

## ENHANCED MAXIMUM FREQUENCY AND FORCE DEVELOPMENT OF FISH HEARTS FOLLOWING TEMPERATURE ACCLIMATION

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### Summary

Inotropic and chronotropic responsiveness of yellow perch (*Perca flavescens* Mitchill) and smallmouth bass (*Micropterus dolomieu* Lacépède) hearts, following low temperature acclimation, was assessed with ventricle strips mounted for isometric force recording. Animals were acclimated to 20° and 5°C, and the performance of ventricle strips from both acclimation temperatures was monitored at 20° and 5°C.

Ventricle strips from yellow perch acclimated to 20°C showed an increase in resting tension when tested at 5°C in the presence of high levels of extracellular calcium. An increase in resting tension did not occur in preparations from 5°C-acclimated fish tested at 5°C. This suggests failure of intracellular calcium regulation which may be ameliorated following an acclimation period.

Ventricle strips were subjected to a force–frequency challenge over the range of 12–48 contractions min<sup>-1</sup> at 1 mmol l<sup>-1</sup> and 3 mmol l<sup>-1</sup> extracellular calcium. Time to 50% relaxation of ventricle strips tested at 5°C was significantly lower for hearts from perch acclimated at 5°C than from those acclimated at 20°C. This was associated with an ability to maintain function at higher pacing rates. Similar trends were exhibited by hearts from smallmouth bass. As calcium extrusion is a prime determinant of relaxation time, these findings further suggest an enhancement in calcium handling capabilities following acclimation to low temperature.

Ventricle strips from both species acclimated to 20°C and tested at 20°C were able to maintain force development to the highest contraction frequencies. Hearts from specimens acclimated to 5°C and tested at 20°C showed a negative force–frequency relationship at low extracellular calcium levels. In yellow perch, this effect was minimized by an increase in calcium availability. This is considered to be a potential mechanism by which animals acclimated to low temperature could make transient excursions into warm water.

### Introduction

A change in environmental temperature has an immediate and substantial effect

Key words: yellow perch (*Perca flavescens*), smallmouth bass (*Micropterus dolomieu*), temperature acclimation, force development, contraction and relaxation times, extracellular calcium, teleost hearts.

upon heart performance in fish. A decrease in temperature results in a decrease in resting heart rate and subsequent cardiac output in many species (Farrell, 1984). At least in trout, low temperature also results in a lowering of maximum heart rate induced by swimming activity (Priede, 1974). Lowering of temperature leads to a decreased frequency of contraction by isolated heart preparations (Randall, 1971; Graham and Farrell, 1985; Tsukuda *et al.* 1985) and to a lowering of stroke volume by isolated sea raven hearts perfused with Ringer's solution containing physiological levels of calcium and paced at rates of 12–48 contractions  $\text{min}^{-1}$  (Bailey and Driedzic, 1989). The cumulative evidence shows that in the absence of intervening factors an acute change in temperature has a direct impact on both the chronotropic and the inotropic responsiveness of the fish heart.

There is a complex interplay amongst force development, the frequency of contraction and calcium availability to the contractile apparatus. In teleosts, increases in isometric contraction frequency of ventricle strips result in a decrease in tension development which may be ameliorated by increased extracellular calcium concentration (Driedzic and Gesser, 1985, 1988). In some teleosts, exercise is associated with an increase in heart rate (Jones and Randall, 1978) and plasma total calcium levels are increased by as much as  $1.5 \text{ mmol l}^{-1}$  following vigorous activity (Ruben and Bennett, 1981). The impact of temperature on the interplay between calcium concentration and the force–frequency relationship has not been investigated.

Chronic exposure to low temperature may invoke alterations in heart performance. Perfused isolated hearts from sea raven, tested in summer months, were more sensitive to increases in filling pressure than hearts removed from animals during winter months when both groups of hearts were tested over a wide thermal range (Graham and Farrell, 1985). Perfused isolated hearts from goldfish acclimated to  $10^\circ\text{C}$  exhibited higher rates of contraction than hearts removed from animals acclimated to  $25^\circ\text{C}$  when both groups of hearts were tested at low temperatures (Tsukuda *et al.* 1985). The object of the current investigation was to assess the impact of an acute temperature transition on the inotropic and chronotropic potential and calcium sensitivity of hearts from animals acclimated to different temperatures.

Two north temperate freshwater species, yellow perch (*Perca flavescens*) and smallmouth bass (*Micropterus dolomieu*), were used. Both species are aggressive carnivores and have similar lifestyles, moving into shallow water to feed during the day and into deeper water at night. Yellow perch and smallmouth bass are both active in summer water temperatures of  $20\text{--}25^\circ\text{C}$ . Yellow perch maintain active swimming at winter temperatures (Magnusson *et al.* 1979; Otto and Rice, 1974), whereas smallmouth bass become torpid at temperatures below about  $5^\circ\text{C}$  (Coble, 1975). We hypothesized that hearts from yellow perch would be able to maintain performance better than hearts from smallmouth bass following a transition from warm to cold temperature and acclimation of yellow perch to low temperature would improve cardiac performance relative to an acute transfer to low temperature.

The problem was approached by monitoring the isometric tension development of ventricle strips at different rates of contraction, external calcium load and temperature. Heart preparations were subjected to a step change in temperature between 5 and 20°C and the force–frequency response was monitored over the physiological range of heart rates. Studies were conducted at low and high physiological levels of extracellular calcium. The most salient findings were (a) elevated calcium levels, at high temperatures, provide protection from the negative inotropic effect of contraction frequency and (b) acclimation of yellow perch to low temperature results in an approximately 50% decrease in relaxation times which, in turn, allows higher maximal rates of contraction at low temperature.

