

Cardiorespiratory responses of the common carp (*Cyprinus carpio*) to severe hypoxia at three acclimation temperatures

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Summary

In vivo measurements of the cardiovascular responses of anoxia-tolerant teleosts to severe prolonged hypoxia are limited. Here, we report the first direct measurements of cardiac output (Q), heart rate (f_H) and stroke volume during prolonged severe hypoxia ($<0.3 \text{ mg O}_2 \text{ l}^{-1}$) in common carp (*Cyprinus carpio* L.) that had been acclimated to 6, 10 and 15 °C. While routine Q and f_H values varied with temperature under normoxic conditions (Q_{10} values of 1.7 and 2.6, respectively), severe hypoxic exposure significantly depressed f_H and Q to similar minimum values that were largely independent of acclimation temperature (Q_{10} values of 1.2). In contrast, the duration of cardiac depression and the subsequent time period during which carp could tolerate severe

hypoxia were inversely related to acclimation temperature (24 h at 6 °C, 6 h at 10 °C, and 2.5 h at 6 °C). Likewise, respiration rate during hypoxia showed a temperature dependence. An unusual finding was that cardiorespiratory status partially recovered during the latter stages of severe hypoxic exposure. We conclude that the cardiorespiratory responses to severe prolonged hypoxia in common carp involved a mixture of temperature-independent, temperature-dependent and time domain phases.

Key words: common carp, *Cyprinus carpio*, heart rate, stroke volume, cardiac output, respiration rate, temperature, hypoxia.

Introduction

Common carp (*Cyprinus carpio* L.) are exposed to a wide range of ambient oxygen levels as a result of their geographic distribution and habitat. Common carp are often found living in shallow waters that are highly eutrophic (Scott and Crossman, 1973) and low in dissolved oxygen levels, especially in summer when aquatic plant respiration can be high (Beamish, 1964). For instance, diurnal water tension fluctuations in a pond where common carp were found to live have been documented to range from 30.66 kPa in the afternoon to 6.66 kPa during the pre-dawn hours during the summer (Garey and Rahn, 1970). Additionally, the distribution of common carp extends into northern latitudes where ice and snow cover may occur for prolonged periods of time during the winter months (McCrimmon, 1968). Extensive ice formation and snow cover over a body of water may cause 'winterkill' conditions, during which a depletion of the dissolved oxygen in the water may occur (Ultsch, 1989). Thus, common carp may be exposed to hypoxic conditions on both a daily and a seasonal basis, and subsequently over a wide range of temperatures.

Currently, a large amount of information exists on the cardiorespiratory responses of teleosts, including carp, to short-term moderate hypoxia (Randall and Shelton, 1963; Holeyton and Randall, 1967a,b; Randall et al., 1967; Randall and Smith, 1967; Lomholt and Johansen, 1979; Wood and

Shelton, 1980; Farrell, 1982; Hughes et al., 1983; Glass et al., 1990, 1991; Perry et al., 1999). However, *in vivo* measurements of the cardiorespiratory responses of anoxia-tolerant teleosts, such as the common carp, during a prolonged exposure to severe hypoxia are limited to indirect measurements of cardiac output (Q). For example, Garey (1970) reported that, at 9–11 °C, Q of two carp decreased by 26% and 39% when the water oxygen partial pressure gradually decreased to below 4.40 kPa (approximately $1.8 \text{ mg O}_2 \text{ l}^{-1}$) over 2 h. In contrast, Itazawa and Takeda (1978) reported that Q increased from 20.2 to 26.6 ml min^{-1} in carp exposed to hypoxia (approximately $1.5 \text{ mg O}_2 \text{ l}^{-1}$) at a temperature of 24.5 °C.

Energy conservation during anoxia is normally reflected by a decreased Q (Jackson, 2000). Therefore, the measured cardiac responses in common carp seem unusual given the very large depression of f_H (Herbert and Jackson, 1985) and Q (Hicks and Wang, 1998; Hicks and Farrell, 2000a,b) (J. A. W. Stecyk, J. Overgaard, T. Wang and A. P. Farrell, unpublished data) normally observed when turtles (*Trachemys scripta*), for example, are exposed to prolonged anoxia. The small changes in Q certainly appear to be counterproductive for long-term survival under oxygen-limiting conditions because survival is dependent upon the ability to depress energy demand relative to the glycolytic potential for ATP production. In addition,

there are also conflicting opinions on whether metabolic depression occurs at the cellular level in severely hypoxic common carp. *In vivo* ^{31}P -NMR studies at 20 °C indicate that carp depleted their phosphocreatine (PCr) and ATP stores, but increased anaerobic glycolysis without metabolic suppression, during both an 11 h stepwise reduction in water oxygen concentration to approximately $1\text{ mg O}_2\text{ l}^{-1}$ (van Ginneken et al., 1995) and after a 1 h anoxic exposure (van Waarde et al., 1990). In contrast, a decreased rate of muscle phosphofructokinase-1 activity along with relatively constant glycogen and ATP levels during a 7 day hypoxic exposure ($0.5\text{ mg O}_2\text{ l}^{-1}$) led Zhou et al. (2000) to conclude that carp depressed their metabolic rate.

In long-term anoxic studies with mussels (*Mytilus edulis*), there appears to be a three-stage metabolic response (anaerobiosis, adaptation and a new steady state) (Livingstone and Bayne, 1977). Similarly, cardiac performance in anoxic turtles (*Trachemys scripta*) initially decreases rapidly before stabilizing at a depressed level (Hicks and Wang, 1998; Hicks and Farrell, 2000a,b) (J. A. W. Stecyk, J. Overgaard, T. Wang and A. P. Farrell, unpublished data). In fact, the depressed cardiac performance in cold anoxic turtles was such that the myocardial tissue did not require a Pasteur effect to support its energy needs (Hicks and Farrell, 2000a,b), a conclusion in line with earlier findings of metabolic depression occurring in the brain (Lutz and Nilsson, 1997) and the whole animal (Jackson, 2000). Consequently, the apparent absence of a metabolic depression in previous studies with common carp could simply reflect the short duration of hypoxia used in some of the studies, compounded perhaps by an agitated state in the animals. We aimed to resolve this uncertainty by directly quantifying the degree of cardiac downregulation in carp during prolonged severe hypoxic conditions that approached anoxia, at three acclimation temperatures (6, 10 and 15 °C). We find that, in contrast to earlier studies with common carp, severe hypoxia leads to a large cardiac downregulation that shows some independence of acclimation temperature.

