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Long-term observations of selected heavy metals contained in otoliths of cod from the Southern Baltic

M. Protasowicki[1] and M. Kosior[2]

[1] Academy of Agriculture, Faculty of Marine Fisheries and Food Technology, Szczecin, Poland

[2] Sea Fisheries Institute, Gdynia, Poland

Abstract

Cadmium, lead, copper, chromium .and zinc contents were determined in otoliths of the cod *Gadus morhua* L. from the Southern Baltic. It was shown that the levels of these heavy metals fluctuated during the period of investigation (1969–1985). It was found that levels of Cd, Cu, Cr and Zn decreased with age and age-dependent morphometric parameters, while the amount of lead increased. Sex of cod did not affect the level of bioaccumulation of these five trace elements in the otoliths. With the exception of Cu no differences were found between heavy metal contents in otoliths of cod from the Gdańsk and Bornholm Deep.

Introduction

The Baltic Sea, like many other seas, is subject to anthropogenic pollution with heavy metals. An increase in the level of these trace elements is best visible in studies of bottom sediments. SUESS and ERLENKEUSER (1975) reported that the concentration of heavy metals in sediments increases distinctly towards surface layers. This phenomenon was observed, depending on the area, beginning with sediment layers situated at a depth of 6–20 cm, that is, representing the beginning of our century.

Some authors believe that pollution with heavy metals in the Baltic Sea is steadily increasing and their concentration might increase two- or threefold by the end of this century (JANKOWSKI et al. 1982). Some marine organisms, including fish, are characterized by their ability of bioconcentrating these elements from the environment. The levels of these in the tissues and organs of plants and animals depend on their concentrations in the water. Numerous publications have pointed to the possible use of fish as indicator organisms in investigations of pollution in aquatic environments (JOHNELS et al. 1967, CHODYNIECKI and PROTASOWICKI 1978, PROTASOWICKI and CHODYNIECKI 1980).

In our own past studies on the distribution of trace metals in fish, special attention was paid to the particularly high content of these microelements in fish otoliths (PROTASOWICKI 1986, 1987). A conclusion was put forward that they may constitute good indicator material for investigating trends on the changes of heavy metal levels. An additional factor in favour of this solution is the possibility of obtaining certain quantities of this easy-to-store material from research institutions engaged in the study of fish populations. They also do not require special conservation procedures.

The aim of this paper was to study the changes in the level of Cd, Pb, Cu, Cr and Zn in otoliths of cod from the Southern Baltic within the past 17 years and determine, if the level of these metals in the organism is dependent on site, sex, age, weight and length, as well as otolith weight.

Material and methods

The material for this study consisted of otoliths of cod *Gadus morhua* L. caught in March of 1969–1985 in the eastern part of the Polish fishery zone (Gdańsk Deep). Samples from 1969 were represented by 25 females and 25 males. Their age ranged from 1 to 7 years, total length from 29 to 76 cm, and weight from 225 to 3 670 g. The remaining otolith material came from 3-year old females whose total length ranged from 35 to 42 cm and weight from 355 to 760 g. Material from fish caught in 1975 and 1980 in the Bornholm Deep was used for comparison. Samples for each of these years consisted of otoliths from five fish.

In order to determine dry weight, the otoliths were dried at a temperature of 105° C. Heavy metal levels were determined by flame atomic absorption spectrometry after solution of dried otoliths in 15 % HNO_3. Final volume of the sample was 5 cm^3. Heavy metal contents were calculated on the basis of a calibration curve. The results were subjected to statistical analysis with F test, $F_{max.}$ Hartley's test, Student's t, Welch and Aspin's test, one-way analysis of variance, two-way analysis of variance, correlations and regressions.

Results and discussion

Heavy metal levels were related to dry weight, which constituted between 99.02 and 99.74 % of the material examined. Dehydration of otoliths was a result of long storage at room temperature.

