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Review

Thermal plasticity of skeletal muscle phenotype in ectothermic vertebrates and its significance for locomotory behaviour

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Summary

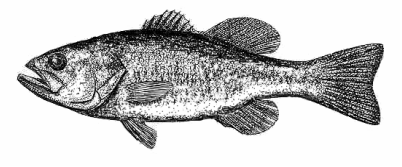
Seasonal cooling can modify the thermal preferenda of ectothermic vertebrates and elicit a variety of physiological responses ranging from winter dormancy to an acclimation response that partially compensates for the effects of low temperature on activity. Partial compensation of activity levels is particularly common in aquatic species for which seasonal temperature changes provide a stable cue for initiating the response. Thermal plasticity of locomotory performance has evolved independently on numerous occasions, and there is considerable phylogenetic diversity with respect to the mechanisms at the physiological and molecular levels. In teleosts, neuromuscular variables that can be modified include the duration of motor nerve stimulation, muscle activation and relaxation times, maximum force and unloaded shortening velocity (V_{max}), although not all are modified in every species. Thermal plasticity in V_{max} has been associated with changes in myosin ATPase activity and myosin heavy chain (MyHC) composition and/or with a change in the ratio of myosin light chain isoforms. In common carp (*Cyprinus carpio*), there are continuous changes in phenotype with acclimation temperature at lower levels of organisation, such as MyHC composition and V_{max}, but a distinct threshold for an effect in terms of locomotory performance. Thus, there is no simple relationship between whole-animal performance and muscle phenotype. The nature and magnitude of temperature acclimation responses also vary during ontogeny. For example, common carp acquire the ability to modify MyHC composition with changes in acclimation temperature during the juvenile stage. In contrast, the thermal plasticity of swimming performance observed in tadpoles of the frog *Limnodynastes peronii* is lost in the terrestrial adult stage. Although it is often assumed that the adjustments in locomotory performance associated with temperature acclimation enhance fitness, this has rarely been tested experimentally. Truly integrative studies of temperature acclimation are scarce, and few studies have considered both sensory and motor function in evaluating behavioural responses. Developmental plasticity is a special case of a temperature acclimation response that can lead to temporary or permanent changes in morphology and/or physiological characteristics that affect locomotory performance.

Key words: temperature acclimation, muscle fibre type, phenotypic plasticity, ectotherm, locomotion.

Introduction

Ectotherms living at temperate latitudes often show phenotypic plasticity, which is thought to buffer the effects of varying environmental temperature on physiological processes. Temperature can vary over short, seasonal or developmental time scales, eliciting responses at levels of organisation ranging from the gene to the whole animal. Rising or falling body temperatures lead to an increase or decrease in metabolic rate, respectively, reflecting changes in the balance between ATP consumption and generation as well as the direct effects of temperature on ligand binding, diffusion and enzyme catalysis (Cossins and Bowler, 1987). Rapid changes in body temperature affect ionic equilibrium and acid–base balance, and behavioural mechanisms are of prime importance in restoring metabolic homeostasis (Crawshaw et al., 1982). Over longer time scales, seasonal temperature changes can modify thermal preferenda (Clark and Green, 1991) and elicit a variety of physiological responses, ranging from winter dormancy (Lemons and Crawshaw, 1985) to acclimation responses (Fry and Hart, 1948). Dormancy and acclimation are not necessarily mutually exclusive. Largemouth bass (*Micropterus salmoides*) maintain similar levels of spontaneous swimming activity at acclimation temperatures of 7–18 °C, but enter a state of winter dormancy at lower temperatures (Fig. 1).

The nature and magnitude of temperature acclimation responses observed at the whole-animal level and the underlying mechanisms at lower levels of organisation vary



Micropterus salmoides

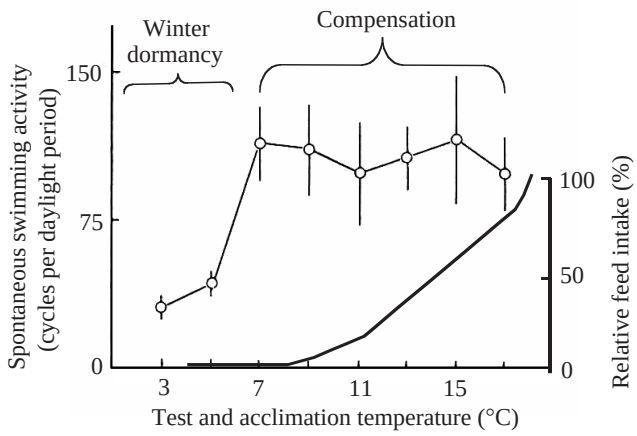


Fig. 1. Influence of acclimation temperature on relative feed intake (solid line) and spontaneous swimming activity (open circles) for largemouth mass (*Micropterus salmoides*). The test temperature was the same as acclimation temperature. Values are means ± S.E.M. (*N*=6–9 fish). Adapted from Lemons and Crawshaw (1985) with permission.

among species and during ontogeny, probably reflecting differences both in ecology and in habitat temperatures (Wakeling et al., 2000; Cole and Johnston, 2001). Relatively little is known about the evolutionary significance of temperature acclimation responses. It has often been assumed that acclimation responses are adaptive and enhance the fitness of an organism in its new environment, the so-called 'beneficial acclimation hypothesis' (Leroi et al., 1994; Huey and Berrigan, 1996). Yet several studies that have set out to test this hypothesis have rejected it (Leroi et al., 1994; Zamudio et al., 1995; Bennett and Lenski, 1997; Gibbs et al., 1998; Huey et al., 1999). However, many of these studies have examined acclimation during early development, leading to some discussion of the validity of the tests because of the confounding effects of developmental plasticity (Wilson and Franklin, 2001).

For many organisms, locomotion is the key to survival. It provides prey, enables migration and facilitates mating and the avoidance of predators. The majority of studies on skeletal muscle performance have focused on the two main types of muscle fibres, fast- and slow-twitch. The fast muscle fibres are generally recruited for high-force, anaerobic activities such as prey capture and escape responses, whilst the slow fibres are recruited during low-force contractions (Bone, 1966; Josephson, 1993). Many studies have used forced exercise models to investigate the thermal plasticity of sustained activity involving flumes (e.g. Fry and Hart, 1948; Johnston, 1993; Rome and Swank, 2001) or treadmills (Feder, 1986). However, during foraging, many ectotherms utilise intermittent patterns of locomotion in which pauses significantly increase the detection rates of prey items (Getty and Pulliam, 1991). Responsiveness to external stimuli during escape behaviour is also a function of acclimation temperature (Tiiska and Lagerspetz, 1999). It is therefore important to consider the sensory and brain function in addition to motor processes to evaluate the behavioural significance of temperature acclimation responses.

In this review, we consider the relationship between the thermal plasticity of muscle phenotype and locomotory performance at the whole-animal level in ectothermic vertebrates and discuss the variety and evolutionary significance of the responses observed. Unfortunately, integrative studies at the gene, protein, muscle and whole-animal level are rare. Even those investigations focused on one or more levels of organisation are restricted to a relatively few species.

Integrative approaches for investigating the thermal plasticity of locomotor performance

Myofibrillar ATPase

To assess contractile phenotype, early studies measured the ATPase activity of isolated myosin or myofibril preparations (Johnston et al., 1975; Sidell, 1980). It should be borne in mind, however, that the values obtained depend on the pH and ionic strength of the assay medium and are sensitive to the mechanical constraints on the myofibrils (Johnston and Sidell, 1984). ATPase activity is usually measured over minutes or tens of minutes, whereas the actin and myosin filaments will shorten to very short sarcomere lengths in milliseconds, reducing the measured activity. Some authors have measured ATPase activity in intact fibres under isometric conditions (Altringham and Johnston, 1986), but the results are not directly applicable to locomotion.

Biomechanics – the work loop technique

The work loop technique, developed by Machin and Pringle (1959), is the main approach used to investigating the biomechanics of muscle under conditions simulating normal locomotion. In some cases, muscles undergo cyclical length changes at a constant frequency. In a classic paper, Josephson (1985) studied one such case, the synchronous muscle of a moth, and exploited the work loop technique to estimate the power requirements of flight. Isolated fibre bundles were subjected to sinusoidal length changes at 25 Hz, corresponding to the wingbeat frequency at 30 °C, and the muscle was phasically stimulated during each length change cycle. By optimising the strain and phase of stimulation, Josephson (1985) was able to estimate the power output by plotting muscle force against fibre length to produce a work loop. The area of the loop represents the work performed during shortening minus the work put into the muscle during the lengthening phase. The mechanical power output was calculated as the net work per cycle multiplied by the frequency.

Altringham and Johnston (1990) used the same approach to investigate the performance of isolated slow and fast muscle fibres in the short-horn sculpin *Myoxocephalus scorpius* over the range of frequencies used during (steady) swimming. The number and timing of stimuli were adjusted at each cycle frequency to maximise power output at 5 °C. Slow and fast muscle fibres produced their maximum power outputs of 5–8 and 25–35 W kg^{-1} wet muscle mass at 2 and 5–7 Hz, respectively. In the case of the slow muscle, its contraction kinetics was too slow to generate any positive work above 8 Hz. This study illustrated why it is necessary to have muscle fibre types with a spectrum of contraction kinetics for locomotion over a wide range of speeds. The intrinsic unloaded shortening speed (V_{max}) is an important parameter because the ratio of shortening speed (V) to V_{max} determines the force generated. Potentially, activation times, relaxation rates, V_{max} and the duration and phase of stimulation all contribute to the shape of the work loop obtained and, hence, to the power delivered by the muscle (Josephson, 1993).

In vivo muscles may not function under the conditions required for maximum power generation. Rome and Swank (1992) optimised work loop parameters for red myotomal muscle in the scup (*Stenotomus chrysops* L.) at 20 and 10 °C. At 20 °C, maximum power output was 27.9 W kg^{-1} wet muscle mass at 5 Hz compared with 12.8 W kg^{-1} wet muscle mass at 2.5 Hz at 10 °C, resulting in a Q_{10} of 2.2. Using the sarcomere length changes and tailbeat frequencies measured for scup during steady swimming, it was concluded that the red muscle probably produces close to its optimum power at 20 °C. In contrast, although the optimum frequency at 10 °C was 2.5 Hz, scup do not swim at such low tailbeat frequencies. Thus, it is important to consider the actual constraints under which the muscle is operating *in vivo* to assess performance at different temperatures and/or acclimation states.

