

The biomechanics and evolutionary significance of thermal acclimation in the common carp *Cyprinus carpio*

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Wakeling, James M., Nicholas J. Cole, Kirsty M. Kemp, and Ian A. Johnston. The biomechanics and evolutionary significance of thermal acclimation in the common carp *Cyprinus carpio*. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R657–R665, 2000.—The effects of thermal acclimation were investigated in the common carp *Cyprinus carpio* L. Acclimation and acute temperature effects were tested during ontogeny from larval [9.5 mm total length (*L*)] to juvenile (69.0 mm *L*) stages and between 8 and 21°C. The myosin heavy chain (MHC) composition, myofibrillar Mg^{2+} - Ca^{2+} -ATPase activity, and muscle strains showed significant thermal acclimation effects. MHCs were only expressed in an acclimation temperature-dependent fashion in fish longer than 37 mm. During fast starts, the temperature had a significant effect on the white muscle strain (33% increase and 50% decrease with increasing acclimation and acute temperature, respectively) and contraction duration (25% decrease with increasing acute temperature). Increases in hydrodynamic efficiency (0.19 to 0.38) and hydrodynamic power requirements ($Q_{10} = 3.2$) occurred with increasing acute temperature (10 to 20 °C). Competing hypotheses about the evolutionary significance of the temperature acclimation response were tested. Acclimation extended the temperature range for fast-start behavior, but no improvements in performance at the whole animal level were found between 8 and 21°C.

acute temperature; fast start; kinematics; hydrodynamic efficiency; muscle mechanics

THERMAL ACCLIMATION IS THE process by which an organism adjusts its physiology or performance in response to an imposed change in environmental temperature and is a particular case of phenotypic plasticity. The temperature ranges for heat and cold stress, feeding, and growth are often modified by a period of acclimation in ectotherms (3). Changes in competitive fitness with thermal acclimation state have been studied with direct competition experiments for both *Escherichia coli* (17) and *Drosophila melanogaster* (36), and thermal acclimation was not found to be beneficial in either case. However, for higher animals, it is more practical to test for a performance parameter that is a plausible correlate of fitness, such as the fast starts used to escape predators (28, 29). Swimming performance has

been shown to be function of acclimation temperature in several fish species (7, 11, 12, 29), however, fast-start behaviors were only considered by two of these studies (11, 29).

Phenotypic plasticity is not only an attribute of the adult phenotype, but the ontogenetic features of the reaction norm (26) should also be considered and may even be the basis of selection (27). For instance, in the carp, a minimum temperature of 15–18°C is required for normal embryonic development after which larval and juvenile stages gradually develop tolerance to lower temperatures (25). By their adult stage, carp are active and feed between 12 and 32°C and are able to enter a state of winter dormancy below 8°C (5). Acclimation of swimming ability is only significant in evolutionary terms if the modified performance confers a selective advantage (9) at temperatures experienced by natural populations.

To determine the significance of acclimation responses, a set of competing a priori hypotheses must be tested (9). In this study, we have tested three such hypotheses about thermal acclimation based on the natural history of the common carp: 1) a beneficial acclimation hypothesis, which predicts that acclimation to a particular temperature gives an organism a performance advantage over another organism that has not had the opportunity to acclimate to that particular environment (17, 36); 2) an optimal developmental temperature hypothesis, which predicts that fish acclimated to one “optimal” temperature will perform best at all acute temperatures (2, 36); and 3) a no-advantage hypothesis, which predicts that acclimation temperature will have no effect on the performance of the fish across the range of temperatures it is normally active. The carp were acclimated to the range of temperatures over which they actively forage for food in the British Isles. One group was hatched and reared at a typical summer temperature of 21°C, a second group was slowly cooled to 15°C after hatching, and a third cooled further to 8°C to mimic the seasonal cooling down to fall temperatures in the wild. It was also hypothesized that the early larval stages would not show temperature acclimation of fast-start perfor-

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mance together with associated changes in muscle properties, but rather the capacity to modify these parameters would be acquired in juveniles concomitant with the onset of seasonal cooling.

The hypotheses were tested by analyzing the fast-start swimming performance of these carp at various stages during ontogeny. Fast starts are rapid acceleration maneuvers used for both escape and prey-capture behaviors in fish, and the acceleration, and therefore velocity, achieved during a fast start is likely to affect survival for both activities (28). However, the mass-specific inertial power required to accelerate a fish in its direction of travel may be a better measure of the fast-start performance as this parameter is the product of the forward components of both the velocity and acceleration (6, 30). An integrative approach was used to identify possible mechanisms for thermal acclimation and so the muscle function during swimming; contractile properties, myosin heavy chain (MHC) composition and Mg^{2+} - Ca^{2+} -ATPase activities were also measured.

METHODS

Fish and acclimation groups. Eggs of common carp *Cyprinus carpio* L. from more than 10 females were fertilized at 23°C and transferred 5 days posthatch to different temperature regimens. The fish were reared for 29 wk at either a constant temperature of 21°C or at temperature regimens designed to simulate a seasonal cooling down to either 15 or 8°C (see Fig. 1). Experiments were conducted on 9.5-mm larvae to 69.0-mm juveniles as detailed in the text. In all cases, the photoperiodic regimen was 12:12-h light to dark. Fish were fed ad libitum on live artemia for the first month and then weaned onto proprietary dry food [British Oil Cate Mills (BOCM) Pauls, UK].

A second set of acclimation experiments was carried out on juvenile carp, 70–140 mm total length (L), that had been reared at ambient environmental conditions in outside ponds. Fish were acclimated for a minimum of 6 wk before experiments to either 10, 15, 20, or 30°C (12:12-h light-dark cycle). All groups were fed ad libitum on proprietary dry food (BOCM Pauls).

