

The relationship between the mercury concentration in fish muscles and scales/fins and its significance

Research Article

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Abstract: The determination of mercury in fish typically involves analysis of muscles. For predicting the concentration of mercury in fish muscle on the basis of the analysis of fish scales or fins, the relationship between total mercury concentrations in fish muscles and in fish scales and fins was studied. Mercury content in fish muscles, scales and fins was determined by atomic absorption spectrometry with thermal decomposition of the sample in a flow of oxygen. A number of scale treatments were applied in order to remove impurities and to enhance the prediction quality. For scale treatment, 40 min of washing with DI water in an ultrasonic bath is recommended. A coefficient of determination $r^2 = 0.93$ for the relationship between Hg concentrations in muscles and scales was achieved for 40 fish among the different fish species tested (European bream, perch, roach) from the Hamry Reservoir, Czech Republic. With respect to fin sampling, the coefficient of determination r^2 for these fish was 0.86. The analysis of fish scales and caudal fins is a useful screening tool for assessing the relative mercury contamination of monitored fish. The method of sampling scales is not suitable for fish species with small scales such as brown trout.

Keywords: Mercury determination • Fish muscle • Scale • Fin

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1. Introduction

Because of the high toxicity of mercury compounds and the capacity for their bioaccumulation among organisms, mercury is considered a highly dangerous element. Methylmercury (CH_3Hg^+), the most toxic species of mercury, is formed in aquatic systems and biomagnified in the food chain from bacteria to plankton and consequently to fish. Fish muscle consumption is an important exposure pathway of mercury to humans [1-3].

Mercury is an element that exists as a monatomic vapour at room temperature. Therefore, one of the most widely used techniques for the determination of mercury in biological materials is the cold vapour (CV) technique, often in combination with atomic absorption

spectrometry (AAS) or atomic fluorescence spectrometry (AFS). In CVAAS and CVAFS, mercury present in the sample solution is reduced to the elemental form. In the CV technique, mercury vapour is liberated from the solution and introduced into the optical path of a spectrometer. To increase sensitivity, preconcentration of mercury vapour onto a gold or gold/platinum trap and subsequent release by thermal desorption are employed [4,5]. For the analysis of solid samples, the conversion of the solid matrix into an aqueous form is necessary. This is achieved by heating the sample with concentrated acid at either atmospheric or elevated pressure. Another approach is the direct analysis of the solid sample by means of combustion in an oxygen atmosphere and collection of the resulting mercury vapour on the surface of a gold amalgamator prior to its thermal release and

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determination by AAS. The determination is direct without separate sample decomposition and is free from matrix interference [4]. This pyrolysis AAS approach was used in this work for the determination of total mercury content in fish muscles, scales and fins.

A combination of gas chromatography (GC) or high-performance liquid chromatography (HPLC) with element specific detection methods such as atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), or inductively coupled plasma mass spectrometry (ICP-MS) is the most commonly used combined technique for the separation and detection of methylmercury. In this work, the GC-AFS combination was used for the determination of methylmercury in fish muscles. The determination of methylmercury in fish tissues involves several analytical steps, including extraction, derivatization, preconcentration into the organic solvent or into the solid phase, separation by GC and detection by AFS. Extraction is one of the most critical steps. The most frequently used procedures for the extraction of mercury species are based on alkaline and acidic leaching. To increase extraction efficiency, ultrasound or microwave assisted extraction is used. Derivatization by using tetraalkylborates, often tetraethylborate, has significant advantages in comparison with Grignard reagents since the reaction can be performed in the aqueous phase [3,6-11]. In this study the preconcentration of derivatives using solid phase microextraction (SPME) in head-space variant and liquid-liquid extraction into hexane were used.