The trend over the last 10 years has been to combine work loop experiments with studies of whole-animal performance to provide more realistic values of muscle strain and stimulation patterns (Josephson, 1993; Franklin and Johnston, 1997; James and Johnston, 1998; Swank and Rome, 2001). Several approaches are available for relating muscle function to body movements. Sarcomere length changes can be either calculated from changes in limb angles or body shape (e.g. Rome and Sosnicki, 1991) or measured directly using surgically implanted sonomicrometry crystals (Franklin and Johnston, 1997; James and Johnston, 1998). The distance between two piezoelectric crystals during lengthening and shortening of the muscle is recorded using alterations in the transit time of ultrasound. Sonomicrometry can be synchronised with electromyographic (EMG) electrodes, which provide information on the phase and duration of muscle activation.

Fish swimming involves alternate contractions of serially arranged myotomes, which have a complex internal and external geometry that varies along the length of the trunk (van Leeuwen, 1999). EMG duty cycle and the timing of muscle activation vary along the body and between fibre types (Coughlin and Rome, 1990). The use of *in vivo* parameters of muscle strain and stimulation in work loop experiments enables the role of muscles at different body positions to be investigated. In several species, muscle fibres in myotomes towards the caudal fin were activated earlier in the cycle than more anterior muscles, resulting in force generation during lengthening for both steady swimming (Altringham et al., 1993) and fast-starts (Johnston et al., 1995b; Wakeling et al., 1999). As a result, the muscle initially does negative work, but the resulting increase in stiffness is thought to play a role in power transmission down the trunk. Elastic energy storage is probably important in most locomotory activities, particularly during running and jumping in terrestrial animals (Biewener and Roberts, 2000). In work loop experiments, muscle fibres are usually attached so as to minimise the influence of serial elastic structures such as tendons, and this is a potential limitation of such experiments.

Whole-animal performance

In fish, locomotory performance is usually studied using high-speed cinematography or video films enabling the velocity and acceleration during steady swimming or fast-starts to be determined. More recent studies have examined the velocity of the wave of curvature travelling down the body of the fish because this is linked to the speed of swimming, particularly during fast-starts (Wakeling and Johnston, 1998; Temple et al., 2000). The power for swimming is generated by the muscles. Estimates of the hydrodynamic power requirements for swimming can therefore be used to predict a minimum value for muscle power output (van Leeuwen et al., 1990; Frith and Blake, 1995; Wakeling and Johnston, 1998; Wakeling et al., 1999). Values of predicted muscle power requirements using this approach have been found to correlate well with measurements of power output involving work loop experiments (Wakeling and Johnston, 1998; Temple et al., 2000).

Short-term responses to temperature change

Studies with teleosts have shown that short-term compensations for the effects of low temperature on muscle power output can be programmed by the central nervous system. The slow and fast muscles in fish are anatomically segregated into distinct layers. Electromyographic studies have shown that there is a systematic recruitment of muscle fibre types in the order slow aerobic (red), fast aerobic (pink) and fast anaerobic (white) as swimming speed increases (Johnston et al., 1977). Fish use the same tailbeat frequency to swim at a given speed over a wide range of temperatures and must therefore generate the same muscle power output (Sisson and Sidell, 1987). However, as temperature decreases, the power output available from the muscle fibres falls with a Q_{10} of 2 or more (Rome, 1990). This affects both the maximum speed attainable and the maximum sustainable speed. The latter is a function of the power output of the aerobic muscle fibre types.

In a study with common carp subjected to forced exercise,

it was shown that the threshold speed at which fast muscle fibres were first recruited decreased as the temperature was lowered (Rome et al., 1984). Thus, over the short term, the carp were able to achieve the same level of performance by recruiting faster-contracting muscle fibre types, but at the expense of relatively rapid fatigue. In the scup, pink muscle, which has a faster relaxation rate and is active for a shorter time than the red muscle, was found to be recruited at lower swimming speeds following an acute drop in temperature (Rome et al., 2000). The effects of an acute decrease in temperature on fast-start performance have also been studied in detail for the common carp (Wakeling et al., 2000). At low temperatures, the magnitude of body bending, muscle strain and contraction duration all increased, and this was associated with a lower hydrodynamic efficiency (Wakeling et al., 2000).

The reduced locomotory performance of some anuran amphibians at low temperature is also at least partially explained by a decrease in muscle power (Hirano and Rome, 1984; John-Alder et al., 1989). However, in the frog *Rana temporaria*, the Q_{10} for both jump take-off velocity and mean swimming velocity was shown to be lower than the Q_{10} for muscle power output determined from work loop experiments (Navas et al., 1999). The mean muscle power output during take-off at 10 °C was only 34 % of the calculated requirements for the whole animal, suggesting the involvement of the storage of elastic strain energy (Navas et al., 1999), as has been suggested for the Cuban tree frog *Osteopilus septentrionalis* (Peplowski and Marsh, 1997). Short-term changes in acid–base balance following a temperature change have the potential to affect muscle performance. Boutilier et al. (1987) reported nonlinear changes in intracellular pH (pHi) with temperature (T) in various striated muscles of the toad *Bufo marinus*, equivalent to a $\Delta pHi/\Delta T$ of –0.14 to –0.023 °C min^{-1} over the range 10–30 °C. Renaud and Stevens (1984) working with isolated sartorius muscle from *Bufo bufo*, calculated that the predicted change in intracellular pH on cooling from 25 to 5 °C was sufficient to increase maximum force and, hence, power during isotonic shortening, perhaps providing a short-term mechanism for compensation to low temperature.

Seasonal acclimatisation of muscle phenotype and locomotory behaviour

Temperature acclimation responses require a stable environmental cue and, consequently, are more prevalent in aquatic species (Scheiner, 1993). There is considerable phylogenetic diversity in the extent to which thermal plasticity of locomotion occurs and also in the underlying mechanisms at the systemic, tissue and molecular levels.

Fish

During the adaptive radiation of the teleosts, temperature acclimation responses in muscle have presumably evolved independently on numerous occasions, and a diversity of molecular mechanisms is therefore to be expected. It is important to include a phylogenetic perspective in analysing the thermal plasticity of locomotory behaviour in terms of the underlying physiological and molecular mechanisms, although such an approach has rarely been adopted in the literature.

Cypriniformes

Cypriniformes of the genus *Cyprinus* and *Carassius* (Family Cyprinidae) are temperate freshwater fish with a wide geographic distribution in temperate regions. Fry and Hart (1948) were among the first to document the remarkable thermal plasticity of swimming performance in the goldfish (*Carassius auratus* L.) on the basis of experiments in a rotating chamber. Goldfish were acclimated to different temperatures for several weeks prior to measuring maximum sustained cruising speed over a 2 min period at a range of temperatures (5–35 °C). It is likely that both slow and fast muscle fibres would have made significant contributions to total power requirements in these experiments. Acclimation to a higher temperature extended the thermal range for activity and shifted the optimum temperature for performance (Fig. 2). Cold acclimation improved locomotory performance at low temperatures but was associated with a marked trade-off in performance at high temperatures and *vice versa* (Fig. 2). The relationship between test temperature and the maximum swimming speed over the first two tail beats of an escape response in goldfish acclimated to either 10 or 35 °C is shown in Fig. 3A. Escape responses are probably exclusively powered by contraction of the fast myotomal muscle.

Several weeks after transfer from 20 to 10 °C, the swimming speed at which common carp first recruit their fast muscle fibres increased relative to that of fish acutely exposed to 10 °C (Rome et al., 1985). Similar results were obtained in striped bass (*Morone saxatilis*) swimming at 15 °C, resulting in a threshold speed for fast muscle recruitment of 2.46 $L\,s^{-1}$ in

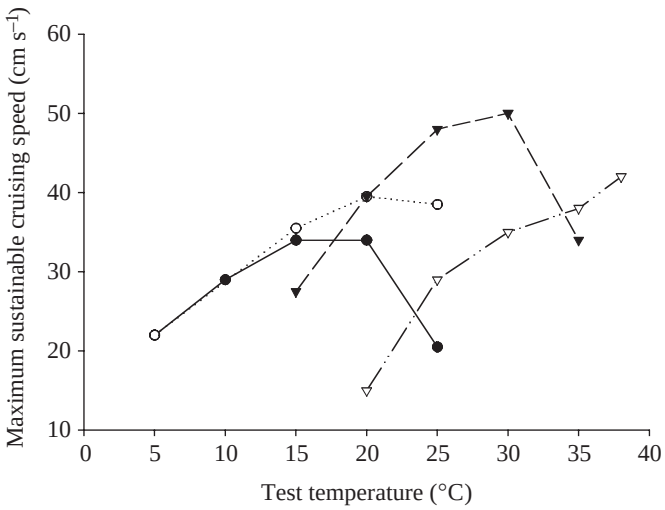


Fig. 2. The forced exercise model used to examine the thermal plasticity of swimming in the goldfish (*Carassius auratus* L.). The maximum speed that could be sustained for 2 min in a rotating chamber is shown as a function of test temperature for fish acclimated to 5 °C (●), 15 °C (○), 25 °C (▼) and 35 °C (▽). Adapted from Fry and Hart (1948) with permission.

9 °C-acclimated individuals and 1.84 $L\,s^{-1}$ in 25 °C-acclimated individuals (Sisson and Sidell, 1987), where L is body length. In both these species, the relationship between tailbeat frequency and swimming speed was independent of acclimation temperature (Sissons and Sidell, 1987; Rome et al., 1985).

