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# The correlation between fish mercury liver/muscle ratio and high and low levels of mercury contamination in Czech localities

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The aim of our study was to divide selective Czech locations into sites that were either heavily or lightly contaminated with mercury on the basis of results of the total mercury liver/muscle ratio in fish. A total of 660 fish of 6 predatory and 8 non-predatory species were collected from 1974 to 2011 at 19 locations of surface water in the Czech Republic. Muscle and liver of fish were analyzed for total mercury content and then the total mercury liver/muscle ratio was calculated. Firstly, indicator chub was studied at fourteen sites and the total mercury liver/muscle ratio was not found to exceed a value of 1. The ratio (about 0.8) from the Odra-Bohumín site was significantly higher in comparison with other sites. Secondly, significantly higher ratios were found for both predators and non-predators from the Skalka Reservoir (>1)in comparison with other studied sites (Lužnice, Otava, Jordán, Berounka; <0.5). The obtained results show that the Skalka Reservoir is still heavily contaminated by mercury. Finally, total mercury liver/muscle ratios of five predator fish were evaluated for the man-made Želivka Reservoir from 1974 to 2011. Mercury contamination was confirmed in the period after impoundment (the total mercury liver/muscle ratio was higher than 1) and also twenty-five years after flooding. The obtained results confirm that the total mercury liver/muscle ratio can be used for the assessment of mercury contamination, a ratio higher than 1 indicating that a location is heavily contaminated with mercury.

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**Keywords:** Czech rivers, Skalka Reservoir, Želivka Reservoir, chub

## 1. INTRODUCTION

It is well know that fish, as an important constituent of the human diet, can represent a dangerous source of certain heavy metals, especially mercury, and can be used as an indicator of environmental contamination. It is historically confirmed that consumption of fish with elevated mercury levels causes severe human intoxication (e.g. Minamata disease). For this reason, it is necessary to monitor and evaluate mercury contamination of the aquatic ecosystem. The concentration of mercury in organisms has been studied extensively and it is known that mercury levels depend on several factors, namely trophic concentration, the size/age of fish, biochemical reactions inside organisms, binding, the release and distribution of mercury in the environment, and some specific environmental factors such as temperature, pH, alkalinity, dissolved organic carbon concentrations, and turbidity [1].

Both inorganic and organic mercury may be taken up by aquatic organisms directly from water or through their diet. Studies have shown that repeated or continuous exposure to any form of mercury can result in the accumulation of mercury in the body. The mercury amount in the organism is affected by its position in the food chain [2-4], its size, age [3, 6, 7] and duration of exposure [8]. There is also an association between Hg concentrations and fis weight [9, 10].

The digestive tract is the main pathway for inorganic Hg intake into fish and skin and gills are other possible organs. Mercury is transported within the organism bound to blood plasma proteins with thes trong binding with –SHgroup.The target organs for mercury are muscle, liver and kidney. In fact, a higher accumulation of mercury in the liver may be considered the primary signal of exposure to metals [10]. Havelkova et al. [11]show on thirteen sites along the Elbe River that mercury accumulation and its distribution is dependent on the degree of mercury contamination - on heavily contaminated site is target organ liver, on the other hand on the lightly contaminated site it is muscle. This is calculated using total mercury liver to muscle ratio (index). Ratio higher than 1 means very contaminated location, when the ratio is lower than 1 it is contamination immaterial. This is confirmed by some authors [12, 13-16] but introvert by others [17, 8, 18].Nevertheless, there is still insufficient understanding of the mercury distribution in fish from heavily and lightly contaminated sites. Therefore, the main aim of this study was to collected and analyzed data of mercury content in the liver and muscle of both predator and non-predator fish from twenty sites and found out if the THg liver/muscle ratio can be used for the assessment of mercury pollution. The other aim is to divide selective Czech locations into sites that were either heavily or lightly contaminated with mercury on the basis of results of the total mercury (THg) liver/muscle ratio in fish.