Monitoring mercury content in fish involves sampling a representative number of fish from a particular population, the killing of the fish, the treatment of samples and the analysis of muscle tissue [12]. Nets, traps and electrofishing are used for fish sampling. The use of these classic methods in repeated monitoring of one locality could potentially decline a small fish population. Therefore, it is sometimes more suitable to use non-lethal methods. Materials such as fish scales, fins, tissue grafts or drops of blood can all be used in non-lethal sampling [12-18]. The analyses of blood and axial muscle obtained by biopsy provide reliable estimates of total mercury in fillets and may require the application of anesthetics or antiseptics to maintain fish health [12]. Baker demonstrated by using lake whitefish (*Coregonus clupeaformis*) and northern pike (*Esox lucius*) that reliable measurements of fish muscle Hg concentrations can be performed from small samples (<100 mg) harvested with biopsy tools. A field study of the effects of the dermal punch biopsy method on the survival of northern pike showed that tissue harvesting did not reduce survival. The analysis of Hg content in muscle harvested with biopsy tools provides Hg measurements

comparable in accuracy to traditional, whole-fish methods but without causing mortality [12]. Samples of muscle collected with a biopsy needle and dermal punch were used for the prediction of mercury concentration in the axial fillet with a coefficient of determination r^2 ranging from 0.93 to 0.97 [12]. Schmitt and Brumbaugh found that concentrations of total mercury in fillets of smallmouth bass (*Micropterus dolomieu*) could be accurately predicted from total mercury concentrations in biopsy plugs ($r^2 = 0.98$), biopsy needles ($r^2 = 0.99$), and blood ($r^2 = 0.92$) [13]. Lake *et al.* evaluated scales of largemouth bass (*Micropterus salmoides*) as predictors of tissue mercury concentration, and found a coefficient of determination of 0.90 by using a mild soap solution with heating and ultrasonication for the preliminary washing treatment of scales [14]. According to Lake *et al.* the biopsy method yields more accurate predictions of Hg concentrations in muscle tissue than those of the scale model. The sampling by biopsy did not decrease the survival of the fish studied, but sampling by scale is probably a less harmful technique than taking biopsy plugs, especially for small fish. Therefore, scale sampling may offer some advantages over the biopsy technique [14]. By means of fin clip analysis, Rolffus *et al.* found that the concentration in selected fin clips was a better predictor of mercury in fillets for individual Arctic grayling (*Thymallus arcticus*, $r^2 = 0.84$) and winter flounder (*Pseudopleuronectes americanus*, $r^2 = 0.94$) than for individual northern pike (*Esox lucius*, $r^2 = 0.62$) or walleye (*Sander vitreus*, $r^2 = 0.63$) from several lakes [16]. The clipping of pelvic and caudal fins is commonly performed to mark fish, and fin clips are rapidly collected with minimal harm to the organism [16]. Partially clipped fins usually regenerate and the repeated clipping of fins from the same individual fish may allow monitoring of changes in mercury, especially in small populations. By measuring the total mercury in caudal fins and fillets of small numbers of walleye and northern pike, it was found that the mercury concentration in the caudal fin was a good predictor of mercury levels in the fillet [19]. Also in selected northern pike of restricted size, the mean concentrations of total mercury in caudal fins and fillets were strongly correlated ($r^2 = 0.95$) [16]. However, the applicability of these methods to other scales or fins, species, or geographical areas is unknown.

The focus of this work is to investigate the relationship between total mercury concentrations in fish muscles and in fish scales or fins and to determine whether the analysis of Hg in fish scales or fins would allow the useful estimation of Hg in fish muscles. If so, the sampling of scales or fins may be the best non-lethal method of sample collection to determine mercury content in fish muscles. Fish species were chosen to cover different trophic levels

of fish: perch (*Perca fluviatilis*) as a predatory fish, roach (*Rutilus rutilus*) as a planktivorous fish and European bream (*Abramis brama*) as an omnivorous fish; they were also chosen because they were species which had not been previously tested. Brown trout (*Salmo trutta* m. *fario*) was chosen as a species obtainable in large numbers in various localities. The aim of this paper is also to develop procedures for determining mercury levels in these monitored specimens.

2. Experimental procedure

2.1. Instrumentation

The total contents of mercury in the monitored materials were determined using the single-purpose AMA 254 (Altec) analyser. This method is based on the thermal decomposition of a sample in a flow of oxygen, the capture of mercury by a gold amalgamator, and measurements of the mercury vapour absorbance after thermal release from the amalgamator. Each time, 40–100 mg of a sample were weighed or 10–200 μL of solution were dosed in nickel boats. The absolute limit of detection for all measurements on the AMA 254 analyser was 3 pg Hg . The limit of detection is defined as the threefold standard deviation of the blank.