Materials and methods

Experimental animals

Thirty-one common carp (*Cyprinus carpio* L.) with a mean body mass of $1.06\pm 0.05\text{ kg}$ (mean \pm S.E.M.) were used in this study. Carp utilized in the 15 °C experiments were significantly smaller ($0.79\pm 0.06\text{ kg}$) than fish used in both the 6 °C ($1.11\pm 0.08\text{ kg}$) and 10 °C ($1.23\pm 0.07\text{ kg}$) experiments. Carp were captured using traps by a local fisherman and held indoors in a 2000 l flow-through fibreglass tank receiving aerated dechlorinated water. The fish were fed *ad libitum* with commercial trout food pellets (ProForm Aquaculture Feeds, Chilliwack, British Columbia, Canada), but were not fed during the experimentation periods. Room lights were maintained on a 12h:12h L:D photoperiod while water temperature varied seasonally. All experiments were conducted between February and August 2000 and at

the acclimation temperatures of the fish ($6.1\pm 0.01\text{ °C}$, $10.2\pm 0.05\text{ °C}$ and $15.3\pm 0.01\text{ °C}$).

Surgical procedures

Carp were anaesthetized with buffered tricaine methanesulphonate (MS-222) (0.2 g l^{-1} MS-222 + 0.2 g l^{-1} NaHCO_3) until opercular movements were no longer observed. The animals were then weighed and placed ventral side up on an operating table, and the gills continuously irrigated with a chilled recirculating weaker anaesthetic solution (0.1 g l^{-1} MS-222 + 0.1 g l^{-1} NaHCO_3). A 0.5 cm incision was made in the skin along the inside of the opercular cavity wall slightly dorsal to the ventral aorta, and the connective tissue and pericardium surrounding the vessel were carefully teased aside. An ultrasonic flow probe (Transonic Systems, Ithaca, New York, USA) was positioned around the ventral aorta immediately after the bulbus arteriosus and anchored in place with 3-0 gauge silk thread sutures. The lead of the flow probe was led out of the opercular cavity and securely fastened with several skin sutures, including one to the base of the dorsal fin.

Recovery of the fish from anaesthetic began during the final stages of the operation when gill irrigation was switched from the anaesthetic solution to aerated fresh water. As soon as the fish began opercular movements, which usually occurred within 5 min of the commencement of the freshwater irrigation, they were transferred to the experimental holding tube. This was constructed from a 65 cm long, 12 cm diameter non-transparent polyvinyl chloride pipe that was compressed widthwise into an oval shape, preventing the fish from reversing direction. One end of the tube was constructed from clear Plexiglas to permit observation of the fish when needed, but was otherwise covered over. The flow probe lead exited the top of the experimental holding tube through a stoppered opening. The freshwater supply to the tube was flow-through (1.51 min^{-1} for normoxia; 0.51 min^{-1} for hypoxia) and at the same temperature as that of the water in the large holding tank.

Instrumentation and terminology

Blood flow through the ventral aorta (Q) was measured by the Transonic flow probe and the signals were preamplified and displayed on a Gould chart recorder (model 2202, Cleveland, Ohio, USA). Heart rate (f_H) was determined by counting the number of systolic peaks over a 3 min period, and stroke volume (V_S) was calculated as the quotient of Q and f_H over a complete cardiac cycle. Both V_S and Q were normalized for body mass. Respiration rate (f_R) and ventilation amplitude were determined visually by counting the number of buccal pumps occurring over 1 min and by assessing the degree of buccal and opercular flaring using an arbitrary 0–5 ranking scale, respectively.

Experimental protocol

After implantation of the flow probe, carp were given a 5-day post-operative recovery period during which cardiorespiratory recordings were acquired at the same time each day. Carp were then exposed to severely hypoxic

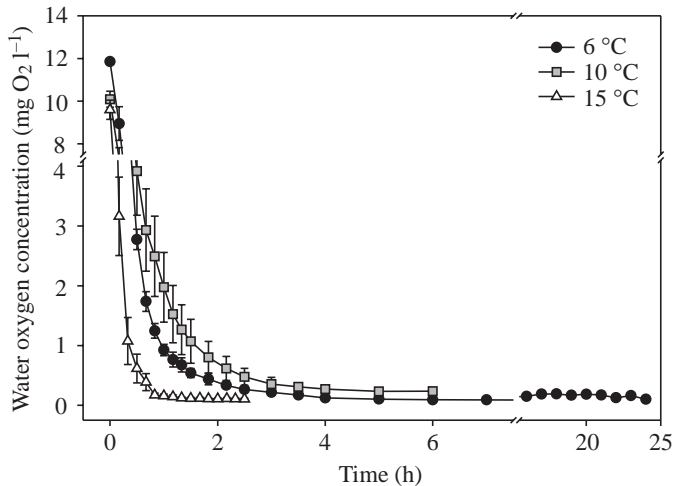


Fig. 1. Water oxygen concentration as a function of exposure time for the three groups of common carp exposed to severe hypoxia. Values are means \pm S.E.M.; $N=8$ (6 °C), 15 (10 °C) and 8 (15 °C).

conditions for either 24 h at 6 °C, 6 h at 10 °C or 2.5 h at 15 °C, within 4 days of the conclusion of the post-operative recovery period. Severe hypoxia was achieved and maintained by continuously bubbling the water entering the experimental holding tube with 100% N₂ in an exchange column. For each temperature group, an attempt was made to maintain the oxygen concentration of the water below 0.5 mg O₂ l⁻¹ and often below 0.3 mg O₂ l⁻¹ (Fig. 1). Finally, once the hypoxic period was completed, normoxia was restored over a 15 min period and cardiorespiratory status further monitored during a 5 day post-hypoxic exposure recovery period.

