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ASSESSMENT OF MERCURY IN MUSCLES, LIVER AND GILLS OF MARINE AND FRESHWATER FISH*

Joanna Łuczyńska[1], Marek Jan Łuczyński[2], Beata Paszczyk [1]

[1]Chair of Commodity and Food Analysis
University of Warmia and Mazury in Olsztyn
[2]Inland Fisheries Institute in Olsztyn

Abstract

In this study, the total mercury concentration was determined in the muscles, liver and gills of six fish species (rainbow trout *Oncorhynchus mykiss* Walb., carp *Cyprinus carpio* L., bream *Abramis brama* L., perch *Perca fluviatilis* L., ide *Leuciscus idus* L. and flounder *Platichthys flesus* L.). The fish were acquired from October to November 2012. Mercury was analyzed by atomic absorption spectrometry using thermal decomposition, compounds of mercury and amalgamation. The mercury content in fish organs reached 0.006-0.168 (in mg kg^{-1} wet weight) in muscles, 0.001-0.027 in gills and 0.003-0.045 in the liver. The muscles of perch and ide had significantly more Hg compared to the other fish's muscles ($P \leq 0.05$). The liver and gills of perch, ide and flounder contained more Hg than the same tissues of the other fish ($P \leq 0.05$). Generally, the highest Hg content was determined in muscles (except rainbow trout) ($P \leq 0.05$), whereas the lowest Hg content was found in gills (except perch) ($P \leq 0.05$). The content of Hg in gills of perch did not differ from the one in the liver ($P > 0.05$). There was a positive correlation between the weight or length of a fish and the Hg concentration in its tissues, except for the length and Hg in the gills of carp. However, a statistically significant positive correlation between the body weight and the Hg levels in fish appeared only in the case of the organs of ide ($P \leq 0.004$) and muscles of carp ($P \leq 0.038$). The correlation between the factor condition and the content of Hg, albeit positive ($0.106 < r < 0.811$) except for the organs of flounder, was not statistically significant ($P > 0.05$).

Keywords: total mercury, freshwater fish, muscles, liver, gills, body weight, total length, biotic factors, condition factor.

dr inż. Joanna Łuczyńska, Chair of Commodity and Food Analysis, University of Warmia and Mazury in Olsztyn, Heweliusza 6, 10-756 Olsztyn, Poland, phone: +48 89 5234165, e-mail: jlucz@uwm.edu.pl

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INTRODUCTION

Repeated pathways of mercury transmission through air, food, water (sea, rivers, lakes and groundwater), pharmaceuticals, cosmetics, etc., account for its easy accessibility to man, but factors such as the biomagnification of mercury in a food chain make this problem more intricate (Castro-González, Méndez-Armenta 2008, Zahir et al. 2005). According to Stankovic et al. (2014), microbes, fungi, plants, animals and humans are used as bioindicators of heavy metals originating from air, water, sediment, soil and food web. Therefore, fish could be a good and effective indicator of these elements in an aquatic environment. Like all living organisms in an aquatic ecosystem, fish represent a specific level of the trophic pyramid and a link in the bioaccumulation or biomagnification of heavy metals. According to Dallinger et al. (1987), bioaccumulation means that heavy metals in biota are concentrated in relation to abiotic environment components like water or sediment, whereas biomagnification is defined as a progressively increasing concentration of metals along the food web. Kehrig et al. (2009) showed that the total mercury concentration increased from the lower trophic level (prey) to the top-level (predator). Similar observations were made by Da Silva et al. (2005) and Ikemoto et al. (2008). In turn, Boyd (2010) reported that heavy metals in freshwater habitats can modify chemical communications between individuals and affect ecological relationships both within and between species. Processes of bioaccumulation and biomagnification depend on environmental and intrinsic factors (Jezierska, Witeska 2006). Literature data, both in some earlier (Berninger, Pennanen 1995, Voigt 2000, Farkas et al. 2001, Łuczyńska 2005, Łuczyńska, Brucka-Jastrzębska 2006) and in the latest reports (2012-2013) (Mazej et al. 2010, Burger et al. 2012, Hosseini et al. 2013, Järv et al. 2013, Zrnčić et al. 2013), show that the concentration of mercury in fish is affected by many factors, such as species, body weight, total length and factors connected with fish condition. In view of the above, the objective of this study was to estimate the effect of species on the total mercury content in muscles, gills and liver of fish, and to determine differences between the content of this metal in organs of the same species. An additional aim was to evaluate the dependence between the size (body weight and total length) or the factor condition of fish and the concentration of mercury in fish tissues. Another justification of the study was that there are only a few study conducted on fish from commercial sources.

MATERIAL AND METHODS

Samples

All samples (n = 53) of rainbow trout (*Oncorhynchus mykiss* Walb.), carp (*Cyprinus carpio* L.), bream (*Abramis brama* L.), perch (*Perca fluviatilis* L.),

Table 1

Body weight, total length (min-max, mean± SD) and content of mercury in fish organs (min-max), mg kg^{-1} wet weight

