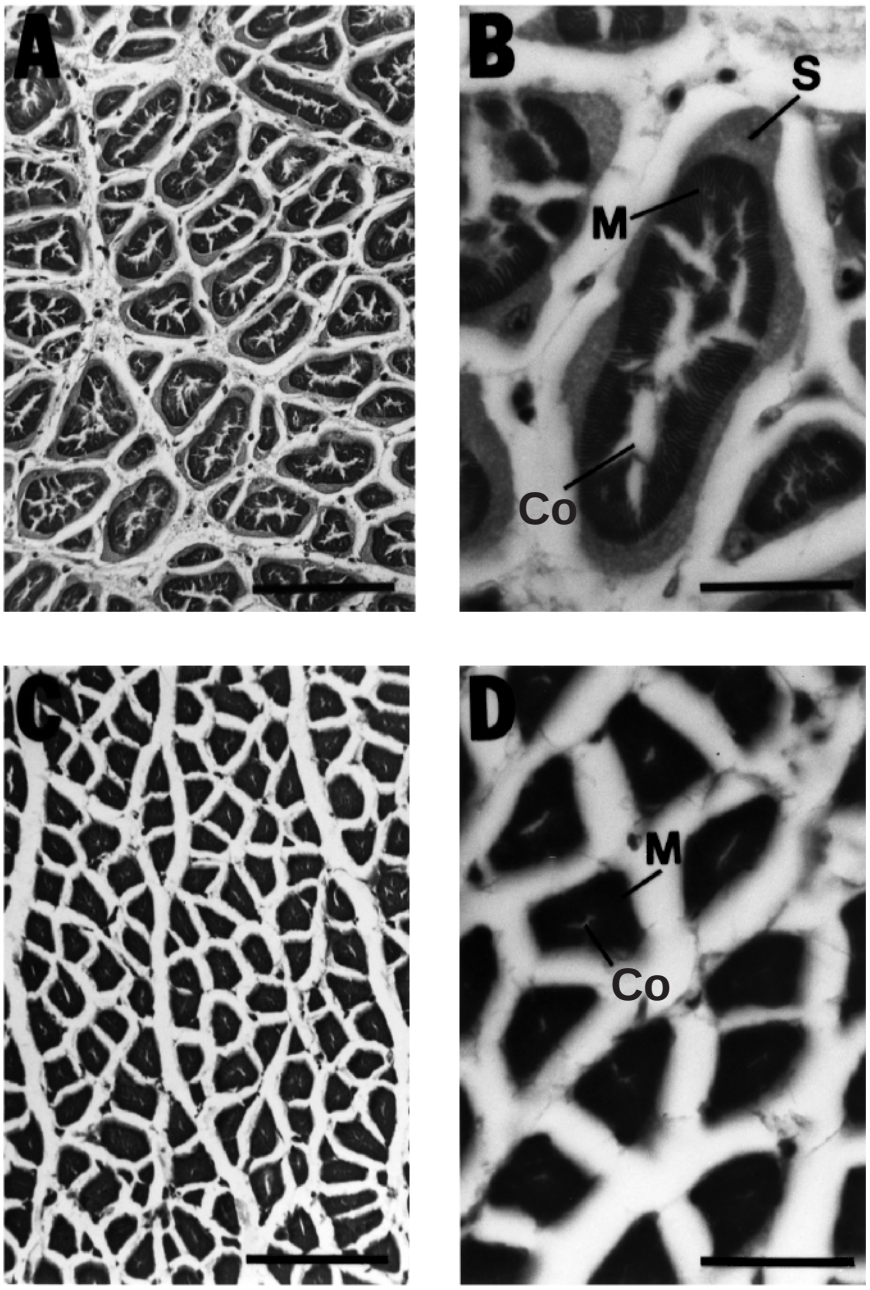

Fig. 2. Photomicrographs of sonic muscle fibers from fish collected during and following the spawning season. Sections were stained with hematoxylin and eosin. (A,B) Sonic muscle fibers in mid-June, during the spawning season and the period of maximal sonic muscle mass. (C,D) Sonic muscle fibers in early August, following the spawning season and at a time of reduced sonic muscle mass. Note the seasonal difference in the areas of the peripheral sarcoplasm (S), myofibrillar region (M) and central core (Co). Scale bar, 80 μm (A,C) or 20 μm (B,D).

