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Nitrite Intoxication of Common Carp (*Cyprinus carpio* L.) at Different Water Temperatures

H. KROUPOVÁ[1], J. MÁCHOVÁ[1], V. PIAČKOVÁ[1], M. FLAJŠHANS[1], Z. SVOBODOVÁ[1, 2], G. POLESZCZUK[3]

[1]University of South Bohemia České Budějovice, Research Institute of Fish Culture and Hydrobiology, Vodňany, Czech Republic
[2]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[3]Szczecin University, Szczecin, Poland

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Abstract

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Common carp (*Cyprinus carpio* L.) were exposed to nitrite (1.45 mmol·l^{-1} NO_2^-) for 48 hours at 14 °C and 20 °C, in order to investigate the mechanism of nitrite poisoning at these water temperatures. The effect of nitrite exposure on fish was assessed on selected haematological and biochemical indicators of the blood. Moreover, nitrite accumulation in the blood, liver and muscle was measured. Nitrite exposure produced high levels of methaemoglobin (88.2 ± 3.3% and 92.9 ± 6.1%) at both water temperatures compared with controls (0.3 ± 0.6% and 2.6 ± 3.0%). High fish mortality occurred in experimental groups (30% and 51%) compared with controls (0%). Nitrite exposure also resulted in an accumulation of nitrite in the fish body. The highest nitrite levels developed in the blood plasma, followed by the liver and muscle, respectively. Carp concentrated nitrite in the blood plasma and tissues to markedly higher levels at higher temperature (20 °C). The plasma nitrite concentrations (10.5 ± 1.9 mmol·l^{-1}) were in this case more than 7 times higher than the environmental one. At lower temperature (14 °C), plasma nitrite concentration reached 5.0 ± 1.5 mmol·l^{-1}. In either event, plasma K^+ levels increased and Cl^- levels and osmolality remained unchanged. Plasma Na^+ levels slightly decreased at the higher temperature. Nitrite-exposed fish showed lower haematocrit values (PCV) at both experimental temperatures compared with controls. At 20 °C, the blood haematocrit decrease (0.20 ± 0.02 l·l^{-1}) was accompanied by a low erythrocyte count (1.05 ± 0.12·10^{12} l^{-1}) and by a low haemoglobin level (51 ± 11 g·l^{-1}). At the lower temperature (14 °C), the haematocrit decrease (0.25 ± 0.02 l·l^{-1}) was caused by a low mean corpuscular volume (167 ± 27 fl). No significant changes were observed in the mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), or selected erythrocyte dimensions (major axis, minor axis and aspect ratio).

Fish, nitrite accumulation, haematology, biochemical indices, methaemoglobinaemia

An intensive culture of several fish species in recirculation systems has become very popular in Europe during the last few decades. This kind of aquaculture relies on recirculating water systems that remove waste ammonia from water. Ammonia, i.e. the main product of protein metabolism of fish (Wood 1993), is removed from the tanks by nitrification (conversion to nitrites and subsequently to nitrates) by means of bio-filters. Imbalance in the nitrification process can often lead to an increase in the nitrite concentrations by up to 1 mmol·l^{-1} NO_2^- or more (Kamstra et al. 1996). This may result in mass fish mortality (Svobodová et al. 2005a).

Nitrite is actively taken up through the gills and enters the blood stream (Margiocco et al. 1983; Jensen et al. 1987). From the blood plasma, nitrite diffuses into red blood cells, where it oxidises the iron in haemoglobin to the +3 oxidation state to produce methaemoglobin, which lacks the capacity to bind oxygen (Bodansky 1951). Methaemoglobin turns the blood to a chocolate-brown colour. So, nitrite poisoning is

Address for correspondence:
Hana Kroupová
University of South Bohemia
Research Institute of Fish Culture and Hydrobiology
Zátiší 728/II, 389 25 Vodňany, Czech Republic

Phone: +420 383 382 402
Fax: +420 383 382 396
E-mail: kroupova@vurh.jcu.cz
http://www.vfu.cz/acta-vet/actavet.htm

sometimes called a brown blood disease. However, recent studies on fish have discovered that nitrite is a disrupter of multiple physiological functions and that the toxicity of nitrite results from a combination of effects rather than from any single effect in particular (Jensen 2003).

