

AN A JOHNSTON and DEREK BALL

Thermal stress and muscle function in fish

Introduction

Temperature is one of the most important extrinsic factors determining muscle performance in ectotherms. In considering the effects of temperature on muscle contraction in fish it is important to distinguish between sustainable and maximum effort, since they are supported by different muscle fibre types. A great variety of muscle phenotypes are observed, brought about by the differential expression of different protein isoforms, and by varying the amounts of ion channels, membrane pumps and cellular organelles (see Johnston & Altringham, 1991, for a recent review). Slow swimming activity largely involves the recruitment of slow twitch muscle fibres, which are dependent on aerobic metabolism, and complex respiratory and circulatory support systems to supply oxygen and substrates (Bone, 1966; Johnston, Davison & Goldspink, 1977). Slow twitch fibres, with their high concentrations of myoglobin and mitochondria and well developed capillary supply, are the main fibre type in red muscle (Johnston, 1981; Altringham & Johnston, 1988a).

As swimming speed increases, faster contracting muscle fibres are recruited (Johnston *et al.*, 1977; Rome, Loughna & Goldspink, 1984). Maximum performance is achieved during fast-starts associated with escape responses and predation and involves the recruitment of the entire white muscle mass (Johnston, Franklin & Johnson, 1993). The white muscle is typically composed of a single fibre type expressing fast isoforms of the myofibrillar proteins and containing high concentrations of the cytoplasmic Ca²⁺-binding protein parvalbumin (Gerday *et al.*, 1979; Rowlerson *et al.*, 1985; Crockford & Johnston, 1993). White fibres have larger average diameters than red fibres and contain higher volume densities of myofibrils, and a more extensive sarcoplasmic reticulum for faster calcium cycling (Akster, Granzier & ter

Keurs 1985; Fleming *et al.*, 1990). Slow muscle fibres are orientated parallel to the longitudinal axis of the trunk whereas fast muscle fibres are arranged in complex helical patterns (Alexander, 1969). Rome and Sosnicki (1991) showed that fast fibres have a higher gearing ratio, needing to shorten by only 25% as much as the slow fibres to produce a given change in body curvature.

Muscle performance varies with both the passive properties of the musculoskeletal system and the active properties of the nervous system (Johnston, 1991; Van Leeuwen, 1995). In general, fast muscle is dependent on fewer other physiological systems than is slow muscle since it functions largely independently of the circulation (except for recovery) and utilizes anaerobic metabolism and endogenous fuel supplies (phosphocreatine and glycogen) (Johnston & Altringham, 1991).

Because of these differences the factors limiting muscle performance at the whole animal level may well vary with the type of swimming activity. Thus, although many properties of muscle can best be studied using *in vitro* preparations, it is essential to relate any results to how the muscle functions in the intact animal. It is also important to consider realistic levels of thermal stress. A major problem in considering the likely impact of global warming is that most studies on the effect of temperature on muscle contraction have been directed towards understanding cold-adaptation rather than responses to thermal stress. Similarly, although there is a growing literature on the phenotypic responses to temperature change, the main focus of this research has been on eurythermal species rather than on stenothermal fish which are more likely to be vulnerable to any detrimental effects of global warming.

This chapter starts with a review of the phenotypic and genotypic responses of fish muscle to temperature change and concludes with some speculation on the likely impact of global warming on swimming performance. Experimental paradigms for future research are also discussed.

Phenotypic and genotypic responses to temperature

Whole animal studies

Rome and co-workers recorded electromyograms (EMGs) to investigate the effects of temperature on muscle recruitment in the common carp (*Cyprinus carpio*) and the scup (*Stenotomus chrysops*). The maximum speed the fish could swim using the red muscle alone was found to increase with warming, reflecting its higher power output. The threshold speed for the recruitment of white muscle fibres was correspondingly

increased. Indeed in all species studied, warming causes an increase in maximum sustainable swimming speed until some limit is reached, after which performance usually declines (see Fig. 1). The temperature at which swimming performance levels off is a function of habitat temperature. For example, the Antarctic species, *Pagothenia borchgrevinski* reaches its maximum cruising speed (U_{crit}) at -0.8°C and is unable to swim above 2°C (Wohlschlag, 1964), whereas in the largemouth bass (*Micropterus salmoides*) from North America U_{crit} reaches a maximum at $25\text{--}30^{\circ}\text{C}$ (Beamish, 1970). The reason for the drop-off in sustainable performance at temperatures approaching the upper thermal tolerance of a species (Fig. 1) is probably complex, reflecting a failure in one or more of the support systems to the muscle fibres and/or a drop in muscle power output (see Egginton *et al.*, this volume).

There are relatively few data on the effects of thermal stress on escape behaviour in fish. The typical response of a fish to a noxious stimulus or predator is a C-start in which the head and the tail rotate in the same direction away from the centre of mass during the first tailbeat. Batty and Blaxter (1992) studied the maximum speed of fish larvae following C-start escape responses using a high speed video recording. The maximum speed (U_{max}) of newly hatched herring (*Clupea harengus*) larvae increased up to 15°C , whereas U_{max} in plaice larvae

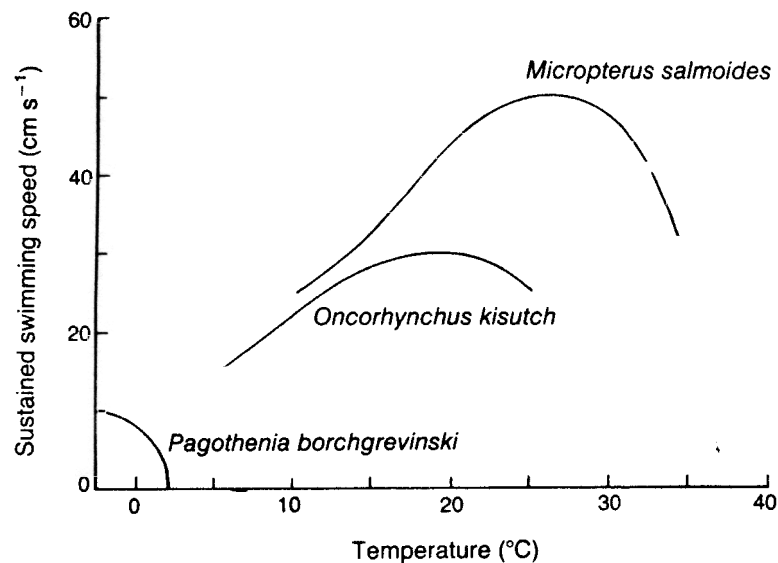


Fig. 1. Influence of temperature on the maximum sustainable swimming speed of fish (adapted from Beamish, 1978).

(*Pleuronectes platessa*) reached a plateau between 8 and 12 °C, due to a reduction in stride length.

Properties of isolated proteins and muscles

The temperature tolerance of extant teleost species ranges from -2 °C in Antarctica to 43–45 °C for fish living in geothermal hot-springs. Studies with isolated proteins and muscle preparations have been used to gain some insight into the limits and mechanisms underlying evolutionary adjustments to different thermal environments.

Antarctic species living permanently between -2 and 1 °C have the lowest upper lethal temperatures reported for fish of around 6 °C (Somero & DeVries, 1967). The species *Notothenia rossii* has been reported to synthesize heat shock proteins in response to thermal stress at temperatures as low as 4 to 5 °C (Maresca *et al.*, 1988). Myosin from coldwater fish has an unstable tertiary structure and it readily aggregates and loses its ATPase activity on purification (Connell, 1960; Johnston *et al.*, 1975a). Johnston and Walesby (1977) showed a strong correlation between the half-life of thermal de-activation of white muscle myofibrillar ATPase and habitat temperature. Under identical conditions of protein concentration, ionic strength and pH, the half-time for thermal deactivation at 37 °C increased from little over a minute in several Antarctic fish to over 500 minutes in the hot-spring fish, *Oreochromis alcalicus grahami* from Lake Magadi, Kenya.

Studies with live and skinned (demembrated) muscle fibres isolated from fast muscle have shown that maximum isometric tensile stress is a function of both experimental and adaptation temperature (Johnston & Brill, 1984; Johnston & Altringham, 1985; Johnson & Johnston, 1991a). Skinned fibres from Antarctic fish produce 5–10 times more tension at 0 °C than fibres from tropical species (Fig. 2a). However, when measured at the normal body temperature of each species maximum tensions are comparable, and may even be somewhat higher for coldwater species (Fig. 2a). At temperatures above 5–10 °C skinned muscle fibres from the Antarctic icefish (*Chaenocephalus aceratus*) failed to relax completely following maximal activations, generating Ca²⁺-insensitive residual tension (Fig. 3).

