

# MECHANICAL PROPERTIES OF RED AND WHITE SWIMMING MUSCLES AS A FUNCTION OF THE POSITION ALONG THE BODY OF THE EEL *ANGUILLA ANGUILLA*

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## Summary

The way in which muscles power steady swimming depends on a number of factors, including fibre type and recruitment, muscle strain, stimulation pattern and intensity, and the intrinsic mechanical properties of the muscle fibres. For a number of undulatory swimming fish species, *in vivo* studies have shown that muscles at different positions along the body are stimulated during different phases of the strain cycle. Moreover, some intrinsic contractile properties of the muscles have been found to vary according to their position along the body.

We report the first results on the mechanical properties of the red and white muscles of an anguilliform swimmer, *Anguilla anguilla*. Small preparations (0.147–1.335 mg dry mass) were dissected from positions at fractions of 0.2, 0.4, 0.6 and 0.8 of total body length (*BL*). We determined the time to 50% and 100% peak force and from the last stimulus to 50% relaxation for isometric contractions; we measured the sarcomere lengths that coincided with *in situ* resting length. None of these quantities varied significantly

with the longitudinal position from which the fibres were taken. We also measured power and work output during contractions under conditions approximating those used *in vivo* (cycle frequency, 1 Hz; strain amplitude,  $\pm 10\%$   $L_0$ , where  $L_0$  is the length giving maximum isometric force). During these experiments, work output was affected by stimulation phase, but did not depend on the longitudinal position in the body from which the muscles were taken.

Our results indicate that red and white eel muscles have uniform properties along the body. In this respect, they differ from the muscle of most non-anguilliforms, in which muscle kinetics varies in a systematic way along the body. Uniform properties may be beneficial for anguilliform swimmers, in which the amplitude of the travelling wave can be pronounced over the entire body length.

Key words: European eel, *Anguilla anguilla*, swimming, muscle mechanics, red muscle, white muscle, kinetics, power, work.

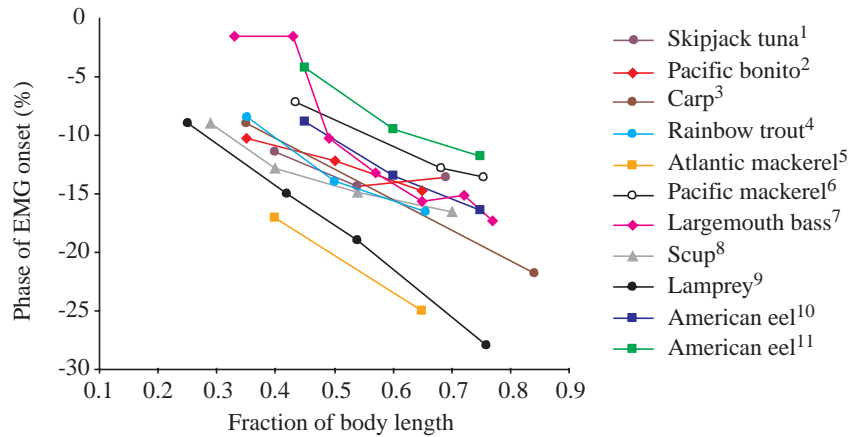
## Introduction

Undulatory swimming fish use a backward-travelling wave of lateral curvature to propel themselves through the water. This mechanical wave corresponds to the occurrence of rhythmic, nearly sinusoidal, changes in length in the swimming muscle fibres. Observations of intact swimming fish (Fig. 1) have shown that the phase of electromyographic (EMG) activity relative to the sinusoidal movement of the muscle fibres varies systematically along the length of the fish. At the rostral end of the fish, EMG activity starts just before the time at which the muscle fibres begin to shorten. EMG activity starts progressively earlier in muscle fibres closer to the caudal end of the fish, i.e. stimulation starts earlier in the stretch part of the movement cycle. This pattern seems to be consistent among several species, including fish with different swimming styles and body shapes.

How does this pattern influence the capacity of axial muscle

from different longitudinal positions to generate force, work and power? Experiments on isolated muscle (white muscle, e.g. Altringham et al., 1993; Curtin and Woledge, 1996; James et al., 1998; Johnson and Johnston, 1991; red muscle, e.g. Altringham and Johnston, 1990; Coughlin, 2000; Rome et al., 1992; pink muscle, e.g. Coughlin and Rome, 1996) have shown that the power output from muscle is strongly dependent on stimulation phase during sinusoidal movement. When stimulus phase is varied at constant stimulus duty cycle and movement frequency, an optimum phase can be identified at which maximum power is produced; at higher or lower phases, power output is lower. This relationship is found in both fast- and slow-twitch fibres (Curtin and Woledge, 1993a; Curtin and Woledge, 1993b). Thus, stimulus phase is an extrinsic factor, imposed on the muscle of a swimming fish, that determines how much power the muscle contributes to locomotion.

Fig. 1. Phase of onset of electromyographic (EMG) activity recorded at different locations along the body of fish during steady swimming. From published data, we calculated phase as the time from the start of shortening to the start of EMG activity, expressed as a percentage of the cycle time (see Fig. 3). <sup>1</sup>Skipjack tuna *Katsuwonus pelamis*, red muscle (Shadwick et al., 1999); <sup>2</sup>Pacific bonito *Sarda chiliensis*, slow-twitch muscle (Ellerby et al., 2000); <sup>3</sup>carp *Cyprinus carpio*, red muscle (van Leeuwen et al., 1990); <sup>4</sup>rainbow trout *Oncorhynchus mykiss*, slow muscle (Hammond et al., 1998); <sup>5</sup>Atlantic mackerel *Scomber scombrus*, red muscle (Wardle and Videler, 1993); <sup>6</sup>Pacific mackerel *Scomber japonicus*, red muscle (Shadwick et al., 1998); <sup>7</sup>largemouth bass *Micropterus salmoides*, red muscle (Jayne and Lauder, 1995); <sup>8</sup>scup *Stenotomus chrysops*, red muscle (Rome et al., 1993); <sup>9</sup>lamprey *Lampetra fluviatilis* (Williams et al., 1989); <sup>10</sup>American eel *Anguilla rostrata* swimming at  $1 \text{ BL s}^{-1}$ , red muscle (Gillis, 1998); <sup>11</sup>American eel *Anguilla rostrata* swimming at  $0.75 \text{ BL s}^{-1}$ , red muscle (Gillis, 1998). *BL* is body length.



Stimulus duty cycle and strain pattern are other examples of extrinsic factors that influence power.

