Stress Response to Long Distance Transportation of Common Carp (*Cyprinus carpio* L.)

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Abstract

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The stress responses and changes in biochemical and haematological indices were investigated in three-year-old common carp (*Cyprinus carpio* L.) during a long-distance transportation in special truck tanks. Twelve-hour transportation caused a significant increase in ammonia ($p < 0.01$), mean corpuscular volume MCV ($p < 0.01$), metamyelocytes ($p < 0.05$) and band neutrophils ($p < 0.01$), and a significant decrease in Cl^- ($p < 0.05$), lactate ($p < 0.05$), ALT ($p < 0.05$) and ALP ($p < 0.01$) levels. The values of LDH ($p < 0.01$), AST ($p < 0.05$), CK ($p < 0.01$) and haematocrit PCV ($p < 0.05$) were also significantly influenced by the transportation, but no time-dependent relation was found. On the contrary, the levels of cortisol, glucose and total protein in the biochemical profile, and the values of erythrocyte count (RBC), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), leukocyte counts (WBC) and leukogram (except for metamyelocytes and band neutrophils) in the haematological profile were not significantly influenced by the transportation. Results showed that pre-transport fish manipulation (hauling, netting, handling, loading) was found to be an important stressor for fish. Long-distance transportation itself was relatively considerate for the common carp tested.

Fish, transport, haematological parameters, biochemical parameters

In food animal industries, handling and transport are often stressful events. In fish aquaculture, the processes of grading, capturing (most commonly by netting), transferring out of water and transporting fish are inherently stressful, and lead to a "fight or flight" stress response (Barton et al. 1980). This response causes the primary release of adrenalin and cortisol, which is followed by secondary changes in blood and tissues, such as hyperglycemia, hyperlactemia, hypercholesterolemia, changes in blood plasma enzyme activity and ion concentrations, reduced glycogen content in muscle and liver, an increase in metabolic rate, and also shifts in haematological profile and immunological capacity (Mazeaud et al. 1977; Staurnes et al. 1994; Stave and Robertson 1985).

Transporting live fish is a multiple-phase operation that should be designed to minimize stress and the costs involved. Transport of fish by tank trucks requires a special care to ensure that water quality parameters (temperature and oxygen content, in particular) and fish density requirements are maintained. Transport stress may be often caused by low oxygen levels, temperature differences between storage pond and tank water, or poor transport water quality due to inadequate water exchange that cause accumulation of carbon dioxide and ammonia (Erikson et al. 1997). Excretory products, mucus and regurgitated food degrade water quality. Respiration causes a decrease in dissolved oxygen levels and an increase in carbon dioxide levels in the transport medium. The excretion of nitrogenous wastes increases the level of ammonia in the transport medium. The increase in carbon dioxide concentration causes water pH to decrease.

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Stress and muscle activity during transportation procedures usually shorten the time to the onset of rigor mortis, which is essentially triggered by the depletion of glycogen and ATP in muscle cells. Handling and processing of fish during rigor mortis can result in a loss of quality and lower fillet yield. The pre-rigor period must be long enough to ensure that bleeding, gutting, washing, chilling and packing take place before the onset of rigor mortis. Ante-mortem handling stress has also adverse effects on product quality such as reducing fish freshness and softening muscle texture (Izquierdo-Pulido et al. 1992; Nakayama et al. 1994).

The objective of this study was to assess the transport stress response, i.e. the effect of pre-transport manipulation procedures (hauling, netting, handling and loading) and transportation itself on changes in selected biochemical and haematological indices of three-year-old common carp (*Cyprinus carpio* L.) during a long-distance transportation. Transport water quality parameters were also monitored.

Materials and Methods

The experiment was carried out during a regular transport (April 29, 2005) of fish from storage ponds at a commercial fish farm in Hluboká nad Vltavou via Brno (Czech Republic) to a fish-pond in Boheľov (Slovakia). Carp were held in a freshwater flow-through storage pond for three days prior to transport, and then transferred to 2.4 m^3 transport tanks (loading density of 334 kg body weight·m^{-3}). Fish were transported in long-distance transporting tanks (AGK Kronawitter GmbH, Germany) that were insulated and continuously aerated with gas oxygen during the transport. The transport took 12 hours in total, 7 hours from Hluboká nad Vltavou to Brno and 5 hours from Brno to Boheľov.

Water quality variables were registered throughout the experiment, the results are given in Table 1. The values of temperature and oxygen content were measured *in situ* (WTW pH 235), and pH, NH_4^+, Cl^-, acid neutralisation capacity ($ANC_{4.5}$) and chemical oxygen demand (COD_{Mn}) were measured in laboratory.