Species	Body weight	Totat length	Muscles	Gills	Liver
Bream n = 12	129-594 323.1±146.5	25.5-32.0 28.6±2.3	0.006 – 0.028 0.018±0.008 *c*	0.001 – 0.006 0.003±0.001 *c*	0.002 – 0.013 0.006±0.003 *c*
Ide n = 12	742-1266 970.7±147.1	36.0-44.0 40.3±2.6	0.046 – 0.221 0.124±0.052 *a*	0.005 – 0.026 0.014±0.007 *a*	0.015 – 0.086 0.045±0.021 *a*
Rainbow trout n = 12	136-238 195.5±30.5	23.1-27.2 25.1±1.2	0.013 – 0.016 0.015±0.001 *c*	0.005 – 0.008 0.006±0.001 *b*	0.014 – 0.019 0.016±0.002 *b*
Carp n = 6	938-1432 1191.7±178.4	34.0-36.2 34.8±0.8	0.004 – 0.009 0.006±0.001 *d*	0.001 – 0.002 0.001±0.000 *c*	0.003 – 0.004 0.003±0.000 *c*
Flounder n = 5	245-369 310.4±50.8	24.2-31.7 28.2±2.8	0.038 – 0.084 0.057±0.017 *b*	0.010 – 0.022 0.015±0.005 *a*	0.016 – 0.075 0.037±0.025 *a*
Perch n = 6	296-704 578.7±145.2	28.0-34.5 32.8±2.4	0.078 – 0.336 0.168±0.124 *a*	0.007 – 0.029 0.027±0.035 *a*	0.021 – 0.074 0.040±0.024 *a*

n – number of fish; *a*, *b*, *c* – significant differences between the same organs of fish species ($P \leq 0.05$) (in columns). The same letter indicates the absence of significant differences ($P > 0.05$).

ide (*Leuciscus idus* L.) and flounder (*Platichthys flesus* L.) were bought from October to November 2012 in big discount shops operating on the Polish market. On the day of purchase, the fish were taken to the laboratory, where the body weight (± 1 g) and total length (± 0.1 cm) of each specimen were measured (Table 1). The muscle tissue from the dorsal part, the liver and the gills were dissected, placed in polypropylene bags and stored at -30°C until analysis. For all the fish species, each sample was prepared from organs taken from one specimen.

Determination of mercury

Duplicate samples of up to 270 mg (± 0.0001 g) from all organs of fish were weighed into a quartz boat and analyzed according to the recommended procedure. Total mercury was measured by atomic absorption thermal decomposition using a Milestone DMA-80 (with dual-cell). The first step involved drying at 200°C (for samples, including fish, with high water content). The purging time (the time between the end of drying/decomposition and the start of Hg measurement) was 60 s. The amalgam heater time (the time necessary for mercury release and its collection into an absorption cuvette) was 12 s. The signal recording time was 30s. The parameters for drying and decomposition (temperature/time, respectively) were as follows: max. start temp. 200°C/60 s, drying temperature 160°C/60 s; decomposition (burned in an oxygen flow) at 650°C/60 s. The time between the termination of drying and the onset of decomposition (650°C) was 120 s. The absorption wavelength was 253.65 nm (at a detection limit of 0.005 ng Hg) and the detector consisted of UV enhanced

photodiodes. The method was verified by measuring the elements in the reference material BCR CRM 422 (muscles of cod *Gadus morhua* L.) with a certified value of mercury. The per cent recovery rate was 100.2% (n = 4).

Statistical analysis

The data were calculated using one-way analysis of variance Anova (the Duncan's test) to evaluate significant interspecific differences in the content of mercury both between species and organs of the same species. The Bartlett's test showed that the variances were heterogeneous, hence mean values in particular groups were transformed ($\log \bar{x}$). Differences were found to be significant at $P \leq 0.05$. The correlation coefficients between the content of Hg and condition factor FCF, body weight and total length of fish were calculated using a Statistica 10 programme. The significance levels of $P \leq 0.01$ and $P \leq 0.05$ were used. The condition of the fish examined was calculated with the Fulton's condition factor (FCF).

$$FCF = 100\ W\ L^{-3},$$

where: W – total body weight of fish (g), L – total length of fish (cm).

RESULTS AND DISCUSSION

Differences between fish species

The mean and standard deviation values of the total mercury content in the muscle tissue, liver and gills are presented in Table 1. Muscles of perch contained significantly more mercury (0.168 mg kg^{-1}) than muscles of the other fish (0.006 - 0.057 mg kg^{-1}) ($P \leq 0.05$), except ide (0.124 mg kg^{-1}) and the differences between the Hg content in the muscles of perch and ide were not statistically significant ($P > 0.05$). The concentration of total mercury in the muscles of the analyzed fish could be ordered as follows: perch ≈ ide > flounder > bream ≈ rainbow trout > carp ($P \leq 0.05$). Cultured fish as well as carp and rainbow trout were characterized by the lowest content of this metal. The content of mercury in the liver of the fish species studied decreased as follows: ide ≈ perch ≈ flounder > rainbow trout > bream ≈ carp ($P \leq 0.05$). Significant differences in the levels of mercury were found between rainbow trout (0.016 mg kg^{-1}) and the other fish, and between bream (0.006 mg kg^{-1}) and the other fish (except carp). There were no significant differences between the content of mercury in the gills of perch (0.027 mg kg^{-1}), flounder (0.015 mg kg^{-1}) and ide (0.014 mg kg^{-1}) ($P > 0.05$) or between bream (0.003 mg kg^{-1}) and carp (0.001 mg kg^{-1}). The value of total mercury in the gills of the analyzed fish could be ordered as follows: perch ≈ flounder ≈ ide > rainbow trout > bream ≈ carp ($P \leq 0.05$).

