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Mutually exclusive muscle designs: the power output of the locomotory and sonic muscles of the oyster toadfish (*Opsanus tau*)

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Animals perform a vast array of motor activities. Although it has generally been accepted that muscles are well suited to the function that they must perform, specialization for performing one function may compromise their ability for carrying out another. We examined this principle in the toadfish muscular system: slow-twitch red and fast-twitch white myotomal muscles are used for powering swimming at relatively low frequencies, while the superfast swimbladder muscle powers mating calls by contracting at 100 Hz. We measured muscle power output over a wide range of frequencies. The red and white locomotory muscles could not generate power over *ca.* 2.2 and 12 Hz, respectively and, hence, could not power sound production. In contrast, the swimbladder muscle has many specializations that permit it to generate power at frequencies in excess of 100 Hz. However, these specializations drastically reduce its power output at low frequencies: the swimbladder muscle generated only one-twentieth of the power of the red muscle and one-seventh of the power of the white muscle at the frequencies used during swimming. To generate the same total power needed for swimming would require unfeasibly large amounts of swimbladder muscle that could not fit into the fish. Hence, the designs of the swimbladder and locomotory muscles are mutually exclusive.

Keywords: locomotory muscle; swimbladder muscle; oyster toadfish; muscle design; power; work

1. INTRODUCTION

Animals perform a vast array of motor activities over a large range of speeds and frequencies. These tasks require very different mechanical contributions from the muscles that drive them. It has generally been accepted that muscles are well suited to the function that they must perform (Rome *et al.* 1988). Since the experiments of Hill (1938, 1964) it has been recognized that maximum power and optimal efficiency of muscle contraction are achieved when muscles shorten at a V/V_{max} of between 0.15 and 0.4 (where V is the muscle shortening velocity and V_{max} is the maximum velocity of shortening). Hill (1950) predicted that, during *in vivo* function, muscles would shorten within this optimal range of V/V_{max}. Therefore, because there is a wide range of different movement speeds (Vs) that animals have to perform, there must be a similarly wide range of muscle V_{max} values in order to maintain an optimal V/V_{max}. Although this 'optimization theory' was a very attractive idea and explained the large variation in the mechanical properties of muscle observed in the animal kingdom, there was little direct support for it.

In the last decade or so, new techniques for measuring muscle function during normal motor activity have provided more robust evidence for this theory. For instance, in studies of fish swimming, Rome *et al.* (1988) found that fast-twitch white muscle fibres powered fast swimming while operating at an optimal V/V_{max} and that slow-twitch red fibres powered slow, steady swimming while they operated at an optimal V/V_{max}. One particularly interesting result was that although the fast-twitch white muscle could generate power at low swimming speeds, this would require the fibres to shorten at a very low V/V_{max} where they would be energetically inefficient. This probably explains why they are not active during this activity. More compellingly, it was found that slow-twitch muscle would not only be inefficient at powering fast swimming, but that it simply cannot shorten fast enough to power fast movements. These results suggested a principle, namely that the specializations of a muscle enabling it to perform one function may compromise its ability to carry out another function (Rome & Lindstedt 1998).

In this study, we examine this principle in one of the fastest vertebrate muscles known: the toadfish swimbladder muscle. Toadfish, like normal fish, swim slowly using their slow-twitch red muscle and make quicker movements using their fast-twitch white muscle. However, male toadfish also produce a loud, hooting mating call, which is commonly termed a 'boat whistle', in order to attract females to their nest (Skoglund 1961; Fine 1978). This call is produced by oscillatory contractions of the swimbladder muscle at 100 Hz (15 °C) and over 200 Hz (25 °C), thereby vibrating their large gas-filled swimbladder.

Recent studies have demonstrated that a number of important specializations in protein isoforms and the distribution of intracellular components take place in the swimbladder muscle in order to enable it to contract and relax at these high frequencies (Rome *et al.* 1996, 1999). However, these specializations come at a cost. The reduced myofilament volume (due to a high sarcoplasmic reticulum volume) and a lower proportion of attached cross-bridges than in locomotory fibres (due to an increased cross-bridge detachment rate constant) result in the toadfish swimbladder muscle only being able to

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generate one-tenth of the isometric force of normal locomotory fibres (Rome *et al.* 1999). This presents a new possibility that not only are the slow fibres unable to produce the very fast movements, but, in addition, the superfast fibres may be mechanically incapable of producing the slow movements used during normal swimming. That is, the mechanical properties of the locomotory and swimbladder muscles may be so dramatically different that one muscle cannot perform the function of the other and, hence, the muscles' designs are mutually exclusive (Rome & Lindstedt 1998).