Cardiorespiratory variables were recorded at predetermined intervals. Control normoxic values were recorded immediately preceding the commencement of N₂ bubbling (time zero). For the 6 °C and 10 °C carp, cardiovascular recordings were then taken every 10 min for the first 1.5 h, every 20 min for the next hour, every 30 min for the next 1.5 h, and finally every hour thereafter until the end of the exposure period. Cardiovascular readings were collected every 10 min over the entire 2.5 h exposure period for the 15 °C carp. During each recording, continuous measurements of blood flow were taken for 3.5 min, during which period care was taken not to disturb the carp in any way. All procedures were in accordance with Simon Fraser University Animal Care Guidelines.

Data analysis

Values (mean \pm S.E.M.) are presented for cardiorespiratory variables at each sample time. Within-temperature-group comparisons were determined using either a one-way repeated-measures analysis of variance (ANOVA) or a paired *t*-test when appropriate. Comparisons among the three temperature groups were performed using a one-way ANOVA. Multiple comparisons were performed using Student–Newman–Keuls tests and in all instances $P \leq 0.05$ was used as the level of significance.

Visual analysis of the cardiovascular responses to severe

prolonged hypoxia revealed a three-phase pattern of change at all acclimation temperatures (see Fig. 2). Cardiac status initially decreased concurrent with decreases in water oxygen concentration to approximately 1 mg O₂ l⁻¹ over 2 h at 6 °C, 1 h at 10 °C and 20 min at 15 °C. As water oxygen concentration further decreased to less than 0.3 mg O₂ l⁻¹, cardiac status was maintained at a relatively stable level compared to the acute response. Finally, cardiac status increased towards normoxic levels during the final stages of severe hypoxic exposure as water oxygen concentration was maintained at a near-anoxic level. Q_{10} values were calculated for specific mean values during each of the three phases using data from the 6 °C and 15 °C animals (i.e. normoxic control, hypoxic minimum and hypoxic maximum; minimum and maximum referring to cardiovascular status). The water oxygen concentrations for the minimum hypoxic cardiovascular Q_{10} values were 0.33 ± 0.04 mg O₂ l⁻¹ (6 °C) and 0.38 ± 0.14 mg O₂ l⁻¹ (15 °C) and were between 2.0 and 3.0 mg O₂ l⁻¹ for the hypoxic minimum Q_{10} of f_R .

Results

Post-operative recovery period

Daily recordings of cardiorespiratory variables were stable by the second day of post-operative recovery, showing only minor fluctuations for the remainder of the recovery period (data not shown). Cardiorespiratory status remained stable for the following 4 days (6 of 31 fish were monitored this long), as cardiorespiratory variables at the start of the hypoxic exposure (Table 1) were not significantly different compared with those recorded on the fifth day of the post-operative recovery period with the exception of f_R at 15 °C, which increased significantly from 12.3 ± 4.6 min⁻¹.

Effect of acclimation temperature on normoxic cardiorespiratory status

Control, normoxic cardiorespiratory status for fish at the three acclimation temperatures is presented in Table 1. Cardiorespiratory status was clearly temperature-dependent under normoxic conditions. Routine Q , f_H and f_R values decreased significantly with temperature with Q_{10} values of 1.7, 2.6 and 3.5, respectively.

Cardiorespiratory responses to severe hypoxia

As shown in Fig. 2, cardiac activity was clearly depressed during severe hypoxia at all three acclimation temperatures. Q decreased to minimum values approximately 2.5 h at 6 °C, 2 h at 10 °C, and 50 min at 15 °C after the onset of hypoxia and remained significantly lower than normoxic control values throughout the remainder of the hypoxic exposure (except the $N=3$ data points in the 6 °C group). The minimum level for cardiac depression was largely independent of hypoxia duration as well as acclimation temperature because minimum hypoxic values were not significantly different among the acclimation temperatures (Fig. 3B; Table 1). In fact, the Q_{10} value for minimum Q during severe hypoxia was 1.2.