According to the European Food Safety Authority (EFSA), provisional tolerable weekly intake (PTWI) for inorganic mercury is 4 μg kg^{-1} body

weight – b.w. (Efsa Journal 2012). The fish consumption in 2012 was 12.1 kg per capita (an adult of the body weight 70 kg) (Statistical Yearbook of Agriculture 2013) and a 100 g portion of bream, ide, rainbow trout, carp, flounder and perch contained 1.81 μg, 12.4 μg, 1.5 μg, 0.60 μg, 5.70 μg and 16.80 μg of mercury, which corresponded to 0.15%, 1.03%, 0.12%, 0.05%, 0.47% and 1.40%, respectively, of the PTWI reference dose.

Jakimska et al. (2011) claimed that the most important factor affecting the amount of metals in the tissues of a given animal, including fish, was a diet. Generally, bioaccumulation of metals in fish depends on biotic factors, including the fish species, age, size (body weight and total length) and feeding habits (Jezierska, Witeska 2006, Polak-Juszczak 2012). In the present study, the content of mercury in fish varied between organs and some species. Has-Schön et al. (2006) reported large differences in the heavy metal content, including mercury, depending on a fish species and tissue analyzed. Omnivorous species did not correlate significantly ($P > 0.05$) with either a predator or benthophagous groups (Dušek et al. 2005). The same authors observed that benthophagous species strongly correlated with predatory fish only in the case of young individuals. The content of mercury in perch, a representative species of predatory fish, from the Bay of Puck in Poland (0.110 mg kg^{-1}) was higher than in the other species examined by Boszke et al. (2003). These results coincide with the current findings. The content of mercury in muscles of fish from Skalka Reservoir (the Czech Republic) decreased as follows: asp > eel > big head carp (*Aristichthys nobilis* L.) ≈ bream ≈ roach ($P < 0.05$) (Maršálek et al. 2005). The predatory fish asp and eel had more mercury than non-predatory species. Similar observations were made by Łuczyńska and Brucka-Jastrzębska (2006). There were also some significant differences in the total mercury content among five fish species studied by Andreji et al. (2006). According to these authors, predatory fish (wels catfish, *Silurus glanis* L.) had the highest content of Hg whereas the lowest values of Hg were found in omnivorous fish, such as Prussian carp, *Carassius gibelio* L. ($P < 0.001$). According to Cizdziel et al. (2002), the mercury concentration increased in higher trophic levels, whereas Richard et al. (2000) noted that carnivorous fish had more mercury than non-carnivorous species (South America). Ruelas-Inzunza et al. (2008) also found a higher content of Hg in muscle tissue of carnivorous fish than in non-carnivorous species. On the other hand, the same authors reported an opposite regularity in the case of liver. Hosseini et al. (2013) noted that mercury can be transferred into higher levels of the trophic pyramid by biomagnification, and its accumulation in a high level of the food chain depends on the organisms from the lowest trophic level.

Mercury distribution in different organs

The total mercury concentration in the muscles, liver and gills varied within the species and between the organs (Figure 1). The highest statistically significant content of this heavy metal ($P \leq 0.05$) was found in muscles,

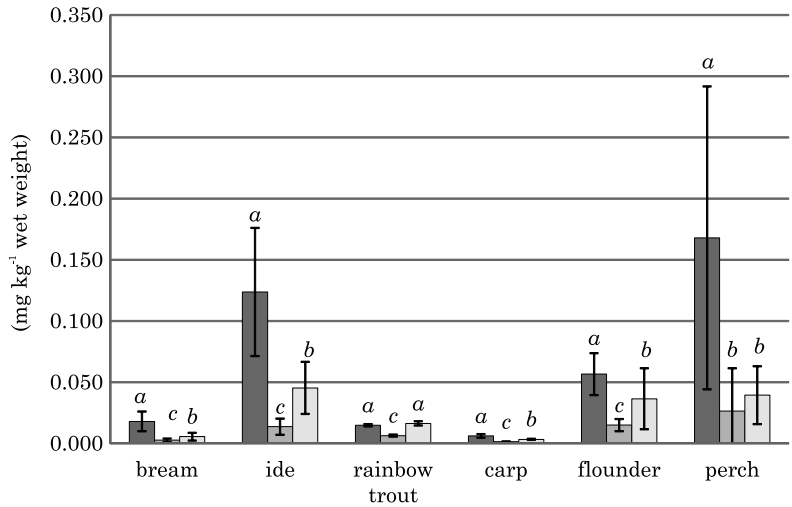


Fig. 1. Comparison of mercury in different organs of the same fish species:
a, *b*, *c* – significant differences ($P \leq 0.05$). The same letter indicates the absence of significant differences ($P > 0.05$)

followed by the liver and gills. Rainbow trout was an exception in that that the content of Hg in this fish decreased in the order: liver ≈ muscles > gills ($P \leq 0.05$). Significantly the lowest content of Hg was found in gills in all the fish examined except perch. The concentration of Hg in gills was statistically similar to that in the liver ($P > 0.05$). Al Sayegh-Petkovšek et al. (2012) found that generally a higher content of Hg was determined in muscles and liver than in gills. Mercury was primarily accumulated in muscles of fish ($P < 0.05$) (Ružín Water Reservoir, Slovakia), followed by the liver and kidney (Brázová et al. 2012). Łuczyńska and Krupowski (2009) observed higher values of mercury in fish muscles (except mackerel and flounder) than in the liver and gills. In the case of mackerel and flounder, the content of Hg rose in the following sequence: liver ≈ muscles > gills and muscles ≈ liver ≈ gills, respectively ($P \leq 0.05$). The fish species studied by Maršálek et al. (2005) were characterized by diversified accumulation of mercury in organs, because asp, bighead carp and bream had significantly more mercury ($P \leq 0.05$) in the liver followed by muscles and the gonads, whereas roach had significantly higher Hg ($P \leq 0.05$) in muscles and the the liver than in the gonads. A higher content of mercury in muscles than in the kidneys, liver, spleen, ovaries, testes and gills of carp was found by other authors (Čelechovská et al. 2007).