Here we test this hypothesis using the work loop technique (Josephson 1985) on three fibre types of the toadfish. We ask whether the muscle in question can generate the same total mechanical power at the appropriate frequency of operation as the muscle that normally performs that activity. We conclude that muscle used to power low-frequency movement cannot power high-frequency movement and vice versa; hence, the designs are indeed mutually exclusive.

2. MATERIAL AND METHODS

The toadfish were kept in flow-through seawater tanks at ambient ocean temperature and were fed *ad libitum* throughout their captivity. In accordance with federal recommendations and with the approval of the Marine Biological Laboratory Institutional Animal Care and Use Committee, the toadfish were sedated by lowering their temperature on ice until they became unresponsive. They were then killed by severing their spinal columns and destroying their brains and spinal cords (double pithing).

In common with other fish, the anatomical segregation of different fibre types allowed us to dissect small intact bundles of fibres (pure in fibre type) from the slow-twitch red and fast-twitch white trunk musculatures and the superfast swimbladder muscles of the toadfish (Rome *et al.* 1996). The swimbladders were cut in half longitudinally, and fibre bundles (1 mm diameter) were taken from the middle third of each half. These preparations were used for up to 3 days *post-mortem* as their stress and twitch kinetics were found to be unaltered. Superficial red and white muscles (0.5 mm diameter) were taken from a region approximately half way down the tail, between 0.65 and 0.8 of body length (the toadfish head occupies *ca.* 50% of its total body length).

The muscle bundles were tied by their tendons between the arm of a Cambridge Technology (Watertown, MA, USA) 602X servomotor and either a Cambridge 400 or a Konigsburg Instruments (Pasadena, CA, USA) F5A-2 force transducer as previously described in Rome & Swank (1992) and Rome *et al.* (1996). The muscle chamber was filled with Ringer's solution (132 mM NaCl, 2.6 mM KCl, 2.7 mM $CaCl_2$, 10 mM imidazole, 10 mM sodium pyruvate and 1 mM $MgCl_2$, pH 7.7 at 15 °C) (Altringham & Johnston 1990). All experiments were conducted at 15 °C.

Sarcomere length was determined using laser diffraction. The muscle length was adjusted in order to give a starting length (L_o) corresponding to a sarcomere length of 2.3 μm (Rome *et al.* 1999). A series of isometric twitches and brief tetanic contractions was performed in order to measure the muscle's twitch kinetics and maximum isometric force. The activation time was the time it took for the force to increase from 50 to 90% of maximum force ($T_{a,50–90}$). The relaxation time was measured in two ways: the time needed to relax from 90 to 10% of maximum force ($T_{r,90–10}$) and the time needed to relax from 95 to 80% of maximum force ($T_{r,95–80}$). These isometric contractions also provided a means of assessing whether the preparation was healthy. Preparations with low stresses or exceptionally slow-twitch kinetics were rejected

The length change trajectory (strain) and stimulation phase were controlled and the data captured and analysed on-line via a Keithley Instruments (Cleveland, OH, USA) DAS1802AO card and software written in-house in Testpoint (Capital Equipment Corporation, Billerica, MA, USA). The preparations were subjected to sinusoidal strain cycles about L_0 and the amplitude was expressed as $\pm\%L_0$. Stimulation was applied at selected phases during the strain cycle via a pair of parallel-plate platinum electrodes on each side of the chamber. Experiments were carried out over a range of cycle frequencies. The strain, stimulus duration and stimulus phase were manipulated systematically at each frequency in order to yield the maximum net work. Force was plotted against strain for each cycle in order to produce a series of loops and net work was calculated as the area of the loop, which is equal to the difference between the work performed on the muscle during lengthening and the work performed by the muscle during shortening. Power was calculated as the product of the net work and cycle frequency. All work and power results were reported as a function of muscle mass.