Thermal tolerance. The thermal tolerance of fish was determined by heating and cooling groups of 10 carp at 3°C/h from their acclimation temperature. The maximum and minimum swimming temperatures were taken as the temperatures at which the fish lost equilibrium and at which no escape responses could be elicited. Once these maximum or minimum temperatures had been reached, the fish were brought back slowly to their acclimation temperature.

Swimming experiments. Dorsal images of fast-start swimming were filmed using both high-speed ciné and high-speed video cameras operating at 300 and 500 frames/s, respectively. Starts were elicited by startling the fish with a fine rod directed at its snout and were only analyzed in the cases in which a response was elicited without making contact with the rod. Fast starts were filmed at water temperatures of 10, 15, and 21°C, with the acute temperature being changed by 1°C/h between experiments: the times and acclimation ranges for these experiments are shown in Fig. 1. In a second set of experiments, carp of average L (74 mm) that had been acclimated to 10, 15, 20, or 30°C were swum at acute test temperatures of 10, 20, and 30°C using similar procedures. Detailed descriptions of the filming setup are available elsewhere (34).

Kinematic analysis. The spine curvature (reciprocal of the radius of curvature) was calculated for six equally spaced sites along the fish from 0.3 to 0.8 L (30). This range of body locations coincides with the location of the white myotomal muscle (34). The spine curvatures were used to calculate white muscle strain and muscle contraction durations (31).

Fig. 1. *A*: the rearing temperatures of the fish: constant 21°C (solid line, ○), simulated seasonal cooling to 15°C (dashed line, △), and simulated seasonal cooling to 8°C (dotted line, □). The position of the symbols mark the times when swimming performance was tested. *B*: the experimental design for the acute swim temperatures: a 2 × 3 factorial design was used for the first 2 experiments, and then a 3 × 3 factorial design was used when there were 3 distinct acclimation groups.

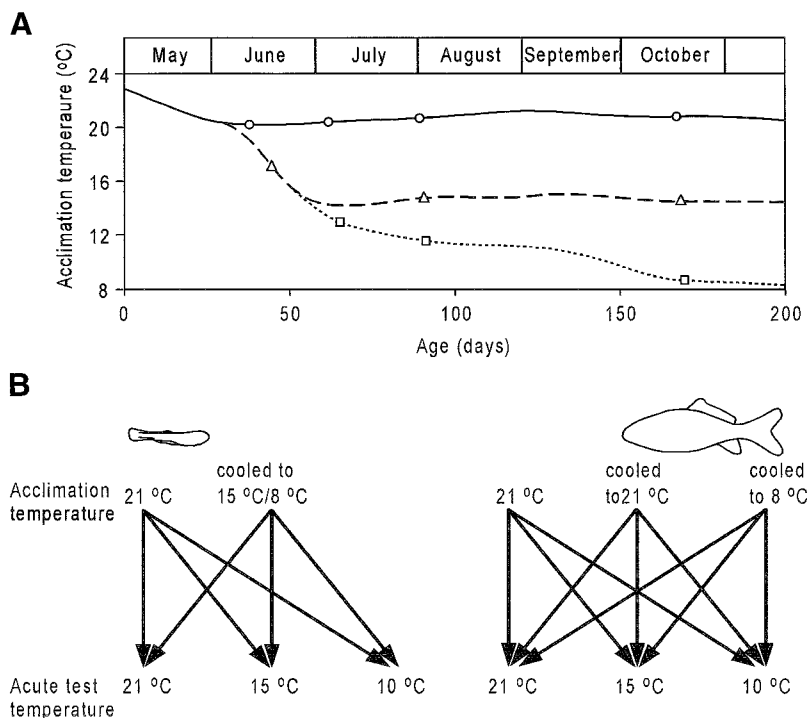


Table 1. Probability values from general linear model analyses of variance

Covariate	Response					
	Log V_{max}	Log \bar{P}	Curvature	Strain	Duration	Strain rate
			0.3–0.8 L	0.3–0.8 L	0.3–0.8 L	0.3–0.8 L
Length	<0.001*	0.015*	0.096	0.299	0.877	0.776
Position			<0.001*	<0.001*	0.662	<0.001*
Length \times position			0.193	0.012*	0.496	0.003*
Acute T	0.002*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Length \times acute T	0.002*	0.003*	0.023*	0.240	0.653	0.205
Acclimation T	0.309	0.625	<0.001*	<0.001*	0.540	<0.001*
Length \times acclimation T	0.124	0.906	0.132	0.400	0.932	0.083
n	144	145	700	700	709	689

n , No. of values tested for each response. Where no value appears, no test was performed between the response and the covariate. Curvature and strain values were tested over the range of longitudinal body positions where the myotomal muscle occurs (0.3–0.8 total length (L)). *Significant terms ($P < 0.05$). T, temperature; V_{max} , maximum velocity; \bar{P} , power requirement.

during the initial tail beat of each fast start. Spine positions from silhouette images of the fish were combined with data of the longitudinal distribution of the body mass to calculate the position of the center of mass (30). Displacements of the center of mass during the first tail beat of each fast start were used to calculate the maximum velocity (V_{max}) and the mean inertial hydrodynamic power requirements (\bar{P}), which is the power needed to accelerate the fish in its direction of travel (30, 34). For each group tested, the five most powerful fast starts were used for further analysis, representing the top one-third of the data.

