

# Thermal Dependence and Acclimation of Fast Start Locomotion and Its Physiological Basis in Rainbow Trout (*Oncorhynchus mykiss*)

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## Abstract

Rainbow trout (family Salmonidae, *Oncorhynchus mykiss*) were acclimated to 5° and 20° C for 4 wk. The acclimatory response of the C-start (fast-escape swimming) and the molecular, biochemical, and contractile properties of fast-twitch muscle were investigated. With peptide mapping, no change was noted in the expression of myosin heavy chains in fast-twitch muscles with thermal acclimation. The myofibrillar ATPase activity of fast-twitch muscle isolated from individuals of each acclimation group was determined at 5° and 20° C. The activity of myofibrillar ATPase was highly temperature dependent ( $Q_{10} = 2.9$ ). Acclimation temperature had no significant effect on ATPase activity measured at 5° or 20° C. The thermal stability of the myofibrillar ATPase, however, was significantly increased following a period of acclimation to a higher temperature. Fast-twitch muscle contraction time (force onset to 50% relaxation) decreased from 32 ms to 12 ms with an acute change in temperature from 5° to 20° C ( $Q_{10} = 1.9$ ). Acclimation to low temperature resulted in only a small increase in twitch contraction speed (11%). After specimens were exposed to either 5° or 20° C, C-starts were elicited at both acclimation temperatures. The linear speed (m/s) and angular velocity (degrees/s) attained by the fish at each temperature were both temperature-dependent ( $Q_{10} = 1.2$ ). The total distance moved in 40 ms and the angular velocity of the fish at 20° C increased slightly (24% and 15%, respectively) following a period of acclimation to the higher temperature. Acclimation temperature had no statistically significant effect on maximum linear velocity. Thus, trout are capable of only minor acclimatory adjustments in response to exposure to high temperature. Further, it appears that this acclimatory response has not been facilitated by the differential expression of temperature-specific myofibrillar protein isoforms, as has been previously found in other polyploid fish species.

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## Introduction

Cyprinid fishes, such as the carp (*Cyprinus carpio* L.) and goldfish (*Carassius auratus* L.), are capable of significant phenotypic adaptations of fast muscle function in response to temperature change over seasonal timescales (see Johnston, Fleming, and Crockford 1990). As a foundation to the study of thermal acclimation of fast muscle physiology, Johnston, Davison, and Goldspink (1975) were the first to describe a change in the kinetic properties of a contractile protein in response to thermal acclimation: fast muscle myofibrillar ATPase of goldfish measured at low temperature increased significantly following a period of cold acclimation. Subsequently, this effect has been correlated with the maximum shortening velocity ( $V_{max}$ ) of this muscle fiber type (Crockford and Johnston 1990). Changes in the kinetic properties of the  $\text{Ca}^{2+}$ -ATPase of sarcoplasmic reticulum and isometric twitch contraction speed of fast muscle have also been found in conjunction with temperature acclimation in cyprinids (Fleming et al. 1990).

It has been suggested that carp and goldfish are particularly adept at acclimating to long-term temperature changes owing to their polyploid genetic condition (Sidell and Johnston 1985; Goldspink et al. 1992). It has been proposed that this chromosomal duplication may have facilitated the evolution of new alleles appropriate to different thermal environments. In other words, as with other enzyme systems (see Johnston and Dunn 1987), different isoforms of muscle contractile proteins may be expressed after long-term exposure to different temperatures in order to maintain contractile function and presumably locomotor performance. Indeed, it has been shown that different myosin heavy chains (MHCs) (Gerlach et al. 1990; Hwang, Watabe, and Hashimoto 1990; Goldspink et al. 1992) and myosin light chains (Crockford and Johnston 1990) are expressed by the fast-twitch muscle of carp in response to long-term exposure to low temperature;  $V_{max}$  and presumably power output are thereby adjusted to compensate for the detrimental effects of low temperature on swimming performance (Crockford and Johnston 1990). However, compared with other groups of teleost fishes or even other ectotherms, this pattern of thermal acclimation is apparently unusual. For example, muscle contractile properties are unaffected by acclimation temperature in several noncyprinid fish species (reviewed in Johnston et al. [1990]), reptiles, and amphibians (Renaud and Stevens 1981, 1984; Putnam and Bennett 1982; Rome 1983; Else and Bennett 1987). However, recent studies of a diploid marine teleost, the short-horned sculpin (*Myoxocephalus scorpius*), have shown that fast muscle contractile properties (power output,  $V_{max}$ , force production) change significantly in response to

seasonal changes in temperature (Johnson and Johnston 1991; Beddow and Johnston 1995).