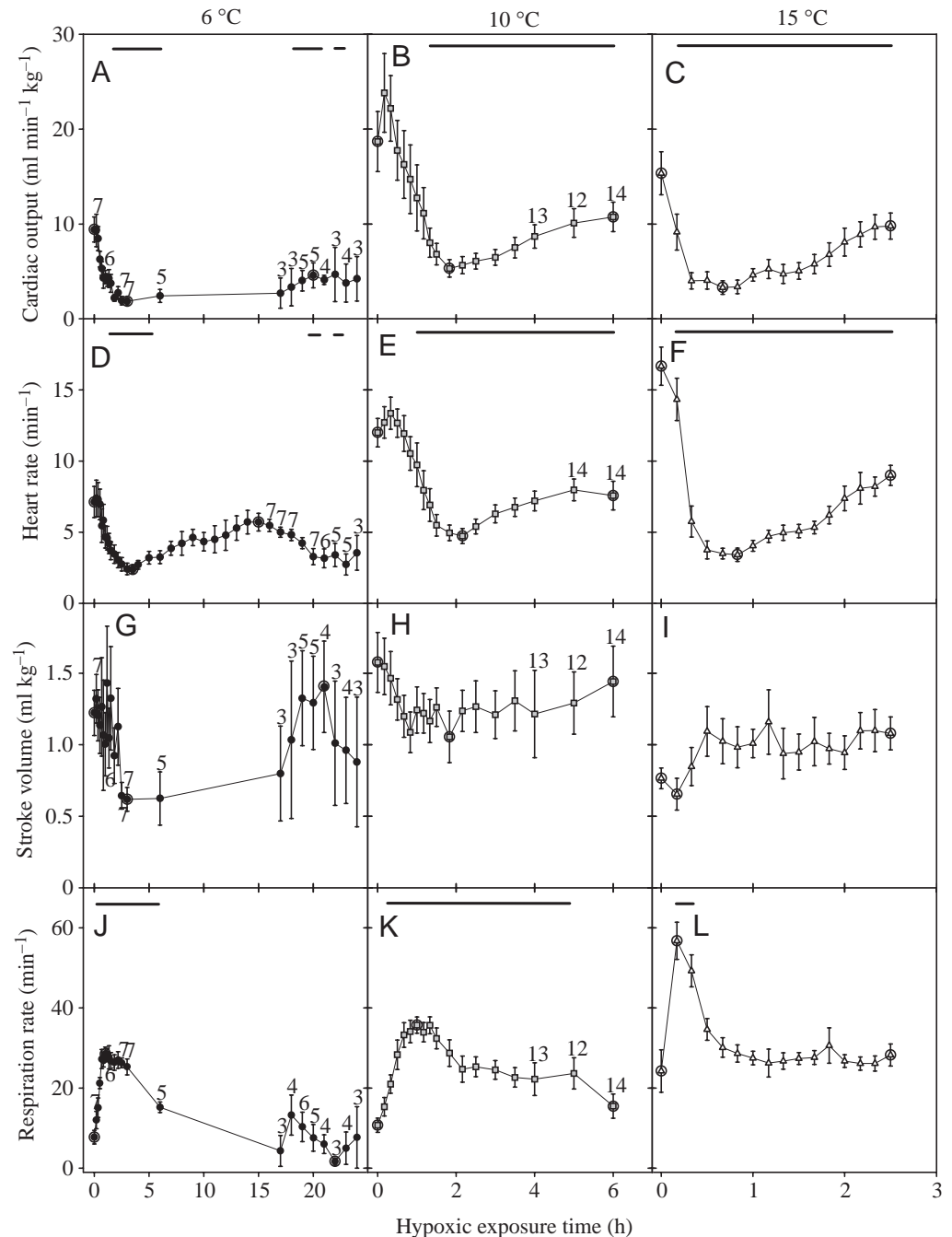


Fig. 2. Chronological changes of the cardiorespiratory status of 6-, 10- and 15°C-acclimated common carp exposed to severe hypoxia. Note the different time scales among temperature groups. Significant differences ($P \leq 0.05$) from time zero are indicated by the solid line above the traces. Circled data points are designated as the normoxic control, hypoxic minimum, and hypoxic maximum values and correspond with those given in Table 1. Values are means \pm S.E.M.; $N=8$ (6°C), 15 (10°C) and 8 (15°C) unless otherwise indicated (numbers above points).

In contrast to the absolute level of cardiac depression, the duration of cardiac depression and the period for which carp tolerated severe hypoxia were inversely related to acclimation temperature. Specifically, Q remained depressed approximately tenfold longer at 6°C (23.5 h) than at 15°C (2.33 h) (Fig. 2A,C) and the time that carp tolerated a water oxygen concentration below $0.3 \text{ mg O}_2 \text{ l}^{-1}$ was approximately 22 h at 6°C, 3.5 h at 10°C, and 2 h at 15°C (Fig. 1). Late in the hypoxic exposure a characteristic increase in cardiac activity was observed at all acclimation temperatures (Fig. 2), resulting in a maximum hypoxic Q significantly greater than minimum hypoxic Q values (except at 6°C) (Table 1). The Q_{10}

for maximum hypoxic Q was 2.3, suggesting a return to a temperature dependence of cardiac activity during prolonged exposure.

f_R increased progressively with hypoxia, reaching maximum values at $2.0\text{--}3.0 \text{ mg O}_2 \text{ l}^{-1}$ before returning to control values (Fig. 2J-L; Table 1). f_R increased maximally by 3.7-fold at 6°C, 3.3-fold at 10°C, and 2.3-fold at 15°C, representing a Q_{10} of 2.2. The lowest hypoxic f_R values corresponded to a Q_{10} of 17. Therefore, contrary to f_H (see below), the f_R response during hypoxia was temperature-dependent.

Despite the general patterns of change described above, some important quantitative differences in the cardiovascular

Table 1. Cardiorespiratory status of 6-, 10- and 15 °C-acclimated carp before, during and after severe hypoxic exposure

Temperature (°C)	Time	Cardiac output (ml min ⁻¹ kg ⁻¹)	Heart rate (min ⁻¹)	Stroke volume (ml kg ⁻¹)	Respiration rate (min ⁻¹)
6	Normoxic control	9.4±1.3 (8) ^a	7.1±1.1 (8) ^a	1.22±0.16 (8) ^{a,d}	7.8±1.7 (8)
	Hypoxic minimum	1.9±0.4 (7) ^b	2.4±0.3 (8) ^b	0.62±0.08 (7) ^{b,c}	28.5±1.7 (8) ^a
	Hypoxic maximum	4.6±1.3 (5) ^{b,c}	5.7±0.6 (8) ^a	1.41±0.32 (4) ^a	1.7±0.8 (3)
	Post-hypoxia	7.0±0.7 (4) ^{a,c}	11.5±1.2 (4) ^c	0.68±0.04 (4) ^{c,d}	9.4±1.7 (5)
10	Normoxic control	18.7±3.2 (15) ^{a,d}	12.0±1.0 (15) ^a	1.58±0.21 (15)	10.7±1.8 (15) ^{a,c}
	Hypoxic minimum	5.3±0.9 (15) ^b	4.7±0.5 (15) ^b	1.06±0.18 (15)	35.7±2.0 (14) ^b
	Hypoxic maximum	10.8±1.5 (14) ^c	7.6±1.0 (14) ^c	1.44±0.25 (14)	15.5±3.0 (15) ^a
	Post-hypoxia	14.0±3.1 (8) ^{c,d}	10.8±1.7 (8) ^{a,c}	1.37±0.34 (8)	7.0±1.0 (8) ^c
15	Normoxic control	15.3±2.3 (8) ^a	16.7±1.3 (8) ^a	0.77±0.07 (8) ^a	24.3±5.3 (8)
	Hypoxic minimum	3.3±0.7 (8) ^b	3.4±0.5 (8) ^b	0.65±0.11 (8) ^a	56.8±4.6 (8) ^a
	Hypoxic maximum	9.8±1.4 (8) ^c	9.0±0.7 (8) ^c	1.16±0.23 (8) ^b	26.1±1.9 (8)
	Post-hypoxia	25.9±7.2 (7) ^a	28.1±4.9 (7) ^d	0.79±0.12 (7) ^{a,b}	31.4±7.5 (7)

Values are means ± S.E.M. (N).