Muscle mechanics. Bundles of 15–80 white muscle fibers were isolated from the caudal myotomes of carp of average total L (108.0 mm; ± 0.6 SE, $n = 21$). The bundles were attached to an isometric force balance in a Ringer solution of the following composition (in mM): 115.7 NaCl, 8.4 sodium pyruvate, 2.7 KCl, 1.2 $MgCl_2$, 5.6 $NaHCO_3$, 0.64 NaH_2PO_4 , 2 H_2O , 2.1 $CaCl_2$, 3.2 HEPES sodium salt, and 0.97 HEPES, pH 7.4 at 20°C (based on data from Ref. 8). Detailed descriptions of the dissection techniques and force balance have been described elsewhere (33). The length of the preparation was set to give the maximal twitch force equivalent to a resting sarcomere L of 1.96 μm (21). Muscle force production during twitches was measured at the three acute temperatures of 10, 15, and 20°C, with the temperature of the Ringer solution being changed at 1.5°C/h between experiments. The pH of the Ringer solution was allowed to vary freely with temperature ($\Delta pH/\Delta^\circ C = -0.0088$ per $^\circ C$).

Muscle power output during the fast starts for the 15°C-acclimated group of juvenile fish from this study was determined using a muscle model that had been previously validated for white myotomal muscle of carp acclimated to 15°C (33, 34). The initial input parameters were the muscle strain

and contraction duration for the initial contraction of the fast start appropriate to each longitudinal position along the fish. The muscle activation was assumed to be simultaneous with the onset of shortening (32), and the stimulus duration was numerically optimized to yield the greatest power output. The total muscle power output was calculated as the mean power output generated at all sites along the trunk weighted by the proportion of white muscle mass (34) located at each of those sites.

Hydrodynamic efficiency. The total hydrodynamic power requirements are generated by the mechanical power output from the muscle. The muscle power output is a good estimate of these total hydrodynamic requirements (34). The hydrodynamic efficiency (η) during a fast start was therefore estimated by the ratio of the inertial power required to accelerate the fish in its direction of travel to the total muscle power output (34).

Myofibrillar ATPase assays. The white muscle of larvae and juvenile carp (10–120 mm total L) was dissected using a binocular microscope. Myofibrils were prepared in a medium containing (in mM) 10 Tris \cdot HCl (pH 7.4), 50 NaCl, and 1 EDTA, as described previously (13). The protein concentration of myofibrils was determined using the Lowry method (19). ATPase activity was determined by measuring the production of inorganic phosphate in a medium containing (mM) 62.5 Tris \cdot HCl (pH 7.5), 3.8 $MgCl_2$, 5 ATP, and 0.2 $CaCl_2$, as previously described (1).

MHC composition. Myofibrils were adjusted to 2 mg/ml and the proteins separated on a 0.75-mm-thick polyacrylamide gel containing SDS-PAGE, as described by Laemmli (16) but with the inclusion of 10 mM DL-dithiothreitol in the sample buffer. The bands were identified in unfixed gels by rapid staining in 0.01% (mass/vol) Coomassie blue colloidal stain (20). The gel segments containing the MHC were cut out with a razor blade and placed into the wells of a 1-mm-

Table 2. Probability values from general linear model analyses of variance

Covariate	Response			
	Log ATPase	Time for 50% twitch	Time for peak twitch	Time for 50% twitch relaxation
Length	0.703			
Acute T		<0.001*	<0.001*	<0.001*
Acclimation T	0.015*	0.233	0.111	0.062
Length \times acclimation T	0.015*			
n	354	140	140	140

n , No. of values tested for each response. Where no values appear, no test was performed between the response and the covariate. *Significant terms ($P < 0.05$).

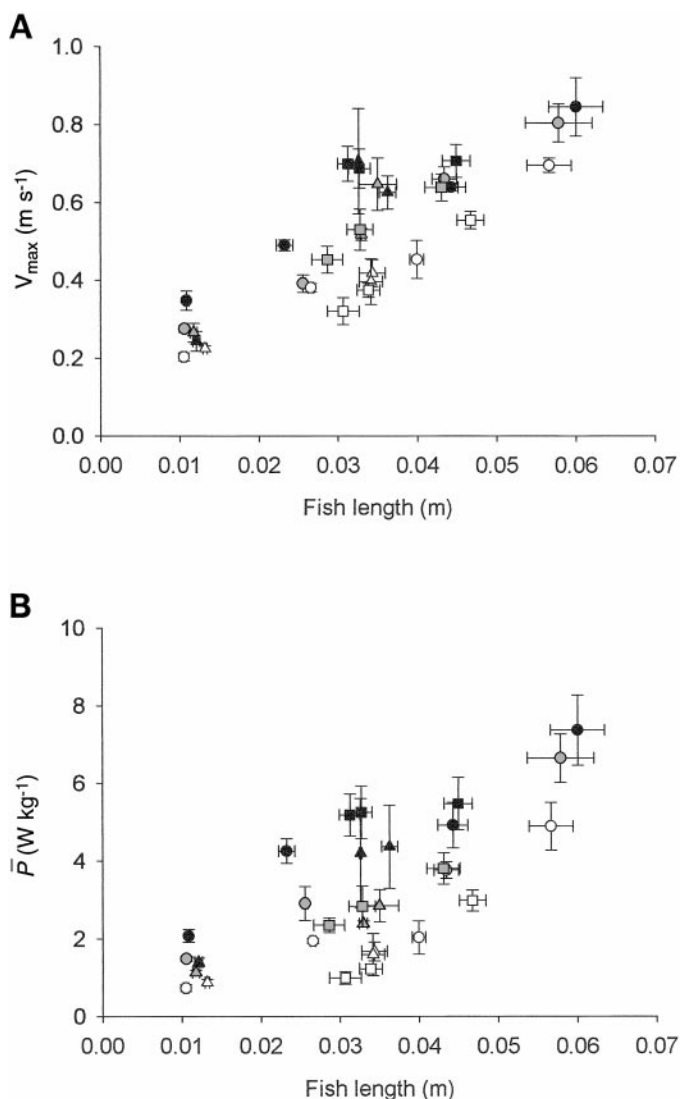


Fig. 2. Maximum velocity (V_{\max} ; A) and mean inertial hydrodynamic power requirements (\bar{P} ; B) during the first tail beat of the fast starts (means \pm SE; $n = 5$). The rearing temperature of the fish is distinguished by the symbol shape with circles for the 21°C group, triangles for medium-acclimated group, and squares for cold-acclimated group. The acute swimming temperature is denoted by the color of the symbol: black, 21°C; gray, 15°C; white, 10°C acute temperatures.