Experiments on isolated muscle have also shown that among fish, as among other animals, the intrinsic properties of the muscle fibres vary widely. In other words, when an identical extrinsic challenge is imposed (stimulus and strain pattern), the contractile response depends on the source of the muscle. The fast white fibres and slow red fibres are the most obvious examples of fibres with different intrinsic properties. However, intrinsic properties also vary even within one fibre type, fast or slow. In addition, there is clear variation in the intrinsic properties of fibres from different locations along the length of the body in some, but not all, fish species. The most commonly reported differences in fish muscle are in the kinetics of isometric twitches or tetani. As the examples in Fig. 2 show, there are striking differences in the duration of relaxation (the time required for force to decline after the end of stimulation). In species in which variation is found, muscle fibres from the caudal end take longer to relax. Altringham et al. (Altringham et al., 1993) and Rome et al. (Rome et al., 1993) discuss how variations in intrinsic properties are matched to the extrinsic factors operating in the fish, resulting in enhanced muscle performance compared with what could be achieved with uniform intrinsic properties. In the case of the saithe *Pollachius virens* (Altringham et al., 1993), the slower relaxation of caudal muscle ensures that it is active while it is being lengthened and, thus, because of its stiffness, that it can act as an effective transmitter of power. In the case of scup *Stenotomus chrysops* (Rome et al., 1993), the variation in relaxation time contributes, along with other factors, to enabling net positive work to be done by muscle at all locations along the body.

Experiments such as those discussed above have not previously been reported for isolated muscle from anguilliform swimmers, in which the travelling wave of curvature may be pronounced and of large amplitude along the whole length of the body during some of the many different swimming modes

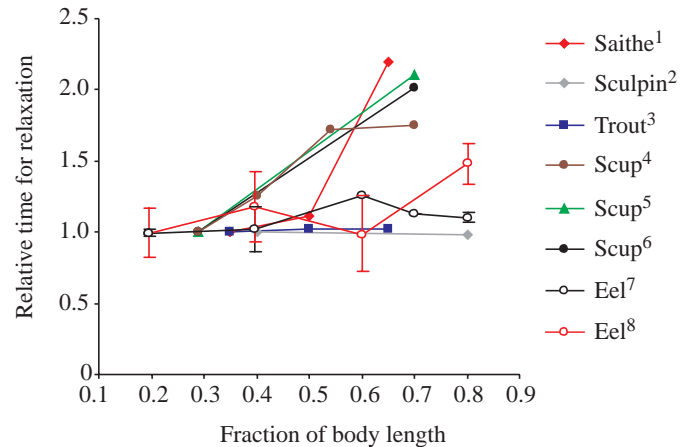


Fig. 2. Time required for relaxation of force of muscle fibres isolated from different locations along the length of the fish. We calculated relaxation times as multiples of the relaxation time for the muscle from the rostral end of the fish. <sup>1</sup>Time from twitch stimulus to 90% relaxation of white muscle from saithe *Pollachius virens* (Altringham et al., 1993); <sup>2</sup>time from last stimulus to 50% relaxation after a brief tetanus of fast fibres from short-horned sculpin *Myoxocephalus scorpius* (Johnston et al., 1993); <sup>3</sup>time from twitch stimulus to 90% relaxation of slow fibres from trout *Oncorhynchus mykiss* (Hammond et al., 1998); <sup>4</sup>time for relaxation from 90% to 10% of force after a brief tetanus of red muscle from scup *Stenotomus chrysops* (Rome et al., 1993); <sup>5</sup>time for relaxation from 90% to 10% of force after a brief tetanus of red muscle from scup *Stenotomus chrysops* (Swank et al., 1997); <sup>6</sup>time from twitch stimulus to 90% relaxation of red muscle from scup *Stenotomus chrysops* (Swank et al., 1997); <sup>7</sup>time from last stimulus to 50% relaxation after a tetanus of red fibres from eel (*Anguilla anguilla*, this study). Values are means  $\pm$  S.E.M.,  $N=3-6$ . See Fig. 5A and text. <sup>8</sup>Time from twitch stimulus to 50% relaxation of white fibres from eel (*Anguilla anguilla*, this study). Values are means  $\pm$  S.E.M.,  $N=6-15$ . See Fig. 5B and text.

employed (D'AOût and Aerts, 1999). In spite of the pronounced difference in the appearance of the mechanical wave, the relative timing between the onset of activation and the

beginning of shortening in anguilliform swimmers (lamprey and eel) is similar to that in carangiform swimmers (see Fig. 1).

In this paper, we address two questions (i): how does stimulus phase influence power output from isolated eel muscle under conditions resembling those seen *in vivo* and (ii) are there differences in the intrinsic properties of muscle isolated from different positions along the body that could influence the power output?

## Materials and methods

### Preparation of the fibre bundles

Live European eels, *Anguilla anguilla* L., ranging from 0.440 to 0.675 m in total body length (*BL*), were bought commercially and housed in freshwater tanks (temperature of the recirculating water, 12 °C) in the animal house at University College London for several days before the start of experiments. Animals were killed by an overdose of anaesthetic (MS-222, 3-aminobenzoic acid ethyl ester, Sigma), followed by decapitation and pithing of the brain and spinal cord. Thin slices of red and white swimming muscle were dissected from one side of the fish and transferred to the laboratory in cold saline.

The *in situ* fibre length was measured in 10 out of 18 experiments. The skin was removed from the intact side of the fish, and fibre lengths were measured using the eyepiece graticule in a dissecting microscope. The mean *in situ* fibre length,  $3.92 \pm 1.19$  mm ( $N=10$ , mean  $\pm$  S.D.), was not significantly different (*t*-test for dependent variables,  $P=0.09$ ) from the  $L_0$  deduced from the length/tension curve ( $4.13 \pm 1.32$  mm,  $N=10$ , mean  $\pm$  S.D.;  $L_0$  is the length giving maximum isometric force).

Small preparations for the experiments on contractile properties were obtained by further dissection under saline. These preparations contained a bundle of parallel fibres attached at either end to a patch of myosepta. Care was taken to make these preparations in such a way that all fibres would remain parallel and stretch equally when the preparation as a whole was stretched. The patches of myosepta on either side of the preparation were clamped and glued (using cyanoacrylic glue) in aluminium foil clips that allowed the preparation to be mounted in the experimental apparatus.

During transport, dissection and experiments, the fibres were kept in saline solution containing (in  $\text{mmol l}^{-1}$ ): NaCl, 150; KCl, 2.6; MgCl<sub>2</sub>, 1.0; sodium pyruvate, 10.0; CaCl<sub>2</sub>, 2.7, NaH<sub>2</sub>PO<sub>4</sub>, 0.7; Na<sub>2</sub>HPO<sub>4</sub>, 2.7 (pH 7.20 in equilibrium with air).

### Experimental apparatus

The experimental apparatus consisted of a bath of recirculating, aerated saline (20.0 °C). In the bath, one end of the preparation was attached to a motor (Cambridge Technology, Inc., model 300B) and the other was kept in a fixed position attached to a strain-gauge force transducer. The fibre preparation was electrically stimulated (Digitimer, model DS7) *via* two platinum wire electrodes in the bath. The duration of each stimulus pulse was 1 ms.