The thermal range over which live fibres produce maximum tension is also highly correlated with habitat temperature, but differs from the pattern observed with skinned muscle fibres (Fig. 2b). At temperatures beyond the upper and lower thermal limits for each species live fibre preparations become progressively inexcitable, leading to a reduction in maximum tension. In contrast, maximum tension either rises or stays

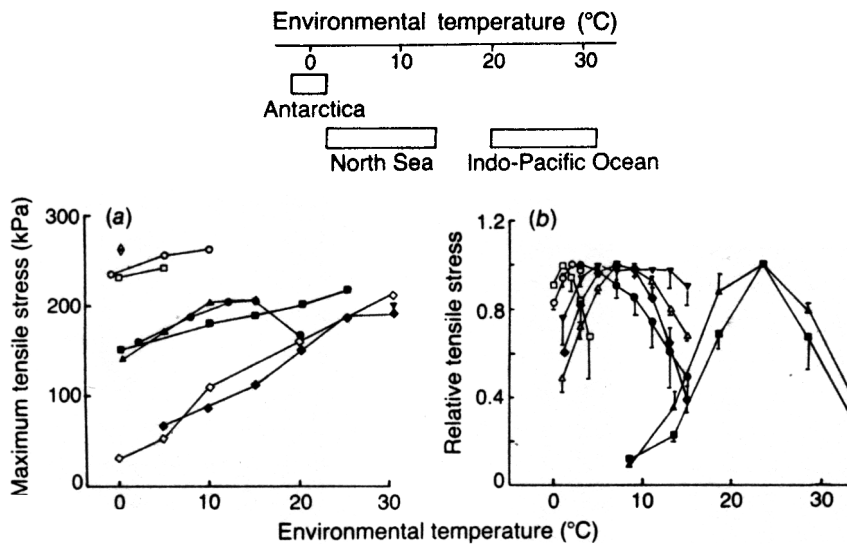


Fig. 2. Effects of temperature on the maximum tensile stress generated by (a) skinned and (b) live fibres isolated from the fast myotomal (myo) and pectoral fin adductor (m.add.p.) of teleost fish. Symbols refer to the following: (a) Antarctic fish: Δ , *Trematomus hansonii* (myo); ∇ , *Notothenia rossii* (myo); \circ , *Chaenocephalus aceratus* (myo); \square , *Notothenia coriiceps* (myo). North Sea fish: \bullet , *Gadus morhua* (myo); \blacktriangle , *Myoxocephalus scorpius* (myo); \blacksquare , *Platichthys flesus* (myo). Tropical fish: \diamond , *Makaira nigricans* (myo); \blacklozenge , *Carangus melampygus* (myo); \blacktriangledown , *Eurythunnus affinis* (myo). (b) Antarctic fish: \circ , *Trematomus lepidorhinus* (m.add.p.); \square , *Notothenia coriiceps* (m.add.p.). North Sea fish: Δ , *Pollachius virens* (myo); \bullet , *Limanda limanda* (myo); \blacklozenge , *Callionymus lyra* (myo); \blacktriangledown , *Aggonis cataphractus* (m.add.p.). Tropical fish: \blacktriangle , *Abudefduf abdominalis* (m.add.p.); \blacksquare , *Thalassoma duperreyi* (myo). Values represent mean \pm SE. See Johnston & Altringham (1985) and Johnson & Johnston (1991a) for original data and numbers of fish examined.

relatively constant as temperature is increased in skinned muscle fibres (Fig. 2a). The mechanism underlying the loss of excitability of live fibres with thermal stress is likely to be complex, involving a failure of activation processes and/or excitation-contraction coupling (Johnson & Johnston, 1991a). It is also important to bear in mind that in the intact animal the muscle fibres are activated by the motor neurons and the relationship between maximum tensile stress and temperature *in vivo* may well differ from that observed with isolated live fibres.

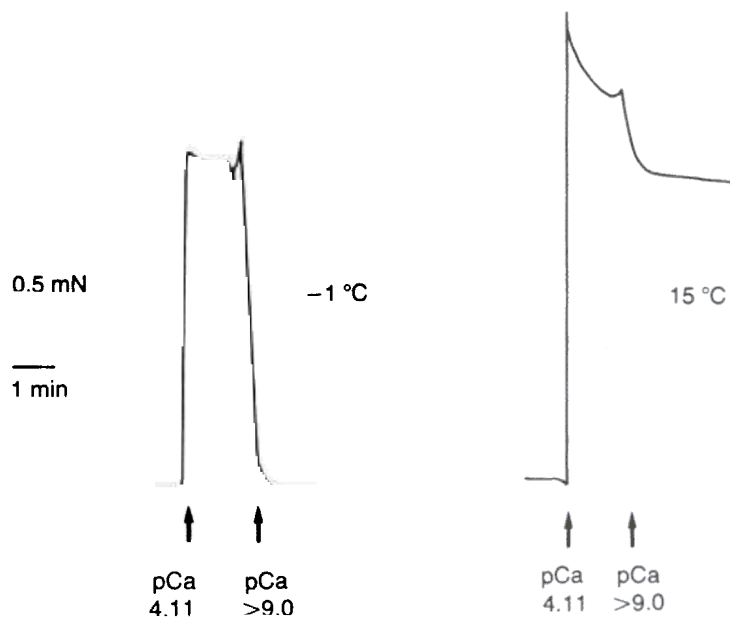


Fig. 3. Skinned fast muscle fibres from the Antarctic icefish (*Chaenocephalus aceratus*) fail to relax completely following activations at high temperatures. The figure shows the tension record from a fibre successively transferred between relaxing ($pCa^{2+} > 9.0$), activating ($pCa^{2+} 4.11$), and relaxing solutions. (Original data from Johnston, 1985.)

Time-dependent muscle contractile properties are highly temperature-dependent and show much less evidence of evolutionary adjustments to different thermal habitats than does maximum tensile stress (Johnston & Altringham, 1985; Johnson & Johnston, 1991a). For example, the unloaded contraction speed of muscle fibres has a Q_{10} of 1.5 to 2.0, and is relatively independent of the temperature at which the fish is living (Fig. 4). Although the rate of relaxation of isometric twitches is greatly prolonged at low temperatures in muscle fibres from tropical species it does show some limited evidence for temperature compensation (Johnson & Johnston, 1991a). This reflects higher rates of calcium pumping by the sarcoplasmic reticulum at low temperatures in coldwater relative to warmwater fish (McArdle & Johnston, 1980).

The power output of muscle fibres during shortening under constant load can be determined from the force-velocity relationship. The best fit of experimental data is usually obtained if the points are not con-

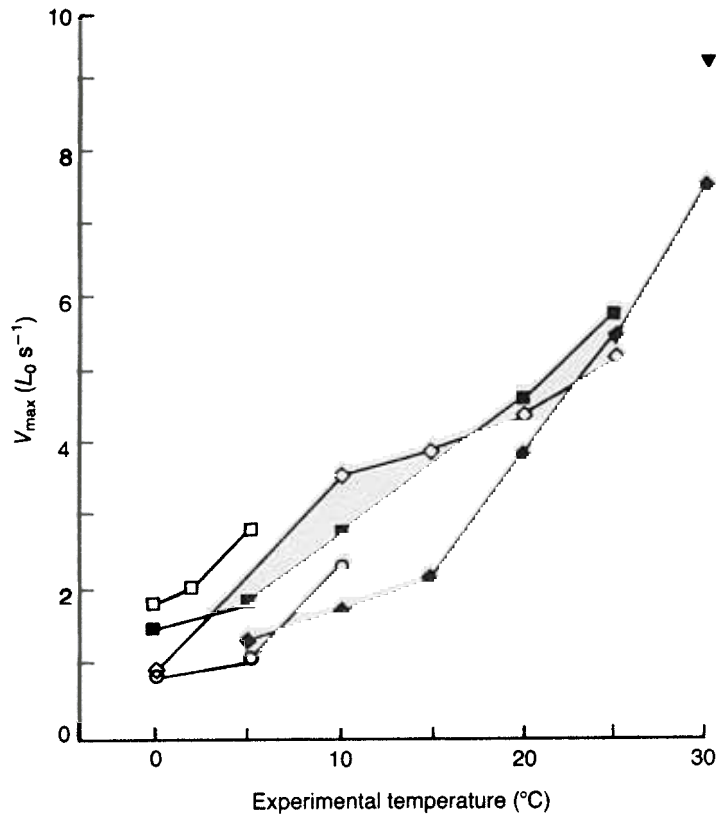


Fig. 4. Effects of temperature on the unloaded contraction velocity (V_{max}) of skinned fast myotomal muscle fibres of teleosts fish. Antarctic: ○, *Chaenocephalus aceratus*; □, *Notothenia corriiceps*. North Sea: ■, *Platichthys flesus*. Tropical: ◇, *Makaira nigricans*; ◆, *Carangus melampygus*; ▼, *Eurythunnus affinis*. (Data from Johnston, 1990.)