thick 13% (mass/vol) acrylamide SDS-PAGE gel and digested with 4 μ l of a 25- μ g/ml solution of *Staphylococcus aureus* V8 protease (Sigma Chemicals, Dorset, UK) according to the methods in Ref. 4. Gels were fixed and stained with PlusOne silver stain (Amersham Pharmacia Biotech, Uppsala, Sweden). Differences in the banding pattern between lanes were detected using gel-analysis software from Scion Image (<http://www.scionimage.com>).

Statistics. Body L , longitudinal body position, acclimation, and acute temperature were used as covariates for general linear model analyses of variance on the measured parameters. L was additionally used as an interaction term to show whether the effect of the covariate on each parameter varied with L and thus ontogeny. Where parameters were heteroscedastic, they were log-transformed before analysis. Tables 1 and 2 highlight which covariates are significant in shaping the pattern of the response. Hypotheses were tested using 95% confidence levels.

Table 3. V_{\max} and hydrodynamic power for 74-mm-long carp ($L = 74.1 \pm 0.3$ mm)

Acute Temperature, °C	Response and Acclimation Temperature, °C			
	V_{\max} (m/s)		\bar{P} (W/kg fish)	
	9	30	9	30
9*†	0.61 ± 0.08	0.34 ± 0.02	2.78 ± 0.46	0.68 ± 0.15
19	0.57 ± 0.05	0.76 ± 0.09	3.87 ± 0.37	4.19 ± 0.57
30*†	0.35 ± 0.07	1.06 ± 0.11	1.15 ± 0.66	9.72 ± 0.92

Values are means \pm SE ($n = 5$). Significant differences between the acclimation groups are denoted by * for V_{\max} and † for \bar{P} .

RESULTS

Swimming performance. The thermal limits for fast-start behavior were determined in fish subjected to a simulated seasonal cooling to 8°C (Fig. 1). Initially, fast-start behavior was observed between 7.4 ± 0.1 and

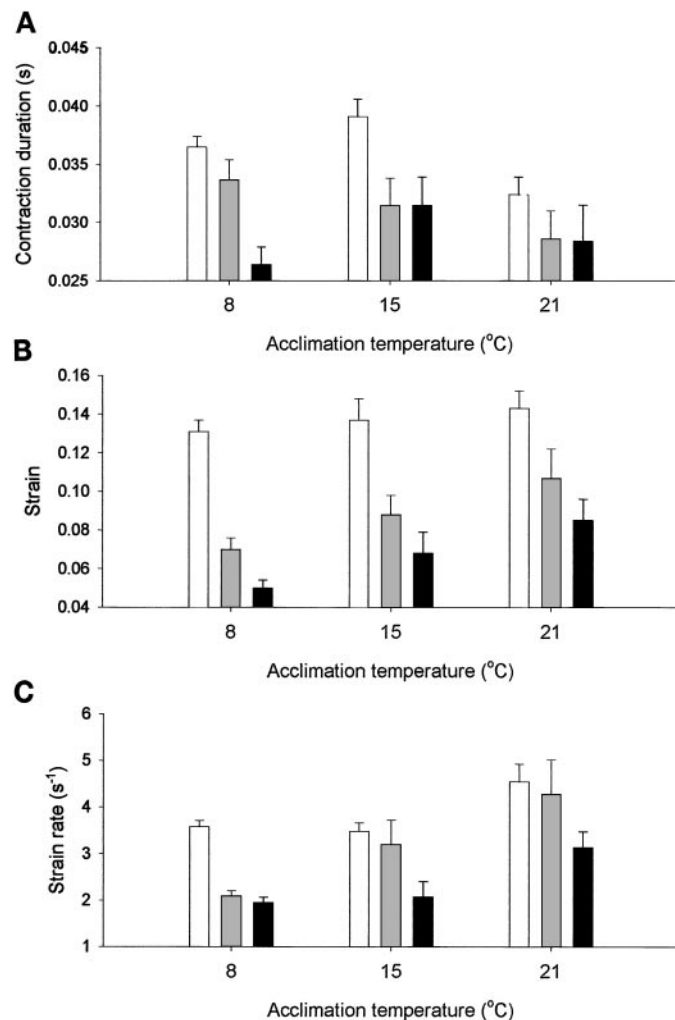


Fig. 3. White muscle contraction duration (A), strain (B), and strain rate (C) during the initial tail beat of the fast start (means \pm SE; $10 < n < 14$ for each bar). Longitudinal body position and body length (L) has a significant effect on these parameters (Table 1) (31), and thus data are shown for the central body region 0.3 to 0.5 L for the size range between 23 and 47 mm. The color of each bar denotes the acute test temperature: black, 21°C; gray, 15°C; white, 10°C.

$35.4 \pm 0.2^\circ\text{C}$ in 10-mm-long larvae acclimated to $21\text{--}23^\circ\text{C}$. In 40-mm-long juveniles acclimated to 10°C , the temperature range for escape behavior had increased at low temperatures and decreased at high temperatures, with a range of 0.6 ± 0.1 to $28.8 \pm 0.2^\circ\text{C}$ (all means \pm SE, $n = 10$).

