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# Physiological responses of over-wintering common carp (*Cyprinus carpio*) to disturbance by Eurasian otter (*Lutra lutra*)

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**Abstract** Using a tame animal, the impact of otter (*Lutra lutra*) disturbance on over-wintering carp (*Cyprinus carpio*) was monitored in two experiments, 133 and 140 days, respectively, over two consecutive winters (November–April). The level of stress in over-wintering carp exposed to various intensities of disturbance by otters was quantified using biological indicators of stress (cortisol, cortisone, indices of nitrogen, carbohydrate, lipid and mineral metabolism and activity of basic blood plasma enzymes) taken from blood plasma of stocked carp at the end of the winter seasons (when the photoperiod was 12 light:12 dark, respectively, 13L:10D). Moreover, condition (Fulton's coefficient of condition and fat content in muscles) and mortality rate of that carp were measured after over-wintering and also after the subsequent vegetation period. The analysis of blood and tissue samples of experimental fish showed changes in nitrogen, carbohydrate and mineral metabolism as well as levels of hormones and fat reserves. Higher response to stress in metabolism of carp with lower intensity of disturbance by otter suggests that high level of disturbance can lead to metabolic adaptation of carp to stress. The effect of stress on the mortality rate of carp during the over-wintering is not clear. Nevertheless, the negative effect of stress on survival, condition and growth rate of carp in the subsequent vegetation period was not observed.

**Keywords** Cortisol · Cortisone · Predator–prey interaction · Stress

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## Introduction

In the aquatic environment fish are exposed to numerous adverse impacts that can cause stress reactions in them. The responses to stress typically involve all levels of animal organisation and are collectively called the integrated stress response (Wendelaar Bonga 1997). For the integrated stress response in fishes, the distinction between primary,

secondary and tertiary responses has been introduced (e.g. Pickering 1981; Wedemeyer et al. 1990; Pickering and Pottinger 1995). Primary responses are activation of brain centres, resulting in the massive release of catecholamines and corticosteroids (Mazeaud et al. 1977; Wendelaar Bonga 1997). Secondary responses usually are defined as the manyfold immediate actions and effects of these hormones at blood and tissue level resulting in, e.g., hyperglycaemia, hypercholesterolaemia, reduced chloride levels (Wedemeyer 1983; Järvi 1990), changes in activity of various enzymes (e.g., dehydrogenase, creatine kinase and the transaminases (Cairns and Christian 1978; Navrátil et al. 1998) and mobilisation of energy substrates (Vijayan et al. 1996; Barton et al. 2002). Tertiary responses extend to the level of the organism and population: inhibition of growth, reproduction, as well as immune response and reduced capacity to tolerate subsequent or additional stressors (e.g. Smart 1981; Schreck 1981; Campbell et al. 1994; Ruane 2002). Besides the changes in the physical environment (e.g. temperature, oxygen level, salinity and water pollution) (e.g., Lusková et al. 2002; Engelsma et al. 2003), handling, transport (Svobodová et al. 1999), crowding (Ruane 2002) and noise (Wysocki et al. 2006), the disturbance of fish by predators may also be an important stressor (Rehnberg and Schreck 1987; Woodley and Peterson 2003; Breves and Specker 2005).

In Central Europe there is a long tradition to grow carp (*Cyprinus carpio* L.) in ponds for human consumption. These are usually stocked in spring and harvested in autumn. During winter many ponds remain empty, while carp that have not been sold are kept in ponds suitable for over-wintering. Such ponds have to provide special conditions in respect to water quality and depth in order to guarantee the survival of carp in good condition for the next season, when they are redistributed in other ponds for growing. Wintering ponds in which fish are stocked over the winter period represent an essential part of the system of carp pond culture.

Eurasian otter (*Lutra lutra* L.), along with Great cormorant (*Phalacrocorax carbo* L.), are the two species of predators most commonly blamed for the serious damage that affects fish stocks in ponds in Central Europe (Kranz 2000). Apart from the consumption of the stocked fish, surplus killing and disturbance of the over-wintering populations are considered a major problem by local fishermen (Kranz 2000). The intensive disturbance of resting fish by otters can lead to stress resulting in loss of body condition and, ultimately, the demise of the total fish stock. Moreover, disturbed fish become more active, lose condition and are more susceptible to parasites and diseases (Kranz 2000; Adámek et al. 2003). However, information concerning the primary response of carp affected by stress from exposure to otter, as well as information concerning the metabolic and health changes following the stress, are lacking.

Therefore experiments with a tame otter, under controlled conditions, were carried out. Determination of cortisol (F) and cortisone (E) levels in peripheral blood was used to evaluate stress exposure, while biological indicators in the area of blood plasma biochemistry were used to evaluate consecutive metabolic and enzymatic changes. A clinical patho-anatomical examination was used to describe the morphological changes due to the wounds caused by otter. The length–weight index and fat content in muscles were used to describe the condition of the fish.