The determination of methylmercury was performed using the Agilent Technologies 6890 N Network GC System with a PSA 10.750 fluorescence detector. The PSA detector was coupled via a pyrolysis oven held at 800°C. An HP-5 silica capillary analytical column with dimensions of 30 m \times 0.32 mm i.d. \times 0.25 μm film thickness was used. The column temperature was held at 50°C for 1 min, programmed at 15°C min^{-1} to 150°C, then programmed at 30°C min^{-1} to a final temperature of 270°C and held for 5 min. A split/splitless injector was used in the splitless mode and maintained at 220°C. The injection volume of hexane extract was 5 μL . The carrier gas flow rate was 0.9 mL min^{-1} of argon. Additional equipment included an SPME (Supelco) fiber holder for manual use, a fiber coated with a 100 μm thickness of poly(dimethylsiloxane), a 10-mL glass vial and a 15 \times 6 mm PTFE-coated magnetic stirring bar used for headspace SPME extraction.

2.2. Samples

Samples of muscles, scales and fins from 10 specimens of roach, the representative of planktivorous fishes, 20 spec. of European bream, the representative of omnivorous fishes, and 10 spec. of perch, the representative of predatory fishes, were analysed. The fish were collected from the Hamry fresh water reservoir on the Chrudimka River, Czech Republic in 2009.

In addition, samples of muscles and scales from 14 spec. of brown trout collected from the Fryšavka River and 6 spec. of chub (*Leuciscus cephalus*) collected from the Dyje River in the localities of Tasovice and Dyjákovice were analysed. The fish from the Hamry Reservoir were sampled using a 50 m seine net while the fish from the Fryšavka River, Dyje River and the localities of Tasovice and Dyjákovice were collected by electrofishing. The fish were sacrificed by cutting the cervical spine immediately after the sampling and stored frozen in plastic bags in a conventional freezer at -20° C. Fish sacrifice was necessary to verify that non-lethal methods of scale/fin analysis are sufficient for the prediction of mercury content in fish muscles. For studying the process of scale treatment and for determining the correctness of the analytical procedure for individual scales, muscles and scales from pike, carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*) and sander (*Sander lucioperca*) from a commercial source were also used.

The samples of fish muscles and scales were deprived of water using lyophilization for 48 h at -52°C in a Christ Alpha 1-2 device. The muscle samples were then homogenized on a Retsch MM 301 mill by grinding in a chamber made from wolfram carbide. Fish scales were then placed in a glass vial and washed with deionized water or other agents for 40 min in a Transsonic 570/H ultrasonic bath. After treatment, the scales were air dried before analysis at ambient temperature in filter paper envelopes. Fin clips were rinsed only with deionized water and dried in filter paper envelopes. In the determination of total mercury content in caudal fins using this treatment, the relative standard deviations (RSDs) were 2–7%. DORM-2 (dogfish muscle, National Research Council of Canada, Ottawa, Canada) was used as the reference material.

2.3. Procedures

For the determination of total mercury content, 100 mg of fish muscle were weighed in nickel boats and analysed on the AMA 254 analyser. The muscle samples were dried at 120°C for 30 s and decomposed at 650°C for 120 s. The AMA 254 analyser was regularly calibrated using standard solutions of 1 - 1000 $\mu\text{g L}^{-1}$ of mercury for the first (0 – 6 ng Hg) and second (0 - 200 ng Hg) calibration interval. The calibration solutions were prepared by diluting primary calibration standard (1000 mg L^{-1} Hg, Sigma Aldrich, Germany) with 0.05% (m/v) $\text{K}_2\text{Cr}_2\text{O}_7$ and 0.6% HNO_3 to improve their stability. The accuracy of the results was controlled by analysis of the standard reference material DORM-2. The relative standard deviation (RSD) was 2.02% (at $4.64 \pm 0.25 \text{ mg kg}^{-1}$ Hg, $n=10$).