An experimental run of 10 loops was performed on each preparation at each cycle frequency and the mean net work was calculated as the mean of the work performed per cycle between the fourth and ninth loops. The first two or three loops were often inconsistent, particularly at high frequencies in the swimbladder muscle. Loops 4–9 were generally consistent and, therefore, were considered to be representative of a steady-state condition. The muscle was allowed to recover for 5 min between runs. Control runs were performed after every fourth experimental run using parameters yielding maximum work close to the middle of the frequency range employed. These controls were used for monitoring the condition of the muscle and, if necessary, for calculating and compensating for any decline in the work output between runs (Altringham & Young 1991). The control work output typically declined by less than 10% during the course of the experiment.

3. RESULTS

In agreement with earlier studies (Rome *et al.* 1999) we found that the swimbladder muscle activated and relaxed very rapidly but generated very low forces compared with the red and white locomotory muscles. The mean maximum isometric stresses (mean ± s.e.) of the red muscle ($204.40 \pm 16.67\ \mathrm{kN\,m^{-2}}$) ($n = 5$) and white muscle ($249.67 \pm 16.94\ \mathrm{kN\,m^{-2}}$) ($n = 4$) were normal for locomotory muscles. In contrast, the swimbladder muscle generated less than one-tenth of their stresses ($15.36 \pm 1.56\ \mathrm{kN\,m^{-2}}$) ($n = 5$). The relative rates of activation and relaxation of the different muscle fibre types were assessed by measuring the time-intervals between different levels of force during isometric twitches. The activation times in the swimbladder muscle were considerably shorter than those for the myotomal muscle types. The $T_{a,50–90}$ in the swimbladder muscle was 2.9 ± 0.2 ms ($n = 6$), as compared with 64 ± 20 ms ($n = 4$) for red muscle and 17.2 ± 4.8 ms ($n = 5$) for white muscle. However, the most marked differences were in the relaxation rates.