### Materials and methods

#### *Animals*

Yellow perch (*Perca flavescens*) ( $82 \pm 7.5$  g) were captured in ponds and rivers near Sackville, New Brunswick, and randomly divided into two groups of equivalent size range; one group, YP20, was acclimated to 20°C while the other group, YP5, was acclimated to 5°C. Smallmouth bass (*Micropterus dolomieu*) ( $138 \pm 46$  g) were captured at Wheaton Lake near St Andrews, New Brunswick, and transported to Mount Allison University where they were randomly divided into two groups of equivalent size range. One group, SB20, was acclimated to 20°C and the other group, SB5, was acclimated to 5°C. There was no effect of body size on any of the parameters measured in this study. Both perch and bass were kept in filtered, partially recirculating water and fed a diet consisting of earthworms and/or ground meat. Specimens were collected during July and August; the acclimation period extended into September and October. All animals were maintained at these thermal regimes for at least 4 weeks under a 12 h low light: 12 h dark photoperiod.

#### *Experimental preparations*

Fish were selected at random from either of the two acclimation temperatures and killed by a sharp blow to the head. The hearts were quickly excised and placed in ice-cold bathing medium containing  $1 \text{ mmol l}^{-1}$   $\text{CaCl}_2$ . Ventricle strips of approximately 1–2 mm diameter were mounted for isometric force recording as described by Gesser (1977). The strips were stimulated to contract *via* two Ag/AgCl electrodes, one on each side of the strip. Strips were stretched until peak force did not increase and allowed to stabilize at  $12 \text{ contractions min}^{-1}$  at the acclimation temperature. Isometric force development was recorded using a Harvard model 363 isometric force transducer and a Biotronix BL882 strip chart recorder.

*Protocols**Calcium saturation studies*

Paired strips from either species were allowed to stabilize at 12 contractions  $\text{min}^{-1}$  at the acclimation temperature. The temperature of the medium for one strip was reset to either 5 or 20°C, depending on the initial temperature. The preparations were then allowed a stabilization period of 15 min at 12 contractions  $\text{min}^{-1}$  and 1  $\text{mmol l}^{-1}$   $\text{CaCl}_2$ . Following the stabilization period, the isometric force development of both strips was recorded for 3 min at 12 contractions  $\text{min}^{-1}$  and 1  $\text{mmol l}^{-1}$   $\text{CaCl}_2$ . At the end of the 3 min period, 15–20 s of high-speed recordings were taken for contraction time measurements. Thereafter  $\text{CaCl}_2$  concentration was increased in steps of 1  $\text{mmol l}^{-1}$   $\text{CaCl}_2$  at 3 min intervals until a final concentration of 5  $\text{mmol l}^{-1}$  was reached. Recordings were taken as before. Frequency was kept constant at 12 contractions  $\text{min}^{-1}$ . The 3 min period was sufficient to allow for a full impact of the altered  $\text{CaCl}_2$  concentration.

Plasma calcium levels in yellow perch and smallmouth bass are not known. In other freshwater teleosts, total plasma calcium ranges from about 1.3 to 4.1  $\text{mmol l}^{-1}$  (Andreasen, 1985; Ruben and Bennet, 1981). The highest levels are found immediately after activity in some species (Ruben and Bennet, 1981). In trout about 60% of the calcium is dissociated (Andreasen, 1985). Free calcium levels therefore range from about 0.8 to 2.5  $\text{mmol l}^{-1}$  in a wide variety of species. In the low- $\text{HCO}_3^-$  Ringer's solution utilized in the present studies essentially all the calcium is dissociated, as measured by calcium electrode. Calcium levels in the titration studies therefore encompassed the physiological to supraphysiological range.

*Force–frequency relationships*

Paired strips from either species were allowed to stabilize in bathing medium containing 1  $\text{mmol l}^{-1}$   $\text{CaCl}_2$ , at the acclimation temperature. Thereafter the temperature of the bathing medium for one strip was reset to either 5 or 20°C depending on the initial temperature. The strip was then allowed to stabilize for 15 min at the new temperature. Following this second stabilization period, both strips were stimulated at a frequency of 12 contractions  $\text{min}^{-1}$  for 3 min. At the end of the 3 min period 15–20 s of high-speed recordings were taken for contraction time measurements. The frequency was then increased in steps of 6 contractions  $\text{min}^{-1}$ , at 3 min intervals, up to 48 contractions  $\text{min}^{-1}$ . During these increases in frequency, recordings were taken as before. Following the 48 contractions  $\text{min}^{-1}$  period, the frequency was decreased to 12 contractions  $\text{min}^{-1}$  for 3 min. Any preparation that did not show at least 90% of the initial twitch force was discarded. Sufficient  $\text{CaCl}_2$  was then added to the bathing medium of both strips to give a final  $\text{CaCl}_2$  concentration of 3  $\text{mmol l}^{-1}$ . The strips were then allowed a stabilization period of 15 min at the new  $\text{CaCl}_2$  concentration and the frequency study repeated. In both species frequency shifts resulted in a new steady-state level within 5–10 contractions. The  $\text{CaCl}_2$  levels used were assumed to approximate the

extremes of free calcium in plasma. Only data obtained under steady-state conditions are presented.

Heart rates in yellow perch and smallmouth bass are not known. In the present experiments, ventricle strips were induced to contract at frequencies ranging from 12 to 48 contractions  $\text{min}^{-1}$ . Numerous studies with other species suggest that the frequencies used here are within the range normally expressed *in situ* by species that do not exhibit high levels of sustained activity (Farrell, 1984; Roberts, 1973).

#### *Bathing media*

In force–frequency and calcium saturation protocols the bathing medium used initially contained (in  $\text{mmol l}^{-1}$ ): NaCl, 110; KCl, 2.0;  $\text{CaCl}_2$ , 1.0;  $\text{MgSO}_4$ , 1.0;  $\text{NaHCO}_3$ , 1.0;  $\text{NaH}_2\text{PO}_4$ , 0.43; and glucose, 5.0. The medium was vigorously gassed with 99.5%  $\text{O}_2$ –0.5%  $\text{CO}_2$  and pH was adjusted to 7.4 with NaOH. The pH of the medium was constant between 5 and 20°C and fresh medium was prepared prior to each experiment. The  $\text{CaCl}_2$  concentrations were altered during the studies as stated above.