strained to pass through the maximum tensile stress (P_0) (Altringham & Johnston, 1988b; Rome & Sosnicki, 1990). Rome and Sosnicki (1990) calculated that the power output ($W kg^{-1}$ wet muscle mass) of red fibres from carp was 60 at $10^{\circ}C$, rising to 94 at $20^{\circ}C$ ($Q_{10} = 1.6$). The power output ($W kg^{-1}$ wet muscle mass) of fast muscle fibres in the short-horned sculpin (*Myoxocephalus scorpius*) increased from 140 at $1^{\circ}C$ to 313 at $8^{\circ}C$, but was similar (292) at $12^{\circ}C$ (Langfeld, Attringham & Johnston, 1989). The plateau in power output towards

the upper temperature limit of this species largely reflected a decrease in the tensile stress generated. P_o reached its maximum value of 315 kPa at 8 °C, declining to only 172 kPa at 16 °C (Langfeld *et al.*, 1989). In both the above studies the force-velocity relationship was found to become progressively more curved at higher temperatures (Langfeld *et al.*, 1989; Rome & Sosnicki, 1990). A more curved force-velocity relationship yields a lower velocity and thus a reduced power output for a given load. For fast muscles from the short-horned sculpin, Hill's constant a/P_o , which is inversely related to the curvature, was 0.27 at 1 °C, 0.24 at 8 °C and 0.17 at 12 °C. Langfeld *et al.* (1989) quantified the effect by normalizing the curves for P_o and V_{max} at each temperature and found that the change in curvature was sufficient to decrease the relative power output by 15% on increasing the temperature from 1 to 8 °C.

In vivo studies suggest that animals use their muscle fibres over a relatively narrow range of values of V/V_{max} (0.17–0.36) where power is maximum (Rome *et al.*, 1988), and efficiency is within 90% of the optimal value (Curtin & Woledge, 1991). Thus as the V/V_{max} of red fibres exceeds around 0.36 then faster contracting fibre types are recruited. For interspecific comparisons the relationship between swimming speed and muscle contraction speed (V) is complicated. For example, scup use a less undulatory style of swimming and have a lower gearing ratio than carp and can consequently swim at higher speeds for the same velocity of muscle shortening (Rome *et al.*, 1992). It should be noted that whereas V_{max} is determined by myosin heavy and light chain composition (Greaser, Moss & Reiser, 1988), V is a function of the rate of activation and relaxation of muscle fibres, tail beat amplitude and the orientation (and hence gearing) of the muscle fibres.

Muscle function during swimming

Isometric contractions cannot be directly related to swimming since both the development of tensile stress and relaxation are speeded up in cyclical contractions (Altringham & Johnston, 1990; Rome & Swank, 1992). Similarly, *in vivo*, muscle fibres seldom shorten under constant load and cross-bridge kinetics are sensitive to the impact of previous contraction cycles, particularly the effects of prestretch (Johnston, 1991; Van Leeuwen *et al.*, 1990; Van Leeuwen, 1995). During steady swimming the myotomal muscle fibres undergo repeated cycles of shortening and lengthening. Under these circumstances the length changes of the myotomal muscles are approximately sinusoidal (Hess & Videler, 1984).

A number of studies have imposed sinusoidal length changes about the *in situ* resting fibre length and stimulated the preparation physically during each cycle (Altringham & Johnston, 1990; Anderson & Johnston, 1992; Rome & Swank, 1992; Altringham, Wardle & Smith, 1993). The work done by the muscle can be calculated from plots of tensile stress and muscle fibre length (work loops). Power output is calculated from the work per cycle multiplied by the cycle frequency. The work output of a muscle is critically dependent on such factors as its starting length, the number and timing of stimuli, the amount of shortening and the contraction frequency (Johnston, 1991; Van Leeuwen, 1995). In some species, such as the short-horned sculpin, muscle fibres in rostral and caudal myotomes have identical mechanical properties and have similar power outputs under *in vivo* conditions (Johnston *et al.*, 1993; Johnston, Van Leeuwen & Beddow, 1995). However, in saithe (Altringham *et al.*, 1993) and Atlantic cod, *Gadus morhua* (Davies, Johnston & Van der Wal, 1995), muscle fibres in caudal myotomes have longer twitch contraction times and probably function somewhat differently during swimming compared with fibres in rostral myotomes.

The various parameters influencing work can be systematically optimized to determine the maximum power output, taking advantage of any available information on cycle frequencies and the stimulation duty cycle during swimming (Rome & Swank, 1992; Altringham *et al.*, 1993; Johnston *et al.*, 1993). Rome and Swank (1992) used the work loop technique to measure the power output of red muscle fibres from the scup at 10 and 20 °C. They selected values of stimulation duty cycle (0.35–0.5) on the basis of EMG recordings made during steady swimming. The optimal strain for maximum work was 0.05–0.06 of resting fibre length. Net positive work per cycle decreased with increasing oscillation frequency and was significantly higher at 20 °C than at 10 °C, except at 1 Hz. Net power output increased to a maximum value and then decreased as the oscillation frequency increased. The maximum power was obtained at 2.5 Hz at 10 °C (12.8 W kg⁻¹ wet muscle mass) and 5 Hz at 20 °C (27.9 W kg⁻¹ wet muscle mass). The maximum speed that scup can swim using their red muscle fibres alone was found to increase from 60 cm s⁻¹ at 10 °C to 84 cm s⁻¹ at 20 °C (for fish 17–20 cm in length) (Rome *et al.*, 1992). Assuming the power required to swim increased with the 2.5 exponent of swimming speed (Webb, 1975), this would require around a 2.7-fold increase in mechanical power. Rome and Swank (1992) showed that the Q₁₀ for muscle power production was a function of oscillation frequency. Comparing muscle power at the highest frequencies observed during swimming, of 4.5 Hz at 10 °C (9 W kg⁻¹ wet muscle mass) and 7.5 Hz at 20 °C (27 W kg⁻¹

wet muscle mass), gave a Q_{10} of around 3, which was in reasonable agreement with the predicted power requirements. Scup use the same tailbeat frequency at any given speed regardless of temperature (Rome *et al.*, 1992). Therefore, in order to swim at their maximum sustainable speed at 20 °C the fish would need to recruit only one-third as much red muscle as at 10 °C, which is the main reason why U_{crit} increases at high temperatures (Rome & Swank, 1992).

Phenotypic plasticity to temperature change

Sustained swimming

Following a period of warm-acclimation lasting several weeks, the maximum sustainable swimming speed of carp (Fig. 5) and goldfish

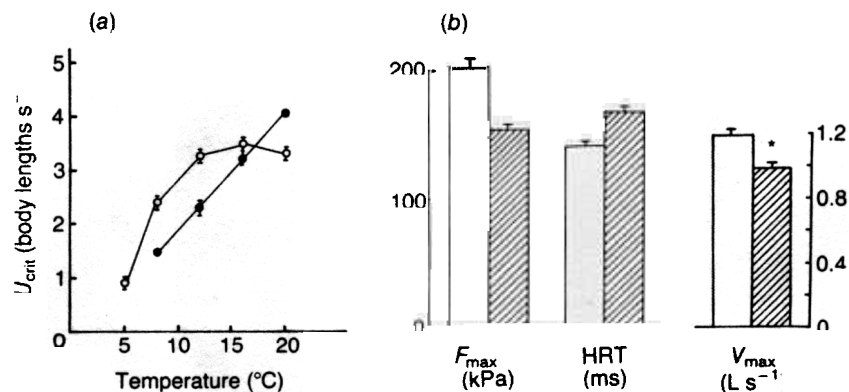


Fig. 5. (a) Effects of temperature on the maximum sustainable swimming speed (U_{crit}) over a 60 min period for common carp (*Cyprinus carpio*) acclimated to either 8 °C (open symbols) or 20 °C (closed symbols) for a minimum of 2 months. Error bars represent mean \pm SE, $n = 88$ fish at each temperature. Note that the swimming performance of the cold-acclimated fish begins to decline above 15 °C. (Original data from Johnston, 1993.)