Nitrite toxicity in fish depends on a large number of external and internal factors. Among the most important ones is water quality, especially the chloride concentration in water (Crawford and Allen 1977; Lewis and Morris 1986; Svobodová et al. 2005b; Pištěková et al. 2005). However, little is known about the influence of temperature on nitrite toxicity. Only a few reports have appeared on this topic and a definite conclusion has not been reached (Colt and Tchobanoglous 1976; Huey et al. 1984). Generally, the water temperature is an important factor that determines chemical toxicity (Cairns et al. 1975) and it has multiple effects on both water characteristics and fish physiology. Therefore, the aim of our study was to discover the influence of water temperature on the nitrite toxicity mechanism in common carp (*Cyprinus carpio* L.). The effect of nitrite exposure on fish at different temperatures was assessed on selected haematological and biochemical indicators of the blood. Moreover, nitrite accumulation in the blood and in selected tissues was measured.

Materials and Methods

Experimental animals

Common carp (*Cyprinus carpio* L.; weight 280 ± 52 g, mean ± SD) were obtained from a local fish hatchery and maintained for 2 weeks in aquaria with dechlorinated tap water. Four days before the start of the experiment, the fish were divided into four groups and acclimated to 14 °C and 20 °C. Temperatures between 14 - 20 °C prevail during the growing season (4 - 5 months) in the Czech Republic. During acclimation and experimental period, the fish were not fed.

Experimental protocol and fish sampling

The test was performed in a semistatic assay for 48 h. Fish were kept in tanks each containing 200 l of test solution. During the acclimation and experimental period, the basic chemical indices of water were as follows: $ANC_{4.5}$ (acid neutralisation capacity) 1.15 $mmol{\cdot}l^{-1}$; COD_{Mn} (chemical oxygen demand) 1.5 $mg{\cdot}l^{-1}$; total ammonia 0.04 $mg{\cdot}l^{-1}$; NO_3^- 7.8 $mg{\cdot}l^{-1}$; PO_4^{3-} 0.01 $mg{\cdot}l^{-1}$; sum of Ca^{2+} + Mg^{2+} 14 $mg{\cdot}l^{-1}$, Cl^- 11 $mg{\cdot}l^{-1}$. Oxygen saturation of the water ranged from 81 to 92% and the pH ranged from 7.08 to 7.57. Four groups each containing 30 specimens of two-year-old carp were exposed to nitrite at different water temperatures (14 °C and 20 °C):

Group E1: 1.45 $mmol{\cdot}l^{-1}$ (67 $mg{\cdot}l^{-1}$) NO_2^-, t = 14.2 ± 0.2 °C
Group C1 (control 1): traces NO_2^-; t = 14.2 ± 0.2 °C
Group E2: 1.45 $mmol{\cdot}l^{-1}$ (67 $mg{\cdot}l^{-1}$) NO_2^-, t = 20.6 ± 0.5 °C
Group C2 (control 2): traces NO_2^-; t = 20.6 ± 0.5 °C

The nitrite concentration was obtained by adding $NaNO_2$ to dechlorinated tap water. The dose of environmental nitrite represented the median lethal concentration for common carp at a similar chloride water concentration (Máchová and Svobodová 2001). Nitrite and chloride content was checked twice during the test and measured values did not differ from the nominal value by more than 10 percent.

A total of 28 carp (7 from each group) was examined to determine the haematological and biochemical profiles of blood. The blood samples were taken from the heart after 48 hour of nitrite exposure. The blood was stabilised by 40 IU of sodium heparin per 1 ml blood. The erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), methaemoglobin (MetHb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC) were determined in the blood samples (Svobodová et al. 1991). To measure the dimensions of native erythrocytes, 5 µl of native blood of each fish analysed was pipetted into 1 ml of isotonic physiological saline (osmolality 289 ± 5 $mmol{\cdot}kg^{-1}$) . The sample was gently vortexed and 20 µl was immediately pipetted on to a clean microscopic slide. No coverslip was used because drift of cells in the sample would make difficult the precise scoring of cells in captured images. The slide was placed on the stage of an Olympus BHS microscope of which the focus level had been pre-adjusted to the slide surface. Erythrocytes sedimented on the slide surface were recorded with a 3CCD Sony DXC-9100P colour camera coupled to the microscope, and processed by the Olympus MicroImage v. 4.0 software in order to measure erythrocyte major and minor axes, and to compute the aspect ratio (relationship between horizontal and vertical axes).