The effect of body weight, total length and condition factor on the content of mercury in selected organs

Among the biological factors, such as life strategies, availability of food or growth rates, the age of an animal as well as the age-related body weight and length are influential. Table 2 shows correlations between the body

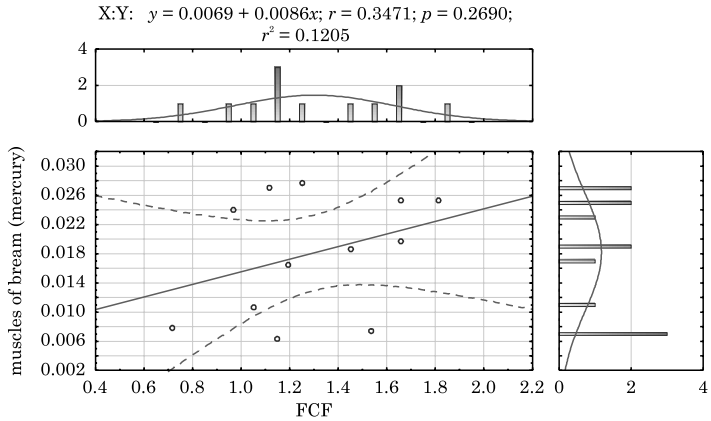
Table 2

Regression equations and linear correlation coefficients (r) between content of mercury in fish organs and body weight or total length

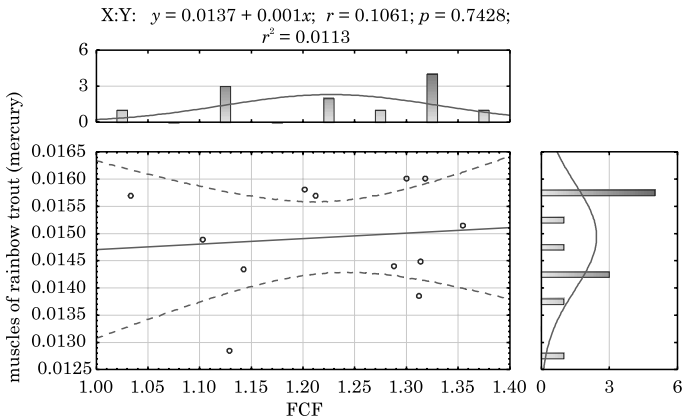
Species	Organs	Body weight (r)	p	Regression equations	Total length (r)	p	Regression equations
Bream $n = 12$	muscles	0.424	0.170	$y = 0.0105 + 2.3544 \cdot 10^{-5}x$	0.499	0.099	$y = - 0.032 + 0.0017x$
	gills	0.322	0.308	$y = 0.0018 + 2.9898 \cdot 10^{-6}x$	0.304	0.336	$y = -0.0023 + 0.0002x$
	liver	0.219	0.495	$y = 0.0041 + 4.7421 \cdot 10^{-6}x$	0.175	0.587	$y = -0.0012 + 0.0002x$
Ide $n = 12$	muscles	0.760	**0.004**	$y = -0.1392 + 0.0003x$	0.086	0.790	$y = 0.0532 + 0.0018x$
	gills	0.795	**0.002**	$y = -0.0209 + 3.5733 \cdot 10^{-5}x$	0.220	0.492	$y = -0.0089 + 0.0006x$
	liver	0.777	**0.003**	$y = -0.0636 + 0.0001x$	0.089	0.782	$y = 0.0157 + 0.0007x$
Rainbow trout $n = 12$	muscles	0.294	0.354	$y = 0.0131 + 9.4599 \cdot 10^{-6}$	0.298	0.346	$y = 0.0086 + 0.0003x$
	gills	0.424	0.169	$y = 0.0042 + 1.1933 \cdot 10^{-5}x$	0.270	0.396	$y = 0.0015 + 0.0002x$
	liver	0.294	0.353	$y = 0.0132 + 1.6567 \cdot 10^{-5}$	0.210	0.512	$y = 0.0087 + 0.0003x$
Carp $n = 6$	muscles	0.836	**0.038**	$y = -0.0018 + 6.7475 \cdot 10^{-6}$	0.529	0.280	$y = -0.0264 + 0.0009x$
	gills	0.317	0.540	$y = 0.0008 + 5.2499 \cdot 10^{-7}$	-0.491	0.322	$y = 0.0077 – 0.0002x$
	liver	0.657	0.156	$y = 0.0013 + 1.7657 \cdot 10^{-6}x$	0.009	0.987	$y = 0.0032 + 5.0302 \cdot 10^{-6}x$
Flounder $n = 5$	muscles	0.773	0.125	$y = -0.024 + 0.0003$x	0.578	0.307	$y = -0.044 + 0.0036x$
	gills	0.661	0.224	$y = -0.0049 + 6.432 \cdot 10^{-5}x$	0.757	0.139	$y = -0.0231 + 0.0014x$
	liver	0.798	0.104	$y = -0.0846 + 0.0004x$	0.618	0.267	$y = -0.1202 + 0.0056x$
Perch $n = 6$	muscles	0.605	0.203	$y = -0.1302 + 0.0005x$	0.420	0.407	$y = -0.5444 + 0.0217x$
	gills	0.514	0.297	$y = -0.045 + 0.0001x$	0.393	0.442	$y = -0.1614 + 0.0057x$
	liver	0.604	0.204	$y = -0.0174 + 9.8366 \cdot 10^{-5}x$	0.409	0.421	$y = -0.0931 + 0.004x$