(b) Effects of temperature acclimation on the contractile properties of live slow muscle fibre bundles isolated from the pectoral fin muscles of common carp acclimated to either 8 °C (open bars) or 20 °C (cross-hatched bars) for a minimum of 2 months. The measurements were made at an experimental temperature of 8 °C. F_{max} , maximum tetanic tension (kPa); HRT, time (ms) from last stimuli to 50% maximum tension; V_{max} , unloaded shortening velocity in fibre lengths s⁻¹. Error bars represent mean \pm SE, $n = 6$ fish at each temperature. (Original data from Langfeld *et al.*, 1991.)

(Fry & Hart, 1948) decreased at low temperatures and increased at high temperatures, although the tailbeat frequency and amplitude required to swim at any given speed remains the same (Johnston, 1993). In many species the relative proportion of red muscle fibres in the myotome varies with acclimation temperature (Johnston & Lucking, 1978; Sidell, 1980). As acclimation temperature increases, the amount of red muscle decreases, presumably since a smaller volume of red muscle is required to swim at a given speed because of its higher power output.

The contractile properties of slow muscle in common carp vary with acclimation temperature (Johnston, Sidell & Driedzic, 1985). Maximum tensile stress and contraction velocity at 7 °C were found to be 1.5 times higher for skinned fibres from carp acclimated to 7 °C than to 23 °C. Fibres from 7 °C-acclimated carp failed to relax completely following maximal activations at 23 °C. The resulting Ca²⁺-insensitive force component (50–70% Po) built up with successive activations and resulted in very low contraction velocities, presumably as a result of the formation of abnormal cross-bridge linkages. In contrast, red fibres isolated from 23 °C-acclimated fish relaxed completely following up to 5 maximal activations each of up to 3 min duration (Johnston *et al.*, 1985). The maximum power output was more than 3-fold higher at 23 °C in 23 °C-acclimated than in 7 °C-acclimated fish. However, following acclimation to 7 °C maximum power output increased to values which were similar to that for warm-acclimated fish at 23 °C, indicating perfect thermal compensation.

Similar changes, but of a more modest nature, have been found for live slow muscle fibre bundles isolated from carp pectoral fin *abductor superficialis* muscle (Fig. 5b). Following acclimation from 8 to 20 °C, the maximum isometric tensile stress (F_{max}) at 8 °C decreased from 202 kPa to 153 kPa, but was unchanged at 20 °C. Twitch half-relaxation time was significantly longer at all temperatures in 20 °C-acclimated than in 8 °C-acclimated fish. Langfeld *et al.* (1989) studied the force-relationship of slow fibres isolated from the pectoral fin abductor muscle at 8 °C. Maximum shortening speed was 17% higher in 8 °C-acclimated than in 20 °C-acclimated fish at 8 °C (Fig. 5b), whereas the curvature of the P-V relationship was independent of acclimation temperature (Langfeld, Crockford & Johnston, 1991). The net result was that slow fibres produced 47% more power at 8 °C following cold-acclimation, largely as a result of increased force production. Since myofibrillar volume density is relatively independent of acclimation, these changes in force production are unlikely to reflect differences in the number of myosin cross-bridges per unit fibre cross-sectional area (Johnston & Maitland, 1980).

The myosin molecule is made up of heavy and light chain sub-units which are present as different isoforms in fast and slow muscle fibres. The globular head of myosin contains one alkali light chain (LC1_f and LC3_f in fast muscles and LC1_s in slow muscles) and one regulatory light chain (LC2_f and LC2_s in fast and slow muscles, respectively). Fibre bundles from 8 °C-acclimated carp were found to contain a higher content of myosin light chain isoforms normally associated with faster contracting muscle fibre types (Langfeld *et al.*, 1991). Since the small (3%) percentage of fast fibres in the preparations did not vary with acclimation temperature, it would appear that LC1_f and LC2_f are co-expressed with LC1_s and LC2_s in the slow fibres of 8 °C-acclimated fish, contributing to the observed increase in shortening speed at low temperatures.

Maximum swimming speeds

Johnston, Davison & Goldspink (1975b) discovered that in goldfish the Mg²⁺/Ca²⁺-activated ATPase activity of white muscle myofibrils increased at low temperatures following several weeks cold-acclimation. The ATPase activity in warm-acclimated fish was also less sensitive to thermal denaturation than in cold-acclimated goldfish. Similar changes in ATPase activity with thermal acclimation have been reported for other fish including common carp (Crockford & Johnston, 1990; Hwang, Watabe & Hashimoto, 1990), roach (*Rutilus rutilus*) and tench (*Tinca tinca*) (Heap, Watt & Goldspink, 1985). Changes in myofibrillar ATPase activity are reversible, take around 4 weeks to complete, and are inhibited in starved fish in which protein synthesis has been reduced to a very low level (Heap, Watt & Goldspink, 1986).

Fleming *et al.* (1990) used a nerve-muscle preparation to investigate twitch contraction kinetics following thermal acclimation. At 8 °C, the half-times for both twitch activation and relaxation were 2 to 3 times faster in preparations from 8 °C-acclimated than 20 °C-acclimated fish. The half-time for relaxation at 20 °C was also shorter for warm- than cold-acclimated fish, indicating plasticity of deactivation rates at both low and high temperatures (Fleming *et al.*, 1990). The pCa²⁺-tensile stress relationship (Johnston, Fleming & Crockford, 1990), parvalbumin content and the surface and volume density of the sarcoplasmic reticulum (SR) (Fleming *et al.*, 1990) were not altered by temperature acclimation in the common carp. The faster relaxation of twitch tensile stress in muscle fibres from cold-acclimated carp has been correlated with an increase in SR Ca²⁺-ATPase activity (Fleming *et al.*, 1990; Ushio & Watabe, 1993). Studies with skinned fibres have shown that

both P_o and V_{max} in white muscle fibres of carp are altered by thermal acclimation (Johnston *et al.*, 1985). ATPase activity (Penney & Goldspink, 1979), maximum tension and contraction velocity (Crockford & Johnston, 1990) do not vary continuously with acclimation temperature but reach upper and lower limits. For example, P_o for fast myotomal fibres at 0 °C is similar in carp acclimated to 2–11 °C, but declines progressively at higher temperatures (Crockford & Johnston, 1990).

The maximum contraction velocity of isolated single fibres has been correlated with the expression of specific myosin heavy chain isoforms (Lannergren, 1987; Bottinelli *et al.* 1994a) and alkali light chain isoforms (Bottinelli *et al.* 1994b). The molecular mechanisms underlying changes in P_o and V_{max} with thermal acclimation in carp include altered expression of myosin heavy chain genes (Gerlach *et al.*, 1990; Hwang *et al.*, 1991) and myosin light chain isoforms (Crockford & Johnston, 1990). Gerlach *et al.* (1990) reported that carp had at least 28 different myosin heavy chain (MHC) genes and found evidence for altered expression with temperature acclimation. The expression of different MHC isoforms with temperature acclimation is supported by peptide mapping studies (Hwang *et al.*, 1991).