The work was divided into three component studies. The first study entitled *Indicator chub study* was focused on comparing the THg liver/muscle ratio of chub from 15 locations. The second study, *Czech rivers and reservoirs study (2003 and 2011),* was focused on 5 Czech locations, where predators and non-predators were caught and their THg liver/muscle ratios were compared among the locations. Finally, the *ŽelivkaReservoir study* was focused on changes in the THg liver/muscle ratio in predator fish from the ŽelivkaReservoir over a 37-year period.

## 2. EXPERIMENTAL PART

*2.1. Indicator chub study*

Firstly, a total of 139 indicator fishes, the non-predator chub (*Leuciscuscephalus* L.), were collected using electrofishing in the spring of 2011 at 14 locations on Czech rivers. The rivers and their sampling locations with the frequency of sampled chub are shown in Table 1.

**Table 1.**Numberof sampled chub (*Leuciscuscephalus* L.) from Czech rivers and their location in 2011

| River – location | Number of sampled chub |
|---|---|
| Berounka – Srbsko | 12 |
| Dyje – Pohansko | 12 |
| Labe – Děčín | 10 |
| Labe – Obříství | 9 |
| Lužnice – Soběslav | 5 |
| Lužnice – Bechyně | 12 |
| Morava – Lanžhot | 9 |
| Odra – Bohumín | 9 |
| Ohře – Louny | 8 |
| Otava – Strakonice | 5 |
| Otava – Topělec | 12 |
| Sázava – Nespeky | 12 |
| Svratka – Židlochovice | 12 |
| Vltava – Vraňany | 12 |
| **TOTAL** | **139** |

*2.2 Czech rivers and reservoirs study (2003 and 2011)*

**Table 2.** Structure and number of sampled fishes from *Czech rivers and reservoirs (2003 and 2011)*

| Fish species | Latin | Number of sampled fishes | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Skalka 2003 | Skalka 2011 | Lužnice Soběslav | Otava Strakonice | Jordán | Berounka Černošice | TOTAL |
| **PREDATORS** | | 4 | 20 | 9 | 9 | 8 | 9 | 59 |
| asp | *Aspius aspius* | 3 | 5 | 4 | 0 | 4 | 4 | 20 |
| barbel | *Barbus barbus* | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| perch | *Perca fluviatilis* | 0 | 5 | 0 | 5 | 4 | 0 | 14 |
| pike | *Esox lucius* | 0 | 5 | 5 | 4 | 0 | 5 | 19 |
| pikeperch | *Stizostedion lucioperca* | 0 | 5 | 0 | 0 | 0 | 0 | 5 |
| **NON-PREDATORS** | | 13 | 27 | 18 | 19 | 15 | 14 | 106 |
| rudd | *Scardinius erythrophthalmus* | 0 | 4 | 0 | 0 | 0 | 0 | 4 |
| big head | *Aristhichthys nobilis* | 5 | 0 | 0 | 0 | 0 | 0 | 5 |
| bream | *Abramis brama* | 5 | 5 | 5 | 4 | 5 | 5 | 29 |
| carp | *Cyprinus carpio* | 0 | 4 | 3 | 0 | 5 | 4 | 16 |
| roach | *Rutilus rutilus* | 3 | 5 | 5 | 5 | 5 | 5 | 28 |
| tench | *Tinca tinca* | 0 | 0 | 0 | 5 | 0 | 0 | 5 |
| chub | *Leuciscus cephalus* | 0 | 4 | 5 | 5 | 0 | 0 | 14 |
| silver bream | *Abramis bjoerkna* | 0 | 5 | 0 | 0 | 0 | 0 | 5 |
| **TOTAL** | | **17** | **47** | **27** | **28** | **23** | **23** | **165** |

Secondly, a total of 165 fishes belonging to 13 fish species (5 predators and 8 non-predators) were collected at 5 different locations during 2011 and also from the Skalka Reservoir in 2003. In this study, fishes from the following localities were sampled: the Berounka, Lužnice, and Otava rivers, and the Skalka and Jordán Reservoirs. Fishes were sampled using electrofishing mainly in the spring. Fish species described by their absolute frequencies are shown in Table 2.

