

## EFFECT OF SEASON AND TEMPERATURE ACCLIMATION ON THE FUNCTION OF CRUCIAN CARP (*CARASSIUS CARASSIUS*) HEART

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### Summary

The temperature dependence of heart rate and isometric contractile properties of cardiac tissue was determined in thermally acclimated crucian carp (*Carassius carassius* L.). Fish were adapted for more than 3 weeks to 5 and 15°C during both summer and winter. At all but the lowest experimental temperatures, heart rate ( $f_H$ ), maximal isometric force ( $F_{max}$ ) and pumping capacity were higher in summer fish acclimated to 15°C than in winter fish acclimated to 5°C. Except for  $f_H$ , similar acclimation effects were observed when comparing cold- and warm-acclimated crucian carp in both seasons. In contrast, the kinetics of isometric contraction was not modified either by seasonal acclimatization or by thermal acclimation within a given season: the temperature dependence of time to peak force (TPT) and time to half-relaxation ( $T_{1/2R}$ ) was independent of the previous thermal history of the fish. The hearts of winter fish were about 10 % smaller than those of summer fish. These results show that acclimation to cold does not induce positive compensatory changes in function or size of crucian carp heart, as happens in many other teleost fishes. Instead, an inverse or noncompensatory acclimation was found. Owing to the inverse thermal acclimation, in winter (at 4°C) the contractile performance of the crucian carp heart is less than one-third of that in summer (at 20°C). The low cardiac activity in cold-acclimated crucian carp seems to be associated with its inactive life style, which is necessary during the winter months to minimize energy consumption in a severely hypoxic environment.

### Introduction

Temperature strongly influences the contractile performance of skeletal and cardiac muscles (Bennett, 1984; Farrell, 1984; Rall and Woledge, 1990). In many ectothermic animals the adverse effects of low temperature are alleviated by compensatory changes in metabolic, contractile or morphological properties of

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muscle, which are induced during graded seasonal adaption to changes in environmental temperature (Penney and Goldspink, 1980; Johnston *et al.* 1985; Egginton and Sidell, 1989; Johnston *et al.* 1990; Bailey and Driedzic, 1990; Bowler and Tirri, 1990). As a result of thermal acclimatization, muscular performance at low temperatures is usually better during the cold season than in the summer months (Fry and Hart, 1948; Rome *et al.* 1985; Bailey and Driedzic, 1990). Improved performance is expressed as the increased oxygen consumption of different muscles and of the whole animal after cold-acclimation (Tsukuda *et al.* 1985; Bowler and Tirri, 1990; Graham and Farrell, 1990) and is supported by enhanced aerobic production of ATP in mitochondria (Egginton and Sidell, 1989; Johnston *et al.* 1985).

Crucian carp (*Carassius carassius* L.) is a cyprinid fish that inhabits small, shallow ponds in Northern Europe and is exposed to large seasonal fluctuations in temperature. In winter the water temperature is just above zero, and during the summer months it may rise above 25°C in the small brown-water ponds. During winter, crucian carp are not only exposed to low temperature but are also faced with oxygen shortage in the severely hypoxic environment under the ice (Holopainen and Hyvärinen, 1985; Piironen and Holopainen, 1986). This is a paradoxical situation in which positive temperature compensation of muscle function would improve locomotor capacity in cold water, but oxygen shortage would limit the usage of these compensatory improvements in metabolism and in the muscular system. The heart is especially interesting in this situation since it is a strictly aerobic tissue, which needs a continuous supply of oxygen for proper functioning. Because compensatory changes in muscle function are typical of cyprinid fishes, including crucian carp (Johnston, 1982), and since the function of the heart is to support the metabolism of skeletal muscles and other tissues, the aim of this study was to determine the effects of temperature acclimation on heart muscle in this paradoxical situation. We tried to ascertain whether temperature acclimation induces compensatory changes in the size and function of the heart of crucian carp, as happens in many other teleost species. Temperature dependence of the  $f_H$  and the contractile properties of isolated cardiac tissue were studied in crucian carp acclimated to 5 and 15°C during the summer and winter months.

### Materials and methods

Crucian carp weighing 13–32 g, were caught in local ponds near Joensuu in eastern Finland. Winter fish were captured during late October 1989 and summer fish at the end of May 1990. In the laboratory they were kept in large tanks (500 l) filled with tap water. During both seasons the fish were divided randomly into two groups, which were acclimated for more than 3 weeks to 5 and 15°C. Crucian carp acclimated to 15°C were given commercial fish food twice a week. In contrast, fish acclimated to 5°C refused to eat and were therefore not fed.

$f_H$  was measured both in isolated hearts and in intact fish. For *in vivo* recording

of  $f_H$ , small needle electrodes were inserted through the ventral wall of the chest on both sides of the pericardium. Fine flexible leads, connecting the electrodes to the oscilloscope, were sutured with surgical thread under the belly of the fish. The fish was placed in a perforated plastic beaker (3 l), which was immersed in a larger water bath with a total volume of 6 l and was supplied with a thermostatted water flow. Usually the fish immediately settled on the bottom of the beaker and remained stationary during the experiment. The temperature of the bath at the onset of the experiment was the same as the acclimation temperature of the fish and it was gradually reduced to the lowest experimental temperature (2°C). When  $f_H$  had stabilized at 2°C, the water temperature was raised at a rate of 3° per 10 min and the electrocardiogram (ECG) was recorded at 3° intervals until 30°C was reached.

For *in vitro* recording of  $f_H$  and contractile properties, whole hearts with intact atrium and sinus venosus were gently excised and transferred to a tissue bath (15 ml) filled with a well-oxygenated physiological solution of the following composition (mmol l<sup>-1</sup>): NaCl 150, KCl 5, MgSO<sub>4</sub> 2, Na<sub>2</sub>HPO<sub>4</sub> 0.5, NaHCO<sub>3</sub> 11, CaCl<sub>2</sub> 2, and glucose 10. To keep the pH at 7.5 during the experiments, the solution was gassed with 95 % O<sub>2</sub>/5 % CO<sub>2</sub>. From the apex of the ventricle the heart was fixed to the bottom of the tissue bath with a needle electrode and from the bulbus arteriosus with a braided silk thread to a force-displacement transducer (Grass FT03). By changing the position of the force transducer with a micromanipulator, the ventricular muscle was stretched to the length where maximal force was produced. Heart rate, contractile force and its first derivative (recorded variables are listed in the next paragraph) were recorded on paper with a polygraph (Grass 7D) and appropriate amplifiers (Fig. 1). Signals were also led to an oscilloscope and selected tracings were photographed with a polaroid camera.