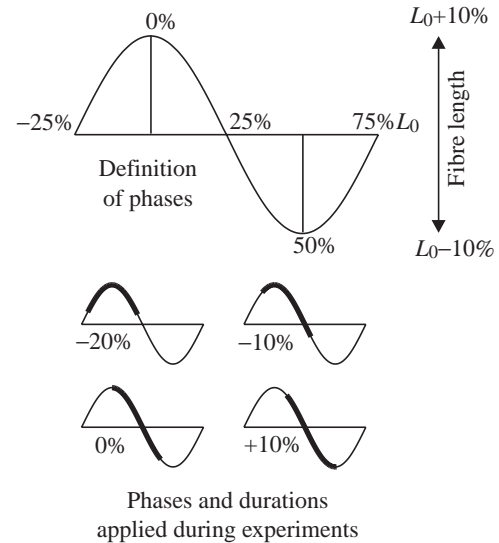


Fig. 3. Strain and stimulation pattern during the sinusoidal strain experiments. Strain was constant at  $L_0 \pm 10\%$ , where  $L_0$  is the length giving maximum isometric force. Stimulation duration was 0.4 s (0.4 cycle) and was given at four different phases,  $-20\%$ ,  $-10\%$ ,  $0\%$  and  $+10\%$ . Phase is the time from the start of shortening to the time of the first stimulus, expressed as a percentage of cycle duration.

A custom-designed data-acquisition program (written in ViewDac, Keithley) was used to control stimulation and motor arm position and to record force and motor arm position.

### Experimental protocol

Our aim was to gather data from red and white fibres taken from four positions along the fish, 0.2, 0.4, 0.6 and 0.8 *BL*. In one case, we used red fibres from all four body positions in the same fish (body length 0.550 m), and in another case we used

Table 1. Preparations of *Anguilla anguilla* muscle fibre bundles used in the work experiments

Fibre type	Position (%)	<i>M</i> (mg)	<i>L</i> <sub>0</sub> (mm)
Red	20	0.360±0.058 ( <i>N</i> =2)	3.27±0.58 ( <i>N</i> =2)
	40	0.592 ( <i>N</i> =1)	3.7 ( <i>N</i> =1)
	60	0.534 ( <i>N</i> =1)	3.92 ( <i>N</i> =1)
	80	0.203±0.056 ( <i>N</i> =2)	2.04±0.16 ( <i>N</i> =2)
White	20	0.360±0.058 ( <i>N</i> =2)	3.27±0.58 ( <i>N</i> =2)
	40	0.798±0.234 ( <i>N</i> =5)	4.78±0.55 ( <i>N</i> =5)
	60	0.523±0.061 ( <i>N</i> =3)	4.76±0.48 ( <i>N</i> =3)
	80	0.417±0.050 ( <i>N</i> =2)	2.70±0.41 ( <i>N</i> =2)

Position indicates approximate longitudinal position as a percentage of total body length *BL* (the tail tip would be 100%).

*M*, mass of the experimental preparation with as much of the non-fibre material removed as possible.

*L*<sub>0</sub>, the fibre length corresponding with maximal isometric 400 ms tetanic force (for the red fibres) or isometric twitch force (for the white fibres). The sarcomere lengths at *L*<sub>0</sub>, measured using laser diffraction, are given in Fig. 4.

Values are mean  $\pm$  S.D.

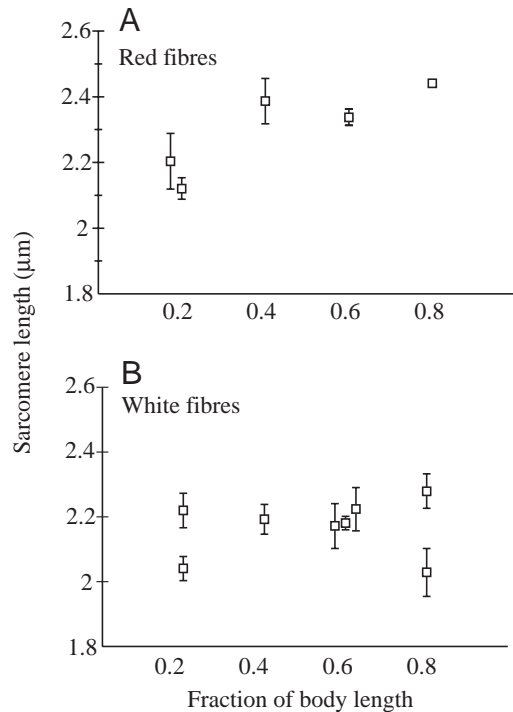


Fig. 4. Sarcomere lengths from different locations along the body length. Sarcomere lengths were measured by laser diffraction. Values are means  $\pm$  S.E.M. for six fibres randomly selected from the fibre preparation concerned. 1–3 fibre preparations were measured. (A) Red fibres; (B) white fibres.

white fibres from all four positions in the same fish (body length 0.586 m). Ten additional experiments were performed in which we studied fibres from only one or two longitudinal positions per fish (see Table 1 for an overview). Sample kinetic data for one additional red fibre preparation (mass, 0.613 mg;  $L_0$ , 2.58 mm) taken from 0.7 BL are added in Fig. 2 and Fig. 5, but this preparation has not been analysed further.

#### Isometric contractions: supramaximal stimulation and $L_0$

After the fibre preparation had been mounted in the experimental apparatus, the following protocol was used. (i) The stimulation current was adjusted to give maximal muscle force. This supramaximal stimulus strength was used during the rest of the experiments. (ii) The length/force relationship was investigated by varying the distance between the two clips at the ends of the preparation and measuring isometric force. We used 400 ms tetani at a stimulation frequency of 20 Hz for the red fibres, and twitch stimuli for the white fibres. The length giving maximum isometric force was taken as  $L_0$ . (iii) The fusion frequency was determined with 400 ms tetani at  $L_0$  by increasing the stimulus frequency in steps of 10 Hz starting with 20 Hz. Fused tetanus was attained at 60–90 Hz for the red fibres and at 70–110 Hz for the white fibres. A slightly higher stimulation frequency (usually 100 Hz) was used to obtain supramaximal tetani of red fibres. There was a 3 min rest period between tetani. (iv) Recordings were made of isometric tetani with a stimulation pattern like that used in the sinusoidal strain experiments (see below).