The significance of this pattern of thermal acclimatory ability at the sub-organismal level for locomotor performance is a question that is only now being addressed (Beddow, van Leeuwen, and Johnston 1995; Johnson and Bennett 1995). Integrated studies of physiology and behavior at all levels of organization are essential to the understanding of complex organismal phenomena: "Individual traits do not evolve independently, rather they evolve only in the context of a complete, functioning organism" (Bennett 1989). Thus, in this study we set out to determine the thermal acclimatory response of burst escape swimming (C-starts) in rainbow trout (*Oncorhynchus mykiss* L.) and its basis in the underlying properties of fast muscle function at the molecular, biochemical, and cellular levels of organization. Trout, as all the extant members of the family Salmonidae, are polyploid. Therefore, this investigation in companion with others (see Johnson and Bennett 1995) serves as a further test to the idea that ploidy level may be an important factor in the evolution of thermal acclimatory ability.

## Material and Methods

### *Fish*

Rainbow trout (family Salmonidae, *Oncorhynchus mykiss*) were obtained from Oregon State University at Corvallis. Animals were maintained in 30-gal glass aquaria with fresh aerated water under ambient light conditions (16L:8D) and were fed blood worms and trout chow ad lib. Trout were divided into two acclimation groups and maintained at 5° and 20°C ( $\pm 0.5^\circ\text{C}$ ) for at least 4 wk prior to experimentation. Fish were all of similar size, with a mean total length of  $5.9 \pm 0.4$  cm (SD,  $n = 50$ ).

### *Electrophoretic Analysis of Myofibrillar Proteins*

Fish were killed by a blow to the back of the head and decapitated. Fast-twitch muscle myofibrils were isolated for the determination of myofibrillar ATPase activity (see methods described below). Myofibril samples were stored in glycerol at  $-80^\circ\text{C}$  for up to 4 wk.

Myofibrils were prepared for SDS-polyacrylamide electrophoresis (SDS-PAGE) by boiling for 3 min in a solution containing 60 mM Tris-HCl, pH 6.75, 2% SDS, 10% glycerol, 1% 2-mercaptoethanol, and 0.002% bromophenol blue. Gels with SDS-PAGE were run using a Bio-Rad Mini Protean II system with vertical slab gels (80 mm  $\times$  73 mm  $\times$  0.5 mm). Samples from

both acclimation groups ( $5^{\circ}$  and  $20^{\circ}\text{C}$ ,  $n = 3$  for each) were run on separating gels of various acrylamide concentrations (10%–18%) and a stacking gel of 4%.

Changes in the activity of fast-twitch muscle myofibrillar ATPase with thermal acclimation have previously been associated with a change in the expression of MHCs (Johnson and Bennett 1995). Therefore, MHCs were isolated from fast-twitch muscle fiber samples of both acclimation groups ( $n = 3$  for each). Myofibril samples were run on SDS-PAGE gels (7.5% separating, 4% stacking) and stained briefly (30 s) in 0.25% Coomassie blue, 40% methanol, and 10% glacial acetic acid. The MHC bands were cut from the gel and homogenized with a hand-held glass rod in approximately 2–3 vol of 0.5% SDS, 50 mM Tris-HCl, pH 7.5. Samples were incubated on ice for 1 h and spun for 15 min in an Eppendorf bench-top centrifuge. The top 100–200  $\mu\text{L}$  of sample were removed without contamination by gel fragments. The MHCs isolated in this way ran as single bands on re-electrophoresis. The MHCs were digested with type 1-S  $\alpha$ -chymotrypsin from bovine pancreas (Sigma) by adding 200 ng of enzyme to 50  $\mu\text{L}$  of sample. Digestion was allowed to proceed for 30 min at  $25^{\circ}\text{C}$  and stopped by the addition of 2-mercaptoethanol (2%) and SDS (2%) and heating to  $100^{\circ}\text{C}$  for 3 min. The resulting peptides were visualized by running the samples on 12% SDS-PAGE gels and staining with a Bio-Rad Silver Stain Plus kit.

#### *Myofibrillar ATPase Activity*

After they were killed, fish were skinned and the majority of fast-twitch (white) muscle was removed, with care taken to avoid contamination with slow-twitch (red) fibers from the region around the lateral line. All procedures involved in the isolation of myofibrils were performed on ice. The muscle samples were homogenized in an extraction buffer containing Triton X-100, 1% (wt/vol); KCl (100 mM); ethylenediaminetetraacetic acid (EDTA) (5 mM); and Tris-HCl (10 mM), with a pH of 7.2 at  $0^{\circ}\text{C}$ . Myofibrils were isolated by centrifuging the samples at 10,000  $\text{g}$  for 10 min. This procedure was repeated five times. A small sample was removed and suspended in glycerol for electrophoretic analysis (see above), and the remaining pellet suspended in an equal volume of KCl (150 mM) and Tris-HCl (30 mM), with a pH of 7.2 at  $0^{\circ}\text{C}$ . Protein concentration in the final sample was determined with a Bio-Rad Detergent Compatible Protein Assay kit (modification of the Lowry determination). Samples were typically 5–10 mg protein/mL.