Few studies have examined thermal acclimation in marine species that are subject to more limited seasonal temperature changes. The most studied species is the short-horned sculpin which is an essentially coldwater species with a distribution in the eastern Atlantic from the Arctic to the northwest coast of France. Beddow, Van Leeuwen and Johnston (1995) found that the kinematic parameters of fast-starts elicited by predation are modified at high temperatures in summer-acclimated fish (Fig. 6). Fast-starts are used in prey capture and are highly variable depending on the position of the fish at the start of the attack and distance to the prey. The sculpin creep towards their prey using a combination of their pectoral and pelvic fins. For 20 cm fish the mean strike distance was 10 cm for 15 °C-acclimated fish and 5 cm for 5 °C-acclimated fish. Initially the body is bent into an S-shape, the pectoral fins rapidly adducted and the dorsal and ventral fins partially erected. This is followed by a complete tailbeat and an unpowered glide of variable duration. Towards the end of the powered phase of the fast-start the jaws are expanded and protruded, and the fish attempts to suck in the prey, greatly increasing the velocity of the attack. The average velocities reached during the complete tail beat were found to be relatively independent of temperature (Q_{10} 1.2–1.3) (Fig. 6). For 15 °C-acclimated fish average values for maximum acceleration were 16.2 m s⁻² at 5 °C and 18.0 m s⁻² at 15 °C. Following 6 to 8 weeks acclimation to 15 °C, the maximum velocity of the fast-start

at 15 °C was 33% faster than in 5 °C-acclimated fish acutely exposed to the same temperature (Fig. 6). This increase in performance with warm-acclimation was sufficient to increase the percentage of successful attacks during prey capture from 23.2 to 73.4% (Beddow *et al.*, 1995).

Fast muscle fibres isolated from fish acclimated to 5 °C generated maximum tetanic tensions of 139 kPa at 5 °C, but force generating capabilities declined above 10 °C and P_o at 15 °C was only 78 kPa (Fig. 7a). In contrast, P_o in fibres from 15 °C-acclimated fish increased from 125 kPa at 5 °C to 282 kPa at 15 °C (Fig. 7a). The low tensile stresses generated by fibres from winter-acclimated fish at summer temperatures probably reflect a partial failure of excitation–contraction coupling, since peak force at 15 °C was increased 2.2 times following depolarization with a high potassium solution (Beddow & Johnston, 1995).

Ball and Johnston (1996) have also shown that the force–temperature characteristics of skinned muscle fibres are relatively independent of acclimation temperature (Fig. 7b). The maximum contraction speeds of fast muscle fibres in the short-horned sculpin are also modified at high (Fig. 8) and low temperatures following thermal acclimation (Beddow & Johnston, 1995). Only relatively minor differences in contractile properties were evident between freshly caught sculpins and fish acclimated to

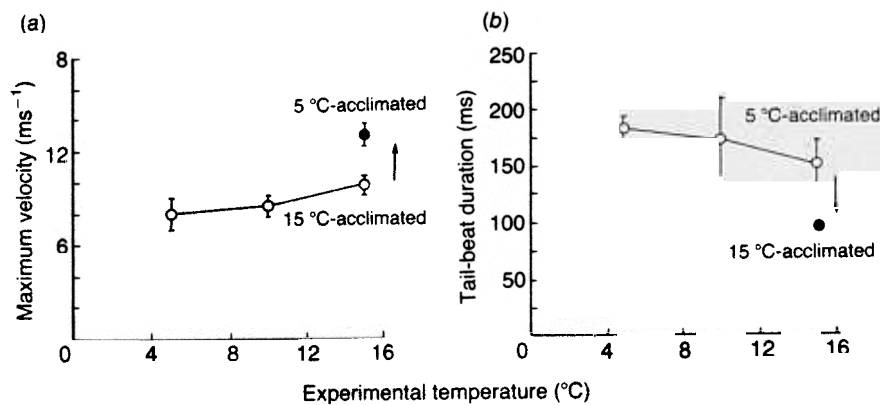


Fig. 6. Influence of temperature and thermal acclimation on (a) maximum velocity and (b) tailbeat duration in predation fast-starts in the short-horned sculpin (*Myoxocephalus scorpius*). Fish were acclimated to either 5 °C (○) or 15 °C (●) for a minimum of 2 months. Values represent mean \pm SE, $n = 6-8$ fish at each temperature. (From Beddow *et al.*, 1995.)

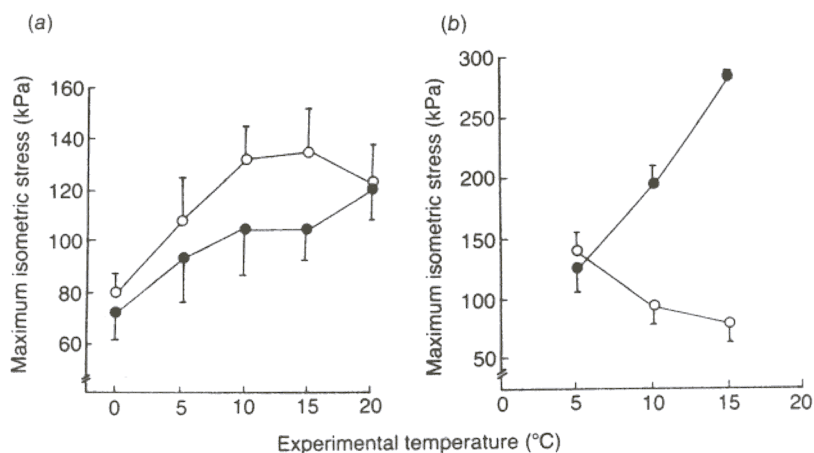


Fig. 7. Maximum isometric tensile stress generated by (a) skinned and (b) live fibres isolated from the fast myotomal muscle of short-horned sculpin acclimated to either 5 °C (○) or 15 °C (●) for a minimum of 2 months. Values represent mean \pm SE, $n = 7-11$ fish at each temperature. (From Ball & Johnston, 1996 and Beddow & Johnston, 1995, respectively).

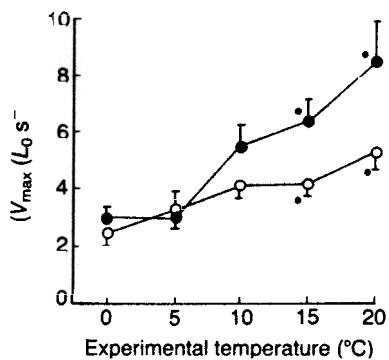


Fig. 8. Influence of temperature and thermal acclimation on the maximum contraction velocity of skinned fibres isolated from the fast myotomal muscle of the short-horned sculpin. Fish were acclimated to either 5 °C (○) or 15 °C (●) for a minimum of 2 months. Values represent mean \pm SE, $n = 7-11$ fish at each temperature. (From Ball & Johnston, 1996.)

the same temperatures under a constant photoperiodic regime (12 h dark: 12 h light), indicating that temperature was the most important environmental variable influencing the results. Johnson and Johnston (1991b) investigated the effects of temperature and thermal acclimation on the ability of fast muscle fibres from the short-horned sculpin to do oscillatory work. Under optimal conditions of stimulation and strain the cycle frequency required for maximum work per cycle increased from 4 Hz at 5 °C to 9 Hz at 15 °C (Fig. 9). At 15 °C, the peak force generated per cycle and the average power output were significantly higher in summer- than winter-acclimated fish (Fig. 9).

For unsteady swimming muscle, length changes deviate significantly from sine waves and vary from tailbeat to tailbeat (Van Leeuwen 1995; Johnston *et al.*, 1995). Johnston *et al.* (1995) calculated the changes in strain of the fast muscle in short-horned sculpin during the first tailbeat of fast-starts associated with prey capture. Isolated fibres were subjected to the strains calculated for the first tailbeat of the fast-start (abstracted cycle) and stimulated at a range of phases with the *in vivo* duty cycle determined from EMG recordings. For 5 °C-acclimated fish, the average power per cycle (W kg^{-1} wet muscle mass) was 21.7 at 5 °C, falling to 6.9 at 15 °C. Following acclimation to 15 °C, average power output per cycle increased to 22.8 at 15 °C, indicating near perfect thermal compensation of muscle performance with acclimation (Fig. 10).

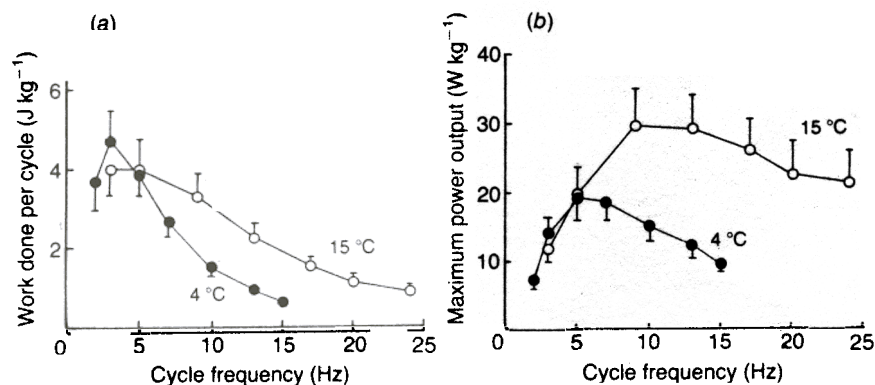


Fig. 9. Influence of thermal acclimation on (a) work and (b) power output during cyclical contractions of fast muscle fibres in the short-horned sculpin. Fish were acclimated to either 4 °C (●) or 15 °C (○) for a minimum of 2 months. (See Johnson & Johnston, 1991b for original data and further details.)