n – number of fish; p – significance levels

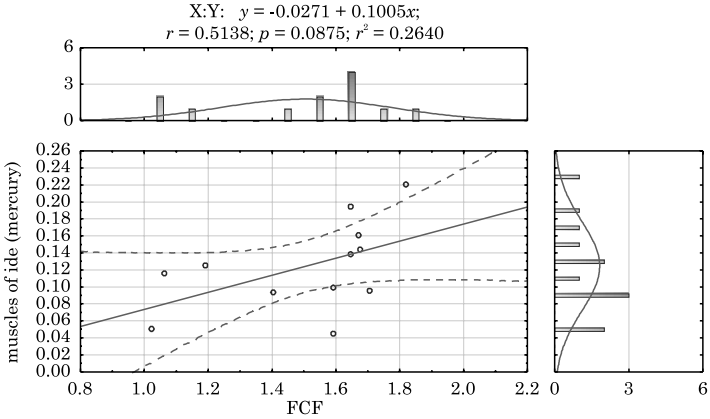
a


X:Y: y = 0.0069 + 0.0086x; r = 0.3471; p = 0.2690;
r² = 0.1205
muscles of bream (mercury)
FCF

b


X:Y: y = 0.0137 + 0.001x; r = 0.1061; p = 0.7428;
r² = 0.0113
muscles of rainbow trout (mercury)
FCF

c


X:Y: y = -0.0271 + 0.1005x;
r = 0.5138; p = 0.0875; r² = 0.2640
muscles of ide (mercury)
FCF

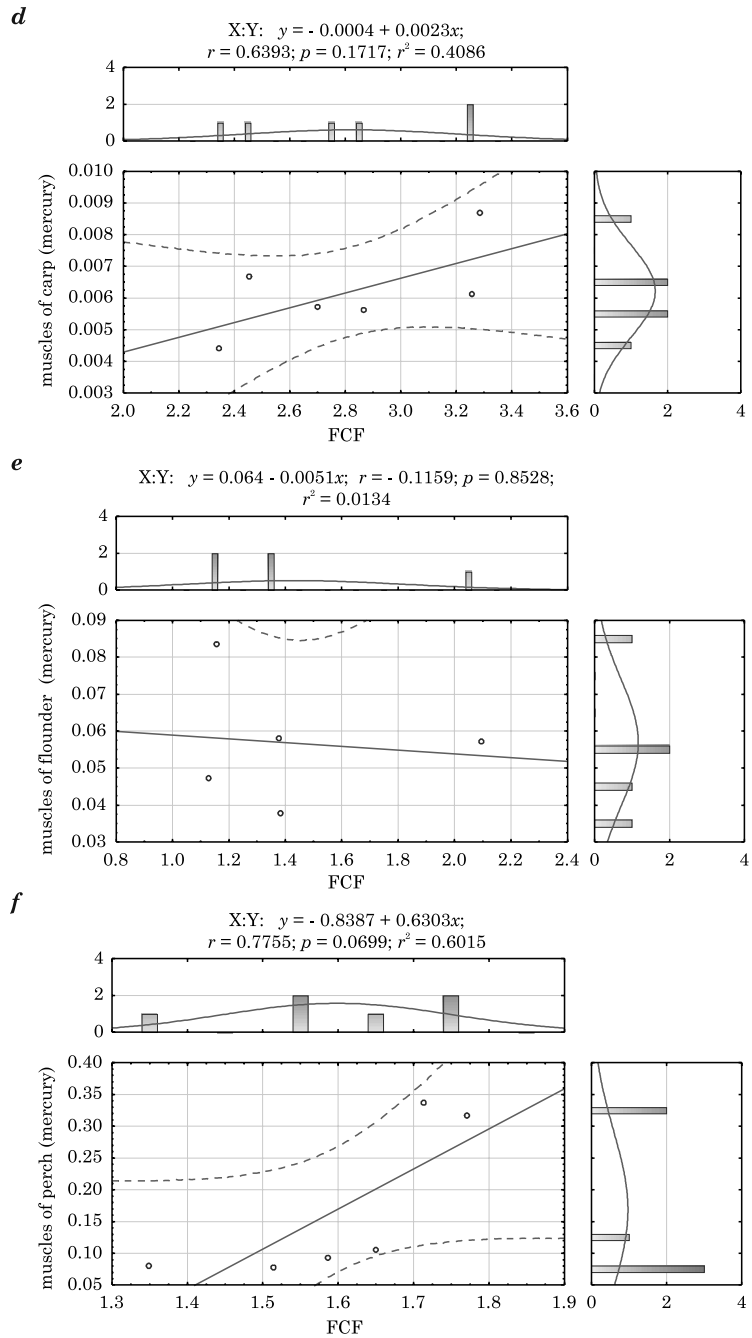
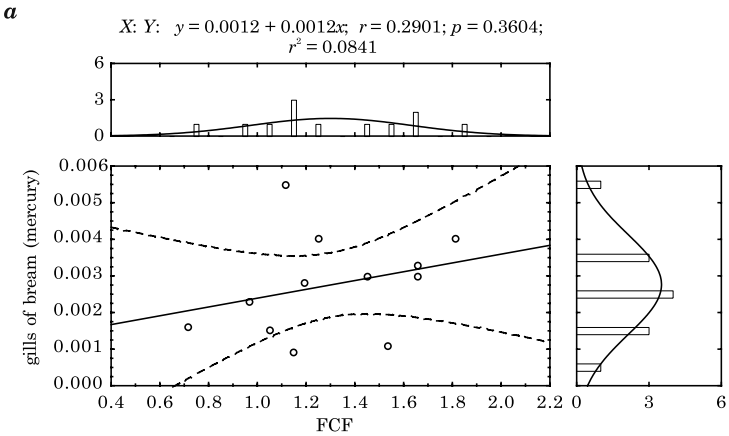
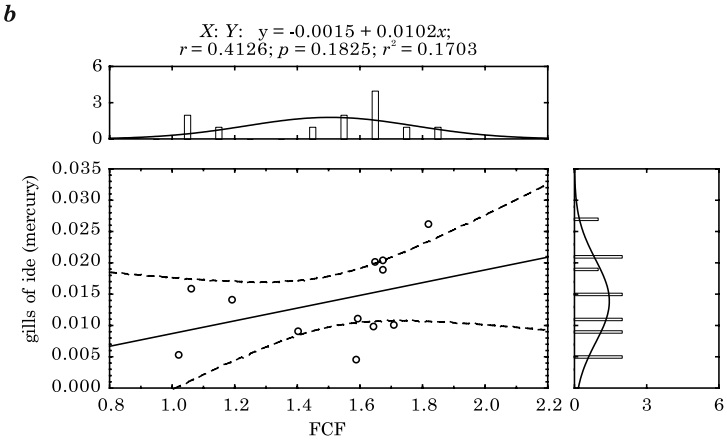