Control normoxic values immediately preceded hypoxic exposure; hypoxic minimum and maximum refer to cardiovascular status during hypoxic exposure, and the values presented correspond to those shown in Fig. 2.

Post-hypoxic exposure values were recorded on the fifth day of the recovery period.

Significant differences ($P \leq 0.05$) between times for each variable at each acclimation temperature are indicated by dissimilar letters.

responses existed among temperatures. Q decreased maximally by 4.9-fold and 4.6-fold for the 6 °C and 15 °C carp, respectively, while the decrease was only 3.5-fold at 10 °C (Table 1). After reaching a minimum value, Q then doubled at 10 °C and tripled at 15 °C. The maximum hypoxic Q values that were reached prior to the termination of the hypoxic period (between approximately 0.1 and 0.3 mg O₂ l⁻¹) were significantly lower by twofold (6 °C), 1.7-fold (10 °C) and 1.6-fold (15 °C) than the respective control normoxic values (Table 1).

Cardiac depression was mainly mediated through chronotropic effects (Fig. 2A–F). Minimum f_H values during severe hypoxia represented reductions of 4.9-fold at 15 °C, 2.6-fold at 10 °C and 3.0-fold at 6 °C (Table 1) and, like Q , resulted in a Q_{10} of 1.2. As with Q , f_H increased during the terminal stage of hypoxia, but remained significantly lower than control normoxic f_H at 10 °C (1.5-fold) and 15 °C (1.9-fold) (Table 1). The Q_{10} for the maximum hypoxic f_H was 1.7.

V_S was more variable compared with Q and f_H . As a result, no consistent pattern of change in V_S emerged among the three temperature groups during severe hypoxia (Fig. 2G–I). V_S at 6 °C initially decreased twofold before returning to the control value by the end of the hypoxic exposure period (Table 1). At 10 °C there was a similar, but less pronounced (no statistically significant changes) pattern of change in V_S . However, V_S only increased (1.5-fold) during the 2.5 h hypoxic exposure at 15 °C, but normoxic V_S was significantly lower than that at either 6 °C or 10 °C.

Recovery from severe hypoxia

At all three acclimation temperatures, a secondary period of decline in f_H was observed late in the hypoxic exposure (data presented for 6 °C only; Fig. 2D). We reasoned that

this secondary decline in f_H was a prelude to death because at 6 °C it was associated with two fish dying and at 10 °C five fish exposed to severe hypoxia for greater than 6 h (1 for 12 h, 1 for 10 h, and 3 for 7 h) also exhibited secondary reductions in f_H and subsequently died. Further experiments were thus terminated when we felt that f_H had reached a hypoxic maximum (6 h at 10 °C). Hypoxic exposure was terminated after 2.5 h at 15 °C as f_H of the first fish exposed to severe hypoxia at this temperature decreased approximately twofold within 10 min of reaching a hypoxic maximum at 2.5 h of exposure. Subsequently, all but one fish survived.

Fish surviving the hypoxic exposure recovered their cardiorespiratory status (Table 1). The majority of cardiorespiratory variables obtained on the fifth day of the post-hypoxic recovery period were not significantly different from normoxic control values. Exceptions were significant elevations of f_H at 6 °C and 15 °C.

Discussion

Normoxic cardiorespiratory status

Carp were given a 5-day post-operative recovery period under normoxic conditions. Since Q , f_H , V_S and f_R all stabilized by the second day of post-operative recovery and cardiovascular status recorded on the last day of the recovery period was not significantly different from control normoxic values recorded up to 4 days later, we are confident that our routine measurements indicate a successful recovery from the operation as well as acclimation to each of the experimental temperatures. Furthermore, the present study compares favourably with previous studies of normoxic cardiovascular status in carp. For instance, routine Q and f_H values of

