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THE THERMAL ACCLIMATION OF BURST ESCAPE PERFORMANCE IN FISH: AN INTEGRATED STUDY OF MOLECULAR AND CELLULAR PHYSIOLOGY AND ORGANISMAL PERFORMANCE

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Summary

Goldfish (Family Cyprinidae, *Carassius auratus*) and killifish (Family Cyprinodontidae, *Fundulus heteroclitus*) were acclimated to 10, 20 and 35 ˚C for 4 weeks. The thermal acclimation of C-start (escape swimming) performance and the physiological properties of fast twitch muscle fibres that underlie it were investigated in these species at the molecular (myosin isoform expression), biochemical (myofibrillar ATPase activity), cellular (contractile kinetics) and organismal levels of organisation. Peptide maps were obtained for fast muscle myosin heavy chains, isolated from 10 ˚C- and 35 ˚C-acclimated fish. Different myosin heavy chain isoforms were expressed in response to a change in acclimation temperature in goldfish, but myosin heavy chain isoform expression was unaffected by acclimation temperature in killifish. Compared with fish acclimated to 35 ˚C, acclimation to 10 ˚C increased the activity of fast muscle myofibrillar ATPase assayed at 10 ˚C fivefold in goldfish and only 50 % in killifish. Muscle twitch contraction time at 10 ˚C decreased significantly in response to acclimation to 10 ˚C in both species; however, the magnitude of this response was much greater in goldfish (100 %) than in killifish (30 % or less). In goldfish, these changes in the physiological properties of fast twitch fibres during 10 ˚C acclimation resulted in a six- to eightfold increase in the speed and turning velocity of fish performing C-starts at 10 ˚C. By comparison, the somewhat smaller acclimatory response of killifish fast muscle properties was accompanied by only a minor (50 % or less) adjustment in locomotor performance. Thermal acclimatory responses of fast muscle at the molecular, biochemical and cellular levels of organisation are clearly reflected in alterations in organismal escape performance.

Key words: thermal acclimation, C-start, fish, goldfish, *Carassius auratus*, killifish, *Fundulus heteroclitus*, muscle physiology, kinematics, polyploidy.

Introduction

Many teleost fish species are often exposed to changes in temperature. These changes may be relatively acute, for example during migration, flooding, tidal changes, etc, or may be seasonal or long-term in nature. Following acute exposure to a decline in temperature, muscle power output (see Rome, 1990) and consequently locomotor performance (both sustained and burst-speed swimming) are severely compromised (reviewed in Beamish, 1978; Guderley and Blier, 1988; Sidell and Moerland, 1989; Bennett, 1990; Johnston *et al.* 1991).

Long-term exposure to cold temperature induces acclimatory changes in muscle contractile properties and enzyme activities, and these have been interpreted as enhancing locomotor performance at low temperatures (Loughna *et al.* 1983). Long-term acclimatory responses in red (slow twitch) muscle and sustained swimming behaviour during cold acclimation are well known and documented in several species of fish (see Johnston and Dunn, 1987). However, temperature acclimation of white (fast twitch) muscle fibres has been found only in a few species of cyprinid fish (Johnston *et al.* 1975; Heap *et al.* 1985, 1986; Crockford and Johnston, 1990; Fleming *et al.* 1990; Gerlach *et al.* 1990; Hwang *et al.* 1990; Goldspink *et al.* 1992) and has been demonstrated to be absent in others (Penney and Goldspink, 1981; Sidell *et al.* 1983; Heap *et al.* 1985; Sidell and Johnston, 1985; Johnston and Wokoma, 1986; Moerland and Sidell, 1986). Since the contractile properties of fast twitch muscle are thought to limit maximum locomotor speed at the organismal level (Wardle, 1975; Marsh and Bennett, 1985, 1986; Marsh, 1990; Swoap *et al.* 1993), the absence of acclimation at the muscle level suggests that organismal escape performance may not acclimate at cold temperatures. In any event, the

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acclimation of burst escape speed in fish has not been investigated. It was therefore the aim of this investigation to determine to what degree thermal acclimatory responses of muscle at the molecular and cellular levels are capable of ameliorating the detrimental effects of exposure to cold temperature on burst escape locomotor performance.

In this paper, we examine the effects of acute temperature exposure and thermal acclimation on the C-start, an escape response in teleost fish initiated by the Mauthner cell (Zottoli, 1977). This locomotor behaviour is ideal for this investigation as it is highly stereotyped and involves the simultaneous recruitment of all motor units, including all the fast twitch fibres. The C-start consists of two stages (Eaton *et al.* 1977). In stage 1, the fish bends into a 'C' as a result of the simultaneous contraction of all muscle fibres along one side of the body; stage 2 consists of an S-shaped propulsive stroke (see Fig. 1A; Eaton *et al.* 1977; Nissanov and Eaton, 1989). In this study, the thermal dependence of C-start performance and the underlying physiology of fast muscle function (myosin isoform expression, myofibrillar ATPase activity, twitch contraction kinetics) were investigated in one species known to demonstrate significant thermal acclimatory ability in these physiological parameters (goldfish) and compared with another species (killifish), previously shown to lack similar responses.

Materials and methods

Fish

Goldfish (Family Cyprinidae, *Carassius auratus*) were obtained from a local fish farm (Bayou Aquatics, Ontario, CA, USA) and killifish (Family Cyprinodontidae, *Fundulus heteroclitus*) from Woods Hole Marine Biological Laboratory (MA, USA). All animals were of similar size, with a mean total length of 6.4±0.8 cm (S.D., N=75) for goldfish and 6.8±0.8 cm (N=64) for killifish. Goldfish were maintained in fresh water and killifish in 100 % sea water under ambient light conditions (16 h:8 h light:dark). Fish were divided into three groups and kept at 10, 20 and 35 °C (±0.5 °C) for at least 4 weeks prior to experimentation. All animals were fed *ad libitum* with fish flake and frozen bloodworms. For experimental procedures, fish were rapidly killed by a blow to the head followed by immediate decapitation.

Polyacrylamide gel electrophoresis

Muscle samples from 10 °C- and 35 °C-acclimated fish were analysed using electrophoretic techniques. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed using small vertical slab gels (80 mm×73 mm×0.5 mm; Bio-Rad Mini-PROTEAN II). SDS–PAGE gels were run using separating gels containing a range of acrylamide concentrations of 10 % to 20 %, with a stacking gel of 4 %. Alkali urea gels were run to characterise the myosin light chains (MLCs) of actomyosin prepared from myofibril samples. MLCs were separated on gels containing 8 mol l^{-1} urea, 10 % acrylamide at pH 8.6 (Perrie and Perry, 1970). Actomyosin was prepared from myofibril samples according to Huriaux *et al.* (1990). For SDS–PAGE and alkali urea gels, protein samples were prepared for electrophoresis by boiling for 3 min in a solution containing 60 mmol l^{-1} Tris–HCl, pH 6.76, 2 % SDS, 10 % glycerol, 1 % mercaptoethanol and 0.002 % Bromophenol Blue (Crockford and Johnston, 1990).

Myofibril samples were also characterised using isoelectric focusing on 125 mm×65 mm×0.4 mm gels (Bio-Rad model 111 Mini IEF Cell), containing Bio-lyte carrier ampholytes (pH range 3.5–5.2, 2.25 %, pH range 3.5–9.5, 0.75 %) (Bio-Rad), 2 % Nonidet P-40, 1 % mercaptoethanol, 4.85 % acrylamide and 0.15 % *N*,*N*′-methylene-bis-acrylamide (Crockford and Johnston, 1990). Samples were prepared by dissolving myofibrils in 8 mol l^{-1} urea, 2 % Nonidet P-40 and 1 % mercaptoethanol.

