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Mercury distribution in muscles and internal organs of the juvenile and adult Baltic cod (*Gadus morrhua callarias* Linnaeus, 1758)

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Abstract

Cod (*Gadus marrhua* L), a fish caught in the Baltic Sea, is very popular with consumers. Research on the distribution of mercury in cod tissues and organs was conducted on a group of adult (27) and juvenile (49) individuals in the years 2006-09. Total mercury concentration values in mature cod were always, on average, 1.7 times higher than those in juveniles. The highest Hg_T concentrations were found in the heart, while the lowest ones were found in the gills and gonads. The essential age-specific differences manifest in a relationship between the mercury concentration in fish muscles and brain. Mature individuals, i.e. of length >80 cm, accumulated Hg in muscles, most likely in an attempt to protect the nervous system from toxic exposure. In young individuals, more mercury was concentrated in the brain than in the muscles.. The distribution of Hg_T in organs as well as the low value of the $[Hg_T]_{liver}/[Hg_T]_{muscle}$ ratio testify to relatively low-level mercury contamination in southern Baltic waters.

INTRODUCTION

The Baltic cod (*Gadus morrhua callarias* Linnaeus, 1758), one of the subspecies of the Atlantic cod, is a predatory fish. Its diet mostly consists of invertebrates and fish. Adult individuals commonly eat members of their own species. Young cod dwell in shallow coastal waters, while the adults stay in the open sea where they usually feed near the boom, moving to deep waters in spring. Optimal water temperature for cod ranges from 2 to 10°C. Cod from the Baltic Sea reach sexual maturity quickly, i.e. between the second and third year of life in males, and between the third and fourth year in females, when the body length is 40-50 cm (Kosior 2001). Cod, sprat, and herring are the three most commercially important species caught in the Polish Exclusive Economic Zone (Polak- Juszczak 2009). Cod is particularly valued because of its delicate and protein-rich meat. On the other hand, cod and other seafood constitute a source of toxic mercury for consumers.

Mercury is transported to the marine environment from natural and anthropogenic sources, mainly by atmospheric deposition or land runoff (Fitzgerald and Clarkson 1991, US EPA, 2002). With the participation of microorganisms, inorganic mercury in the water column and sediment undergoes a conversion into methyl mercury (MeHg). Methyl mercury concentration in marine organisms often exceeds 90% of the accumulated total mercury (Hg_T). The processes of bioaccumulation and biomagnification of this organic form of Hg in the marine trophic chain result in increases in its concentration in predatory fishes, such as cod (Fitzgerald and Mason 1997, Kasper et al. 2009, Morel et al. 1998). Mercury enters fish mainly via a food pathway; mercury is generally identified as forming complexes with L-cysteine,

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which is probably incorporated in large peptides and proteins (Harris et al. 2003, Kasper et al. 2009). Many biotic factors influence the concentration and distribution of this toxin in fish, including trophic level, feeding ground, age (Burger and Gochfeld 2007, Kasper et al. 2009), and probably physiological condition. Some of the abiotic factors influencing the concentration and distribution of mercury are water temperature, salinity, pH, and dissolved organic carbon (DOC) (Belger and Forsberg 2006, Kehrig et al. 2001, Svobodová et al. 1999).

The aim of the presented work was to determine the amount of accumulated mercury in Baltic cod, and to study the differences in mercury distribution in their tissues and organs depending on the age, length, and physiological condition of the fish.

MATERIALS AND METHODS

Seventy-six Baltic cod (*Gadus morrhua callarias*) caught in southern Baltic waters between November 2006 and December 2009 were analyzed. After transporting the fish to the laboratory and rinsing with deionized water, biometric parameters were collected, i.e. total length (precision: 1 mm) and weight (precision: 1 g). A longitudinal incision was made in the fish body and the internal organs were collected; namely the liver, spleen, heart, kidneys, small intestine, gonads, stomach content, and muscles. Brain and otholits were extracted from the cranium, and gills were taken out. The isolated tissues and organs were homogenized mechanically, placed in ziplock polyethylene bags, and stored at -20°C until further analysis. The age determinations were based on otholit analysis. Total mercury concentrations in the investigated tissues were measured by atomic absorption spectroscopy with the use of an AMA – 254 mercury analyzer.