Plasma was separated from cells by centrifugation (10 min at 12,000 × *g*) at 4 °C and plasma Na^+, K^+ and Cl^-, and NO_2^- concentrations were determined. After blood sampling, the fish were quickly stunned with a blow to the head, and approximately 1 g pieces of muscle and liver were taken. In these tissues, the nitrite concentration was also measured. The tissue samples were kept at -80 °C until analysis to prevent nitrite oxidation.

Chemical analyses

Nitrite levels in the blood plasma, liver and muscle were determined as described in Shechter et al. (1972). Plasma Na^+, K^+ and Cl^- concentrations were measured by ion-selective electrodes (ADVIA 1650, Bayer). Osmolality of the blood plasma and physiological saline used as a diluent for the measurement of selected erythrocyte proportions was determined using a vapour pressure osmometer (VAPRO 5520, Wescor).

Statistical analysis

Statistical software STATISTICA (version 6.1 for Windows, StatSoft) was used to determine differences between the test groups. At the beginning, all measured variables were checked for normality (Kolmogorov-Smirnov test) and homoskedasticity of variance (Bartlett's test). If those conditions were satisfied, one-way ANOVA was applied to determine whether there were any significant differences in measured variables between nitrite-exposed fish at different temperatures and control fish. When a difference was detected ($p < 0.05$), Tukey's multiple comparison test was applied. If the conditions for ANOVA were not satisfied, the non-parametric test (Kruskal-Wallis's test) was used.

Contingency tables were applied to determine differences in mortality between test groups (Zar 1996).

Results

Fish mortality

No mortality was observed in the control groups. High fish mortality was noticed during nitrite poisoning in group E1 (30%) and E2 (51%) compared with the control groups. The difference between group E1 and E2 was not statistically significant ($\chi^2 = 3.28$, df = 1, p = 0.07).

Nitrite concentration in selected tissues (blood plasma, liver and muscle)

Exposure of carp to 1.45 mmol·l^{-1} of ambient nitrite resulted in an accumulation of nitrite in the blood plasma to 5.0 ± 1.5 mmol·l^{-1} at 14 °C (group E1) and 10.5 ± 1.9 mmol·l^{-1} at 20 °C (group E2). The nitrite concentration in group E2 was significantly higher ($p < 0.05$) than in group E1 (Fig. 1). The plasma nitrite concentrations were more than 3.5 (group E1) and 7 times (group E2) greater than environmental ones.

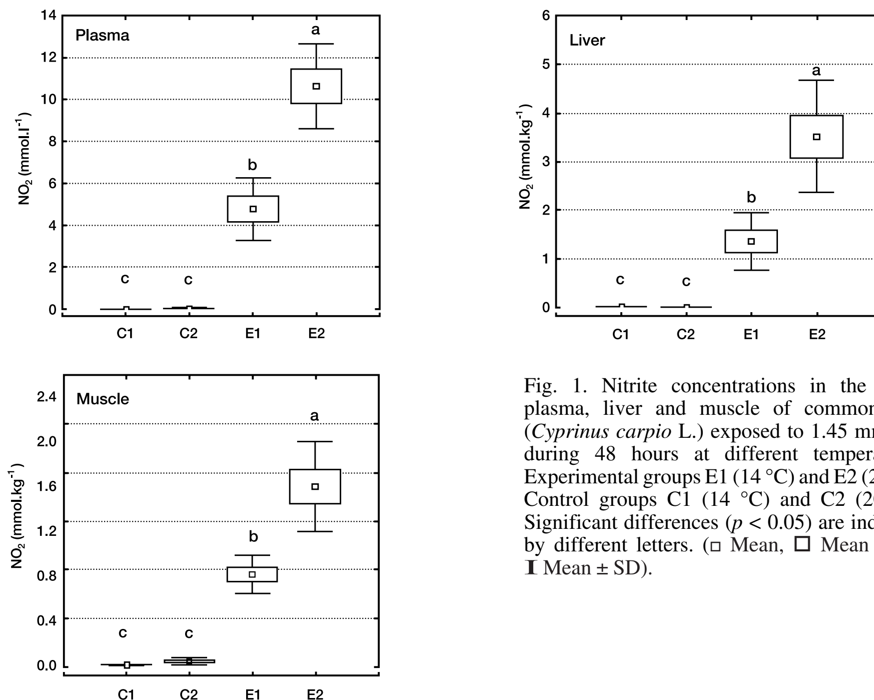

Fig. 1. Nitrite concentrations in the blood plasma, liver and muscle of common carp (*Cyprinus carpio* L.) exposed to 1.45 mmol·l^{-1} during 48 hours at different temperatures. Experimental groups E1 (14 °C) and E2 (20 °C). Control groups C1 (14 °C) and C2 (20 °C). Significant differences ($p < 0.05$) are indicated by different letters. (□ Mean, □ Mean ± SE, I Mean ± SD).