All monitored parameters were used to determine whether or not:

(1) over-wintering carp show changes in blood plasma parameters, indicating stress by otter presence in the pond;
(2) the stress response induced by otter disturbance has no effect on the condition and mortality rate of over-wintering fish during the winter as well as the subsequent period (April–October).

## Materials and methods

### Material

The experiments were carried out over two subsequent winters 2003/2004 (trial I) and 2004/2005 (trial II).

In both years the trials were performed using common carp-average weights of 482 ± 92 g (mean ± SD) and 371 ± 72 g, respectively, and standard lengths ($S_L$) of 242 ± 13 mm and 218 ± 13 mm, respectively. All the fish were of the same origin, in excellent body condition and health (Table 1).

In trial I a female-orphaned otter, 6 months old, was used; in trial II an 18-month-old male was used.

**Table 1** Biochemical and condition parameters in carp before over-wintering in trial I and trial II

| Indicators | Trial I | | | Trial II | | |
|---|---|---|---|---|---|---|
| | *n* | Mean | SD | *n* | Mean | SD |
| TPP (g $l^{-1}$) | 19 | 25 | 3 | 15 | 30 | 2 |
| AL (g $l^{-1}$) | 19 | 10.4 | 1.1 | 15 | 10.5 | 0.7 |
| CREA (μmol $l^{-1}$) | 19 | 14.3 | 2.9 | 15 | 14.3 | 2.5 |
| GL (mmol $l^{-1}$) | 19 | 3.8 | 1.2 | 15 | 4.1 | 1.4 |
| CHOL (mmol $l^{-1}$) | 18 | 3.7 | 0.5 | 15 | 5.5 | 0.6 |
| TGL (mmol $l^{-1}$) | 19 | 1.3 | 0.3 | 15 | 1.7 | 0.4 |
| Ca (mmol $l^{-1}$) | 19 | 2.63 | 0.16 | 15 | 2.22 | 0.11 |
| P (mmol $l^{-1}$) | 19 | 1.60 | 0.42 | 15 | 2.14 | 0.10 |
| AST (μkat $l^{-1}$) | 16 | 2.73 | 0.81 | 14 | 2.50 | 0.95 |
| ALT (μkat $l^{-1}$) | 18 | 0.85 | 0.41 | 14 | 0.48 | 0.16 |
| ALP (μkat $l^{-1}$) | 18 | 1.10 | 0.38 | 15 | 0.53 | 0.16 |
| LD (μkat $l^{-1}$) | 18 | 8.9 | 2.7 | 15 | 15.6 | 7.2 |
| A/G | 18 | 0.73 | 0.08 | 15 | 0.55 | 0.08 |
| AST/ALT | 19 | 4.6 | 2.6 | 13 | 6.0 | 3.0 |
| LD/AST | 19 | 2.7 | 1.3 | 14 | 6.2 | 2.4 |
| FQ | 19 | 3.36 | 0.23 | 15 | 3.56 | 0.24 |
| ML (%) | 18 | 4.6 | 1.5 | 15 | 4.2 | 1.3 |

SD, standard deviation; TPP, total plasma protein; AL, albumin; CREA, creatinine; GL, glucose; CHOL, cholesterol; TGL, triacylglycerol; Ca, total calcium; P, inorganic phosphate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LD, lactate dehydrogenase; A/G, albumin/globulin ratio; AST/ALT, aspartate aminotransferase/alanine aminotransferase ratio; LD/AST, lactate dehydrogenase/aspartate aminotransferase ratio; FQ, Fulton's condition factor; ML, muscular lipid

The otters were kept at the Otter Station, Pavlov, in enclosures with semi-natural ponds. They were tame and trained to catch live fish.

In trial I, the fish ($n$ = 160) were stocked in a chain of experimental ponds of 0.01 ha and depth about 1.5 m located at the village of Kejžlice (49°35′ N, 15°14′ E; 470 m above sea level). In trial II, the fish ($n$ = 290) were stocked in a chain of ponds of 0.3 ha with the depth about 0.8 m from Pavlov (49°41′ N, 15°19′ E; 430 m a.s.l.). The experimental ponds were fenced and contained two lines of electric wire to prevent wild otters and American mink (*Mustela vison* Schreber) entering the experimental areas.

## Experimental design

The fish were stocked in experimental ponds for 134 days (5 November 2003–17 March 2004) in trial I and 140 days (24 November 2004–11 April 2005). Fishing the ponds was carried out 5 weeks after the last visit by the otter. The water temperature, pH and oxygen level in the experimental ponds were regularly monitored during the over-wintering period.

The fish were over-wintered in three experimental ponds, each subject todifferent levels of disturbance by the otter. The first group was not disturbed and served as a control group (CG1). Fish in the second group (experimental group 2, EG2) were in contact with the otter for 1 h once a week, and the third group (EG3) in contact with the otter for 1 h twice a week.

After over-wintering all ponds were fished out using a haul seine and all surviving individuals measured and weighed, as well as being examined for the injuries caused by otter. Subgroups of at least 16 fish were taken from each experimental pond and used for tissue and blood sampling (see below). The remainder were marked by dorsal fin cutting and stocked in one single pond for the duration of the growth period (trial I: 17.3–25.10). The survivors were measured and weighed, while the body condition index, the growth rate and survival rate were calculated for each experimental group of fish.