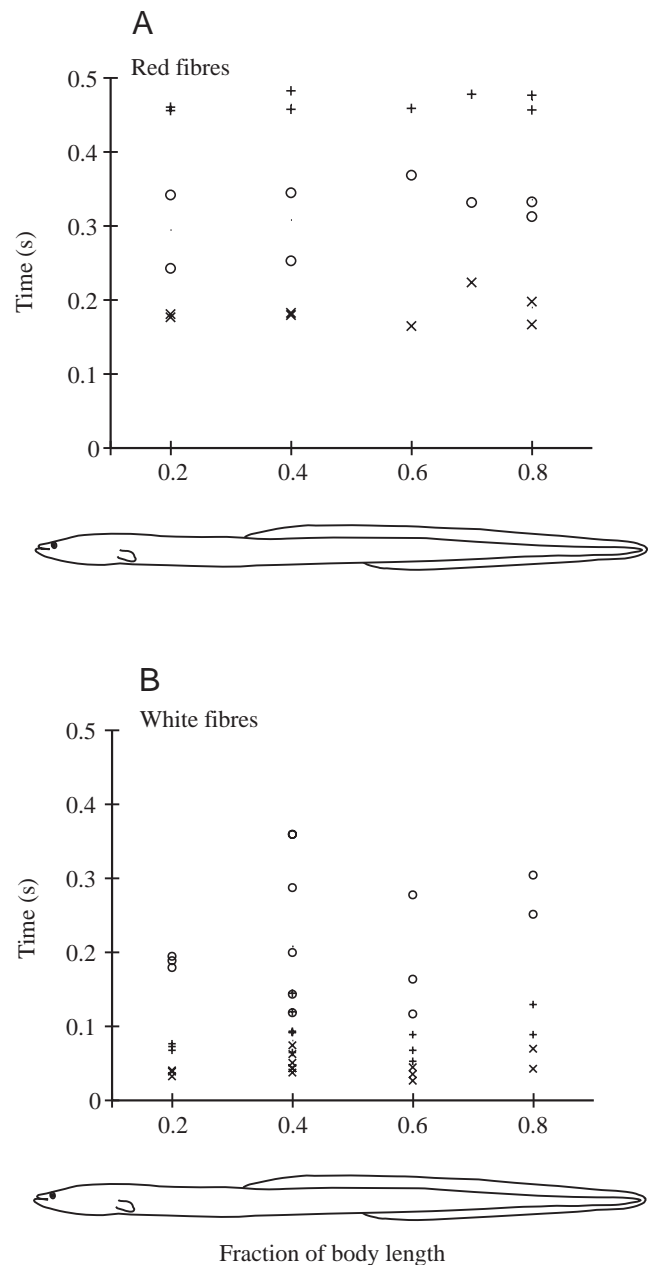
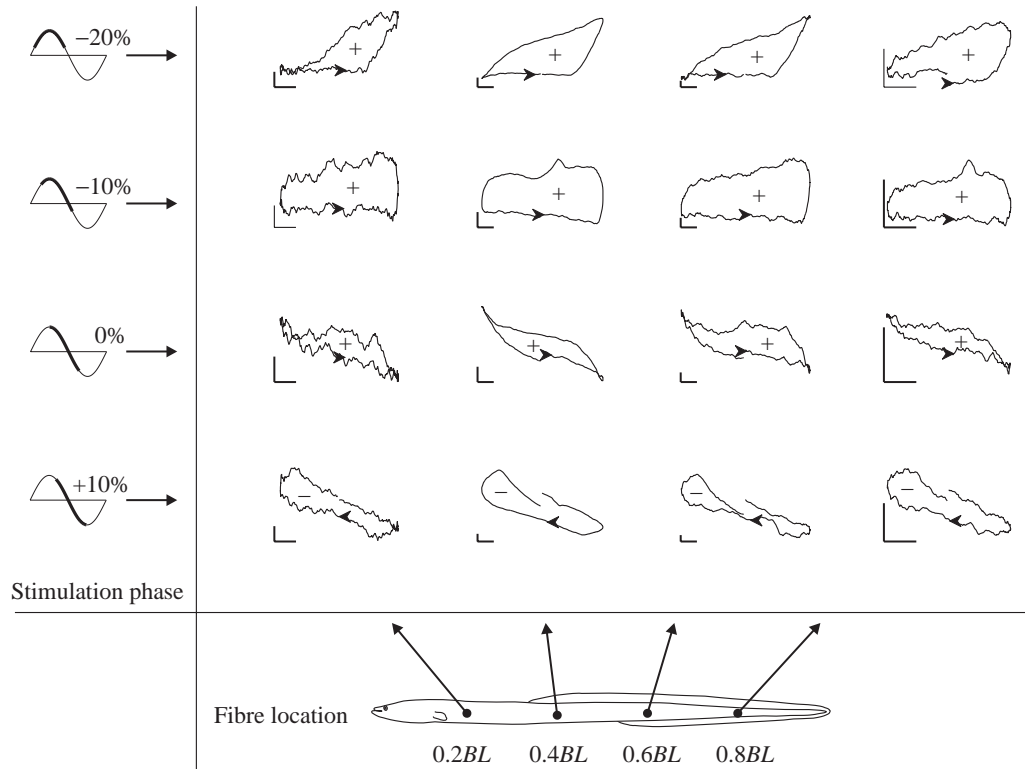


Fig. 5. Time from first stimulus to 50% and 100% peak force and time from last stimulus to 50% peak force during relaxation in isometric stimulations. Each point is the mean of observations made on one fibre preparation. +, time to peak force; x, time to 50% peak force; o, time to 50% relaxation. (A) Results from eight red fibre preparations using tetanus stimulation. Regressions: for time to 50% peak force,  $y=(0.174\pm 0.017)+(0.02\pm 0.03)x$ ,  $r^2=0.067$ ,  $P=0.535$ ; for time to 100% peak force,  $y=(0.459\pm 0.010)+(0.01\pm 0.02)x$ ,  $r^2=0.091$ ,  $P=0.460$ ; for time to 50% relaxation,  $y=(0.281\pm 0.039)+(0.07\pm 0.07)x$ ,  $r^2=0.140$ ,  $P=0.360$ , where  $y$  is time in seconds and  $x$  is fraction of body length. Values are means  $\pm$  S.E.M. (B) Results from 13 white fibre preparations using twitch stimulation. The lines are regressions: for time to 50% peak force,  $y=(0.039\pm 0.011)+(0.0002\pm 0.0002)x$ ,  $r^2=0.050$ ,  $P=0.464$ ; for time to 100% peak force,  $y=(0.075\pm 0.020)+(0.0003\pm 0.0004)x$ ,  $r^2=0.055$ ,  $P=0.441$ ; for time to 50% relaxation,  $y=(0.170\pm 0.054)+(0.0009\pm 0.0011)x$ ,  $r^2=0.066$ ,  $P=0.395$ .



Fig. 6. Red fibre preparations: work loops (force versus length change) during movement at 1 Hz and with a stimulation duty cycle of 0.4 at four different stimulation phases, as shown in the diagrams on the left. Fibres were taken from four different positions along the body, as shown in the diagram at the bottom. *BL*, body length. The scale for the different work loops is given on the left of the loop: vertical, 10 mN; horizontal, 0.1 mm. The arrow on each work loop indicates the movement direction. The sign in the loop indicates whether the net work was positive or negative. The ripple on the force recordings from 0.2 and 0.8 *BL*, where *BL* is body length, is noise from the recording circuit. It is prominent in these recordings because these muscle preparations were small and therefore produced low active force. All recordings are from fibre preparations from the same eel.