The increased swimming speed at which fast muscle fibres are first recruited following cold acclimation partly reflects a greater volume of red muscle, largely due to an increase in the number of slow fibres: goldfish (Johnston and Lucking, 1978); striped bass (Jones and Sidell, 1982). Temperature acclimation responses are reversible and involve changes in the contractile and metabolic phenotype of individual fibres. For example, Langfeld et al. (1991) found that red pectoral fin adductor muscle fibres in the common carp expressed exclusively slow-type myosin light chain isoforms at an acclimation temperature

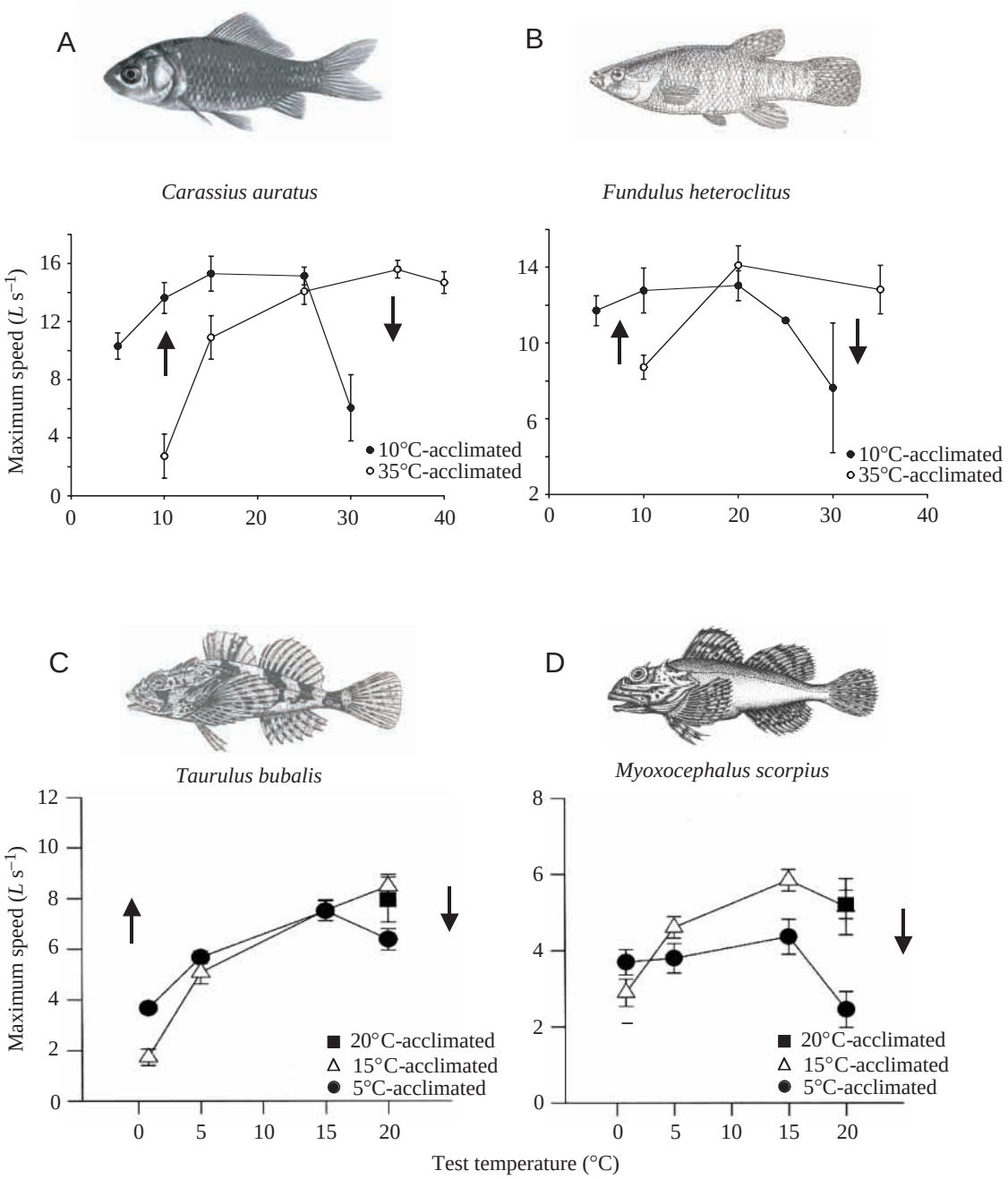


Fig. 3. Thermal plasticity of fast-start behaviour in various fish species. The figure illustrates maximum length-specific speed during escape responses in fish acclimated to different temperatures in (A) goldfish *Carassius auratus*, N=10, (B) killifish *Fundulus heteroclitus*, N=5, (C) long-spined sea scorpion *Taurulus bubalis*, N=7, and (D) short-horn sculpin *Myoxocephalus scorpius*, N=9. Arrows indicate the direction of the acclimation response at a specified test temperature; the horizontal line (in D) indicates no change. Values are means ± S.E.M. See original publications for experimental details. The data are adapted from Johnson and Bennett (1995) and Temple and Johnston (1998). L, total body length.

of 20 °C, but a significant proportion of fast myosin light chains at an acclimation temperature of 8 °C. Maximum shortening velocity (V_{max}) was 17 % higher at 8 °C in the cold- than in the warm-acclimated fish, and this was probably attributable to the expression of fast myosin light chains in slow muscle since there was no evidence for changes in myosin heavy chain composition (Langfeld et al., 1991).

In goldfish and common carp, the contractile performance of fast muscle fibres is improved at low temperatures following cold acclimation. Johnston et al. (1975) found that Mg^{2+}-Ca^{2+}-activated fast muscle myofibrillar ATPase activity was higher at all assay temperatures in 1 °C- than in 26 °C-acclimated goldfish. Cold-acclimation was also associated with an increased susceptibility of the ATPase activity of isolated myofibrils to denaturation at 37 °C, suggesting changes in the tertiary structure of myosin. Similar changes in myofibrillar ATPase activity with temperature acclimation were subsequently reported for common carp (Hwang et al., 1990) and for several other cyprinid species (Heap et al., 1985). Experiments with skinned muscle fibres showed that both maximum force production and maximum shortening velocity increased at low temperatures following cold acclimation in both slow and fast muscle types in the common carp (Johnston et al., 1985). Changes in the relative proportion of myosin light chain 3 ($LC3_f$) to myosin light chain 1 ($LC1_f$) with acclimation temperature are thought to contribute to the observed adjustments in V_{max} of fast muscle fibres (Crockford and Johnston, 1990). The molar ratios of $LC3_f$/$LC1_f$ mRNAs were significantly higher in 30 °C- (3.93) than 10 °C-acclimated (3.10) carp (Hirayama et al., 1997). In addition, Hirayama et al. (1998) found two types of mRNA encoding myosin regulatory light chain in carp fast skeletal muscle that had different numbers of polyadenylation signals prior to the poly(A) tail and showed altered expression following temperature acclimation.

Watabe and co-workers have elegantly shown that fast muscle contains multiple isoforms of myosin heavy chain and that their relative proportions change with acclimation temperature (Watabe et al., 1995). Imai et al. (1997) isolated cDNA clones encoding light meromyosin (LMM) from 10 °C-, 20 °C- and 30 °C-acclimated carp. The type of mRNA transcribed and the proportions of each isoform were found to be a function of acclimation temperature (Imai et al., 1997). In spite of the 95.6 % identity between the 10 °C- and 30 °C-type LMM isoforms (Imai et al., 1997), there were marked differences in thermodynamic properties (Nakaya et al., 1997), which are thought to reflect amino acid substitutions in the C-terminal half of the LMM (Kakinuma et al., 1998). Kakinuma et al. (1998) suggested that the lower thermostability of the 10 °C-type LMM would aid energy transduction from the S1 heads to the thick filaments, thus facilitating contraction in cold-acclimated carp. Hirayama and Watabe (1997) also found isoform differences in the first 60 amino acid residues from the N terminus in the crossbridge head of myosin subfragment-1.

In common carp, acclimation from 20 to 8 °C was shown to result in an increase in the rate of relaxation of the fast muscle fibres at 8 °C (Johnston et al., 1990). This was associated with an increase in the activity of sarcoplasmic reticulum (SR) Ca^{2+}-ATPase but no change in the surface or volume density of SR vesicles (Fleming et al., 1990) or the pCa/tension relationship

Table 1. *Neuromuscular variables reported to change at low temperature following cold-acclimation in teleost fish*

	Species					
Parameter	Common carp *Cyprinus carpio*	Goldfish *Carassius auratus*	Killifish *Fundulus heteroclitus*	Short-horn sculpin *Myoxocephalus scorpius*	Rainbow trout *Oncorhynchus mykiss*	Scup *Stenotomus chrysop*
Motor nerve EMG duration	ND	ND	ND	ND	ND	↓
Muscle						
Activation time	↓	*↓	*↓	–	*↓	↓
Relaxation time	↓	↓	↓	–	↓	–
V_{max}	↑	ND	ND	–	ND	–
P_{max}	↑	ND	ND	–	ND	–
Myofibrillar ATPase activity	↑	↑	↑	–	–	ND
MyHC composition	Yes	Yes	No	No	–	ND
$LC3_f$:$LC1_f$ ratio	Yes	ND	ND	Yes	ND	ND

Cyprinus carpio[1,2,3,4,5,6,7,8,9], *Carassius auratus*[10], *Fundulus heteroclitus*[10], *Myoxocephalus scorpius*[11,12], *Stenotomus chrysops*[13,14], *Oncorhynchus mykiss*[15].

MyHC, myosin heavy chain; $LC3_f$:$LC1_f$ ratio, molar ratio of isoforms of fast myosin light chain 3 to fast muscle myosin light chain 1.

Upward-facing arrows indicate an increase; downward-pointing, a decrease; dashes, no change; ND, not determined; P_{max}, maximum tension; V_{max}, maximum unloaded shortening speed; * indicates a decrease in twitch contraction time (force onset to 50 % relaxation); activation and relaxation times were not separately reported.

Data are taken from [1]Johnston et al. (1985), [2]Crockford and Johnston (1990), [3]Hwang et al. (1990), [4]Johnston et al. (1990), [5]Langfeld et al. (1991), [6]Hirayama et al. (1997), [7]Imai et al. (1997), [8]Wakeling et al. (2000), [9]Cole and Johnston (2001), [10]Johnson and Bennett (1995), [11]Beddow and Johnston (1995), [12]Ball and Johnston (1996), [13]Rome and Swank (2001), [14]Swank and Rome (2001) and [15]Johnson et al. (1996).

Refer to the text for information on the muscle fibre type studied in each species.

of skinned muscle fibres (Johnston et al., 1990). Although adjustments to relaxation rate with cold acclimation are apparently common, there appear to be several ways in which this can achieved (Table 1). For example, in goldfish, the surface density of SR was found to be significantly higher in fish acclimated to 5 °C than in those acclimated to 0 °C (Penney and Goldspink, 1990). In the closely related crucian carp *Carassius carassius*, the specific activity of SR Ca^{2+}-ATPase was increased following acclimation from 22 to 2 °C, but the amount of enzyme was reduced, resulting in no net change in activity (Vornanen et al., 1999). The extent to which these different findings represent genuine species differences in the mechanism of the acclimation response or simply reflect variations in acclimation conditions remains to be ascertained.