Peptide mapping was used to characterise myosin heavy chains (MHCs). SDS–PAGE gels (7.5 %) of myofibril samples (approximately 5 mg ml^{-1}) were run according to the methodology described above, stained briefly in Coomassie Blue (30 s) and washed with distilled water. MHC bands were cut from the gel, chopped finely and homogenised with a glass pestle, in 400 μl of a medium containing 50 mmol l^{-1} Tris–HCl (pH 7.4) and 0.5 % SDS, and left to stand for 60 min (Crockford *et al.* 1991). The samples were spun in an Eppendorf bench-top centrifuge for 15 min and the top 100–200 μl of buffer was carefully removed, avoiding contamination with gel fragments. MHCs purified in this way ran as single bands on re-electrophoresis. Samples of MHCs were digested with Type 1-S chymotrypsin (from bovine pancreas, Sigma; 200 ng of enzyme per 50 μl sample) at 20 °C for 30 min. Digestion was stopped by the addition of 2-mercaptoethanol (2 %) and SDS (2 %) and heating to 100 °C for 3 min (Crockford *et al.* 1991). The resulting peptides were resolved using 18 % to 22 % SDS–PAGE gels and stained using a Bio-Rad silver stain plus kit.

Myofibrillar ATPase activity

After the fish had been killed, the fast (white) epaxial muscle was rapidly removed from the entire length of the fish, taking care to avoid slow twitch muscle fibres along the lateral line. Myofibrils were prepared according to Johnston *et al.* (1975) in a solution containing 1 % (w/v) Triton X-100, 100 mmol l^{-1} NaCl, 5 mmol l^{-1} EDTA, 10 mmol l^{-1} Tris–HCl, pH 7.2 at 0 °C. The protein concentration of the final sample was determined using a Bio-Rad detergent-compatible protein assay kit. A sample of myofibrils was suspended in glycerol and stored at −80 °C for electrophoretic analysis (described above). Myofibrils (approximately 0.2 mg ml^{-1}) were pre-incubated at 10, 20 and 35 °C for 2 min in a solution containing 30 mmol l^{-1} Tris–HCl, 1.17 mmol l^{-1} EGTA, 1.17 mmol l^{-1} $MgSO_4$, 2.8 mmol l^{-1} $CaCl_2$, pH 7.4 at assay temperature. Following the addition of 6 mmol l^{-1} ATP, the activity of myofibrillar ATPase was assayed by measuring the amount of phosphate produced in 2 min. The reaction was terminated by the addition of 33 % (v/v) of 10 % trichloroacetic acid at 0 °C. The final concentration of inorganic phosphate was determined

by the method of Fiske and Subbarrow (1925). To determine the thermal stability of the myofibrillar ATPase, enzyme activities were monitored following pre-incubation at high temperature (40 °C) for between 5 min and 1 h. ATPase activity was then determined at 40 °C according to the methods described above.

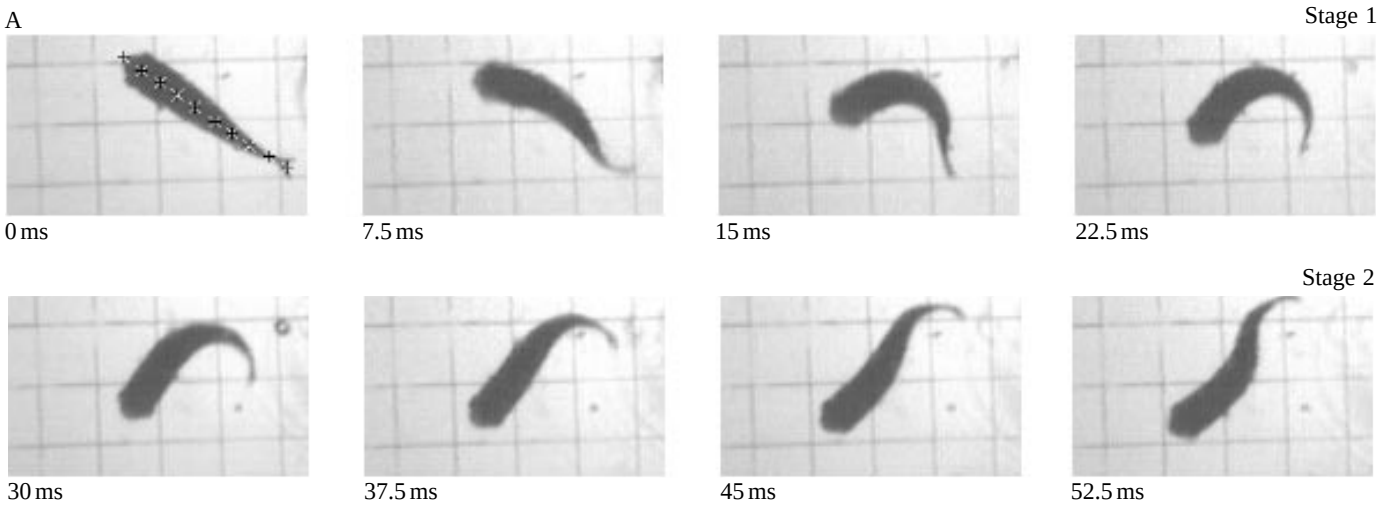
Twitch contraction kinetics

Fast muscle twitch contraction kinetics were measured *in situ* in freshly killed fish using a modification of the method described by Archer *et al.* (1990). The experiments were performed in a Perspex temperature-controlled (±0.1 °C) chamber with a base of Sylgard elastomer on which to secure the specimen with dissection pins. Experimental preparations of goldfish were continually immersed in Ringer's solution containing 119 mmol l^{-1} NaCl, 2.7 mmol l^{-1} KCl, 1 mmol l^{-1} $MgCl_2$, 1.8 mmol l^{-1} $CaCl_2$, 20 mmol l^{-1} $NaHCO_3$ and 10 mmol l^{-1} sodium pyruvate (Johnson *et al.* 1994). Preparations of killifish were maintained in Ringer's solution designed for marine teleosts (Altringham and Johnston, 1988) and containing 132.5 mmol l^{-1} NaCl, 2.6 mmol l^{-1} KCl, 1 mmol l^{-1} $MgCl_2$, 2.7 mmol l^{-1} $CaCl_2$, 18.5 mmol l^{-1} $NaHCO_3$, 3.2 mmol l^{-1} NaH_2PO_4 and 10 mmol l^{-1} sodium pyruvate. The pH of the Ringer's solution was maintained at 7.4 by bubbling with 95 % O_2:5 % CO_2. The abdominal cavity was exposed with a lateral incision, the viscera were carefully removed and the abdominal myotomes pinned back. Abdominal membranes were removed to expose the fast muscle myotomes and a suction electrode was placed on the fourth or fifth spinal nerve close to its origin from the spinal column. During the contraction of the appropriate myotome, force was monitored using a force transducer made from two Entran ESU-060-1000 strain gauges bonded to a spring steel bar. A pin, bonded to the surface of the transducer, was inserted into the adjacent myotome next to the posterior myoseptum. Output signals from the transducer were modified using a custom-designed bridge amplifier and displayed and stored using a Metrabyte A/D converter interfaced to an IBM-PC. Single, supramaximal stimuli (typically 0.1–0.5 ms duration, 20–30 V) were administered *via* the suction electrode using a Grass S48 stimulator connected to a current amplifier. The preparation was allowed to recover for at least 10 min between stimuli. Twitch contraction time (T_c, the time from the onset of force production to 50 % relaxation) was measured in preparations from all acclimation groups and both species at temperatures encompassing the acclimation regimes.

High-speed video analysis

Fish were videotaped performing C-starts (escape responses) in an area 25 cm×20 cm×15 cm using a NAC HSV-400 high-speed video system at 400 frames s^{-1}. Experimental trials were performed in an insulated glass aquarium marked on the base with a 2 cm×2 cm×2 cm grid. Fish were filmed ventrally by placing a front-surface mirror at 45 ° to the horizontal below the tank. Illumination was provided by a combination of 500 W photofloods (Smith Victor) and synchronized strobes (NAC). Temperature was controlled (±0.5 °C) using submersible aquarium heaters and Neslab cryocool immersion coolers. C-starts were elicited either by striking the side of the tank or by dropping a small weight into the tank above the fish. Only C-starts performed from a stationary position were recorded and analyzed. Trials were performed over a broad range of temperatures within the critical thermal limits for the different acclimation groups and species. Critical thermal maximum was defined as the temperature at which fish lost equilibrium and critical thermal minimum as the temperature at which fish failed to respond to tactile stimulation. Fish recovered completely when returned to more moderate temperatures. Water temperature was changed at a rate not exceeding 10 °C h^{-1}. Animals were placed in the experimental arena at the acclimation temperature and allowed to adjust to their new surroundings for no less than 1 h. After recording C-starts at this temperature, the water was either cooled or heated to the next trial temperature. Within acclimation groups, the order in which trials were performed (i.e. above or below acclimation temperature first) was randomized from fish to fish. C-starts at acclimation temperature were recorded again at the end of the experiment to determine repeatability. Up to three C-starts were recorded per fish at each temperature.