In some experiments, the isometric contractile properties of the ventricular muscle were studied from electrically paced tissue. The sinus venosus and atrial tissue were carefully trimmed away to eliminate spontaneous beating. Otherwise, the experimental conditions were the same as those for spontaneously beating hearts. Muscles were stretched to the optimum length and they were paced with a constant frequency of 0.2 Hz. The voltage of the square pulse was twice the threshold amplitude and its duration was kept at 5 ms. The following variables were measured from chart or oscilloscope recordings: maximal isometric force ( $F_{\max}$ ), time to peak force (TPT), time to half-relaxation from the peak force ( $T_{1/2R}$ ), maximal rate of force development ( $F'_{\max}$ ) and maximal rate of relaxation ( $F'_{\min}$ ) (Fig. 1). Temperature dependence of these variables in electrically paced and spontaneously beating hearts was determined in the range 4–35°C.  $Q_{10}$  values were calculated from the equation  $R_2/R_1^{(10/T_1-T_2)}$ , where  $R_1$  and  $R_2$  are the values of the variable at temperatures  $T_1$  and  $T_2$ , respectively. Because time-dependent variables and maximal isometric force decrease with rising temperature, reciprocals of their  $Q_{10}$  value are given to make comparison easier.

Relative heart size was determined for whole hearts and for ventricle and bulbus arteriosus. Hearts were blotted lightly on paper and wet mass was determined to

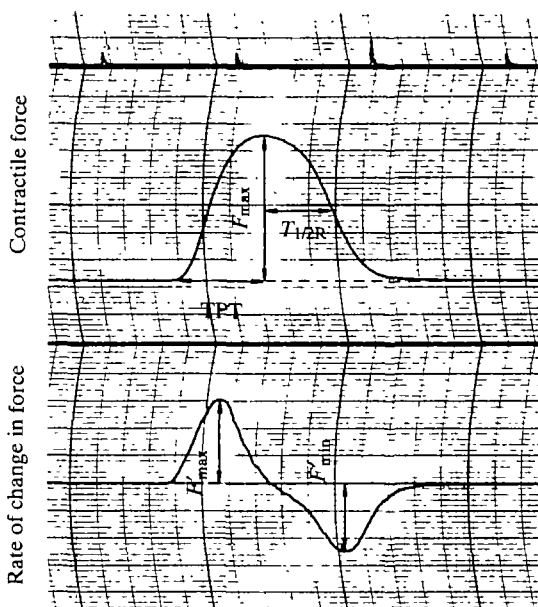


Fig. 1. Contractile variables measured in experiments with isolated fish hearts. The recording is from a warm-acclimated summer fish at the experimental temperature of 15°C.  $F_{\max}$ , maximal developed force; TPT, time to peak force;  $T_{1/2R}$ , time to half-relaxation;  $F'_{\max}$ , maximal rate of force development;  $F'_{\min}$ , maximal rate of relaxation. The top trace shows time in seconds.

the nearest 0.1 mg. Heart:body mass ratio was calculated for 15–18 fish in each acclimation group.

Student's *t*-test was used to compare mean values between acclimation groups. The differences were considered to be significant when  $P < 0.05$ .

## Results

### *Heart rate in vitro*

The beating frequency of excised isolated hearts provides information on temperature-induced changes in the pacemaker cells of the sinus venosus in the absence of interference from the controlling nervous and humoral systems. Because there was no difference in  $f_H$  between acclimation groups within a given season, the results in Fig. 2A are pooled data from both cold- and warm-acclimated fish. At the lowest experimental temperature (4°C), the values of  $f_H$  of winter and summer fish were equal (8–9 beats  $\text{min}^{-1}$ ), but when the temperature was raised, the  $f_H$  of summer fish increased more than that of winter animals. This difference was significant ( $P < 0.05$ ) at 13°C and became more pronounced at higher temperatures. Consequently, the  $Q_{10}$  value for summer fish (2.39) was slightly higher than that of winter fish (2.22) (Table 1). The temperature tolerance of  $f_H$  was higher in summer fish than in winter fish. At temperatures over 28°C the

Table 1.  $Q_{10}$  values for cold- and warm-acclimated crucian carp in winter and in summer

Variable	Winter		Summer	
	5°C	15°C	5°C	15°C
TPT(s) (4–22°C)	3.08		3.10	
(22–37°C)	2.26		2.32	
$T_{1/2R}$ (s) (4–22°C)	3.44		3.03	
(22–37°C)	1.87		1.78	
Heart rate (beats min <sup>-1</sup> )				
<i>In vitro</i> (4–28°C)	2.22		2.39	
<i>In vivo</i> (2–26°C)	2.17		2.27	
$F_{\max}$ (mg mg <sup>-1</sup> tissue) (4–19°C)	1.78	1.49	2.03	1.49
Pumping capacity (g mg <sup>-1</sup> tissue min <sup>-1</sup> ) (4–19°C)	1.59	1.89	1.73	2.03

Values for time-dependent variables and  $F_{\max}$  are given as  $1/Q_{10}$  to make comparisons easier.

For heart rate, time to peak force (TPT) and time to half-relaxation from peak force ( $T_{1/2R}$ ), combined results from both cold- and warm-acclimated animals are given.

Temperature range for each  $Q_{10}$  determination is shown in parentheses.

beating frequency of the winter fish hearts began to decline, whereas the  $f_H$  of summer fish continued to rise up to 34°C without any signs of deterioration (Fig. 2A). Therefore, the maximum values of  $f_H$  were higher in summer fish (88 beats min<sup>-1</sup> at 34°C) than in winter fish (54 beats min<sup>-1</sup> at 28°C). The summer value may not be the true maximum, since temperatures over 34°C were not tested.

#### Heart rate in vivo

$f_H$  measurements from live fish are complementary to *in vitro* experiments in that the neuronal modulation of the basal  $f_H$  is present. ECGs could be measured reliably for several hours in crucian carp, since after the electrodes had been mounted the fish settled down quietly on the bottom of the reservoir. The results from these ECG measurements are qualitatively very similar to the *in vitro* recordings of  $f_H$  (Fig. 2B). Again, the  $f_H$  of summer fish was higher than that of winter fish, but intraseasonal thermal acclimation did not affect  $f_H$ . A clear difference in temperature tolerance was also found. The beating frequency of the hearts of summer fish continued to increase up to 30°C without any indication of arrhythmia, in contrast to the  $f_H$  of winter fish, which reached a maximum at 26°C and then declined at higher temperatures. In general, the resting values of  $f_H$  of live fish were slightly higher than *in vitro* basal values of  $f_H$ . Resting  $f_H$  values of live fish at the lowest test temperature (2°C) were 10–11 beats min<sup>-1</sup>, and

