Proc. Estonian Acad. Sci. Biol. Ecol., 2000, **49**, 4, 327–334
https://doi.org/10.3176/biol.ecol.2000.4.03

CHANGES IN COPPER AND CADMIUM CONTENT OF MALE HERRING IN RELATION TO SPAWNING

Mart SIMM* and Jonne KOTTA

Estonian Marine Institute, Viljandi mnt. 18B, 10617 Tallinn, Estonia

Received 23 March 2000, in revised form 1 June 2000

Abstract. Copper and cadmium concentrations were determined in the muscles, liver, and gonads of male herring caught in the middle part of the Gulf of Finland, eastern Baltic Sea. Fish age did not affect the trace metal concentrations in muscles and gonads, whereas the concentrations were significantly higher in the liver of 5–7 year old fish as compared to 2–4 year old fish. The concentration of trace metals in herring gonads depended on their maturity stage and most pronounced changes occurred during spawning. The excretion of trace metals during spawning comprised 15–30% of the total content of copper and cadmium in the muscles, liver, and gonads of male herring. Relative amounts of copper and cadmium excreted during spawning were higher for 2–4 year old than 5–7 year old fish.

Key words: age, cadmium, copper, herring, maturity stage of gonads, Baltic Sea.

INTRODUCTION

Since the 1970s the concentration of trace metals has been systematically studied in the fish of the Baltic Sea. Traditionally samples of muscle tissue have been used in monitoring (Haahti, 1991; HELCOM, 1996; Jankovski et al., 1996; Polak-Juszczak, 1997; Leivuori et al., 1998). It has been concluded that in order to obtain more accurate data on spatial and temporal trends the concentration of trace metals should be measured in different organs and tissues of fish, e.g. in liver or kidneys, where their concentrations are higher than in muscles.

The concentration of trace metals has been also analysed in the gonads and fry of fish (Seisuma et al., 1984; Draganik et al., 1995; Jankovski et al., 1996). Seasonal variation may be an important factor in these analyses. During the

* Corresponding author; mart@klab.envir.ee

development of gonads, their volume increases considerably, exceeding for example 30% of the total weight of herring just before spawning. Consequently, a significant amount of trace metals is excreted during spawning. Hence, besides trace metal background values in the ambient environment their content in gonads depends on the maturity stage of fish. There is some evidence that the trace metal concentrations reflect the physiological state of fish in terms of breeding cycle (Essink, 1980; Draganik et al., 1995; Larsson et al., 1996; Stange et al., 1996).

The concentration of trace metals can be determined in the eggs or fry of female fish. As to the sexual products of male fish, i.e. milt, this is more complicated. Based on the trace metal concentrations in gonads before and after spawning we estimated the changes in the copper and cadmium content during spawning. The estimates in trace metal concentrations in muscles and liver made it possible to analyse the approximate proportion of the excreted trace metals in their total content in fish tissues.

MATERIAL AND METHODS

Samples of herring (*Clupea harengus membras* L.) were collected from sea areas adjacent to Tallinn Bay, the Gulf of Finland, in different months during 1994–96. The total length and wet weight of fish were determined immediately after the catch and the otholiths for age determination were separated. The fish were kept at –20 °C until further analysis. In the laboratory, the fish were sorted according to sex, age, and developmental stage of gonads. The common classification into six stages was followed. Stage I (juveniles) was neglected due to the uncertainty of sex determination and small size of gonads. Stage II (individuals which are becoming mature) and stage VI (spent individuals) were pooled together as stage (VI)II. Stages III and IV refer to mature but prespawning fish and stage V to spawning individuals.

The concentrations of copper and cadmium were determined in the herring lateral muscle tissues, liver, and gonads. A sample consisted of 10–20 pooled specimens of male herring of the same age. From each specimen we took 3–10 g pieces of the right-hand-side lateral muscle and the entire liver and gonads.

The dry weight of samples was determined after drying the material at 90 °C for 8 h. The samples of dried tissue and organs were homogenized and 2–3 replicate subsamples were analysed. Approximately 0.2–0.3 g of the substance was heated in concentrated nitric acid (suprapur) at 110 °C for 8 h and subsequently the acid was evaporated to a nearly dry residue. Finally, distilled water was added to a final volume of 25 mL. The copper and cadmium concentrations were measured with an atomic absorption spectrophotometer (AAS, Perkin-Elmer 5000; flameless technique; HGA-500). The detection limits for copper and cadmium were 0.05 and 0.01 $\mu g\ g^{-1}$, respectively. Analytical reliability was checked by measurements of commercial certified reference material (CRM 278). Recovery was over 70%.