Since the concentration of heavy metals in some tissues and organs is dependent on the sex of fish (PROTASOWICKI 1986, 1987), a comparison of the level of trace metals in the otoliths of cod males and females was made (Table 1). Differences between mean values

Table 1

Comparison of features of male and female cod *Gadus morhua* L. and heavy metal concentrations in their otoliths

Feature	Mean ± SD		Test result*)
	Females	Males	
Age, years	3.6 ± 1.1	3.5 ± 1.1	–
Total length (cm)	44.6 ± 9.1	43.8 ± 9.3	–
Fish weight (g)	1071 ± 709	984 ± 660	–
Otolith weight (g)	0.242 ± 0.116	0.223 ± 0.107	–
Concentration (μg · g^{-1})			
Cd	4.729 ± 0.681	4.506 ± 0.543	–
Pb	28.1 ± 16.1	27.3 ± 20.6	–
Cu	10.9 ± 0.6	11.1 ± 0.9	–
Zn	3.33 ± 0.65	3.25 ± 0.36	–
Cr	208 ± 14	207 ± 14	–

*) A comparison of results was made by t-Student's test, only in the case of Zn Welch and Aspin test was used because of variance non-homogenity

–: insignificant differences (P = 95 %), number of fish n = 25 turned out to be statistically insignificant. Therefore, subjecting the relationship between heavy metal content and age, length, weight of fish and otolith weight to further examination, the data obtained for samples taken from males and females were combined (Table 2).

It was found that levels of Cd, Cu, Zn and Cr in otoliths decreased with age and other morphometric features dependent on age, while the level of lead increased. This relationship was best expressed in case of Cd and Cr, which is attested to by high determination (r^2) and correlation (r) coefficients. The correlation discovered previously referred to heavy metal levels in fish muscles and its weight (CHOW TONG et al. 1974, PROTASOWICKI and CHODY NIECKI1980, PROTASOWICKI et al. 1983). However, as indicated in earlier publications by the author (PROTASOWICKI et al. 1983), this relationship was not a permanent feature; when analysing levels of Cd, Pb, Cu, and Zn in 13 fish species, negative or positive correlations were found in only 15 cases out of the total of 52. It was also found that the level of copper in Baltic cod muscles decreased slightly with an increase in fish weight. The power of this relationship was quite small (r = –0.196).

Table 2

Relationship between heavy metal levels in cod *Gadus morhua* L. otoliths and age (W), length (L), fish weight (Mc) and otolith weight (Mo), y = a + bx

Relationship		Coefficient*)			
		r^2	r	a	b
Cd	– W	0.716	–0.846	6.328	–0.480
	L	0.724	–0.851	7.170	–0.058
	Mc	0.662	–0.814	5.380	–0.742
	Mo	0.716	–0.846	5.717	–4.720
Pb	– W	0.267	0.517	–3.1	8.6
	L	0.382	0.618	–27.0	1.24
	Mc	0.294	0.542	12.7	14.5
	Mo	0.277	0.526	7.5	8.66
Cu	– W	0.432	–0.657	12.7	–0.5
	L	0.476	–0.689	13.6	–0.06
	Mc	0.482	–0.694	11.8	–0.0008
	Mo	0.518	–0.719	12.2	–5.1
Zn	– W	0.381	–0.617	4.35	–0.30
	L	0.406	–0.637	4.91	–0.04
	Mc	0.344	–0.586	3.76	–0.00045
	Mo	0.392	–0.626	3.98	–2.95
Cr	– W	0.619	–0.787	243	–10
	L	0.683	–0.826	263	–1.3
	Mc	0.673	–0.820	224	–0.0168
	Mo	0.717	–0.846	232	–106

*) r^2 : determination coefficient
r : correlation coefficient
a : free term
b : regression coefficient
number of fish n = 50

Table 3

Heavy metal concentrations in otoliths of 3-year old females of cod *Gadus morhua* L. caught in the Gdańsk Deep in 1969–1985