For the isolation of mercury from fish muscle samples, 250 mg of sample were placed into a glass vial. Then 10 mL of 25% (w/v) methanolic KOH solution were added and the sample was shaken in an ultrasonic bath at 50 – 55°C for 4 h. The fish extract was stored at 4°C prior to GC-AFS analysis.

For headspace SPME sampling by GC-AFS analysis, the magnetic stirring bar, 3 mL of deionized water and 2 mL of acetate buffer solution (pH = 5) were placed in a 10-mL glass vial. A 100 µL aliquot of the fish extract or 100 µL aliquot of mercury standards and 1 mL of 2% NaBH₄ solution were added, and the vial was then closed immediately. The fiber was drawn into the needle of the holder and the needle was used to pierce the septum of the sample vial. The fiber was then lowered into the headspace by depressing the plunger and did not come into contact with the liquid. After 4 min, the fiber was retracted into the needle and immediately inserted into the GC injector for thermal desorption at 220°C for 1 min. For extraction sampling, 3 mL of deionized water, 2 mL of acetate buffer solution (pH = 5), a 100 µL aliquot of the fish extract or 100 µL aliquot of mercury standards, 1 mL of hexane and 0.5 mL of 2% NaBH₄ solution were placed in a 10-mL glass vial. The vial was closed immediately and shaken for 5 min. After phase separation, 5 µL of extract were injected into the GC column. GC-AFS was regularly calibrated using a standard solution containing 1 mg L⁻¹ of methylmercury. Fresh calibration solutions were prepared daily. Calibration curves were strictly linear ($r^2 = 0.9999$ for SPME, $r^2 = 0.9932$ for extraction). Accuracy was controlled using analyses of dogfish muscle SRM (DORM-2) with a MeHg content of 4.47 ± 0.32 mg kg⁻¹ (as Hg).

For the determination of mercury in scales, individual scales or collections of several scales were weighed in nickel boats and analysed on an AMA 254 analyser. The correctness of this procedure was verified by means of comparison with results obtained with pulverized and homogenized samples of scales of carp and grass carp. Before grinding, scales were cut into pieces with a ceramic knife. Mercury content in scales after cleaning treatment was independent of the location of scales on the body of fish. Scales from the dorsal part of the bodies of carp and grass carp were also cut along the scale circuli into three parts. A decrease in mercury content towards the central part was observed. Front and hind parts of scales with the same period of growth exhibited identical contents of mercury. For the determination of mercury in caudal fin parts or in whole fins, these fins/fin parts were weighed in nickel boats and analysed on an AMA 254 analyser.

3. Results and discussion

3.1. Determination of total mercury and methylmercury contents in fish muscle

In the determination of total mercury content in fish muscle samples, the RSDs were 2-3% (n=3). The limit of detection for the total mercury content was 0.03 µg kg⁻¹. The total mercury contents in fish muscles are shown in Figs. 1-5 and for selected samples in Table 1.

For the determination of methylmercury content, the certified reference material DORM-2 and muscle samples from bream and perch were analysed. The extraction efficiency for mercury using ultrasonic extraction with 25% (w/v) methanolic KOH solution was determined using the AMA 254 mercury analyser and was quantitative.

The derivatization process with NaBH₄ was studied and optimized. An important factor in the derivatization is pH. A maximum signal was achieved at pH 5. The optimum extraction time was 4 min for SPME and 5 min for LLE. Using SPME, the conversion of methylethylmercury into diethylmercury occurs after a longer extraction time. The volume of fish extract and total volume of solution are also important factors. The used extract-volume/total-volume ratio did not lead to coagulation and the extraction yields of methylmercury determined by GC-AFS for certified reference material DORM-2 were $94.7 \pm 3.4\%$ (n = 5) for SPME and $98.5 \pm 2.8\%$ (n = 5) for LLE, thus in good agreement with certified values.

The content of MeHg⁺ in the muscle tissue of perch was higher than in bream (Table 1). This is in accord with the higher accumulation of mercury in predatory fish. This is consistent with the findings of other studies [20,21], where approximately 56–100% of the total mercury in the fish was methylmercury. The determined high content of methylmercury in fish muscle indicates that knowledge of the total mercury content can also be useful if it can be obtained without destruction of the fish. Therefore, the mercury content in fish scales and fins was analysed.