Fig. 2. Relationship between the Fulton's condition factor and the content of mercury (mg kg^{-1} wet weight) in muscles of: *a* – bream, *b* – ide, *c* – rainbow trout, *d* – carp, *e* – flounder, and *f* – perch; *p* – significance level; *r* – correlation coefficient

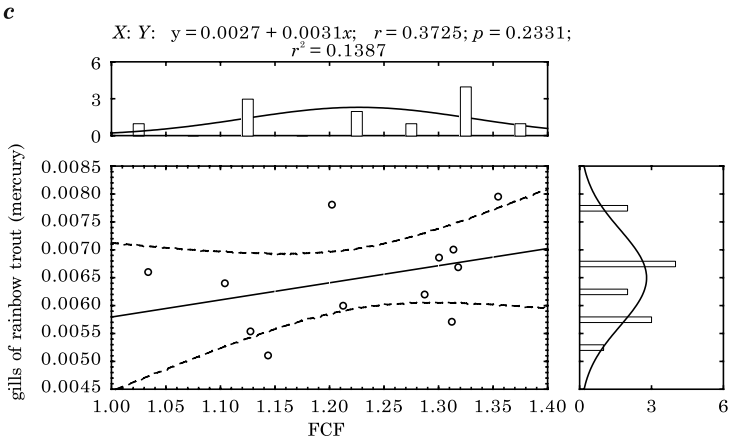
a


X: Y: y = 0.0012 + 0.0012x; r = 0.2901; p = 0.3604;
r² = 0.0841
gills of bream (mercury)
FCF

b


X: Y: y = -0.0015 + 0.0102x;
r = 0.4126; p = 0.1825; r² = 0.1703
gills of ide (mercury)
FCF

c


X: Y: y = 0.0027 + 0.0031x; r = 0.3725; p = 0.2331;
r² = 0.1387
gills of rainbow trout (mercury)
FCF

