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A Negative Correlation between Mercury Content in Muscle and Body Weight in Carp from Uncontaminated Ponds

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Abstract

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The relationship between body weight and total mercury content in the muscle of common carp (*Cyprinus carpio*) from 13 uncontaminated ponds in South and West Bohemia was investigated. In total, 215 three-years-old marketable carps and 26 samples of bottom sediment were analysed. Total mercury contents (THg) in muscle and sediments were determined using an single-purpose atomic absorption spectrophotometer. The correlation between body weight and mercury content in muscle was found to be negative and statistically significant (Pearson correlation: $r_p = -0.269$, $P < 0.001$). THg content in muscle significantly decreased as the body weight of carp from uncontaminated ponds increased. This is likely due to the intensive weight gain of marketable carp.

Keywords: *Cyprinus carpio*; total mercury; sediment

Mercury is a highly dangerous element with an accumulative and persistent character in the environment and in biota (Kothny 1973). Fish are known to be a suitable bioindicator of the seriousness of mercury pollution in freshwater ecosystems (Čelechovská *et al*. 2007; Maršálek *et al*. 2007; Kružíková *et al*. 2008; Kenšová *et al*. 2012). The mercury content increases with age and weight of fish (Cizdziel *et al*. 2002; Jewett *et al*. 2003). The level of accumulated mercury is evaluated in fish muscle tissue. Muscle is the target tissue for mercury accumulation in fish from lightly contaminated localities; on the other hand, the target organ is liver, when fish originate from heavily contaminated localities (Havelková *et al*. 2008). Muscle tissue is a part of the fish most frequently used for human consumption, since muscle tissue is the edible part of the fish (Pirrone & Mahaffey 2005). The maximum allowable levels for heavy metals in foodstuffs are laid down by Commission Regulation (EC) No. 1881/2006, amended by EC 594/2012. Discrete limits for total mercury (THg) concentrations are 1 mg/kg for carnivorous fish and 0.5 mg/kg for non-carnivorous fish.

According to Celo *et al*. (2006), inorganic bivalent mercury is converted to methylmercury by anaerobic sulphate-reducing bacteria in the bottom sediment. The methylation of inorganic mercury in sediments contributes significantly to the total content of methylmercury in the aquatic environment (Eisler 2006). The accumulation of mercury in fish depends on several factors. It depends especially on the amount of available inorganic mercury in the sediment/water column and on trophic interaction. It is also important which microorganism is involved in the conversion of inorganic mercury into its organic form, methylmercury, which is the most toxic form

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of mercury (Jackson 1990; WHO 1990). Methylmercury is bioaccumulated and biomagnified in the food chain (Celo *et al*. 2006).

Mercury content in bottom sediments depends on the degree of environmental load of the locality and on the type of sediment. Sediment samples with a predominance of silt and organic components contain higher mercury levels in most cases compared to samples of a sandy nature. Other important factors of methylation include the existence of anoxic conditions, the presence of sulphide and sulphate, the presence of nutrients, water pH (Beckvar *et al.* 1996) and temperature (Ullrich *et al.* 2001; Huguet *et al.* 2010).