The fish population tested consisted of 47 fish. Blood samples were withdrawn by cardiocenthesis before transport (i.e. during fish transfer from the storage pond to truck tanks) in Hluboká nad Vltavou (16 individuals), and after 7 and 12 hours of transport, in Brno (15 individuals) and Boheľov (16 individuals), respectively. *In situ*, haematocrit (PCV) was measured and leukogram blood smear prepared. A small volume of heparinized blood was reserved at 4 °C for red and white blood cell counts (RBC, WBC) and haemoglobin content (Hb) (Svobodová et al. 1991) . The rest of heparinized blood was centrifuged at 3000 rpm for 10 min and plasma samples were stored at 4 °C in Eppendorf test-tubes until analyses were performed (i.e. within 8 hours after blood sampling).

Plasma biochemical indices (glucose, lactate, LDH, CK, ALT, AST, ALP, ammonia, chloride, total protein) were measured by a biochemical analyzer Cobas EMira using commercial test kits. Plasma cortisol concentration was measured using HPLC/DAD (Waters).

Experimental data were statistically tested using variance analysis of the Statistica 6.0 software (Kruskal Wallis ANOVA).

Results

The results of water samples analysis are given in Table 1 and the values (mean ± SD) of biochemical and haematological indices of the common carp tested are presented in Table 2 and Table 3, respectively.

Table 1. Variables of the transport water tested (controlled aeration).

Parameters	Hluboká	Brno	Boheľov
temperature (ºC)	12.6	12.1	12.0
oxygen (%)	21.9	55.7	116.2
pH	7.39	6.46	6.57
$ANC_{4.5}$ (mmol·l^{-1})	2.0	2.0	2.2
COD_{Mn} (mg·l^{-1})	12.8	29.7	44.8
NH_4^+ (mg·l^{-1})	1.19	8.69	13.00
Cl^- (mg·l^{-1})	27.37	35.42	40.25

Mean values of body length, body weight and spleen weight (41.1 ± 4.13 cm, 1213.1 ± 322.07 g and 3.1 ± 0.93 g in Hluboká nad Vltavou, 39.6 ± 5.41 cm, 1203.3 ± 540.99 g and 4.1 ± 1.74 g in Brno, and 40.0 ± 3.89 cm, 1196.9 ± 367.18 g and 3.9 ± 1.32 in Boheľov, respectively) did not differ statistically among the groups tested.

In the study, the value of SSI (“spleen/soma index”, i.e. relative weight of spleen) was also tested. The values of relative spleen weight were found 0.27 ± 0.088%, 0.34 ± 0.089%, and 0.33 ± 0.077% in Hluboká nad Vltavou, Brno, and Boheľov, respectively. No significant difference in the SSI indice among the groups tested was found.

Results of plasma biochemical profile of experimental groups of common carp exposed to long-distance transportation (Fig. 1) showed a significant increase in ammonia ($p < 0.01$), and a significant decrease in chloride ($p < 0.05$), lactate ($p < 0.05$), ALT ($p < 0.05$) and ALP ($p < 0.01$) values. The values of LDH ($p < 0.01$), AST ($p < 0.05$) and CK ($p < 0.01$) were significantly influenced by the transport, too, but no time-dependent relation was found. The concentrations of cortisol, glucose and total protein were not significantly changed (Table 2).

Table 2. Biochemical indices of common carp during long-distance transportation.

Indices	Hluboká Mean ± SD	Brno Mean ± SD	Boheľov Mean ± SD
Cortisol (ng·ml^{-1})	213.3 ± 61.88 ^{a}	206.6 ± 42.48 ^{a}	201.6 ± 36.38 ^{a}
Glucose (mmol·l^{-1})	8.2 ± 1.50 ^{a}	8.7 ± 2.20^{a}	9.4 ± 2.58 ^{a}
LDH (µkat·l^{-1})	10.2 ± 3.09 ^{a}	14.7 ± 0.89 ^{b}	9.9 ± 6.16 ^{a}
AST (µkat·l^{-1})	2.2 ± 1.17 ^{a}	3.4 ± 0.81 ^{b}	2.5 ± 1.23 ^{a}
CK (µkat.l^{-1})	375.1 ± 137.46 ^{a}	761.8 ± 457.44 ^{bc}	588.2 ± 411.57 ^{ac}
Total protein (g·l^{-1})	29.8 ± 3.43 ^{a}	31.1 ± 5.46 ^{a}	30.1 ± 3.46 ^{a}

Note: Groups with different alphabetic superscripts differ significantly.