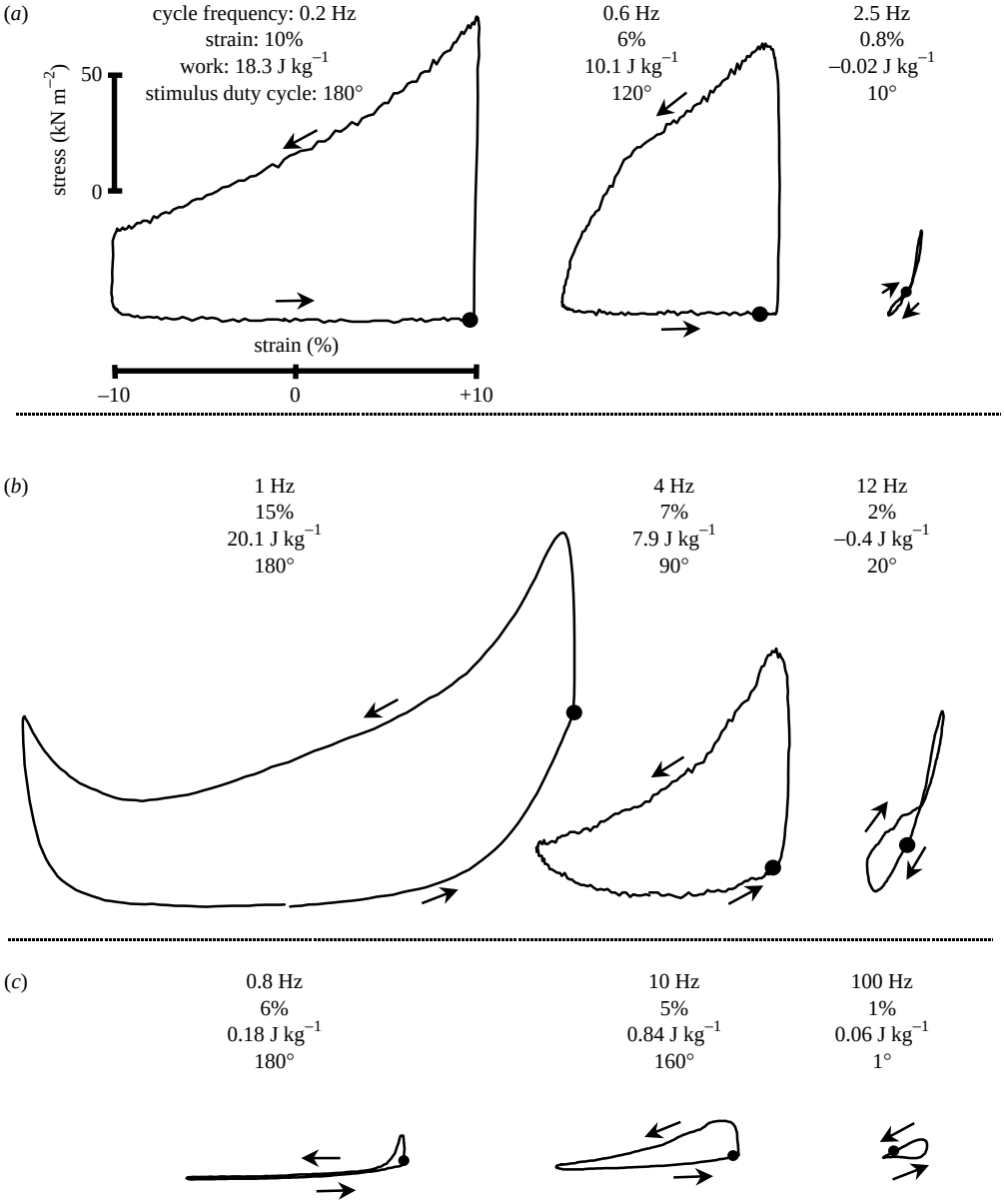

Figure 1. Work loops from toadfish (*a*) red, (*b*) white and (*c*) swimbladder muscles. Note that, for the red and white muscles, as with most muscles, the work (which is signified by the area of the loop) continually increased as the cycle frequency decreased. In contrast, work increased in the swimbladder muscle as the cycle frequency decreased to 10 Hz, but thereafter decreased with decreasing frequencies. Also note the small relative force and work production of the swimbladder muscle as compared with the locomotory muscles. The arrows signify the lengthening and shortening portions of the work loops. The dots signify at what point in the length change cycle that stimulation started. The duty cycle of the stimulus is given in degrees. The muscle generally received one stimulus at the highest frequencies shown for each fibre type.

The initial relaxation time $T_{r,95–80}$ was *ca.* 50 times shorter in the swimbladder muscle than in the red muscle ($T_{r,95–80} = 3.42 \pm 0.25$ ms, $T_{r,95–80} = 171 \pm 45$ ms and $T_{r,95–80} = 20.9 \pm 4.5$ ms for the swimbladder, red and white muscles, respectively). The difference became even larger (70 times) when relaxation was measured at lower force levels ($T_{r,90–10} = 15.2 \pm 2.9$ ms, $T_{r,90–10} = 1103 \pm 199$ ms and $T_{r,90–10} = 118 \pm 37$ ms for the swimbladder, red and white muscles, respectively).

(a) *Work loop measurements and power output*

Work and power output varied with cycle frequency in all muscles. In agreement with many previous studies (Rome & Swank 1992; James *et al.* 1996), work in the locomotory red and white muscles was found to be highest at low frequency (figures 1 and 2). The mean maximum work was 14.94 ± 1.8 J kg^{-1} at 0.2 Hz and 19.92 ± 5.77 J kg^{-1} at 1 Hz for the red and white muscles, respectively. As the frequency was increased, the work per cycle decreased dramatically: the red muscle could not generate work above 2.2 Hz and the white muscle could not generate work above 12 Hz.

We found that the swimbladder muscle generated markedly lower (17–23-fold) maximum work per cycle than the locomotory muscles (figures 1 and 2). Although much of this reduction reflected the lower isometric forces, the swimbladder muscle, in contrast to the other muscles, did not generate the greatest work at the lowest