C-start sequences were downloaded from the video and stored as digital images on an IBM-PC installed with a PC-VISION*plus* framegrabber card (Imaging Technology Inc., MA, USA). Video frames were downloaded from the first detectable signs of movement by the fish following the stimulus, until the animal moved beyond the field of view or slowed significantly; only sequences in which both stage 1 and stage 2 of the C-start were fully visible were used in the analyses. Individual video images were digitized using customized software ['Measurement Television' (MTV), Garr Updegraff, Data Crunch, San Clemente, CA, USA]. The coordinates of ten evenly spaced points along the midline of the fish from the nose to the tip of the caudal fin were digitized for each image (Fig. 1A). The coordinates for images of each trial were downloaded to Microsoft Excel (version 5.0 for Windows) and plotted (Fig. 1B). During the initial stages of the C-start (stage 1), fish bend about a point known as the stretched body centre of mass (SBCM); this region of the body is particularly important as it is believed to be the target site for predatory strikes (Nissanov and Eaton, 1989). Thus, we chose the point along the midline closest to the SBCM for our kinematic measurements. Three kinematic parameters were measured using a customized macro function in Microsoft Excel. Maximum angular velocity during stage 1 (degrees s^{-1}; MaxANG), measured over 5 ms (2 frames), represents the maximum rate of change of angle between the tip of the head and the SBCM. Maximum velocity (m s^{-1}; MaxVEL) represents the maximum forward velocity attained by the SBCM, measured over 5 ms. The third kinematic parameter measured was the total distance moved by the SBCM in 40 ms ($DIST_{40ms}$), which represents the approximate time for the completion of both stage 1 and stage 2 of a C-start performed at 35 °C. The temperature at which maximum values for each of the kinematic parameters



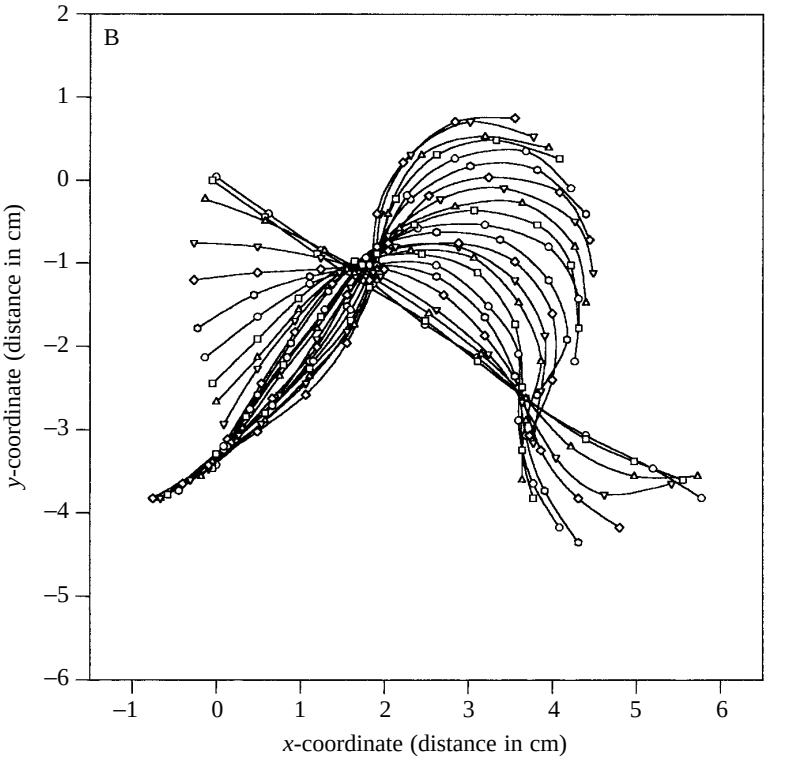


Fig. 1. (A) Video frames taken at 7.5 ms intervals of the C-start of a 35 °C-acclimated goldfish at 35 °C. Note the two stages of the C-start, the initial 'C' (stage 1) and the propulsive stroke of stage 2. The squares represent a scale of 2 cm×2 cm. The midline of the fish in the first frame has been digitized (10 equally spaced points). (B) The midlines of the fish performing the C-start in A, plotted and fitted with a cubic spline function. The origin is the tip of the head at the beginning of the C-start. Data represent the complete C-start sequence, each digitised midline representing consecutive frames 2.5 ms apart.

were obtained was noted and defined as the 'optimum temperature' (cf. Huey and Stevenson, 1979) for that variable.

Statistics

For all experimental variables, the effects of acute temperature change and acclimation were determined using a two-way analysis of variance (ANOVA). The effect of acclimation temperature on the experimental parameters at individual test temperatures was subsequently determined using a one-way ANOVA nested within individuals. The ANOVA was followed by a Tukey's multiple comparison test when it was necessary to isolate specific differences between the values for different acclimation groups. With kinematic data, a Mann–Whitney (non-parametric) *U*-test was used to incorporate data for warm-acclimated individuals that failed to elicit an escape response at low temperature (scored as zero). A Wilcoxon two-sample test (non-parametric) was used to examine differences in the optimum temperature for kinematic parameters between acclimation groups. The Tukey's jackknife method was used to test for differences in the magnitude of the acclimation response between species for each of the experimental parameters (all tests are described in Sokal and Rohlf, 1981).

Results

Polyacrylamide gel electrophoresis

SDS–PAGE gels (10 % to 20 %) of fast muscle myofibrils isolated from animals acclimated to 10 and 35 °C were

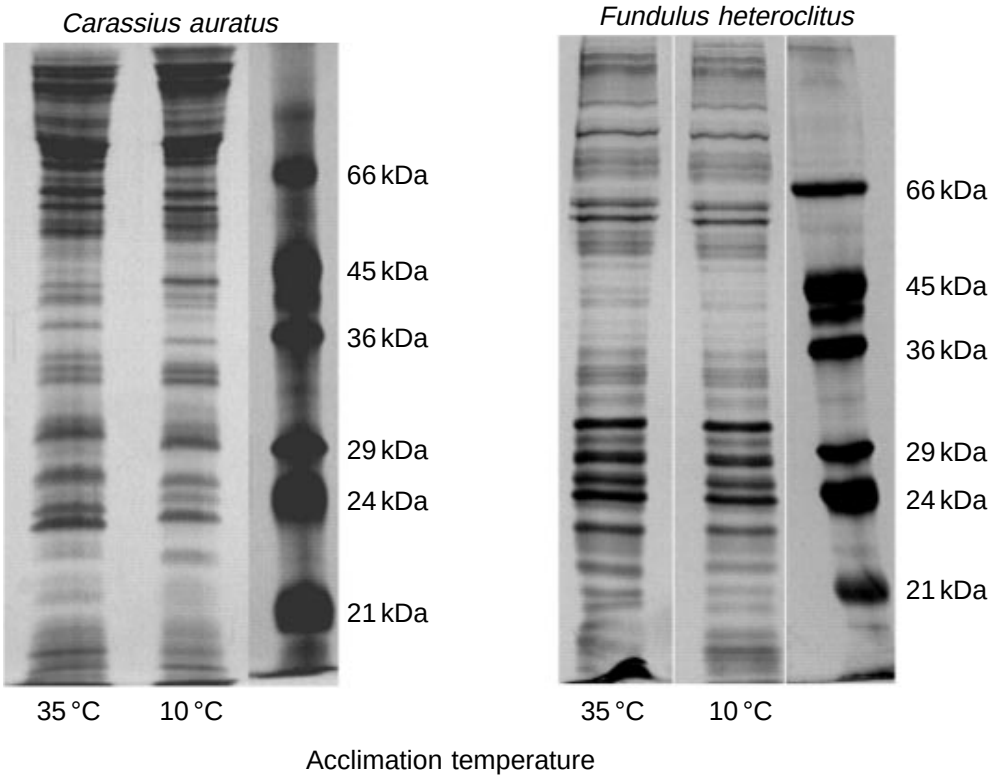


Fig. 2. Peptide maps obtained from myosin heavy chains (MHCs) isolated from goldfish (left) and killifish (right), acclimated to 10 and 35 ˚C. MHCs were digested for 30 min at 25 ˚C and run on SDS–PAGE gels (4 % stacking, 22 % separating gel). The third lane on the right of each gel is a Dalton VII-L (Sigma) molecular mass marker. Note the clear differences in the peptides for 10 ˚C- and 35 ˚C-acclimated goldfish between 50 kDa and 23 kDa.

identical when compared within species. There was no apparent difference in the mobility of myosin heavy and light chains, actins, tropomyosins or troponins. Acclimation temperature also appeared to have no effect on the expression of myosin light chains, visualised using alkali urea gels and isoelectric focusing (IEF), in either goldfish or killifish. However, according to peptide maps obtained for MHCs isolated from 10 ˚C- and 35 ˚C-acclimated fish, there was a consistent and significant shift in the expression of MHCs in the goldfish (N=4) but not in the killifish (N=4). Fig. 2 shows peptide maps obtained for 10 ˚C- and 35 ˚C-acclimated goldfish and killifish (22 % SDS–PAGE).