3.2. Treatment of scales

To remove surface contamination by mercury, but also impurities such as mucus or skin residues or other tissue adhering to scales, and to increase the coefficient of determination, different scale-cleaning treatments were tested. The washing of scales with deionized (DI) water, H₂O₂, HNO₃ and soap were all used in conjunction with an ultrasonic bath. The mercury content in the non-washed scales and the scales cleaned by ultrasonic bath with different types of reagents (DI water, 3% (v/v)

H₂O₂, 0.01 M HNO₃ and 1 g L⁻¹ soap) was compared. The reproducibility of the results was greatest when using DI water. These experiments were performed with scales of pike and sander. By cleaning scales with DI water or soap, the value of mercury content was constant after 30 min; with HNO₃ it was constant after 40 min. In the presence of 3% H₂O₂ (v/v), the mercury content was invariable after 10 min, but the accuracy of determination was low. In H₂O₂ the scales were gradually dissolved. The lowest RSD (2-6%) was obtained by cleaning with DI water. With respect to the visual appearance of scales, the time of cleaning was prolonged to 40 min. Only after this time were the scales clean, without impurities such as mucus or skin residues. Similar results were obtained for scales of chub, bream, perch and roach. With the scales of brown trout, the treatment was complicated because of their small size. The scales flocked in the ultrasonic bath and cleaning was not effective.

3.3. The relationship between total mercury concentrations in fish muscles and in scales

In order to predict the content of mercury in fish muscle, the relationship between mercury content in fish muscles and mercury content in fish scales and fins was studied.

For the scales of brown trout, cleaning was not effective; therefore, the relationship between total mercury concentrations in fish muscles and in unwashed scales was used (Fig. 1). The coefficient of determination r^2 was 0.68. However, the large amount of scales necessary for analysis cannot be removed without causing injury to the trout.

In the investigation of the relationship between total mercury concentrations in fish muscles and in unwashed scales in bream, perch and roach from the Hamry Reservoir, no relationship was detected. If the amount of mercury in individual unwashed scales in place of total mercury concentrations in unwashed scales was included, a relation was found with a coefficient of determination $r^2 = 0.81$ (Fig. 2). The same relationship was observed for chub scales, with $r^2 = 0.46$. A low value of r^2 can be the result of a small number of samples and

Table 1. Results for the determination of methylmercury in muscle samples by GC-AFS.

	total Hg ^a (mg kg ⁻¹)	MeHg ⁺ ^b (mg kg ⁻¹)	
		SPME	LLE
Bream	2.78 ± 0.06	2.53 ± 0.16	2.50 ± 0.11
Perch	6.41 ± 0.13	6.13 ± 0.20	6.39 ± 0.11

^an = 3, ^bn = 5

the existence of different sampling sites. According to Fig. 3, the treatment of scales of bream and perch with DI water and with soap had significant results: for soap, $r^2 = 0.90$, and for DI water, $r^2 = 0.91$. Both methods are suitable for scale treatment; RSD for treatment with DI water was lower. The r^2 values were the same as those for scales of largemouth bass using treatment with soap solution with heating and ultrasonication [14].

The relationship between Hg concentrations in muscles and scales of bream, perch and roach from the Hamry Reservoir, including a 95% confidence band and a 95% band of prediction constructed using SigmaPlot 10.0, is shown in Fig. 4. The term 'confidence band' refers to the region of uncertainties in the predicted values over a range of values for the independent variable. The term 'prediction band' refers to the region of uncertainties in predicting the response for a single additional observation at each point within a range of independent variable values. Prediction bands are always wider than confidence bands. A coefficient of determination $r^2 = 0.93$ for 40 fish across all species tested was achieved, while r^2 for individual species was smaller. The coefficient of determination r^2 for roach was 0.42; for perch, 0.62; for