Biochemical analyses

Sonic muscle protein concentration was significantly correlated with SMSI (r^2=0.23) and changed significantly during the collecting period (P=0.0003). Protein concentration increased from May to June, peaking (15.34±1.77 % tissue wet mass) just after SMSI (Fig. 4A,B), and was similar to that of epaxial muscle (14.59±1.44 % wet mass; N=8). By mid-August, sonic muscle protein concentrations (10.60±1.61 %) had decreased significantly below May levels. Ash content decreased from 1.3 % wet mass in mid-May to 0.4 % as SMSI peaked and then increased significantly to 5.7 % in August (P<0.0001; Fig. 4C). Trunk ash content (1.54 %) was similar to early-season sonic muscle ash content.

Sonic muscle lipid and glycogen concentrations were high in May, decreased precipitously in early June (P=0.0005 for lipid, P=0.0029 for glycogen) and then decreased very little between mid-June and mid-August (Fig. 4D,E). This sharp decrease in lipid and glycogen values occurred during the period of maximal seasonal sound production (Connaughton and Taylor, 1995*b*). Sonic muscle lipid levels dropped from 1.28±0.25 to 0.77±0.14 % wet mass during the collecting season, and glycogen levels dropped from 0.24±0.09 to 0.04± 0.03 % wet mass. Epaxial muscle lipid (0.68±0.37 % wet mass, N=8) and glycogen (0.020±0.008 % wet mass) contents were just below the seasonal minima for the sonic muscles and considerably below peak sonic muscle values. Sonic muscle water content also decreased significantly across the season (P<0.0001; Fig. 4F), dropping from 82.2±0.84 to

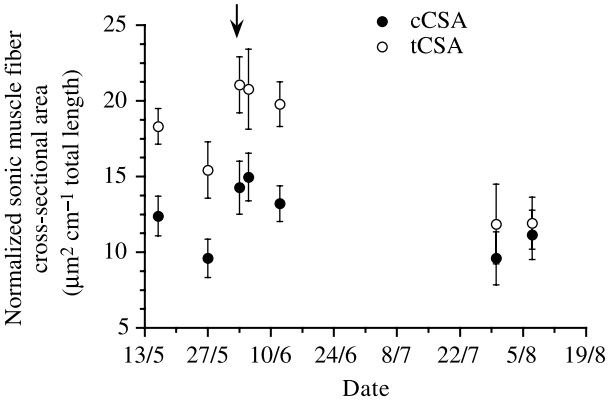

Fig. 3. Normalized sonic muscle contractile cylinder cross-sectional area (cCSA) and total cross-sectional area (tCSA) plotted at different dates. Since fiber CSA varied with fish total length, both of the CSA measurements were normalized by fish total length and are expressed as $\mu m^2\,cm^{-1}$ total length (values are means ± 1 S.E.M. for 4–10 fish, N=12 fibers per fish). Measurement of tCSA included the peripheral ring of sarcoplasm, the myofibrillar region and the fragmented central core noted in many fibers, while cCSA included only the myofibrillar and central core regions. The arrow indicates the time of maximal sonic muscle mass (see Fig. 4B).

79.69±0.78 % wet mass. Water content was also significantly correlated with glycogen concentration (r^2=0.34). Trunk muscle water content was 79.34±1.34 % wet mass.

Acoustic recordings

Both SPL and SMSI decreased significantly over the course of the season (P=0.0007 for SPL and $P \leqslant 0.0001$ for SMSI; Fig. 5A,B); SPL decreased from 65.7±1.8 to 59.8±1.6 dB and SMSI from 2.97±0.29 to 1.07±0.15 % body mass. There was a significant positive correlation between SPL and SMSI (P=0.0027, r^2=0.45; Fig. 5C). Pulse duration exhibited a small increase, from 3.42±0.14 ms during the spawning period to 3.63±0.09 ms in August (P=0.02; Fig. 6A), and was inversely correlated with SMSI (P=0.05, r^2=0.17, Fig. 6B). The dominant frequency ranged from 473 to 573 Hz, with a median value of 528 Hz, and did not vary with SMSI. The pulse repetition rate ranged from 20.1 to 30.0 pulses s^{-1}