The activity of myofibrillar ATPase was determined at  $5^{\circ}$  and  $20^{\circ}\text{C}$  for eight individuals of each acclimation group according to the procedure outlined by Johnston et al. (1975). The activity of ATPase was assayed by

measuring the release of inorganic phosphate ( $P_i$ ) in a medium containing Tris-HCl (30 mM) (pH 7.4 at assay temperature), ethylene glycol-bis(β-aminoethyl ether) (EGTA) (1.17 mM),  $MgSO_4$  (1.17 mM),  $CaCl_2$  (2.8 mM), ATP (2 mM), and 0.5–1.0 mg myofibrillar protein. Protein samples were preincubated at the assay temperature for 3 min. The reaction was started by the addition of ATP and terminated after 2 min by the addition of 0.33 vol of 10% trichloroacetic acid and placed on ice. The final concentration of  $P_i$  was determined by the procedure of Fiske and Subbarow (1925). Values were expressed as the number of micromoles of  $P_i$  released per milligram of protein per min ( $\mu\text{mol } P_i/\text{mg}/\text{min}$ ).

In order to examine the effect of acclimation temperature on the thermal stability of the myofibrillar ATPase, four muscle samples from each acclimation group were preincubated at 32.5°C for 15 min. Activity of ATPase was then assayed at this temperature by the methods outlined above.

#### *Twitch Contraction Kinetics*

An in situ method of measuring contraction kinetics (modified from Archer, Altringham, and Johnston [1990]) was employed in this study, as the small size of the fish made isolated muscle preparations impossible. Direct measurements of force output were not possible with this technique. Muscle twitches were elicited and recorded for the fast abdominal myotomes of four trout from each acclimation temperature. Fish were killed, and the head and viscera removed from the abdominal cavity. The preparation was placed into a Plexiglas chamber containing Ringer's of the following composition:  $NaCl$  (119 mM),  $KCl$  (2.7 mM),  $MgCl_2$  (1 mM),  $CaCl_2$  (1.8 mM),  $NaHCO_3$  (20 mM), and sodium pyruvate (10 mM), with a pH of 7.4 at experimental temperature (Johnson et al. 1994). Temperature was controlled ( $\pm 0.1^\circ\text{C}$ ) through a cooling jacket connected to a Neslab temperature-controlled water bath. The fish were pinned down to secure the abdominal myotomes flush to the surface of the chamber (see fig. 1 of Altringham and Johnston [1988]). The peritoneum and connective tissue were carefully removed to expose the abdominal muscle fibers and associated spinal nerves radiating from the vertebral column (extreme care is required to avoid damaging the nerves and muscle fibers). A suction electrode was placed on the fourth or fifth spinal nerve to stimulate the associated myotomes. Supramaximal stimuli were administered with a Grass S48 stimulator (typically 0.1–0.5 ms duration, 20–30 V). At least 10 min rest was given between each stimulus. Force was measured by inserting a needle connected to a transducer into the muscle against the posterior myoseptum. The force transducer consisted of two Entran ESU-060-1000 strain gauges bonded to a steel bar.

Signals from the transducer were modified with a bridge amplifier (in-house design) and downloaded to an IBM-PC using a Metrabyte A/D board. Force records were visualized and analyzed through customized software (METRA, Paul Steinbach). Twitch contraction time ( $T_c$ ), defined as the time from the onset of force production to 50% relaxation, was measured for preparations acutely exposed to temperatures ranging from 5°C to 30°C. Temperature was varied in a random fashion. Twitch contraction kinetics were highly reproducible during ascending or descending temperature changes.

### C-Start Performance

The C-start is an extremely rapid and highly stereotyped escape response, triggered by the Mauthner cell in teleost fish (Nissanov and Eaton 1989). It is characterized in two stages (fig. 1a). In stage 1, the body of the fish bends into a "C" about a fixed point known as the stretched body center of mass (SBCM) (fig. 1b) as a result of the simultaneous activity of all the muscle fibers along one side of the body. The SBCM represents an important point both biomechanically and ecologically, as it is believed to be the target for predatory strikes (Nissanov and Eaton 1989). Stage 2 of the C-start is characterized by an S-shaped propulsive stroke during which the SBCM is accelerated away from its original position.