Johnson and Bennett (1995) compared temperature acclimation responses in the goldfish and the killifish (*Fundulus heteroclitus*) at the biochemical, cellular and whole-animal levels of organisation in the same set of experiments. When tested at 10 °C, goldfish acclimated to 10 °C showed a six- to eightfold increase in the speed (Fig. 3A) and turning velocity of fast-start escape responses compared with fish acclimated to 35 °C. This was associated with altered myosin heavy chain isoform expression, a fivefold increase in fast muscle myofibrillar ATPase activity and a 100 % decrease in isometric twitch contraction time (Table 1). In contrast, the killifish showed no changes in MyHC isoform expression and a much more modest increase in ATPase activity (55 %) and decrease in twitch contraction time (35 %) following acclimation to the same temperature. Temperature acclimation modified the relationship between maximum swimming speed and test temperature in a similar fashion in the two species, although the magnitude of the response was much smaller in the killifish (Fig. 3A,B). The Q_{10} for maximum velocity in C-start behaviour in 35 °C-acclimated fish, calculated over the range 10–35 °C, was substantially lower (1.2) in the killifish than in the goldfish (2.0) (Johnson and Bennett, 1995). The much better cold-water performance of the killifish than of the goldfish following acute temperature transfer in 35 °C-acclimated individuals was therefore associated with a smaller temperature acclimation response at the tissue level.

Juvenile and adult stages of common carp can survive over-wintering in ice-covered ponds, whereas the embryonic stages require a minimum temperature of at least 15 °C to pass through normal development. Common carp spawn in the summer at or above 20 °C, and the cold-tolerance of the larvae and early juveniles gradually increases as the season progresses. To investigate the development of the acclimation response, Wakeling et al. (2000) reared one group of carp larvae at a constant temperature of 21 °C and cooled other groups to either 8 or 15 °C with a time course that mimicked seasonal cooling. Fast-start performance and fast muscle myofibrillar ATPase activity were found to be independent of acclimation temperature in carp less that 37 mm in total length (*TL*). In carp, greater than 37 mm *TL*, acclimation to 8 °C resulted in an increase in myofibrillar ATPase at all assay temperatures. The MyHC peptide map characteristic of cold-acclimated adult stages was also not observed until 37 mm *TL* in the 8 °C group (Fig. 4).

Cole and Johnston (2001) used a statistical model to compare the time course of the change in fast muscle myofibrillar ATPase activity in fry with mean starting total lengths of 30 and 44 mm. A significant increase in myofibrillar ATPase activity was observed after 2–3 weeks in the 44 mm group, but not until 4–5 weeks in the 30 mm group after they had reached 37 mm *TL*. The development of the temperature acclimation response for myofibrillar ATPase and MyHC expression appears to be a function of maturity state and is presumably established prior to the onset of the first winter (Cole and Johnston, 2001).

Scorpaeniformes

The short-horn sculpin (*Myoxocephalus scorpius*) and long-spined sea scorpion (*Taurulus bubalis*) (Family Cottidae) are shallow-water, temperate, marine teleosts. Adult short-horn sculpin are usually found at a depth of 30–50 m, where temperatures follow seasonal means, whilst juveniles and all stages of long-spined sea scorpion are found in the more variable thermal environment associated with rock pools and the shallow sublittoral zone (Foster, 1969; King and Fives, 1983). In St Andrews Bay, Scotland, the typical mean winter and summer sea temperatures are 5 and 15 °C respectively (Beddow et al., 1995). The ability to modify escape performance following acclimation to the mean winter and summer temperatures was found to vary during ontogeny in the short-horn sculpin but not in the long-spined sea scorpion, perhaps reflecting differences in thermal niche distributions between these two closely related Cottidae species (Temple and Johnston, 1998).

Acclimation to a winter temperature of 5 °C significantly improved escape performance in long-spined sea scorpion measured at 0.8 °C (Fig. 3C), whereas 60 % of fish acclimated to 15 °C could not swim at this temperature. The velocity of the wave of curvature passing down the body was also calculated, and this was higher at 0.8 °C but lower at 20 °C in 5 °C-acclimated than in 15 °C-acclimated fish, indicating a trade-off in performance at high and low temperatures (Fig. 5A–C). Estimates of the useful hydrodynamic power output per unit muscle mass in the direction of travel, calculated from the inertial power requirements, were also significantly affected by acclimation state and were 32 % higher at 0.8 °C in 5 °C- than in 15 °C-acclimated fish (Fig. 5D). In contrast, predicted power requirements at 20 °C (98 W kg^{-1} wet muscle mass) were 90 % higher in 15 °C- than in 5 °C-acclimated fish (Fig. 5D).

In contrast, in the short-horn sculpin, maximum length-specific speed during escape responses was only significantly different between acclimation groups at a test temperature of 20 °C (Fig. 3D). For fast-starts filmed during prey capture, the maximum speed in the direction of the attack was 33 % greater and the tailbeat duration of the propulsive stroke was 37 % shorter at 15 °C in 15 °C- than in 5 °C-acclimated short-horn

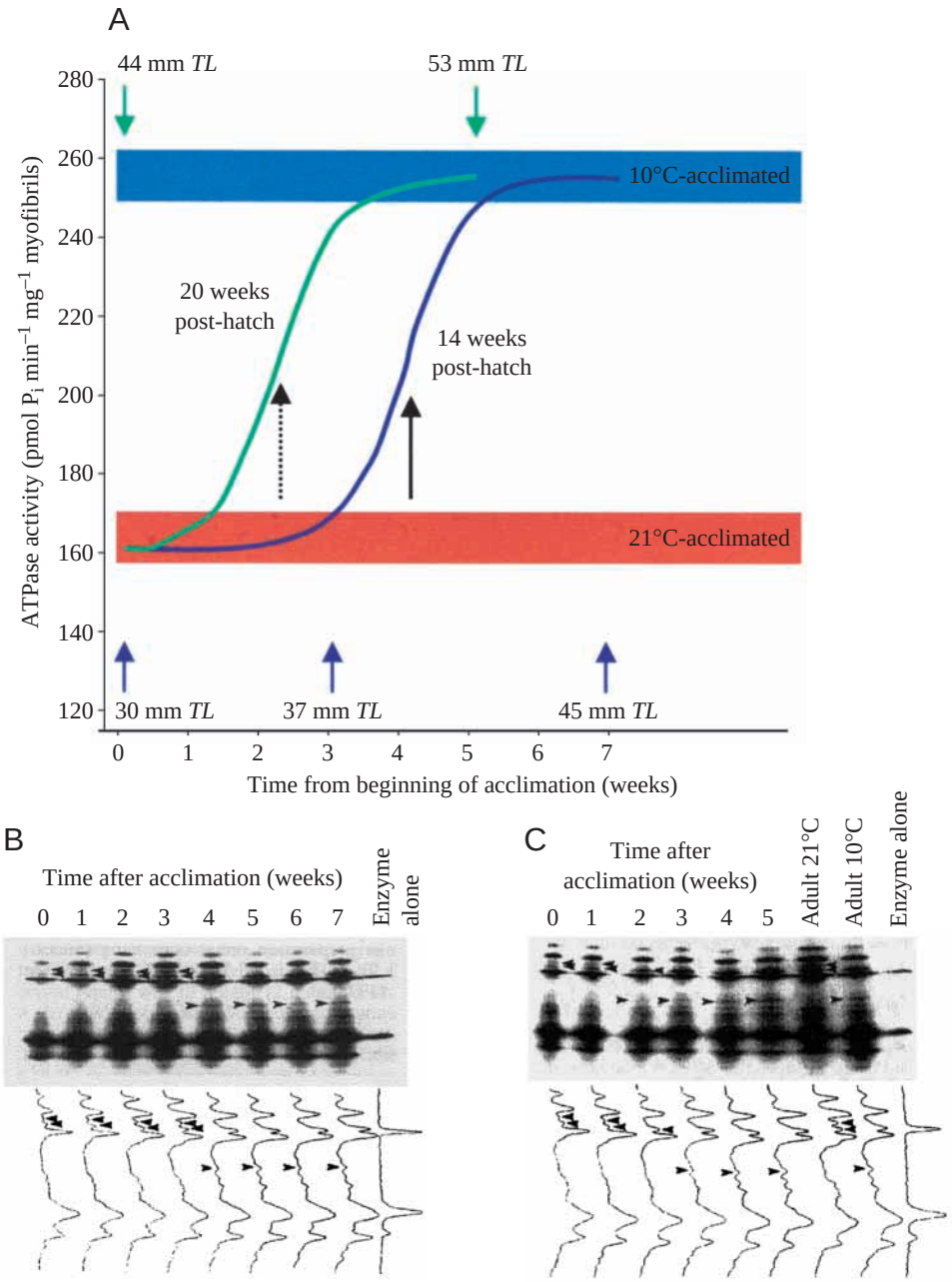


Fig. 4. (A) Thermal plasticity of Mg^{2+}-Ca^{2+} myofibrillar ATPase activity acquired during ontogeny in the common carp. The time course of changes in enzymic activity was longer for juveniles at 14 weeks (purple) than at 20 weeks (green) post-hatch. The blue and red shaded areas represent the myofibrillar ATPase activity of 120 mm total length (*TL*) 10 °C- and 21 °C-acclimated carp respectively. The lengths of the fish during the experiment are indicated with colour-coded arrows. (B,C) Changes in myosin heavy chain (MyHC) peptide maps (top) and their densitometric scans (bottom) with age and acclimation temperature. Bands characteristic of 21 °C-acclimated and 10 °C-acclimated fish are indicated by left- and right-facing arrowheads, respectively. The MyHC composition characteristic of cold acclimation was not observed in any fish greater than 37 mm total length. Adapted from Cole and Johnston (2001).

sculpin (Beddow et al., 1995). The improvement in locomotory performance at 15 °C was sufficient to increase the percentage of successful predation from 23.2 to 73.4 % in summer- compared with winter-acclimatised animals (Beddow et al., 1995). However, acclimation from 15 to 5 °C had little impact on fast-start performance at 5 °C (Beddow et al., 1995; Temple and Johnston, 1998).