### *2.3 Želivka Reservoir study (1974–2011)*

A total of 356 fishes belonging to 5 predatory species were sampled from the Želivka Reservoir between 1974 and 2011. Želivka is a man-made reservoir situated in the central part of the Czech Republic. The frequencies of collected fishes are shown in Table 3. The fishes were caught using electrofishing in the spring, by beach seining nets during the night in summer, and by gill nets from deeper parts of the reservoir in autumn.

**Table 3.**Structure and number of sampled fishes over time from the *Želivka Reservoir*

| Fishspecies | Latin | Number of sampled fishes | | | | | |
|---|---|---|---|---|---|---|---|
| | | Until 1985 | 1986-1990 | 1991-1995 | 1996-2000 | After2000 | Total |
| **PREDATORS**[1] | | | | | | | |
| asp | *Aspius aspius* | 7 | 2 | 22 | 16 | 24 | 71 |
| eel | *Anguilla anguilla* | 0 | 0 | 0 | 0 | 2 | 2 |
| perch | *Perca fluviatilis* | 27 | 31 | 66 | 29 | 28 | 181 |
| pike | *Esox lucius* | 26 | 6 | 0 | 2 | 7 | 41 |
| pikeperch | *Stizostedion lucioperca* | 9 | 4 | 34 | 8 | 6 | 61 |
| **Total** | | **69** | **43** | **122** | **55** | **67** | **356** |

### *2.4 Fish sampling*

The captured fishes from all studied locations were weighed and scales were sampled for age determination. Fish muscle samples (from the cranial area dorsal to the lateral line) and liver samples were removed, placed in polyethylene bags, labeled and stored at -18 °C.

### *2.5 Mercury analysis and calculation of total mercury (THg) liver/muscle ratio*

Total mercury content in the muscle and liver of fish was determined by cold vapor atomic absorption spectrometry using an AMA 254 (Altec Ltd., Czech Republic) analyzer. This method does not require any sample preparation. The limit of detection of THg was 1 μg/kg. The limit of detection was set as the sum of the triple standard deviation of a blank and a blank mean value. The accuracy of THg values was validated using standard reference material BCR-CRM 464 (Tuna Fish, IRMM, Belgium). The total mercury concentration in fish muscle and liver was given in mg/kg fresh weight (FW) and then the THg liver/muscle ratio was calculated. The THg liver/muscle ratio is the ratio of liver to muscle Hg concentrations and is calculated as follows: [THg in liver (mg/kg)/THg in muscle (mg/kg)].THg liver/muscle ratio was calculated from the geometric mean.

### *2.6 Statistical analysis*

Standard descriptive statistics were applied in the analysis; categorical variables were described using absolute frequencies of categories; mercury concentrations and their ratio were described using the geometric mean and its 95% confidence interval due to their lognormal distribution. The relationship between continuous variables was assessed using the Spearman rank correlation. Differences in mercury concentrations and their ratios among reservoirs were analyzed with analysis of variance (ANOVA) followed by the Tukey post hoc test or independent t-test when comparing two sites. A significance ($\alpha$) of 0.05 was used as the level of statistical significance in all analyses. Analyses were performed using SPSS 19.0.1 (IBM Corporation, 2010).

## 3. RESULTS

### *3.1. Indicator chub study*

A total of 139 chub were analyzed for correlation of 1) the THg content in liver and muscle and 2) the liver/muscle ratio with age and weight. Positive correlations ($p<0.001$) for THg muscle and THg liver with age and weight were confirmed and are shown in Table 4. For separately localities, no significant correlation was found out between THg liver/muscle ratio and age and weight (p=0.013-0.85 and p=0.019-0.966, respectively) and it is valid also for all data from the *Indicator chub study* (p=0.133 and p=0.069, respectively); therefore, no conversion to age or weight was necessary for our evaluation.