#### *Data analysis*

In the force–frequency studies, force development between peak and resting level was expressed as a percentage of twitch force developed at 12 contractions  $\text{min}^{-1}$  at the acclimation temperature for each strip. In the calcium saturation studies, force development was expressed as a percentage of force development at 12 contractions  $\text{min}^{-1}$ , in  $1 \text{ mmol l}^{-1}$   $\text{CaCl}_2$  and at the final temperature for each strip. Resting tension was calculated by subtracting percent twitch tension from percent peak systolic tension, each being a function of force developed at 12 contractions  $\text{min}^{-1}$ . Time to peak tension and time to 50% relaxation were calculated from the high-speed recordings. Data are expressed as means  $\pm$  s.e.m. To test differences between groups, comparisons were made with either a Wilcoxin signed-rank test or a Mann-Whitney *U*-test. To test differences within groups a Friedman two-way ANOVA was used. In all cases, a *P* value of less than 0.05 was considered significant.

### **Results**

#### *Calcium saturation studies*

Ventricle strips from group YP20 tested at 20°C showed a significant positive inotropic response to increasing calcium concentration (Fig. 1, top). There was no change in resting tension under these conditions (Fig. 1, middle). Strips run at 5°C showed a small but significant decrease in twitch force and a significant increase in resting tension. Ventricle strips from group YP5 showed significant increases in both twitch and resting tension when tested at either 20 or 5°C. There was no change in time to peak tension with increasing calcium concentration. Ventricle strips from group YP20 had times to peak tension of  $1.42 \pm 0.05$  and  $0.63 \pm 0.04$  s at test temperatures of 5 and 20°C, respectively. Preparations from YP5 had times to

peak tension of  $1.24 \pm 0.04$  and  $0.49 \pm 0.02$  s at test temperatures of 5 and 20°C, respectively. In both groups, ventricle strips run at 5°C had a significantly greater (i.e. slower) time to peak tension than strips run at 20°C. Increasing calcium concentration had no effect on time to 50% relaxation (Fig. 1, bottom) for strips from group YP5 run at 5 or 20°C and strips from group YP20 tested at 20°C.

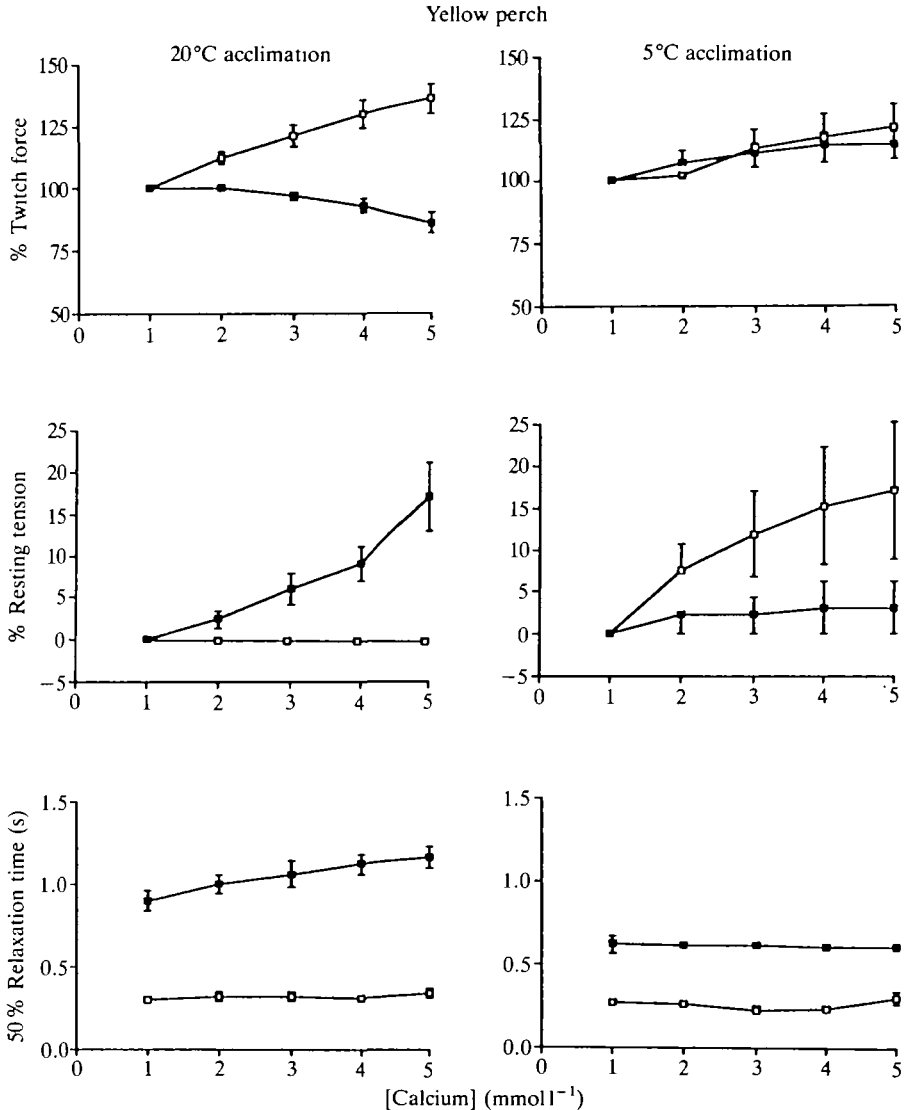


Fig. 1. Percent twitch force (top panels), percent resting tension (middle panels) and time to 50% relaxation (bottom panels) *versus* calcium concentration in isometrically contracting ventricle strips from yellow perch acclimated to 20°C (left side) and 5°C (right side). Strips tested at 20°C are represented by open squares; strips tested at 5°C are represented by closed squares. All values are means  $\pm$  S.E.M.,  $N=7$  for each acclimation group.

However, strips from group YP20 run at 5°C showed a significant positive relationship between time to 50% relaxation and calcium concentration. In addition, relaxation time was 50–80% greater in strips from group YP20 tested at 5°C than in strips from group YP5 tested at 5°C.