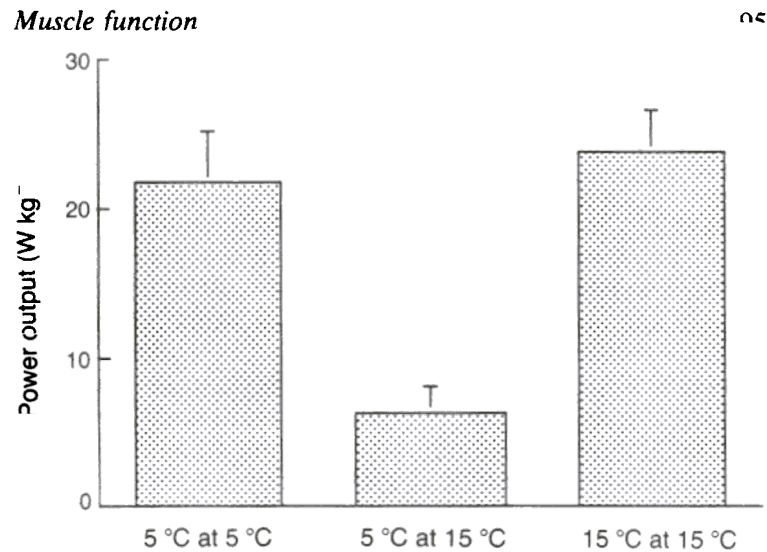


Fig. 10. The effects of temperature and thermal acclimation on the average power output of fast muscle fibres from short-horned sculpin measured under conditions simulating the first tailbeat of a fast-start. Values represent mean \pm SE, $n = 5$ fish at each temperature. (From Johnston *et al.*, 1995.)

Recently, we have investigated the molecular mechanisms underlying changes in V_{\max} with temperature acclimation in the short-horned sculpin (Ball & Johnston, 1996). Peptide mapping of myosin heavy chains with four different enzymes revealed no differences between 5 °C-acclimated and 15 °C-acclimated fish. No myofibrillar protein isoforms unique to either acclimation temperature were detected using 2-dimensional polyacrylamide gel electrophoresis. However, using capillary electrophoresis the ratio of myosin light chains LC3_f to LC1_f was significantly higher ($1:1.66 \pm 0.60$) in muscle from 5 °C-acclimated than from 15 °C-acclimated ($1:0.73 \pm 0.20$) fish (mean \pm SD, 6 fish per acclimation temperature). The results are consistent with the change in V_{\max} with temperature acclimation being due to the altered expression of myosin light chains independently of myosin heavy chain composition. In support of this hypothesis myofibrillar ATPase activity of fast muscle was found to be independent of acclimation temperature (Ball & Johnston, 1996). It is known that myosin heavy chain composition is a major determinant of ATPase activity (Bottinelli *et al.* 1994b), whereas removal of the light chains from myosin decreases contraction velocity without affecting ATPase activity (Lowey, Waller & Trybus, 1993). Thus the molecular mechanisms underlying the plasticity of muscle contractile properties

with temperature acclimation are different in the two species (common carp and short-horned sculpin) that have been examined so far.

Implications for global warming and suggestions for future research

The temperatures of the world's oceans and freshwater bodies have changed constantly since their formation. For example, prior to the breakup of the super-continent Gondwanaland around 35 million years ago, the Southern Ocean had a relatively temperate climate (12–15 °C). The early Cenozoic fish fauna contained representatives of cosmopolitan groups found in temperate seas today, e.g. gadoids, salmonids, etc. Following the establishment of present-day oceanic circulation patterns 20 million years ago there has been a gradual cooling to around –1.86 °C at high latitudes. Although the cooling of the Southern Ocean took place over many millions of years there is recent evidence for periods of particularly sharp cooling and intermittent periods of warming (see Fig. 1 in Clarke & Johnston, 1996). The present-day fauna is highly endemic and lacks such groups as gadoids, clupeoids and salmonids, consistent with the extinction of some fishes. It is dominated by a single suborder of bottom-living perciformes, the Notothenioides, which lack swimbladders. Notothenioid fish have undergone a striking adaptive radiation to fill a range of ecological niches since the early Tertiary. This radiation was driven by climate change, the intermittent availability of new habitats during interglacial periods, and perhaps the lack of competition from other groups due to extinctions (see Clarke & Johnston, 1996, for a review). Thus climate change has historically been a major driver of the evolution of fishes. However, even the most rapid periods of cooling/warming in the past are 10 or 100 times slower than predicted by global warming models for the next 100 years. The rate of evolutionary change that is possible is clearly a function of generation time. The time to sexual maturity in fish ranges from a few weeks in the killifish found in seasonal ponds to 5–8 years in many coldwater marine fish. For a rate of temperature change of 2 °C per 100 years the scope for adaptations in the genotype may be limited for the majority of species. Thus, in considering the worst case scenarios for global warming it is the phenotypic responses to temperature change that species are able to make which will be most critical for survival.

With some important exceptions, such as species living in the deep sea or in polar regions, fish often encounter daily changes in temperature which are far in excess of those expected with global warming. For example, many mesopelagic fishes undergo extensive diurnal vertical

migrations from cold deep water during the day to the warmer surface layers at night. It is also common for fish living at the margins of the ocean to undergo extensive heating or cooling with each tidal cycle. An extreme example is provided by the common killifish (*Fundulus heteroclitus*) which inhabits saltmarshes along the Atlantic coast of North America. On warm sunny days in early summer the temperature of isolated pools can rise from 12 °C at high tide to around 30 °C at low tide, without adversely affecting the swimming abilities of the killifish (Sidell *et al.*, 1983). Freshwater fish living in relatively small bodies of water also frequently experience marked diurnal and seasonal changes in temperature, often in excess of 20 °C. Although some species become torpid at low temperature others can remain active over a wide range of temperatures, indicating considerable phenotypic plasticity (Lemons & Crawshaw, 1985). For eurythermal species living in the middle of their thermal tolerance range the relatively small temperature changes expected with global warming are therefore unlikely to have a major impact on swimming abilities.

Many traits contribute to the thermal tolerance of a species. Heat stress in swimming muscles is unlikely to be a common immediate cause of death, since there are other tissues such as the gill epithelium and respiratory neurones which are probably more sensitive and likely to fail (Cossins & Bowler, 1987). However, any impairment of swimming performance may influence fitness through a decreased ability to forage for food or an increased likelihood of predation. Thus thermal effects on muscle function may in the long-term influence the success of a particular species. We know that species differ markedly both in the extent of their phenotypic responses to temperature change and in the cellular and molecular mechanisms underlying the plasticity of particular traits. In general, species from stenothermal environments show less plasticity in muscular performance with temperature change than do eurythermal fish. Species living towards the upper limit of their zone of thermal tolerance are therefore likely to be more vulnerable to a given rise in temperature than species living in the middle of their thermal range. Thus a 2 °C rise in temperature is likely to have a proportionally greater effect on swimming performance in *P. borchgrevinski* living at -1.86 to 0 °C, than in a temperate species such as the largemouth bass which routinely experiences 4 to 30 °C (Fig. 1).

More research is required specifically directed at the impact of thermal stress on muscle function, particularly in stenothermal species. The starting point for future work should be the responses to temperature at the organismal level, in terms of both sustained and maximum swimming performance. Studies on isolated muscle fibres are valuable

but where possible measurements of contractile performance should be carried out under the constraints operating during swimming. The results from organismal and physiological experiments should be used as the appropriate starting point to select the cellular and molecular techniques needed to elucidate underlying mechanisms.

Finally, the great majority of studies in this field have concentrated on adult stages because of their ease of study. However, we should remember that natural selection operates on all stages of the life/cycle and that temperature tolerance generally rises during ontogeny (see Johnston, Vieira & Hill, 1996; Rombough, this volume). The key to understanding the responses of fish to elevated temperatures associated with global warming may well therefore lie with studies of the early life stages.