## Blood sampling

Blood was sampled from fish caught at random by a haul seine. Samples were collected in both experiments at the same time (from 09.00 to 13.00 h) using the following technique: fish were anaesthetized with Menocaine (Spofa, Prague, the Czech Republic) (3-aminobenzoic acid ethylester natrium hydrogen sulphate) at a concentration of 0.06 g $l^{-1}$ (Král 1988). Blood was collected by puncturing the caudal vessels. Sodium heparin (5,000 IU in a 1 ml injection) was used as anticoagulant. Plasma was obtained by centrifuging the blood at 4,100*g* for 10 min and then separated into plastic syringes. The biochemical tests were performed within 24 h of storage at 4°C; the blood plasma samples for cortisol and cortisone determination were frozen to –22°C and stored for further analysis. Blood sampling in trial I was performed after 17 days and in trial II after 37 days from the end of stress exposure. The photoperiod was 12 daylight:12 darkness (trial I) and 13L:10D (trial II).

The blood plasma was subjected to biochemical examination to determine total plasma protein (TPP, in g $l^{-1}$), albumin (AL, in g $l^{-1}$), albumin/globulin ratio (A/G), blood urea nitrogen (BUN, in mmol $l^{-1}$), urea (UA, in μmol $l^{-1}$), creatinine (CREA, in μmol $l^{-1}$), total bilirubin (T-BIL, in μmol $l^{-1}$), glucose (GL, in mmol $l^{-1}$), cholesterol (CHOL, in mmol $l^{-1}$), triacylglycerol (TGL, in mmol $l^{-1}$), total calcium (Ca, in mmol $l^{-1}$), inorganic phosphate (P, in mmol $l^{-1}$), sodium ($Na^+$, in mmol $l^{-1}$), potassium ($K^+$, in mmol $l^{-1}$), chloride ($Cl^-$, in mmol $l^{-1}$), alanine aminotransferase (ALT, μkat $l^{-1}$), aspartate aminotransferase (AST, in μkat $l^{-1}$), alkaline phosphatase (ALP, in μkat $l^{-1}$), lactate dehydrogenase (LD, in μkat $l^{-1}$), cortisol (F, in nmol $l^{-1}$) and cortisone (E, in nmol $l^{-1}$).

The values of ions $Na^+$, $K^+$ and $Cl^-$ were determined by the ISE, Nova 5 analyser (USA). F was measured by the direct in-the-laboratory developed radioimmunoassay (RIA), using coated tubes with the antiserum to cortisol-3-*O*(carboxymethyl)oxime and bridge- and position-homologous [$^{125}$I] radioiodinated derivative with tyosine methylester as a tracer (Bičíková et al. 1988); E was determined by another in-the-laboratory developed RIA, including the following steps: dichloromethane extraction of the serum, evaporation of the solvent, thin layer chromatography of the extract on TLC plates with a fluorescent indicator (Alufolien Kieselgel $F_{254}$, Merck, Darmstadt, FRG) in system dichloromethane–methanol 97:3 with authentic cortisone standard on each edge, sucking off the zones corresponding to cortisone standard visualised in ultraviolet light ($R_f$ = 0.50), their elution with ethanol, evaporation of the eluate and RIA as an endpoint. For the latter an unspecific antiserum against cortisol-21-hemisuccinate cross-reacting with cortisone by approximately 25% with cortisone, cortisone standard and [$^3$H]cortisone (Radiochemical Center, Amersham, UK) as a tracer were used. Bond from free radioactivity was separated by adsorption on dextran-coated charcoal (Pharmacia, Uppsala, Sweden and Norit. A, Serva, FRG). Radioactivity of tritium was measured after adding of scintillation coctail (OptiPhase Hi Safe 2, Wallac, Finland) on liquid scintillation spectrometer (Beckman LS 6800, USA). The HITACHI 717 analyser (Japan) was used for the determination of the remaining indicators.

## Assessment of condition indices

To evaluate condition, Fulton's condition factor [weight (g) × 100/$S_L^3$ (cm)] (FQ) was calculated and fat content in muscle [ML (%)] determined by the Soxlet method.

## Statistical analysis

Inter-group variability of monitored blood and body condition parameters were compared using one-way ANOVA followed by Tukey test or non-parametric Kruskal–Wallis test followed by post-hoc comparison (Zar 1999). Data were transformed whenever normality of input data was not met using logarithmic or square root transformation. Statistical analyses were conducted using Excel and STATISTICA software (StatSoft Inc. 1999).

The netting and handling of fish can be considered as acute stress for fish. The levels of corticosteroids in blood serum depend strongly on the time interval between exposure to stressor and blood sampling (Pottinger and Moran 1993; Pottinger 1998; Ruane et al. 2002). Non-parametric correlation (Sperman rank correlation) was used for comparison of levels of corticoids in individual fish and time interval between

exposure to potential stressor (netting and handling) and blood sampling.