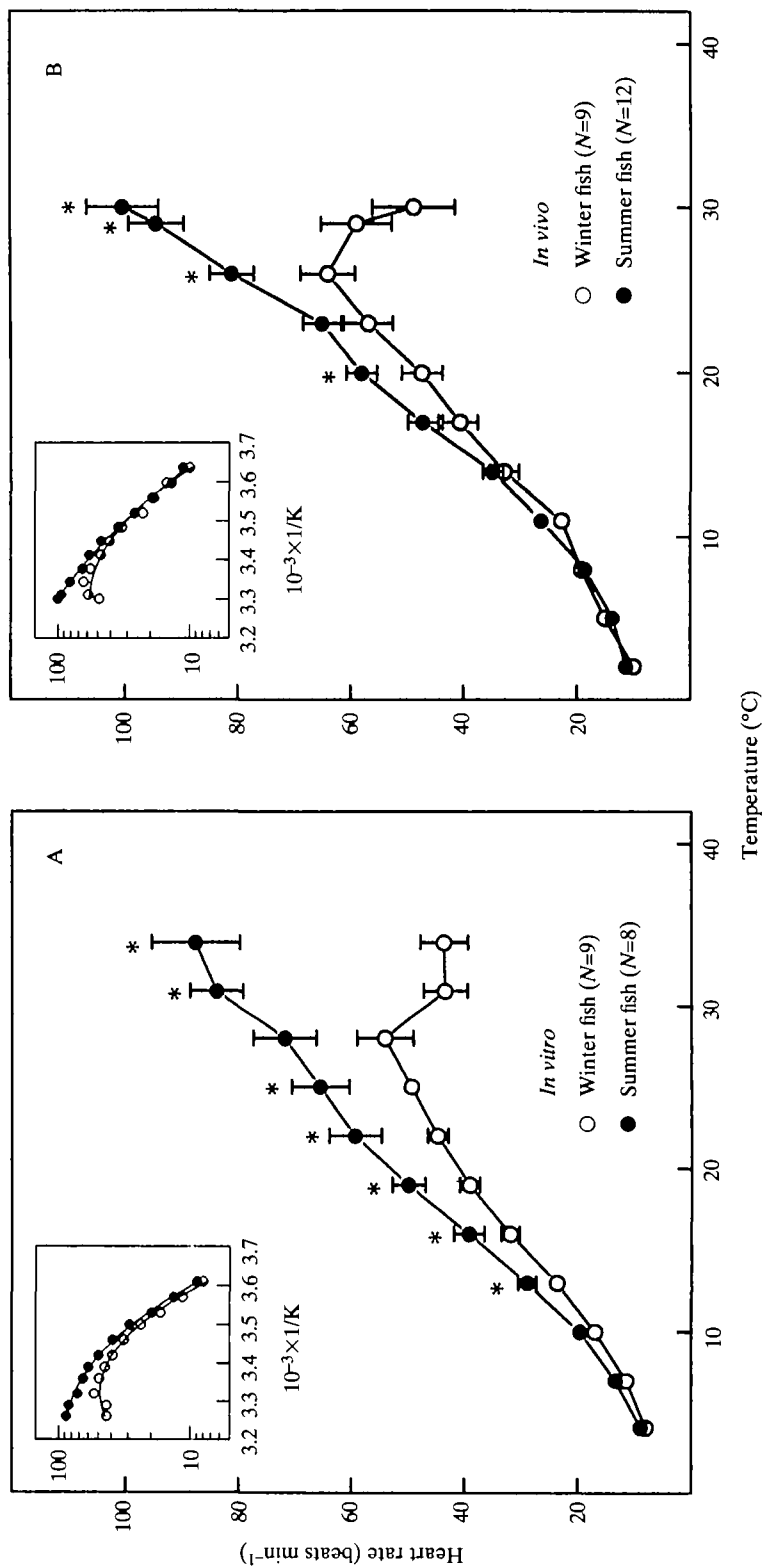


Fig. 2. Temperature dependence of heart rate in crucian carp during summer and winter. (A) *In vivo* experiments with isolated hearts. (B) Results from *in vivo* ECG recordings from live fish. Results are means  $\pm$  s.e. of 8–12 hearts or animals as shown in the figure. Arrhenius plots of the same data are shown in insets. \* indicates a value significantly different from that for winter fish ( $P < 0.05$ ).

maximum values of  $f_H$  were 64 and 100 beats  $\text{min}^{-1}$  for winter and summer fish, respectively.

### *Isometric force*

Unlike  $f_H$ , maximal force of contraction was changed by temperature acclimation during both seasons. The  $F_{\max}$  of spontaneously beating hearts, expressed per gram of tissue (wet mass), was almost the same in warm- and cold-acclimated fish at the lowest experimental temperature (4°C). With increasing temperature,  $F_{\max}$  declined almost linearly but the decrease was steeper in cold-acclimated than in warm-acclimated fish (Fig. 3). This is reflected in the lower  $Q_{10}$  values of fish acclimated to 15°C (Table 1). If winter fish acclimated to 5°C and summer fish acclimated to 15°C are compared, the same phenomenon is noticed: at 4°C, the values of  $F_{\max}$  are equal but, with rising temperature, the force of the contraction in winter fish hearts declines faster than in those of summer fish (see Fig. 9).

It is important to note that the effect of temperature on  $F_{\max}$  of paced ventricles, i.e. in muscles contracting with constant frequency, is biphasic (Fig. 4). When the temperature is raised from 4°C, there is an initial steep decline in  $F_{\max}$ , but above 19°C,  $F_{\max}$  together with  $F'_{\max}$  and  $F'_{\min}$  clearly begin to increase (Fig. 4). This secondary rise in maximal force is absent in spontaneously beating hearts (Fig. 3).

The linear decrease in  $F_{\max}$  in spontaneously beating hearts is probably the combined result of the influence of temperature (as shown in paced hearts) and the negative inotropic effect of increased beating frequency.

Temperature acclimation changes the force–frequency relationship of the crucian carp heart (Figs 5 and 6). In cold-acclimated animals, the typical negative force–frequency relationship (decrease in force with increasing rate) of teleost fishes was evident, but in warm-acclimated crucian carp, a transient (summer) or sustained (winter) positive force–frequency relationship (increase in force with increasing rate) was found. It was characteristic of the hearts of cold-acclimated crucian carp that at a waterbath temperature of 15°C they could not follow the highest pacing rate of 1 Hz (Fig. 5).