Acknowledgements

The authors are grateful to the Natural Environment Research Council of the UK for financial support.

References

- Akster, H. A., Granzier, H. L. M. & ter Keurs, H. E. D. J. (1985). A comparison of quantitative ultrastructural and contractile characteristics of muscle fibre types in the perch. *Perca fluviatilis* L. *Journal of Comparative Physiology B*, **155**, 685–91.
- Alexander, R. McN. (1969). The orientation of muscle fibres in the myomeres of fishes. *Journal of the Marine Biological Association of the United Kingdom*, **49**, 263–90.
- Altringham, J. D. & Johnston, I. A. (1988a). Activation of multiply innervated fast and slow myotomal muscle fibres of the teleost *Myoxocephalus scorpius*. *Journal of Experimental Biology*, **140**, 313–324.
- Altringham, J. D. & Johnston, I. A. (1988b). The mechanical properties of polyneuronally innervated, myotomal muscle fibres isolated from a teleost fish *Myoxocephalus scorpius*. *Pflügers Archives*, **412**, 524–9.
- Altringham, J. D. & Johnston, I. A. (1990). Modelling muscle power output in a swimming fish. *Journal of Experimental Biology*, **148**, 395–402.
- Altringham, J. D., Wardle, C. S. & Smith, C. I. (1993). Myotomal muscle functions at different locations in the body of a swimming fish. *Journal of Experimental Biology*, **182**, 191–206.
- Anderson, M. E. & Johnston, I. A. (1992). Scaling of power output in fast muscle fibres of the Atlantic cod during cyclical contractions. *Journal of Experimental Biology*, **170**, 143–54.

- Ball, D. & Johnston, I. A. (1996). Molecular mechanisms underlying the plasticity of muscle contractile properties in fish following temperature acclimation. *Journal of Experimental Biology* **199**, 1363–73.
- Batty, R. S. & Blaxter, J. H. S. (1992). The effect of temperature on the burst swimming performance of fish larvae. *Journal of Experimental Biology*, **170**, 187–201.
- Beamish, F. W. H. (1970). Oxygen consumption of largemouth bass in relation to swimming speed and temperature. *Canadian Journal of Zoology*, **48**, 1221–8.
- Beamish, F. W. H. (1978). Swimming capacity. In *Fish Physiology*, ed. W. S. Hoar & D. J. Randall, pp. 101–87. New York: Academic Press.
- Beddow, T. A. & Johnston, I. A. (1995). Plasticity of muscle contractile properties following temperature acclimation in the marine fish *Myoxocephalus scorpius*. *Journal of Experimental Biology*, **198**, 193–201.
- Beddow, T. A., Van Leeuwen, J. L. & Johnston, I. A. (1995). Swimming kinematics of fast-starts are altered by temperature acclimation in the marine fish *Myoxocephalus scorpius*. *Journal of Experimental Biology*, **198**, 203–8.
- Bone, Q. (1966). On the function of myotomal muscle fibres in elasmobranch fish. *Journal of the Marine Biological Association of the United Kingdom*, **46**, 321–49.
- Bottinelli, R., Betto, R., Schiaffino, S. & Reffiani, C. (1994a). Maximum shortening velocity and coexistence of myosin heavy chain isoforms in single skinned fast fibres of rat skeletal muscle. *Journal of Muscle Research and Cell Motility*, **15**, 413–19.
- Bottinelli, R., Betto, R., Schiaffino, S. & Reffiani, C. (1994b). Unloaded shortening velocity and myosin heavy chain and alkali light chain isoform composition in rat skeletal muscle fibres. *Journal of Physiology (London)*, **478**, 341–9.
- Clarke, A. & Johnston, I. A. (1996). Evolution and adaptive radiation of Antarctic fish. *Trends in Ecology and Evolution*, **11**, 212–18.
- Cossins, A. R. & Bowler, K. (1987). *Temperature Biology of Animals*. London: Chapman & Hall.
- Connell, J. J. (1960). The relative stabilities of the skeletal muscle myosins of some animals. *Biochemical Journal*, **80**, 503–10.
- Crockford, T. & Johnston, I. A. (1990). Temperature acclimation and the expression of contractile protein isoforms in the common carp (*Cyprinus carpio* L.). *Journal of Comparative Physiology B*, **160**, 23–30.
- Crockford, T. & Johnston, I. A. (1993). Developmental changes in the composition of myofibrillar proteins in the swimming muscles of Atlantic herring, *Clupea harengus*. *Marine Biology*, **115**, 15–22.

- Curtin, N. A. & Woledge, R. C. (1991). Efficiency of energy conversion during shortening of muscle fibres of the dogfish, *Scyliorhinus canicula*. *Journal of Experimental Biology*, **158**, 343–53.
- Davies, M. L. F., Johnston, I. A. & Van der Wal, J.-W. (1995). Muscle fibres in rostral and caudal myotomes of the Atlantic cod (*Gadus morhua* L.) have different mechanical properties. *Physiological Zoology*, **68**, 673–97.
- Fleming, J. R., Crockford, T., Altringham, J. D. & Johnston, I. A. (1990). Effects of temperature acclimation on muscle relaxation in the carp: a mechanical, biochemical and ultrastructural study. *Journal of Experimental Zoology*, **255**, 286–95.
- Fry, F. E. J. & Hart, J. S. (1948). Cruising speed of goldfish in relation to water temperature. *Journal of the Fisheries Research Board of Canada*, **7**, 175–99.
- Gerday, C., Joris, B., Gerardin-Otthiers, N., Collin, S. & Hamoir, G. (1979). Parvalbumins from the lungfish, *Protopterus dolloi*. *Biochimie*, **61**, 589–99.
- Gerlach, G.-F., Turay, L., Malik, K. T. A., Lida, J., Scutt, A. & Goldspink, G. (1990). Mechanisms of temperature acclimation in the carp: a molecular biology approach. *American Journal of Physiology*, **259**, R237–44.
- Greaser, M. L., Moss, R. L. & Reiser, P. J. (1988). Variations in contractile properties of rabbit single muscle fibres in relation to troponin T isoforms and myosin light chains. *Journal of Physiology (London)*, **406**, 85–98.
- Heap, S. P., Watt, P. W. & Goldspink, G. (1985). Consequences of thermal change on the myofibrillar ATPase of five freshwater teleosts. *Journal of Fish Biology*, **26**, 733–8.
- Heap, S. P., Watt, P. W. & Goldspink, G. (1986). Myofibrillar ATPase activity in the carp (*Cyprinus carpio*): interactions between starvation and environmental temperature. *Journal of Experimental Biology*, **123**, 373–82.
- Hess, F. and Videler, J. J. (1984). Fast continuous swimming of saithe (*Pollechiu virens*): a dynamic analysis of bending moments and muscle power. *Journal of Experimental Biology*, **109**, 229–51.
- Hwang, G. C., Watabe, S. & Hashimoto, K. (1990). Changes in carp myosin ATPase induced by temperature acclimation. *Journal of Comparative Physiology B*, **160**, 233–9.
- Hwang, G.-C., Ochiai, Y., Watabe A. S. & Hashimoto, K. (1991). Changes of carp myosin subfragment-1 induced by temperature acclimation. *Journal of Comparative Physiology B*, **161**, 141–6.
- Johnson, T. P. & Johnston, I. A. (1991a). Temperature adaptation and the contractile properties of live muscle fibres from teleost fish. *Journal of Comparative Physiology B*, **161**, 27–36.
- Johnson, T. P. & Johnston, I. A. (1991b). Power output of fish muscle fibres performing oscillatory work: effects of acute and