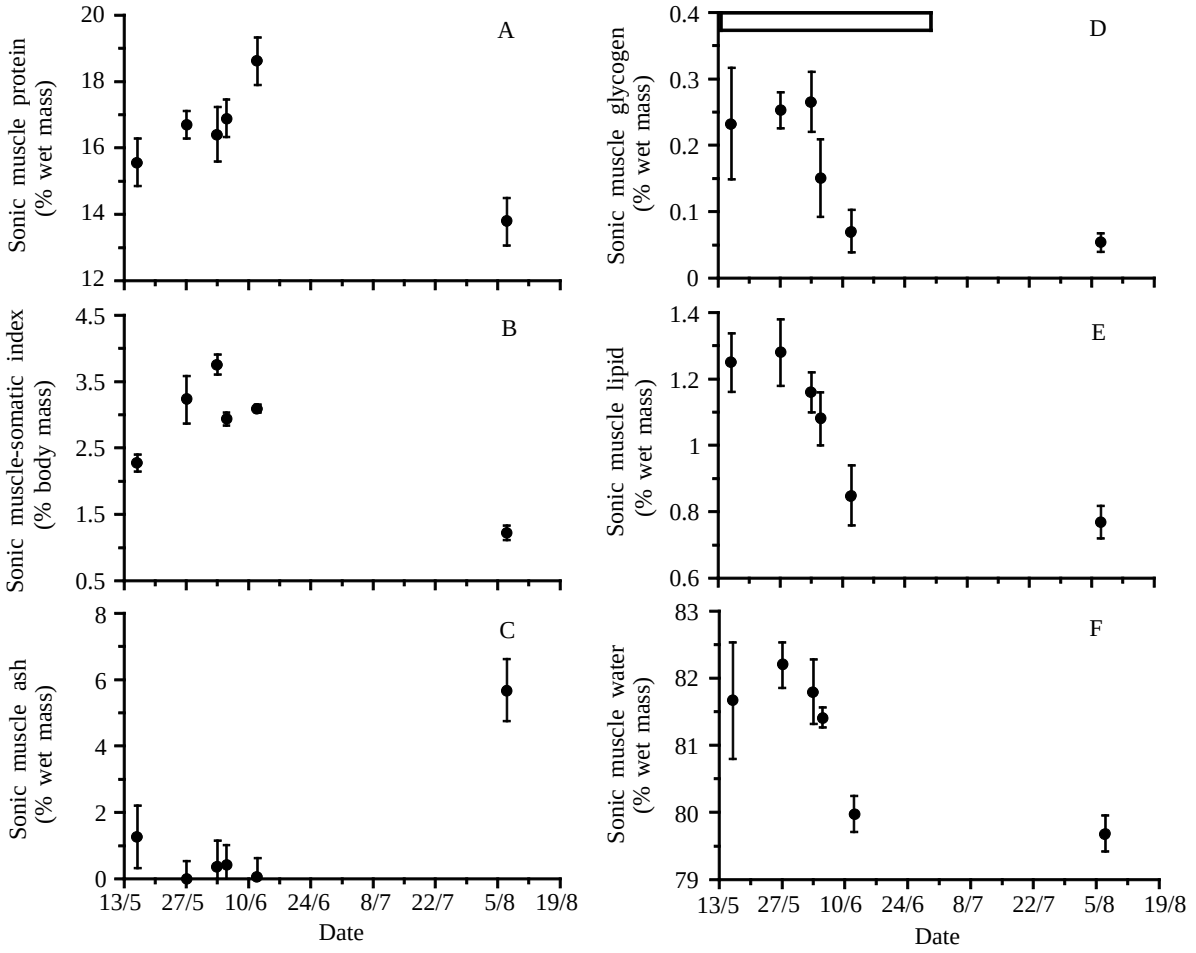

Fig. 4. Sonic muscle protein content (A), muscle-somatic index (SMSI) (B), ash content (C), glycogen content (D), lipid content (E) and water content (F) plotted at different dates. Data are expressed as a percentage of the wet mass of muscle tissue, except for SMSI which is expressed as a percentage of the total mass of the fish (values are means ± 1 S.E.M. for 5–10 fish). The open bar over D–F indicates the period of maximal sonic behavior in the field (Connaughton and Taylor, 1995*b*).