Fig. 2 shows the effect of increasing calcium concentration on ventricle strips run at 5 and 20°C from groups SB5 and SB20. In all cases the strips showed a significant positive inotropic response to increasing calcium concentration (Fig. 2, top). Significant increases in resting tension were found in groups SB20 and SB5 when tested at 5°C (Fig. 2, middle). There was no change in time to peak tension with increasing calcium concentration. Ventricle strips from group SB20 had times to peak tension of  $1.38 \pm 0.09$  and  $0.89 \pm 0.09$  s at test temperatures of 5 and 20°C, respectively. Preparations from SB5 had times to peak tension of  $1.29 \pm 0.08$  and  $0.57 \pm 0.05$  s at test temperatures of 5 and 20°C, respectively. In both groups, ventricle strips run at 5°C had a significantly greater time to peak tension than strips tested at 20°C. Times to 50% relaxation (Fig. 2, bottom) were significantly greater at 5 than at 20°C test temperatures for both acclimation groups. There was no effect of increasing calcium concentration on these times in strips from group SB20. Group SB5 tested at 20°C showed a significant decrease in time to 50% relaxation with increasing calcium concentration, whereas strips tested at 5°C showed a significant increase in time to 50% relaxation.

#### *Force–frequency relationships*

##### *Inotropic responses*

Force–frequency responses of ventricle strips tested with  $1 \text{ mmol l}^{-1}$   $\text{CaCl}_2$  in the bathing medium are presented in Fig. 3. When ventricle strips from group YP20 were run at 20 and 5°C a significant negative inotropic response was observed under both conditions. Cooling resulted in a significantly greater force of contraction at the lower frequencies (12 and 18  $\text{contractions min}^{-1}$ ) and an inability of the cooled strip to maintain the pacing regime at frequencies greater than 30  $\text{contractions min}^{-1}$ . When strips from group YP5 were run at 5 and 20°C, preparations exhibited a significant negative inotropic response with increasing frequency at both temperatures. However, unlike the strips from group YP20, in this case the strips were capable of maintaining the pacing regime at 5°C. Also, warming the strips caused a significant loss of contractile force at lower frequencies (12–24  $\text{contractions min}^{-1}$ ).

Strips from group SB20 run at 20°C and with  $1 \text{ mmol l}^{-1}$   $\text{CaCl}_2$  in the bathing medium, did not show any significant change in contractile force with increasing frequency. But strips run at 5°C showed a significant decline in force of contraction with increasing frequency up to 30  $\text{contractions min}^{-1}$ , after which the strips failed to maintain the pacing regime. Strips from group SB5 showed a significant negative inotropic response with increasing frequency at both test temperatures. The strips tested at 5°C tended to fail to maintain the pacing regime above 30  $\text{contractions min}^{-1}$  with only two of the seven strips continuing to 42  $\text{contractions min}^{-1}$  (Fig. 3).

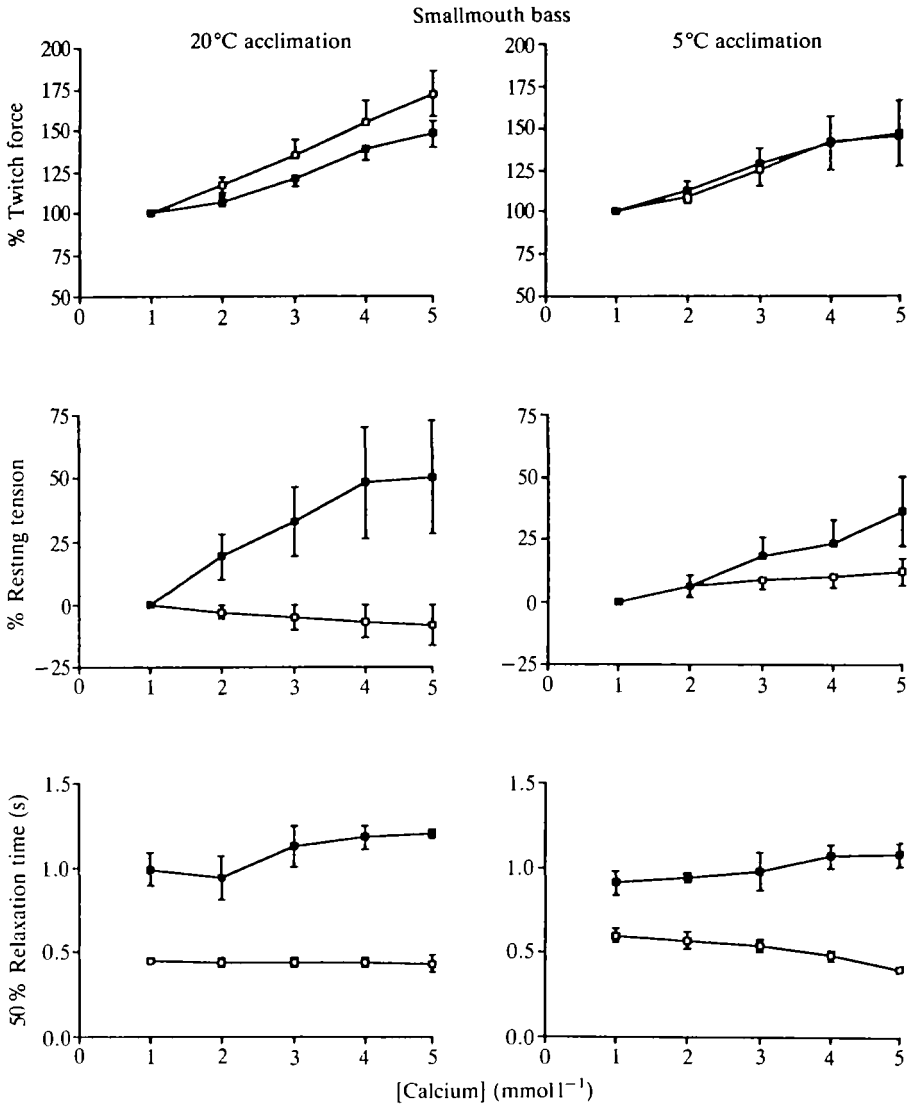


Fig. 2. Percent twitch force (top panels), percent resting tension (middle panels) and time to 50% relaxation (bottom panels) *versus* calcium concentration in isometrically contracting ventricle strips from smallmouth bass acclimated to 20°C (left side) and 5°C (right side). Strips tested at 20°C are represented by open squares; strips tested at 5°C are represented by closed squares. All values are means  $\pm$  S.E.M.,  $N=6$  for each acclimation group.