### *Pumping capacity*

The product of the  $f_H$  and the  $F_{\max}$  of isolated isometrically contracting hearts was used as a measure of the cardiac output or pumping ability of the crucian carp heart and the term ‘pumping capacity’ was used for it. The pumping capacity of the crucian carp heart was very sensitive to temperatures between 4 and 20°C (Fig. 7). Furthermore, its temperature dependence clearly differed in the two acclimation groups. The  $Q_{10}$  value for warm-acclimated fish was higher than that for cold-acclimated fish in both summer (2.03 vs 1.73) and winter (1.89 vs 1.59). As a result, the pumping capacity of warm-acclimated fish was higher than that of cold-acclimated animals. The difference was especially pronounced between 10 and 30°C.

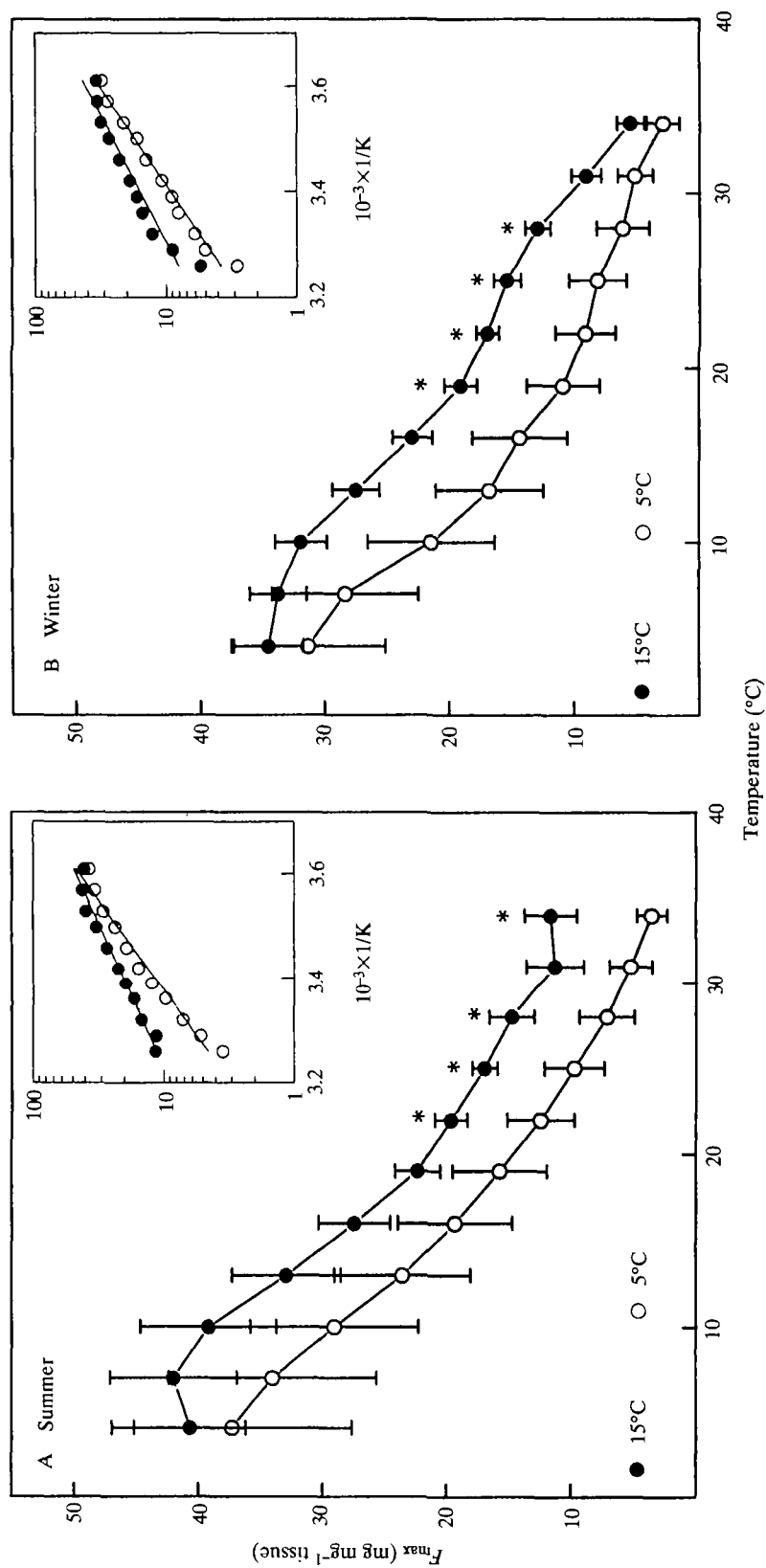


Fig. 3. Maximal isometric force of spontaneously beating hearts from cold-acclimated (5°C) and warm-acclimated (15°C) crucian carp during summer (A) and winter (B) months. Results are means  $\pm$  s.e. of four hearts.  $F_{\max}$  is expressed as mg g<sup>-1</sup> tissue wet mass. \*, significantly different from value for cold-acclimated fish ( $P < 0.05$ ). Insets show Arrhenius plots of the same data.



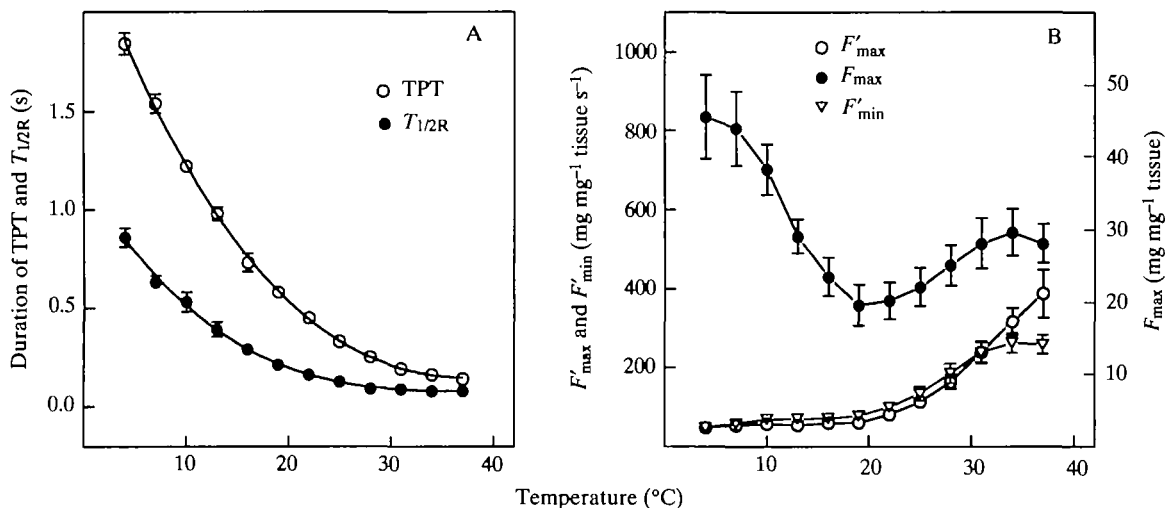


Fig. 4. Temperature dependence of contractile parameters (TPT,  $T_{1/2R}$ ,  $F_{\max}$ ,  $F'_{\max}$ ,  $F'_{\min}$ ) in paced hearts of winter crucian carp. Note the biphasic temperature dependence of  $F_{\max}$ . Results are means  $\pm$  s.e. of four hearts. Pacing frequency was 0.2 Hz.