Myofibrillar ATPase activity

The activity of myofibrillar ATPase was expressed as μmoles of phosphate (P_i) produced per milligram of myofibrillar protein per minute. Both acute temperature exposure and a change in acclimation temperature had a significant effect on myofibrillar ATPase activity in both species (P<0.01, two-way ANOVA). Measured at 10 ˚C, myofibrillar ATPase activity was affected by acclimation temperature for both goldfish (P<0.0001, ANOVA) and killifish (P=0.001, ANOVA) (Fig. 3). However, the magnitude of the effect was much greater in the goldfish (P<0.001, Tukey's jackknife test), ATPase activity measured at 10 ˚C increasing by 530 % in the goldfish and by only 55 % in the killifish, in 35 ˚C-acclimated compared with 10 ˚C-acclimated fish. Acclimation temperature had no significant effect on myofibrillar ATPase activity measured at 35 ˚C (P=0.48, nested ANOVA). Assayed at 35 ˚C, the acclimation of killifish to 35 ˚C did result in a small decrease in ATPase activity compared with those of the 10 ˚C- and 20 ˚C-acclimated groups (P<0.01 ANOVA, P<0.01 Tukey's multiple-comparison test) (Fig. 3).

By pre-incubating myofibrils isolated from goldfish and killifish at 40 ˚C for up to 1 h, we examined the thermal stability of the myofibrillar ATPase. In both species, the ATPase activity of myofibrils isolated from 35 ˚C-acclimated animals was stable throughout the full 60 min exposure to 40 ˚C (Fig. 4). In contrast, ATPase activity declined rapidly in muscle from 10 ˚C-acclimated animals; the responses were almost identical in the goldfish and killifish (Fig. 4), an observation in contrast to previous observations on the latter species (Sidell *et al.* 1983). This shift in thermal stability clearly represents a resistance adaptation and appears to be a widespread phenomenon in teleosts in response to temperature changes over evolutionary as well as seasonal time scales.

Twitch contraction kinetics

Twitch contraction time (T_c) was significantly affected by acute temperature changes and acclimation temperature in both species (P<0.001, two-way ANOVA; Fig. 5). For goldfish, twitch contraction at 10 ˚C was 100 % faster in 10 ˚C-acclimated compared with 35 ˚C-acclimated animals (P<0.001, ANOVA) and 55 % faster than that at 20 ˚C (P<0.001, ANOVA; Fig. 5). There was also a small, but significant, effect of acclimation temperature on T_c measured at 35 ˚C (P<0.01, ANOVA); T_c was 13.4 ms for 10 ˚C-acclimated animals and approximately 10 ms for 20 ˚C- and 35 ˚C-acclimated goldfish (Fig. 5).

The results for the acclimation of killifish followed an almost identical pattern, although the magnitude of the

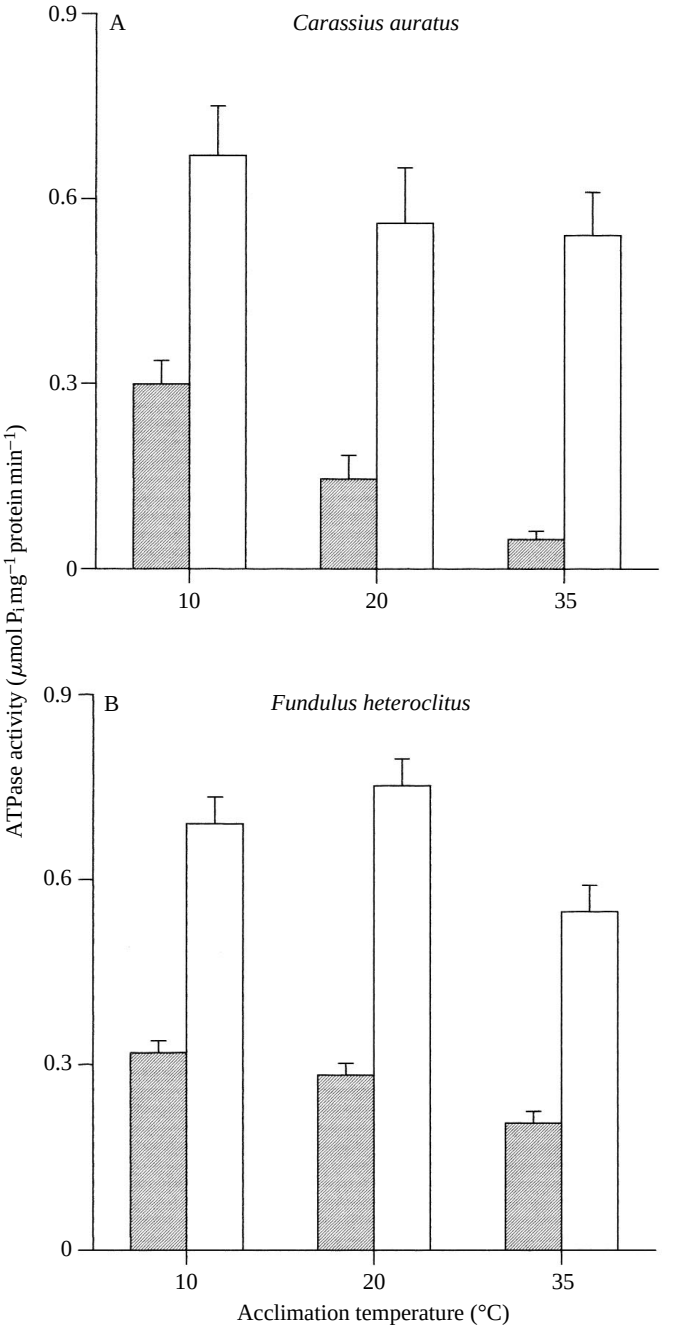


Fig. 3. The activity of myofibrillar ATPase as a function of acclimation temperature for fast muscle myofibrils isolated from goldfish (A) and killifish (B). The shaded bars represent activities measured at 10 ˚C, the unshaded bars represent values obtained at 35 ˚C. Values are expressed as means + S.E.M. (N=8 for each acclimation group and species). Animals used in these experiments were of comparable size (within species). The total lengths (mean ± S.D., N=8) of goldfish were 6.1±0.7 cm, 6.4±0.9 cm and 6.5±0.9 cm and of killifish were 6.5±0.6 cm, 6.8±0.6 cm and 6.6±0.5 cm (10 ˚C-, 20 ˚C- and 35 ˚C-acclimated respectively).