Three isometric tetani were recorded at  $L_0$ , with supramaximal stimuli at tetanic fusion frequency and with a duration of 400 ms. There was an interval of 600 ms without stimulation between these tetani in a set (1000 ms cycle and duty factor of 0.4, as in the sinusoidal strain experiments, see below) and a rest period of 3 min between the sets of tetani to allow complete recovery of the fibre preparation. Isometric tetani were also recorded at fibre lengths of  $L_0-10\%$  and  $L_0+10\%$ .

#### Sinusoidal strain experiments

Next, experiments were performed in which the fibre length was varied sinusoidally (at a frequency of 1 Hz) around  $L_0$  with a standard amplitude of  $\pm 10\% L_0$ . This value was chosen because it is within the range recorded by D'Août and Aerts (D'Août and Aerts, 1999) and matches what we calculated, using the method of D'Août and Aerts (D'Août and Aerts, 1999), from observations at the middle of the body of swimming *Anguilla anguilla* (Grillner and Kashin, 1976). In our experiments, three cycles of movement with stimulation were recorded. The stimulation duty factor was 0.4 (thus, the fibres were stimulated for 400 ms in each 1000 ms cycle), which corresponds with electromyographic data for swimming *Anguilla anguilla* (Grillner and Kashin, 1976). In the American eel *Anguilla rostrata*, Gillis (Gillis, 1998) observed shorter duty factors, typically between 0.2 and 0.3. We used supramaximal stimuli at tetanic fusion frequency and at four different stimulus phases. The stimulus phase (indicating the relative timing of the stimulation and movement) was defined as the time interval measured from

the time at which fibre length was longest to the start of stimulation. Phase is expressed in units of percentage of cycle duration. The following phases were used:  $-20\%$  (stimulation starting 200 ms before maximal length),  $-10\%$  (stimulation starting 100 ms before maximal length),  $0\%$  (stimulation starting at maximal length) and  $+10\%$  (stimulation starting 100 ms after maximal length) (Fig. 3). These phases were chosen because they span the whole range of phases *in vivo* (deduced from Grillner and Kashin, 1976). Before and after the sinusoidal strain experiments, control recordings were made in which the fibres were moved but without stimulation. The passive force output was subtracted from the total force output during stimulation experiments to obtain the active force.

#### Analysis of the recordings

##### Isometric kinetics

During isometric tetani, some white fibre preparations showed a period of slow increase in force following the initial, faster increase in force. We assume this phenomenon to be 'creep' (Gordon et al., 1966; Julian et al., 1978; Edman and Reggiani, 1984) and used an extrapolation method based on that of Gordon et al. (Gordon et al., 1966) to correct for it. The force near the end of stimulation was linearly extrapolated back to the start of stimulation. Similarly, the rapidly rising force near the beginning of stimulation was linearly extrapolated forward. The point of intersection of the two extrapolations was taken as the peak force corrected for creep. We measured the time from the first stimulus to the corrected peak force, the

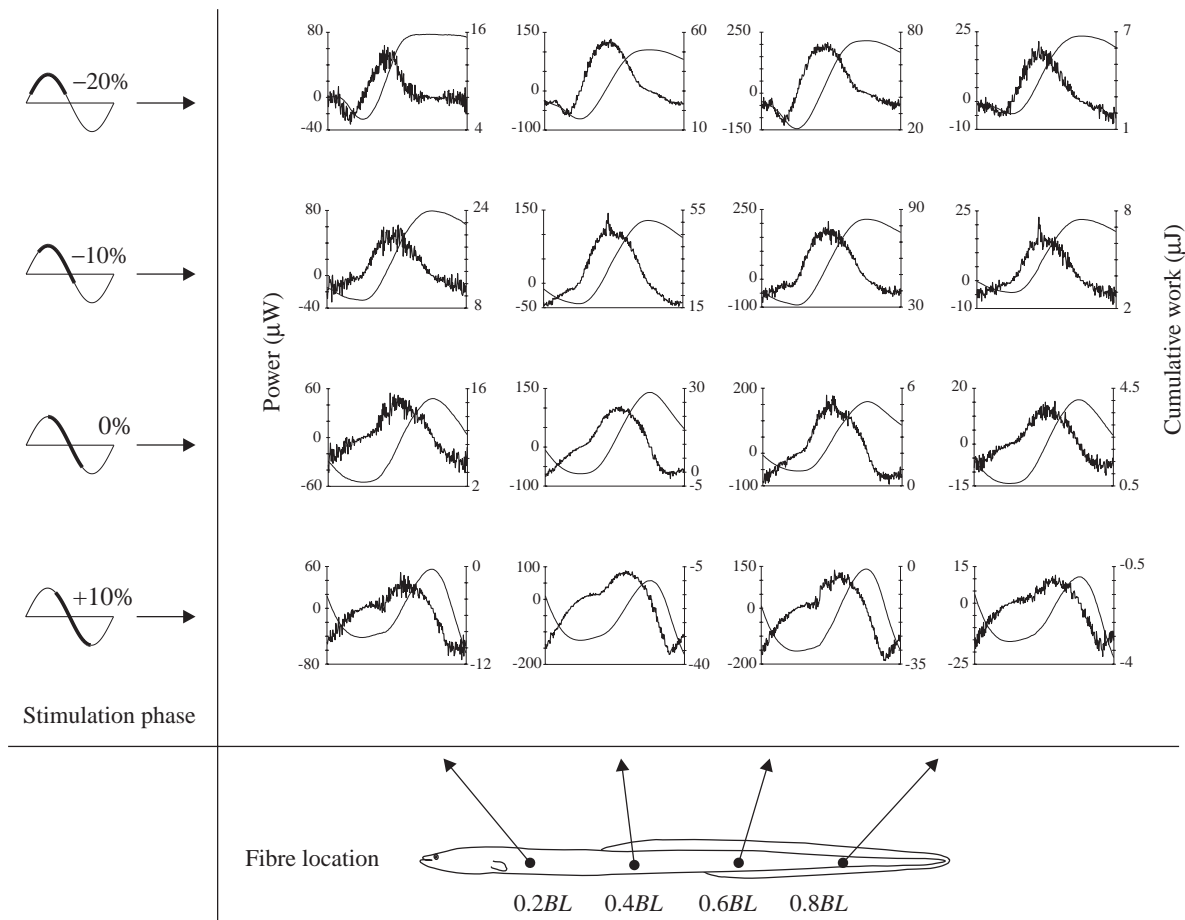


Fig. 7. Red fibre preparations: superimposed time courses of power (thick line) and cumulative work (thin line) during one cycle of movement at 1 Hz. Stimulation with a duty factor of 0.4 was given at four different stimulation phases, as shown in the diagrams on the left. Fibres were taken from four different positions along the body, as shown in the diagram at the bottom.  $BL$ , body length. The power scale is shown on the left axis and the work scale on the right axis. All recordings are from fibre preparations from the same eel.

time from the first stimulus to 50% of the corrected peak force and the time from the last stimulus to 50% relaxation (i.e. to 50% of peak force).