To examine C-start performance, fish were placed into a customized glass aquarium (25 cm × 20 cm × 15 cm) at their acclimation temperature for at least 2 h prior to experimentation. By striking the side of the aquarium, we elicited C-starts and then videotaped them with an NAC-HSV400 video system at 400 frames per second. Fish were then acutely exposed to the acclimation temperature of the other group (5° or 20°C) by heating or cooling the water at a rate no more than 10°C per hour. The C-starts were filmed at this temperature and upon return of fish to their own acclimation temperature. At least three C-starts were filmed following a temperature change for five individuals of each acclimation group. A minimum of 10 min rest was allowed between successive C-starts at each trial temperature.

Video frames from each C-start were downloaded to an IBM-PC using a PCVISIONplus frame grabber card (Imaging Technology, Bedford, Mass.). Frames were downloaded from the first detectable movement following the stimulus to a point at which the fish had noticeably slowed after stage 2 of the C-start. The midlines of the fish were digitized for each frame with customized software called MTV (Measurement Television, Garr Updegraff, Data Crunch, San Clemente, Calif.). Ten equally spaced points were placed along the midline of the fish (fig. 1a), and the coordinates entered into a table in MTV. For each trial (C-start sequence), the table of

**a)****Stage 1**

0 ms



10 ms



20 ms

**Stage 2**

30 ms



40 ms



50 ms

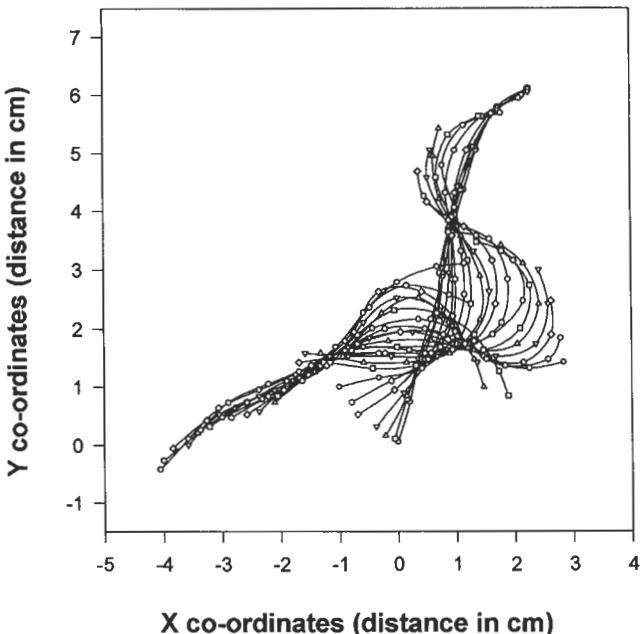
**b)**

Fig. 1. a, Selected video frames from a C-start sequence of a 20° C-acclimated trout swimming at 20° C. Note the two stages of the C-start: stage 1, where the body bends into the "C", and stage 2, where the animal's center of mass is accelerated from its original position. The first video frame has had the midline digitized with 10 equally spaced points from the tip of the head to the tip of the tail. b, A graph showing the digitized midlines for the entire video sequence of the C-start of the fish shown in part a. The origin represents the tip of the head at the start of the sequence. The coor-

coordinates was transferred to Microsoft Excel version 5.0 for Windows and graphed (fig. 1b). With a customized macro for Microsoft Excel, the SBCM was determined and three kinematic parameters were calculated. Maximum velocity (MAXvel, m/s) was calculated from the maximum distance moved by the SBCM in 5 ms (two consecutive video frames). Maximum angular velocity (MAXang, degrees/s [ $^{\circ}$ /s]), was calculated from the maximum change in angle between the tip of the head and the SBCM, turned in 5 ms. The total distance moved by the SBCM in 40 ms (DIST<sub>40ms</sub>), a parameter used in a companion study (Johnson and Bennett 1995), was also determined.

### Statistics

A repeated measures one-way ANOVA was used to determine the effects of acute and acclimation temperature on the experimental parameters. A one-way ANOVA, nested within individuals, was then used to examine the effects of acclimation at each experimental temperature. A *t*-test was also used on a number of occasions (all tests from Sokal and Rohlf [1981]).