When the calcium concentration of the bathing medium was increased to 3 mmol<sup>-1</sup> there were no substantial changes in the relationships observed at 1 mmol<sup>-1</sup> CaCl<sub>2</sub>, for groups YP20 and SB20 (Fig. 4). However, in contrast to the response in 1 mmol<sup>-1</sup> CaCl<sub>2</sub>, strips from group YP5 run in 3 mmol<sup>-1</sup> CaCl<sub>2</sub> a



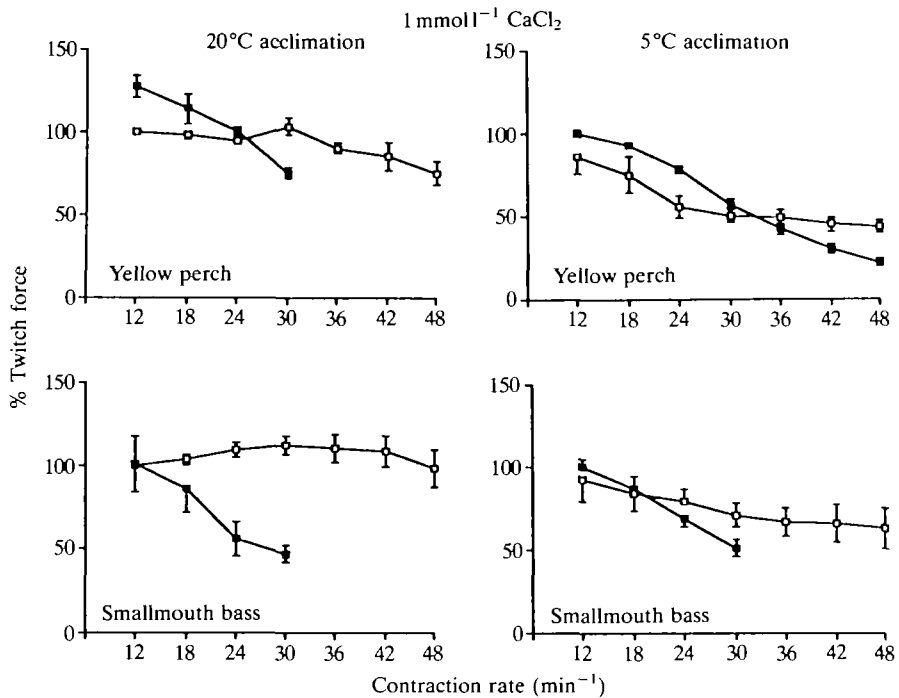


Fig. 3. Force development *versus* increasing frequency of contraction of isometrically contracting ventricle strips in bathing medium containing  $1 \text{ mmol l}^{-1} \text{ CaCl}_2$  from yellow perch (top panels) and smallmouth bass (bottom panels) acclimated to  $20^\circ\text{C}$  (left side) and  $5^\circ\text{C}$  (right side). Strips tested at  $20^\circ\text{C}$  are represented by open squares, strips tested at  $5^\circ\text{C}$  by closed squares. All values are means  $\pm$  s.e.m.,  $N=7$  for all groups.

$20^\circ\text{C}$  did not show any significant deviation from the initial force with increasing frequency. Furthermore, strips from group SB5 tested at  $20^\circ\text{C}$  showed a significant increase in relative force of contraction with increasing frequency at the higher calcium level. The strips run at  $5^\circ\text{C}$  from group YP5 and group SB5 showed a significant decline in force of contraction with increasing frequency, as in the  $1 \text{ mmol l}^{-1} \text{ CaCl}_2$  bathing medium. Only two of the seven strips from group SB5 tested at  $5^\circ\text{C}$  maintained the pacing regime to  $36 \text{ contractions min}^{-1}$ .

#### Time relationships

**Time to peak tension.** Increasing frequency had no effect on time to peak tension at the test temperature of  $20^\circ\text{C}$ , for strips from group YP20. However, strips run at  $5^\circ\text{C}$  from groups YP20 and YP5, and at  $20^\circ\text{C}$  for group YP5, showed a significant decrease in time to peak tension with increasing frequency (Fig. 5) with  $1 \text{ mmol l}^{-1} \text{ CaCl}_2$  in the medium. Group SB20 strips run at either 20 or  $5^\circ\text{C}$  showed a significant decrease in time to peak tension with increasing frequency, as did the strips run at  $5^\circ\text{C}$  from group SB5. Strips run at  $20^\circ\text{C}$  from group SB5 did not show any change in time to peak tension with increasing frequency of contraction.