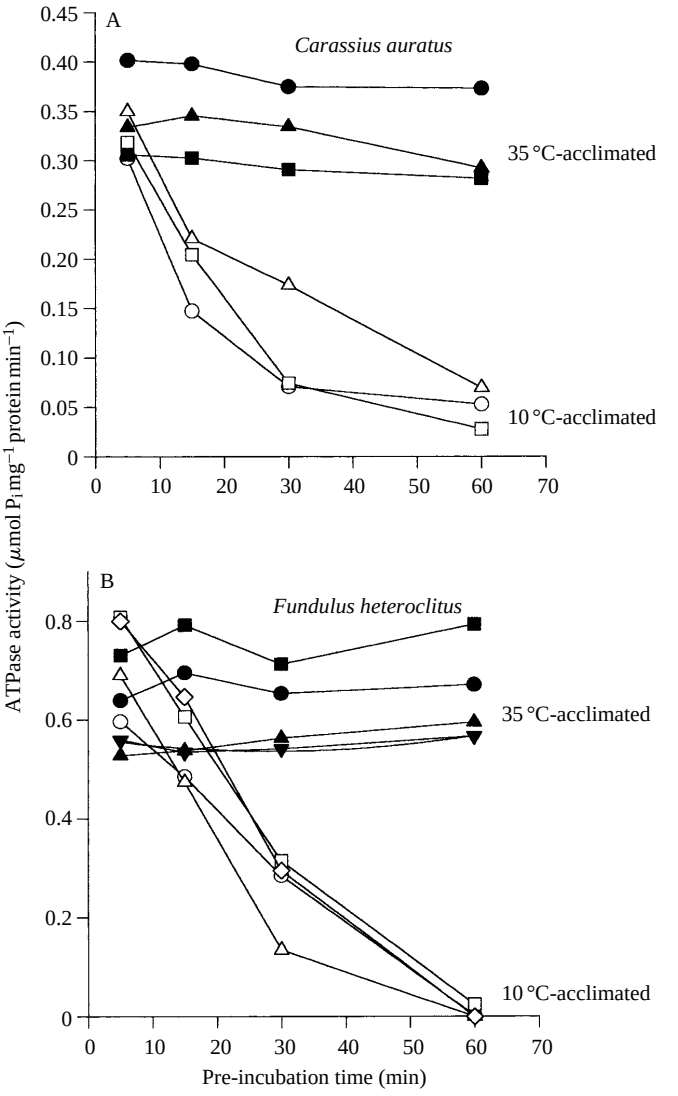


Fig. 4. The activity of myofibrillar ATPase as a function of pre-incubation time at 40 ˚C, for fast muscle myofibrils isolated from goldfish (A) and killifish (B). Myofibrils were pre-incubated for periods of up to 1 h prior to the assay to examine thermal stability. Filled symbols represent values obtained from animals acclimated to 35 ˚C; open symbols represent data obtained from 10 ˚C-acclimated fish (N=3 for goldfish, N=4 for killifish). Warm- and cold-acclimated specimens for each species were of similar size (mean total length±S.D.), for goldfish, 6.2±0.2 cm, 6.4±0.3 cm and for killifish 6.6±0.4 cm, 6.4±0.5 cm (10 °Cand 35 °C-acclimated fish respectively).

response of T_c was much smaller (P<0.001, Tukey's jackknife test on values obtained at 10 ˚C; Fig. 5). At 10 ˚C, T_c decreased by 35 % from 50 ms to 37 ms in killifish acclimated to 35 ˚C and 10 ˚C respectively (P<0.001, ANOVA; Fig. 5). The T_c of animals acclimated to 10 ˚C was significantly slower at 20 ˚C by around 24 % (P<0.001 ANOVA) when compared with those acclimated to 20 and 35 ˚C (P<0.001, Tukey's multiple comparison test). Acclimation temperature had no significant effect on T_c measured at 35 ˚C in killifish (P=0.69, ANOVA; Fig. 5).

High-speed video analysis

Acute temperature exposure had a significant effect on all kinematic variables measured for both species and acclimation groups (P<0.05, two-way ANOVA).

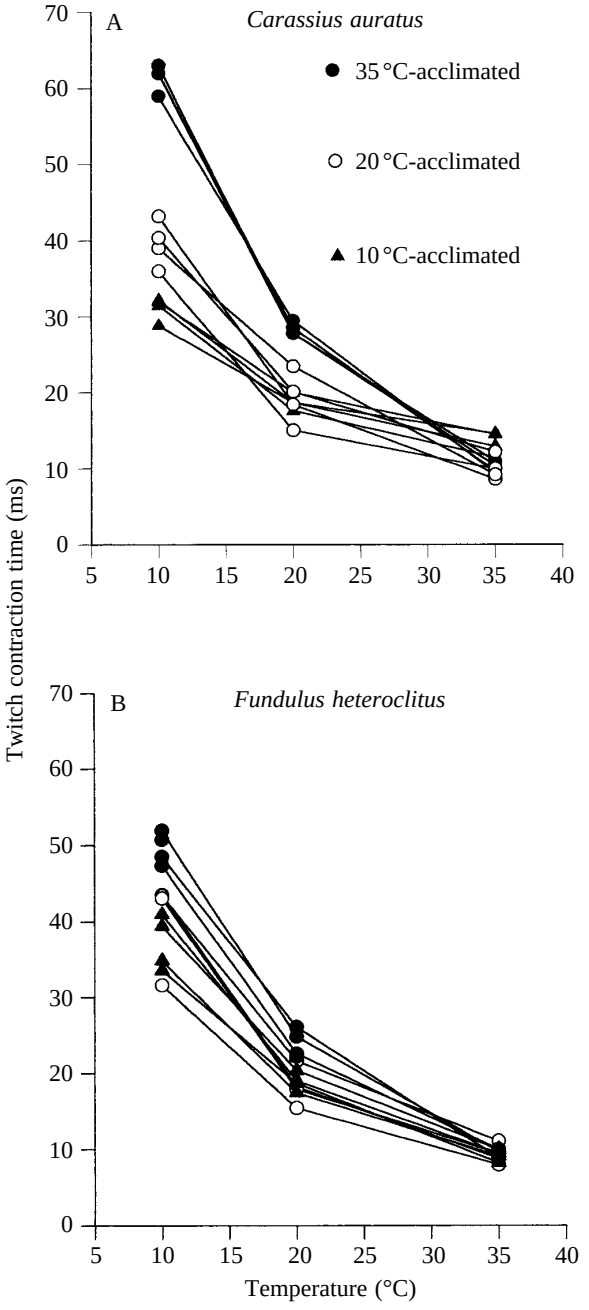


Fig. 5. Twitch contraction time (time from the onset of force to 50 % relaxation) as a function of temperature for goldfish (A) and killifish (B), acclimated to 10, 20 and 35 ˚C. Data represent the mean of three measurements made for each individual at each test temperature (N=4 individuals for each acclimation group and species). All individuals of each species were of comparable size (mean total length ± S.D.); for goldfish, 7.0±0.4 cm, 6.6±1.3 cm and 6.6±0.7 cm and for killifish, 6.3±0.6 cm, 6.0±0.5 cm and 6.4±0.2 cm (10 ˚C-, 20 ˚C- and 35 ˚C-acclimated respectively).

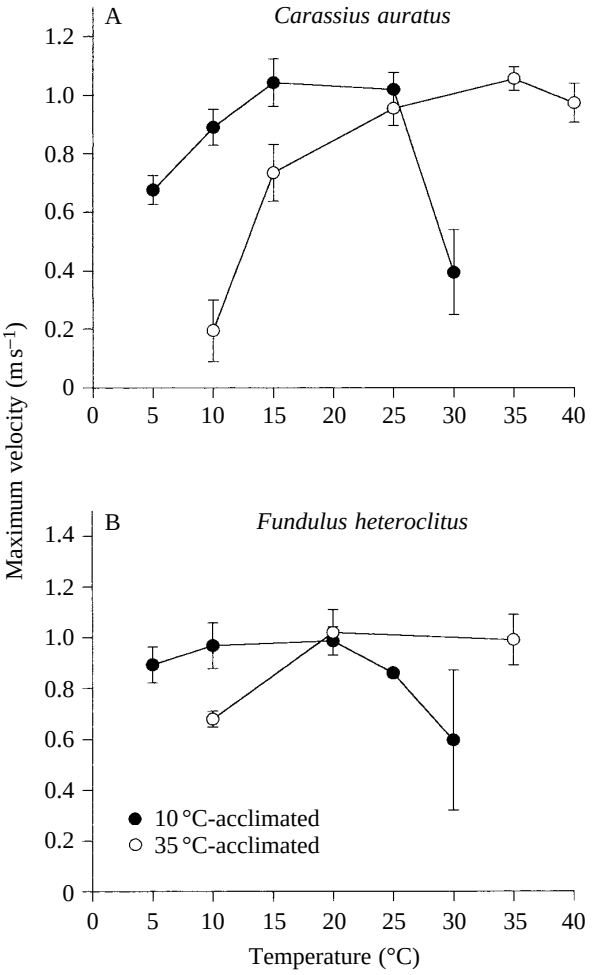


Fig. 6. Maximum velocity attained during C-starts following acute exposure to a range of temperatures (x-axis), for goldfish (A) and killifish (B), acclimated to 10 ˚C (filled circles) and 35 ˚C (open circles). Values represent means ± S.E.M. (N=10 for each acclimation group of goldfish and N=5 for each acclimation group of killifish). Fish that failed to respond to stimuli at a fiven temperature were scored as zero (see text). The size of fish did not differ between acclimation groups of the same species and sizes were as follows (mean total length ± S.D.); for goldfish, 6.6±0.5 cm and 6.6±0.4 cm and for killifish, 7.6±1.2 cm and 7.8±1.1 cm (10 ˚C- and 35 ˚C-acclimated respectively).