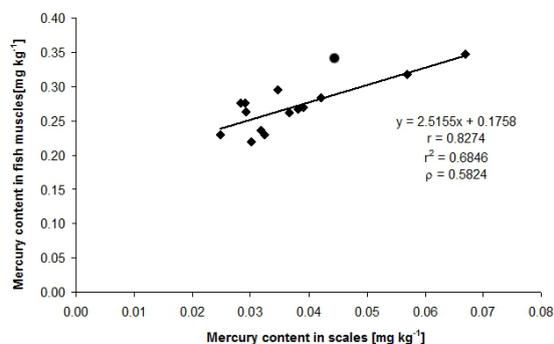


Figure 1. Relationship between mercury concentration in fish muscles and the content of mercury in unwashed scales for trout from the locality of Fryšavka.

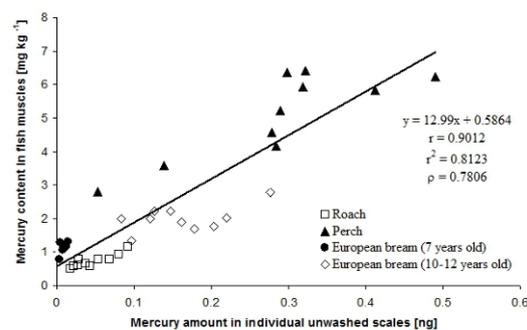


Figure 2. Relationship between mercury concentration in fish muscles and the amount of mercury in individual unwashed scales for bream, perch and roach from the Hamry Reservoir.

7-year-old European bream, 0.60; for 10-12-year-old European bream, 0.35; and for all European bream together, 0.81. This weaker correlation can be affected by the relative small number of specimens and the narrow range of mercury concentration for individual fish species. Higher r^2 for all European bream together indicates this. Therefore r^2 for fish across species was higher than for individual species, although fish species have different dietary strategies.

The relationship between total mercury concentrations in fish muscles and in fish scales or fins was also assessed by using Pearson's (r) and Spearman's ($\rho = \text{rho}$) correlation coefficients, which were calculated using the STATISTICA 9 software (StatSoft Inc., Tulsa, USA). In all cases $r > \rho$ was achieved (Figs. 1,2,4,5) and normal data distribution was demonstrated. A level of $P < 0.05$ was considered statistically significant.

Perch is a predatory fish and accumulates the largest amount of mercury in tissue, but also in scales. Bream is an omnivorous fish exhibiting a medium level of mercury, while roach is a planktivorous fish with a lower amount of Hg in muscle and scales. The concentrations of mercury in scales of roach are similar to those for bream from younger generations (7 years). This is in agreement with a study [22] in which fish species were categorized into four ecological groups (carnivorous, omnivorous, planktivorous and herbivorous fish) according to mercury content in fish muscles. Mercury contents for the carnivorous and herbivorous fish were significantly different [22]. Omnivorous and planktivorous fish were overlapping between the other two. Mercury levels were usually higher in the muscles of older and larger fish than in those of younger specimens, as a consequence of the longer time for bioaccumulation [22]. This is consistent with similar findings from other fish studies [20,21]. Our results suggest, in agreement with [14], that predicting Hg concentrations in the muscle tissue of roach, bream and perch from measured Hg concentrations in their scales may be useful for assessing Hg contamination in fish muscle as a first-level form of screening to determine locations that may require further testing. The method may also be utilized, as in [17], for the examination of trends in Hg contamination in terms of the analysis of scales from archived collections.

3.4. The relationship between total mercury concentrations in fish muscles and in fins

The relationship between mercury concentrations in fish muscles and fins for bream, perch and roach from the Hamry Reservoir is shown in Fig. 5. The mercury concentration in fins increased with the concentration in muscle. The coefficient of determination $r^2 = 0.86$ for

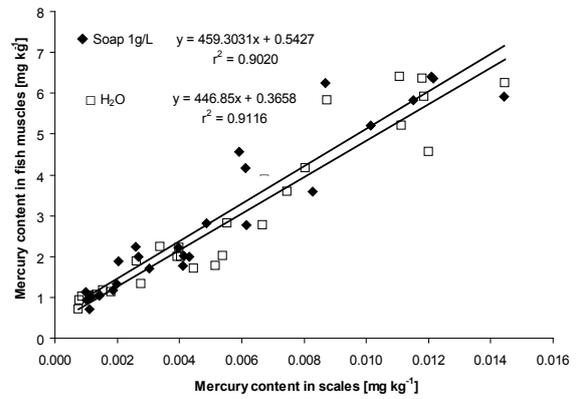


Figure 3. Relationship between mercury concentration in fish muscles and scales for bream, perch and roach from the Hamry Reservoir. Scales were cleaned with DI water or soap in an ultrasonic bath for 40 min.