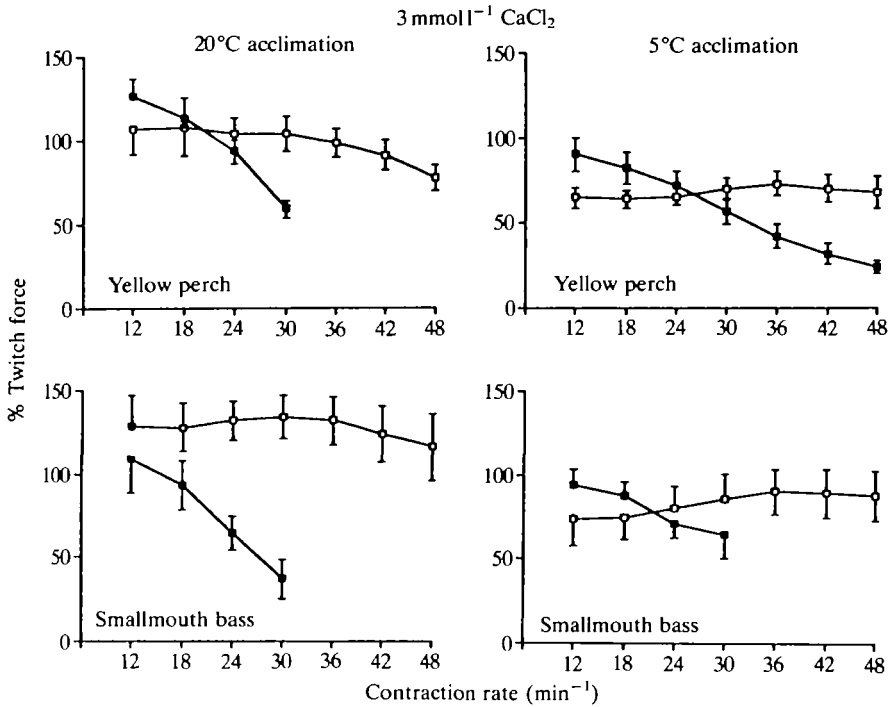


Fig. 4. Force development *versus* increasing frequency of contraction of isometrically contracting ventricle strips in bathing medium containing 3 mmol l<sup>-1</sup> CaCl<sub>2</sub> from yellow perch (top panels) and smallmouth bass (bottom panels) acclimated to 20°C (left side) and 5°C (right side). Strips tested at 20°C are represented by open squares, strips tested at 5°C are represented by closed squares. All values are means  $\pm$  s.e.m.,  $N=7$  for all groups.

The effects of increasing frequency on times to peak tension with 3 mmol l<sup>-1</sup> CaCl<sub>2</sub> in the medium were similar to those that occurred at the lower calcium level (data not shown).

*Time to 50% relaxation.* Increasing frequency had no effect on time to 50% relaxation in strips run at 20°C in medium containing 1 mmol l<sup>-1</sup> CaCl<sub>2</sub> from either group YP20 or YP5 (Fig. 6). In addition, these strips showed similar times to 50% relaxation for both acclimation groups. Time to 50% relaxation was 30–40% less for group YP5 than for group YP20 at the 5°C test temperature. For example, at 12 contractions min<sup>-1</sup>, time to 50% relaxation was 0.98  $\pm$  0.1 s for group YP20 and 0.68  $\pm$  0.08 s for group YP5. Above 30 contractions min<sup>-1</sup>, strips from group YP20 failed to maintain the pacing regime, unlike the strips from group YP5. In both cases there was a significant decline in relaxation time with increased frequency of contraction. In both acclimation groups, time to 50% relaxation was significantly longer at 5° than at 20°C.

Groups SB20 and SB5 showed no significant change in relaxation time with increasing contraction frequency under any test conditions. Relaxation time wa

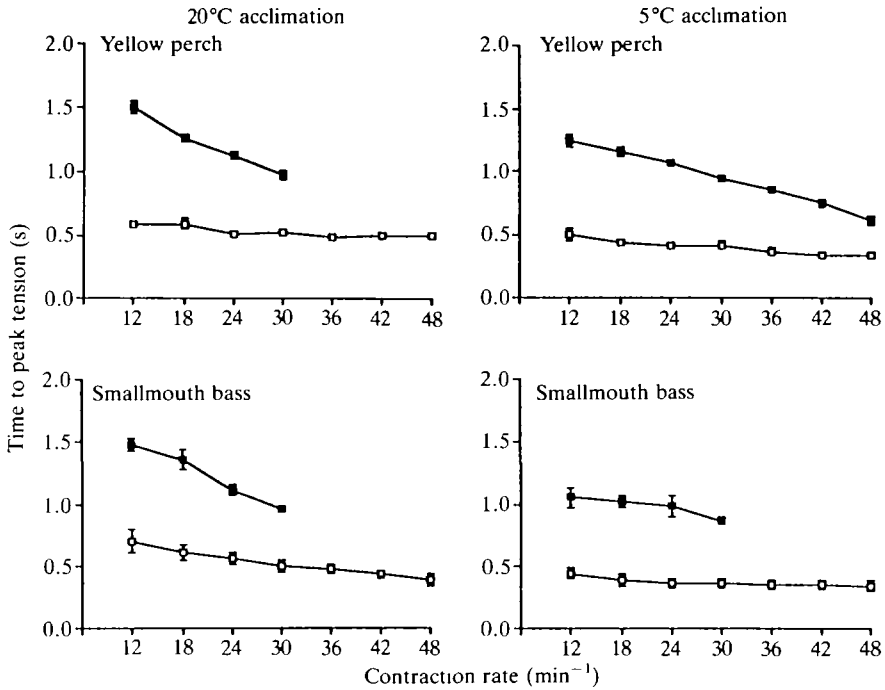


Fig. 5. Time to peak tension *versus* increasing contraction frequency of isometrically contracting ventricle strips in bathing medium containing  $1 \text{ mmol l}^{-1} \text{ CaCl}_2$  for yellow perch (top panels) and smallmouth bass (bottom panel) acclimated to  $20^\circ\text{C}$  (left side) and  $5^\circ\text{C}$  (right side). Strips tested at  $20^\circ\text{C}$  are represented by open squares; strips tested at  $5^\circ\text{C}$  are represented by closed squares. All values are means  $\pm$  s.e.m.,  $N=7$  for all groups.

significantly greater for strips run at  $5^\circ\text{C}$  than for strips run at  $20^\circ\text{C}$ . The strips tested at  $5^\circ\text{C}$  from group SB5 had significantly shorter relaxation times than strips tested at  $5^\circ\text{C}$  from group SB20.

Strips run at  $20^\circ\text{C}$  from either species showed no significant difference in time to 50% relaxation as a function of acclimation temperature (Fig. 6). However, strips run at  $5^\circ\text{C}$  from group YP20 and group SB20 showed significantly longer times to 50% relaxation than the strips run at  $5^\circ\text{C}$  from group YP5 or group SB5, respectively.