## Results

### *Electrophoretic Analysis of Myofibrillar Proteins*

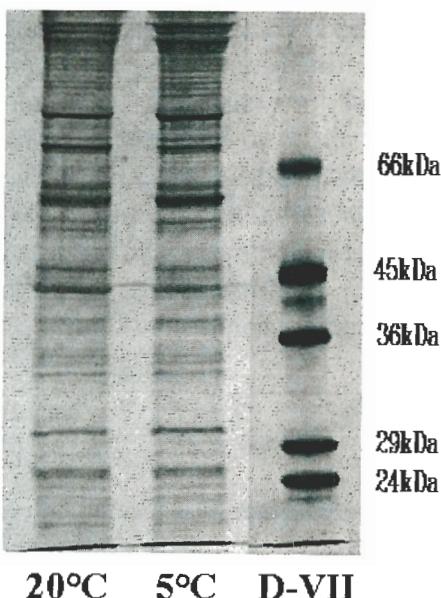
Myofibrillar proteins were characterized on SDS-PAGE gels (10%–18%) according to molecular weight. No differences were found in the migration of MHCs, myosin light chains, actins, tropomyosins, and troponins between muscle samples from the two acclimation groups. Peptide maps of isolated MHCs were also found to be identical for 5°- and 20°C-acclimated fish (fig. 2). This constancy is in contrast to the findings for the MHCs of goldfish acclimated to 10° and 35°C, digested and analyzed under identical conditions (cf. fig. 2 of Johnson and Bennett [1995]).

### *Myofibrillar ATPase Activity*

The activity of myofibrillar ATPase in trout was highly temperature-dependent (repeated measures ANOVA,  $P < 0.001$ ) (fig. 3) with a Q<sub>10</sub> of 2.9.

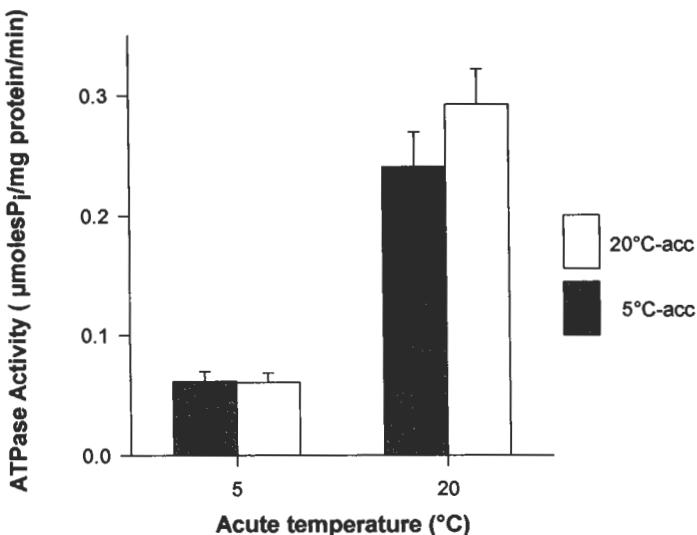
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*dinates from each video frame have been fitted with a cubic spline. Note that the fish initially bends about the third point along the body, representing the SBCM.*



*Fig. 2. Peptide maps of MHCs isolated from fish acclimated to 20°C (left) and 5°C (right). The MHCs were digested for 30 min with type 1-S  $\alpha$ -chymotrypsin and run on 12% SDS-PAGE gels. The third lane to the right is a Dalton VII-L molecular weight marker from Sigma.*

Acclimation state did not significantly affect ATPase activity (ANOVA: 20°C acclimation,  $P = 0.23$ ; 5°C acclimation,  $P = 0.94$ ) (fig. 3). Myofibril samples from 5°C-acclimated trout maintain a stable ATPase activity for up to 60 min at 30°C ( $0.56 \pm 0.05$   $\mu\text{g P}_i/\text{mg}/\text{min}$ , for four specimens). However, even short exposure (5 min) to 32.5°C resulted in a large decline in ATPase activity (typically 75%–85%). At 32.5°C, ATPase activity typically reached a maximum value for both 5°C and 20°C-acclimated trout following a 15-min preincubation. Values of ATPase were therefore measured for the two acclimation groups following a 15-min exposure to 32.5°C. The activity of myofibrillar ATPase was  $0.44 \pm 0.21$   $\mu\text{g}/\text{mg}/\text{min}$  for 20°C-acclimated fish and only  $0.12 \pm 0.05$   $\mu\text{g}/\text{mg}/\text{min}$  ( $\bar{X} \pm \text{SD}$ ) for 5°C-acclimated fish, following a 15-min exposure to 32.5°C ( $t$ -test,  $P > 0.05$ ). This difference suggests a measurable increase in the thermal stability of this enzyme following a period of warm acclimation, in contrast to previous observations on this species (Penney and Goldspink 1981). It is now apparent from this and other recent studies (Johnson and Bennett 1995) that the ability of myofibrillar ATPase to increase thermal stability during long-term exposure to high temperature is a feature of teleosts of wide phylogenetic origins.