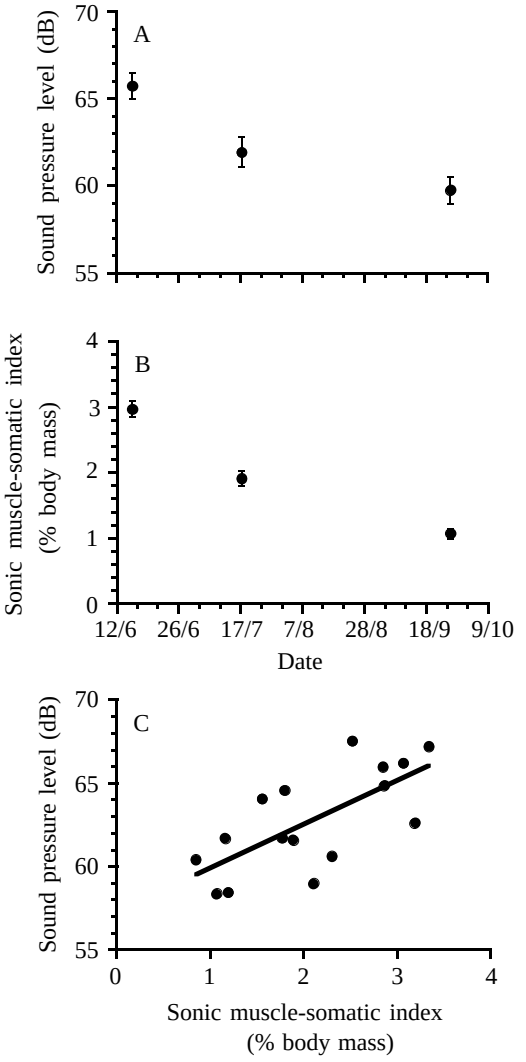

Fig. 5. Mean sound pressure level (A) (SPL re: 20 μPa) and sonic muscle-somatic index (B) (SMSI, expressed as a percentage of body mass) plotted at different dates (values are means ± 1 S.E.M. for six fish). (C) Regression of SPL across SMSI: SPL=1.28SMSI+52.4, r^2=0.45, P=0.0027, N=16.

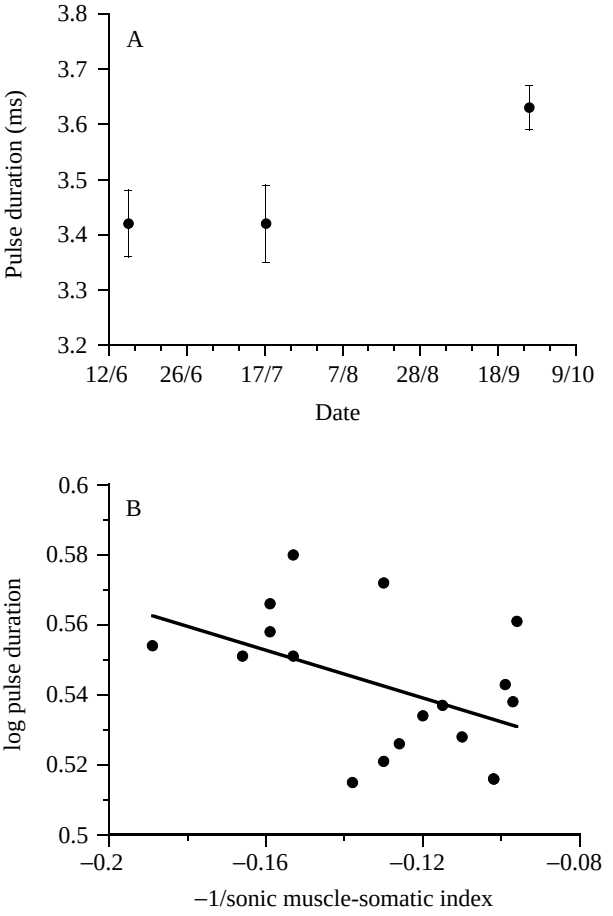

Fig. 6. (A) Mean pulse duration plotted against date (values are means ± 1 S.E.M. for six fish). (B) Regression of log pulse duration (d) against –1/sonic muscle-somatic index (SMSI): d=–0.34SMSI+0.50, r^2=0.17, P=0.05, N=18).

(25.79±2.52 pulses s^{-1}, N=18), and the number of pulses in a train ranged from 2 to 5.5 (median ± twenty-fifth and seventy-fifth percentiles: 3±3 and 4, N=18). Neither of these parameters varied with SMSI.