#### Sinusoidal strain experiments

We scaled the recorded forces to the maximal force during isometric tetani of the same preparation. This gave the information needed to address the questions asked in this study. The absolute values of force were not normalized for preparation size because the methods we had in place for quantifying cross-sectional area could not take account of the following variables. (i) The amount of fat within the muscle tissue, which varied considerably between the individual fish we used and, from visual inspection during dissection, we judged that its contribution to the total cross-sectional area was not negligible. (ii) The proportion of live fibres (and consequently, the cross-sectional area of contracting fibres) probably varied among muscle preparations, and this value could not be obtained using our protocol.

The work and power produced during the second cycle of movement were assessed from work loops (i.e. force *versus* displacement plots) and from the recordings of the time course

of force and length change. Power as a function of time was calculated as the product of instantaneous force and velocity.

#### Post-experiment measurements on the fibre bundles

After experiments, preparations were fixed in ethanol, and  $L_0$  was measured while the preparation was still clamped at  $L_0$  in the experimental apparatus. After removing the fixed preparation from the apparatus, the clips and as much non-fibre material as possible were removed. The fibres were teased apart, dried at room temperature for approximately 1 day and weighed on a microbalance. The sarcomere length of fibres from some of the preparations was measured by laser diffraction (see, for example, Cleworth and Edman, 1972).

## Results

### Red fibre preparations

The mass,  $L_0$  and sarcomere length of the red fibres are given in Table 1 and in Fig. 4A. Sarcomere length at  $L_0$  does not show a clear relationship with the position along the body and averages  $2.3\mu\text{m}$  (two-tailed *t*-tests revealed no significant differences, except between  $0.2BL$  and  $0.4BL$ ,  $P=0.04$ ).

Fig. 5A shows times to 50% and 100% peak force (measured from the first stimulus) and the half-relaxation time (measured from the last stimulus) measured in isometric tetani at  $L_0$ . Values do not show a clear and systematic relationship with position along the body. Regression analysis showed that none of the slopes was significantly different from zero (see legend to Fig. 5). Analysis of variance indicated that the variance between positions was not significantly greater than 'within position' variance. For comparison with other species, the values for half-relaxation times were normalised to the mean value for  $0.2BL$ . Mean values  $\pm$  S.E.M. are plotted in Fig. 2. The results are more like those of trout and sculpin, for which relaxation time is similar in fibres from all body positions, than those of saithe and scup, for which relaxation is much slower in fibres from the caudal end of the fish.

#### Sinusoidal strain experiments

The work produced in the sinusoidal strain experiments and the influence of phase and muscle position can be estimated by constructing work loops for all four longitudinal body positions and all four stimulation phases (Fig. 6). As can be seen from these examples, work loops from experiments with the same stimulation phase, but with fibres from different positions, tend to be very similar in shape. In contrast, work loop shape changes drastically when the stimulation phase changes. Red fibres from all body positions produce net positive work at stimulation phases of  $-20\%$ ,  $-10\%$  and  $0\%$ , whereas with fibres from all body positions net work was negative with a stimulus phase of  $+10\%$ . This phase is not likely to be used *in vivo* during steady swimming (see below). The effect of stimulus phase is not surprising, but the fact that the work loops differ so little between positions contrasts with results from muscles of carangiform fish.

Like the work loops, Fig. 7 shows that power curves (and, hence, the cumulative work) are strongly affected by stimulation phase, but show little dependence on the position in the body from which the muscle fibres came. Power curves from a stimulation phase of  $-20\%$  typically consist of a stage at the beginning of stimulation when the muscle is being stretched and power is negative. This is followed by a stage of high positive power output, and the net effect is positive work for one complete cycle. At a stimulation phase of  $-10\%$ , the amount of negative power is very small and there is large net positive work over one cycle. At stimulation phases of  $0\%$  and  $+10\%$ , negative power results from the muscle being stretched while force is still being produced during relaxation. Note that 300 ms is the typical value for the half-relaxation time from an isometric tetanus (see Fig. 5A). For a complete cycle of movement, there is a small net positive work output at a stimulation phase of  $0\%$ , and net negative work at a phase of  $+10\%$ .

The cumulative work curves (Fig. 7) can be used to calculate the amount of positive and negative work produced progressively during one cycle and the net work per complete cycle. It is clear that muscles from all positions deliver net positive work at stimulation phases of  $-20\%$ ,  $-10\%$  and  $0\%$ .