The method precision was expressed as the variability of repeated determinations, and equaled ca. 10%. Total mercury concentrations were expressed in ng g^{-1} of wet mass. Precision and accuracy of the method was controlled by analyzing the mercury concentration in the certified biological reference material, i.e. QTM057BT and QTM055BT (Quasimene Laboratory Performance Studies Trace Metals in biota). The obtained error equaled 4%. The detection limit for Hg is 0.05 Hg ng g^{-1}.

Data were analyzed by using StatSoft *STAISTICA 8* statistical software. Fish were split into two analytical groups by age. Adult individuals (n=27) that had reached sexual maturity were in the first group. The second group consisted of juvenile fish (n=49). The first group contained two subgroups: individuals caught during the spawning season between March and August (n=17), and those collected outside the spawning season, between September and February (n=10). The Shapiro–Wilk's test was used to assess the normality of the data, while the correlations between ranks were checked with the Spearman's rank correlation test. The Mann-Whitney U test was applied to evaluate the statistical significance of the difference between two independent samples. All null hypotheses were tested at the significance level of $p<0.05$.

RESULTS

Because the distribution of Hg_T in the investigated tissues and organs of the Baltic cod was non-parametric, a median was used for analyzing the data. The presence of mercury was detected in all organs of the analyzed fish (Table 1). The highest Hg_T concentrations in both mature and juvenile cod were observed in the heart, muscles, and brain. The Hg_T concentrations in all tissues of mature fish were higher than those in juvenile tissues; the concentration differences were statistically significant ($p=0.00$).

The increase in Hg_T concentration in mature fish as compared to juveniles was not proportional. For the age ratio of 1.3, the concentrations in the specific organs and tissues of adults were, on average, 1.7 times higher. The largest concentration increases were found in kidneys, muscles, gills, heart, spleen and brain, and amounted to 2.56, 2.07, 1.98, 1.90, 1.71, and 1.54 times, respectively. The concentration increases in the liver and gonads of older fish were lower, and equaled 1.47 and 1.44, respectively. Mercury in the muscle tissues of the Baltic cod in both groups was distributed unevenly, displaying a decreasing trend from the head to the tail. The lowest Hg_T concentrations were found in the gonads and gills. The Hg_T concentrations in the gonads of juvenile and mature fish were 11 and 15 [ng g^{-1} w.w.], respectively.

Fish caught during the reproductive period were characterized by lower total mercury concentrations in tissues and organs compared to individuals collected outside the spawning season (Table 1; Fig. 1). However, the differences in Hg_T distribution in organs were not statistically significant ($p>0.05$).

A ratio of total mercury in liver to total mercury in muscle tissue was calculated for both subgroups

Table 1

Statistical characteristics of biometric parameters of fish and total mercury concentration values, Hg_T [ng g^{-1} w.w.] in the organs and tissues of cod caught in the years 2006-2009 in the coastal zone of the southern Baltic.

	Sexually mature cod					Juvenile cod				
	N	$\bar{x}\pm SD$	M	Min	Max	N	$\bar{x}\pm SD$	M	Min	Max
Age	27	4.6 ±1.8	4	3	8	49	2 ±0.4	2	1	3
Lenght (cm)	27	68.41 ±22	62	43	109	49	39 ±4	40	28	51
Weight (g)	27	4046 ±3408	2115	650	11140	49	671 ±226	632	230	1400
Muscles	27	108 ±99	64	14	390	45	31 ±12	31	9	65
Brain	27	100 ±93	55	21	392	46	34 ±16	35	8	76
Heart	27	101 ±75	81	6	264	43	45 ±26	43	8	116
Gills	27	31 ±26	22	3	106	46	13 ±6	11	2	29
Liver	26	52 ±79	25	5	392	41	21 ±18	17	4	103
Spleen	27	75 ±102	40	15	529	39	24 ±10	23	7	60
Intestine	26	52 ±65	26	4	292	28	16 ±7	17	2	32
Kidneys	27	49 ±33	44	12	129	31	19 ±9	17	2	41
Gonads	26	20 ±14	15	6	62	35	12 ±7	11	3	27
Food	19	24 ±23	16	4	84	21	22 ±29	10	5	134
Liver / muscle	26	0.47 ±0.32	0.39	0.16	1.58	41	0.65 ±0.42	0.57	0.24	2.06