Discussion

There are limited data supporting a direct effect of androgens on striated muscle, and most muscle growth can be attributed to growth hormone and insulin-like growth factors (Florini, 1987; Florini *et al.* 1996). Exceptions to this generalization include several sexually dimorphic muscles affected directly by androgens, such as the syringeal muscles of birds (Luine *et al.* 1980), the laryngeal muscles of anurans (Kelley, 1986; Sassoon and Kelley, 1986; Marin *et al.* 1990) and the bulbocavernosus and levator ani muscles of the perineal complex in male rodents (Venable, 1966; Forger and Breedlove, 1991; Tobin and Joubert, 1991). Androgens also play a role in sonic muscle sexual dimorphism in several fishes, including the weakfish (Connaughton and Taylor, 1995*a*), the toadfish *Opsanus tau* (Fine and Pennypacker, 1986) and the midshipman *Porichthys notatus* (Brantley *et al.* 1993*a*). However, seasonal or activational effects of androgens (Pheonix *et al.* 1959; Adkins-Regan, 1981) on striated muscle are not a generally recognized phenomenon. Seasonal variation has been observed in the frog flexor carpi radialis used in amplexus (Muller *et al.* 1969) and in the mouse perineal muscle complex (Forger and Breedlove, 1987). The sonic muscles of the male weakfish exhibit a dramatic seasonal variation, tripling in mass during the spring spawning season and decreasing to pre-spawning mass by the end of the summer in response to changing androgen titers (Connaughton and Taylor, 1994). Similarly, the sonic muscles of the haddock *Melanogrammus aeglefinus* double in volume during the spawning season (Templeman and Hodder, 1958).

Acoustic data from this study support the hypothesis that increasing sonic muscle mass allows the weakfish to produce a more intense sound during the mating season. Although our

SPL values were recorded in air, an unnatural condition for these sound producers, our seasonal comparisons were unaffected, as all fish were recorded under identical conditions. There was also a minor, but significant, decrease in pulse duration with increasing sonic muscle mass, suggesting that muscle twitches were slightly more rapid, as well as more forceful, during the period of peak sound production. The absence of a seasonal change in pulse repetition rate or in the number of pulses in a train suggests that these parameters, determined by the central nervous system, were not directly affected by seasonally changing steroid levels.

The diameters of the sonic muscle fibers were significantly greater during the spawning season, averaging 39 μm, compared with post-spawning diameters of 23 μm, and both were considerable smaller than trunk epaxial muscle fiber diameters (110 μm). These post-spawning values are somewhat smaller than, but similar to, those reported by Ono and Poss (1982): 29.6 μm for sonic muscle fibers and 131.7 μm for trunk fibers collected during August. Compared with the morphology in late summer, fibers from weakfish collected in June expressed an enlarged myofibrillar region and an expanded peripheral sarcoplasm. In addition, many fibers showed an enlarged, fragmented central core. Both the peripheral and central sarcoplasmic regions were invisible or barely visible in light micrographs of most late-season fibers (Fig. 2C,D). Ono and Poss (1982) described the ultrastructure of sonic muscle fibers from late-season specimens and observed mitochondria-rich sarcoplasm surrounding the myofibrils and within the central cores.

Our observations that the area of the contractile cylinder decreased to approximately half its maximal size (from 555 to 296 μm^2) following the spawning season and that the peripheral ring of sarcoplasm decreased by 32.6 % from late May to August suggest functional changes in fiber contraction capabilities during and following the spawning season. Contractile cylinder area (cCSA) is an imperfect measure of contractile tissue area since it includes both the myofibrillar area and the area of the central core, which changed during fiber hypertrophy and atrophy; however, definitive changes in myofibrillar area can be observed in the sonic muscle fibers during the collecting season (see Fig. 2). An increased myofibrillar area during the spawning season would allow for more forceful contractions, resulting in an elevated amplitude of the sounds produced (Oster and Jaffe, 1980; Barry, 1987; Frangioni *et al.* 1987). We further suggest that increased peripheral and central sarcoplasmic areas will support an even greater volume of mitochondria than that encountered by Ono and Poss (1982). Increased mitochondrial volume would produce more energy and therefore contribute to greater fatigue-resistance during the period of maximal use of these muscles. Abundant mitochondria-rich peripheral sarcoplasm is found all year round in the sonic muscle fibers of Type I male midshipman, which produce long-duration mating calls, compared with the sonic muscle fibers of the non-calling Type II males and females (Brantley *et al.* 1993*b*; Brantley and Bass, 1994).