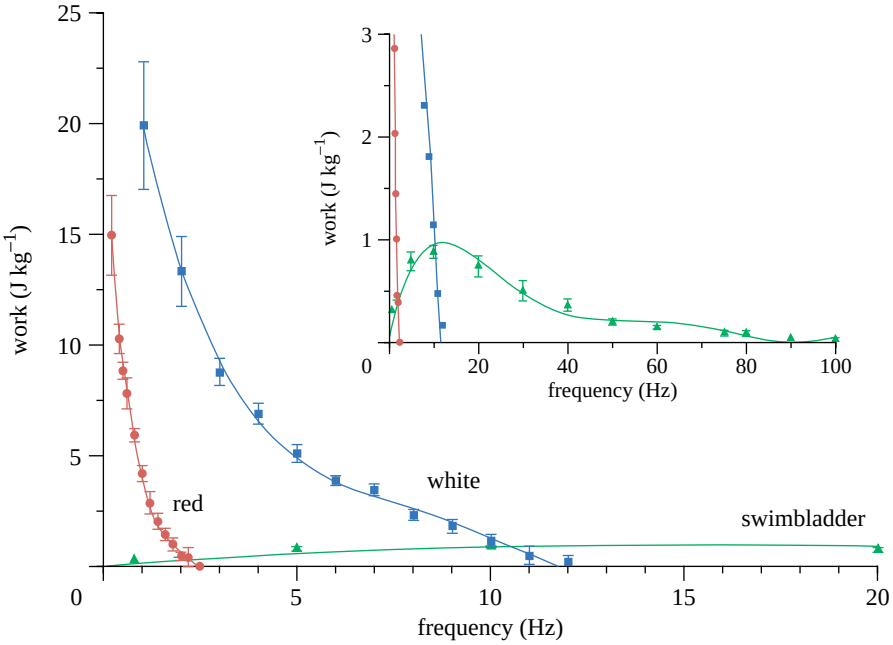

Figure 2. A plot of red muscle (red filled circles), white muscle (blue filled squares) and swimbladder muscle (green filled triangles) mean work (J kg^{-1}) versus cycle frequency (Hz). The error bars show standard errors of the mean. The number of replicates is five or greater for each point on the graph except for the swimbladder at 0.8 Hz ($n = 2$). The curves drawn are third- or fourth-order polynomial, fitted, 'least-squares' regressions in Sigma Plot (SPSS Science, Chicago, IL, USA). Data at 0.8 Hz were excluded from the regression for the swimbladder.

frequencies. Initially, work increased as the frequency decreased down to *ca.* 10 Hz (0.88 ± 0.06 J kg^{-1}) but thereafter work decreased with further decreases in oscillation frequency. Another difference is that, unlike the red and white muscles, which show a very sharp decline in work with increasing frequency, the work output from the swimbladder muscle declined gradually, with positive work being generated up to 100–120 Hz (figure 2).

The relationship between power output and cycle frequency also showed marked differences between the three fibre types. The power output rose to a maximum in all muscles then declined with increasing oscillation frequency (figure 3). Interestingly, whereas the maximum work values per cycle in the red and white muscles were 17- and 23-fold, respectively, more than the swimbladder muscle, the ability of the swimbladder muscle to operate at higher frequencies permitted it to generate a maximum power (14.19 ± 2.09 W kg^{-1} at 20 Hz) three times greater than the red muscle (4.72 ± 0.48 W kg^{-1} at 0.8 Hz) and over half the maximum power of the white muscle (27.64 ± 0.94 W kg^{-1} at 4 Hz) (figure 3).

There is a narrow range of frequencies over which the red and white muscles can generate positive power. Both the red and white muscles show fairly symmetrical power versus frequency curves, where the maximum frequency at which positive power can be obtained is approximately threefold higher than the frequency at which maximum power is generated (f_{opt}) (2.2 versus 0.8 Hz and 12 versus 4 Hz for the red and white muscles, respectively). In contrast, the swimbladder muscle can generate positive power at up to 100–120 Hz. This represents a fivefold higher frequency than the f_{opt}. In addition, because of the very low work per cycle, the swimbladder muscle produces very little power (0.25 W kg^{-1} at 0.8 Hz and 0.84 W kg^{-1} at 2 Hz) at the low frequencies employed by the red and white muscles for powering swimming.