**Table 4.** Correlation between fish characteristics and THg content from the *Indicator chub study*

| | Chub(N=143)[1] | |
|---|---|---|
| | Age | Weight |
| THg muscle | 0.465 (<0.001) | 0.481 (<0.001) |
| THg liver | 0.333 (<0.001) | 0.328 (<0.001) |
| THgliver/muscle | -0.126 (0.133) | -0.153 (0.069) |

[1]*Spearman correlation and its statistical significance*

The values of the THg liver/muscle ratio for indicator chub and statistical differences ($p<0.001$) among studied locations are shown in Fig. 1. Although the geometric mean of the THg liver/muscle ratios in all the studied localities did not exceed 1, significant differences were observed among the studied locations.

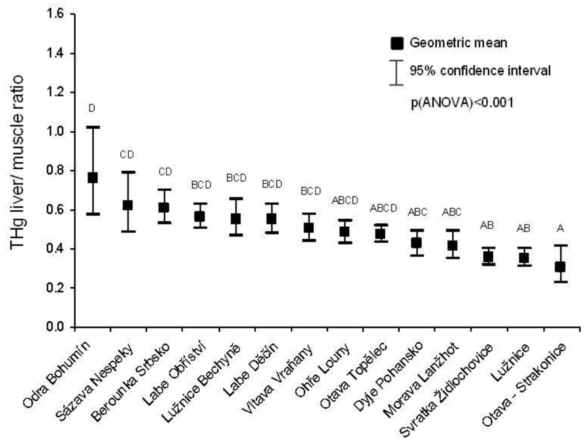


**Figure 1.**THg liver/muscle ratio for indicator chub. *A, B, C, D – homogeneous groups of localities computed Using Tukey post hoc test; the same letters denote groups without statistically significant differences*

*3.2 Czech rivers and reservoirs study (2003 and 2011)*

This study compared both predator and non-predator fish. A dataset consisting of 165 fish was investigated for correlations among THg muscle, THg liver, liver/muscle ratio and the main characteristics of fish (age and weight). Similarly to the *Indicator chub study,* no correlation (Table 5) for ether predators or non-predators was found between THg liver/muscle ratio and age (or weight); thus, no conversion to age or weight was needed. This correlation was not approved in the separately locations.

The geometric mean of mercury content in the muscle and liver of sampled fishes from the each locality is showed in the Fig. 2. The highest accumulation of mercury is express in the Skalka Reservoir both in 2003 and 2011.