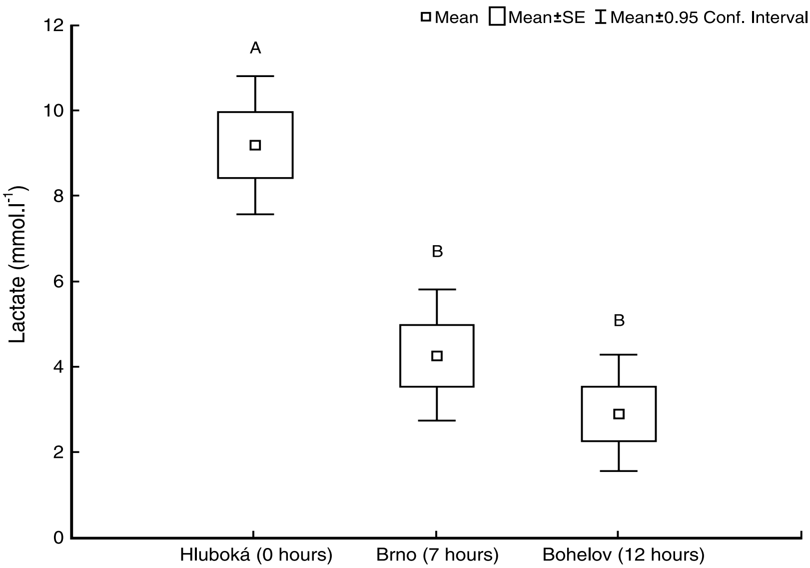


Fig. 1a. Plasma lactate concentration of common carp during long-distance transportation.
Note: Columns with different alphabetic superscripts differ significantly.

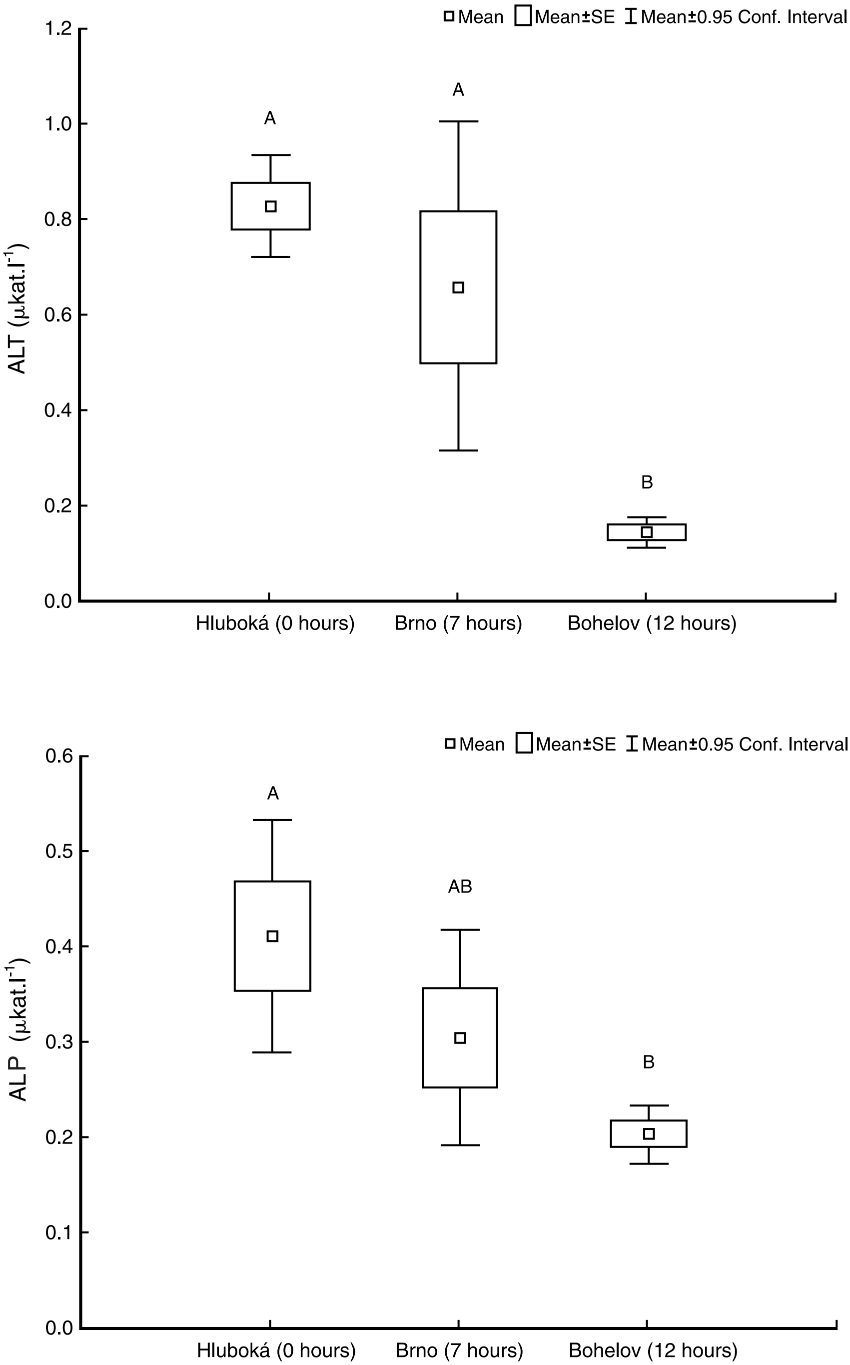
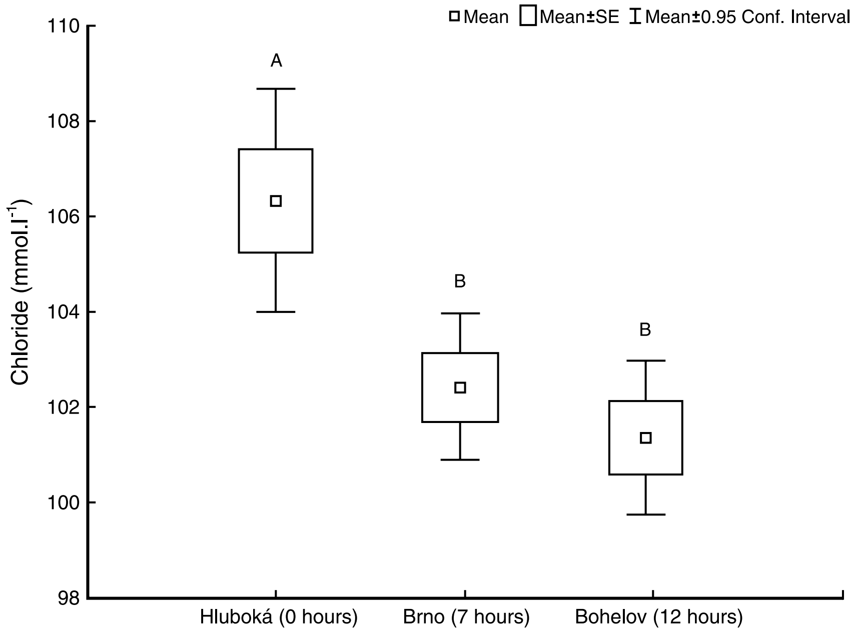