Increasing calcium concentration in the bathing medium to  $3 \text{ mmol l}^{-1}$  did not alter the relationships observed at  $1 \text{ mmol l}^{-1} \text{ CaCl}_2$  between time to 50% relaxation and frequency in any group at either test temperature, except SB20 tested at  $5^\circ\text{C}$ , where a small decrease in relaxation time with increasing frequency was observed from 18 to 30 contractions  $\text{min}^{-1}$  (data not shown).

## Discussion

### Calcium saturation studies

Ventricle strips from yellow perch acclimated to  $5^\circ\text{C}$  when tested at  $5^\circ$  or  $20^\circ\text{C}$ ,

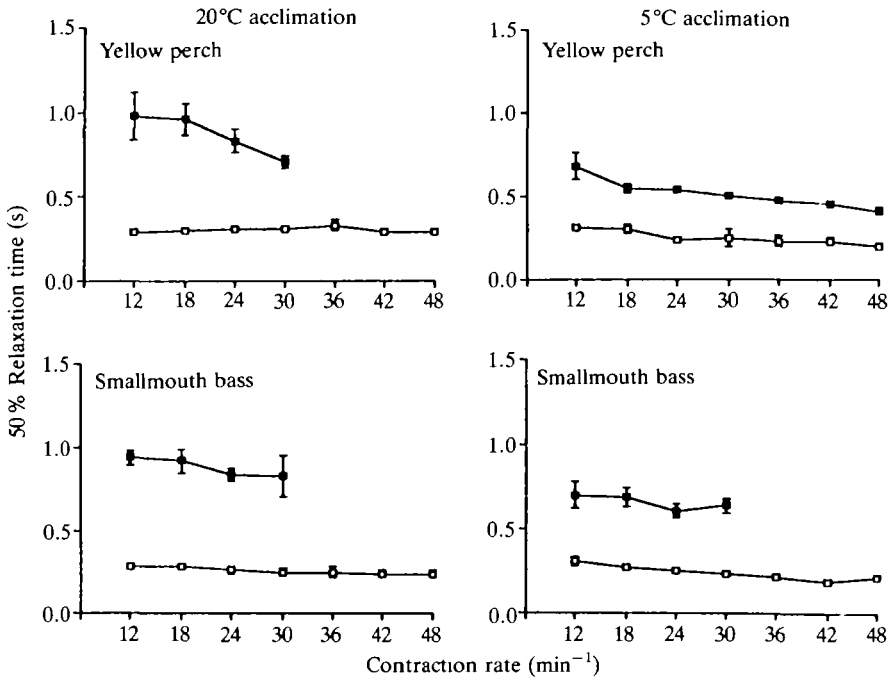


Fig. 6. Time to 50% relaxation *versus* increasing contraction frequency of isometrically contracting ventricle strips in bathing medium containing  $1 \text{ mmol l}^{-1} \text{ CaCl}_2$  for yellow perch (top panels) and smallmouth bass (bottom panels) acclimated to 20°C (left side) and 5°C (right side). Strips tested at 20°C are represented by open squares; strips tested at 5°C are represented by closed squares. All values are means  $\pm$  S.E.M.,  $N=7$  for all groups.

and strips from yellow perch acclimated to 20°C when tested at 20°C, all showed an increased force development when calcium concentration was elevated. However, ventricle strips from yellow perch acclimated to 20°C when tested at 5°C, showed a decreased twitch tension due to elevation in resting tension. In addition, relaxation times are prolonged at the 5°C test temperature for ventricle strips from 20°C-acclimated animals. A contractile event involves calcium recycling to and from the myofibrillar proteins. In amphibian heart, the calcium necessary to support contractility is derived primarily from the extracellular space and is extruded from the cell during relaxation (Morad and Cleeman, 1987). The situation appears to be similar in teleost hearts, which have a low volume of sarcoplasmic reticulum (Santer, 1985) and are insensitive to ryanodine, a known inhibitor of calcium release from sarcoplasmic reticulum (Driedzic and Gesser, 1988). Increases in resting tension and prolonged relaxation times suggest that, in ventricle strips from yellow perch acclimated to 20°C and tested at 5°C, calcium remains available to the contractile apparatus following peak tension development. This implies that calcium extrusion by the myocytes acclimated to 20°C is compromised at the lower test temperature. The basic finding, though, is that

acclimation to low temperature results both in the typical force *versus* calcium concentration curve shown in numerous other species (Driedzic and Gesser, 1985, 1988) and in a decreased time to 50 % relaxation.

Ventricle strips from smallmouth bass acclimated to either 5° or 20°C showed an increase in force development as extracellular calcium concentration was elevated at both test temperatures. Ventricle strips from animals acclimated to 20°C and tested at 5°C showed large increases in resting tension as external calcium concentration was elevated. Again, this is suggestive of a failure to extrude calcium.

#### *Force–frequency response at low temperature*

Ventricle strips from yellow perch acclimated to 5°C and tested at 5°C exhibited a negative force–frequency relationship over the frequency range tested. Ventricle strips from yellow perch acclimated to 20°C and tested at 5°C were unable to follow pacing rates in excess of 30 contractions  $\text{min}^{-1}$ . These relationships were the same at both extracellular calcium loads. The ability of the ventricle strips to maintain contractility at higher frequencies represents a clear alteration in potential function in response to acclimation to low temperature. Relaxation times at the 5°C test temperature were significantly shorter for ventricle strips from fish acclimated to 5°C than for those from fish acclimated to 20°C. This was observed at both 1 and 3  $\text{mmol l}^{-1}$   $\text{CaCl}_2$ . The shorter relaxation times induced by acclimation to low temperature may be a prerequisite for the higher maximal frequencies. At a test temperature of 5°C, a rate of 30 contractions  $\text{min}^{-1}$  and 1  $\text{mmol l}^{-1}$   $\text{CaCl}_2$  in the bathing medium, ventricle strips from 20°C-acclimated fish have a 50 % relaxation time of about 0.75 s and a time to peak tension of 1 s. Given that relaxation decays exponentially, a complete contractile event would take somewhat longer than 2.5 s. The maximum theoretical frequency would be slightly in excess of 24 contractions  $\text{min}^{-1}$ . This implies that the tissue is functioning at the edge of what the maximal achievable rate could be and that for this to increase the myocytes must have a further capacity to decrease either time to peak tension or relaxation time. In yellow perch ventricle strips, tested at 5°C, time to peak tension remains constant at all but the lowest frequencies, regardless of acclimation temperature. But following acclimation to low temperature, time to 50 % relaxation is reduced by about 0.5 s at all frequencies and both levels of extracellular calcium.