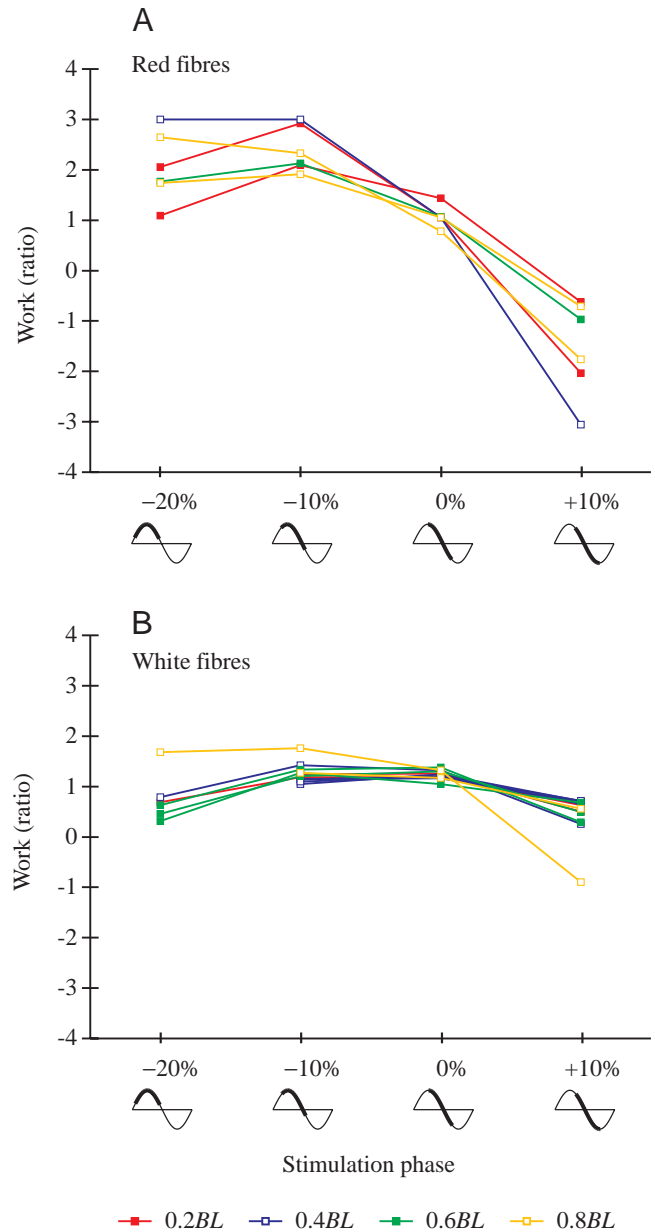
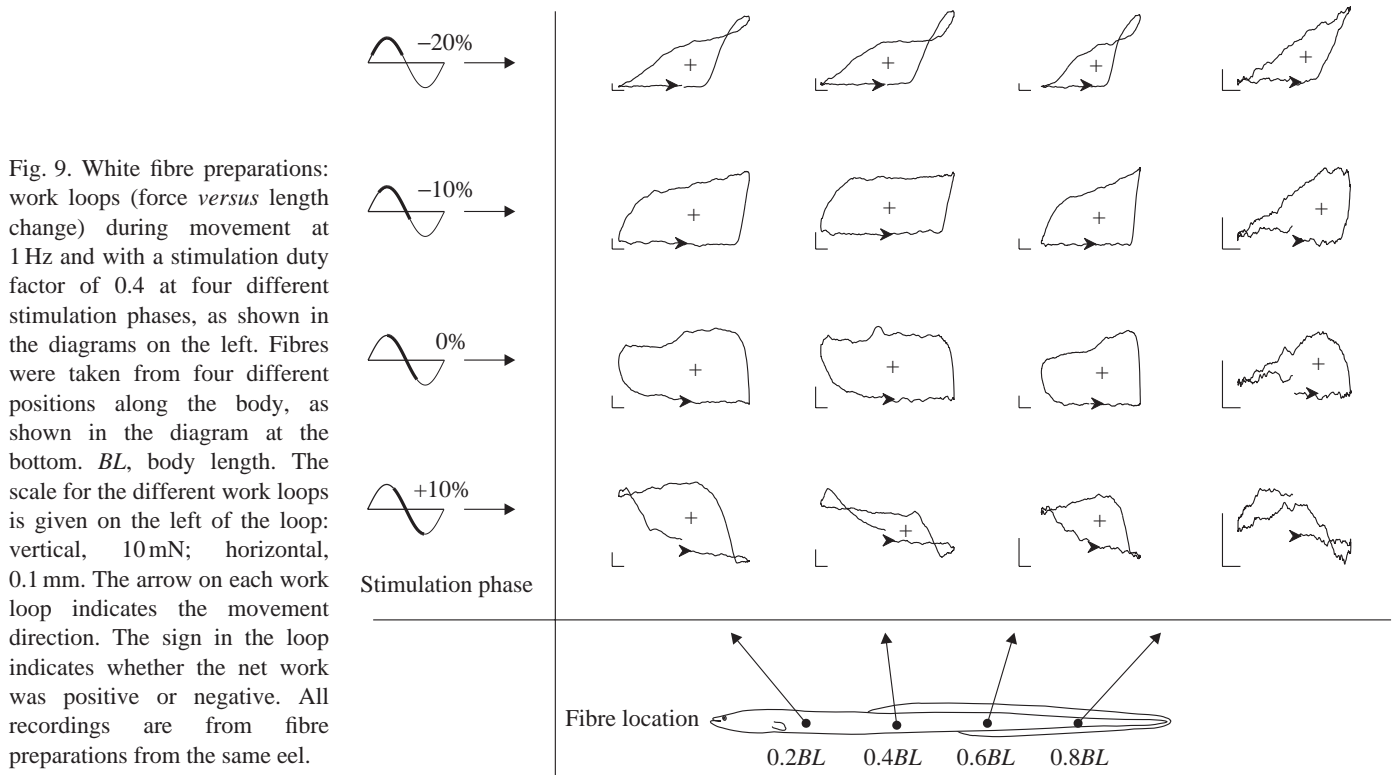


Fig. 8. Net work in one cycle of movement at 1 Hz with a stimulation duty factor of 0.4 at four different stimulus phases. Different colors refer to results for different muscle preparations. Net work is expressed relative to the average work for that fibre preparation over all phase conditions applied. (A) Results for six red fibre preparations; (B) results for six white fibre preparations. *BL*, body length.

This corresponds to phases recorded *in vivo* using electromyography (Gillis, 1998), where EMG activity typically starts between phases of  $-19\%$  and  $-3\%$  (i.e. between  $20^\circ$  and  $80^\circ$  in the phase units used by Gillis, 1998).

Fig. 8A summarizes the values for net work per cycle for all the red muscle preparations. Each value is scaled to the mean value for the fibre preparation concerned. In general, work is highest at stimulation phases of  $-10\%$ . In some cases, net work at a phase of  $-20\%$  is lower as a result of some negative work



at the beginning of stimulation as the muscle starts delivering force while it is being stretched. At a phase of +10%, net negative work is produced because of the large portion of negative work resulting from the muscle being stretched while it is exerting considerable relaxation force (Fig. 7).

#### White fibre preparations

The mass and  $L_0$  values of the fibres are given in Table 1. Fig. 4B shows that sarcomere lengths at  $L_0$  were not significantly different in fibres from different body positions (all combinations of body positions tested, two-tailed *t*-test,  $0.28 < P < 0.94$ ).

Fig. 5B shows times to 50% and 100% peak force (time from first stimulus) and the half-relaxation time (time from last stimulus) measured in isometric tetani at  $L_0$ . As was the case with red fibres, the values do not show a clear and systematic relationship with position along the body. Regression analysis showed that none of the slopes was significantly different from zero (see legend to Fig. 5). Analysis of variance indicated that the variance between positions was not significantly greater than 'within-position' variance. These results suggest that the kinetics of isometric force production does not vary along the length of the fish or that any variations are small and subtle. For comparison with other species, the values for half-relaxation times were normalised to the mean value for 0.2 *BL*. Mean values  $\pm$  S.E.M. are plotted in Fig. 2. As with eel red fibres, the results are more like those for trout and sculpin, for which relaxation time is similar in fibres from all body positions, than those of saithe and scup, for which relaxation is much slower in fibres from the caudal end of the fish.

#### Sinusoidal strain experiments

Fig. 9 shows work loops that give a qualitative view of the work done by the muscle fibres during a complete cycle of movement. In general, net positive work is produced at all four stimulation phases. Clearly, the shape of the work loops obtained with different stimulation phases differs widely. The work loops from fibres taken at different body positions are strikingly similar when the fibres operate under the same strain and stimulation conditions. In agreement with the isometric results, these results indicated that there are no, or only subtle, systematic differences in muscle properties along the body length. Fig. 10 depicts power and cumulative work done during one cycle of movement. These curves show the same trend as the work loops. In addition, it can be observed that, in general, substantial negative power production is only observed at stimulus phases of -20% and +10%. At intermediate stimulus phases, a little negative power is produced and relatively high net positive work per cycle can be expected.