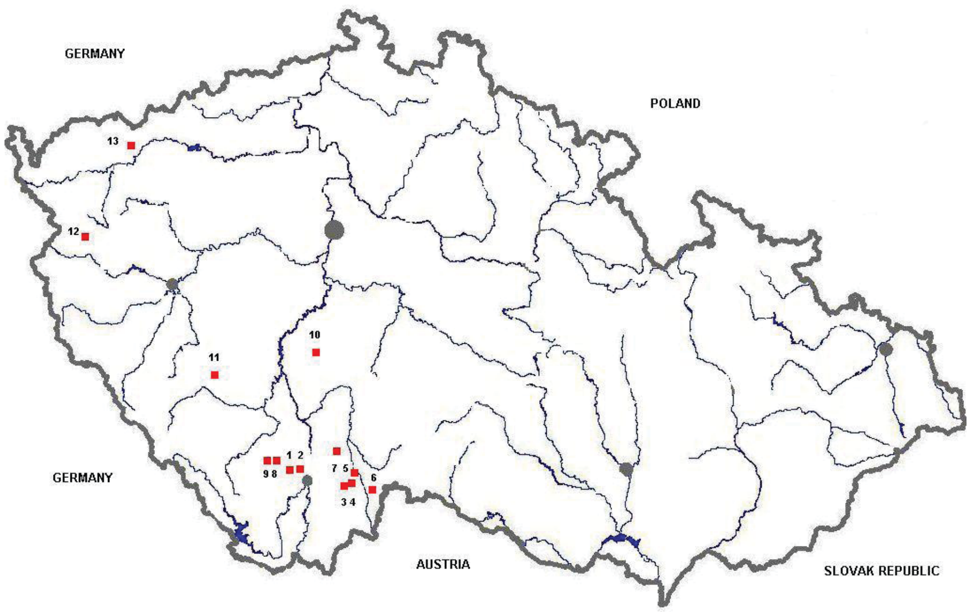
Many studies have addressed fish ponds in the Czech Republic (Svobodová *et al.* 1975, 1999; Maršálek *et al*. 2007). Monitoring of the mercury contamination of fish in ponds in the Czech Republic is of considerable importance, because ponds are the main source of Czech freshwater fish production, especially of common carp. Carp plays an economically important and traditional role in regional food culture. In the Czech Republic, it is its massive consumption during Christmas. The aim of this study was to evaluate the relationship between mercury content in muscle and total body weight in carp from 13 uncontaminated ponds in South and West Bohemia.

MATERIAL AND METHODS

Common carp. A total of 215 common carps (*Cyprinus carpio*) were caught during August and September each year from 2001 to 2003. The collected fish were obtained from a total of 13 fish ponds in South and West Bohemia (Figure 1).

In all monitored ponds, semi-intensive pond farming conditions were applied, i.e. combination of natural food and supplemental feeding with cereals (triticale, wheat, barley) (Hůda 2009). Feeding of cereals in ponds is responsible for 25–50% of biomass gain, while the remaining production arises from the natural food intake (Szumiec 1999; Adámek *et al*. 2008). For carp's fry, cereals were adjusted to coarser pieces by grinding, in two- and three-years-old carp the whole grains of cereals were used, both unprocessed and mechanically adjusted (e.g. by pressing). This technological procedure was identical in all monitored ponds. The ponds and their sampling locations are shown in Figure 1. The fish were caught with a catching net. The fish were then weighed, scale samples were taken to determine their age, and muscle tissue was collected for analysis. The muscle samples were collected from the cranial area dorsal to the lateral line. They were placed in polyethylene bags, labelled, and stored at –18°C until the analyses were carried out. Characteristic data from the observed ponds, such as number of sampled carps and carp weight are shown in Table 1. In this work we considered only three-years-old carp. The parameters of weight and age are comparable.

Sediments. At all the sites, sediment samples (comprising a total of 26 samples) were taken and analysed for total mercury content both in 2001 and 2003. Samples were taken from the surface layer of the sediment to a depth of 3 to 10 cm (depending on the thickness of the deposited sediment) from at least



1 – Dehtář (246 ha); 2 – Bezdrev (450 ha); 3 – Svět (201.5 ha); 4 – Spolský (137 ha); 5 – Rožmberk (489 ha); 6 – Starý Kanclíř (33 ha); 7 – Horusický (416 ha); 8. – Dřemliny (75 ha); 9 – Čežárka (5.5 ha); 10 – Tovaryš (15 ha); 11 – Buzický (55.8 ha); 12 – Regent (52 ha); 13 – Nejda (9.6 ha)

Figure 1. Sampling ponds

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Table 1. Main characteristics of carp and total mercury contents in the muscle tissue of common carp from 13 ponds (overall significance of ANOVA for both variables $P < 0.001$)