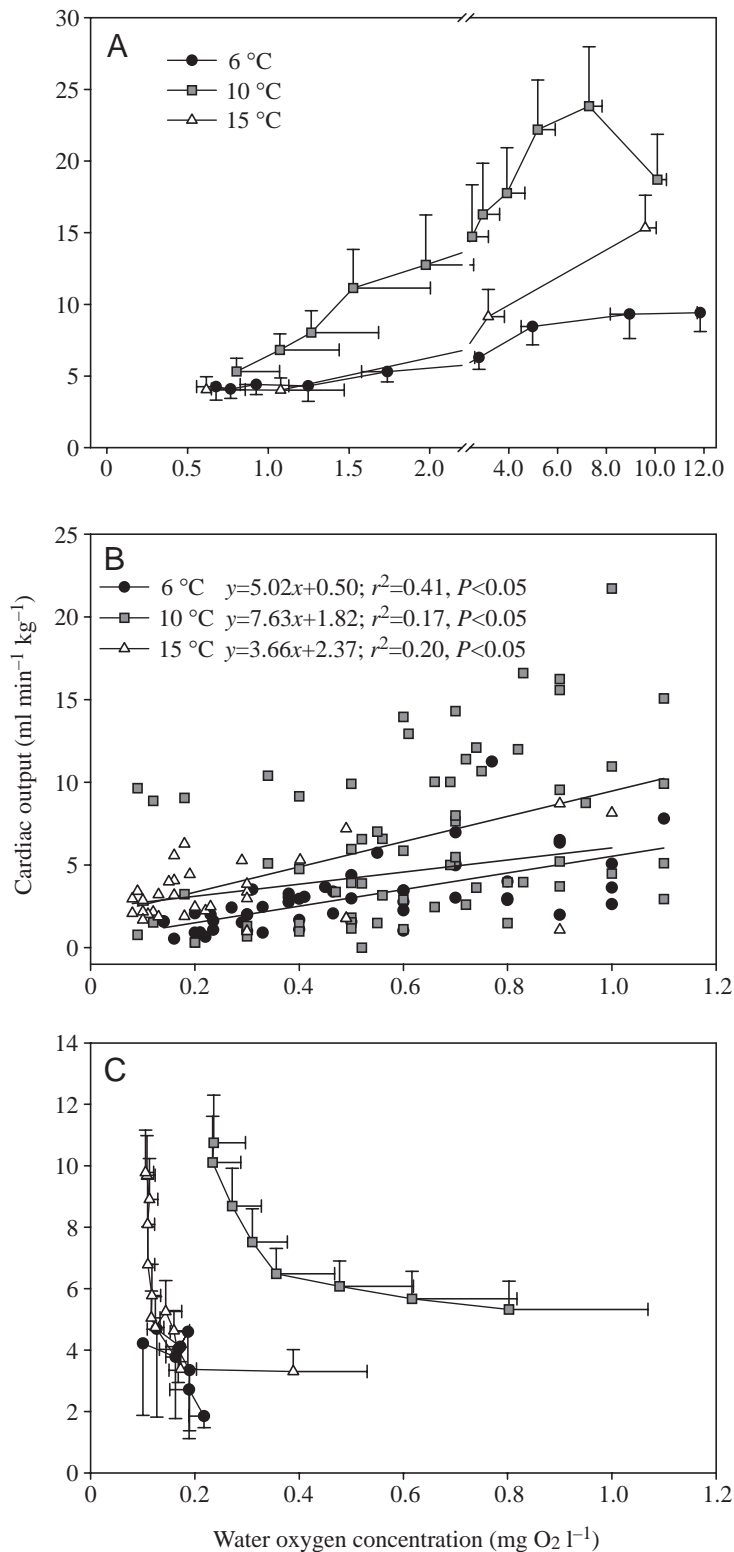


Fig. 3. Cardiac outputs of 6-, 10- and 15°C-acclimated common carp to severe prolonged hypoxia expressed as a function of water oxygen concentration. (A) Acute response with values presented to one value after the end of the acute phase. (B) Prolonged response presented to minimum hypoxic Q values. (C) Development of the third phase from minimum hypoxic Q . Values in A and C are means \pm S.E.M ($N=3-8$ (6°C), 12–15 (10°C) and 8 (15°C); values in B are from individual fish.

$18.7 \pm 3.2 \text{ ml min}^{-1} \text{ kg}^{-1}$ and $12.0 \pm 1.0 \text{ min}^{-1}$, respectively, at 10°C are comparable with Q ($18.3 \pm 1.87 \text{ ml min}^{-1} \text{ kg}^{-1}$) and f_H (16 min^{-1}) values reported by Garey (1970) for resting common carp under normoxic conditions at 9–11°C. Itazawa (1970) reported a Q of $20.7 \text{ ml min}^{-1} \text{ kg}^{-1}$ for normoxic common carp at 15–16°C. Carp acclimated to 15°C in the present study had a Q of $15.3 \pm 2.3 \text{ ml min}^{-1} \text{ kg}^{-1}$. An f_H value of 16.2 min^{-1} , calculated from the mean circulation time, mass and Q of carp (Itazawa, 1970) almost exactly matches the f_H of 16.7 min^{-1} for the similarly sized 15°C fish in the present study.

Routine normoxic cardiorespiratory status was clearly temperature-dependent, as expected (Table 1). Corresponding Q_{10} values for Q and f_H agree well with those observed in other teleost species (for a review, see Farrell and Jones, 1992). As indicated by the higher Q_{10} for f_H than Q , V_S at 6°C was significantly greater than that at 15°C, thus partially compensating the negative chronotropic effect of cold acclimation. This finding means that inferences as to the effectiveness of cardiovascular temperature compensation that are based solely on f_H could be misleading for common carp. f_R and ventilation amplitude also decreased with cold acclimation. These findings agree with the observation that oxygen consumption in common carp changes with temperature (Beamish, 1964). Presumably, the observed decreases in Q , f_R and ventilation amplitude with temperature in normoxic common carp reflect parallel changes in oxygen consumption.

Cardiorespiratory responses to severe hypoxia

The present study, which is the first to measure directly the progressive changes in Q of an anoxia-tolerant teleost exposed to severely hypoxic conditions over prolonged periods, revealed large decreases (3.5–4.9-fold) in Q . Our findings clearly contrast with earlier indirect measurements of Q for common carp exposed to shorter term and less severe hypoxia (approximately $1.5-1.8 \text{ mg O}_2 \text{ l}^{-1}$), in which only a small decrease (26–39%) (Garey, 1970) or an increase (30%) (Itazawa and Takeda, 1978) in Q was reported. Even though the cardiac depression in common carp was large, it did not reach the level (8- to 13-fold decrease) observed in turtles *Trachemys scripta* (Hicks and Farrell, 2000a,b), which are more anoxia-tolerant than common carp. Nevertheless, the cardiac depression we observed at all three acclimation temperatures resembled the three-phase response displayed by other anoxia-tolerant species exposed to oxygen-limiting conditions. Specifically, our extended monitoring revealed that the cardiorespiratory response could be divided into acute, prolonged and tertiary expiratory phases. Analysis of each of these phases provides insight into the metabolic status of the animal and also lends indirect information about cardiovascular control.