#### Duration of contraction

Time to peak force and time to half-relaxation were used to characterize the duration of the contraction and relaxation phases of the cardiac cycle. Thermal acclimation had no effect on these time-dependent variables of cardiac contraction. The most clear-cut finding from these experiments was the strong temperature sensitivity of the variables below 22  $^{\circ}\text{C}$  (Fig. 8), the  $Q_{10}$  values at this temperature range being over 3 (Table 1). Above 22  $^{\circ}\text{C}$  the duration of the contraction-relaxation cycle was only slightly shortened by rising temperature. The temperature sensitivity of the relaxation phase was especially reduced:  $Q_{10}$  values above 22  $^{\circ}\text{C}$  were 1.9 and 1.5 for TPT and  $T_{1/2R}$ , respectively. This pattern of temperature effect is in sharp contrast to the temperature dependence of  $F_{\max}$ ,  $F'_{\max}$  and  $F'_{\min}$  (shown in Fig. 4) for paced ventricles of the winter fish. Neither season nor temperature acclimation had any major effects on the time-dependent contractile properties. The slightly lower TPT of the summer fish is consistent with the higher  $f_H$  in this group. A general tendency was that the duration of contraction at low temperatures was longer in spontaneously beating hearts than in paced muscles. This is readily understandable, since the frequency of paced ventricles (12  $\text{min}^{-1}$ ) is clearly higher than the inherent rhythm of the spontaneously beating hearts (8  $\text{min}^{-1}$ ) at low temperatures, while a high rate of pacing tends to shorten the duration of contraction.

#### Heart size

Cold-acclimation was associated with a slight increase in the size of the whole

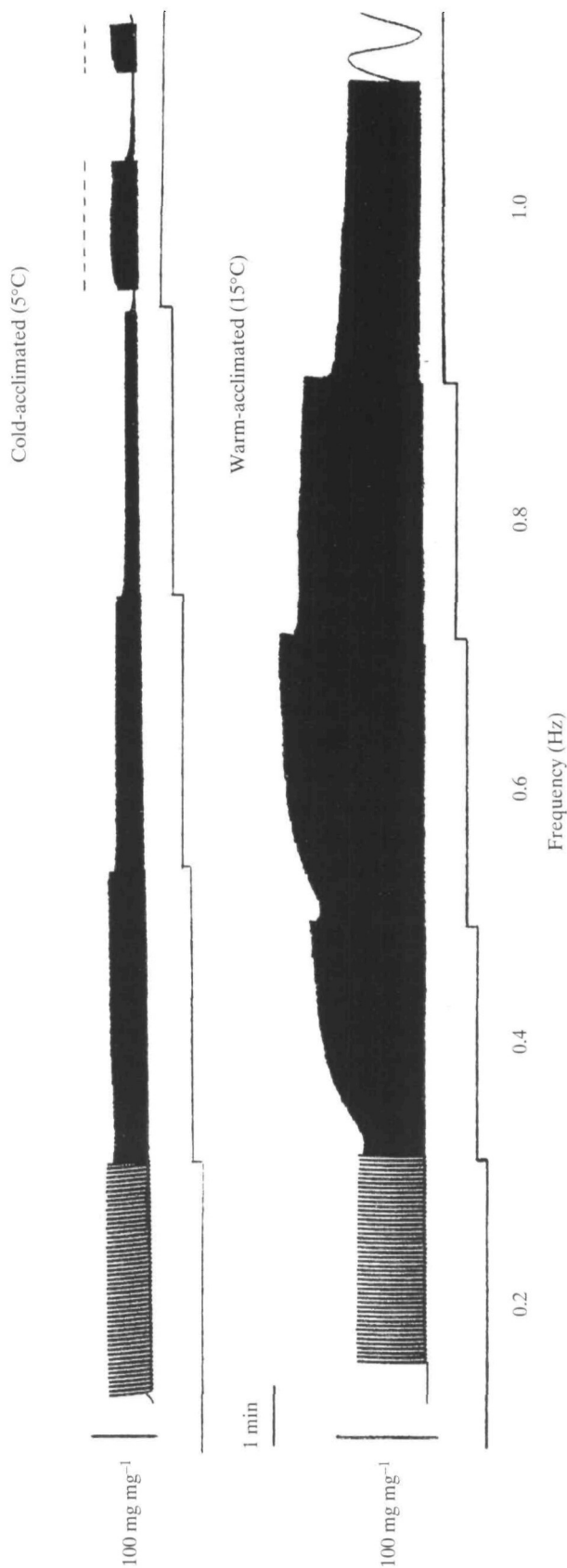


Fig. 5. Original polygraph recordings of the force-frequency relationship for hearts of warm- and cold-acclimated crucian carp during summer. Pacing rate was raised from 0.2 to 1.0 Hz at 0.2 Hz intervals. The heart of the cold-acclimated carp could not follow the highest pacing rate (dashed line). The vertical bar indicates 100 mg force per mg tissue wet mass. The temperature of the tissue bath was 15°C.

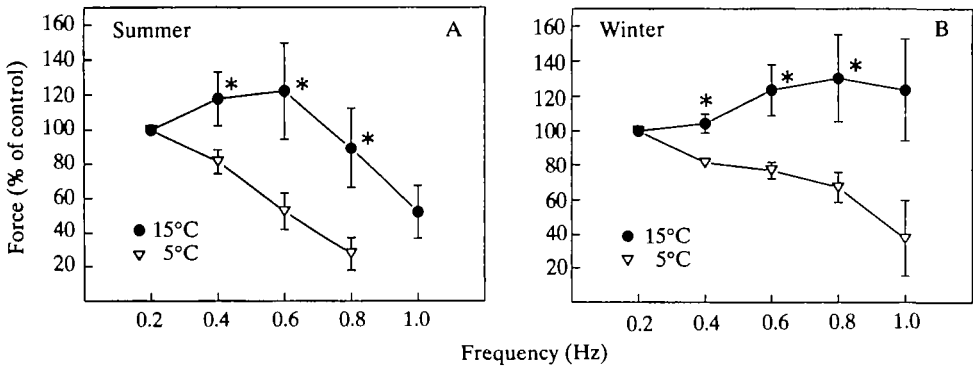


Fig. 6. Force–frequency relationship of cold- and warm-acclimated crucian carp hearts in summer (A) and winter (B). The results are means  $\pm$  s.e. of 4–5 fish. \* indicates a significant difference ( $P < 0.05$ ) between warm- and cold-acclimated animals.