4. DISCUSSION

(a) *Specializations and trade-offs for high-frequency performance*

The swimbladder muscle can generate positive power at up to 100–120 Hz. This is *ca.* 10 times the maximum frequency at which the white muscle can generate positive power (12 Hz) and *ca.* 50 times the maximum frequency at which the red muscle can generate power (2.2 Hz). Fundamental to this capability is the ability of the swimbladder muscle to relax from isometric contractions *ca.* 50 times faster than the red muscle. It has been shown previously that rapid relaxation kinetics are dependent on having (i) a very fast Ca^{2+} transient, (ii) a fast cross-bridge detachment rate constant g, and, probably, (iii) a high off rate K_{off} of Ca^{2+} from troponin-C (Rome *et al.* 1996, 1999). The first two of these modifications result in performance trade-offs (Rome & Lindstedt 1998; Rome *et al.* 1999). The fast Ca^{2+} transient requires a high density of SR (Appelt *et al.* 1991), thereby leaving less room for the force-generating myofibrils. Further, the fast detachment rate constant (coupled with a normal attachment rate constant f) results in the swimbladder muscle having a fivefold lower proportion of myosin heads attaching and generating force as compared with locomotory muscles. These two factors combine to reduce the force generation of the swimbladder muscle to approximately one-tenth to one-fifteenth of that generated by locomotory muscle (Rome *et al.* 1999; this study). This trade-off of force for speed reduces the work per cycle.

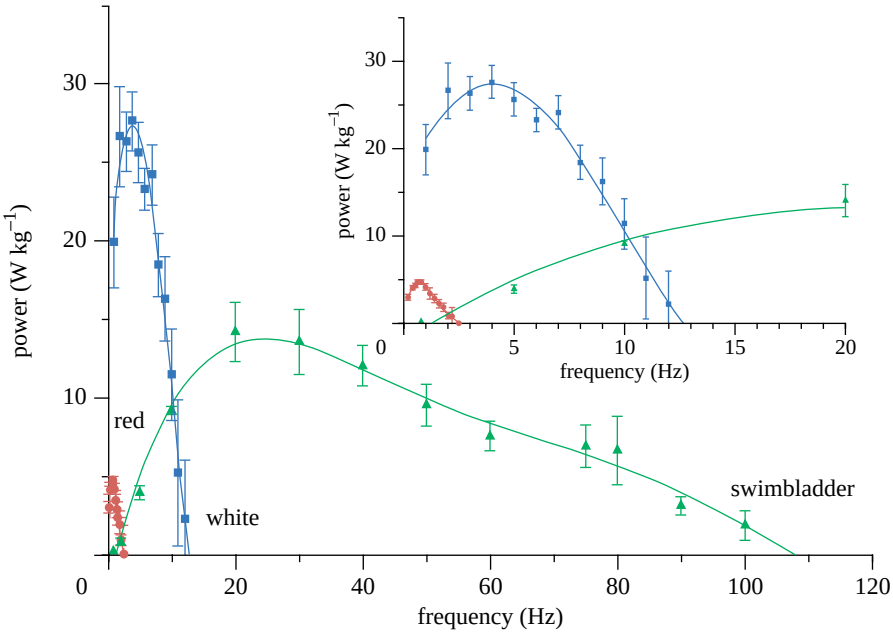

Figure 3. A plot of red muscle (red filled circles), white muscle (blue filled squares) and swimbladder muscle (green filled triangles) mean power output (W kg^{-1}) versus cycle frequency (Hz). The error bars show standard errors of the mean. The number of replicates used to calculate each point was five or greater except for the swimbladder at 0.8 Hz ($n = 2$). The curves drawn are third- or fourth-order polynomial, fitted, 'least-squares' regressions in SIGMA PLOT (SPSS). Data at 0.8 Hz were excluded from the regression for the swimbladder.

The reduction in mechanical power output due to this trade-off is particularly marked at low frequencies. The red and white muscle curves in figures 2 and 3 show the differences between slow-twitch and fast-twitch muscles. At any given frequency, the fast-twitch muscle generates more work and, thus, more power than the slow-twitch muscle. This is because the fast-twitch muscle has a higher V_{max}, which permits it to shorten with a lower V/V_{max} and generate more force. In addition, at the higher frequencies the fast-twitch muscles' ability to relax faster permits longer stimulation, which increases the power output further. Unlike the myotomal muscles, the swimbladder muscle produces very little work at low frequencies, *ca.* 20-fold less than the locomotory muscles. Much of this reduction is due to the low force. However, another factor is that the swimbladder muscle cannot maintain activation during a long duration contraction. When normal locomotory muscle such as toadfish red and white muscle undergoes a tetanic contraction, the muscle generates a nearly constant force for several seconds. Thus, during low-frequency work loops (where the kinetics of relaxation are usually not limiting), maximum work is obtained by starting stimulation just prior to the beginning of shortening and then maintaining this stimulation throughout shortening. In this case the muscle generates a nearly square work loop, representing a large quantity of work (figure 1).