Fig. 1b, c. Plasma ALT and ALP activities of common carp during long-distance transportation.
Note: Columns with different alphabetic superscripts differ significantly.



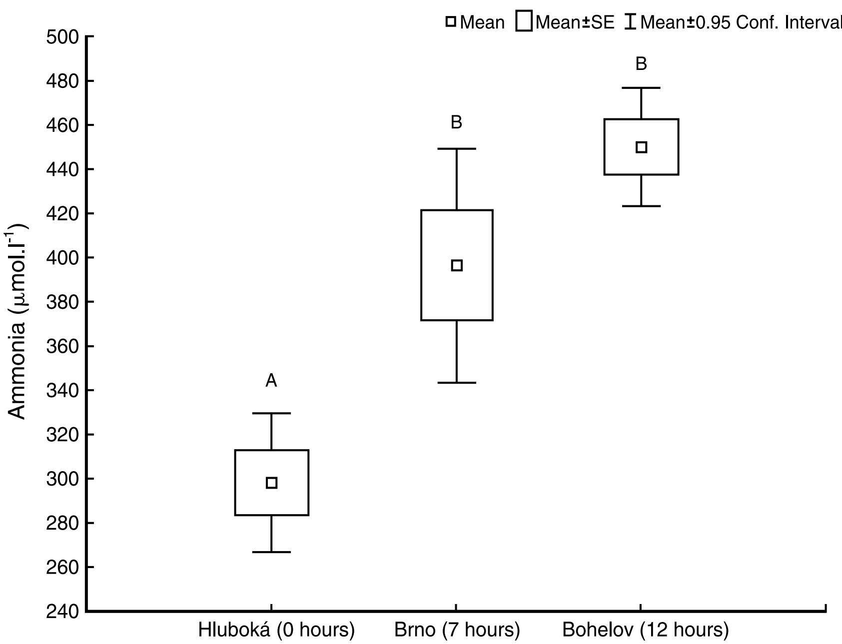


Fig. 1 d, e. Plasma chloride and ammonia concentrations of common carp during long-distance transportation.
Note: Columns with different alphabetic superscripts differ significantly.

As far as the haematological indices are concerned (Fig. 2), the long-distance transport caused a significant increase in MCV ($p < 0.01$), metamyelocytes counts ($p < 0.05$) and band neutrophils counts ($p < 0.01$). The values of PCV ($p < 0.05$) were also significantly changed without the confirmation of time-dependent relation. The other haematological indices tested were comparable within the groups (Table 3).

Table 3 Haematological indices of common carp during long- distance transportation.