Ponds	*n*	Mean weight (95% CI) (g)	Mean Hg content (95% CI) (mg/kg)
Dehtář	15	1387 (1184–1591)[abd]	0.104 (0.077–0.132)[e]
Bezdrev	20	1858 (1392–2324)[a]	0.030 (0.023–0.037)[b]
Svět	15	1046 (693–1398)[bcd]	0.037 (0.031–0.042)[bdf]
Spolský	16	1377 (1170–1583)[abd]	0.039 (0.031–0.047)[bdf]
Rožmberk	18	1383 (1180–1586)[ab]	0.032 (0.025–0.040)[b]
Starý Kanclíř	15	1435 (1256–1615)[ab]	0.032 (0.027–0.038)[bd]
Horusický	15	1744 (1328–2161)[ac]	0.020 (0.017–0.023)[b]
Dřemliny	19	1295 (1079–1511)[abd]	0.022 (0.019–0.025)[b]
Čežárka	13	665 (496–833)[de]	0.060 (0.053–0.066)[cd]
Tovaryš	20	1209 (1025–1394)[bcd]	0.026 (0.021–0.031)[b]
Buzický	20	1208 (836–1579)[bcd]	0.026 (0.022–0.030)[b]
Regent	15	983 (705–1262)[bdf]	0.071 (0.055–0.087)[c]
Nejda	14	940 (850–1030)[be]	0.061 (0.048–0.074)[cf]

Homogeneous groups of samples are defined on the basis of Tukey's HSD post hoc test and denoted by letters a–f; *n* – number of sampled fish; CI – confidence interval

5 sampling sites within one locality. After sampling from several sites, the sediment was mixed, homogenised, and stored in plastic, glass or stainless steel sample containers. Then, at a temperature of 4°C, the sample containers were transported to a workplace, where they were immediately frozen. In their frozen state, the samples were transferred to laboratories.

Determination of total mercury (THg). The contents of THg in the muscle of common carp and in bottom sediment were determined using an AMA 254 (Altec Ltd., Prague, Czech Republic) analyser. This method does not require any sample preparation. The limit of THg detection was 1 μg/kg. The limit of detection was set as the sum of the triple standard deviation of a blank and the blank mean value. The accuracy of THg values was validated using the BCR-CRM 464 standard reference material (Tuna Fish, IRMM, Belgium).

The wavelength employed was 253.65 nm, and reproducibility was below 1.5%. The temperature program parameters for sediment were set to the values 10/150/45, meaning 10 s for drying, 150 s for decomposition, and 45 s waiting time. The temperature program parameters for muscle were 60/150/45. For each sample, two independent measurements were performed. The AMA 254 computed the mean and the standard deviation. If the standard deviation was higher than 10%, the measurement was repeated.

Determination of organic matter in sediment. In a dried and weighed Petri dish, about 5 g of sediment was weighed to the nearest 10 mg. The open Petri dish containing the sediment sample was placed into an oven at 105°C and dried, alongside the lid, to a uniform weight (heating time, about 3 h). After drying, the closed Petri dishes were placed in a desiccator. After cooling (for 45 min at least), the Petri dishes were weighed to the nearest 10 mg. This was followed by the combustion of organic matter in the dry matter. Dry matter in Petri dish was combusted in a furnace where the temperature was gradually growing up to 550°C. At this temperature, the Petri dish was kept for 2 hours. This temperature causes the combustion of organic substances. The difference between dry matter weights before and after combustion expresses an amount of organic matter.

Statistical analysis. Standard parametric descriptive statistics were applied in the analysis – the distribution of the data allowed the provision of a mean supplemented by a 95% confidence interval. Analysis of variance (ANOVA) was followed by Tukey's HSD test for the identification of homogeneous groups of ponds and was used for analysis of the statistical significance of differences in weight and Hg content between the ponds. The expected relationship between the total weight of each individual fish and the content of Hg in its muscle was tested using the Pearson and Spearman correlation coefficients both for the entirety of the data and individually for each site, yielding very close results that excluded a statistically significant dependence between body weight and the Hg content in muscles. $P < 0.05$ was used as the level of statistical significance in all analyses. The analysis

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was conducted in R studio version 0.96.331 of the R statistical programming language version 2.15.1.