Nitrite also accumulated in the muscle and liver of carp, but these tissues showed lower accumulated levels of the toxicant compared with the blood plasma (Fig. 1). The levels of nitrite in the liver in group E1 and E2 were as follows: 1.4 ± 0.6 $mmol \cdot kg^{-1}$ and 3.5 ± 1.1 $mmol \cdot kg^{-1}$, respectively, and in the muscle 0.76 ± 0.16 $mmol \cdot kg^{-1}$ and 1.5 ± 0.4 $mmol \cdot kg^{-1}$, respectively. Nitrite levels, both in the liver and in the muscle, were significantly higher ($p < 0.05$) at the higher temperature.

Haematological variables

Nitrite exposure affected the following haematological variables: methaemoglobin and haemoglobin concentrations, haematocrit values, erythrocyte count and mean corpuscular volumes (Fig. 2).

The methaemoglobin content increased markedly ($p < 0.01$) in both experimental groups E1 and E2 (88.2 ± 3.3% and 92.9 ± 6.1%, respectively) compared with the control groups (C1: 0.3 ± 0.6%; C2: 2.6 ± 3.0 %) (Fig. 2). The haematocrit values (Fig. 2) were significantly lower ($p < 0.01$) in the carp of group E1 (0.25 ± 0.02 $l \cdot l^{-1}$) compared with control group C1 (0.33 ± 0.04 $l \cdot l^{-1}$) and values in the carp of group E2 (0.20 ± 0.02 $l \cdot l^{-1}$) were significantly lower compared with control group C2 (0.31 ± 0.03 $l \cdot l^{-1}$). The haematocrit values were significantly lower in group E2 compared with group E1. The erythrocyte counts decreased significantly ($p < 0.01$) only in group E2 (1.05 ± $0.12 \cdot 10^{12}$ l^{-1}) (Fig. 2). In group E1, the erythrocyte counts (1.56 ± $0.21 \cdot 10^{12}$ l^{-1}) did not differ significantly from the control groups (C1: 1.70 ± $0.20 \cdot 10^{12}$ l^{-1}; C2: 1.54 ± $0.24 \cdot 10^{12}$ l^{-1}). In group E2, a markedly lower haemoglobin concentration (51 ± 11 $g \cdot l^{-1}$, $p < 0.01$) was found compared with groups E1 (75 ± 5 $g \cdot l^{-1}$), C1 (84 ± 9 $g \cdot l^{-1}$), and C2 (76 ± 10 $g \cdot l^{-1}$) (Fig. 2). Significantly lower ($p < 0.05$) MCV values were found in carp of groups E1 (167 ± 27 fl) compared with groups E2 (190 ± 25 fl), C1 (195 ± 24 fl), and C2 (195 ± 16 fl) (Fig. 2). No significant changes were observed in the MCH and MCHC. No significant differences were also found in selected erythrocyte proportions: major axis (C1: 13.4 ± 1.0 µm; C2: 14.2 ± 0.4 µm; E1: 13.8 ± 1.1 µm; E2: 14.1 ± 0.5 µm), minor axis (C1: 9.3 ± 0.8 µm; C2: 10.5 ± 0.3 µm; E1: 9.6 ± 0.9 µm; E2: 10.4 ± 0.6 µm), and aspect ratio (C1: 1.5 ± 0.2 µm; C2: 1.4 ± 0.1 µm; E1: 1.4 ± 0.2 µm; E2: 1.4 ± 0.1 µm).