Plasticity of swimming behaviour in short-horn sculpin is associated with adaptations in the biomechanics of isolated muscle fibres. Johnson and Johnston (1991) used the work loop technique to investigate the maximum power output of fast muscle during sinusoidal muscle length changes in winter- and summer-caught fish. Following optimisation of the strain and stimulation parameters, the mean power output delivered per cycle by the muscle at 15 °C was more than twice as high in summer-caught (30 W kg^{-1} wet muscle mass) as in winter-caught (9 W kg^{-1} wet muscle mass) short-horn sculpin, largely because of an increase in force during the shortening phase of the cycle (Fig. 6A). Temple et al. (2000) used *in vivo* information on muscle strain and the timing and duration of stimulation during fast-starts (Fig. 6B) in conjunction with work loop experiments with isolated muscles and obtained broadly similar results, although somewhat higher absolute values of power (Fig. 6C). At 15 °C, the maximum instantaneous power delivered by the fast fibres was 275.6 W kg^{-1} wet muscle mass in 15 °C-acclimated fish compared with 178.5 W kg^{-1} wet muscle mass in 5 °C-acclimated fish (Fig. 6D). In contrast, contractile performance

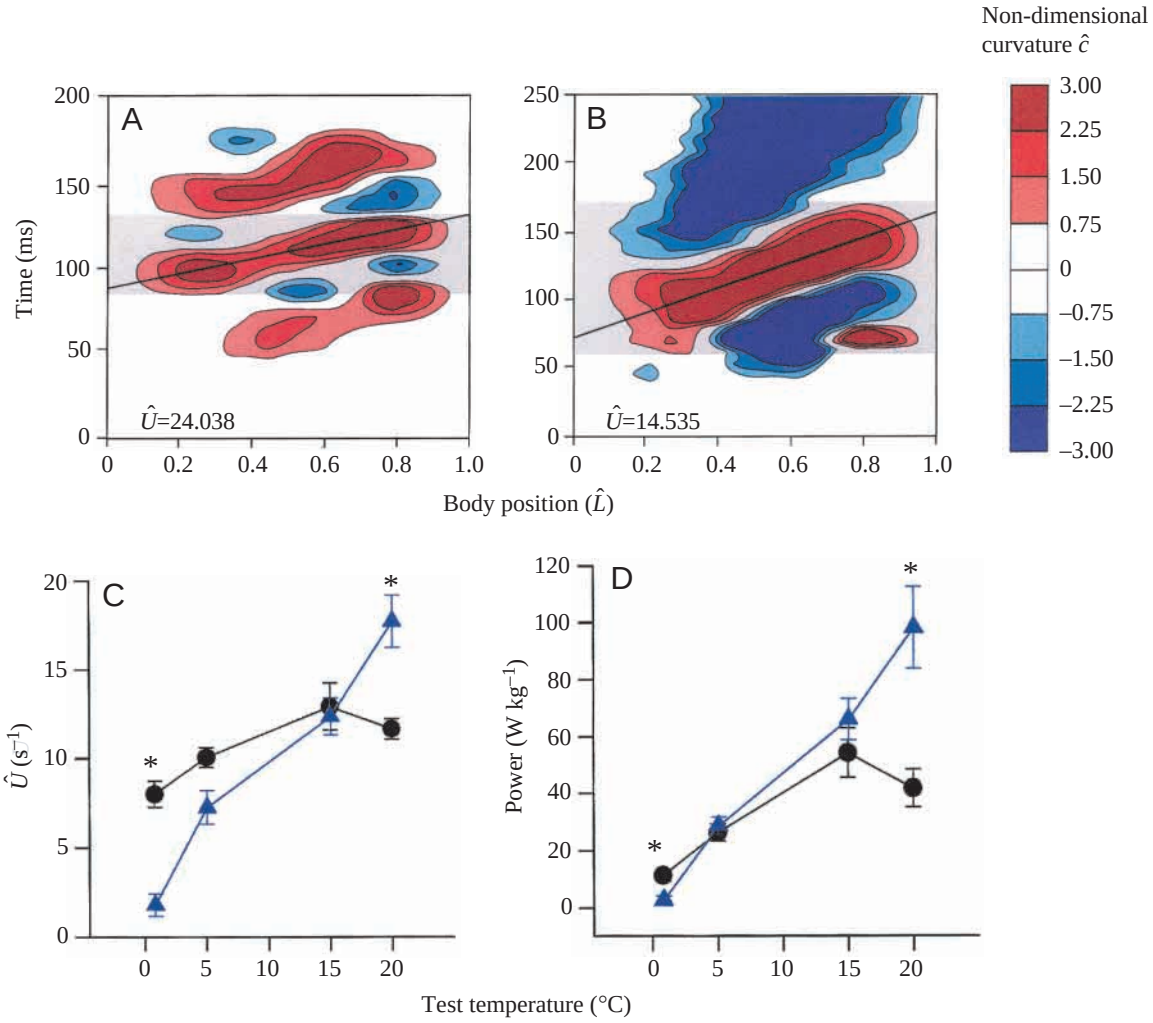


Fig. 5. Thermal plasticity of fast-start behaviour in the long-spined sea scorpion (*Taurulus bubalis* Euphr.). (A,B) Representative contour plots showing how the non-dimensional body curvature, $\hat{c}$, changes with time and body position (0=snout, 1.0=tail) during fast-starts. *L*, body length. (A) Results for a 15 °C-acclimated fish tested at 20 °C; (B) results for a 5 °C-acclimated fish tested at 20 °C. The slope of the solid line is inversely proportional to the rate at which the wave of curvature ($\hat{U}$, s^{-1}) travels down the fish. The grey area indicates the period over which the mean inertial power requirements were calculated. (C) The velocity of the wave of curvature ($\hat{U}$) travelling down the body of the fish for 5 °C-acclimated (circles) and 15 °C-acclimated (triangles) individuals. (D) The power requirements of the contralateral contraction of escape responses. An asterisk indicates significant differences between acclimated groups at 0.8 and 20 °C. Values are means ± S.E.M. (*N*=7). From Temple (1998).

at 5 °C was relatively independent of temperature acclimation state, as was found for locomotory behaviour (Fig. 6A,C,D).

V_{max} and the unloaded contraction velocity of skinned fast-muscle fibres were 2.4 and 2.8 times higher, respectively, measured at 15 °C in 15 °C- than in 5 °C-acclimated short-horn sculpin (Beddow and Johnston, 1995; Ball and Johnston, 1996). In contrast to temperature acclimation in common carp and goldfish, two-dimensional gel electrophoresis and peptide mapping of purified fast muscle myosin heavy chains revealed no myofibrillar isoforms unique to 5 °C- and 15 °C-acclimated fish and no change in myofibrillar ATPase activity (Table 1). The molar ratio of myosin light chain isoforms ($LC3_f$:$LC1_f$) determined by capillary electrophoresis was significantly lower in fast muscle from 15 °C-acclimated (0.73) than from 5 °C-acclimated fish (1.66) (Ball and Johnston, 1996). Thus, adjustments in muscle shortening velocity at high temperatures following summer acclimatisation in sculpin are probably achieved *via* changes in the expression of myosin light chain isoforms (Table 1).

Salmoniformes

The salmoniformes are a diverse group of mostly northern-hemisphere freshwater or andromous fishes. Johnson et al. (1996) investigated muscle protein composition, contractile properties and fast-start performance in rainbow trout (*Oncorhynchus mykiss*) acclimated to either 5 or 20 °C for 4 weeks. Acclimation from 5 to 20 °C produced no statistically significant effect on maximum linear swimming velocity at 20 °C and resulted in only small improvements in distance moved in 40 ms (24 %) and in angular velocity (15 %). There

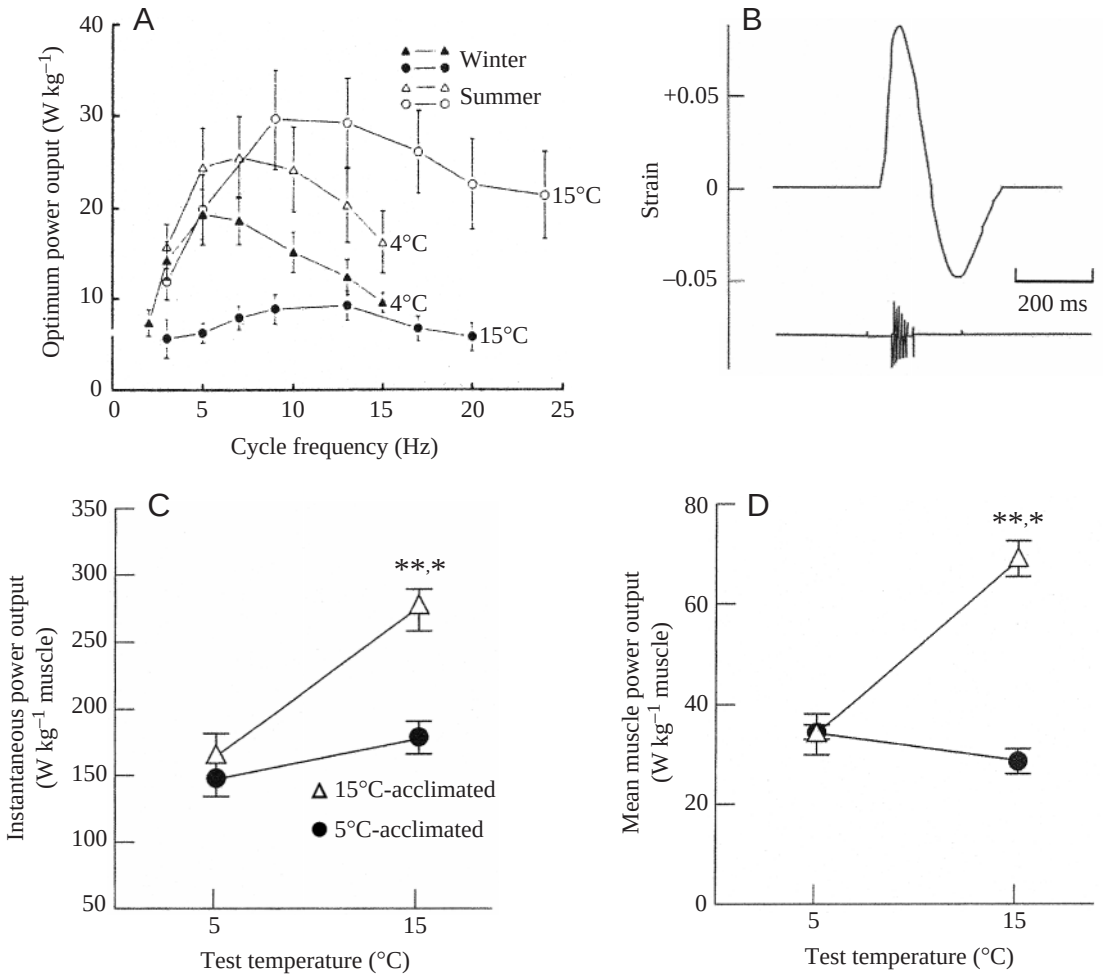


Fig. 6. Influence of seasonal acclimatisation (A) and laboratory acclimation (B,C,D) and on the power output of fast muscle fibres from the short-horn sculpin (*Myoxocephalus scorpius*). (A) The relationship between cycle frequency and power output (W kg^{-1} wet muscle mass) of fast fibre bundles undergoing sinusoidal length changes. From Johnson and Johnston (1991). (B) Strain. (C) Maximum instantaneous and (D) mean power (W kg^{-1} wet muscle mass) output of fast fibres undergoing length change patterns recorded during the contralateral contraction of a fast-start escape response. Asterisks indicate significant differences between acclimation groups: **P*<0.05, ***P*<0.001. Values are means ± S.E.M. (*N*=6 for 5 °C-acclimated fish and *N*=7 for 15 °C-acclimated fish). From Temple et al. (2000).

were no changes in the expression of the myosin heavy chains or myofibrillar ATPase activity with acclimation. Only a minor decrease in fast muscle twitch contraction time (11 %) was found following acclimation to 5 °C (Table 1). Interestingly, however, the myofibrillar ATPase activity became less resistant to thermal denaturation at high temperatures following warm-acclimation, although the mechanism was not investigated. The Q_{10} for the maximum velocity of fast-starts was only 1.2 in rainbow trout (Johnson et al., 1996), a value similar to that reported for the killifish (Johnson and Bennett, 1995). The mechanistic basis of the low thermal dependence of locomotion in certain species and its relationship to the magnitude of the temperature acclimation response is a promising area for future research.