## Results

During the experiments the water temperature in ponds fluctuated between 1.5°C and 4.9°C and pH between 6.9 and 7.2.

Clinical analysis of the blood plasma and tissue of the fish from trial I revealed changes in the metabolism of nitrogen (UA, CREA), carbohydrates (GL), minerals (Ca, P) and hormones (E) and in energy reserves (ML) in carp exposed to disturbance by the otter (Table 2). The comparison of blood samples of fish from different experimental groups showed that the values of UA, CREA, GL, Ca and P were significantly higher in EG2 than in CG1, while the same indices did not differ between EG3 and CG1. The levels of E increased in both experimental groups exposed to otter disturbance (EG2, EG3). The ratio F/E was in EG2 lower than in CG1, while EG3 did not differ again. The levels of muscle lipids were highest in EG2 and lowest in EG3. The values of other blood plasma indices (TPP, AL, BUN, CHOL, TGL, $Na^+$, $Cl^-$, ALT, AST, ALP, LD, A/G, AST/ALT, LD/AST, F and FQ) did not differ significantly amongst experimental groups (Table 2).

In the trial II changes in carp exposed to otter disturbance occurred mainly in mineral metabolism (Ca, P, $Na^+$, $K^+$, $Cl^-$) (Table 3). Changes were found also in UA, TGL, ratio AST/ALT and levels of E. The values of other indices (TPP, AL, BUN, CREA, GL, CHOL, ALT, AST, ALP, LD, A/G, LD/AST, F, FQ, ML) did not differ significantly amongst experimental groups (Table 3). The values of E increased only in case of EG2, and also F/E was lowest in the same experimental group of fish. As for lipid metabolism, a decrease occurred in TGL in both experimental groups. In contrast to findings from trial I, the levels of UA in trial II decrease in blood plasma of disturbed fish. In fish disturbed by the otters, the values of minerals (Ca, P, $Na^+$, $K^+$ and $Cl^-$) were reduced when compared with the control group. Differences were found mainly between groups CG1 and EG2.

No significant changes in levels of corticoids were observed when compared with the time interval between the exposure to acute stressor (netting and handling) and blood sampling (Sperman rank correlation; trial I: CG1/F: $n = 15$, $P > 0.05$; CG1/E: $n = 13$, $P > 0.05$; EG2/F: $n = 16$, $P > 0.05$; EG2/E: $n = 16$, $P > 0.05$; EG3/F: $n = 16$, $P > 0.05$; EG3/E: $n = 16$, $P > 0.05$; trial II: CG1/F: $n = 15$, $P > 0.05$; CG1/E: $n = 15$, $P > 0.05$; EG2/F: $n = 15$, $P > 0.05$; EG2/E: $n = 15$, $P > 0.05$; EG3/F: $n = 15$, $P > 0.05$; EG3/E: $n = 15$, $P > 0.05$).

From 160 fish stocked in the experimental ponds for trial I, 135 fish survived the winter. The mortality of carp in different experimental groups varied slightly, ranging from 6% to 11% of stocked fish ($\chi^2 = 5.4$, df = 2, NS). Superficial skin injuries, namely in the head, jaw and anal fin regions, were revealed by macroscopic inspection of fish bodies in 17% of individuals of EG3. In two individuals, where head lesions were particularly deep, these injuries were accompanied by changes in blood plasma (hyponatraemia and decreased concentration of chloride ions; high levels of F).

Fish mortality recorded in trial II was much higher than in trial I. Of 290 stocked carp, only 114 individuals survived the winter. While in CG1 the mortality was only 11% in the groups visited by otters, it was 79% (EG2) and 83% (EG3), the difference being significant ($\chi^2 = 123.6$, df = 2, $P < 0.001$). No skin injuries were recorded in any of the experimental groups of fish.

Comparison of growth rates (ANOVA: $F = 0.438$, df = 2.56, $P = 0.65$) and survival of fish ($\chi^2 = 3.13$, df = 2, NS) in trial I during the subsequent vegetation period did not show any differences amongst non-disturbed or low and high-disturbed fish. Due to low number of surviving fish from trial II after winter period stocking of fish for vegetation period and comparison of growth rates and survival was not possible.

## Discussion

Monitored physical and chemical properties of water (temperature, pH, oxygen levels) of all experimental ponds were within the range ensuring good over-wintering conditions for the experimental carp and confirmed by good body condition of the fish in the control groups after the winters.