Symbols: N- number of samples, $\bar{x}$ - mean value, SD – standard deviation, Min – minimal value, Max – maximal value

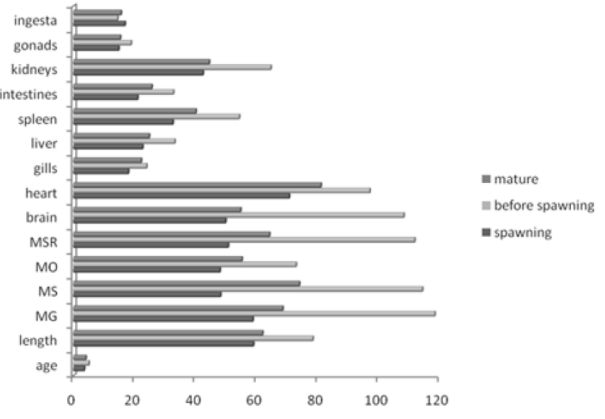

Fig. 1. Median Hg_T [ng g^{-1} w.w.] concentration in the tissues and organs of mature fish, during the spawning season and outside the reproductive period, and mean fish length [cm]. MG – cephalad muscle, MS- mesial muscle, MO- caudal muscle, MSR- mean Hg concentration in the three separate muscle parts

of fish. The highest value of the ratio 0.65, was observed in sexually immature individuals. The ratio decreased with increased fish age. In mature fish the ratio equaled 0.47; during the spawning season it reached 0.55, which was higher by 0.37 compared to the values obtained for the remaining part of the year. The differences between the ratios of total mercury concentration in liver and in muscles were statistically significant.

DISCUSSION

Mercury in predatory fish mainly occurs in the form of methyl mercury, which accounts for 80 – 90% of total mercury present (Boudou and Ribeyre 1983, Houserova et al. 2007, Kasper et al. 2009). Many factors influence the concentration and distribution of mercury in the tissues and organs, including trophic level, feeding habits, metabolic rate (Kasper et al. 2009, Leaner and Mason 2004), and structural and physiological features of each species which may change mercury distribution under similar exposure conditions (Falkowska et al. 2010, Kasper et al. 2009). At this point, the influence of biological barriers, such as intestines, skin, and gills, which control the processes of adsorption and absorption of mercury compounds followed by their transport with blood to target organs, should be stressed (Falkowska et al. 2010, Mason et al. 2000, Wiener et al. 2003).

A food pathway is the main route for methyl mercury to enter the fish body. A high coefficient of transfer across the intestinal wall is characteristic for MeHg; it ranges from 56 to 95%, and in some cases, up to 100%. The coefficient value is influenced by the factors that control the bioavailability of MeHg in the diet of fish (Leaner and Mason 2002). Bioavailability of inorganic mercury is lower and ranges from 10 to 27% (Kasper et al. 2009, Wang and Wong 2003, Wiener et al. 2003). Methyl mercury is transported via blood, mainly in the red blood cells, to other cells in the organism. The spleen directly reflects the mercury content in blood (Baatrupand Danscher 1987, Ciardullo et al. 2008, Giblin and Massaro 1973). The Hg_T distribution in organs of mature Baltic cod was as follows:

heart > muscles > brain > kidneys > spleen > intestines > liver > gills > gonads

In juvenile individuals, the pattern of mercury distribution was the following:

heart > brain > muscles > spleen > kidneys > liver > intestines > gills > gonads

Both patterns show that the waters of the southern Baltic are slightly contaminated. Foster et al. (2000) and Svobodová et al. (1995), who researched the influence of environmental pollution on the mercury distribution in fish organs, have established that in individuals dwelling in slightly contaminated areas the

pattern is as follows:

muscles > kidneys > liver > gonads

Almud et al. (2007), during experimental studies conducted on the Atlantic cod (*Gadus morhua*), proved that MeHg accumulation in muscles begins ca. 10 days after the exposure. At first, the process occurs in internal organs, as confirmed by Leaner and Mason (2004). Methyl mercury, which has a high affinity for thiol groups, is transported in the form of complexes with L-cysteine from other internal organs to muscle tissue where it becomes incorporated into protein chains (Clarkson 1997, Leaner and Mason 2004). In Atlantic cod (*Gadus morhua*) more than 99% of the accumulated mercury was present in the protein fraction (Almud et al. 2007, Harris et al. 2003). This suggests that muscle tissue is a storage place for methyl mercury (Downs et al. 1998, Kasper et al. 2009, Leaner and Mason 2004, Wiener et al. 2003), and therefore a target organ in fish originating from areas with low levels of contamination (Havelková et al. 2008).