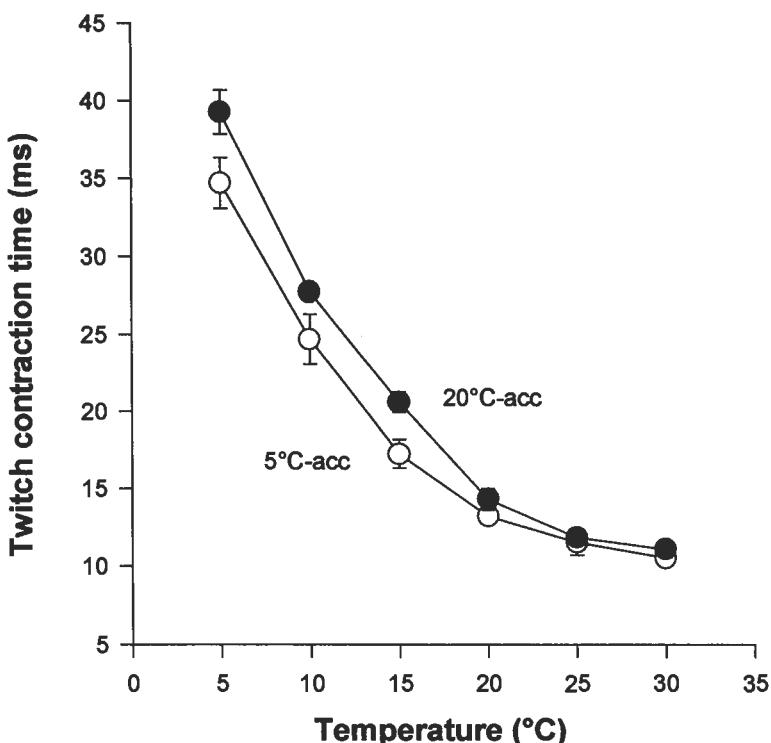
*Fig. 3.* The activity of myofibrillar ATPase measured at 5° and 20°C (X-axis) for fast muscle myofibrils isolated from trout. The shaded bars represent data obtained from 5°C-acclimated fish, and unshaded bars represent values for 20°C-acclimated fish. Values are expressed as means  $\pm$  SE ( $n = 8$  for each acclimation group). The sizes of warm- and cold-acclimated specimens used in this assay were not significantly different.

#### Twitch Contraction Kinetics

Twitch contraction time was also found to be highly temperature-dependent (repeated measures ANOVA,  $P < 0.001$ )—typically 32 ms at 5°C and 12 ms at 20°C ( $Q_{10} = 1.9$ ) (fig. 4). Acclimation to cold temperature did result in a minor (approx. 11%) but significant decrease in  $T_c$  measured at temperatures below 15°C (repeated measures ANOVA,  $P = 0.05$ ), with a significant interaction between acute temperature exposure and acclimation temperature (repeated measures ANOVA,  $P = 0.01$ ) (fig. 4). By comparing data obtained at 5° and 20°C separately, acclimation temperature was also found to have a significant effect on twitch contraction speed measured at 5°C ( $t$ -test,  $P = 0.05$ ) but not at 20°C ( $t$ -test,  $P = 0.18$ ).

#### C-start Performance

Acute temperature exposure had a significant effect on all three kinematic variables (repeated measures ANOVA,  $P \leq 0.001$ ), with a  $Q_{10}$  of 1.2–1.3 (fig. 5). Acclimation temperature had no significant effect on MAXvel (repeated measures ANOVA,  $P = 0.64$ ) tested at a trial temperature of 5°C (ANOVA,