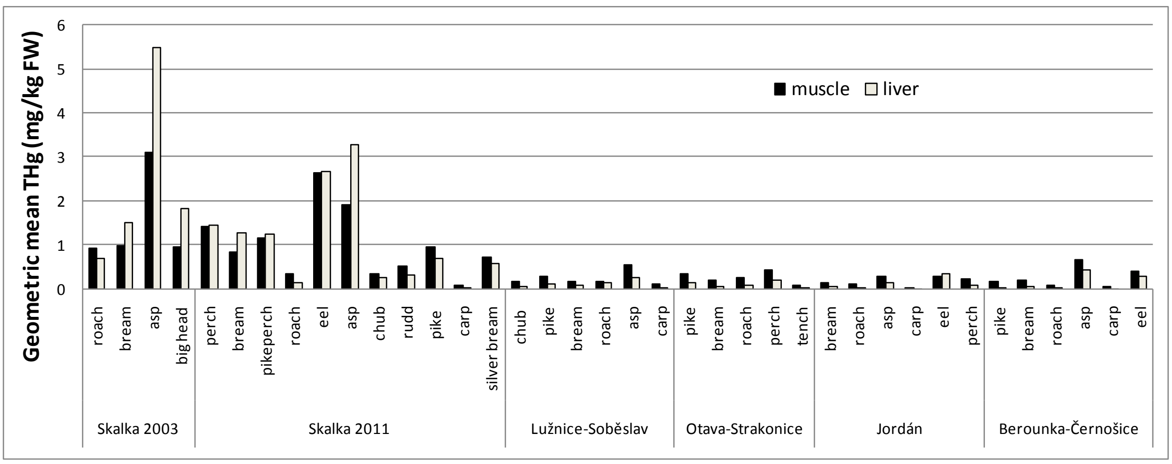


**Figure 2.** Geometric mean of THg in the muscle and liver of sampled fishes from the analyzed locations

**Table 5.** Correlation between fish characteristics and THg content from the *Czech rivers and reservoirs study*

| | Predators (N=59)[1] | | Non-predators (N=106)[1] | |
|---|---|---|---|---|
| | Age | Weight | Age | Weight |
| **THg muscle** | 0.337 (p=0.005) | 0.401 (p=0.001) | 0.500 (p<0.001) | 0.242 (p<0.001) |
| **THg liver** | 0.357 (p=0.003) | 0.344 (p=0.004) | 0.428 (p<0.001) | 0.136 (p=0.033) |
| **THg liver/muscle** | 0.105 (p=0.392) | 0.036 (p=0.772) | 0.132 (p=0.038) | -0.079 (p=0.215) |

[1] *Spearman correlation and its statistical significance*

The THg liver/muscle ratio according to locality and the food preference of fishes as well as their statistical differences (p<0.001) among locations are shown in Fig. 3. The statistically highest THg liver/muscle ratio was found in the Skalka Reservoir in 2003 followed by the Skalka Reservoir in 2011.

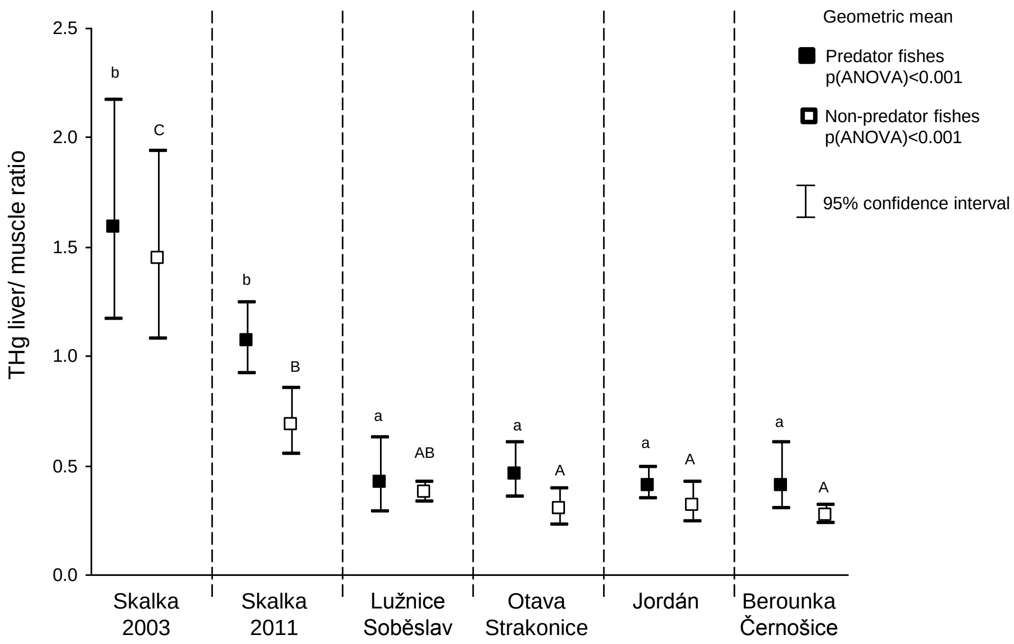


**Figure 3.** THg liver/muscle ratio for fishes from the Czech rivers and reservoirs; *a, b– homogeneous group of predator fish computed using Tukey post hoc test; the same letters denote groups without statistically significantdifferences;A, B, C–homogeneous groups of non-predator fish computed using Tukey post hoc test; the same letters denote groups without statistically significant differences*