Otter entering a pond repeatedly can be considered as a chronic stressor, and it can vary in intensity and

**Table 2** Final examination in trial I: haematological and condition indicators in carp after 133 days of over-wintering (17 March 2004)

| Indicators | Experimental groups | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CG1 | | | EG2 | | | EG3 | | |
| | *n* | Mean | SD | *n* | Mean | SD | *n* | Mean | SD |
| TPP (g $l^{-1}$) | 15 | 26.5 | 1.6 | 15 | 26.4 | 2.0 | 15 | 27.5 | 2.7 |
| AL (g $l^{-1}$) | 15 | 8.7 | 0.8 | 15 | 8.6 | 0.5 | 16 | 8.8 | 1.1 |
| BUN (mmol $l^{-1}$) | 15 | 0.4 | 0.2 | 15 | 0.5 | 0.1 | 16 | 0.4 | 0.2 |
| UA (μmol $l^{-1}$) | 15 | $3^{a}$ | 4 | 13 | $17^{a}$ | 13 | 16 | 19 | 24 |
| CREA (μmol $l^{-1}$) | 15 | $15.5^{a}$ | 1.8 | 15 | $19.5^{ab}$ | 2.4 | 16 | $17.0^{b}$ | 2.7 |
| GL (mmol $l^{-1}$) | 15 | $3.6^{a}$ | 1.0 | 15 | $5.6^{ab}$ | 1.1 | 14 | $3.5^{b}$ | 1.4 |
| CHOL (mmol $l^{-1}$) | 15 | 4.2 | 0.8 | 15 | 4.1 | 0.5 | 16 | 4.1 | 0.6 |
| TGL (mmol $l^{-1}$) | 15 | 1.4 | 0.4 | 14 | 1.4 | 0.2 | 16 | 1.3 | 0.3 |
| Ca (mmol $l^{-1}$) | 15 | $2.50^{a}$ | 0.14 | 15 | $2.64^{a}$ | 0.13 | 16 | 2.53 | 0.18 |
| P (mmol $l^{-1}$) | 15 | $1.46^{a}$ | 0.19 | 14 | $1.80^{ab}$ | 0.34 | 16 | $1.49^{b}$ | 0.36 |
| $Na^{+}$ (mmol $l^{-1}$) | 15 | 137 | 3 | 15 | 138 | 3 | 15 | 138 | 5 |
| $Cl^{-}$ (mmol $l^{-1}$) | 15 | 108 | 3 | 15 | 107 | 2 | 15 | 107 | 3 |
| ALT (μkat $l^{-1}$) | 14 | 0.11 | 0.04 | 15 | 0.13 | 0.05 | 16 | 0.18 | 0.11 |
| AST (μkat $l^{-1}$) | 15 | 1.63 | 0.57 | 15 | 1.77 | 0.45 | 16 | 1.58 | 0.45 |
| ALP (μkat $l^{-1}$) | 15 | 0.59 | 0.19 | 15 | 0.97 | 0.46 | 16 | 1.24 | 1.20 |
| LD (μkat $l^{-1}$) | 14 | 7.0 | 2.8 | 14 | 7.7 | 1.9 | 16 | 6.7 | 2.6 |
| A/G | 15 | 0.49 | 0.05 | 15 | 0.49 | 0.05 | 16 | 0.49 | 0.04 |
| AST/ALT | 15 | 14.9 | 5.6 | 15 | 14.3 | 4.6 | 16 | 11.3 | 5.5 |
| LD/AST | 15 | 4.8 | 1.4 | 15 | 4.9 | 1.5 | 16 | 4.3 | 1.5 |
| F (mmol $l^{-1}$) | 15 | 351 | 117 | 16 | 404 | 202 | 16 | 503 | 238 |
| E (mmol $l^{-1}$) | 13 | $20^{ab}$ | 7 | 16 | $54^{a}$ | 21 | 16 | $52^{b}$ | 17 |
| F/E | 15 | $17.9^{a}$ | 10.1 | 16 | $9.1^{a}$ | 6.3 | 16 | 10.9 | 7.2 |
| FQ | 15 | 3.15 | 0.17 | 15 | 3.18 | 0.20 | 16 | 3.09 | 0.22 |
| ML (%) | 15 | 1.9 | 0.9 | 15 | $2.7^{a}$ | 1.2 | 16 | $1.6^{a}$ | 0.7 |

SD, standard deviation; TPP, total plasma protein; AL, albumin; BUN, blood urea nitrogen; UA, urea; CREA, creatinine; GL, glucose; CHOL, cholesterol; TGL, triacylglycerol; Ca, total calcium; P, inorganic phosphate; $Na^{+}$, sodium; $Cl^{-}$, chlorine; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LD, lactate dehydrogenase; A/G, albumin/globulin ratio; AST/ALT, aspartate aminotransferase/alanine aminotransferase ratio; LD/AST, lactate dehydrogenase/aspartate aminotransferase ratio; F, cortisol; E, cortisone; F/E, cortisol/cortisone; FQ, Fulton's condition factor; ML, muscular lipid

Figures marked with the same letter are significantly different from each other ($P < 0.05$)

duration, with the result that fish often show a variety of responses. To understand the response to chronic stress, it is important to look closely at the stressor (Ruane 2002). In the natural environment, otters usually visit several ponds during 1 night (Kranz 1995; Poledník 2005), spending from a few minutes to several hours there, and they can revisit particular ponds more often one to two times per week. Thus, otter disturbance is basically a multiple acute stressor. In the case of the presented experiments the intensity of stressor was also not constant; in principle it was a series of short-term acute stress caused by otter presence and finished by possible acute stress caused by netting and handling.