Sonic muscle protein concentration increased significantly during mid-June and was paralleled by increasing SMSI and fiber CSA. Increased CSA of both the myofibrils and sarcoplasm suggest that rising protein concentrations may reflect increases in both contractile and mitochondrial proteins, enabling the sonic muscle to contract more forcefully and for longer periods. The continued rise in protein concentration for approximately 1 week following maximal SMSI and fiber CSA may only be an apparent change, resulting from constant protein concentrations and decreasing lipid, glycogen and water concentrations. Alternatively, the sonic muscles may be adding protein without an increase in muscle mass or fiber CSA, as levels of lipid, glycogen and water are decreasing at this time. Increasing protein concentrations are probably the result of both the direct effects of increasing androgen titers (Lamb, 1975) and increased protein synthesis resulting from the use of the muscle (Goldberg *et al.* 1975) during the period of seasonal sound production (Connaughton and Taylor, 1995*b*).

Lipid and glycogen levels were examined since these are potential metabolic substrates for the sonic muscle, and levels of both decreased in June during the period of maximal acoustic behavior (Connaughton and Taylor, 1995*b*). Maximal lipid content of the sonic muscles (1.28 % wet mass) was twice that of the epaxial muscle, but was low compared with values for other lipid-rich muscles in fishes; typical fish dark (red) muscle may have a lipid content of up to 30 % wet mass (Love, 1970), and the lipid content of the toadfish sonic muscle is 15.64 % wet mass (Fine *et al.* 1986). In teleosts, glycogen levels are typically higher in dark muscle than in white muscle (Love, 1970, 1980), and maximal levels in the weakfish sonic muscles were an order of magnitude greater than in white epaxial muscle. Glycogen levels of 5.05 $\mu m\,mg^{-1}$ wet tissue were noted in the sonic muscles of the toadfish (Fine *et al.* 1986), and maximal levels in weakfish were just over half of this value (approximately 2.67 $\mu m\,mg^{-1}$ wet tissue). Lower concentrations of lipid and glycogen in weakfish sonic muscles than in the same muscles of the toadfish correlate with the duration of sustained contraction these muscles experience during sound production: weakfish acoustic trains consist of spaced pulses, each caused by single, simultaneous twitches of the bilateral sonic muscles (M. A. Connaughton, M. L. Fine and M. H. Taylor, in preparation), whereas the toadfish boatwhistle results from a sustained contraction, often over 200 Hz in frequency (Skoglund, 1961; Fine, 1978; Rome *et al.* 1996) and ranging from 200 to 600 ms in duration (Gray and Winn, 1961; Fish and Mowbray, 1970; Fine, 1978).

Ash values increased dramatically after the spawning season, when concentrations of organic molecules (protein, glycogen, lipid) decreased. We suggest that this increase reflects a passive maintenance of levels of important ions such as Ca^{2+} and Mg^{2+}, which would cost energy to re-accumulate the following season, during the next period of muscle hypertrophy.

Sound production plays an important role in the reproductive behavior of the weakfish, as indicated by field and laboratory observations (Connaughton and Taylor, 1995*b*,

1996). The absence of external sexual dimorphism (Mercer, 1983) and the evening spawning habits (Taylor and Villoso, 1994) of the weakfish would diminish the value of visual cues in reproductive behavior, particularly in the turbid, inshore waters in which the species spawns (Biggs *et al.* 1983; Mercer, 1983). Sound stimuli, which can be localized in the dark, would therefore be adaptive, and sound production in weakfish courtship may function in mate attraction (Connaughton and Taylor, 1996), as has been observed in the toadfish (Gray and Winn, 1961) and the midshipman (Ibara *et al.* 1983; Brantley and Bass, 1994). Ultimately, the driving force behind the seasonal changes in weakfish sonic muscle is likely to be sexual selection in the form of apparent reproductive fitness and female mate choice. We hypothesize that females would be more likely to hear, and might also preferentially mate with, males that produced more intense calls and that called more frequently. Call amplitude is increased seasonally by increasing myofibrillar area and contractile protein content. Increased size of the individual also leads to increased sound amplitude, as larger individuals have a greater sonic muscle mass (M. A. Connaughton, M. L. Fine and M. H. Taylor, in preparation). This matter was not addressed in the present study because the fish recorded here were all of similar size. Increased call frequency would be supported by greater metabolic substrate stores (glycogen and lipids) and an increased mitochondrial content in expanded peripheral and central sarcoplasm.