Concentration of plasma K^+, Na^+, Cl^- and plasma osmolality

Major changes were observed in plasma K^+ values (Fig. 3). Significant differences ($p < 0.05$) were found among all of the test groups. In the carp of group E1, nitrite exposure caused a significant ($p < 0.01$) increase of 121% in the plasma K^+ concentration compared with control group C1. In the carp of group E2, an increase ($p < 0.01$) of 112% compared with control group C2 was observed. However, we also observed a significant difference between control groups C1 (2.2 ± 0.60 $mmol \cdot l^{-1}$) and C2 (1.8 ± 0.39 $mmol \cdot l^{-1}$).

A slight (only 5%) but significant ($p < 0.05$) decrease in plasma Na^+ values was observed in group E2 compared with the control (group C2) (Fig. 3). No statistically significant differences were observed in the plasma Na^+ concentration at the lower temperature. However, a significant ($p < 0.01$) difference was found between control groups C1 and C2. No statistically significant differences were observed in plasma Cl^- concentrations among all of the test groups (Fig. 3). Finally, no significant differences were observed in plasma osmolality (C1: 287 ± 7 $mmol \cdot kg^{-1}$, C2: 287 ± 6 $mmol \cdot kg^{-1}$, E1: 286 ± 7 $mmol \cdot kg^{-1}$, E2: 287 ± 5 $mmol \cdot kg^{-1}$).

Discussion

High fish mortality was observed during nitrite poisoning at both 14 °C (group E1 - mortality 30%) and 20 °C (group E2 - mortality 51%). However, no significant influence of temperature on mortality in common carp was found.

Nitrite exposure of common carp resulted in nitrite accumulation in the fish body. The