Year	Fish length (cm)	Weight (g) fish	Weight (g) otolith	Concentration ($\mu g \cdot g^{-1}$ dry wt) Cd	Pb	Cu	Zn	Cr
1969	38 ± 1.2	571 ± 46	0.175 ± 0.018[b]	5.16 ± 0.19[bc]	17.9 ± 2.5[d]	11.1 ± 0.6[b]	3.47 ± 0.29[ab]	215 ± 8[a]
1970	38 ± 1.1	532 ± 51	0.155 ± 0.011[c]	5.34 ± 0.27[b]	54.8 ± 4.2[b]	11.5 ± 0.2[ab]	3.19 ± 0.33[b]	207 ± 7[ab]
1971	37 ± 1.6	510 ± 32	0.133 ± 0.019[c]	6.02 ± 0.45[a]	61.5 ± 4.8[a]	11.4 ± 0.5[ab]	3.65 ± 0.53[ab]	210 ± 8[ab]
1972	38 ± 0.9	556 ± 85	0.154 ± 0.016[c]	5.60 ± 0.24[a]	55.3 ± 2.0[b]	11.5 ± 0.4[ab]	3.47 ± 0.16[ab]	210 ± 4[ab]
1973	39 ± 2.4	623 ± 146	0.166 ± 0.026[b]	5.41 ± 0.35[b]	54.8 ± 3.0[b]	11.9 ± 0.3[ab]	3.30 ± 0.28[b]	212 ± 7[ab]
1974	37 ± 1.3	569 ± 64	0.146 ± 0.016[c]	5.51 ± 0.15[b]	57.5 ± 1.7[ab]	12.5 ± 0.2[ab]	3.38 ± 0.11[ab]	217 ± 7[a]
1975	38 ± 1.5	577 ± 73	0.174 ± 0.016[b]	5.31 ± 0.10[b]	54.5 ± 2.2[b]	12.2 ± 0.4[ab]	3.24 ± 0.22[b]	211 ± 3[ab]
1976	37 ± 1.1	575 ± 42	0.179 ± 0.033[b]	5.40 ± 0.43[b]	50.1 ± 4.5[c]	11.4 ± 0.7[ab]	3.88 ± 0.60[ab]	206 ± 7[ab]
1977	37 ± 1.0	577 ± 63	0.152 ± 0.016[c]	5.58 ± 0.26[a]	52.3 ± 5.0[c]	12.5 ± 1.2[ab]	3.38 ± 0.34[b]	203 ± 4[ab]
1978	38 ± 1.1	514 ± 74	0.163 ± 0.021[bc]	5.56 ± 0.37[ab]	54.1 ± 3.0[b]	12.9 ± 0.4[ab]	3.27 ± 0.15[b]	204 ± 7[ab]
1979	38 ± 1.1	519 ± 91	0.178 ± 0.045[b]	5.06 ± 0.63[bc]	48.0 ± 5.7[c]	11.8 ± 0.6[ab]	3.00 ± 0.36[b]	196 ± 3[ab]
1980	37 ± 2.2	510 ± 96	0.189 ± 0.019[b]	5.01 ± 0.48[c]	48.4 ± 1.3[ab]	12.0 ± 1.3[ab]	3.27 ± 0.40[b]	206 ± 26[ab]
1981	38 ± 1.5	586 ± 97	0.223 ± 0.036[a]	4.77 ± 0.31[c]	50.6 ± 3.3[c]	11.5 ± 0.4[ab]	2.90 ± 0.27[b]	196 ± 4[ab]
1982	38 ± 1.1	569 ± 83	0.152 ± 0.014[c]	5.54 ± 0.30[ab]	62.0 ± 5.4[a]	12.7 ± 0.6[ab]	3.22 ± 0.43[b]	213 ± 18[ab]
1983	37 ± 1.3	540 ± 100	0.140 ± 0.017[c]	5.72 ± 0.28[a]	62.1 ± 4.0[a]	13.0 ± 0.8[ab]	4.54 ± 1.35[a]	208 ± 11[ab]
1984	38 ± 1.7	593 ± 116	0.159 ± 0.027[bc]	5.72 ± 0.89[a]	62.0 ± 9.3[a]	13.2 ± 2.5[a]	3.76 ± 0.42[ab]	212 ± 39**
1985	37 ± 1.5	628 ± 89	0.171 ± 0.048[b]	5.31 ± 0.42[b]	58.0 ± 4.2[b]	12.6 ± 0.6[ab]	3.28 ± 0.26[b]	190 ± 11[b]

Symbols used: mean ± standard deviation (letters a–d denote groups of homogeneous means, when letter absent, means do not differ statistically, ** : mean excluded from analysis because of its excessive variance – F_{max} Hartley's test), number of fish n = 5

Knowning about the above relationships, it was decided to collect material from 3-year old females, characterized by the smallest gap between total length and weight, for long-term observations.