Analysing the data with a two-way analysis of variance, all kinematic parameters were highly significantly affected by acclimation temperature in the goldfish (P<0.005). For goldfish acclimated to 35 ˚C, seven out of 10 individuals failed to respond to stimuli and elicit a C-start response at 10 ˚C. All goldfish acclimated to 10 ˚C responded with a C-start response at 10 ˚C. Using the available data at 10 ˚C for C-starts of 10 ˚C- and 35 ˚C-acclimated goldfish (N=10 and N=3, respectively), MaxVEL increased by 35 % (P=0.05, ANOVA; Fig. 6), $DIST_{40ms}$ by 52 % (P=0.01, ANOVA; Fig. 7) and MaxANG by 42 % (P=0.07, ANOVA; Fig. 8) with a decrease in acclimation temperature from 35 to 10 ˚C. Alternatively, by scoring zero for 35 ˚C-acclimated individuals that would not C-start at 10 ˚C, a Mann–Whitney U-test resulted in a highly significant effect of acclimation temperature for all three kinematic variables measured at 10 ˚C.

For killifish, acclimation temperature had a significant effect on MaxANG only (P<0.01, two-way ANOVA), although the

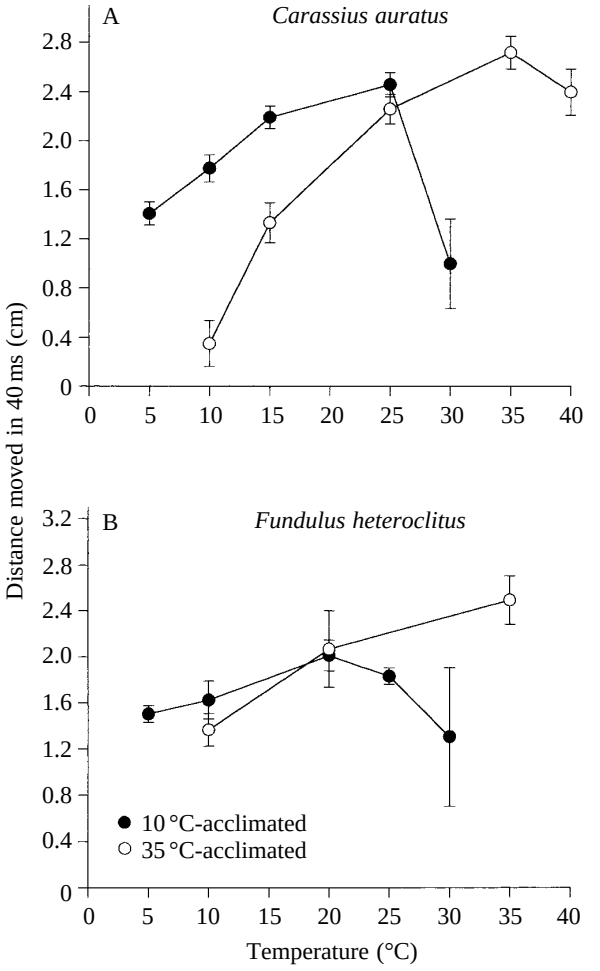


Fig. 7. The total distance moved in 40 ms by fish in C-starts following acute exposure to a range of temperatures (*x*-axis), for goldfish (A) and killifish (B), acclimated to 10 ˚C (filled circles) and 35 ˚C (open circles). Values represent means ± S.E.M. (*N*=10 for each acclimation group of goldfish and *N*=5 for each acclimation group of killifish). Fish that failed to respond to stimuli were scored as zero (see text). Total lengths for fish are given in the legend to Fig. 6.

interaction between acute temperature exposure and acclimation temperature was significant for MaxVEL (*P*=0.02, two-way ANOVA). All killifish acclimated to 10 and 35 ˚C (*N*=5 at both temperatures) elicited C-starts at 10 ˚C. Measured at 10 ˚C, MaxVEL and MaxANG were significantly greater in 10 ˚C-acclimated compared with 35 ˚C-acclimated killifish (*P*=0.009 and *P*=0.004 respectively, ANOVA; Figs 6, 8). Acclimation temperature had no significant effect on $DIST_{40ms}$ measured at 10 ˚C (*P*=0.22, ANOVA; Fig. 7).

In Figs 6–8, the three kinematic variables are plotted against temperature for goldfish and killifish acclimated to 10 and 35 ˚C. Note the shift of the curves to the right with an increase in acclimation temperature (i.e. an increase in optimum temperature). In goldfish, this increase in optimum temperature was significant for all kinematic parameters; optimum temperature increased by 21 ˚C for MaxVEL (*P*=0.004, Wilcoxon), 13 ˚C for MaxANG (*P*=0.01, Wilcoxon) and 14 ˚C for $DIST_{40ms}$ (*P*=0.004, Wilcoxon; Figs 6–8). For killifish, acclimation temperature had a significant effect on the optimum temperature for MaxANG (*P*=0.04, Wilcoxon), increasing it from 22 to 35 ˚C. The changes in optimum temperature were only 8 ˚C for MaxVEL and 4 ˚C for $DIST_{40ms}$ with an increase in acclimation temperature from 10 to 35 ˚C, which are not significant (*P*=0.197 and *P*=0.102 respectively, Wilcoxon).

Table 1. *Values of Q_{10} for experimental parameters calculated from mean values over equivalent temperature ranges (10–35 °C)*

	Goldfish		Killifish	
	10 °C-acclimated	35 °C-acclimated	10 °C-acclimated	35 °C-acclimated
Myofibrillar ATPase	1.4	2.6	1.4	1.5
Twitch contraction speed	1.4	2.1	1.8	1.9
Kinematics				
MaxVEL	*	2.0	*	1.2
MaxANG	*	2.0	*	1.3
$DIST_{40ms}$	*	2.3	*	1.3

*Values not available as animals would not C-start at 35 °C.

MaxVEL, maximum velocity attained by the stretched body centre of mass; MaxANG, maximum angular velocity during stage 1 of the C-start; $DIST_{40ms}$, time taken to complete stages 1 and 2 of a C-start at 35 °C.

Discussion

Acute responses to temperature change

The biochemical and contractile properties of fast muscle of goldfish and killifish are profoundly affected by acute changes in temperature (Table 1). This thermal dependence of muscle function agrees with many previous observations (Bennett, 1990; Johnston *et al.* 1990). Despite the significant thermal dependence of isolated muscle function, very little is known about the thermal dependence of C-start escape behaviour at the organismal level. Some earlier studies reported burst speed to be relatively independent of temperature (Beamish, 1966; Brett, 1967, 1970), but more recent investigations have found a significant thermal effect (Rulifson, 1977; Webb, 1978; Fuiman, 1986; Batty and Blaxter, 1992). The present investigation is the first strictly integrative study to examine the thermal dependence of C-start performance at all levels of organisation from molecular to organismal. For both species (goldfish and killifish), the thermal dependence of myofibrillar ATPase activity and twitch contraction kinetics is reflected in the thermal dependence for all organismal kinematic parameters measured for C-starts (Table 1). The thermal dependence of myofibrillar ATPase and twitch contraction kinetics are thus potentially important determinants of *in vivo* muscle function and C-start performance.