The present data on stress indicator levels of experimental fish show a large difference in results obtained from experiments from different years. Experimental ponds, duration of experiments and behaviour of the otters are main differences between the two trials. However, some trends were obvious in both years. Analysis of the fish revealed changes in the metabolism of nitrogen (UA, CREA),

**Table 3** Final examination in trial II: haematological and condition indicators in carp after 140 days of over-wintering (11 April 2005)

| Indicators | Experimental groups | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CG1 | | | EG2 | | | EG3 | | |
| | *n* | Mean | SD | *n* | Mean | SD | *n* | Mean | SD |
| TPP (g $l^{-1}$) | 15 | 32.4 | 3.2 | 15 | 29.7 | 4.4 | 15 | 30.5 | 3.2 |
| AL (g $l^{-1}$) | 15 | 10.4 | 1.2 | 15 | 10.0 | 0.9 | 15 | 10.2 | 0.9 |
| BUN (mmol $l^{-1}$) | 15 | 1.5 | 0.5 | 15 | 1.1 | 0.4 | 15 | 1.5 | 0.6 |
| UA (μmol $l^{-1}$) | 14 | 59[a] | 19 | 12 | 40[a] | 16 | 15 | 47 | 21 |
| CREA (μmol $l^{-1}$) | 15 | 17.3 | 3.2 | 15 | 16.4 | 2.8 | 15 | 16.6 | 2.2 |
| GL (mmol $l^{-1}$) | 15 | 3.7 | 1.8 | 15 | 3.3 | 0.8 | 15 | 3.9 | 1.3 |
| CHOL (mmol $l^{-1}$) | 15 | 5.7 | 1.4 | 15 | 5.9 | 1.4 | 15 | 6.2 | 1.6 |
| TGL (mmol $l^{-1}$) | 14 | 2.9[ab] | 1.2 | 15 | 2.0[a] | 0.8 | 15 | 1.7[b] | 0.8 |
| Ca (mmol $l^{-1}$) | 15 | 2.63[a] | 0.12 | 15 | 2.40[a] | 0.12 | 15 | 2.51[a] | 0.13 |
| P (mmol $l^{-1}$) | 14 | 2.42[ab] | 0.25 | 15 | 1.74[a] | 0.44 | 15 | 2.01[b] | 0.41 |
| $K^+$ mmol $l^{-1}$ | 15 | 2.6[a] | 0.63 | 15 | 2.1 | 1.49 | 15 | 1.6[a] | 0.38 |
| $Na^+$ (mmol $l^{-1}$) | 14 | 144[a] | 3 | 15 | 130[a] | 20 | 15 | 139 | 5 |
| $Cl^-$ (mmol $l^{-1}$) | 14 | 113[a] | 3 | 15 | 101[ab] | 15 | 15 | 110[b] | 3 |
| ALT (μkat $l^{-1}$) | 13 | 0.62 | 0.34 | 15 | 0.74 | 0.51 | 15 | 0.46 | 0.28 |
| AST (μkat $l^{-1}$) | 15 | 2.08 | 0.62 | 15 | 1.93 | 0.77 | 15 | 2.30 | 1.22 |
| ALP (μkat $l^{-1}$) | 15 | 0.47 | 0.12 | 15 | 0.64 | 0.43 | 15 | 0.56 | 0.24 |
| LD (μkat $l^{-1}$) | 14 | 14.7 | 6.4 | 15 | 13.3 | 5.7 | 15 | 16.6 | 14.4 |
| A/G | 15 | 0.46 | 0.05 | 15 | 0.51 | 0.08 | 15 | 0.48 | 0.09 |
| AST/ALT | 15 | 3.7[a] | 2.2 | 15 | 3.3[b] | 1.7 | 15 | 6.8[ab] | 6.0 |
| LD/AST | 15 | 7.6 | 3.0 | 15 | 7.4 | 2.9 | 15 | 7.2 | 3.0 |
| F (mmol $l^{-1}$) | 15 | 315 | 97 | 15 | 416 | 267 | 15 | 373 | 119 |
| E (mmol $l^{-1}$) | 15 | 29[a] | 9 | 15 | 58[a] | 31 | 15 | 46 | 22 |
| F/E | 15 | 11.4[a] | 4.3 | 15 | 7.8[a] | 4.1 | 15 | 9.2 | 4.1 |
| FQ | 15 | 3.35 | 0.21 | 15 | 3.12 | 0.45 | 15 | 3.17 | 0.16 |
| ML (%) | 15 | 3.9 | 1.3 | 15 | 4.4 | 1.4 | 15 | 4.7 | 2.2 |

SD, standard deviation; TPP, total plasma protein; AL, albumin; BUN, blood urea nitrogen; UA, urea; CREA, creatinine; GL, glucose; CHOL, cholesterol; TGL, triacylglycerol; Ca, total calcium; P, inorganic phosphate; $K^+$, potassium; $Na^+$, sodium; $Cl^-$, chlorine; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LD, lactate dehydrogenase; A/G, albumin/globulin ratio; AST/ALT, aspartate aminotransferase/alanine aminotransferase ratio; LD/AST, lactate dehydrogenase/aspartate aminotransferase ratio; F, cortisol; E, cortisone; F/E, cortisol/cortisone; FQ, Fulton's condition factor; ML, muscular lipid

Figures marked with the same letter are significantly different from each other ($P < 0.05$)

carbohydrates (GL) and minerals (Ca, P, $Na^+$, $Cl^-$), in the activities of enzymes (ALT, ALP), levels of the hormones (E) and in energy reserves (ML) in fish disturbed by otter.