Perciformes

The Perciformes, as currently recognised, represent the largest and most diverse order of vertebrates; they are found in almost every aquatic habitat from the poles to the equator and include both eurythermal and stenothermal species. Rome and Swank (2001) investigated the recruitment of red muscle during steady swimming in scup (*Stenotomus chrysops*) (family Sparidae) acclimated to either 10 °C (cold-acclimated) or 20 °C (warm-acclimated) for 6 weeks. At 10 °C, they found that tailbeat frequency, muscle strain and stimulation phases were independent of acclimation temperature, whereas the EMG duty cycle was approximately 20 % shorter in cold- than in warm-acclimated fish (Table 1). In contrast, at 20 °C, all the measured variables were similar between acclimation groups. It was suggested that reductions in EMG duty cycle might result from altered functioning of the pattern generator during cold-acclimation, indicating that the nervous system has the ability to adjust to cold temperatures. In an accompanying paper, work loop experiments were used to test the hypothesis that the shorter stimulus duty cycle would increase muscle power output (Swank and Rome, 2001). The results differed according to the

anatomical position of the muscle along the body. Integrated over the length of the fish, the power output at 10 °C was 2.7, 8.9 and 5.8 times higher in cold- than in warm-acclimated fish swimming at 30, 40 and 50 cm s^{-1}, respectively. Temperature acclimation had no effect on maximum tension, relaxation rate or unloaded shortening speed, but resulted in an approximately 50 % faster activation rate in cold- than in warm-acclimated individuals at 10 °C (Table 1). By studying cold-acclimated muscle under warm and cold *in vivo* acclimation conditions, they were able to attribute 60 % of the improved power output to adjustments in activation rate and 40 % to the reduction in stimulus duty cycle. Such alterations following cold acclimation were thought also to increase the efficiency of the muscle (Swank and Rome, 2001).

Amphibians

Studies on the thermal acclimatory capacity of amphibians at the levels of the whole animal and isolated muscle indicate little or no phenotypic plasticity to seasonal temperature change in most species (Putman and Bennett, 1981; Miller, 1982; Renaud and Stevens, 1983; Rome, 1983; Else and Bennett, 1987; Knowles and Weigl, 1990). However, Wilson and Franklin

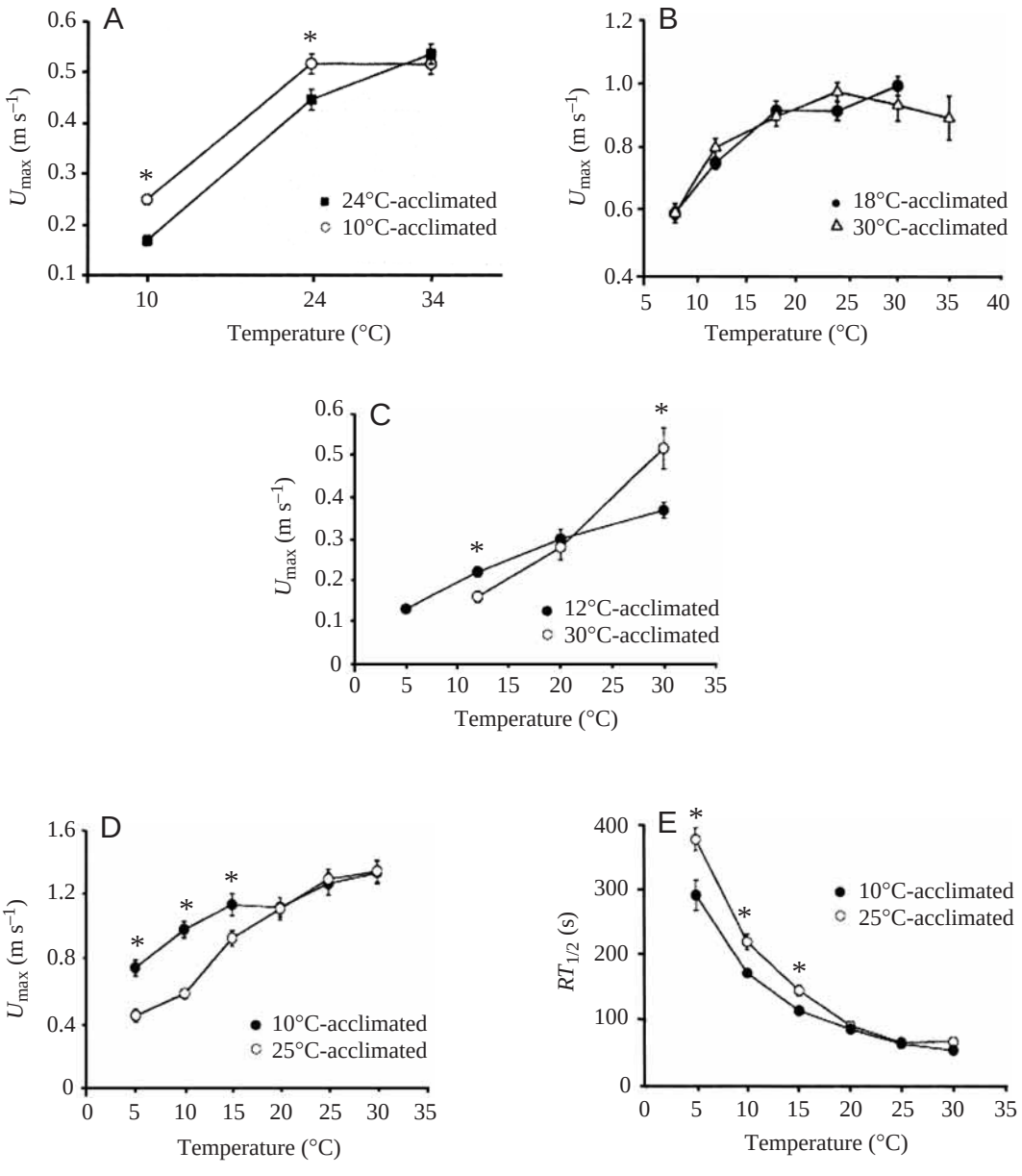


Fig. 7. (A–D) Influence of temperature acclimation on maximum speed during locomotion, U_{max}, in (A) tadpoles and (B) adults of the striped marsh frog (*Limnodynastes peronii*) and (C) tadpoles and (D) adults of the African clawed frog (*Xenopus laevis*). (E) Influence of temperature acclimation on the time from last stimulus to 50 % tetanic relaxation, $RT_{1/2}$, of isolated gastrocnemius muscle from adult *X. laevis*. From Wilson and Franklin (1999, 2000) and Wilson et al. (2000) with permission. Values are means ± S.E.M. (A, N=15; B–E, N=10–12). Asterisks denote significant differences between groups (P<0.05).

(1999) found that tadpoles of the striped marsh frog (*Limnodynastes peronii*) acclimated to 10 °C for 6 weeks had maximum swimming speeds and accelerations at 10 °C that were 47 % and 53 % higher, respectively, than those of tadpoles acclimated to 24 °C. In contrast, the adult stages showed no temperature acclimation response (Fig. 7A,B). Both tadpoles and adult stages of the fully aquatic amphibian *Xenopus laevis* showed improvements in maximum swimming performance following a period of temperature acclimation (Fig. 7C,D). For example, the minimum temperatures for burst swimming were 5 and 10 °C in tadpoles acclimated to 12 and 30 °C, respectively. At 12 °C, maximum speed was 38 % higher in 12 °C- than in 30 °C-acclimated individuals. Conversely, at a test temperature of 30 °C, the maximum swimming speed was 41 % faster in the warm- than in the cold-acclimated tadpoles, indicating a trade-off between performance at high and low temperatures (Wilson et al., 2000). At 10 °C, the maximum swimming velocity of adults acclimated to 10 °C was 67 % faster than that of adults acclimated to 25 °C, although there was no difference between acclimation groups at higher temperatures (Fig. 7C,D). Isolated gastrocnemius muscle fibres from adult *X. laevis* showed higher tetanic tension and decreased relaxation times at 10 °C in 10 °C- than in 25 °C-acclimated frogs, indicating some thermal plasticity of muscle contractile properties (Wilson et al., 2000) (Fig. 7E). One plausible hypothesis is that acclimation responses are observed only in fully aquatic amphibians or tadpole stages that have sufficiently stable temperature cues to initiate an acclimation response.