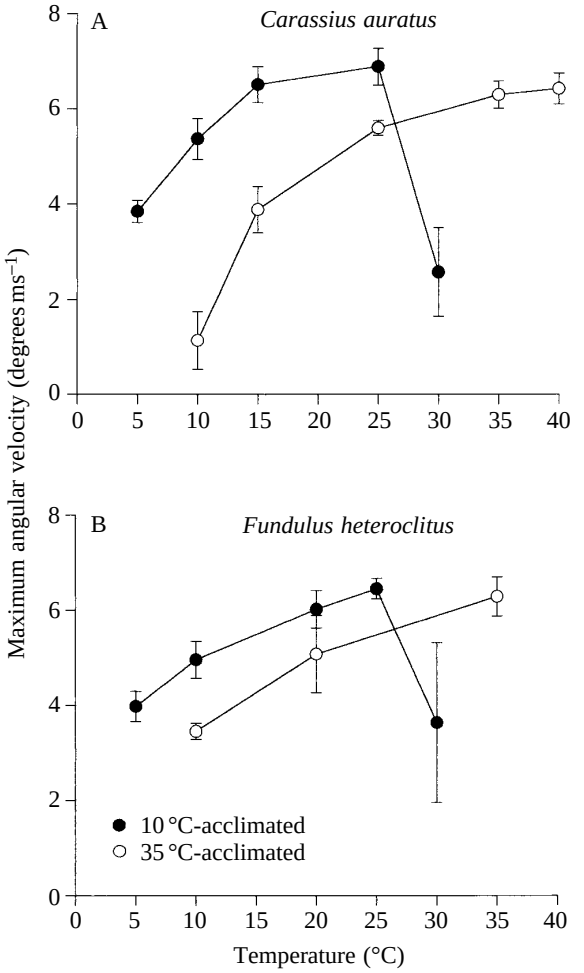


Fig. 8. The maximum angular velocity for fish during C-starts following acute exposure to a range of temperatures (*x*-axis), for goldfish (top) and killifish (bottom), acclimated to 10 ˚C (filled circles) and 35 ˚C (open circles). Values represent means ± S.E.M. (*N*=10 for each acclimation group of goldfish and *N*=5 for each acclimation group of killifish). Fish that failed to respond to stimuli were scored as zero (see text). Total lengths of fish are given in the legend to Fig. 6.

Acclimatory responses to long-term temperature change

In addition to the acute effects of temperature, prolonged exposure to low temperature results in a significant increase in myofibrillar ATPase activity and twitch contraction speed in at least in some teleost species, notably the cyprinids (see Figs 3, 5). In relation to organismal performance, goldfish acclimated to 35 ˚C can swim at approximately 1.0 m s^{-1} and yet may achieve only 0.2 m s^{-1} at 10 ˚C, if they swim at all (a majority of our 35 ˚C-acclimated fish did not; Fig. 6). However, following a period of acclimation to 10 ˚C for 4 weeks, animals may swim at speeds up to 0.9 m s^{-1}, demonstrating almost perfect temperature compensation (Fig. 6). The results of this study are unique in that they corroborate the hypothesis that the thermal acclimatory responses in the cyprinids previously demonstrated at the biochemical and tissue level represent a mechanism of maintaining locomotor performance at low temperature.

Both goldfish and killifish are known to be relatively eurythermal species, capable of tolerating a wide seasonal range of temperatures (Elliot, 1981; Sidell *et al.* 1983). However, killifish, being an estuarine species, are commonly subjected to large and rapid reductions in temperature of up to 10–15 ˚C in less than an hour (Sidell *et al.* 1983). Consistent with previous observations, myofibrillar ATPase activity is comparatively insensitive to changes in temperature in killifish (Q_{10}=1.4–1.5) (Table 1; Sidell *et al.* 1983). From observations in the wild (Sidell *et al.* 1983) and in the laboratory on C-start performance (this study; Figs 6–8), locomotor capability is not substantially impaired by the acute changes in temperature commonly experienced in the summer months (12–35 ˚C). However, a significant effect of acclimation temperature on myofibrillar ATPase activity was found in this study across a broader acclimation regime (10–35 ˚C) than previously studied (5–25 ˚C for Sidell *et al.* 1983). Killifish acclimated to 35 ˚C, compared with 10 ˚C- and 20 ˚C-acclimated fish, apparently have a significantly lower ATPase activity. No apparent changes in the expression of myofibrillar protein isoforms were found, so the response of ATPase to acclimation appears to be achieved through a mechanism other than differential isoform expression (reviewed in Johnston and Dunn, 1987). A small acclimatory response was also demonstrated for twitch contraction kinetics (Fig. 5). Apparently there are some compensatory mechanisms to maintain locomotor ability in this species at low temperature. In comparison with goldfish, however, acclimatory mechanisms are clearly different and the resulting compensation in locomotor performance is substantially less.

Polyploidy and acclimatory ability

The unique thermal acclimatory ability of carp and goldfish to undergo significant physiological changes at biochemical and cellular levels of organisation has received considerable attention (reviewed in Johnston and Dunn, 1987: Johnston *et al.* 1990). This ability has been attributed to their polyploid genetic structure (Sidell and Johnston, 1985; Goldspink *et al.* 1992). Specifically, the multiple gene copies afforded by chromosomal duplication are presumed to permit the evolution of alleles appropriate to different thermal environments, as demonstrated in the studies of Baldwin and Hochachka (1970) and Somero (1975). Differential expression of temperature-specific isoforms is, of course, not the only mechanism by which enzymatic changes in response to thermal acclimation may be mediated (for discussion, see Johnston and Dunn, 1987). However, the thermal acclimation of fast twitch muscle myofibrillar ATPase and twitch contraction speed in carp and goldfish appear to be mediated through the differential expression of temperature-specific isoforms of myosin heavy chains (Gerlach *et al.* 1990; Hwang *et al.* 1990; Goldspink *et al.* 1992; Fig. 2 this study) and myosin light chains (Crockford and Johnston, 1990), consistent with the hypothesis that polyploidy is important for the formation of temperature-specific isoforms in response to thermal acclimation in these fish.

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References

ALTRINGHAM, J. D. AND JOHNSTON, I. A. (1988). The mechanical properties of polyneuronally innervated myotomal muscle fibres isolated from a teleost fish (*Myoxocephalus scorpius*). *Pflügers Arch.* **412**, 524–529.

ARCHER, S. D., ALTRINGHAM, J. D. AND JOHNSTON, I. A. (1990). Scaling effects on the neuromuscular system, twitch kinetics and morphometrics of the cod, *Gadus morhua*. *Mar. Behav. Physiol.* **17**, 137–146.

BALDWIN, J. AND HOCHACHKA, P. W. (1970). Functional significance of isozymes in thermal acclimatization: acetylcholinesterase from trout brain. *Biochem. J.* **116**, 883–887.

BATTY, R. S. AND BLAXTER, J. H. S. (1992). The effects of temperature on the burst swimming performance of fish larvae. *J. exp. Biol.* **170**, 187–210.

BEAMISH, F. W. H. (1966). Swimming endurance of some Northwest Atlantic fishes. *J. Fish. Res. Bd Can.* **23**, 341–347.

BEAMISH, F. W. H. (1978). Swimming capacity. In *Fish Physiology*, vol. VII (ed. W. S. Hoar and D. J. Randall), pp. 101–187. New York: Academic Press.

BENNETT, A. F. (1990). Thermal dependence of locomotor capacity. *Am. J. Physiol.* **259**, R253–R258.

BRETT, J. R. (1967). Swimming performance of sockeye salmon in relation to fatigue time and temperature. *J. Fish. Res. Bd Can.* **24**, 1731–1741.

BRETT, J. R. (1970). Fishes. In *Marine Ecology*, vol. 1, *Environmental Factors* (ed. O. Kinne), pp. 513–560. New York: John Wiley and Sons.

CROCKFORD, T. C. AND JOHNSTON, I. A. (1990). Temperature acclimation and the expression of contractile protein isoforms in the skeletal muscles of common carp (*Cyprinus carpio* L.). *J. comp. Physiol.* B **160**, 23–30.

CROCKFORD, T. C., WOMACK, K. E., JOHNSTON, I. A., MCANDREW, B. J., MUTUNGI, G. AND JOHNSON, T. P. (1991). Inter- and intra-specific variation in myosin light chain and troponin I composition in fast muscle fibres from two species of fish (genus *Oreochromis*) which have different temperature-dependent contractile properties. *J. Muscle Res. Cell Motility* **12**, 439–446.