Primary response of fish was measured by levels of plasma F and E. The fish exposed to disturbance by otter showed only increased levels of E and correspondingly decrease of F/E ratios in blood plasma, although only in EG2 was it statistically significant when compared with the control groups of both years. The values of E were always higher in fish exposed to a lower intensity of disturbance than those exposed to high intensity. This result suggests that a higher frequency of exposure to disturbance by otter can lead to metabolic adaptability of the fish. It is suggested that the E in teleost fish arises from F (Patino et al. 1985, 1987; Balm 1986). But it is not clearly established whether it possess biological activity in fish or it is present only as an inactive metabolite of F (Potinger and Moran 1993). Despite

the role of E in fish organisms, raised levels of E in the EG2 group suggest that before blood sampling there were higher concentrations of F there. Oxidation of F to inactive E by 11$\beta$-hydroxysteroid dehydrogenase type 2, at least in higher vertebrates, is believed to be one of the counter-regulatory mechanisms protecting the organism from an excess of biologically active corticoids, occurring at (chronic) stress (Vierhapper et al. 1991). It may be the case of elevated levels E found in this experiment.

Measured levels of F did not differ between experimental groups. However, its concentrations in blood plasma of fish of all experimental groups reached values (114–182 ng $ml^{-1}$) about the level found in unstressed fish (10–50 ng $ml^{-1}$, Dabrowska et al. 1991; Roelants et al. 1993; Yin et al. 1995; Pottinger 1998; Ruane et al. 2001), but being much lower than those reported for acutely stressed carp (up to 300 ng $ml^{-1}$, Ruane et al. 2001). It was shown that the magnitude extent of the F response to stress in roach (*Rutlius rutilus* L.) was dependant on water temperature (Pottinger et al. 1999), being lower in low temperatures. Thus, the moderate increase of F levels in all experimental fish can be attributed to acute stress from netting during low water temperature. Other explanations of moderate increase of F levels lay in fasting and physiological changes of carp connected with over-wintering. The effect of fasting on F levels in otherwise unstressed fish is not clear, and various responses of fasting fish were recorded including reduction (e.g. Farbridge and Leatherland 1992), increase (Jørgensen et al. 2002) or no changes (e.g. Pottinger et al. 2003) in blood plasma F levels. Moreover, Ruane et al. (2002) showed differences in levels of F as the response to acute confinement stress in two groups of carp with different feeding history. F is considered as the principal corticosteroid secreted by the teleost fish adrenal system in response to acute stress, but some difficulties arise when trying to quantify chronic stress levels (Mommsen et al. 1999). Chronic stress dealing with continuous and periodic (weekly or annual) exposure to a stressor is a complex process, and the fish can respond physiologically in a number of ways, through cumulative responses, additive responses and/or subclinical responses (Moberg 2000).

The secondary responses are usually defined as the manyfold immediate actions and effects of stress. The changes in fat reserves in experimental groups of carp did not follow any explainable pattern. Greater decrease of ML in EG3 compared to EG2 recorded in the trial I may be related to the greater energy output due to the more frequent disturbance of the over-wintering fish by the otter. However, fat reserves in the control group also decreased even to the same level as fish in EG3. Moreover, in the trial II no changes in fat reserves of carp from all experimental groups were recorded. Increased glycaemia in carp of EG2 in trial I may be considered as a response to stress, and it is attributed to immediate effects of catecholamines and corticosteroids on glycogenolysis (Wendelaar Bonga 1997). It was often observed in exposure to acute stress caused by confinement (Pottinger 1998; Ruane et al. 2001) or pollutants (Srivastava 1981; Singh and Srivastava 1982; Natarajan 1989; Gill et al. 1990; Sancho et al. 1997; Lusková et al. 2002). However, these findings were observed neither in the other fish group (EG3) nor in trial II. On the other hand in trial II, the changes in the lipid spectrum, manifested in lower TGL values in disturbed fish, were recorded. This might be related to a higher energy output due to stress due to increased lipolysis of TGL to free fatty acids serving as energy source. Aerial exposure and net confinement of hatchery-reared turbot (*Scophthalmus maximum* L.) led to raised plasma-free fatty acid (FFA) levels instead of GL and lactate (Waring et al. 1996). Despite the year-to-year differences in changes of glycid and lipid metabolisms of disturbed carp, a common trend is obvious: observed changes lead to mobilisation of energy reserves in fish disturbed by otter compared to the control group. The increase in uricaemia in group EG2 of carp disturbed by the otter in trial I may be caused by insufficient UA excretion through the kidneys by stressed fish. However, just opposite findings in trial II (higher levels of UA in the control group) make such a conclusion unreliable and reasons for changes in UA levels in blood plasma of carp after over-wintering remain unclear. The increased levels of CREA appear to be connected with disorder in glomerular filtration of kidneys in stressed fish. However, the significantly higher CREA level was observed in fish of EG2, compared with fish of EG3. In trial II the levels of CREA did not differ amongst experimental groups of fish.