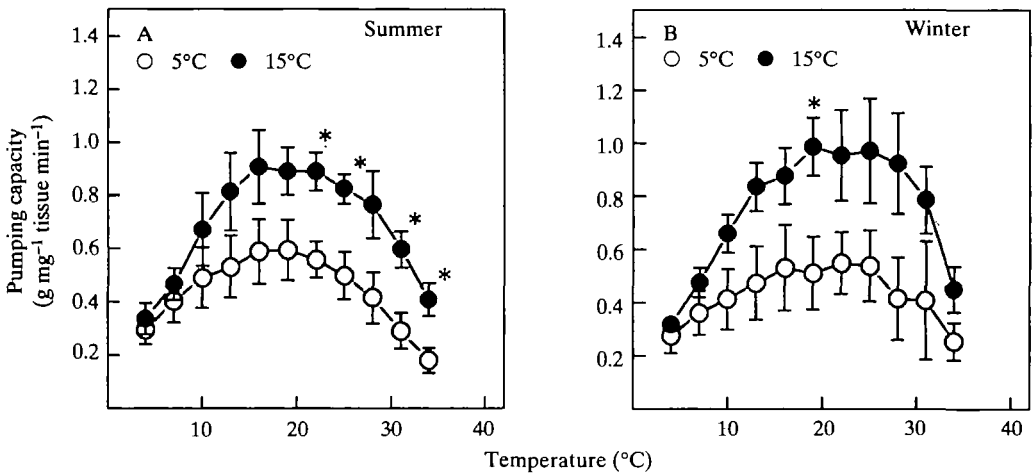


Fig. 7. Pumping capacity, calculated as the product of heart rate and maximal isometric force, of the hearts of cold- and warm-acclimated crucian carp in summer (A) and winter (B). \* significantly different from value for cold-acclimated carp ( $P < 0.05$ ). Results are means  $\pm$  s.e. of four fish.

heart and the ventricle but not in the size of the bulbus arteriosus in both summer and winter. The effect of 10°C differences in acclimation temperature on heart size was, however, minor. The increase in ventricular mass due to cold-acclimation was 5.5% in winter and 17.2% in summer, the latter value being statistically significant. Seasonal acclimatization had an opposite effect on heart size, winter fish having about 10% smaller hearts than summer fish (Table 2).

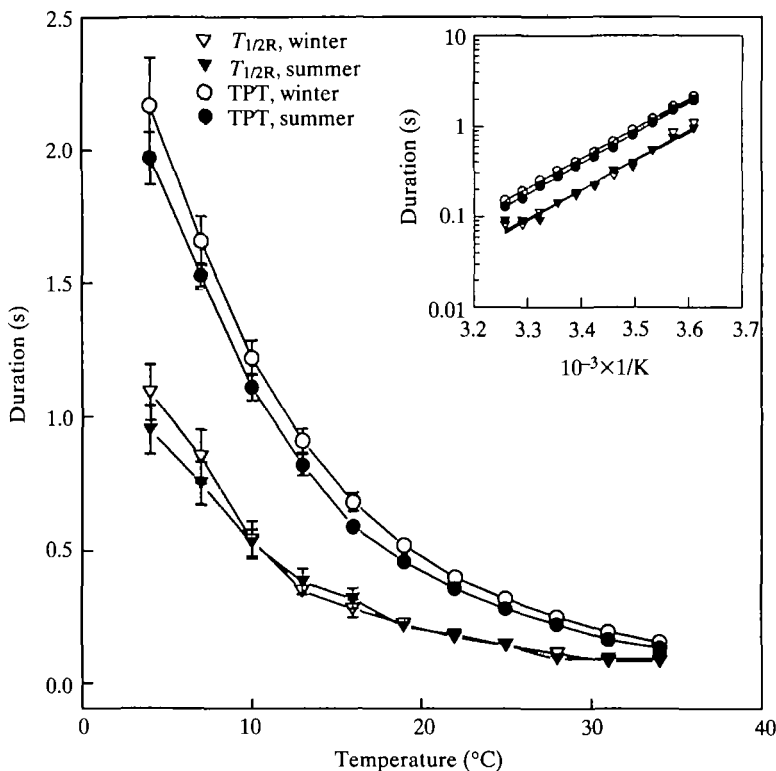


Fig. 8. Temperature dependence of the duration of isometric contraction in spontaneously beating hearts of crucian carp in winter and summer. The results are means  $\pm$  S.D. of eight fish. The inset shows Arrhenius plots of the data.

## Discussion

### *Direct effects of temperature on heart function*

Rising temperature had a positive chronotropic but a negative inotropic effect on the spontaneously beating heart of crucian carp. Because  $f_H$  has a direct effect on contractility, the temperature-induced changes in inotropy and kinetics of contraction were also studied on paced preparations. According to the results of these experiments, the durations of activation and relaxation are shortened by rising temperature, especially at temperatures between 4 and 20°C. Above 20°C, the duration of isometric contraction was reduced only slightly. These effects of temperature on the time-dependent variables of contraction in the crucian carp heart are consistent with the general finding that TPT and  $T_{1/2R}$  in vertebrate muscles are markedly temperature-dependent over the entire temperature range, and that this dependence is weaker at higher temperatures (Bennett, 1984). Shortening of contraction duration (below 20°C) was associated with a prominent decline in  $F_{\max}$ ; whereas, above 20°C, temperature had a positive inotropic effect. Similarly,  $F'_{\max}$  and  $F'_{\min}$  were only increased slightly in the temperature range 4–20°C, but above 20°C, both variables increased very steeply. Taken together,

Table 2. *Effect of season and temperature acclimation on relative heart size of crucian carp*

	Winter		Summer	
	5°C	15°C	5°C	15°C
Fish	21.9±4.57 (15)	22.4±4.24 (16)	21.9±3.50 (18)	20.7±4.51 (16)
Whole heart	0.093±0.009	0.091±0.017	0.107±0.016	0.096±0.011
Ventricle	0.054±0.005	0.050±0.008	0.060±0.011*	0.053±0.006
Bulbus	0.029±0.004	0.029±0.009	0.029±0.006	0.030±0.006

Values for whole fish are given as mass (g). Values for whole heart, ventricle and bulbus are given as a percentage of body mass.

The results are means±s.d. of 15–18 animals as indicated in parentheses.

\* significantly ( $P<0.05$ ) different from the value of warm-acclimated summer fish.

these findings suggest that the negative effect of temperature on the contractility of the crucian carp heart is closely correlated with shortening of the active state as has been suggested previously (Vornanen, 1989). When shortening of the contraction duration ceases, at around 20°C, the positive inotropic effects of rising temperature are revealed.