However, in the case of the swimbladder muscle the force during a tetanic contraction rises, stays constant for only tens of milliseconds and then declines rapidly to between 10 and 20% of the maximum force, despite being stimulated continuously (see Fig. 1 in Rome *et al.* 1999). The significance of this is illustrated during the work loop at 0.8 Hz (figure 1*c*). The force remains elevated for a short time corresponding to only *ca.* 15% of the shortening portion of the work loop. Because the muscle shortens such a short distance during the elevated force portion (*ca.* 1% L_o), very little work is done. However, at 10 Hz (figure 1*c*), the duration of force generation (*ca.* 50 ms) is equal to the duration of the shortening stroke. Thus, the muscle can shorten a far greater distance (5% L_o) during force generation and, accordingly, it generates far more work (Layland *et al.* 1995). Consequently, 10 Hz represents the peak in the work–frequency relationship (figure 2). Therefore, although the underlying mechanism for the swimbladder muscle's inability to maintain high force for long periods of time is unknown, it contributes significantly to the reduced work and power at low frequencies.

(b) *Mutually exclusive designs: can the locomotory red and white muscles power high-frequency sound production?*

The designs of different muscles are mutually exclusive if a given muscle type (type a) is incapable of performing a task normally carried out by another muscle type (type b) and if type b muscle is incapable of performing the task normally carried out by type a muscle. Therefore, in order to assess whether the designs are mutually exclusive, we need to determine whether each muscle type can generate the same total mechanical power as the normal muscle type does at the appropriate frequency of operation. We have shown that locomotory muscle cannot power high-frequency sound production at *ca.* 100 Hz. The red muscle is incapable of generating any positive power over *ca.* 2.2 Hz and the white muscle is incapable of producing positive power over 12 Hz. Further, when driven at 100 Hz, not only was the white muscle unable to generate positive work, but this treatment resulted in permanent damage. However, this alone should not

convince us of the mutually exclusive design of the muscle fibre types we have examined. We also need to consider whether the swimbladder muscle could perform the role of the myotomal muscles in locomotion.

(c) *Mutually exclusive designs: can superfast muscle power swimming?*

Although the swimbladder muscle is capable of generating some mechanical work and power at the low frequencies used during swimming (figure 3), it is not sufficient for powering swimming. In order to generate the same total power as the red or white muscle, such a large quantity of extra swimbladder muscle would be needed that it could not physically fit into the fish. To illustrate this point, video tapes showed that toadfish use their white muscle at a frequency of *ca.* 4 Hz during rapid swimming. At this frequency, the mass-specific power output of the swimbladder muscle is approximately one-seventh that of the white muscle (figure 3). Therefore, in order to generate the same total power, the mass of the swimbladder muscle would have to be approximately seven times that of the white muscle. Because the white muscle constitutes over half of the total mass of fish (Zhang *et al.* 1996), increasing the swimbladder muscle mass to sevenfold that of the white muscle would result in a threefold more massive fish.

A similar analysis shows that the swimbladder muscle cannot replace the red muscle either. At 0.8 Hz (the optimum for the red muscle), the power generated per kilogram of red muscle is *ca.* 20-fold greater than that of the swimbladder muscle. Thus, in order to generate the same total power the swimbladder muscle would have to occupy a 20-fold greater volume than the red muscle. The magnitude of this required augmentation increases further (to 40–65-fold) when one accounts for the volume of mitochondria necessary for providing the swimbladder muscle with an aerobic capacity similar to that of the red muscle. Again, the toadfish's body would not be able to accommodate this large muscle mass. Thus, we conclude that the swimbladder muscle is incapable of powering swimming and, hence, that the designs of the sonic and the locomotory muscles are mutually exclusive.

These findings show that powering the wide range of activities in which animals engage requires considerable specialization of the molecular components of muscle and that these modifications in turn entail trade-offs in mechanical performance. Further, our results show that these trade-offs can be so extreme that they can lead to mutually exclusive designs (i.e. the swimbladder is designed solely for high-frequency contractions while the locomotory muscles are designed solely for low-frequency contractions). Hence, no one muscle can perform all motor activities, thus explaining the multitude of different fibre types found in the animal kingdom.

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