RESULTS

In 2001 and 2003, sediment samples were obtained from 13 fish ponds in South and West Bohemia. The mercury content of the sediment ranged from 0.5 to 2 mg/kg of organic matter. The highest total mercury contents were measured in sediments from the Čežárka pond in 2001 and the Tovaryš pond in 2003 (Figure 2).

Fish age and fish weight was comparable. Carp from the Bezdrev pond (1858 g) had the highest mean weight. The lowest mean weight was measured in the Čežárka pond (665 g).

Total mercury content was measured in the muscle of common carp. Carp is an omnivorous fish that prefers benthic foods. The contents of THg in the muscle of marketable common carp from 13 fish ponds are shown in Table 1. Total mercury values ranged from 0.017 mg/kg to 0.137 mg/kg. The highest content of total mercury was determined in carp from the Dehtář pond (mean 0.104 mg/kg). The lowest was in carp from the Horusický pond (mean 0.020 mg/kg). Fish from the Horusický pond had the greatest weight and the lowest content of total mercury. Fish from the Čažárka, Regent, and Nejda ponds had the lowest weight and the highest total mercury content. All measured values of total mercury in the muscles of carp were below the hygienic limit.

The correlations between total fish weight and total muscle mercury content from 13 fish ponds are shown in Table 2. In all ponds the correlations were negative, with the correlations statistically significant in the Dehtář (r_p=–0.603, P = 0.017), Tovaryš (r_p = –0.557, P = 0.011) and Regent ponds (r_p = –0.523, P = 0.045).

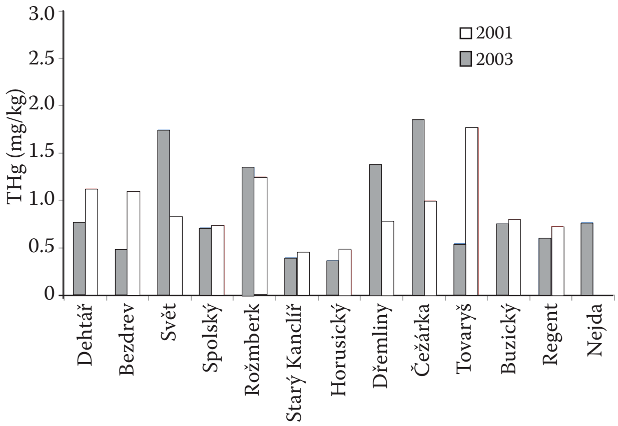

Figure 2. Total mercury contents (mg/kg of organic matter) in sediments from 13 fish ponds

THg content in muscle significantly decreased as the body weight of carp increased (Figure 3). The Pearson and Spearman correlation coefficients were computed. The total Pearson correlation was r_p = –0.269, P < 0.001; the total Spearman correlation was r_s = –0.382, P < 0.001. A negative correlation was confirmed.

DISCUSSION

In 2001, the lowest concentration of total mercury was found in sediment recovered from the Horusický

Table 2. Correlations between the total fish weight of each pond group and the content of total mercury in muscle

Ponds	Pearson correlation r_p	P	Spearman correlation r_s	P
Dehtář	–0.603	0.017	–0.660	0.007
Bezdrev	–0.357	0.122	–0.574	0.008
Svět	0.240	0.390	0.099	0.727
Spolský	–0.247	0.357	–0.237	0.378
Rožmberk	–0.161	0.522	–0.347	0.159
Starý Kanclíř	–0.142	0.614	–0.256	0.357
Horusický	–0.449	0.094	–0.523	0.045
Dřemliny	–0.007	0.978	–0.110	0.654
Čežárka	–0.316	0.292	–0.330	0.271
Tovaryš	–0.557	0.011	–0.588	0.006
Buzický	–0.386	0.092	–0.492	0.028
Regent	–0.523	0.045	–0.509	0.052
Nejda	–0.400	0.157	–0.446	0.112