#### *Effect of season and temperature acclimation on heart function*

Considering the effects of seasonal acclimatization, the most relevant comparison is made between 5°C-acclimated winter fish and 15°C-acclimated summer fish (Fig. 9). Note that at the lowest experimental temperatures (2 or 4°C) three of the physiological variables ( $f_H$ ,  $F_{\max}$ , pumping capacity) were almost equal for both groups, but at higher temperatures the values for all three variables were higher in warm-acclimated summer fish than in cold-acclimated winter fish. This type of temperature acclimatization, which includes rotation of the curves of the temperature-dependent variables and where the cold-acclimated animal has the lower  $Q_{10}$  value, is designated as noncompensatory or inverse acclimation by Prosser (1973). Owing to the inverse acclimation, the contractile performance of the crucian carp heart is more than threefold higher in summer (at 20°C) than in winter (4°C).

Shortening of the activation and relaxation phases of muscle contraction has been shown to occur in both skeletal and cardiac muscles of some fish species after cold-acclimation (Heap *et al.* 1987; Fleming *et al.* 1990; Bailey and Driedzic, 1990). The changes were suggested to be due either to more efficient handling of  $\text{Ca}^{2+}$  by the sarcoplasmic reticulum or to reduced diffusion distance for  $\text{Ca}^{2+}$  in the cold-acclimated fish (Penney and Goldspink, 1980; Fleming *et al.* 1990; Bowler and Tirri, 1990). According to the present study, a 10°C difference in environmental temperature does not alter the time course of contraction in the crucian carp heart.

An increase in isometric force production after cold-acclimation has been demonstrated in skinned skeletal muscles of several cyprinid fishes, but not those of flounder (*Platichthys flesus*) (Johnston *et al.* 1990; Johnston and Wokoma,

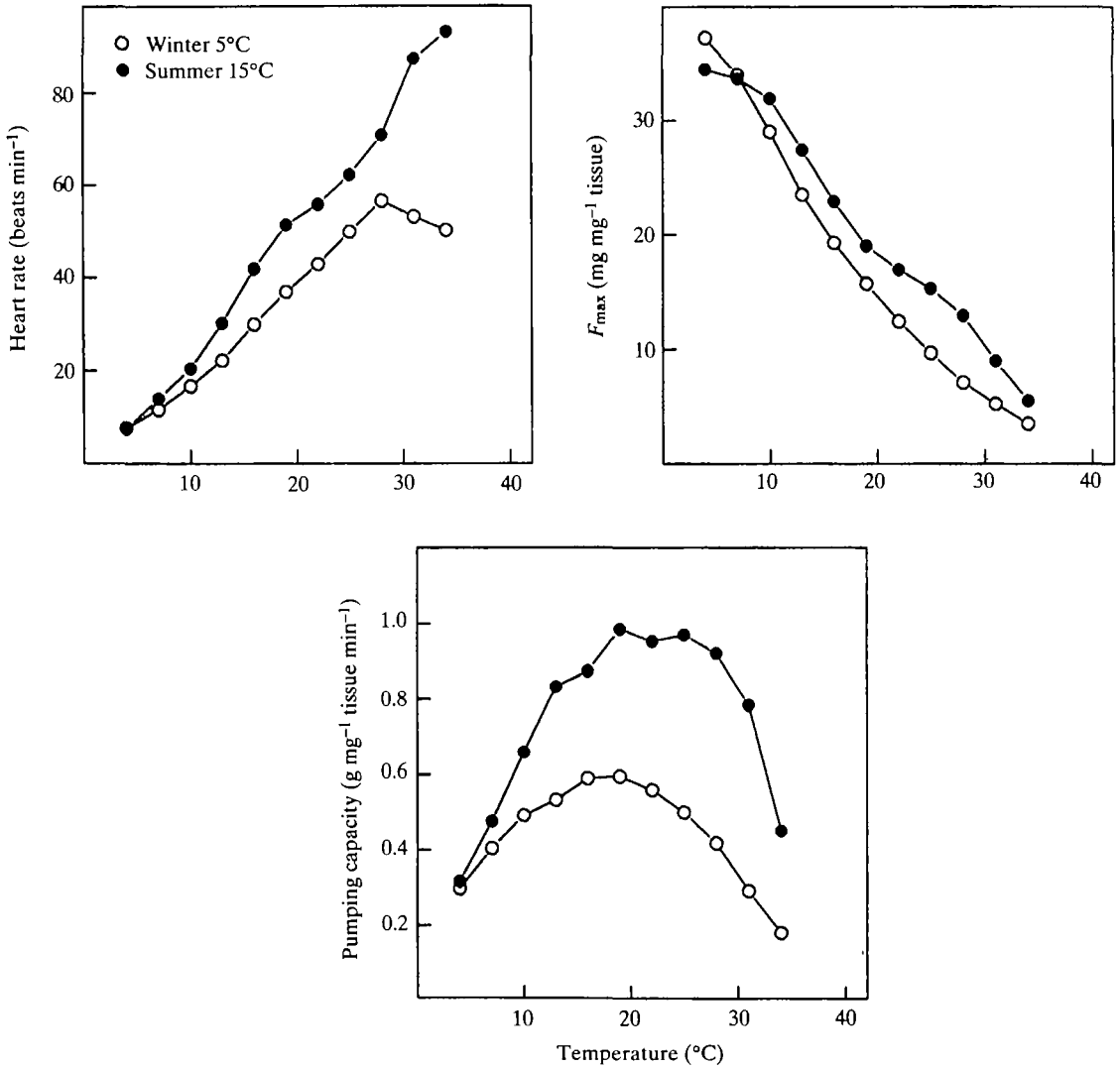


Fig. 9. Summary of the differences in cardiac contractile function between cold-acclimated (5°C) winter fish and warm-acclimated (15°C) summer fish. Results are means of four fish for each group. Error bars are not shown.

1986). This is thought to involve changes in myofibril composition. In contrast to these findings, acclimation to cold induces an inverse acclimation in the force production of the heart muscle of crucian carp. The higher  $F_{\max}$  of summer fish became more evident at higher temperatures, i.e. at higher beating rates. Therefore, the difference seems to be explained by the temperature-induced change in the force–frequency relationship. The contraction force of warm-acclimated fish hearts increased with increasing beating rate, but the contractility of the hearts of cold-acclimated fish decreased when the pacing rate was increased.