This study describes a seasonal, androgen-driven hypertrophy in the periphery of the weakfish sonic motor system and the resultant changes in sonic muscle morphology, biochemistry and acoustic output. The changes in acoustic characteristics observed here appear to be the direct result of the hypertrophy of the sonic muscles and may have repercussions on reproductive success.

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References

ADKINS-REGAN, E. (1981). Early organizational effects of hormones. In *Neuroendocrinology of Reproduction* (ed. N. T. Adler), pp. 159–228. New York: Plenum Press.

BARRY, D. T. (1987). Acoustic signals from frog skeletal muscle. *Biophys. J.* **51**, 769–773.

BIGGS, R. B., SHARP, J. H., CHURCH, T. M. AND TRAMONTANO, J. M. (1983). Optical properties, suspended sediments and chemistry associated with the turbidity maxima of the Delaware estuary. *Can. J. Fish. aquat. Sci.* **40** (Suppl. 1), 172–179.

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*a*). Androgen effects on vocal muscle structure in a teleost fish with inter- and intra-sexual dimorphism. *J. Morph.* **216**, 305–318.

BRANTLEY, R. K., TSENG, J. AND BASS, A. H. (1993*b*). The ontogeny of inter- and intrasexual vocal muscle dimorphisms in a sound-producing fish. *Brain Behav. Evol.* **42**, 336–349.

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. AND TAYLOR, M. H. (1995*a*). The effects of exogenous testosterone on sonic muscle mass in the weakfish, *Cynoscion regalis. Gen. comp. Endocr.* **100**, 238–245.

CONNAUGHTON, M. A. AND TAYLOR, M. H. (1995*b*). Seasonal and daily cycles in sound production associated with spawning in the weakfish, *Cynoscion regalis. Env. Biol. Fish* **42**, 233–240.

CONNAUGHTON, M. A. AND TAYLOR, M. H. (1996). Drumming, courtship and spawning behavior in captive weakfish, *Cynoscion regalis. Copeia* **1996**, 195–199.

DEMSKI, L. S., GERALD, J. W. AND POPPER, A. N. (1973). Central and peripheral mechanisms of teleost sound production. *Am. Zool.* **13**, 1141–1167.

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

FINE, M. L. AND PENNYPACKER, K. R. (1986). Hormonal basis for sexual dimorphism of the sound producing apparatus of the oyster toadfish. *Exp. Neurol.* **92**, 289–298.

FINE, M. L., PENNYPACKER, K. R., DRUMMOND, K. A. AND BLEM, C. R. (1986). Concentration and location of metabolic substrates in fast toadfish sonic muscle. *Copeia* **986**, 910–915.

FISH, M. P. AND MOWBRAY, W. H. (1970). *Sounds of Western North Atlantic Fishes*. Baltimore: The Johns Hopkins Press.

FLORINI, J. R. (1987). Hormonal control of muscle growth. *Muscle & Nerve* **10**, 577–598.

FLORINI, J. R., EWTON, D. Z. AND COOLICAN, S. A. (1996). Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr. Rev.* **17**, 481–517.

FORGER, N. G. AND BREEDLOVE, S. M. (1987). Seasonal variation in mammalian striated muscle mass and motoneuron morphology. *J. Neurobiol.* **18**, 155–165.

FORGER, N. G. AND BREEDLOVE, S. M. (1991). Steroid influences on a mammalian neuromuscular system. *Sem. Neurosci.* **3**, 459–468.

FRANGIONI, J. V., KWAN-GETT, T. S., DOBRUNZ, L. E. AND MCMAHON, T. A. (1987). The mechanism of low-frequency sound production in muscle. *Biophys. J.* **51**, 774–783.

GIVEN, M. F. (1988). Growth rate and the cost of calling activity in male carpenter frogs, *Rana virgatipes. Behav. Ecol. Sociobiol.* **22**, 153–160.

GOLDBERG, A. I., ETLINGER, J. D., GOLDSPINK, D. F. AND JABLECKI, C. (1975). Mechanism of work-induced hypertrophy of skeletal muscle. *Med. Sci. Sport* **7**, 248–261.

GOODE, G. B. (1884). *The Fisheries and Fisheries Industries of the United States*, section I, *Natural History of Useful Aquatic Animals*. Washington, DC: US Government Printing Office.