Heavy metal levels in otoliths of fish caught in the Gdańsk Deep in 1969–1985 are presented in Table 3. The above data indicate that the length of fish from which the otoliths examined were taken, ranged from 34 to 42 cm and weight from 390 to 760 g, with otolith weight being 0.102 – 0.283 g. Cadmium levels in the otoliths were 4.28 – 7.23, lead 14.5 – 78.1, copper 10.4 – 17.6 zinc 2.52 – 6.25, and chromium 172 – 238 $\mu g \cdot g^{-1}$ dry weight.

One-way variance analysis revealed statistically significant differences in mean heavy metal levels in otoliths of cod taken in 1969–1985. Therefore, on the basis of Tukey's multiple confidence interval, groups of homogeneous means were distinguished (P = 95 %). The greatest differences between mean minimum and maximum concentrations were revealed in the case of lead. It is, nevertheless, difficult to explain the sudden rise in the level of this trace element from 17.9 ± 2.5 $\mu g \cdot g^{-1}$ in otoliths from 1969 to 54.8 ± 4.2 $\mu g \cdot g^{-1}$ in samples from the following year. It should also be added that after 1970, mean lead levels in the otololiths fluctuated between 48 ± 5.7 and 62.1 ± 4.0 $\mu g \cdot g^{-1}$ dry weight. The changes in the levels of the other elements were much smaller and no rapid increase similar to that of lead after 1969 was observed.

Beginning the analysis of trends in changes of heavy metal levels in 1969–1985, a check was made whether the assumption about homogenity of the material was fulfilled. Data presented in the table 3 indicate that 3-year old cod females, from which the otoliths studied were collected, were homogeneous with regard to total length and weight. It turned out, however, that otoliths weights from different years exhibited statistically significant differences. Therefore, taking into account the relationship between otolith weight and level of heavy metals, mentioned above (Table 2), mean values of trace elements in a "standard otolith" of 3-year old females were calculated in order to eliminate the influence of changes caused by the different weight of these structures. The calculations were carried out according to linear regression equations determined for each root (Table 2) with a general formula $y = a + bx$; coefficient "a" was replaced by mean metal level in otoliths from a given year, "x" was the difference between mean weight of those otoliths and the weight of a "standard otolith". The weight of a "standard otolith" was assumed to be the mean value calculated on the basis of the weight of 85 otoliths examined, equalling 0.165 g.

The results of these calculations presented in Table 4 served for an analysis of the trend in changes of heavy metal levels in otoliths in the period under study, with the independent variable, that is, time, being assigned the values from 1 to 17.

The analysis revealed that changes in cadmium and zinc levels in otoliths of cod from the Gdańsk Deep in 1969–1985 did not exhibit any permanent trend (Table 5). The trend

Table 4

Mean levels of heavy metals in "standard otoliths" of 3-year old females of cod *Gadus morhua* L. caught in the Gdańsk Deep in 1969–1985

Year	Cd	Pb	Cu	Zn	Cr
1969	5.21	17.8	11.2	3.50	216
1970	5.29	54.9	11.4	3.16	206
1971	5.87	61.8	11.2	3.56	207
1972	5.55	55.4	11.4	3.44	209
1973	5.41	54.8	11.9	3.30	212
1974	5.42	57.7	12.4	3.32	215
1975	5.31	54.4	12.2	3.27	212
1976	5.47	50.0	11.5	3.92	207
1977	5.52	52.4	12.4	3.34	202
1978	5.55	54.1	12.9	3.26	204
1979	5.12	47.9	11.9	3.00	197
1980	5.12	48.2	12.1	3.34	209
1981	5.04	50.1	11.8	3.07	202
1982	5.48	62.1	12.6	3.18	212
1983	5.60	62.3	12.9	4.47	205
1984	5.69	62.1	13.2	3.74	211
1985	5.34	57.9	12.6	3.30	191

Standard otolith: otolith with a weight of 0.165g (mean for 85 fish examined)

Table 5.

Estimation of trends in changes of hearvy metal levels (y) in otoliths of cod *Gadus morhua* L. caught in the Gdańsk Deep in the 1965–1985 period.