Indices	Hluboká Mean ± SD	Brno Mean ± SD	Boheľov Mean ± SD
RBC ($T \cdot l^{-1}$)	1.46 ± 0.175^{a}	1.46 ± 0.193^{a}	1.38 ± 0.225^{a}
PCV ($l \cdot l^{-1}$)	0.32 ± 0.027^{a}	0.36 ± 0.044^{b}	0.36 ± 0.028^{b}
Hb ($g \cdot l^{-1}$)	94.74 ± 28.047^{a}	92.68 ± 15.463^{a}	93.43 ± 9.875^{a}
MCH (pg)	65.49 ± 21.108^{a}	64.02 ± 9.772^{a}	69.11 ± 12.329^{a}
MCHC ($l \cdot l^{-1}$)	0.30 ± 0.084^{a}	0.27 ± 0.048^{a}	0.26 ± 0.028^{a}
WBC ($G \cdot l^{-1}$)	57.56 ± 32.932^{a}	76.33 ± 45.613^{a}	79.94 ± 28.963^{a}
Lymphocytes ($G \cdot l^{-1}$)	51.02 ± 33.147^{a}	65.32 ± 42.681^{a}	63.74 ± 26.855^{a}
Monocytes ($G \cdot l^{-1}$)	0.91 ± 0.678^{a}	0.73 ± 0.968^{a}	0.52 ± 1.494^{a}
Myelocytes ($G \cdot l^{-1}$)	1.98 ± 1.944^{a}	2.17 ± 1.500^{a}	2.74 ± 1.995^{a}
Segmented neutrophils ($G \cdot l^{-1}$)	0.82 ± 1.047^{a}	0.92 ± 1.003^{a}	0.62 ± 0.988^{a}
Basophils ($G \cdot l^{-1}$)	0.03 ± 0.089^{a}	0.00 ± 0.000^{a}	0.13 ± 0.392^{a}

Note: Groups with different alphabetic superscripts differ significantly.

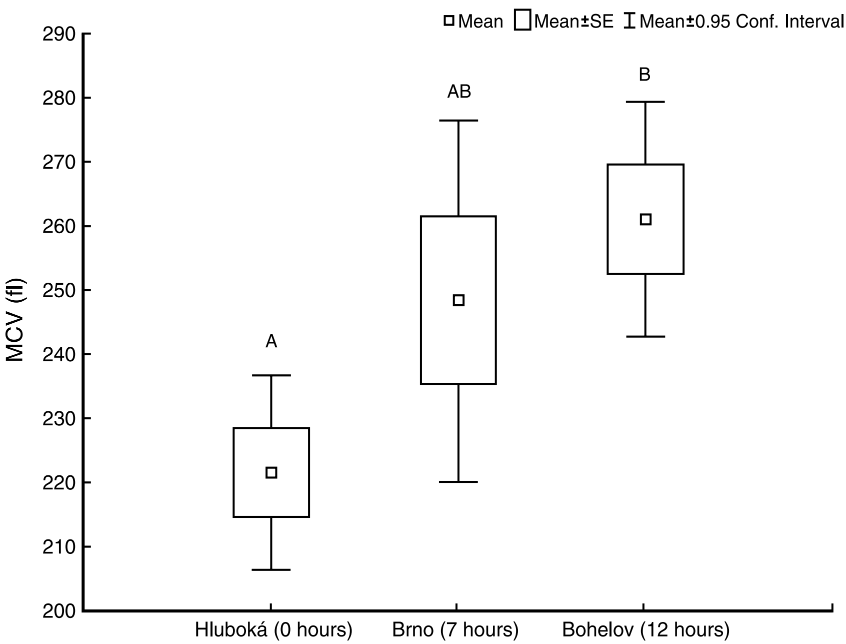


Fig. 2a. MCV value of common carp during long-distance transportation.
Note: Columns with different alphabetic superscripts differ significantly.