There were increases in both the V_{\max} and the inertial \bar{P} with body L and acute swimming temperature (Fig. 2). However, there was no significant effect of thermal acclimation over a 13°C range on fast-start performance in either of the two measures (V_{\max} and \bar{P}) over the range $8\text{--}21^\circ\text{C}$ (Table 1; Fig. 2).

A second series of experiments tested a wider range of acclimation temperatures. Carp reared at ambient environmental temperatures and acclimated to either 9 or 30°C for 6 wk were tested at either 9 , 19 , or 30°C (Table 3). Again, no significant difference in V_{\max} and \bar{P} was detected between acclimation groups at a test temperature of 19°C . However, at 9°C , V_{\max} was 79% higher and \bar{P} was 309% higher in 9°C - than in 30°C -acclimated fish (Table 2; 1-tailed t -tests, $P < 0.01$). In contrast at 30°C , V_{\max} was 203% higher, and \bar{P} was 745% higher in 30°C - than in 9°C -acclimated fish (Table 3). Thus fast-start behavior was modified by thermal acclimation over a 21°C range. Improved performance at the acclimation temperature was accompanied by a trade-off in performance at the other end of the thermal range.

Muscle kinetics and hydrodynamic efficiencies during fast starts. Significant acclimation responses were detected in the mode of muscle shortening during the fast starts (Table 1). The degrees of strain, strain rate, and contraction duration are shown in Fig. 3. The cold-acclimated fish swam with reduced muscle strain and strain rates compared with both the medium- and hot-acclimated groups (Fig. 3). These differences with acclimation temperature were independent of fish L ,

however, and so were not gradually acquired during ontogeny.

Changes in the mode of muscle shortening were more pronounced after an acute change in temperature than after a change in acclimation state, reflecting how rapid (hourly) changes in temperature alter the swimming response in a different manner to more gradual (weekly) changes. For fish of any acclimation state, there were acute changes in the white muscle strain, contraction duration, and strain rate during the initial tail beat of the fast start (Table 1). Reduction of the acute swimming temperature resulted in increases in the magnitude of body bending and thus muscle strain and in the contraction duration. The net muscle-strain rate also increased for the colder swimming temperatures (Fig. 3).

The total muscle power output during the initial tail beat of the fast start increased with rising acute swimming temperature for the 15°C -acclimated fish; however, these increases were not as great as those for the inertial hydrodynamic \bar{P} . There was thus an increase in the η (Fig. 4) with increasing acute temperature. Planar least-squares regression analysis of the η revealed a slight, but not significant, decrease in η with increasing body L ; however, the increase in η with increasing acute temperature was significant ($P < 0.001$). These increases in the η reflect the temperature-induced changes in the extent and duration of body bending during the fast starts.

Muscle contractile properties. The time course for twitch contractions was measured for white myotomal muscle in 100- to 120-mm-long carp. There was a significant effect of the acute temperature on the three time courses measured (Table 2). This was due to increasing acute temperatures causing a pronounced decrease in the times for the muscle to develop 50% of its peak force, its peak value, and for the drop from

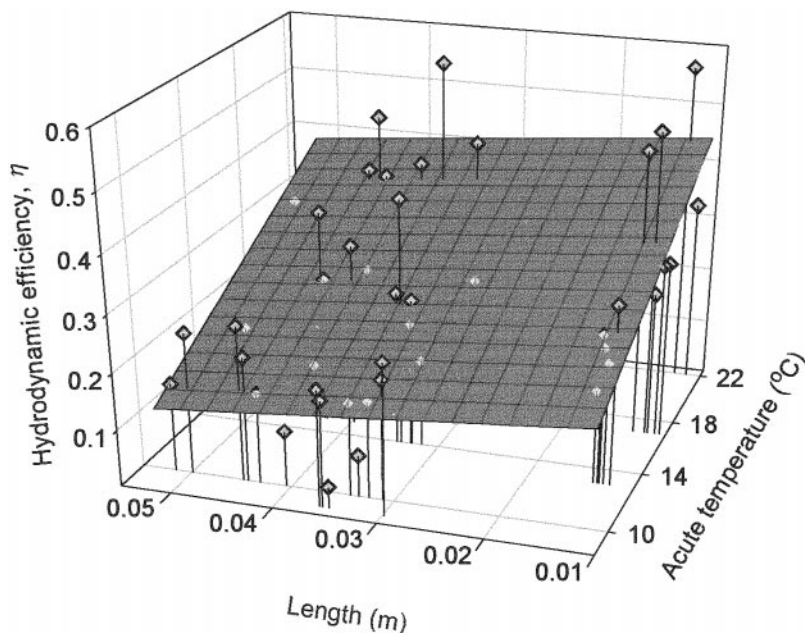


Fig. 4. Hydrodynamic efficiencies for the 15°C -acclimated fish shown with a mesh depicting the least-squares planar regression through these data ($n = 45$). Points appearing above the mesh have a black outline, whereas those below have no outline. The illustrated plane takes the form: hydrodynamic efficiency (η) = $c_0 + c_1T + c_2L$, where $c_0 = 0.050$ ($P = 0.480$), $c_1 = 0.019$ ($P < 0.001$), $c_2 = -1.602$ ($P = 0.168$), and T denotes acute swimming temperature.