EATON, R. C., BOMBARDIERI, R. A. AND MEYER, D. H. (1977). Mauthner initiated startle response in teleost fish. *J. exp. Biol.* **66**, 65–81.

ELLIOT, J. M. (1981). Some aspects of thermal stress on freshwater teleosts. In *Stress and Fish* (ed. A. D. Pickering), pp. 209–245. London: Academic Press.

FISKE, C. H. AND SUBBAROW, Y. J. (1925). The colorimetric determination of phosphorus. *J. biol. Chem.* **66**, 374–400.

FLEMING, J. R., CROCKFORD, T., ALTRINGHAM, J. D. AND JOHNSTON, I. A. (1990). The effects of temperature acclimation on muscle relaxation in the carp, a mechanical, biochemical and ultrastructural study. *J. exp. Zool.* **255**, 286–295.

FUIMAN, L. A. (1986). Burst-swimming performance of larval danios and the effects of diel temperature fluctuations. *Trans. Am. Fish. Soc.* **115**, 143–148.

GERLACH, G., TURAY, L., MAILIK, K., LIDA, J., SCUTT, A. AND GOLDSPINK, G. (1990). The mechanisms of seasonal temperature acclimation in carp: a combined physiological and molecular biology approach. *Am. J. Physiol.* **259**, R237–R244.

GOLDSPINK, G., TURAY, L., HANSEN, E., ENNION, S. AND GERLACH, G. (1992). Switches in fish myosin genes induced by environment temperature in muscle of the carp. *Symp. Soc. exp. Biol.* **46**, 139–149.

GUDERLEY, H. AND BLIER, P. (1988). Thermal acclimation in fish: Conservative and labile properties of swimming muscle. *Can. J. Zool.* **66**, 1105–1115.

HEAP, S. P., WATT, P. W. AND GOLDSPINK, G. (1985). Consequences of thermal change on the myofibrillar ATPase of five freshwater teleosts. *J. Fish Biol.* **26**, 733–738.

HEAP, S. P., WATT, P. W. AND GOLDSPINK, G. (1986). Myofibrillar ATPase activity in the carp *Cyprinus carpio*: interactions between starvation and environmental temperature. *J. exp. Biol.* **123**, 373–382.

HUEY, R. B. AND STEVENSON, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *Am. Zool.* **19**, 357–366.

HURIAUX, F., VANDEWALLE, P., PHILIPART, J. C. AND FOCANT, B. (1990). Electrophoretic comparison of myosin light chains and parvalbumins of trunk and head muscles from two barbel (*Barbus barbus*) populations. *Comp. Biochem. Physiol.* B **97**, 547–553.

HWANG, G. C., WATABE, S. AND HASHIMOTO, K. (1990). Changes in carp myosin ATPase induced by temperature acclimation. *J. comp. Physiol.* B **160**, 233–239.

JOHNSON, T. P., SYME, D. A., JAYNE, B. C.. LAUDER, G. V. AND BENNETT, A. F. (1994). Modeling red muscle power output during steady and unsteady swimming in largemouth bass (*Micropterus salmoides*): A comparison of *in vitro* and *in vivo* function. *Am. J. Physiol.* **267**, R481–R488.

JOHNSTON, I. A. AND DUNN, J. F. (1987). Temperature acclimation and metabolism in ectotherms with particular reference to teleost fish. In *Temperature and Animal Cells* (ed. K. Bowler and B. J. Fuller), pp. 67–93. Cambridge: Symposia of the Society for Experimental Biology XXXXI.

JOHNSTON, I. A., FLEMING, J. D. AND CROCKFORD, T. (1990). Thermal acclimation and muscle contractile properties in cyprinid fish. *Am. J. Physiol.* **259**, R231–R236.

JOHNSTON, I. A., JOHNSON, T. P. AND BATTRAM, J. C. (1991). Does low temperature limit burst swimming performance in Antarctic fish ? In *Second International Conference: Biology of Antarctic Fishes* (ed. G. di Prisco, B. Maresca and B. Tota), pp. 179–190. Heidelberg: Springer Verlag.

JOHNSTON, I. A., WALESBY, N. J., DAVISON, W. AND GOLDSPINK, G. (1975). Temperature adaptation in myosin of Antarctic fish. *Nature* **254**, 74–75.

JOHNSTON, I. A. AND WOKOMA, A. (1986). Effects of temperature and thermal acclimation on contractile properties and metabolism of skeletal muscle in the flounder (*Platichthys flesus* L.). *J. exp. Biol.* **120**, 119–130.

LOUGHNA, P., ROME, L. C. AND GOLDSPINK, G. (1983). The effects of temperature acclimation upon fibre type recruitment in fish myotomal muscle. *Am. Zool.* **23**, 887.

MARSH, R. L. (1990). Deactivation rate and shortening velocity as determinants of contractile frequency. *Am. J. Physiol*. **259**, R223–R230.

MARSH, R. L. AND BENNETT, A. F. (1985). Thermal dependence of isometric contractile properties of skeletal muscle and sprint performance of the lizard *Dipsosaurus dorsalis*. *J. comp. Physiol*. **155**, 541–551.

MARSH, R. L. AND BENNETT, A. F. (1986). Thermal dependence of isotonic contractile properties of skeletal muscle from the lizard *Sceloporous occidentalis* with comments on methods for fitting and comparing force–velocity curves. *J. exp. Biol*. **126**, 79–87.

MOERLAND, T. S. AND SIDELL, B. D. (1986). Biochemical responses to temperature in the contractile protein complex of striped bass, *Morone saxatilis*. *J. exp. Zool*. **238**, 287–295.

NISSANOV, J. AND EATON, R. C. (1989). Reticulospinal control of rapid escape turning manoeuvres in fishes. *Am. Zool*. **29**, 103–121.

PENNEY, R. K. AND GOLDSPINK, G. (1981). Temperature adaptation by the myotomal muscle of fish. *J. therm. Biol.* **6**, 297–306.

PERRIE, W. T. AND PERRY, S. V. (1970). An electrophoretic study of the low molecular-weight components of myosin. *Biochem. J*. **119**, 31–38.

ROME, L. C. (1990). Influence of temperature on muscle recruitment and muscle function *in vivo*. *Am. J. Physiol*. **259**, R210–R222.

RULIFSON, R. A. (1977). Temperature and water velocity effects on the swimming performance of young of the year striped mullet, spot and pinfish. *J. Fish. Res. Bd Can*. **34**, 2316–2322.

SIDELL, B. D. AND JOHNSTON, I. A. (1985). Thermal sensitivity of contractile function in chain pickerel *Esox niger*. *Can. J. Zool.* **63**, 811–816.

SIDELL, B. D., JOHNSTON, I. A., MOERLAND, T. S. AND GOLDSPINK, G. (1983). The eurythermal protein complex of the mummichog (*Fundulus heteroclitus*): adaptation to a fluctuating thermal environment. *J. comp. Physiol.* B **153**, 167–173.

SIDELL, B. D. AND MOERLAND, T. S. (1989). Effects of temperature on muscular function and locomotory function in teleost fish. *Adv. comp. env. Physiol*. **5**, 115–156.

SOKAL, R. R. AND ROHLF, F. J. (1981). *Biometry*, 2nd edition. New York: W. H. Freeman and Co.

SOMERO, G. N. (1975). The roles of isozymes in adaptation to varying environments. In *Isozymes II. Physiological Function* (ed. C. L. Market), pp. 221–234. New York: Academic Press.

SWOAP, S. J., JOHNSON, T. P., JOSEPHSON, R. K. AND BENNETT, A. F. (1993). Temperature, muscle power and limitations on burst locomotor performance in the lizard *Dipsosaurus dorsalis*. *J. exp. Biol*. **174**, 185–197.

WARDLE, C. S. (1975). The limit of fish swimming. *Nature* **255**, 725–727.

WEBB, P. W. (1978). Effects of temperature on fast-start performance of rainbow trout, *Salmo gairdneri*. *J. Fish Res. Bd Can*. **35**, 1417–1422.

ZOTTOLI, S. J. (1977). Correlation of the startle reflex and the Mauthner cell auditory responses in unrestrained goldfish. *J. exp. Biol*. **66**, 243–254.