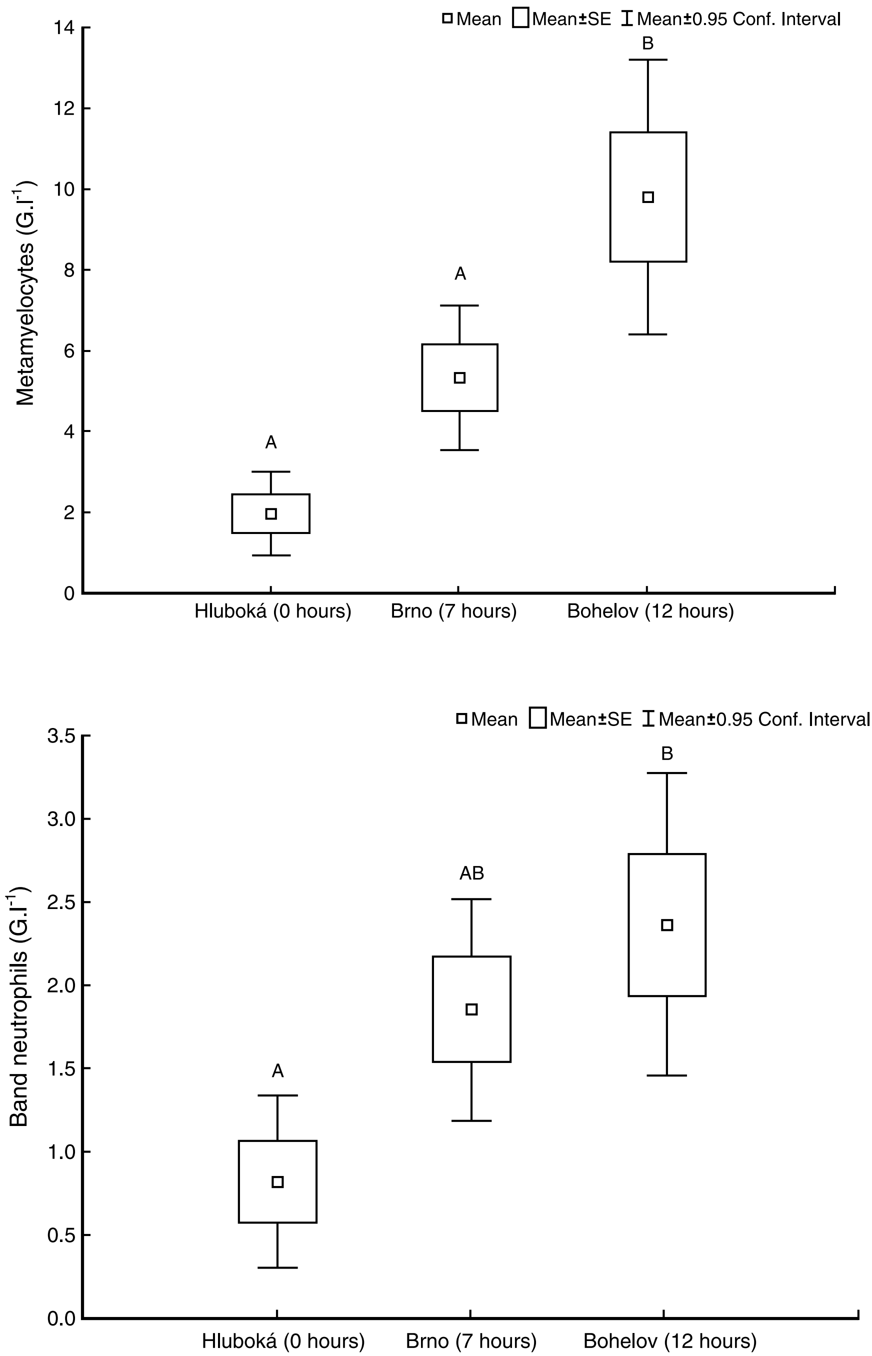


Fig. 2b, c. Metamyelocytes counts, and band neutrophil counts of common carp during long-distance transportation.
Note: Columns with different alphabetic superscripts differ significantly.

Discussion

Recent studies show that one of the most important factors that affect the fish welfare during short- or a long-distance transport, is an adequate adjustment and stress reduction in fish by minimization of manipulation procedures, pre-transport defecation to prevent the accumulation of metabolites in transport medium, as well as the avoidance of temperature and light changes, using anaesthetics, etc. (Svobodová et al. 1999) .

None of the water-quality parameters measured (Table 1) suggested that adverse water quality prevailed at any time during long-distance transportation.

Being vulnerable to short-term temperature fluctuations, necessary care must be taken when transferring fish to new ponds or new culture or transport media until acclimatized (Roberts and Shepherd 1997). In our experiment, storage pond and truck tank water temperatures were found similar (i.e. approximately 12.5 °C). As for common carp, a critical value of oxygen saturation in water is 12.0% and 15.3% at 10 and 15 °C, respectively (Svobodová et al. 2003). In the study, transport water oxygen content increased markedly during the fish transportation due to continual aeration, and was sufficient for the fish.

The value of pH slightly decreased during the transport, nevertheless, it held within the optimum range 6.4 - 8.4 (Roberts and Shepherd 1997). The elevated COD_{Mn} value and the levels of ammonia and chloride in transport medium may be ascribed to higher fish metabolic rate in a relatively high-density tank volume.

Pre-transport manipulation, i.e. capturing and reloading fish into transport tanks, appeared to be a severe stressor to fish. In accordance with our findings, some previous studies proved that capture-loading manipulation (hauling, netting, catching, reloading) is the major cause of the transport stress response (Schreck et al. 1989; Weireich and Tomasso 1991). Robertson et al. (1987), Pottinger (1998) and Ruane et al. (2001) reported that manipulation with fish in general (capturing, confinement, increased stocking density, transportation, etc.) lead to the elevation of plasma cortisol, glucose and lactate concentrations. Ruane et al. (2001) reported that during net-confinement, plasma cortisol levels increased rapidly after 30 min to reach the concentrations of 240 - 320 $ng \cdot ml^{-1}$. Robertson et al. (1987) reported that cortisol concentrations increased with the increase of transport time. On the basis of our study results, we assume that pre-transport manipulation lead to the increase of plasma cortisol. The increased concentration of cortisol lasted throughout the transportation.