- seasonal temperature change. *Journal of Experimental Biology*, **157**, 409–23.
- Johnston, I. A. (1981). Structure and function of fish muscles. *Symposium of the Zoological Society of London*, **48**, 71–113.
- Johnston, I. A. (1985). Effects of temperature on force–velocity relationship of skinned fibres isolated from Icefish (*Chaenocephalus aceratus*) skeletal muscle. *Journal of Physiology (London)*, **361**, 40P.
- Johnston, I. A. (1990). Cold-adaptation in marine organisms. *Philosophical Transactions of the Royal Society of London, Series B*, **326**, 655–67.
- Johnston, I. A. (1991). Muscle action during locomotion: a comparative perspective. *Journal of Experimental Biology*, **160**, 167–185.
- Johnston, I. A. (1993). Phenotypic plasticity of fish muscle to temperature change. In *Fish Ecophysiology*, ed. J. C. Rankin & F. B. Jensen, pp. 321–40. London: Chapman & Hall.
- Johnston, I. A. & Altringham, J. D. (1985). Evolutionary adaptation of muscle power output to environmental temperature: force–velocity characteristics of skinned muscle fibres isolated from Antarctic, temperate and tropical marine fish. *Pflügers Archives*, **405**, 136–40.
- Johnston, I. A. & Altringham, J. D. (1991). Movement in water: constraints and adaptations. In *Biochemistry and Molecular Biology of Fishes*, Vol. 1, ed. P. W. Hochachka & T. Mommsen, pp. 249–68. Amsterdam: Elsevier.
- Johnston, I. A. & Brill, I. A. (1984). Thermal dependence of contractile properties of single skinned muscle fibres isolated from Antarctic and various Pacific marine fishes. *Journal of Comparative Physiology B*, **155**, 63–70.
- Johnston, I. A., Davison, W. & Goldspink, G. (1975b). Adaptations in Mg^{2+} -activated myofibrillar ATPase activity induced by temperature acclimation. *Federation of European Biochemical Societies Letters*, **50**, 293–5.
- Johnston, I. A., Davison, W. & Goldspink, G. (1977). Energy metabolism of carp swimming muscles. *Journal of Comparative Physiology B*, **144**, 203–16.
- Johnston, I. A., Fleming, J. D. & Crockford, T. C. (1990). Thermal acclimation and muscle contractile properties in cyprinid fish. *American Journal of Physiology*, **259**, R231–6.
- Johnston, I. A., Franklin, C. E., & Johnson, T. P. (1993). Recruitment patterns and contractile properties of fast muscle fibres isolated from rostral and caudal myotomes of the short-horned sculpin. *Journal of Experimental Biology*, **185**, 251–65.
- Johnston, I. A. & Lucking, M. (1978). Temperature induced variation in the distribution of different muscle fibre types in the goldfish (*Carassius auratus*). *Journal of Comparative Physiology B*, **124**, 111–16.

- Johnston, I. A. & Maitland, B. (1980). Temperature acclimation in crucian carp: a morphometric study of muscle fibre ultrastructure. *Journal of Fish Biology*, **17**, 113–25.
- Johnston, I. A., Sidell, B. D. & Driedzic, W. R. (1985). Force-velocity characteristics and metabolism of carp muscle fibres following temperature acclimation. *Journal of Experimental Biology*, **119**, 239–49.
- Johnston, I. A., Van Leeuwen, J. & Beddow, T. (1995). How fish power predation fast-starts. *Journal of Experimental Biology*, **198**, 1851–61.
- Johnston, I. A., Vieira, V. L. A. & Hill, J. (1996). Temperature and ontogeny in ectotherms: muscle phenotype in fish. In *Phenotypic and Evolutionary Adaptations of Animals to Temperature*, ed. I. A. Johnston & A. F. Bennett. *Society for Experimental Biology Seminar Series*. pp. 153–81. Cambridge: Cambridge University Press.
- Johnston, I. A. & Walesby, N. J. (1977). Molecular mechanisms of temperature adaptation in fish myofibrillar adenosine triphosphatases. *Journal of Comparative Physiology B*, **119**, 195–206.
- Johnston, I. A., Walesby, N. J., Davison, W. & Goldspink, G. (1975a). Temperature adaptation in myosin of Antarctic fish. *Nature*, **254**, 74–75.
- Langfeld, K. S., Altringham, J. D. & Johnston, I. A. (1989). Temperature and the force-velocity relationship of live muscle fibres from the teleost *Myoxocephalus scorpius*. *Journal of Experimental Biology*, **144**, 437–48.
- Langfeld, K. S., Crockford, T. & Johnston, I. A. (1991). Temperature acclimation in the common carp: force-velocity characteristics and myosin sub-unit composition of slow muscle fibres. *Journal of Experimental Biology*, **155**, 291–304.
- Lannergren, J. (1987). Contractile properties and myosin isoenzymes in various kinds of *Xenopus* twitch muscle fibres. *Journal of Muscle Research and Cell Motility*, **8**, 260–73.
- Lemons, D. E. & Crawshaw, L. I. (1985). Behavioural and metabolic adjustments to low temperature in the largemouth bass (*Micropterus salmoides*). *Physiological Zoology*, **58**, 175–80.
- Lowey, S., Waller, G. S. & Trybus, K. M. (1993). Skeletal muscle myosin light chains are essential for physiological speeds of shortening. *Nature*, **365**, 454–6.
- McArdle, H. J. & Johnston, I. A. (1980). Evolutionary temperature adaptation in fish muscle sarcoplasmic reticulum. *Journal of Comparative Physiology B*, **135**, 157–64.
- Maresca, B., Patriarca, E., Goldenberg, C. & Sacco, M. (1988). Heat shock and cold-adaptation in Antarctic fish: a molecular approach. *Comparative Biochemistry and Physiology*, **90B**, 623–9.

- Penney, R. K. & Goldspink, G. (1979). Compensation limits of fish muscle myofibrillar ATPase to environmental temperature. *Journal of Thermal Biology*, **4**, 269–72.
- Rome, L. C., Choi, I.-H., Lutz, G. & Sosnicki, A. (1992). The influence of temperature on muscle function in the fast swimming scup. I. Shortening velocity and muscle recruitment during swimming. *Journal of Experimental Biology*, **163**, 259–79.
- Rome, L. C., Funke, R. P., Alexander, R. McN., Lutz, G., Aldridge, H., Scott, F. & Freadman, M. (1988). Why animals have different muscle fibre types. *Nature*, **335**, 824–7.
- Rome, L. C., Loughna, P. T. & Goldspink, G. (1984). Muscle fibre recruitment as a function of swim speed and muscle temperature in carp. *American Journal of Physiology*, **247**, R272–9.
- Rome, L. C. & Sosnicki, A. J. (1990). The influence of temperature on mechanics of red muscle in carp. *Journal of Physiology*, **427**, 151–69.
- Rome, L. C. & Sosnicki, A. J. (1991). Myofilament overlap in swimming carp. II. Sarcomere length changes during swimming. *American Journal of Physiology*, **260**, C289–96.
- Rome, L. C. & Swank, D. (1992). The influence of temperature on power output of scup red muscle during cyclical length changes. *Journal of Experimental Biology*, **171**, 261–81.
- Rowlerson, A., Scapolo, P. A., Mascarello, F., Carpena, E. & Vegetti, A. (1985). Comparative study of the myosins present in the lateral muscle of some fish: species variations in myosin isoforms and their distributions in red, pink and white muscle. *Journal of Muscle Research and Cell Motility*, **6**, 601–40.
- Somero, G. N. & De Vries, A. L. (1967). Temperature tolerance of some Antarctic fishes. *Science*, **15**, 257–8.
- Sidell, B. D. (1980). Response of goldfish (*Carassius auratus*) to temperature acclimation: adaptations in biochemistry and proportions of different fibre types. *Physiological Zoology*, **53**, 948–57.
- Sidell, B. D., Johnston, I. A., Moerland, T. S. & Goldspink, G. (1983). The eurythermal myofibrillar complex of the mummichog (*Fundulus heteroclitus*): adaptations to a fluctuating thermal environment. *Journal of Comparative Physiology B*, **153**, 167–73.
- Ushio, H. & Watabe, S. (1993). Effects of temperature acclimation on Ca²⁺-ATPase of the carp sarcoplasmic reticulum. *Journal of Experimental Biology*, **265**, 9–17.
- Van Leeuwen, J. L. (1995). The action of muscles in swimming fish. *Experimental Physiology*, **80**, 177–91.
- Van Leeuwen, J. L., Lankheet, M. J. M., Akster, H. A. & Osse, J. W. M. (1990). Function of red axial muscles of carp (*Cyprinus carpio*): recruitment and normalized power output during swimming in different modes. *Journal of Zoology (London)*, **220**, 123–40.

- Webb, P. W. (1975). Hydrodynamics and energetics of fish propulsion. *Bulletin of the Fisheries Research Board of Canada*, **190**, pp. 159.
- Wohlschlag, D. E. (1964) Respiratory metabolism and ecological characteristics of some fishes in McMurdo Sound, Antarctica. *Antarctic Research Series*, **1**, 33–62.