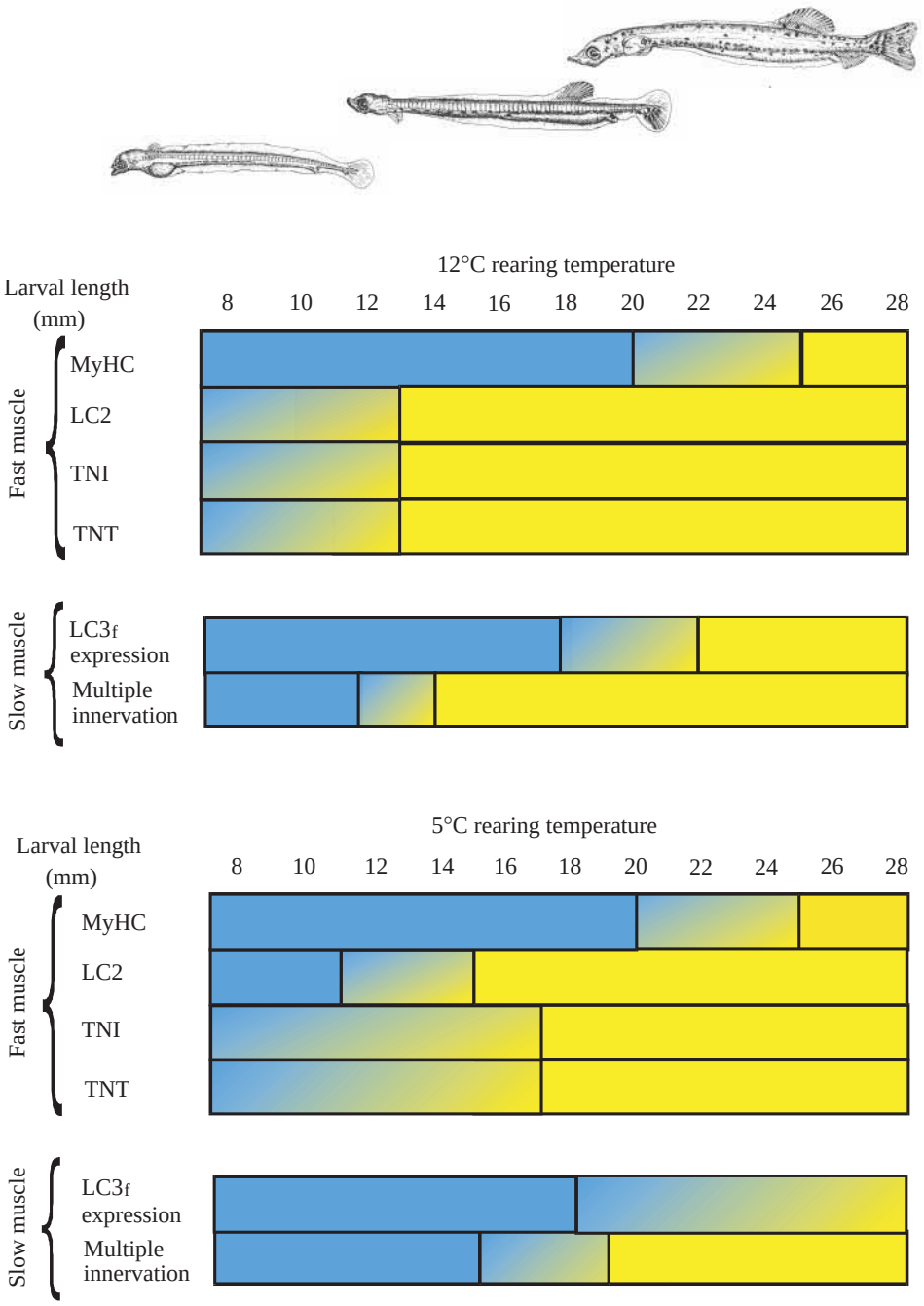


Fig. 8. Developmental plasticity of myofibrillar protein composition and slow muscle innervation patterns in the myotomal muscles of Atlantic herring (*Clupea harengus*) reared at 12 or 5 °C. After hatching, temperature was allowed to rise, mimicking a seasonal warming. MyHC, myosin heavy chains; LC2, myosin light chain 2; TNI, troponin I; TNT, troponin T; LC3f, myosin light chain 3. Blue, embryonic pattern; yellow, adult pattern. Data from Johnston et al. (1997, 1998).

Evolutionary significance of thermal plasticity of locomotion

Wakeling et al. (2000) tested three *a priori* hypotheses about the evolutionary significance of the temperature acclimation response of fast-starts in the common carp *Cyprinus carpio* on the basis of their known natural history. Juvenile fish were acclimated to 8 and 21 °C or 9, 19 and 30 °C for 2 months and then tested at each temperature. The three hypotheses were (i) a 'beneficial acclimation' hypothesis, which predicts that acclimation to a particular temperature will give the organism a performance advantage relative to a non-acclimated individual, (ii) an 'optimal development' hypothesis, which

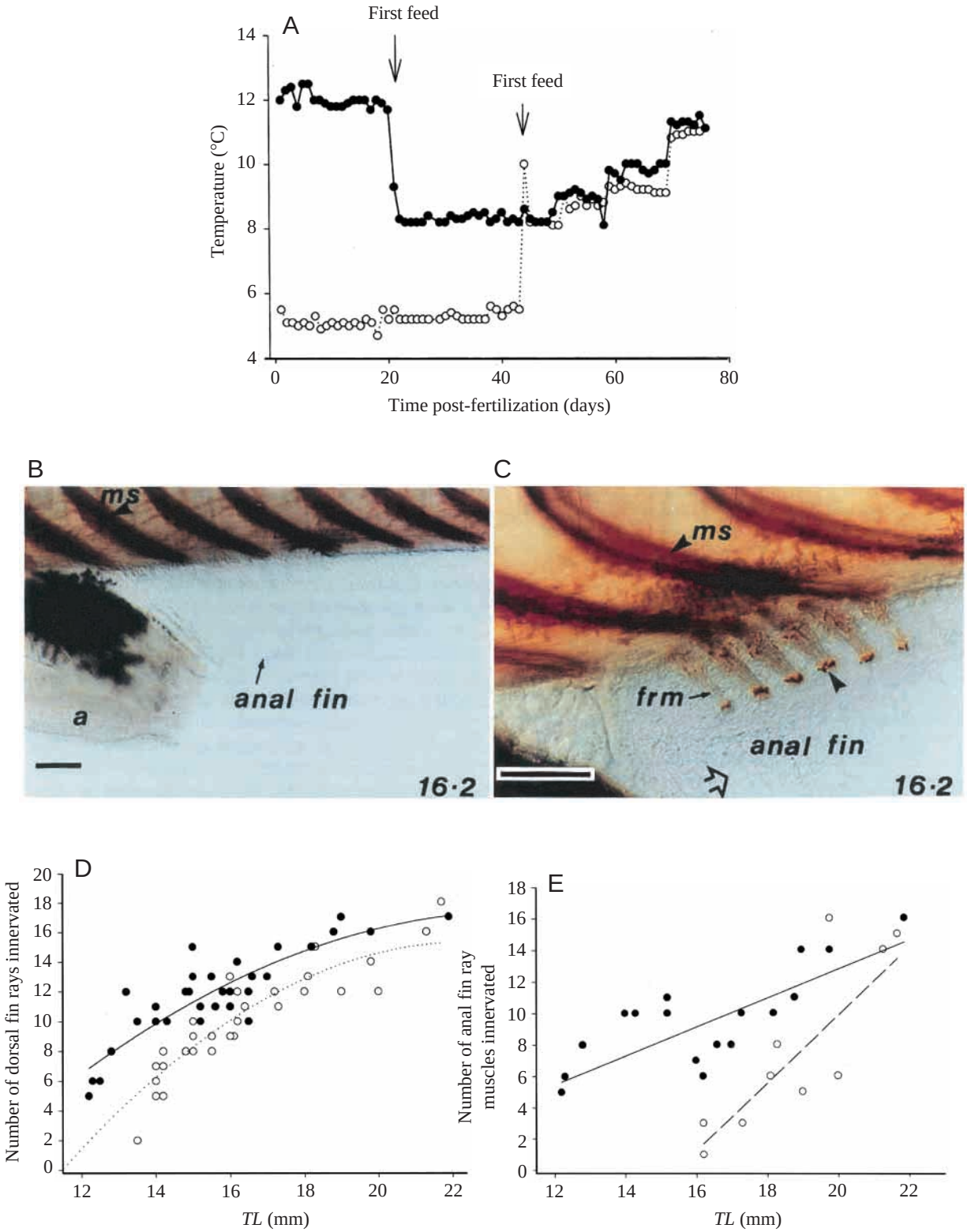


Fig. 9. Influence of rearing temperature on dorsal and anal fin ray muscle development in Atlantic herring (*Clupea harengus*). (A) Embryos were incubated at 5 °C (open circles) or 12 °C (filled circles) until first feeding and then reared at ambient temperature. (B,C) Whole-mount larvae of 16.2 mm total length stained for acetylcholinesterase activity following the 5 °C (B) and 12 °C (C) rearing regimes. From Johnston et al. (2001). *a*, anus; *frm*, fin ray muscles; *ms*, myosepta. (D) Number of dorsal fin ray muscles (second-order polynomials were fitted to the data) and (E) number of anal fin ray muscles innervated in relation to total body length (*TL*) in larvae reared under the two thermal regimes shown in A. First-order linear regressions were fitted to the data.

predicts that fish acclimated to one 'optimal' temperature will perform best at all temperatures tested, and (iii) a 'no-advantage' hypothesis, which predicts that acclimation temperature will have no effect on the performance of the fish over the range of temperatures within which it is normally active. In the British Isles, common carp actively forage for food between 8 and 30 °C, although summer temperatures rarely exceed 21 °C. Temperature acclimation was found to alter the MyHC composition, ATPase activity, spine curvature, fast muscle strain and muscle strain rate but had no effect on fast-start swimming performance between 8 and 21 °C. In a second series of experiments involving a wider range of temperatures (9, 19 and 30 °C), a significant acclimation response was observed. At 9 °C, maximum swimming speed was 79 % higher and predicted hydrodynamic power requirements were 309 % greater in 9 °C- than in 30 °C-acclimated fish. Thus, whether the 'beneficial acclimation hypothesis' was accepted or rejected depended on the nature of the test and the range of acclimation temperatures studied (Wakeling et al., 2000).

Acclimation responses are usually tested by comparing the performance of fish acclimated to a particular temperature with that of fish acutely transferred to the same temperature over less than 24 h. In the case of *Cyprinus carpio*, it would appear that quite large differences in acclimation temperature were required to observe a modification in fast-start performance at the whole-animal level. In contrast, differences in myofibrillar ATPase activity and MyHC composition were apparent with smaller differences between acclimation groups (Wakeling et al., 2000). Studies with skinned fibres isolated from fast muscle have shown that maximum unloaded shortening speed and maximum tension generation vary continuously with acclimation temperature (Crockford and Johnston, 1990). Thus, it seems that there are continuously variable acclimation responses at lower levels of organisation, e.g. in MyHC expression patterns and shortening speed, with a threshold for an observable effect at the whole-animal level. This is perhaps not surprising given the large number of energy-transfer steps between the mechanochemical transduction of chemical energy by actomyosin ATPase and the transmission of thrust along the surface of the body (Wakeling et al., 2000).