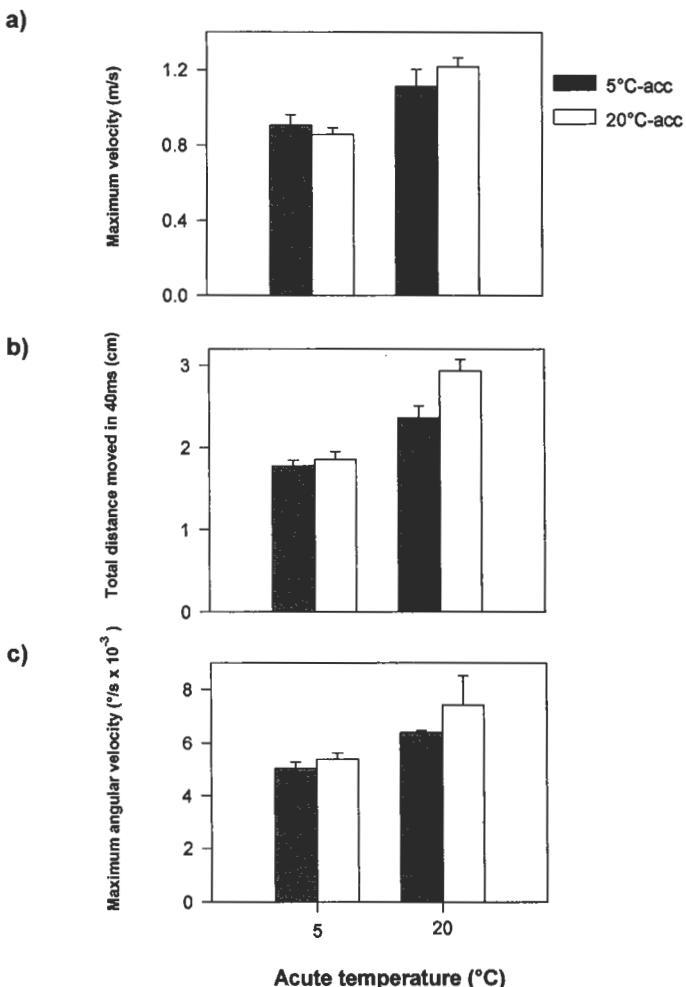
*Fig. 4. Twitch contraction time (time from the onset of force to 50% relaxation) as a function of temperature for trout acclimated to 5°C (open circles) and 20°C (solid circles). Values represent means  $\pm$  SE ( $n = 5$  for each acclimation group). There was no significant difference in the size of individuals from each acclimation group.*

$P = 0.42$ ) or 20°C (ANOVA,  $P = 0.29$ ) (fig. 5). However, acclimation to 20°C did result in a 15% increase in MAXang and a 24% increase in DIST<sub>40ms</sub> measured at 20°C (ANOVA,  $P = 0.04$  and  $P = 0.02$ , respectively) (fig. 5). A significant interaction between acclimation temperature and acute temperature for DIST<sub>40ms</sub> was found (repeated measures ANOVA,  $P = 0.03$ ). No kinematic parameters measured at 5°C were significantly affected by acclimation (ANOVA,  $P = 0.28$ ,  $P = 0.42$ , and  $P = 0.46$  for MAXang, MAXvel, and DIST<sub>40ms</sub>, respectively).

## Discussion

### Effects of Acute Temperature Change

The burst escape swimming speeds of a number of teleost species have recently been shown to be significantly affected by an acute decline in tem-



*Fig. 5. Kinematic parameters obtained from 5°C-acclimated (shaded bars) and 20°C-acclimated fish (unshaded bars) performing C-starts at 5° and 20°C (X-axis). The three parameters measured were (a) the maximum velocity (m/s), (b) the distance moved by the SBCM in 40 ms (cm), and (c) the maximum angular velocity (°/s). Values represent means  $\pm$  SE for five fish of each acclimation group. Fish from different acclimation groups were of similar size.*

perature (fig. 5, this study; Johnson and Bennett 1995). For example, the maximum velocity of trout during a C-start declines by 30% following an acute drop in temperature from 20°C to 5°C ( $Q_{10} = 1.2\text{--}1.3$ ) (fig. 5). This reduction in swimming speed is also accompanied by a significant decline in the twitch contraction kinetics ( $Q_{10} = 1.9$ ) and myofibrillar ATPase activity

( $Q_{10} = 2.9$ ) of fast-twitch muscle fibers recruited for this locomotor behavior (figs. 3 and 4; Johnson and Bennett 1995). The effects of temperature on the contractile and enzymatic properties of muscle, however, are greater than the observed effects on locomotor performance. Thus, although the integration of studies at the organismal and suborganismal levels provide us with some insight into the physiological basis of C-start performance, one must still remain cautious when extrapolating from the isometric and enzymatic properties of muscle *in vitro* to muscle function *in vivo* (Beddow et al. 1995). The dynamic properties of muscle *in vivo* are clearly more complex, as muscle length and force change continuously during each tail beat (Beddow et al. 1995).