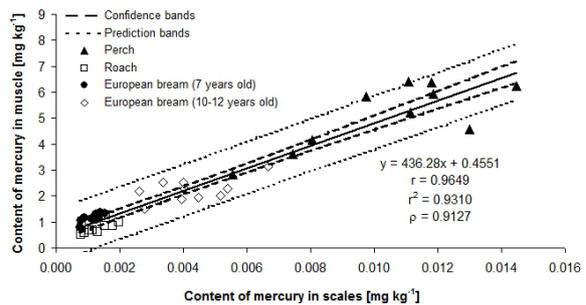


Figure 4. Relationship between mercury concentration in fish muscles and scales for bream, perch and roach from the Hamry Reservoir. Scales were cleaned with DI water in an ultrasonic bath for 40 min.

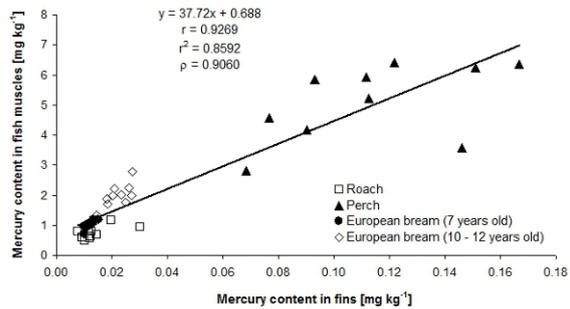


Figure 5. Relationship between mercury concentration in fish muscles and fins for bream, perch and roach from the Hamry Reservoir. Fins washed with DI water.

40 fish across fish species was relatively high, but r^2 for individual species was mostly smaller. The coefficient of determination r^2 for roach was 0.39; for perch, 0.28; for 7-year-old European bream, 0.71; for 10-12-year-old European bream, 0.51; and for all European bream together, 0.86. The analysis of caudal fins from fish is a useful screening tool for assessing the relative mercury

contamination of monitored fish. However, the longer time needed for healing after fin sampling means that the analysis of scales is preferable for the prediction of total mercury in muscles.

4. Conclusion

Typically, the analysis of muscles provides accurate determination of mercury levels in fish. In this study, the relationship between total mercury concentrations in fish muscles and in fish scales and fins was investigated in order to assess whether the analysis of scales or fins could provide a nonlethal approach for predicting mercury content in fish muscles. For the determination of mercury in individual fish scales and fins, atomic absorption spectrometry with the thermal decomposition of a sample in a flow of oxygen, e.g. using an AMA 254 analyser, is available. The treatment of scales to remove mucus and scraps of skin, or other tissue adhering to them, plays a significant role. For the optimal treatment of scales, washing with DI water in an ultrasonic bath for 40 min is recommended. Using this treatment of scales, a coefficient of determination $r^2=0.93$ for the relationship between Hg concentrations in muscles and scales was achieved by 40 fish among fish species from the Hamry Reservoir, Czech Republic (European bream,

perch, roach). The method of sampling scales is not suitable for fish species with small scales such as brown trout. Obtaining the required amount of scales might significantly affect the health of the trout specimens. With respect to caudal fin sampling, the coefficient of determination r^2 for 40 fish from the Hamry Reservoir was 0.86. Although fish species play an important role in the accumulation of mercury, the locality can have an important influence on the relationship between mercury content in fish muscles and mercury content in fish scales and/or fins across fish species. The analysis of fish scales and caudal fins is a useful screening tool for assessing the relative mercury contamination of monitored fish. However, the preferred method appears to be scale analysis because of the shorter time required for healing after scale sampling.

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