Acute phase of severe hypoxia

With the onset of hypoxic exposure, f_H and Q decreased considerably to similar minimum levels independent of acclimation temperature (Table 1). These initial acute cardiovascular responses observed in common carp are similar to those observed in other fish exposed to short-term moderate hypoxia. Specifically, the 25–35% reductions in Q occurring at water oxygen concentrations around $3.5 \text{ mg O}_2 \text{ l}^{-1}$ in carp at all three acclimation temperatures (Fig. 3A) compare very well with the 31%, 37% and approximately 32% reductions in Q observed in lingcod *Ophiodon elongatus* (Farrell, 1982), eels *Anguilla anguilla* (Peyraud-Waitzenegger and Soulier, 1989) and rainbow trout *Oncorhynchus mykiss* (Perry et al., 1999), respectively, with moderate hypoxic exposure. Likewise, the immediate augmentation in f_R in response to hypoxic exposure agrees well with past studies investigating the respiratory responses of carp to hypoxia (Itazawa and Takeda, 1978; Lomholt and Johansen, 1979; Hughes et al., 1983; Glass et al., 1990, 1991). Maximum increases in f_R (Table 1) correspond especially well with the 3.2-fold f_R increase displayed in carp exposed to a moderate level of hypoxia (approximately $3.2 \text{ mg O}_2 \text{ l}^{-1}$) at 24.5°C (Itazawa and Takeda, 1978). The concurrent increase in ventilation amplitude previously observed with hypoxic exposure (e.g. Itazawa and Takeda, 1978; Lomholt and Johannesen, 1978; Hughes et al., 1983; Glass et al., 1990) was also apparent from visual observations in the present study. The similarity of the present acute cardiorespiratory values with those of past acute studies suggests that the small changes in Q of carp observed by Garey (1970) and Itazawa and Takeda (1978) were a consequence of moderate hypoxia levels and short exposure times.

The acute phase of cardiac depression in common carp was clearly dependent on water oxygen concentration (Fig. 3A). This initial oxygen-dependent decrease lasted until oxygen levels were less than $1 \text{ mg O}_2 \text{ l}^{-1}$ and therefore also occurred during the first part of the prolonged phase, as indicated by the positive associations between Q and water oxygen concentration in Fig. 3B. Interestingly, the state of severe hypoxia was achieved at a slower rate at 10°C (Fig. 1) and this appeared to have two effects on the cardiac status of the fish. First, 10°C fish exhibited a stress response at the onset of hypoxia, leading to an initial slight increase in Q above normoxic levels. As a result, subsequent reductions in Q during hypoxia did not occur as quickly nor to the same magnitude as in the 6°C and 15°C fish. Secondly, the elevated Q and associated activity during the early phase of hypoxia would have depleted anaerobic energy stores faster and this may explain why the increase in Q towards normoxic levels occurred at a much higher water oxygen concentration at 10°C ($0.4\text{--}0.6 \text{ mg O}_2 \text{ l}^{-1}$) than at 6°C and 15°C ($0.1\text{--}0.2 \text{ mg O}_2 \text{ l}^{-1}$) (Fig. 3C).

Taken together, the acute cardiorespiratory responses to severe hypoxia in carp and other teleosts may be best explained as an attempt to maintain oxygen transfer at the gills and peripheral tissues in the face of declining oxygen availability. The occurrence of hypoxic bradycardia has been suggested to

benefit gas exchange at the gill surface through the slowing of blood flow through the branchial circulation (Randall and Shelton, 1963) and by promoting cardiac filling (Farrell, 1984), which in turn augments stroke volume, leading to the recruitment of the secondary gill lamellae, and thus increasing the functional surface area of the gills available for gas exchange (Farrell, 1980; Soivio and Tuurala, 1981). Additionally, a decreased Q would increase capillary transit time in peripheral tissues and residence time of blood in the heart, thus aiding diffusional gas exchange in the tissues and heart, respectively (Farrell, 1982).

Prolonged phase of severe hypoxia

The prolonged cardiorespiratory response consisted of a sustained depression of Q at water oxygen concentrations less than $1 \text{ mg O}_2 \text{ l}^{-1}$. During the prolonged phase, the absolute Q value appeared to be largely independent of acclimation temperature (Table 1). Nevertheless, the duration of the prolonged phase was clearly inversely related to temperature (Fig. 2). Common carp rely on classical glycolysis in conjunction with the use of phosphocreatine stores through the creatine kinase equilibrium for energy during both hypoxic and anoxic exposure (van Waarde et al., 1990; van Raaij et al., 1994; van Ginneken et al., 1995, 1998). Length of survival under severely hypoxic conditions then becomes dependent upon the amount of energy stores available prior to exposure and the rate at which the stores are utilized.

The present study provides a quantitative insight into the problem of cardiac energy supply and demand during anoxia as the work performed by the cardiac muscle can be expressed as ATP demand, which in turn can be related to rates of glycolysis (Hicks and Farrell, 2000a,b). An estimate of the routine myocardial power output of 1.2 mW g^{-1} can be made using normoxic routine values of $53 \text{ cmH}_2\text{O}$ ($=5.194 \text{ kPa}$) (Farrell, 1982) for mean ventral aortic pressure (common carp acclimated to $9\text{--}10^\circ\text{C}$) (Garey, 1970), $18.7 \text{ ml min}^{-1} \text{ kg}^{-1}$ for Q (Table 1) and $1.38 \pm 0.17 \text{ g}$ ($N=15$) for mean ventricular wet mass (10°C carp; present study) (i.e. $18.7 \times 5.194 \times 0.0167 / 1.38$, where 0.0167 is a conversion to mW). This estimate fits nicely within the range for routine cardiac power output measured in other teleost hearts ($0.5\text{--}3.0 \text{ mW g}^{-1}$) (Farrell, 1984). With a 3.5-fold reduction in Q during hypoxia, estimated power output would decrease to 0.33 mW g^{-1} , assuming that ventral aortic pressure did not change. However, Burggren et al. (1997) have suggested that anaerobic myocardial ATP generation can routinely fuel a maximum cardiac power output of only approximately $0.1\text{--}0.2 \text{ mW g}^{-1}$. Therefore, carp would have to reduce the ventral aortic pressure by 33–66% to achieve this level of power output. Ventral aortic pressure decreased by 23–58% in other anoxia-tolerant species when exposed to anoxia for prolonged time periods (Herbert and Jackson, 1985; Hicks and Farrell, 2000a,b). Therefore, a 50% decrease in ventral aortic pressure in carp does not seem unreasonable and would place the value for minimum cardiac power output within the known capabilities for maximum anaerobic ATP production of cardiac tissue. However, even

with a 50% decrease in ventral aortic pressure the estimated sevenfold reduction in power output at 10 °C, elevenfold at 6 °C and twelvefold at 15 °C [calculated using Table 1 values for normoxic and hypoxic minimum Q , 53 cm H₂O (=5.194 kPa) and 26.5 cm H₂O (=2.597 kPa) for normoxic (Garey, 1970) and presumed hypoxic ventral aortic pressure, respectively, and 1.5±0.18 g ($N=8$) and 0.78±0.08 g ($N=8$) for mean ventricular wet mass (6 °C and 15 °C carp, respectively; present study)] indicates that a Pasteur effect is needed in the cardiac tissue, because glycolysis supplies 18 times less ATP per mole glucose than does oxidative metabolism.