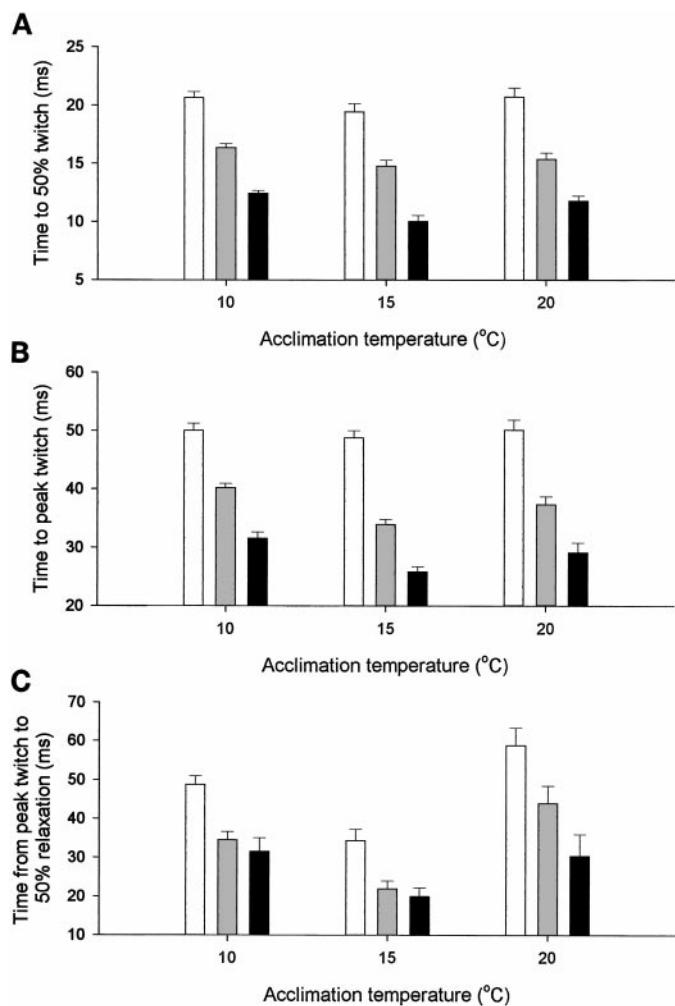


Fig. 5. The time from the onset of stimulation to 50% peak twitch tension (A) and peak twitch tension (B). C: time from peak twitch tension to 50% relaxation. The color of each bar denotes the acute test temperature: black, 20°C; gray, 15°C; white, 10°C.

peak to 50% force during relaxation (Fig. 5). However, there was no significant effect of acclimation temperature for these three rates across the range of temperatures tested (10–20 °C; Table 3).

MHC composition. Peptide maps produced by the digestion of the MHCs from the fast myotomal muscle revealed a series of isoforms that were characteristic of both body *L* and acclimation state (Fig. 6A). The relationship between acclimation temperature and body *L* is shown in Fig. 6B. Juvenile isoforms replaced larval isoforms at a body *L* >15 mm; however, this did not re-