The difference in force staircase between acclimation groups may reflect temperature-induced changes in  $\text{Ca}^{2+}$  regulation of the fish myocytes, as is also suggested by the differential sensitivity of the contractile force to external  $\text{Ca}^{2+}$ . The force production of the hearts of winter fish at  $2 \text{ mmol l}^{-1}$  external  $[\text{Ca}^{2+}]$  was about 90 % of the maximum but in summer fish it was only about 60 %. The pumping capacity of an isolated heart was measured as the product of  $f_H$  and  $F_{\max}$ . As noted earlier, temperature has opposite effects on these variables: rising temperature increases  $f_H$  but decreases force production. Furthermore, increasing  $f_H$  often has a negative inotropic effect because of the negative force staircase of teleost fishes (Driedzic and Gesser, 1985; Vornanen, 1989). Because there were no changes in  $f_H$  between acclimation groups within a given season, the higher pumping capacity of warm-acclimated animals was due solely to changes in  $F_{\max}$ . Much of the difference is explained by the steeper negative staircase of cold-acclimated crucian carps. It should be noted, however, that if we compare the pumping capacity of the hearts of winter fish acclimated to  $5^\circ\text{C}$  with that of summer fish acclimated to  $15^\circ\text{C}$ , the higher pumping capacity of the latter is due to both higher  $f_H$  and higher  $F_{\max}$  in summer.

In many teleost fishes cold-acclimation is associated with an increase in relative heart mass, which partially compensates for the adverse effects of low temperature on contractile performance (Goolish, 1987; Tsukuda *et al.* 1985; Graham and Farrell, 1990). In crucian carp the size of the heart is only slightly affected by long-term exposure to low temperatures. In both winter and summer seasons, cold-acclimation increased heart size slightly, showing a 10–20 % compensation of this variable. Seasonal changes in heart size were also minor, winter fish having slightly smaller hearts than summer fish. Again, this is an example of inverse temperature acclimation in the cardiovascular system of crucian carp.

The  $f_H$  of teleost fishes is determined by the intrinsic rate of pacemaker cells in the sinus venosus and its extrinsic control by the autonomic nervous system or humoral factors (Laurent *et al.* 1983). The contribution of intrinsic and extrinsic components to temperature-induced changes in the  $f_H$  of crucian carp was studied by recording the beating of isolated hearts and the ECG of intact fishes. Two seasonal effects on  $f_H$  were noted: the beating frequency of summer fish was higher than that of winter fish at temperatures above  $8^\circ\text{C}$ , and the pulsation rate of the heart of summer fish was much more tolerant to high temperatures than was that of winter fish. The presence of these effects in isolated as well as in intact fish suggests that they are determined primarily by seasonally induced changes in the temperature sensitivity of the pacemaker cells. Thus, the inverse acclimation of  $f_H$  in crucian carp seems to be mediated by temperature-induced changes at the level of cardiac tissue, whereas compensatory acclimation of the  $f_H$  of other fish species is caused mainly by alterations in cholinergic or adrenergic control of pacemaker cells.

The absence of compensation at low temperatures and inverse compensation at higher temperatures of  $f_H$  in crucian carp is in sharp contrast to the reported compensatory changes of  $f_H$  in many other fish species. In trout, the acute effects

of temperature on  $f_H$  are reduced by tonic adrenergic stimulation as a result of cold-acclimation (Graham and Farrell, 1989). Cold-acclimated sole (*Solea vulgaris*) and eel (*Anguilla anguilla*) have higher  $f_H$  values than do warm-acclimated animals of the same species, and this difference is caused largely by the higher cholinergic tone in warm-acclimated animals (Seibert, 1979; Sureau *et al.* 1989). In both species, the compensation effect was more pronounced at temperatures above 12°C. Partial thermal compensation of  $f_H$  has also been found in cold-acclimated perch (*Perca fluviatilis*) (Bowler and Tirri, 1990). In this species, in contrast to sole and eel, the difference in  $f_H$  between warm- and cold-acclimated fish was more pronounced at lower temperatures. The sea raven (*Hemitripterus americanus*) displays no significant temperature compensation for intrinsic  $f_H$  (Graham and Farrell, 1985). In isolated hearts of goldfish (*Carassius auratus*), the pulsation rates for warm- and cold-acclimated fish were the same at 5°C but significantly higher in warm-acclimated fish at 25 and 30°C (Tsukuda, 1990). Our results with crucian carp are similar to those obtained with goldfish. Unlike us, however, Tsukuda (1990) compared 'relative' values and concluded that partial adaptive compensation of  $f_H$  occurs in goldfish. The great variation in acclimation responses between species may be caused by differences in the autonomic nervous system, which controls  $f_H$ , or by differences in locomotor activity.

#### *Physiological significance of inverse temperature acclimation*

Inverse acclimation of  $f_H$ , contraction force, pumping capacity and size of the heart in crucian carp differs from the general compensatory acclimation found in other fish species. Absence of compensatory acclimation in heart size and function means that the performance of cardiovascular systems decreases steeply in winter with declining water temperature and that the ability of the cardiovascular system to support metabolism and locomotor activity weakens accordingly. Hazel and Prosser (1970) have suggested that animals that become lethargic or torpid in the cold show no or inverse acclimation in metabolism and enzyme activities. Consistent with this supposition, acclimation-induced changes in heart function seem to be more pronounced in species that are active at low temperatures than in more sedentary species or in species that enter into a hypometabolic state (Bailey and Driedzic, 1989). Crucian carp inhabit small ponds that freeze nearly to the bottom during the winter season. This species has an excellent capacity to withstand prolonged hypoxia, which is accomplished by efficient anaerobic metabolism and especially by low activity during unfavourable conditions (Blazka, 1958; Shoubbridge and Hochachka, 1981; Piironen and Holopainen, 1986; Nilsson, 1989). Behaviourally, crucian carp respond to hypoxia by settling quietly in one place and making only necessary respiratory movements and balancing movements of the fins. Accordingly, during anoxia the energy consumption of crucian carp has been shown to decline by 70 % from the normoxic value at 20°C (van den Thillart *et al.* 1989). In natural conditions, the energy demand is further reduced by cold water. Accordingly, the requirements set by whole-body metabolism on the function of the heart muscle are far lower in winter than in summer, especially



when energy is produced by anaerobic routes in the hypoxic environment. Therefore, the reason for the absence of positive temperature compensation in heart function may be because the fish are inactive in winter. In fact, positive temperature compensation could be a nonadaptive change for crucian carp, since increased heart size and performance would increase energy (oxygen) consumption, which might not be possible in severely hypoxic conditions. It has been shown that goldfish, when exposed to hypoxia, save energy by voluntarily moving to a colder environment (Rausch and Crawshaw, 1990). The absence of temperature compensation in the crucian carp heart is analogous to behavioural regulation of metabolism in that low temperature is used to reduce cardiac performance, thereby reducing oxygen consumption in a severely hypoxic environment.

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