Plasma glucose levels are elevated in stressed fish as a consequence of the increase of blood catecholamine levels. Nevertheless, the index is a more equivocal index of stress than cortisol, as plasma glucose is a function of many factors (diet, age, season, etc.). In our study, glucose levels were found very high at the beginning, and slightly (non-significantly) increased during the test. The increase of glucose level at the beginning of the test may be ascribed to an enhanced stress reaction of carp to reloading processes, when fish were kept in nets in a high density. Glucose level in unstressed carp reported in previous studies ranged within 2.8 - 5.6 $mmol \cdot l^{-1}$ (Hertz et al. 1989), 2.0 - 5.0 $mmol \cdot l^{-1}$ (Svobodová et al. 1991) and 2.5 - 3.6 $mmol \cdot l^{-1}$ (Blasko et al. 1992). Carp exposure to prolonged hypoxia, crowding stress and confinement in anglers' keepnets were reported to increase blood glucose levels from 5.0 to 10.0 $mmol \cdot l^{-1}$ (van Raaij et al. 1996), from 4.7 to 6.9 $mmol \cdot l^{-1}$ (Yin et al. 1995), and from 5.1 $mmol \cdot l^{-1}$ (3.6 and 3.3 $mmol \cdot l^{-1}$, respectively) to 8.0 $mmol \cdot l^{-1}$ (7.3 and 6.8 $mmol \cdot l^{-1}$, respectively) in three experiments of Pottinger (1998).

The elevation of plasma lactate levels follows on respiratory activity under anaerobic conditions and reflects the imposition of severe exercise (Pottinger 1998). In our study, the highest level of plasma lactate (9.17 $mmol \cdot l^{-1}$) was found at the beginning of the test, when the fish were stressed by hauling and exhausted by physical exercise in relatively hypoxic conditions. In Pottinger's study (1998), the confinement of fish in keepnets caused significant elevation of plasma lactate from 3.0 to 13.9 mmol·l^{-1} and from 2.0 to 3.7 mmol·l^{-1}. In our study, lactate levels decreased significantly ($p < 0.05$) to baseline levels (2.89 mmol·l^{-1}) during the transport, that indicates that the fish became calm after reloading procedure. The decrease of plasma lactate concentration during transportation also corresponds to the increase of water oxygen content in truck tanks.

At the end of the transport, a significant decrease in ALT ($p < 0.05$), ALP ($p < 0.01$) and chloride ($p < 0.05$) levels, and a significant increase in ammonia ($p < 0.01$) concentration were found. Initial high ALT, ALP and chloride levels may be ascribed to the enhancement of fish basal metabolism during stressful reloading. Subsequent modulation of fish during transport leads to a decrease in their levels. Davison et al. (1994) reported that hypoxic conditions caused an increase in chloride levels in fish. That corresponds to the results of our study, in which the highest chloride concentration was found in fish during hauling procedure, when the lowest water oxygen content was measured. During the transport, the oxygen content increased rapidly and chloride levels dropped significantly.

During exhaustive exercise (attack of prey, escape from predators, locomotion and migration processed, starvation), an increase of active energy demand occurs in fish (Smutná et al. 2002). Direct deamination of important tissue energy sources, i.e. amino acids, especially glutamate and aspartate (liver, muscle), histidine (kidney, muscle), serine (kidney) and glutamine (liver, kidney, muscle), leads to energy production (catabolism of glutamine and amino acids provides ATP molecules), and simultaneously to an increase of the ammonia level in fish (Smutná et al. 2002; Philip and Rajasree 1996). Production of an active actine-myosine complex, necessary for muscle contraction, leads to the splitting of ATP to ADP and P_i. Deamination of the AMP molecule, produced from the ADP molecule, leads to the production of IMP and ammonia. It means that in fish, burst exercise (i.e. high muscle activity) results in the production of ammonia. When the detoxification capabilities of the fish are exceeded, ammonia cumulates in the fish organism and acts as a toxicant (Smutná et al. 2002). In our study, the increase of ammonia level was caused by the enhancement of basic energy demand of fish transported (the increase of deamination processes in tissues), and by higher muscle activities of fish in space-quartered transport tanks.

Changes in haematological profile of peripheral blood have often been used as an indicator of stress exposure, though haematological indices results reported are found equivocal. Serious changes in haemogram are found in fish exposed to acute or chronic stress (Svobodová et al. 1994). As far as haematological indices of our study are concerned, twelve-hour transportation caused a significant increase in MCV level ($p < 0.01$), metamyelocytes ($p < 0.05$) and band neutrophils ($p < 0.01$) .