Trace metal	Coefficient			
	r^2	r	a	b
Cd	0.002	–0.045	5.43	–0.002
Pb	0.178	0.422	45.40	0.860
Cu	0.606	0.778++	11.20	0.097
Zn	0.025	0.158	3.32	0.011
Cr	0.236	–0.486+	212	–0.620

r^2 : determination coefficient
r : correlation coefficient:
+ significant correlation, ++ highly significant correlation, no sign insignificant correlation:
a : free term
b : regression coefficient
n = 17
y = a + bt, where t should be assigned values from 1 upwards

pointing to an increase in lead level turned out to be statistically insignificant (P = 95 %). On the basis of the other data one may speak of a slight upward trend in copper level and a downward one in chromium level. Thus, if trace metal level in cod otoliths is assumed to be an indicator of marine environmental pollution, one may speak of an increase in copper concentration and a decrease in chromium concentation in the waters of this part of the Baltic. This does not mean, however, that the concentration of the other metals does not undergo changes. One should remember that a fish organism may control absorption of metals, as a result of which there is no discernible increase in bioaccumulation of these elements although their concentration in water increases to a certain limit.

On the basis of the results obtained for cod otoliths from the Gdańsk and Bornholm Deeps caught in 1975 and 1980, a comparison of cadmium, lead, copper, zinc and chromium levels was made (Table 6).

Two-way variance anylysis according to a random sample model revealed that cod specimens constituted homogenous material as regards total length and weight. The differences among mean otolith weights turned out to be statistically insignificant. As regards heavy metal levels, the comparison revealed that only the differences between areas observed in the case of copper were statistically significant. Otoliths of fish from the Gdańsk Deep exhibited a slightly higher level of this metal. Changes in heavy metal concentrations in time were discussed above. The observed absence of differences between the area of capture is in agreement with the results of earlier studies of concentrations of Cd, Pb and Zn in the muscles of Baltic cod by the author (PROTASOWICKI 1982, 1987). Unlike the present study, the papers mentioned above revealed also the absence of any significant differences in copper levels in cod from the same capture sites.

In conclusion it should be said that otoliths are good material for studying trends in changes of trace metal levels in fish as well as the differences in their concentration among different areas.

Table 6

Comparison of features of 3-year old cod *Gadus morhua* L. from the Gdańsk Deep (1) and Bornholm Deep (2) and heavy metal levels in their otoliths

Feature	Year	Mean ± SD: Gdańsk Deep	Mean ± SD: Bornholm Deep	Significance of differences depending on: capture site	Significance of differences depending on: capture time
Total length (cm)	1975	38 ± 1.5	38 ± 3.7	–	–
	1980	37 ± 2.2	39 ± 1.1		
Fish weight (g)	1975	577 ±73	558 ±140	–	–
	1980	510 ±96	572 ± 45		
Otolith weight (g)	1975	0.174 ± 0.016	0.180 ± 0.037	–	–
	1980	0.189 ± 0.019	0.216 ± 0.036		
Concentration ($\mu g \cdot g^{-1}$)					
Cd	1975	5.31 ± 0.10	5.30 ± 0.47	–	–
	1980	5.01 ± 0.48	4.53 ± 0.30		
Pb	1975	54.5 ± 2.2	54.6 ± 2.9	–	+
	1980	48.4 ± 1.3	49.2 ± 3.0		
Cu	1975	12.2 ± 0.4	11.6 ± 0.5	+	–
	1980	12.0 ± 1.3	11.4 ± 0.2		
Zn	1975	3.24 ± 0.22	3.47 ± 0.52	–	–
	1980	3.27 ± 0.40	2.95 ± 0.22		
Cr	1975	211 ± 3	207 ± 6	–	–
	1980	206 ±26	194 ± 6		

Comparison was made on the basis of two-way variance analysis according to random model, –: insignificant differences, +: significant differences (P = 95 %), n = 5

Conclusions

1. The sex of cod does not affect the level of Cd, Pb, Cu, Zn and Cr in their otoliths.
2. The levels of Cd, Cu, Zn and Cr in the otoliths decreases with an increase in age, length, weight of fish and their otoliths, while the level of lead content increases.
3. The level of these metals in otoliths of cod from the Gdańsk Deep fluctuate considerably during the 17 years considered. A statistically significant trend is revealed in the case of Cu (increase) and Cr (decrease). Contents of cadmium and zinc do not exhibit any trends while an upward trend in lead levels turned out to be statistically insignificant.
4. Except for Cu, no differences are observed between the levels of these metals in otoliths of fish from the Gdańsk Deep and Bornholm Deep.

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