The changes in the metabolism of Ca and P draw attention to the possible disturbance in osteogenesis

and negative effect on the cardiovascular system and the maintenance of the irritability of the heart, muscles and nerves of fish. However,experiments I and II provided totally contradictory and confusing results, making any general conclusion about the potential effect of otter disturbance on metabolism of these two elements not possible.

The concentrations of plasma $Cl^-$, $K^+$ and $Na^+$ ions significantly decreased only in the EG2 group in trial II. A similar decrease of $Cl^-$ levels in blood plasma of carp was recorded following acute stress by confinement (Ruane et al. 2001). The net loss of ions in fresh-water fish is known to be due to an increased permeability of the gills to water (Wendelaar Bonga 1997). As pronounced hypochloraemia can be related to severity of the stressor (van Dijk et al. 1993), the results suggest a higher impact of stress on low-intensity disturbed fish.

The stronger response of the metabolism of nitrogen, carbohydrates and minerals to stress exposure in the fish with less frequent contacts with otter may suggests that a higher frequency of exposure to disturbance by otter leads to adaptability of the fish, resulting in lower impact of stress on metabolism and homeostasis of fish.

Tertiary responses of fish to stressor extend to the level of the organism and population: inhibition of growth, sexual maturation and reproduction (e.g. Pickering 1990; Goos and Consten 2002), and immune response (Wedemeyer 1997), reduced resistance of the fish to infections (Barton and Iwama 1991) and reduced capacity to tolerate subsequent or additional stressors. Changes in immunity and susceptibility to pathogens were monitored indirectly by growth and survival of fish during the exposition to a stressor and in the subsequent period.

Striking differences in survival of fish in the first and second trials were observed. In trial I, the mortality was low, but in trial II it dramatically increased in those fish groups exposed to otter disturbance. The main explanation lies in the different foraging behaviour of the otters used for the experiments. While in trial I, the female otter did not kill the fish she caught, she just pulled it onto the ice and left it; live fish could thus possibly return to the water. The male used in trial II regularly killed the caught fish. Thus, the mortality of fish due to the predation by the otter increased greatly in trial II. Unfortunately it was not possible to quantify correctly the number of fish predated by the otter, and therefore the mortality of carp caused by otter disturbance is not clear. However, the results from trial I, where actual otter predation was considered neglectable, suggest that the disturbance of overwintering fish by otters has no effect on their winter survival.

The comparison of survival, condition and growth rate of carp from different experimental groups after subsequent vegetation period did not reveal any differences between groups.

The wounds caused by otter pursuing the fish are an important economic aspect connected directly with the predation. Such damages of skin are sensitive to bacterial infection or acaulinosis. Moreover, deep lesions can induce disorders in osmoregulation of wounded individuals, thus significantly reducing their health conditions.

In conclusion, the results of both trials showed that repeated disturbance of fish by otters during wintering affects the basic metabolic functions of the fish and gives rise to hormonal response to stress (increased E levels) followed by changes in metabolism of other substances. Despite this, it appears to have no negative effect on survival, condition and grow rate in the vegetation period following the overwintering.

Beside the understanding of the basic facts about the relationship between predator and prey, another-reason for conducting the study is the economical aspect of the phenomenon. Conflicts, arising from otter predation on commercial fish, are nowadays a common phenomenon in many Central European countries (Bodner 1998; Kranz 2000; Myšiak et al. 2004; Kloskowski 2005). The damages on fish caused by otters in ponds and rivers were assessed in 2001 in the Czech Republic as being 3.8 million Euro (Czech Anglers' Association, unpublished data). Apart from damages caused by eating the fish, the secondary damages (namely in terms of mass death due to stress caused by otter) are often mentioned by fish farmers and anglers, and their range exceeds the consumption damages by a factor of 1.9 (Czech Anglers' Association, unpublished data). These economical indicators show that data collected in the present study has high economical importance (not only in the Czech Republic) and could help in conflict mitigation. Although both trials were conducted under slightly different conditions and show slightly different

physiological reaction of disturbed fish, they do not prove any extreme damages as mass death or mass loss of fish biomass. Indeed there isdamage caused by otter eating the fish, but no secondary damage has occurred. Since physiological response of carp to disturbance by otter is a complex reaction under natural conditions, other external factors (e.g. other predators) may interact to reinforce the negative impact on over-wintering fish; thus the existence of the secondary losses cannot be completely excluded.

Another kind of economical loss is decrease of economical value of fish due to the injuries caused by otters. The fish can be seriously injured by otters; however the experiments show good healing of such a wounds during the growth period. Considering that the main market season for carp is Christmas, this economic lossprobably has a low impact on total profit of selling carp.

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