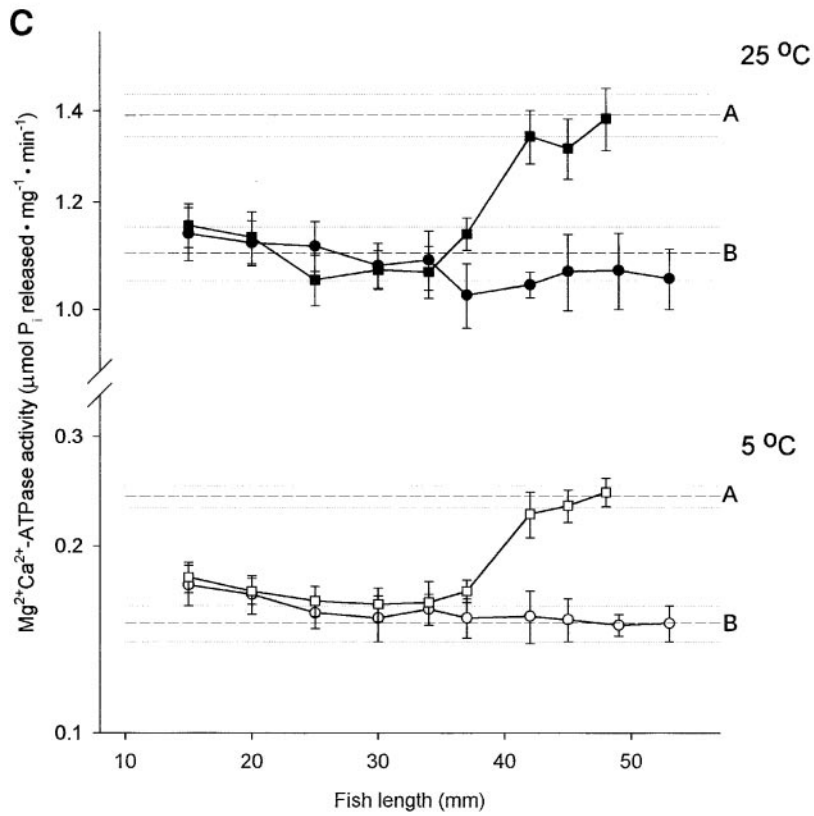
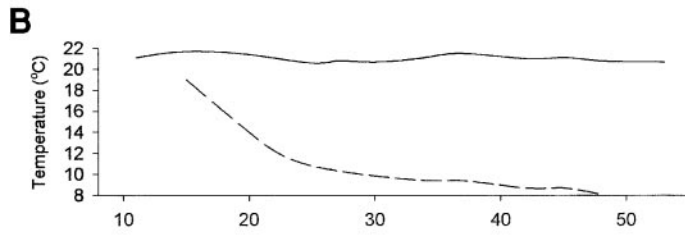
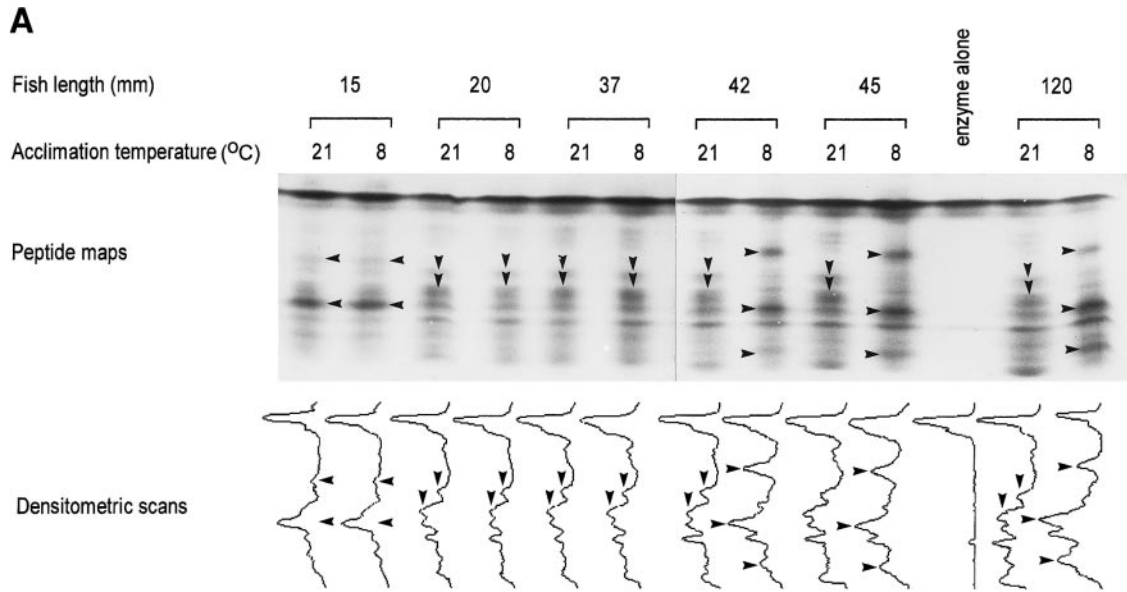
sult in a change in the myofibrillar Mg^{2+} - Ca^{2+} -ATPase activity (Fig. 6C). The MHC pattern was different for the 8°C- and 21°C-acclimated groups in fish greater than 37 mm *L* (Fig. 6A). The main differences in the peptide maps of MHCs isolated from early larval stages (*L* < 15 mm), 21°C-acclimated juveniles, and 8°C-acclimated juveniles are illustrated on the densitometric traces in Fig. 6A by leftward-facing horizontal and vertical and rightward-facing horizontal arrows, respectively. There was also a significant temperature-acclimation response in Mg^{2+} - Ca^{2+} -ATPase activity of fish white muscle myofibrils that was dependent on the fish *L* ("*L* × acclimation temperature" term; Table 3). Changes in MHC composition were correlated with a higher Mg^{2+} - Ca^{2+} -ATPase myofibrillar ATPase activity under steady-state conditions in the 8°C- than in 21°C-acclimated fish (37% higher at 5°C and 23% higher at 25°C; Fig. 6C). Changes in MHC expression and myofibrillar ATPase activity were not directly related to fast-start swimming performance, however, because the swimming performance did not show an acclimation response for the larger fish (*L* >37 mm) over the range of 8–21°C.

DISCUSSION

Acclimation versus acute temperature effects. The time course of temperature change is an important determinant of the phenotypic response observed. Acute temperature changes, on the order of 1°C/h, can alter the rates of enzyme reactions. Acclimation responses occur over a longer period, and studies in cyprinid fish have shown that several weeks are required for adjustments to myofibrillar ATPase (14), MHC composition (10, 35), and contractile performance (11, 15).

The body temperature of fish living in large bodies of water is buffered by the thermal inertia of the water. Carp live in relatively large freshwater ponds and lakes where, during the course of a day, the acute temperature experienced by a fish is never far from the seasonal mean. Seasonal temperature changes occur over a longer time period (a matter of weeks) and can be matched by a changing acclimation state. Acclimation responses are tested by comparing the performance of fish acclimated to a particular temperature with fish acutely transferred to the same temperature. The magnitude of any response observed is therefore dependent on the difference between the acclimation and test temperatures.

Fig. 6. A: peptide maps of myosin heavy chains isolated from carp white muscle. Bands characteristic of larval (<15 mm total *L*), 21°C-acclimated (120 mm total *L*), and 8°C-acclimated (120 mm total *L*) fish are indicated by left-facing, downward, and right-facing arrows, respectively. Densitometric scans of the peptide maps are also shown. The main differences in the banding patterns on the densitometric scans are shown with arrows (left-facing arrow for fish <15 mm *L*, vertical arrows for fish greater than 15 mm acclimated to 21°C and right-facing for fish >37 mm acclimated to 8°C). B: fish were acclimated to a constant 21°C (solid line) or at temperatures reducing to 8°C (dashed line). C: Mg^{2+} - Ca^{2+} -ATPase activities of white muscle myofibrils in relation to body *L* for fish acclimated to the temperature regimens illustrated in Fig. 6B. The results for assays at 5°C (open symbols) and 25°C (solid symbols) are illustrated. Values represent means ± SE of 6 fish/group. In addition, the horizontal dashed line represents the mean values of myofiber ATPase activity for 8°C-acclimated (A) and 21°C-acclimated (B) fish of 120 mm total *L* (SE of the mean is shown by a dotted line).