GRAY, G.-A. AND WINN, H. E. (1961). Reproductive ecology and sound production of the toadfish, *Opsanus tau. Ecology* **42**, 274–282.

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.

HILL, G. L., FINE, M. L. AND MUSICK, J. A. (1987). Ontogeny of the

sexually dimorphic sonic muscle in three sciaenid species. *Copeia* **1987**, 708–713.

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.

KELLEY, D. B. (1986). Neuroeffectors for vocalization in *Xenopus laevis*: hormonal regulation of sexual dimorphism. *J. Neurobiol.* **17**, 231–248.

LAMB, D. R. (1975). Androgens and exercise. *Med. Sci. Sport* **7**, 1–5.

LOVE, R. M. (1970). *The Chemical Biology of Fishes*, vol. 1. New York: Academic Press.

LOVE, R. M. (1980). *The Chemical Biology of Fishes*, vol. 2. New York: Academic Press.

LUINE, V., NOTTEBOHM, F., HARDING, C. AND MCEWEN, B. S. (1980). Androgen affects cholinergic enzymes in syringeal motor neurons and muscle. *Brain Res.* **192**, 89–107.

MARIN, L. L., TOBIAS, M. L. AND KELLEY, D. B. (1990). Hormone-sensitive stages in the sexual differentiation of laryngeal muscle fiber number in *Xenopus laevis. Development* **110**, 703–712.

MARSH, R. L. AND DAWSON, W. R. (1982). Substrate metabolism in seasonally acclimatized American goldfinches. *Am. J. Physiol.* **242**, R563–R569.

MERCER, L. P. (1983). *A Biological and Fisheries Profile of Weakfish*, Cynoscion regalis. Special Scientific Report no. 39. North Carolina Dept. Nat. Resour. Comm. Div. Mar. Fish.

MERRINER, J. V. (1976). Aspects of the reproductive biology of the weakfish, *Cynoscion regalis* (Sciaenidae), in North Carolina. *U.S. Fish. Bull.* **74**, 18–26.

MULLER, E. R. A., GALAVAZI, G. AND SZIRMAI, J. A. (1969). Effects of castration and testosterone treatment on fiber width of the flexor carpi radialis muscle in the male frog (*Rana temporaria* L.). *Gen. comp. Endocr.* **13**, 275–284.

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.

OSTER, G. AND JAFFE, J. S. (1980). Low frequency sounds from sustained contraction of human skeletal muscle. *Biophys. J.* **30**, 119–127.

PHEONIX, C. H., GAY, R. W., GERALL, A. A. AND YOUNG, W. C. (1959). Organizational action of prenatally administered testosterone proprionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinol.* **65**, 369–382.

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. natn. Acad. Sci. U.S.A.* **93**, 8095–8100.

SASSOON, D. AND KELLEY, D. B. (1986). The sexually dimorphic larynx of *Xenopus laevis*: development and androgen regulation. *Am. J. Anat.* **177**, 457–472.

SCHNEIDER, H. (1967). Morphology and physiology of sound-producing mechanisms in teleost fishes. In *Marine Bio-Acoustics*, 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.

SMITH, H. M. (1905). The drumming of the drum-fishes (Sciaenidae). *Science* **22**, 376–378.

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 Bio-Acoustics*, vol. 1 (ed. W. N. Tavolga), pp. 195–211. New York: Pergamon Press.

TAYLOR, M. H. AND VILLOSO, E. P. (1994). Daily ovarian and spawning cycles in weakfish. *Trans. Am. Fish. Soc.* **123**, 9–14.

TEMPLEMAN, W. AND HODDER, V. M. (1958). Variation with fish length, sex, stage of sexual maturity and season in the appearance and volume of the drumming muscles of the swim-bladder in the haddock, *Melanogrammus aeglefinus* (L.). *J. Fish. Res. Bd Can.* **15**, 355–390.

TOBIN, C. AND JOUBERT, Y. (1991). Testosterone-induced development of the rat levator ani muscle. *Dev. Biol.* **146**, 131–138.

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.

VENABLE, J. H. (1966). Morphology of the cells of normal, testosterone-deprived and testosterone-stimulated levator ani muscles. *Am. J. Anat.* **119**, 271–302.

ZAR, J. H. (1984). *Biostatistical Analysis.* Englewood Cliffs, NJ: Prentice-Hall, Inc.