By contrast, significantly lower THg liver/muscle ratios were detected in the Berounka-Černošice, Jordán Reservoir, Otava-Strakonice and Lužnice-Soběslav locations for both predators and non-predators in comparison with Skalka (2003 and 2011), except for non-predators in Lužnice-Soběslav (Fig. 3). Even the THg liver/muscle ratio for non-predators in Skalka 2011 was higher in comparison with Lužnice-Soběslav the difference is not statistically significant which can be caused by both variability and amount of samples. THg liver/muscle ratios in all locations with the exception of the Skalka Reservoir were about 0.4, which corresponds with lightly contaminated locations.

*3.3 Želivka Reservoir study*

In the *ŽelivkaReservoir study*, the assessment of changes in the THg liver/muscle ratio over time was undertaken (Fig 4). The highest THg liver/muscle ratio was found in the first period after impoundment (up to 1985) and 20–26 years after flooding. Total mercury content in the muscle and liver of fishes from the Želivka Reservoir has been published [19].

Similarly to the study mentioned above, the acquired data for predators were correlated with age and weight. Statistical significance ($p<0.001$) was found for both THg muscle and liver in predators when correlated with age and weight, whereas no correlation was found in the case of the THg liver/muscle ratio in relation to age and weight. For this reason, no conversion to age or weight was needed. The results from all Spearman correlations and their statistical significance are shown in Table 6.

**Table 6.** Correlation between fish characteristics and THg content from the *Želivka Reservoir study*

| | Predators (N=356)[1] | |
|---|---|---|
| | **Age** | **Weight** |
| **THg muscle** | 0.086 (p=0.105) | 0.330 (p<0.001) |
| **THg liver** | 0.340 (p<0.001) | 0.482 (p<0.001) |
| **THg liver/muscle** | -0.059 (p=0.272) | -0.080 (p=0.134) |