In the blood of stressed fish, an increase in RBC, haemoglobin concentration and haematocrit level are observed (Doubek et al. 2003). Haemoglobin and haematocrit are often elevated during stress situations to increase oxygen carrying capacity and oxygen supply to the major organs in response to higher metabolic demands (Ruane et al. 1999). In our study, the highest RBC value was found in fish blood samples withdrawn during the transfer from storage ponds to transport tanks in Hluboká, when stress-induced RBC release from spleen to blood circulation was reported. RBC values then slightly decreased. Hb level was not affected, haematocrit and MCV values increased significantly.

Ruane et al. (2001) tested the effect of three-hour net confinement on common carp and found no specific changes in haemoglobin content. Haematocrit values decreased non-significantly after 0.5h and 1.0h confinement. During the recovery, the haematocrit levels increased and became comparable to control levels. The lowest haematocrit values of carp tested in our study were found during reloading fish into transport tanks. Haematocrit values increased significantly during the transportation.

Ruane et al. (2002) exposed common carp to high stocking densities of 56.8 and 113.6 kg·m^{-3} for a period of 87 hours without finding any significant high-density effect on fish haematocrit and haemoglobin values. No changes in haematocrit values were also recorded in the Atlantic cod transport experiment by Staurnes et al. (1994).

Physiological responses of matrinxa juveniles (*Brycon cephalus*) juveniles were determined after the procedures of capturing, loading and 4h transport at different densities (Urbinati et al. 2004). Haematocrit increased after loading, maintaining levels slightly high until the end of the experiment. No differences were verified in RBC number. Acerete et al. (2004) found an increase in haematocrit values and RBC after acute handling stress.

Results of white blood line observation showed a significant increase in counts of metamyelocytes ($p < 0.05$) and band neutrophils ($p < 0.01$), as well as a slight increase in leukocytes and myelocytes, and monocytes count reduction (both non-significant).

An important secondary effect of stress reported in fish (as a consequence of stress-related release of catecholamines) is immunosuppression (lymphocytopenia) and neutrophilia (Wiik et al. 1989; Svoboda 2001; Engelsma et al. 2003). Transport- and handling-stress leads to the elevation of plasma cortisol that is reported to have a direct cytolytic effect on lymphocytes (Wiik et al. 1989). These findings are in agreement with the data published by Wendelaar (1997), who reported that stress caused a rapid increase in neutrophils and a reduction of lymphocytes in peripheral blood. Reduction in lymphocyte count in stressed fish may partly be due to the extravasation of the cells and their penetration to the epithelium of gills, skin or intestine.

In our experiment, long-distance transport in high density tanks led to a slight increase of leukocyte count in the fish tested. The stress was also expressed in the increase in neutrophil granulocytes, mainly of juvenile forms (metamyelocytes and band neutrophils). Ortuno et al. (2001) reported that intense short-term crowding stress caused leukocytes release from head-kidney and their cumulation in blood circulation. Pulsford et al. (1994) detected an increased number of leukocytes, particularly phagocytes and damaged cells, in peripheral blood of stressed dab, *Limanda limanda*, along with a decrease in lymphocyte, thrombocyte and erythrocyte counts. In Espelid et al. (1996) study, after cortisol administration, a marked increase in the relative number of thrombocytes was reported, whereas granulocyte, monocyte and lymphocyte counts remained still relatively constant in Atlantic salmon.

In conclusion, pre-transport manipulation procedures were found to be very stress-inducing and transport itself was relatively considerate for common carp. Although carp may quickly adapt to ambient environment, disturbances should be kept to a minimum to ensure optimal transport conditions, and subsequently high quality standards of fish products.

Stresové zatížení kapra obecného (*Cyprinus carpio* L.) při dlouhodobém transportu

U tříletého kapra obecného byla sledována odpověď, tj. změny vybraných biochemických a hematologických parametrů, na stresové zatížení při dlouhodobém transportu ve speciálních transportních tancích. Dvanáctihodinový transport vedl k signifikantnímu zvýšení koncentrace amoniaku ($p < 0.01$), hodnoty MCV ($p < 0.01$), počtu metamyelocytů ($p < 0.05$) a neutrofilů - tyček ($p < 0.01$) a k signifikantnímu poklesu koncentrace Cl^- ($p < 0.05$) a laktátu ($p < 0.05$) a katalytické koncentrace ALT ($p < 0.05$) a ALP ($p < 0.01$). Katalytická koncentrace LDH ($p < 0.01$), AST ($p < 0.05$) a CK ($p < 0.01$) a hodnota PCV ($p < 0.05$) byly transportem statisticky významně ovlivněny, avšak nebyla prokázána časová závislost na době transportu. Hladiny kortisolu, glukosy a celkových proteinů v biochemickém profilu a hodnoty Hb, MCH, MCHC a počet erytrocytů, leukocytů a leukogram (vyjma metamyelocytů a neutrofilů - tyček) v hematologickém profilu ryb nebyly transportem signifikantně ovlivněny. Manipulační procesy spojené s nakládkou ryb byly pro testované ryby stresujícím faktorem, vlastní transport byl relativně šetrný.

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