#### *Response to Long-Term Temperature Change*

The ability of goldfish to change the enzymatic and contractile properties of fast-twitch muscle fibers has recently been shown to represent an important mechanism of enhancing locomotor performance during long-term exposure to low temperature (Johnson and Bennett 1995). Despite this significant acclimatory response in cyprinids, it remains that several species of teleost, including the rainbow trout (figs. 2 and 3; Penney and Goldspink 1981) and the killifish *Fundulus heteroclitus* (Johnson and Bennett 1995), appear to show only very minor adjustments in the activity of myofibrillar ATPase and the rate of twitch contraction during long-term exposure to cold temperature. For example, the activity of fast-twitch muscle myofibrillar ATPase increases fivefold in goldfish and by only 50% in the killifish (Johnson and Bennett 1995). The increase in the activity of fast-twitch muscle myofibrillar ATPase with cold acclimation in the goldfish is known to be associated with the differential expression of MHC isoforms (Johnson and Bennett 1995). In neither the killifish (Johnson and Bennett 1995) nor the trout (fig. 2) is thermal acclimation associated with a change in the expression of fast-twitch muscle MHC isoforms. Thus, in comparison to the goldfish (see figs. 6–8 of Johnson and Bennett [1995]), locomotor (C-start) performance in the trout is severely compromised by an acute and long-term reduction in temperature from 20° to 5°C (fig. 5). It is interesting that, in contrast to observations on the carp and goldfish (Johnston et al. 1990; Johnson and Bennett 1995), it is at higher temperatures that we see acclimatory adjustments (albeit minor ones) in the C-start performance of trout (fig. 5). A similar pattern has been recently observed for burst swimming in the short-horned sculpin: at 15°C, sculpin acclimated to 15°C swim 30%–40% faster than those acclimated to 5°C (Beddow et al. 1995). These results have been correlated with changes in the contractile properties of fast-twitch

muscle fibers (Johnson and Johnston 1991; Beddow and Johnston 1995). In particular, there is a failure of excitation-contraction coupling at high temperature in cold-acclimated fish, resulting in a significant decrease in isometric force; twitch contraction kinetics at high temperature were also found to increase with warm acclimation. No significant acclimatory changes in the twitch contraction kinetics of trout muscle were found at 20°C (fig. 4). Acclimatory changes in the C-start performance of trout at high temperature may also reflect, at least in part, a failure of excitation-contraction coupling in fast-twitch muscle fibers in cold-acclimated fish, but this assertion remains to be tested. At 15°C, the  $V_{max}$  of fast-twitch muscle is 2.4 times higher in warm- than cold-acclimated scorpion fish (Beddow and Johnston 1995). Since  $V_{max}$  is significantly correlated with the activity of myofibrillar ATPase (Barany 1967), Beddow and Johnston (1995) suggested, but did not demonstrate, that temperature acclimation of sculpin results in altered myosin gene expression, as has been found in the carp (Crockford and Johnston 1990; Hwang et al. 1990; Gerlach et al. 1990) and goldfish (Johnson and Bennett 1995). In trout, we found no significant effect of acclimation to 5° or 20°C in myofibrillar ATPase, although we note that the mean value of the 20°C-acclimated group was 20% above the 5°C-acclimated group at 20°C. It is possible that higher acclimation temperatures might reveal a significant acclimation effect on ATPase activity in these fish.

#### *Polyplody and Thermal Acclimatory Ability*

It has been hypothesized (Sidell and Johnston 1985; Goldspink et al. 1992) that polyplody is an important determinant of thermal acclimatory ability. That is, multiple gene copies afforded by chromosomal duplication may have permitted the evolution of different temperature-specific alleles appropriate to different thermal environments. This hypothesis found support in observations of differential myosin isoform expression in polyploid cyprinid fish acclimated to different temperatures (Crockford and Johnston 1990; Hwang et al. 1990; Johnson and Bennett 1995). Such differential expression is generally absent in diploid fish and other ectothermic vertebrates. The hypothesis was further supported by the presence of different, thermally appropriate isoforms of acetylcholine esterase in brains of rainbow trout, which are polyploid, acclimated to different temperatures (Baldwin and Hochachka 1970). However, the generality of the latter observation in other polyploid fish or the absence of such isoforms in diploid fish has not been examined.

We tested the generality of the association between polyplody and acclimation ability by measuring differential myosin expression and its phys-

iological and organismal consequences in another species of polyploid fish, rainbow trout. Polyploidy in the salmonid clade arose independently from that in the cyprinids and is thus a separate evolutionary development (Allendorf and Thorgaard 1984). By comparison with recent observations on the polyploid goldfish (Johnson and Bennett 1995), we were unable to detect significant acclimatory effects on myosin isoform expression or major changes in other physiological and organismal characters associated with fast muscle contraction kinetics. We conclude, therefore, that there is not a necessary association between polyploidy and acclimation ability. The presence of polyploidy may predispose a group to differential isoform evolution but does not guarantee that it will occur. The formation of temperature-specific isoforms is only one mechanism by which ectotherms might manifest long-term temperature changes (reviewed in Johnston and Dunn [1987]); for instance, changes in pH associated with temperature acclimation can have important effects on enzyme function (e.g., Somero 1981). The fact remains, however, that the most pronounced thermal acclimation of fast muscle contraction is manifest in cyprinid fish. Whether this is a specific association with polyploidy or is a more general property of this clade of fish (which also contains diploid groups) is a topic for further investigation.

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