[1] *Spearman correlation and its statistical significance*

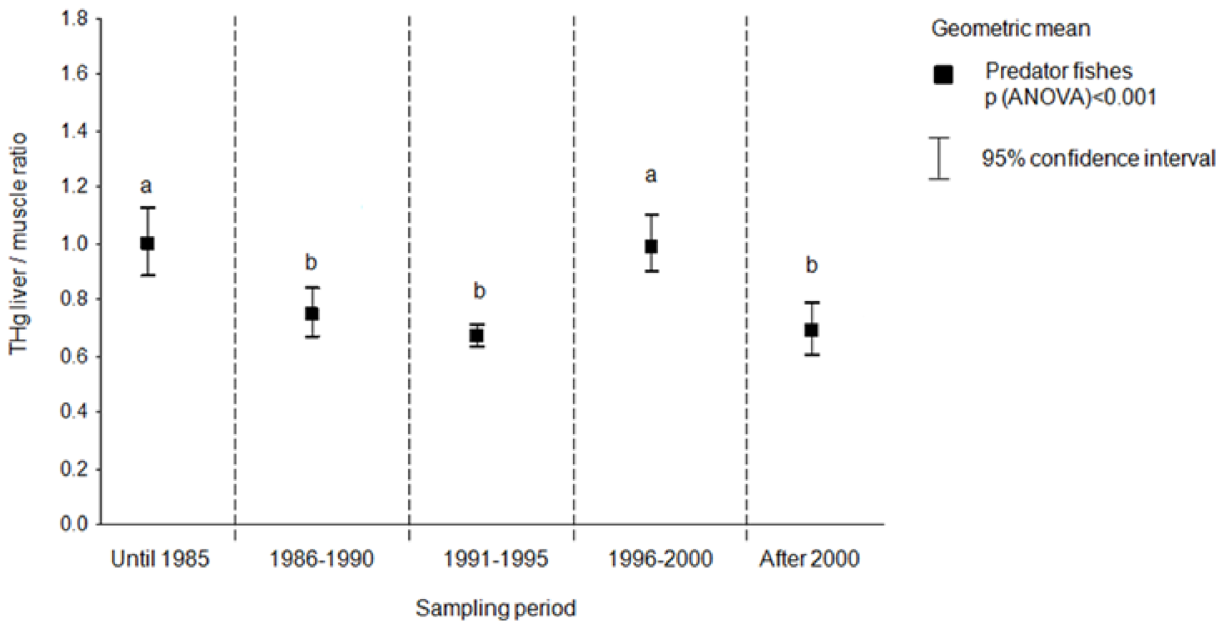


**Figure 4.**THg liver/muscle ratio according to the year of sampling from the Želivka Reservoir. *a, b– Homogeneous groups of predator fish computed using Tukey post hoc test; the same letters denote groups without statistically significant differences*

## 4. DISCUSSION

*4.1 Indicator chub study*

Chub is used as an indicator fish species for assessment of the river contamination in the Czech Republic because is prolific in basin of Elbe and Morava River [20, 21, 11]. The THg liver/muscle ratio in chub was followed on thirteen localities of Czech rivers. Even all the rivers are lightly polluted, the statistical difference was found out. For example, the Odra-Bohumín (geometric mean of THg in muscle = 0.14 mg/kg FW and geometric mean of THg in liver = 0.11 mg/kg FW) site exhibited a significantly higher ratio in comparison with the Otava-Strakonice (THg in muscle = 0.13 mg/kg FW and THg in liver = 0.04 mg/kg FW) site, which means that the mercury contamination at these sites is very different. Whereas the Odra Bohumín site is situated close to the cities of Ostrava and Bohumín, which are heavily industrialized, the Otava-Strakonice site is assumed to be largely uncontaminated and was used as a control site in the study by Kenšová et al. [22].

The mercury content in the muscle did not exceed hygienic limit for mercury (0.5 mg/kg FW) in any analyzed locations and thus locations embody low level of mercury contamination.

The target organ of mercury accumulation in heavily contaminated locations is the liver, because the liver is the main detoxifying organ in living organisms. Conversely, higher mercury levels in the muscle of fish compared to the liver are found in lightly contaminated locations. According to the obtained results from the studied locations, muscle is the target tissue for mercury deposition when the locality is lightly contaminated by mercury. This is in agreement with Havelková et al. (2008) [11], who compared localities that had been heavily and lightly contaminated by mercury. In their study, as in ours, a statistical significance of $p<0.001$ was found for indicator chub, followed by perch and roach.

*4.2 Czech rivers and reservoir study (2003 and 2011)*

Data of THg in liver and muscle for predator and non-predator fishes were collected from the five sites and THg liver/muscle ratio was calculated. The THg liver/muscle ratio for fish from the Skalka Reservoir (both in 2003 and 2011) exceeded 1, which means that mercury accumulates preferentially in the liver. The liver plays an important role in the storage, detoxification and transformation of pollutants, especially metals [23]. The high THg liver/muscle ratio confirmed that the Skalka Reservoir is still a highly contaminated location which confirms also results of total mercury content in the muscle and liver of sampled fishes (Fig 2). The historical mercury contamination of the Skalka Reservoir via waste water from German chemical plants is still persisting and should be monitored in the future. Abreu et al. (2000) [24] also confirmed the preferential accumulation of mercury in the liver in contaminated locations. In their study, THg liver/muscle ratios in three locations of the Ria de Aveiro Lagoon in Portugal were assessed. Mercury was determined in the liver and muscle of sea bass (*Dicentrarchus labrax*) as a top predator species from contaminated and un-contaminated locations. The THg liver/muscle ratio at the contaminated Largo do Laranjo site was 2.0, while at non-contaminated sites in Muranzel and Costa Nova it was 0.9 and 0.4, respectively. Surface water in the Largo do Laranjo is contaminated by industrial mercury.