From our experiments rearing carp at different temperatures ranging from 21 to 8°C, we concluded that acclimation extends the thermal range for fast-start behavior but that there was no evidence for “beneficial improvements” in performance. The limited range of acclimation temperatures used corresponds to those for feeding activity by common carp in the British Isles, where the average summer temperature rarely exceeds 21°C. Thus for these experiments, the beneficial acclimation and optimal developmental temperature hypotheses must thus be rejected in favor of the no advantage in performance hypothesis. However, the beneficial acclimation hypothesis would be accepted for the experiments reported in Table 3. The range of acclimation temperatures used in these experiments may be of ecological relevance in continental regions where summer temperatures are significantly higher than in the British Isles. Similar acclimation responses for escape swimming behavior after relatively large temperature changes have previously been reported in the goldfish *Carassius auratus* and killifish *Fundulus heteroclitus* (11) and two species of Cottidae (29). In all these latter cases, swimming performance was measured after fish had experienced at least a 20°C temperature change in less than 10 h. Caution should be used when making quantitative conclusions from such experiments, because acclimation effects that appear beneficial in the laboratory situation may not be realized in the wild.

Temperature induced changes in swimming biomechanics. Reduction in acute swimming temperature resulted in an increase in the spine curvatures and white muscle strains (Fig. 3). Similar patterns have been observed across a range of fish species tested at their habitat temperature when these temperatures ranged from 0 to 25°C (30), and so this may be a characteristic response to swimming at different temperatures. The decrease in muscle strain rate (V) with increasing acute swim temperatures (Fig. 3) occurs in contrast to increases in the maximum unloaded shortening velocity V_0 of carp muscle with increasing temperature (15, 33). V/V_0 is an important determinate of the pattern of force generation by a muscle (22, 23) and must decrease at higher acute temperatures for these carp. Such decreases in V/V_0 result in an increase in the maximum stress generated, the time course for force generation, and the relative duration of the muscle deactivation compared with the activation (33). These changes in the mode of force generation by the muscle promote the increases in power output at the higher acute temperatures. However, it is not known why the strain rate should change at the different temperatures.

The rates of body bending and thus muscle shortening during the initial tail beat of a fast start are governed by the balance between the bending torque on the spine produced by the muscles and the inertial resistance of the body mass and added mass of water that moves with the fish (32). The higher strain rates that occurred at the cold swimming temperatures could be due to greater muscle force and thus torque on the spine or to a decreased inertial resistance. The slower twitch activation rates at the cold temperatures

(Fig. 6) result in the stress produced by each individual fiber being initially lower than for the warmer temperatures and thus would not promote faster bending. By contrast, greater muscle force could be produced at the colder temperatures if there were a larger proportion of muscle fibers activated, and this has been shown to be the case for steady swimming in carp (22, 24). However, fast-start escape responses are assumed to be maximum-performance activities, recruiting the maximum available myotomal muscle irrespective of the temperature, and it is unlikely that the proportion of muscle fibers activated would be temperature dependent. The mass distribution and thus inertial resistance of the body does not change with temperature; however, increases in the rates of bending could be achieved with a decrease in the added mass of water that must be moved at the colder temperatures. Such a decrease could result from the fins being held in a less erect posture, thus decreasing the depth of the fish, and would be most prominent for muscle fibers in the caudal region of the fish. Indeed, the initial angular acceleration on the spine at 0.6 L would increase by 9% if the dorsal and anal fins were not extended and by 56% if the caudal fins were also not spread (using the arguments in Ref. 32). These possible mechanisms for altering the rates of body bending with change in acute swimming temperature could be investigated further by detailed electromyogram analysis and by the simultaneous use of lateral and planform images during swimming to measure the instantaneous fish depth.

The larger degrees of body bending at the cold acute temperatures concur with a decreased hydrodynamic efficiency. This decrease may be a result of the larger lateral accelerations of the water accompanied by greater parasitic losses due to vortex formation at edges in the cross flow (18). However, such a decrease in efficiency may be a necessary consequence of maintaining a substantial inertial hydrodynamic power output despite the decrease in the contractile performance of the muscle fibers at those temperatures.

Integration of the acclimation response. Short-term temperature changes may produce pathological decreases in performance that are not necessarily related to the function of the neuromuscular system. Caution should therefore be applied in attributing decreased performance at test temperatures far removed from the acclimation temperature in terms of muscle function. However, strong evidence was obtained for temperature acclimation responses at the level of the muscle tissue even where whole animal performance was unaltered (Tables 1 and 2). MHC composition and myofibrillar ATPase activity were independent of acclimation temperature in carp larvae (<37 mm L) over the range 8–21°C. However, for later stages, MHC composition and myofibrillar ATPase activity were a function of the acclimation state of the animal. In contrast, twitch times of white muscle fibers showed no acclimation response over the range of temperatures tested (10–20°C), although the maximum unloaded shortening velocity of skinned carp white muscle fibers does change over the wider range of acclimation states from 2 to 23°C (4). The muscle strain

and strain rates showed differences with acclimation (Table 1); however, these occurred at all stages during ontogeny and thus are not due to the same mechanisms as the changing MHC.

The duration of the initial contraction in fast starts is shorter than the time that would be required for the muscle to reach its fully active state (32, 33). Therefore, muscle force is only produced while the muscle is in unsteady activating and deactivating states. This muscle force is thus dependent on the rates of activation and deactivation (see Table 2) as well the effect of the shortening velocity and the V_0 (33). Muscle force is only converted to hydrodynamic propulsion after transmission through the skeletal elements of the fish. There are many levels of integration that occur between the discrete steps quantified in this study, and each level may have a modulating effect of the whole animal performance. Thus there may be continuously varying acclimation responses at low levels of organization, e.g., in MHC expression patterns, with a threshold for an observable effect at the whole animal level.

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