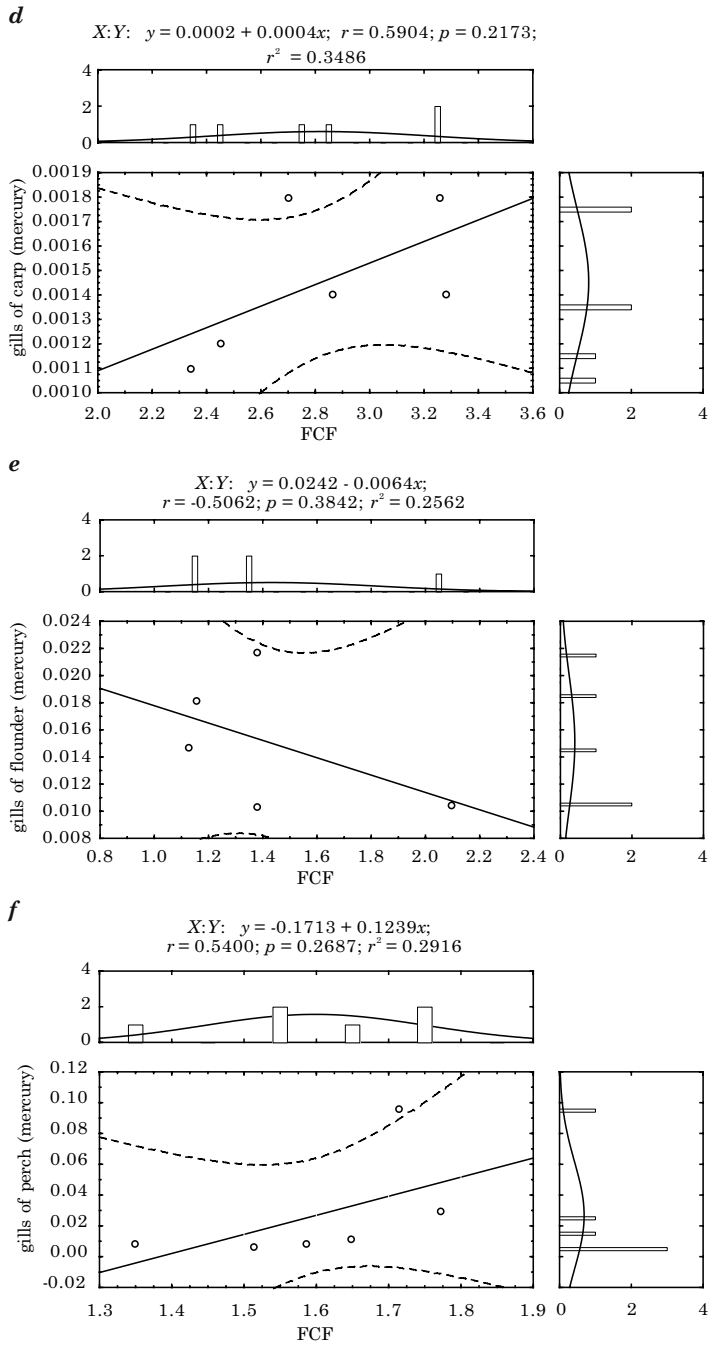
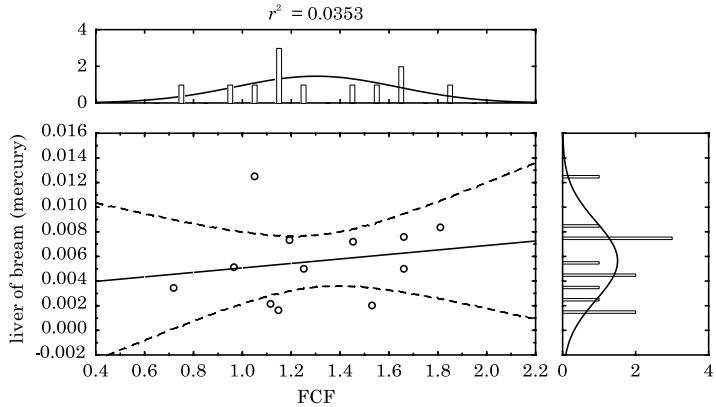
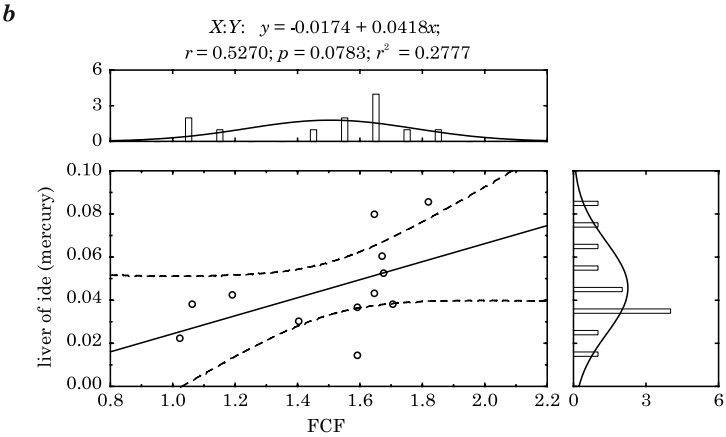


Fig. 3. Relationship between the Fulton's condition factor and the content of mercury (mg kg^{-1} wet weight) in gills of: *a* – bream, *b* – ide, *c* – rainbow trout, *d* – carp, *e* – flounder, and *f* – perch; *p* – significance level; *r* – correlation coefficient

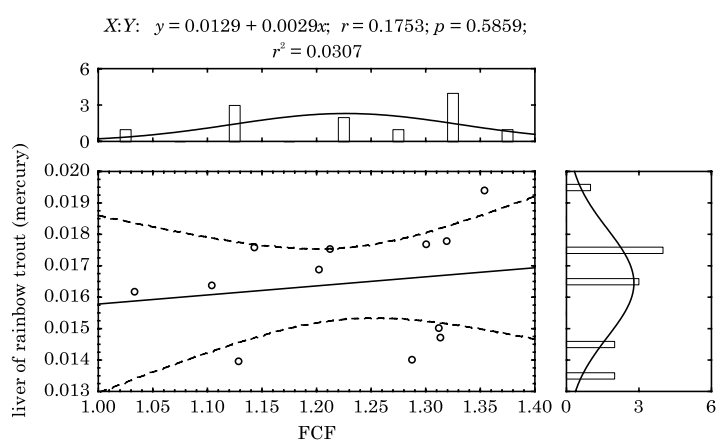
a


X:Y: y = 0.0032 + 0.0018x; r = 0.1879; p = 0.5586;
r² = 0.0353
liver of bream (mercury)
FCF

b


X:Y: y = -0.0174 + 0.0418x;
r = 0.5270; p = 0.0783; r² = 0.2777
liver of ide (mercury)
FCF

c


X:Y: y = 0.0129 + 0.0029x; r = 0.1753; p = 0.5859;
r² = 0.0307
liver of rainbow trout (mercury)
FCF