Therefore, the above estimates of cardiac power output lead to the conclusion that common carp exposed to severe hypoxia are unable to reduce cardiac energy demands to a level that can be supported without an upregulation of anaerobic metabolism. As a result, cardiac energy stores will be depleted more rapidly than if there was no Pasteur effect. This conclusion is consistent with the finding that phosphocreatine and ATP levels continuously decrease throughout anoxic exposure in common carp without reaching a plateau, as found in other anoxia-tolerant teleosts (van Waarde et al., 1990). The differences in the length of near-anoxic survival among acclimation temperatures, however, may be associated with temperature-related differences in the absolute cardiac power output during the acute and expiratory phases, since depressions of myocardial power output would be similar at 6 °C and 15 °C during the prolonged phase provided that presumed changes in ventral aortic pressure are independent of temperature. For example, the temperature-dependent increase in cardiac activity during the expiratory phase would require an augmentation of cardiac glycolytic energy supply, resulting in an increased depletion of energy stores and, consequently, a reduced survival time.

The gradual decrease in f_R with prolonged exposure may be viewed as an energy-conserving mechanism. Specifically, the metabolic cost of continuously pumping large volumes of water across the gills will at some point outweigh the energetic benefits gained from extracting a submaximal amount of oxygen from the water. P_{50} values for carp blood of 0.13 to 1.07 kPa have been reported over a range of temperatures (Garey and Rahn, 1970; Itazawa and Takeda, 1978; Albers et al., 1983; Wurm and Albers, 1989; Glass et al., 1990) and, thus, the carp in this study may have been able to extract a small amount of oxygen from the water. As the following shows, however, the very low levels of water oxygen concentration that carp were exposed to in the present study were unlikely to fuel aerobic energy pathways to a significant degree. Carp in the present study were exposed to water oxygen concentrations well below 0.3 mg O₂ l⁻¹ for the majority of the exposure time (Fig. 1) and the survival time of carp at 15 °C (2.33 h) is more similar to the survival time of carp exposed to complete anoxia at 20 °C (1.5 h) (van Raaij et al., 1994) than to the seven days survival limit observed at a constant hypoxic level of 0.5 mg O₂ l⁻¹ at 22.5 °C, in which carp maintained an oxygen consumption two- to fourfold lower than normoxic controls (Zhou et al., 2000). Also, even if the carp were able to extract

a small amount of oxygen from the water, the cardiac tissue would have been anoxic because the oxygen would have been utilized at the peripheral tissues before reaching the cardiac muscle, due to the heart's location in the circulatory system and its reliance on venous blood.

Expiratory phase

Truly anoxia-tolerant species are able to maintain a new steady state with hypoxic or anoxic exposure (Livingstone and Bayne, 1977). For instance, once depressed, cardiovascular status in turtles only gradually declines as anoxic exposure time increases (Hicks and Wang, 1998; Hicks and Farrell, 2000a,b) (J. A. W. Stecyk, J. Overgaard, T. Wang and A. P. Farrell, unpublished data). In contrast to turtles, common carp at all three acclimation temperatures displayed an increase and then a secondary decline in Q with prolonged hypoxic exposure despite a stable water oxygen concentration and a preceding stable cardiac activity (secondary decline presented for 6 °C fish only; Fig. 2D). The secondary decline was associated with the fish dying. We propose that this tertiary cardiovascular response may reflect an important difference in cardiac function between truly anoxia-tolerant species such as the turtle, and moderately anoxia-tolerant species such as the common carp.

The processes determining Q during prolonged hypoxia are unclear. Catecholamine release, observed after 3 h of hypoxic exposure at 20 °C, has previously been documented in the common carp (van Raaij et al., 1995, 1996a,b). Elevated catecholamine levels, initially hypothesized to be important for stimulating hepatic glycogenolysis, could also mobilize cardiac glycogen stores, stimulate cardiac inotropy and chronotropy, and account for the tertiary increase in cardiac activity. Additionally, common carp do not retain large amounts of lactate in the skeletal muscle mass during hypoxic exposure (Driedzic and Hochachka, 1975). Thus, under prolonged severe hypoxia, the cardiovascular system no longer acts as a conduit for, nor is regulated by, oxygen demand, but rather assumes a secondary role of transporting nutrients and wastes to and from the tissues.

The final decline in cardiac activity could reflect cellular collapse and death either peripherally or centrally, or both. Cardiac performance may ultimately decline because of depleted energy fuels combined with the adverse effects on contractility of a build-up of anaerobic waste products such as H⁺. Compromised brain integrity, as the energy status of the carp brain significantly decreases under anoxia (van Raaij et al., 1994; van Ginneken et al., 1996), could also affect cardiac control mechanisms.

Concluding remarks

In summary, the cardiac responses of a moderately anoxia-tolerant teleost to prolonged severe hypoxic exposure were directly monitored for the first time. In contrast to earlier studies with carp during hypoxia, we observed an appreciable cardiac depression at three acclimation temperatures. In addition, at all three acclimation temperatures we found a

three-phase cardiac response to severe hypoxia consisting of (i) an acute response consisting of approximately fivefold reductions in Q and f_H , (ii) a prolonged response of a stable depressed cardiac status whose duration was inversely related to temperature even though the absolute Q appeared to be temperature-independent, and (iii) a tertiary response that was associated with fish dying. Estimates of cardiac power output suggest that a Pasteur effect was needed to maintain cardiac function despite the large reductions in cardiovascular status, a finding in agreement with past studies investigating the metabolic responses of common carp exposed to both hypoxic and anoxic conditions.

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