Nevertheless, the available evidence suggests that locomotory performance is enhanced at least under some conditions following temperature acclimation. Implicit in the 'beneficial acclimation hypothesis' is the idea that improvements in physiological variables following acclimation confer a fitness advantage. Direct evidence for this supposition is somewhat limited with regards to locomotory performance. Jayne and Bennett (1990) showed that the burst speed and stamina of garter snakes released into the wild was correlated with their survival a year after testing. Tadpoles with a high burst swimming performance were also more likely to survive encounters with garter snakes in experimental mesocosms (Watkins, 1996). Swain (1992) found superior burst swimming performance in sticklebacks (*Gasterosteus aculeatus*) by certain vertebral phenotypes which was matched by increases in the frequencies of those phenotypes in the wild. It is entirely possible, however, that increased locomotory performance following temperature acclimation may incur other costs such as increasing encounter rates with predators, which offset any direct fitness gains. Presumably, there must also be a trade-off between the benefits of increased performance and the energetic costs associated with the restructuring of myofibrils, sarcoplasmic reticulum membranes and metabolic pathways. In no case of temperature acclimation has it been established that the performance gains are sufficient to make a critical contribution to survival in predator–prey encounters,

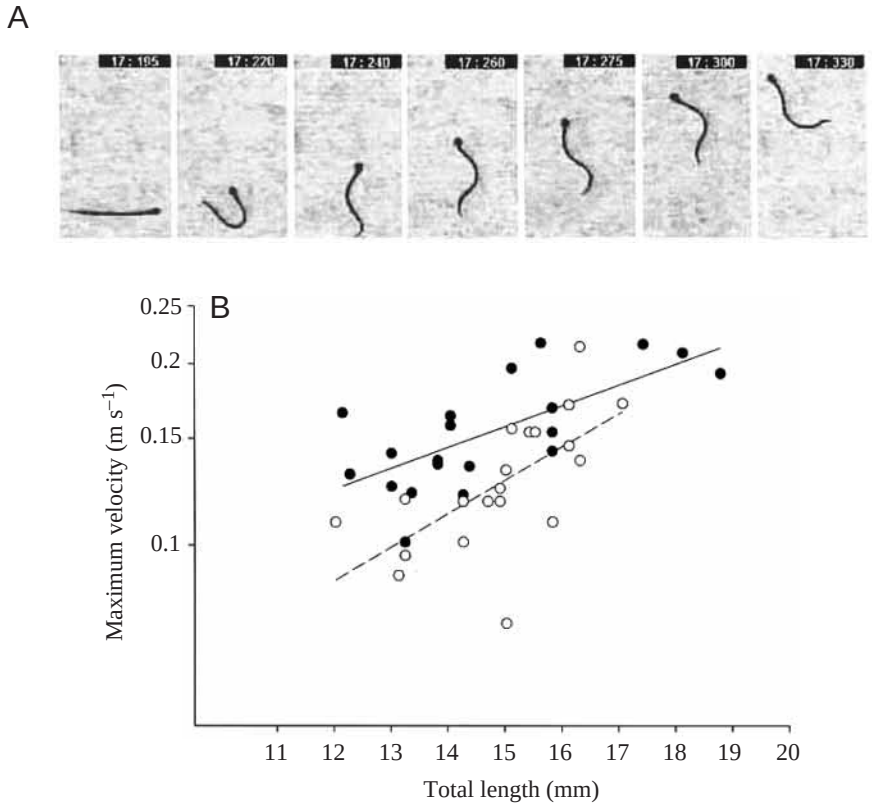


Fig. 10. (A) Escape response of Clyde herring larva (*Clupea harengus*) 18 mm total length, filmed at 200 frames s^{-1}. (B) Maximum velocity attained during escape responses in Clyde herring larvae reared at 5 °C (open circles, dashed line) and 12 °C (filled circles, solid line) until first feeding, when they were transferred to ambient seawater temperatures. Each point represents an escape response from one larva. First-order linear regressions were fitted to the data. 5 °C, U_{max}=–3.12$TL^{1.92}$, r^2=0.42, P=0.002; 12 °C, U_{max}=2.20$TL^{1.21}$, r^2=0.48, P=0.001), where U_{max} is maximum swimming velocity (m s^{-1}) and TL is total length (mm). From Johnston et al. (2001).

although this may well be the case. It is also entirely possible that factors such as responsiveness to predators may be equally important to survival as maximum locomotory performance, and this has yet to be investigated.

Developmental plasticity to temperature

The embryonic stages of ectotherms are frequently exposed to seasonally rising or falling temperatures or may experience changes in thermal regime as a result of migration. Temperature has a major impact on the rate of development, and temperature change is also associated with developmental plasticity leading to temporary and/or long-lasting changes in tissue phenotype (Huey and Berrigan, 1996). Temporary changes in phenotype are relatively common and result from alterations in the relative developmental timing of different tissue components (Fukuhara, 1990; Johnston et al., 1995a). Perhaps the best studied example of an irreversible change in phenotype is temperature-dependent sex determination, which is common in crocodiles and turtles (Janzen and Paukstis, 1991). Embryonic temperature regime has also been shown to influence the growth and locomotory performance of juvenile wall lizards (*Podarcis muralis*) (Braña and Xiang, 2000) and the number of vertebrae in a wide range of fish larvae (Tåning, 1952).

There have been relatively few studies on the developmental plasticity of muscle in relation to locomotion. We have investigated developmental plasticity to temperature in the spring-spawning Clyde stock of Atlantic herring (*Clupea harengus* L.). Clyde herring deposit their eggs on the seabed at a depth of 15–20 m at a time when sea temperatures are rising and range from 4 to 12 °C, depending upon the inter-annual variation in oceanographic conditions. Embryos were found to emerge from the egg capsule after around 9 days at 12 °C, with this increasing to 28 days at 5 °C (Johnston et al., 1995b). At hatching, the transparent herring larvae are 7–9 mm in total length (*TL*) with a prominent primordial fin extending along the dorsal and ventral margins of the trunk (Batty, 1984). At this stage, the larvae swim using an anguilliform mode of locomotion in which the amplitude of the trunk movements increases linearly along the body. Muscle differentiation and much of organogenesis occurs over a protracted period following yolk-sac absorption (Blaxter, 1988; Johnston et al., 1997). As the adult pattern of paired and medial fins and associated muscles gradually develops (12–22 mm *TL*), the trunk becomes more laterally compressed, and the larvae adopt a progressively more carangiform mode of swimming in which the head is kept relatively still and the amplitude of trunk movements increases markedly towards the caudal fin (Batty, 1984). Thus, the transfer of force to the water shifts from the whole surface of the trunk to the caudal fin, which acts as a flexible paddle. The larvae metamorphose into the silver juvenile stage over the size range 33–42 mm *TL*, by which time development is essentially complete.

Laboratory experiments indicate that differentiation of several of the tissues involved in locomotion is uncoupled from somatic growth in larvae reared at different temperatures. Notochord flexion and the development of the medial fins occurred at longer body lengths in larvae reared at 5–8 °C than in those reared at 12–15 °C (Johnston et al., 1998). The slow muscle fibres responsible for sustained activity are initially innervated by *en grappe* endplates at the myosepta. The adult multi-terminal pattern of slow muscle innervation was established in larvae of 12–14 mm *TL* at 12 °C, but was it delayed until 16–19 mm *TL* at 5 °C (Fig. 8).

The majority of the myofibrillar proteins exist as developmental-stage-specific isoforms resulting from multi-gene families or from the alternative splicing of mRNA transcripts. The body length at which the transition from embryonic/larval isoforms to adult isoforms varies with temperature, but is not the same for all proteins (Fig. 8). Thus, at 12 °C, the adult isoform of myosin light chain 2 (LC2) largely replaces the embryonic LC2 in larvae of 13 mm *TL*, whereas this transition is delayed until the larvae are 15 mm *TL* at 5 °C (Fig. 8). The fast muscle in herring embryos and larvae contains a large number of isoforms of troponin T (TnT), which are thought to arise from alternative splicing of a single gene. The number of TnT isoforms decreases during ontogeny, with the adult patterns of isoforms being established by 13 mm *TL* at 12 °C, but not until 17 mm *TL* at 5 °C (Fig. 8). The slow muscle fibres of herring larvae express the adult fast muscle isoform of myosin light chain 3 ($LC3_f$) until 22 mm *TL* at 12 °C, but retain $LC3_f$ until 28 mm *TL* at 5 °C (Fig. 8). Thus, the constituent proteins of the myofibril show considerable variation with respect to body length depending on the prevailing sea temperature.

We would predict from such results that the performance and energetic cost of swimming would be a function of the thermal history of the larvae. Remarkably, the temperature prior to the onset of endogenous feeding was sufficient to influence the relative timing of development of components of the locomotory system more than a month later (Johnston et al., 2001). Clyde herring were reared at 5 or 12 °C until first feeding and then transferred to a common ambient temperature (Fig. 9A). The development of the dorsal and anal fin rays and associated stiffening muscles occurred at shorter body lengths in fish initially reared at 12 °C than in those reared at 5 °C (Fig. 9B–E). This morphological variation was associated with the adult swimming style being adopted at shorter body lengths and with significantly improved fast-start performance in larvae that had experienced warmer temperatures earlier in development (Johnston et al., 2001) (Fig. 10). Once larvae reach 22 mm *TL*, the development of the motor system and associated morphological characters is essentially complete (Batty, 1984) and differences between temperature groups are no longer apparent (Johnston et al., 2001). Developmental plasticity and its associated phenotypic variation can therefore influence locomotory performance and may be of considerable ecological importance during early larval stages when mortality is high because of starvation and predation.

Perspective

The response of burst swimming speed to temperature acclimation in the anadromous Isle Verte population of three-spine sticklebacks (*Gasterosteus aculeatus*) was shown to differ depending on whether the experiments were conducted in the spring or late autumn, when water temperatures were rising or falling, respectively (Guderley et al., 2001). Such studies show that careful attention to experimental design is required if genuine differences between species are to be distinguished from variation due to environmental conditions and factors associated with fish condition and/or reproductive state. Future research should attempt to provide a much stronger ecological and evolutionary framework for the studied behaviours as well as attempting to integrate information from different physiological systems, e.g. sensory and motor. Such a balanced approach should enable the biological significance of different patterns of response to be evaluated. It is already clear that the molecular and physiological mechanisms underlying the thermal plasticity of locomotion vary among species, but the relationship between particular muscle phenotypes and whole-animal performance is much less clear. For the future, proteomics opens up the possibility of investigating all the proteins transcribed under particular environmental conditions and oligonucleotide array technology will allow coordinated gene expression to be studied, potentially at the whole-genome level.

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