In the presented study it has been proven that mercury concentration in the muscles of Baltic cod – as in the case of the Atlantic cod (*Gadus morhua*) (Almud et al. 2007, Luten et al. 1987, Polak-Juszczak 2009) and the Pacific cod (*Gadus macrocephalus*) (Burger and Gochfeld 2007) – is correlated to fish length. The correlation coefficient increased with the increasing length of the Baltic cod, and in the group of mature individuals it reached 0.75 ($p<0.00$). Staveland et al. (1993) demonstrated that mercury concentration in muscles increases with increasing age ($r=0.57$, $p<0.02$), while age shows a statistically significant correlation with fish length ($r=0.74$, $p<0.00$) (Burger and Gochfeld 2007). In the group of mature Baltic cod, the correlation coefficient between fish age and length amounted to 0.93 ($p=0.00$).

Young fish are characterized by a fast growth rate prior to reaching sexual maturity. They inhabit the coastal zone and feed on food that is relatively low in MeHg (Burger and Gochfeld 2007). Considering their diet as well as the fast growth of muscle mass and ca. 10-day delay in MeHg accumulation in muscles, all the listed factors probably influence in a significant way the lower level of MeHg in skeletal muscles of the juvenile Baltic cod. This hypothesis has been confirmed by Chou Chiu (2007) in a study conducted on the Atlantic salmon (*Salmo salar*). Shorter exposure time is also significant. Simoneau et al. (2005) discovered that for a given body length the concentration of mercury in muscles was higher in the fast growing pike (*Sander vitreus*) than in the slower growing individuals. The concentration of Hg_T in muscles of mature Baltic cod was ca. 48% higher than in the juvenile group. Muscles constitute over 60% of the total body weight (Maury-Brachet et al. 2006). Due to the high capacity of muscle tissue for accumulating MeHg, it plays a protective role in fish by lessening the exposure risk to the central nervous system (Falkowska et al. 2010, Wiener and Spry 1996). In the mature cod the concentration of Hg_T in the brain was strongly correlated with the Hg_T concentration in muscles: $r = -0.82$ ($p=0.00$) (Fig. 2). In juvenile fish the relationship between the concentration of Hg_T in brain and in muscles was linear; in mature fish smaller than 80 cm the Hg_T concentration in the brain was characterized by an exponential growth in relation to the Hg_T concentration in muscles. The protective influence of muscles has been demonstrated in fish that were 6 years old or older, and were more than 80 cm long. For these particular individuals, the relationship was defined by the decreasing linear function. The detected regularities should, however, be confirmed by a larger number of analyses.

The Baltic cod spawn from March until August, frequently in the Bornholm Deep area where they find temperature, water oxygenation, and salinity adequate for maintaining the buoyancy of the laid eggs. During the reproduction season the cod's internal body cavity is, to a large degree, filled with gonads. Therefore, the volume of the digestive tract and consequently the amount of food eaten is limited. Lower total mercury concentrations were found in the spleen and intestines during the spawning season even though diets of comparable Hg_T concentrations were consumed. The increased feeding intensity outside the reproduction season resulted in a two-fold increase of Hg_T in muscles; the increase was somewhat lower in the heart, liver, and kidneys. Moreover, the large increase in Hg_T in muscles outside the spawning season might confirm the hypothesis that mercury re-distribution occurs in organs during starvation periods (Boudou and Ribeyre 1996, Wiener et al. 2003).