Statistical analyses comparing predators and non-predators within a given locality using the independent t-test were done. Statistical differences was observed in Berounka-Černošice (p=0.033) and Skalka 2011 (p=0.045). In both cases, a higher THg liver/muscle ratio was found for predator fish species. The same holds true for data published by Havelková et al. (2008) [11], even when the THg liver/muscle ratio was adjusted for age. According to Berzas Nevado et al. (2011) [25], the THg liver/muscle ratio varies substantially among species and also among sampling points. In their study, samples of non-predator carp taken from contaminated sites had a relatively high ratio (mean=2.2) in comparison with the control site. However, this tendency was not observed in the THg liver/muscle ratio for catfish from the same sites.

The THg liver/muscle ratios in all locations with the exception of the Skalka Reservoir were about 0.4, which corresponds with lightly contaminated locations. Similar trend was observed in fish from Otradovice which is lightly contaminated site in the River Jizera[26]. The higher THg in muscle in comparison with THg in liver was found out in other lightly contaminated site, e.g. in bream from the Lake Balaton in Hungary [15].When THg in fish muscle are low (below approx. 0.5 mg/kg FW), mercury concentration in muscle is twice that in liver and THg liver/muscle ratio is low. When mercury concentration in muscle is about 1 mg/kg FW, the THg ratio is reverse and mercury in liver is many times higher than in muscle and it seems to be a highly contaminated site [27].

In our study, predator and non-predator fishes were analyzes on mercury concentration. Values of THg in muscle and liver in carp on all analyzed sites are very low in comparison with other watched fishes with is caused by fact that carp is prick in from the unpolluted ponds [22]. Mercury content in predators and non-predators both in liver and muscle from the same location is differ because accumulation of mercury is related to the position of the fish in the food chain. It is well documented that predator fish have higher mercury content in comparison with non-predators. Despite of this fact, no significant differences in THg liver/muscle ratio between predator and non-predator fish were found in the other locations (with the exception of Skalka and Berounka, which are mentioned above). Thus, the THg liver/muscle ratio of both predator and non-predator fish can be used for the evaluation of contamination in a locality.

### *4.3 Želivka Reservoir study*

For evaluation of the changeover of THg liver/muscle ratio over time for predator fish, the monitored time was split into five periods (up to 1985; 1986–1990; 1991–1995; 1996–2000; after 2000). Changes in the geometric mean of the THg liver/muscle ratio over time and their statistical significances are given in Fig. 4. The highest THg liver/muscle ratio (ranging from 0.88 to 1.12) was found in the first period after impoundment (up to 1985) and 20–26 years after flooding. A very high mercury value in fish was determined after impoundment, although the Želivka Reservoir was supposed to be a mercury-free locality because no mercury source was known [19]. Mercury levels in fish were the highest during the first 15–20 years after flooding because there were favourable conditions for the transfer of mercury from flooded farmland and forest to the river sediment and consequently to the food chain. Very high levels of mercury were detected in fish organs, which exceeded the hygienic safety limit of 0.5 mg/kg (for fish products) and in most cases also 1 mg/kg (the limit for certain predator fish such as pike) [19]. A significantly higher ratio was also found in the period 1996–2000 when the water level rose. It seems, according to the obtained results for the THg liver/muscle ratio, that there were changeful conditions after flooding which alternated in time.

## 5. CONCLUSIONS

On the base of THg liver/muscle ratio the contamination of the selected rivers and reservoirs of the Czech Republic was assessed. When the THg liver/muscle ratio is higher than 1, fish are likely to come from a location heavily contaminated by mercury. By contrast, when mercury is accumulated mainly in the muscle of fish (that is, the THg liver/muscle ratio is lower than 1), the fish are likely to come from a lightly contaminated location. Skalka Reservoir, which is anthropic polluted, still belong to heavily contaminated localities. Želivka Reservoir in the fast period after impoundment (up to 1985) and 20-26 years after flooding as well belonged to heavily contaminated localities. Chosen analyzed Czech rivers and Želivka Reservoir at the present time fall into lightly contaminated localities.

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