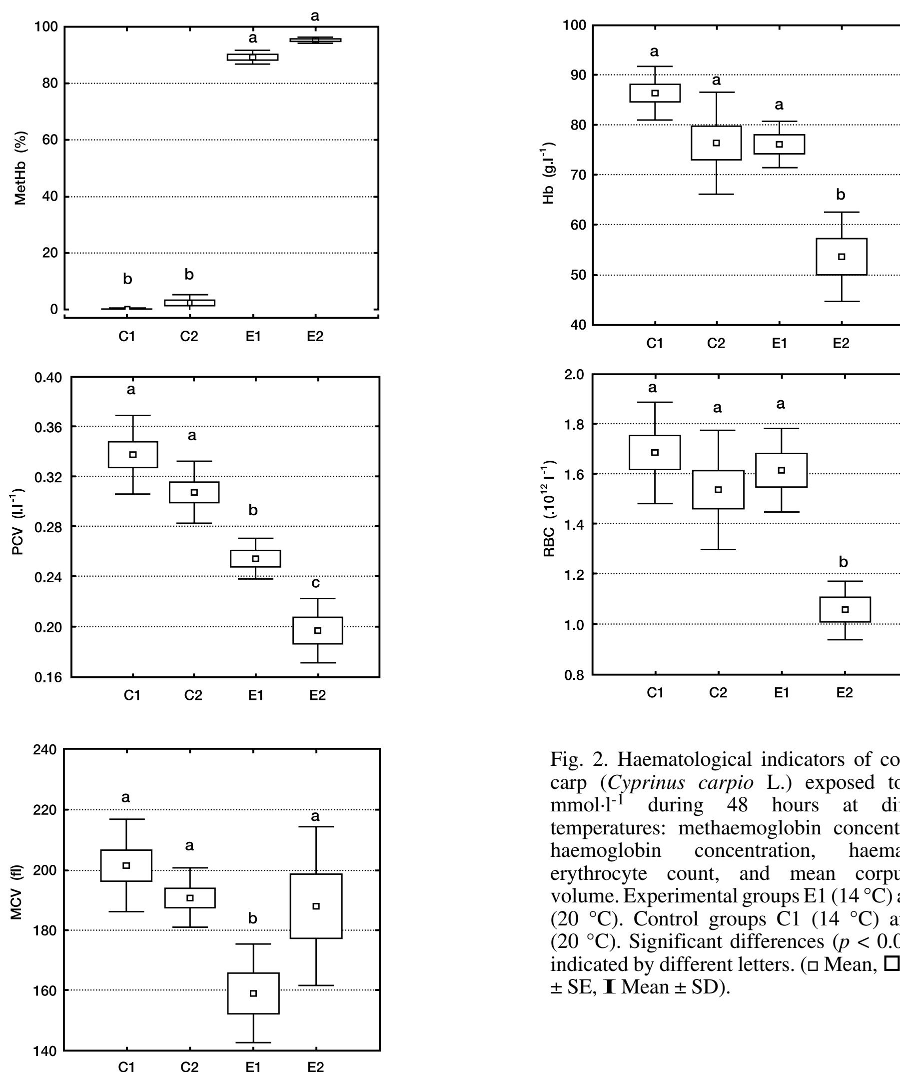

Fig. 2. Haematological indicators of common carp (*Cyprinus carpio* L.) exposed to 1.45 mmol·l^{-1} during 48 hours at different temperatures: methaemoglobin concentration, haemoglobin concentration, haematocrit, erythrocyte count, and mean corpuscular volume. Experimental groups E1 (14 °C) and E2 (20 °C). Control groups C1 (14 °C) and C2 (20 °C). Significant differences ($p < 0.05$) are indicated by different letters. (□ Mean, ☐ Mean ± SE, I Mean ± SD).

highest nitrite levels developed in the blood plasma, followed by the liver and muscle, respectively. Similar results were found by Margiocco et al. (1983) in rainbow trout (*Oncorhynchus mykiss,* Walbaum), showing that relatively low nitrite values developed in the muscle and high nitrite concentrations developed in the liver, brain, and gills. On the other hand Gisbert et al. (2004) observed a higher nitrite content in the skeletal musculature than in the blood plasma of Siberian sturgeon (*Acipenser baerii*, Brandt). In our experiment, plasma nitrite concentrations reached 5.0 mmol·l^{-1} and 10.5 mmol·l^{-1} at 14 °C and 20 °C, respectively. This represents a concentration 3.5 times, respectively 7 times higher than in the surrounding medium. It is well in line with the revealed results of Jensen et al. (1987) on common carp. In this case, exposing carp to 1 mmol·l^{-1} of nitrite for 24 hours resulted in increased blood plasma nitrite to 3.2 mmol·l^{-1} at 15 °C. Our results also suggest that nitrite accumulated to significantly higher levels at higher temperature in both the blood

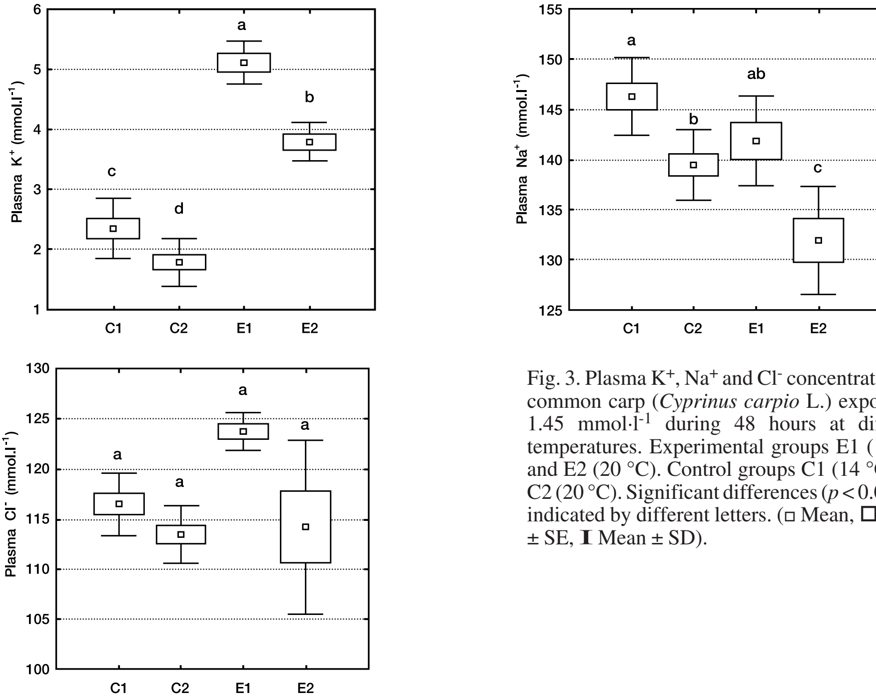

Fig. 3. Plasma K^+, Na^+ and Cl^- concentrations in common carp (*Cyprinus carpio* L.) exposed to 1.45 mmol·l^{-1} during 48 hours at different temperatures. Experimental groups E1 (14 °C) and E2 (20 °C). Control groups C1 (14 °C) and C2 (20 °C). Significant differences ($p < 0.05$) are indicated by different letters. (□ Mean, ☐ Mean ± SE, **I** Mean ± SD).