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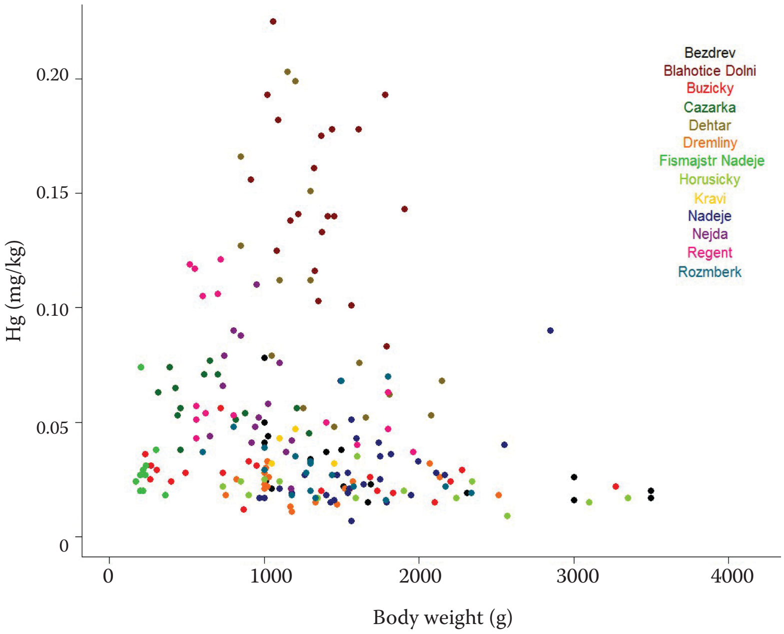

Figure 3. Correlation plot of body weight (m) and THg content in muscle

Pearson correlation: $r_p = -0.269, P < 0.001$
Spearman correlation: $r_s = -0.382, P < 0.001$

pond, and later from the Starý Kanclíř and Horusický ponds in 2003. All the analysed sediment samples were classified as uncontaminated by mercury. Rozanski (2009) stated that it is an uncontaminated pond if the total mercury content does not exceed 2 mg/kg.

Maršálek *et al.* (2007) reported results of mercury content monitoring in the carp (*Common carp*) muscle from four ponds of the Czech Republic (Rožmberk, Spolský, Nezmar, and Velký Bědný). The mercury content in muscle was very low (0.019–0.063 mg/kg). These ponds are uncontaminated by mercury, as well as those in our work.

It is obvious from the results that total mercury content in the body of carp depends on its weight. Carp with a high weight have low total mercury contents. Hejtmánek and Svobodová (1978) provided similar results. They carried out an experiment in which fish were fed mercury-contaminated feed for a certain time. They observed a gradual relative decrease in total mercury content in the organs, but did not detect a decrease in the absolute amount of total mercury in the hepatopancreas or in the kidneys. The decrease in the relative quantity of total mercury was likely due to an increase in the weight of these organs in the period of feeding with feed uncontaminated by mercury. It follows that the fixed levels of mercury in the hepatopancreas and in kidneys were not excreted under the given experimental conditions. The negative correlation between total mercury content and fish weight found in our work is not consistent with the work of various authors who have shown a positive correlation between the concentration of total mercury and age (Cizdziel *et al.* 2002; Jewett *et al.* 2003) and between the concentration of total mercury and body weight (Farkas *et al.* 2003; Kenšová *et al.* 2012) in fish. Mercury bioaccumulation in predators is the highest one due to their position in the food chain (Yingcharoen & Bodaly 1993). Kružíková *et al.* (2011) also reported in their work that high values of Hg in older fish of higher weight, especially predatory species, are not surprising.

CONCLUSIONS

It follows from the results that total mercury content in the muscle of marketable carp from uncontaminated ponds decreases with increasing body weight. This is likely due to the intensive weight gain.

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