Liver is a target organ with regard to mercury accumulation in fish dwelling in contaminated areas (Havelková et al. 2008). Higher values of mercury concentration in liver and muscles were found in the Pacific cod, which feeds in deeper water than the Baltic cod (Burger and Gochfeld 2007). The ratio of

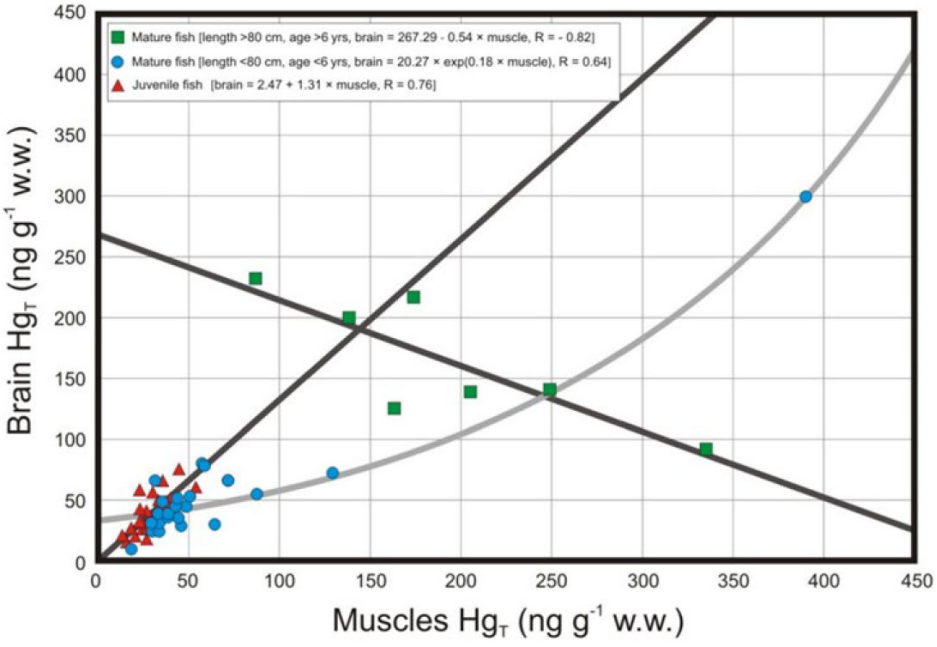

Fig. 2. A relationship between Hg_T concentration in the brain and Hg_T concentration in muscles of the Baltic cod in individuals of different ages and lengths.

Hg_T in liver to Hg_T in muscles, obtained in order to evaluate the Hg accumulation in fish, is <1. A ratio below 0.5 suggests a decrease of Hg in the analyzed fish (Cizdziel et al. 2003). The pattern of Hg_T distribution, as well as the low $[Hg_T]_{liver}/[Hg_T]_{muscle}$ ratio, testify to the fact that the analyzed cod lived in locations with relatively low pollution levels (Foster et al. 2000, Svobodowa et al. 1995).

Among the analyzed fish tissues, gonads were characterized by the lowest total mercury concentration. During the spawning season, Hg_T in gonads remained at a low level, despite the significant growth of these organs. Low mercury concentrations in gonads have been confirmed by other researchers (Kasper et al. 2009, Lindqvist et al. 1991, Maury-Brachet et al. 2006, Svobodowa et al. 1999). Mercury measured in gonads is mainly present as MeHg (Kasper et al. 2009, Wiener and Spry 1996), which in the body of fish occurs in the form of water-soluble complexes containing amino acids with thiol groups (Clarkson 2002). Such amino acids constitute ca. 7% of the proteins present in fish eggs (Block and Weiss 1956), therefore mercury absorption into the gonads is hindered. Gonads eliminate the accumulated mercury when eggs are expelled which results in the transfer of this toxin from adult individuals to the offspring. However, this detoxification mechanism most likely does not play a significant role in cod.

CONCLUSIONS

Low total mercury concentrations measured in the tissues and organs of Baltic cod together with the indicators, such as distribution in organs and the $Hg_{T\text{-}liver}/Hg_{T\text{-}muscle}$ ratio, indicate that the level of mercury pollution in the southern Baltic is relatively low. Nevertheless, mercury concentrations increased with increased fish age, and in sexually mature individuals they were on average 1.7 times higher than juveniles.

In juvenile fish the relationship between the concentration of Hg_T in the brain and in muscles was linear; in mature fish the Hg_T concentration in the brain was characterized by an exponential growth in relation to the Hg_T concentration in muscles. Large individuals (length >80 cm) within the mature cod group (age >6 yrs) were activating a mechanism to protect the nervous system against toxic mercury. In order to lessen brain exposure, they probably accumulated mercury in muscles.

Spawning, which results in the removal of mercury absorbed in roe from the body of female fish, most likely has low significance in the detoxification of the Baltic cod.

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