Smallmouth bass exhibit the same trends as the yellow perch, although the alterations induced by temperature acclimation are not nearly as profound. Ventricle strips from animals acclimated to 5°C and tested at 5°C are able to perform marginally better than strips from animals acclimated to 20°C and tested at 5°C. At low calcium concentration, two of seven preparations from specimens acclimated to 5°C were able to follow the pacing rate at 36 and 42 contractions  $\text{min}^{-1}$ ; at high calcium concentration two of seven preparations followed the rate to 36 contractions  $\text{min}^{-1}$ . Whereas, at both calcium loads, strips from 20°C-acclimated fish when tested at 5°C could contract maximally at 30 contractions

$\text{min}^{-1}$ . At low extracellular calcium concentration time to 50% relaxation was decreased by about 30%.

The greater enhancement of contractile performance following acclimation to low temperature in yellow perch than in smallmouth bass is consistent with their behavioural patterns. Yellow perch are active at low temperatures, whereas smallmouth bass are relatively quiescent. The whole-animal activity of perch at low temperature may be dependent upon heart performance to provide oxygen and nutrients to and remove end products from skeletal muscle either during elevated sustained swimming or following bursts of anaerobic activity. The alterations described here would allow animals to achieve higher heart rates, and presumably cardiac output, following acclimation to low temperature.

A faster relaxation time may be due either to the myocytes having less calcium to handle following a contractile event or to faster removal of calcium. The calcium saturation studies show an increase in resting tension (i.e. an inability to return to original resting tension) in strips from 20°C-acclimated animals when tested at 5°C and therefore favour the explanation that calcium sequestering and not the sensitivity of the contractile proteins is the locus of the adaptation to low temperature. As discussed above, in teleost heart, calcium removal from the sarcoplasm following a contractile event is dependent upon extrusion from the cell. The two primary mechanisms for calcium extrusion from mammalian myocytes are the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (Sutko *et al.* 1986) and a sarcolemmal  $\text{Ca}^{2+}$ -ATPase (Caroni and Carafoli, 1981). In amphibian heart, the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger accounts for about 15% of calcium extrusion and the ATP-dependent exchanger(s) accounts for the balance. Calcium efflux *via* the former system is temperature-insensitive; whereas, ATP-dependent calcium efflux proceeds with a  $Q_{10}$  of 1.5 over the temperature range 25–5°C (Brommundt and Kavalier, 1985). A portion of the calcium efflux from fish myocytes occurs *via* a  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (Busselen and Van Kerkhove, 1978; Gesser and Mangor-Jensen, 1984); however, indirect evidence suggests that there is also a  $\text{Na}^+$ -independent active component (Busselen and Van Kerkhove, 1978). One can speculate that these mechanisms are candidates for the low-temperature-induced decrease in relaxation time observed in the present experiments.

Other fish muscles may have a similar system. Fin muscles isolated from goldfish acclimated to 10°C display shorter isometric relaxation times than muscles removed from specimens acclimated to 28°C when both are tested at low temperatures (Heap *et al.* 1987). The authors attribute this to quicker sequestering of calcium by sarcoplasmic reticulum. Calcium removal following contraction in muscle may be a site of positive thermal compensation that has yet to be fully appreciated.

#### *Force-frequency response at high temperatures*

Ventricle strips from yellow perch and smallmouth bass, acclimated to 20°C and tested at 20°C, are able to maintain performance over the entire frequency range tested at both high and low calcium loads. Yellow perch strips acclimated to 5°C

and tested at 20°C showed a negative force–frequency relationship in medium containing 1 mmol l<sup>-1</sup> CaCl<sub>2</sub>. The negative response was ameliorated by an elevation in calcium levels such that strips paced at 48 contractions min<sup>-1</sup> developed as much tension as strips paced at 12 contractions min<sup>-1</sup>. A calcium enhancement of contractility was also noted for smallmouth bass, in that strips from 5°C-acclimated animals tested at 20°C are better able to maintain performance in high than in low extracellular calcium in response to a frequency challenge. Calcium enhancement of contractility could be a short-term mechanism to facilitate performance at high heart rates when an animal acclimated to low temperature is faced with an acute transition from cold to warm temperatures. This need not require an increase in plasma calcium level but only an increase in calcium delivery to the contractile apparatus. External triggers, such as an increase in catecholamines, could serve this purpose. It is not known with certainty if plasma catecholamine levels increase following an acute transition from cold to warm temperature; however, given that adrenaline levels routinely increase with stress, an increase following a temperature challenge is probable.

In yellow perch, temperature acclimation results in a decrease in relaxation time in cold-acclimated hearts at low test temperatures compared with warm-acclimated hearts at the same temperature. In smallmouth bass, which does not remain active at low temperature, this decrease in response time is not as pronounced. Decreased relaxation time is probably the result of an increased rate of calcium movement out of the myocyte. The physiological importance of this possible adaptation is that it would allow higher rates of contraction.

Ventricle strips from animals acclimated to low temperature and tested under conditions of high temperature and low calcium level show a negative force–frequency relationship. An elevation in calcium delivery to the contractile apparatus can ameliorate this response. This could allow animals acclimated to low temperature a means of achieving high heart rates at high temperatures when whole-animal oxygen demand is greatest.

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