plasma and in selected tissues of the carp. Information on the influence of temperature on nitrite accumulation in fish is limited, but in crayfish (*Astacus asctacus* L.) a lowering of temperature significantly slows down nitrite accumulation (Jeberg and Jensen 1994). Since fish and crayfish are poikilothermic, their physiological processes are highly affected by environmental temperature. This means that the metabolic rate, heart rate, enzyme activities, and blood flow accelerate as body temperature increases and slow down at colder body temperature. For example, Metz et al. (2003) observed a temperature-enhanced branchial enzymatic pump activity in common carp. In our experiment, increasing temperature probably also affected the activity of enzymes involved in the ion uptake through the gills, subsequently causing faster nitrite accumulation in the blood plasma and tissues of carp. Furthermore, chloride cell proliferation may by another factor contributing to higher nitrite accumulation at higher temperature (Schmidt et al. 1998).

As predicted, exposure of carp to nitrite caused a significant increase in methaemoglobin levels. Nitrite-treated carp also showed typical symptoms of methaemoglobinaemia, manifested by unresponsive and disoriented behaviour, and brown colouring of the blood and gills. In our experiment, high metHb levels in the blood of both experimental groups were well in accordance with high nitrite concentrations in the water. MetHb content reached approximately 90% of functional haemoglobin. Such value is highly critical for fish survival (Lewis and Morris 1986). Although there were higher nitrite levels in the plasma of fish at 20 °C, the fish continued to live even in those conditions. Also, metHb levels were nearly the same as at the lower temperature. This may be explained by varied efficiency of the methaemoglobin reductase enzyme at different temperatures. The red blood cells of fish contain NADH-methaemoglobin reductase that reconverts methaemoglobin to haemoglobin (Cameron 1971). This occurs steadily and will restore the normal proportion of haemoglobin within 24 - 72 hours if a fish is transferred to water that lacks nitrite (Huey

et al. 1980). When nitrite is present, the ultimate level of methaemoglobin in the blood is a result of the balance between methaemoglobin formation and conversion to haemoglobin by methaemoglobin reductase (Lewis and Morris 1986). As fish are poikilotherms, the reductase enzyme varies in efficiency with seasonal temperatures (Perrone and Meade 1977) and its activity is possibly accelerated by enhanced temperature.

Nitrite-exposed fish showed lower haematocrit values (PCV) at both experimental temperatures compared with controls. Haematocrit is based on both erythrocyte count (RBC) and volume (MCV). Either low RBC or low MCV caused a decrease of haematocrit values in dependence on water temperature. At the higher temperature, a decrease in PCV accompanied by a decrease in RBC and in the haemoglobin concentration (Hb), in addition to the constant MCV, can be possibly attributed to blood cell lysis. Since the high activity of the methaemoglobin-reductase system to convert the methaemoglobin to haemoglobin during nitrite exposure results in a high metabolic cost to the red blood cells, the normal life span of these cells is shortened (Scarano et al. 1984). The activity of the methaemoglobin-reductase system was probably enhanced by the higher temperature as mentioned above. In contrast, at the lower temperature, PCV and MCV decreased, and RBC with Hb concentration remained unchanged. In this case, a decrease in PCV is possibly caused by red cell shrinkage as reported by Jensen et al. (1987). The RBC shrinkage is connected with the efflux of K^+ from the red blood cells. The K^+ efflux seems to result from activation of a K^+/Cl^- cotransporter that is normally involved in cell volume regulation (Jensen 1990). The activation of K^+/Cl^- efflux draws osmotically obligated water out of the cells and hence induces erythrocyte shrinkage (Jensen 1990). However, this hypothesis is not supported by an increase of mean corpuscular haemoglobin concentration (MCHC), which may be elevated by erythrocyte shrinkage. We did not notice any significant differences in selected erythrocyte proportions.