Fig. 8B summarizes the values for net work per cycle for all the white muscle preparations. Each value is scaled to the mean value for the fibre preparation concerned. In general, work is highest at stimulation phases of -10% to 0%. At a phase of -20%, net work is lower because of the negative work done during part of the cycle, i.e. the muscle starts delivering force when it is being stretched during the beginning of the stimulation. At a phase of +10%, little or no net work is produced because of the large amount of negative work resulting from the muscle being stretched while it was exerting considerable relaxation force (Fig. 10).



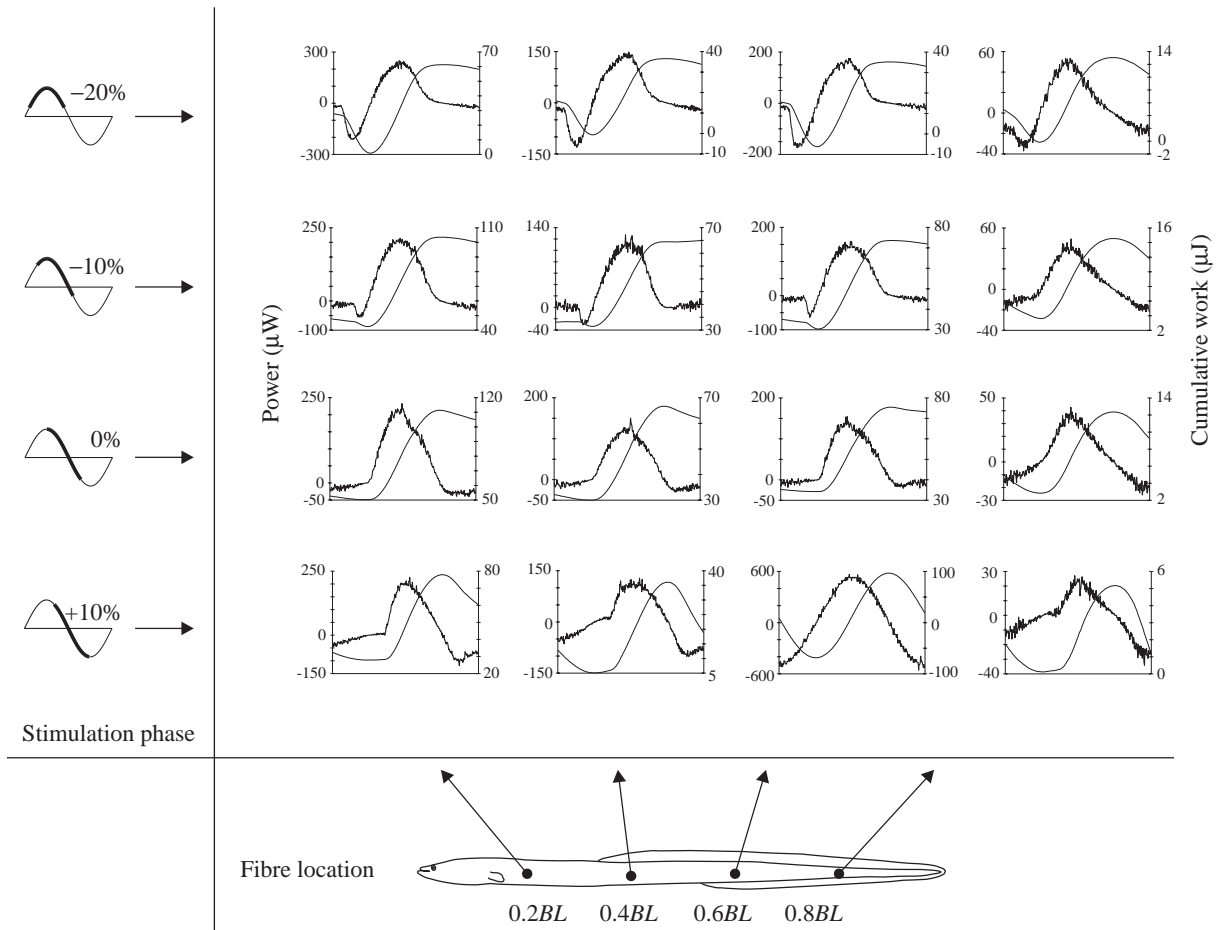


Fig. 10. White fibre preparations: superimposed time courses of power (thick line) and cumulative work (thin line) during one cycle of movement at 1 Hz. Stimulation with a duty factor of 0.4 was given at four different stimulation phases, as shown in the diagrams on the left. Fibres were taken from four different positions along the body, as shown in the diagram at the bottom. The power scale is shown on the left axis and the work scale on the right axis. All recordings are from fibre preparations from the same eel. *BL*, body length.

### Discussion

These results show that the contractile properties of both red and white swimming muscle from the eel *Anguilla anguilla* do not depend significantly on their location along the body length. Isometric kinetics do not differ and, more importantly, during sinusoidal strain experiments (relevant to the *in vivo* regime), muscles from different locations behave similarly when they operate under similar conditions.

*In vivo*, however, the stimulation phase does differ between locations, and our results have shown that in the anguilliform swimmer, as in carangiforms, stimulus phase has a strong impact on the development of force and power. The stimulus phases applied ranged from  $-20\%$  to  $+10\%$ . This covers more than the range of phases recorded *in vivo* for eels, in which EMG activity typically starts between phases of  $-19\%$  and  $-3\%$  (corresponding to  $20^\circ$  and  $80^\circ$ , respectively; Gillis, 1998b). Hence, we conclude that in the eel, as in the scup (Rome et al., 1993) and mackerel *Scomber japonicus* (Shadwick et al., 1998), muscle fibres perform net positive work at all body positions. The results from the present study show that an essential determinant of eel muscle function based on body position is the relative timing of neural activation and

shortening rather than a difference in the intrinsic contractile properties of the muscle fibres. This can be related to eel and, more generally, to anguilliform behaviour. Eel swimming is characterised by a very wide range of swimming styles (Videler, 1993), including backward undulatory swimming (D'Août and Aerts, 1999), forward and backward escape responses, snake-like manoeuvring between vegetation and crevasses and terrestrial crawling. D'Août and Aerts (D'Août and Aerts, 1999) and D'Août (D'Août, 1999) have shown that muscle strain varies greatly between forward and backward swimming, and it is to be expected that still other extrinsic challenges are imposed upon the muscle fibres during various other types of locomotion. Homogeneous ('unspecialised') muscles are probably most suited to deal with the varying demands associated with the wide repertoire of movement associated with the axial system of anguilliforms.

In this context, the variation in intrinsic muscle properties along the length of the body that occurs in several species other than eel can be seen as a regional specialisation that enhances the performance of a specialised swimming type. However, this benefit may be gained at the expense of flexibility in swimming style.

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