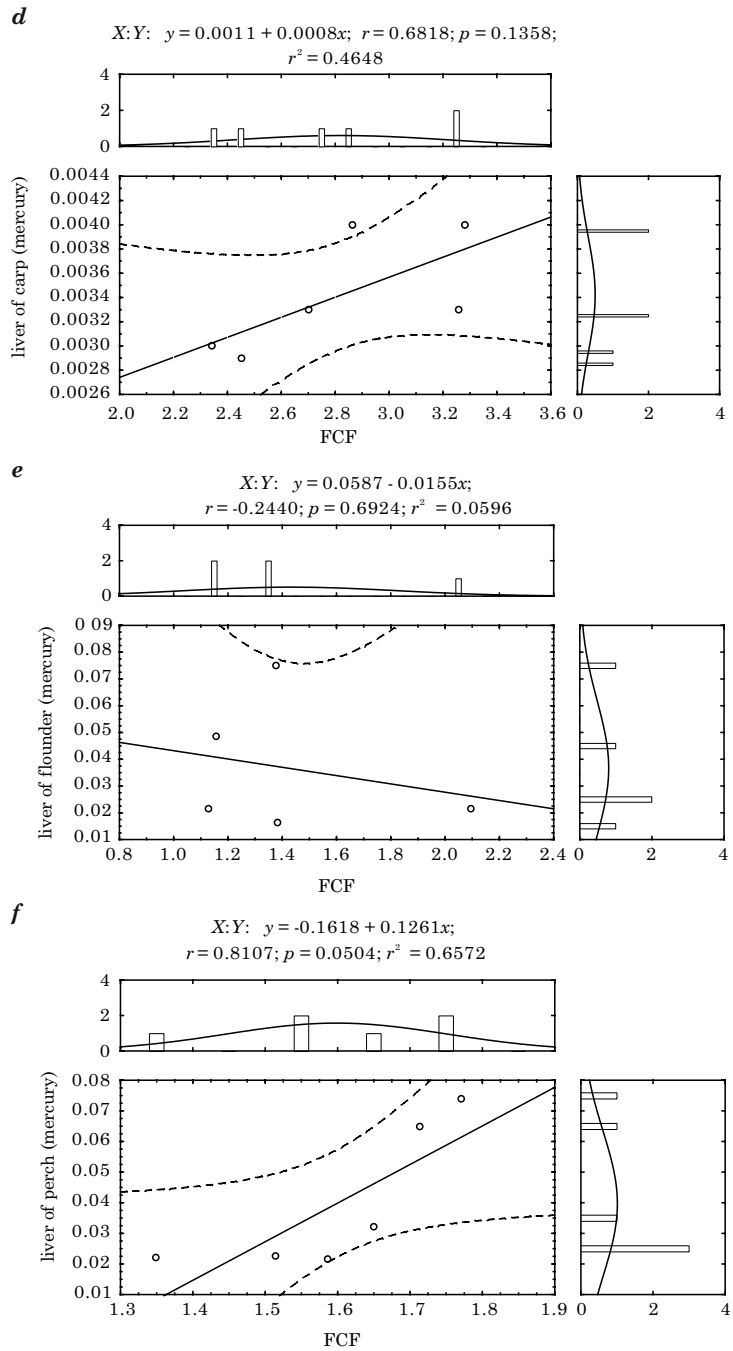


Fig. 4. Relationship between the Fulton's condition factor and the content of mercury (mg kg^{-1} wet weight) in liver of: *a* – bream, *b* – ide, *c* – rainbow trout, *d* – carp, *e* – flounder, and *f* – perch; *p* – significance level; *r* – correlation coefficient

weight and length and the concentration of total mercury in organs of fish. A significantly positive correlation was observed between the weight and levels of muscles, liver and gills of ide ($r = 0.760$, $P \leq 0.004$; $r = 0.777$, $P \leq 0.003$; $r = 0.795$, $P \leq 0.002$, respectively). Moreover, the concentration of Hg increased significantly with the body weight of carp ($r = 0.836$; $P \leq 0.038$). Positive correlation coefficients were determined between the Hg content and body weight ($0.219 < r < 0.798$) and length ($0.009 < r < 0.757$) of the other fish examined (except the gills of carp), but the correlations were weak or statistically insignificant ($P > 0.05$). For the gills of carp, the content of Hg decreased as the total length increased ($P > 0.05$). The content of mercury was most often positively correlated with the factor condition of fish, although the correlation was not statistically significant ($0.116 < r < 0.811$; $P > 0.05$) (Figures 2, 3 and 4). Negative correlation coefficients were determined between the Hg content and the factor condition for flounder, reaching $r = -0.116$, $r = -0.244$, $r = -0.506$ ($P > 0.05$) for the muscles, liver and gills of this fish, respectively. No correlation between the content of Hg in muscles and the length of flounder was determined by Kress et al. (1999). On the other hand, Mazej et al. (2010) reported a positive correlation between the Hg content and length of *Abramis brama danubii* and *Carassius auratus gibonii*. Mercury content also significantly increased with the total length and weight of fish studied by Farkas et al. (2000) and Zrnčić et al. (2013). Generally, larger fish had more mercury than smaller species (Castro et al. 2002, Cizdziel et al. 2002, Jewett et al. 2003). Farkas et al. (2003) observed that the correlation between Hg in the liver and muscles of bream and the factor condition was negative.

CONCLUSIONS

This study showed that the content of mercury varied between the same organs in different species and between different organs in the same species. The muscles of perch, which is the 3rd order consumer in the trophic pyramid, had more mercury than the other examined species. The second highest Hg content in muscles was determined in ide. In contrast, carp, a representative of cultured fish, was less intoxicated by mercury than the other fish. Generally, the muscles of fish contained the highest amount of mercury. The content of mercury was most often positively correlated with the body weight, total length and factor condition of fish, although the correlations were not statistically significant.

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