In carp, plasma osmolality was constant at both experimental temperatures. However, certain alteration in the plasma electrolyte status occurred. Nitrite poisoning caused a marked increase in plasma potassium concentrations in both test groups compared with controls. The divergence between groups C1 - E1 and C2 - E2 reached nearly the same percentage value. Jensen et al. (1987) originally observed that nitrite-induced extracellular hyperkalaemia in carp. The rise in plasma K^+ is due to the nitrite stimulated release of K^+ from skeletal muscle and red blood cells (Jensen 1990; Knudsen and Jensen 1997). In accordance with the findings of Jensen et al. (1987), we found that plasma Na^+ concentrations slightly decreased in group E2 (20 °C). However, in group E1 (14 °C), the values remained unchanged. We also observed that water temperature itself suggested an influence on plasma K^+ and Na^+ levels (significant difference between control group C1 and C2). Our data confirm the results of Metz et al. (2003), who showed that raising temperature induces a decrease in plasma K^+ and Na^+ levels. Plasma Cl^- levels were not affected by nitrite poisoning in either of the experimental groups, which is in contrast with the results of several authors (Jensen et al. 1987; Knudsen and Jensen 1997). Nitrite is a competitive inhibitor of chloride uptake and vice versa (Williams and Eddy 1986); thus, chloride influx is reduced due to the presence of nitrite in ambient water. On the other hand, in fish exposed to nitrite, there is a concomitant loss of K^+ and Cl^- from skeletal musculature (Knudsen and Jensen 1997). Thus, chloride levels remained unchanged, possibly due to the interaction of the above-mentioned effects.

In conclusion, our experiment indicates that mortality and methaemoglobinaemia during nitrite poisoning are not related to water temperature. However, other haematological and biochemical variables (NO_2^- in the blood plasma, muscle, and liver; plasma Na^+ concentration; Hb; PCV; RBC) were more altered during nitrite exposure at higher temperature.

Otrava kapra obecného (*Cyprinus carpio* L.) dusitany při různých teplotách

Dvě skupiny kaprů obecných (*Cyprinus carpio* L.) byly po dobu 48 hodin vystaveny zvýšené koncentraci dusitanů (1,45 mmol·l^{-1} NO_2^-) při dvou různých teplotách (14 °C - 1. skupina a 20 °C - 2. skupina). Vliv zvýšené koncentrace dusitanů na ryby při různých teplotách byl hodnocen pomocí vybraných hematologických a biochemických ukazatelů krve ryb. Navíc byla stanovena koncentrace dusitanů v krvi, játrech a svalovině. Po působení dusitanů došlo při obou teplotách ke zvýšení hladiny methaemoglobinu (88,2 ± 3,3% a 92,9 ± 6,1%) v krvi ryb v porovnání s kontrolními skupinami (0,3 ± 0,6% a 2,6 ± 3,0%). V obou experimentálních skupinách byla navíc zaznamenána vysoká mortalita ryb (30% a 51%). Dusitany byly kumulovány v těle ryb. Nejvyšší koncentrace byly naměřeny v krevní plasmě, nižší v játrech a svalovině ryb, přičemž statisticky významně souvisely s teplotou vody. Při 20 °C dosáhla koncentrace dusitanů v plasmě pokusných ryb hodnoty 10,5 ± 1,9 mmol·l^{-1}, což je 7 krát více než v okolní vodě. Při 14 °C byly naměřeny signifikantně nižší hodnoty (5,0 ± 1,5 mmol·l^{-1}). Expozice ryb dusitanům dále vyvolala v obou případech zvýšení koncentrace draslíku v krevní plasmě, naproti tomu koncentrace chloridů a osmolalita plasmy zůstaly nezměněny. Při vyšší teplotě došlo v plasmě ryb k poklesu obsahu sodíku. Při obou teplotách bylo u pokusných ryb zjištěno snížení hematokritové hodnoty. Při teplotě vody 20 °C byla nízká hodnota hematokritu (0,20 ± 0,02 l.l^{-1}) doprovázena sníženým počtem erytrocytů (1,05 ± 0,12.10^{12} l^{-1}) a sníženou koncentrací hemoglobinu (51 ± 11 g·l^{-1}) v porovnání s kontrolou. Při nižší teplotě (14 °C) byl pokles hematokritu (0,25 ± 0,02 l·l^{-1}) doprovázen nízkými hodnotami středního objemu erytrocytu (167 ± 27 fl). Působení dusitanů na ryby naopak nevyvolalo při žádné z teplot změny v hodnotách hemoglobin erytrocytu, střední barevné koncentrace ani rozměrů nativních červených krvinek proměřovaných v horizontální rovině jako hlavní a vedlejší osy elipsy.

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