The concentration of copper and cadmium was estimated from 275 samples: 113 in muscles, 64 in liver, and 98 in gonads. Weights of tissues and organs are given in grams dry weight. The copper and cadmium concentrations are given on the basis of dry weight of tissue or organ ($\mu g\ g^{-1}$). The term "content" refers to the total amount of copper or cadmium in the tissue or organ of an individual fish (in μg).

Statistical differences in trace metal concentrations between different age and maturity classes were calculated by means of analysis of variance (ANOVA). Analysed data followed the assumptions of normality (Kolmogorov–Smirnov test for goodness of fit) and homoscedasticity (Bartlett's and Hartley's tests).

RESULTS

The concentrations of cadmium and copper in the muscles and gonads of male herring were not significantly different between age classes (ANOVA $p > 0.05$). On the other hand, the concentrations in liver increased with age (ANOVA $p > 0.001$). There was a significant difference in the trace metal concentrations in liver between 2–4-year-old (later referred as young) and 5–7-year-old (later referred as old) herrings. Hence, these year-classes were treated separately in the following statistical analysis.

The average concentration of trace metals was significantly lower in muscles and gonads than in liver (ANOVA $p > 0.001$; Table 1). This difference was more pronounced in older fish. The maturity stage had no effect on the trace metal concentrations in muscles and liver (ANOVA $p > 0.05$). On the other hand, the

Table 1. Concentration of copper and cadmium (mean ± SD, $\mu g\ g^{-1}$ dry weight) in different tissues and organs of male herring at different developmental stages of gonads

Tissue/ organ	Developmental stage	2–4 years old			5–7 years old		
		n	Copper	Cadmium	*n*	Copper	Cadmium
Muscles	III	26	1.6 ± 0.3	0.03 ± 0.01	8	1.8 ± 0.2	0.03 ± 0.02
	IV	24	1.6 ± 0.4	0.03 ± 0.01	9	1.8 ± 0.2	0.04 ± 0.01
	V	10	1.6 ± 0.2	0.06 ± 0.01	6	1.9 ± 0.1	0.04 ± 0.01
	(VI)II	26	1.7 ± 0.4	0.04 ± 0.02	4	2.2 ± 0.1	0.02 ± 0.01
Liver	III	14	12.8 ± 2.9	1.83 ± 0.65	3	32.5 ± 2.9	3.99 ± 0.86
	IV	14	16.4 ± 4.6	2.93 ± 0.90	6	34.0 ± 6.9	5.51 ± 1.17
	V	5	15.7 ± 3.8	2.35 ± 0.60	3	32.0 ± 7.4	3.26 ± 0.89
	(VI)II	17	12.4 ± 2.6	2.00 ± 0.77	2	34.0 ± 6.9	5.04 ± 1.46
Gonads	III	22	3.4 ± 0.8	0.10 ± 0.04	8	3.1 ± 0.5	0.08 ± 0.03
	IV	24	2.8 ± 0.6	0.08 ± 0.03	10	2.7 ± 0.5	0.07 ± 0.03
	V	10	2.5 ± 0.3	0.11 ± 0.02	6	2.5 ± 0.2	0.10 ± 0.02
	(VI)II	14	5.5 ± 1.3	0.64 ± 0.13	4	5.3 ± 0.3	0.37 ± 0.06

concentration of trace metals in gonads was significantly higher after spawning, i.e. in developmental stage (VI)II (ANOVA $p > 0.001$; Table 1). During the development of gonads (from stage (VI)II to stage V) their dry weight increased about 18 times (from 0.029 ± 0.016 to 0.530 ± 0.056 g) for young herring and about 10 times (from 0.120 ± 0.087 to 1.250 ± 0.217 g) for older fish. At the same time the weight of muscles and liver remained practically unchanged. The average weight of muscles and liver of young herring was 1.529 ± 0.337 g and 0.014 ± 0.004 g. The relevant figures for older fish were 3.151 ± 0.642 g and 0.051 ± 0.027 g.

The content of copper was significantly higher in muscles than in liver or gonads (ANOVA $p > 0.001$; Table 2). The content of copper in muscles and liver was not affected by the developmental stage of gonads. On the other hand, the content of copper in gonads increased during their development till stage V, i.e. spawning (Table 2).

The total content of copper in muscles, liver, and gonads of a male herring varied between 2.87 and 4.32 μg (average 3.57 ± 1.12) for younger fish and between 7.79 and 11.55 μg (average 9.41 ± 2.63) for older fish. The amount of copper in gonads comprised 5.6–31.0% of that in muscles, liver, and gonads for younger fish and 7.6–28.9% for older fish. Taking into account the content of copper in gonads before and after spawning, the amounts excreted during spawning

Table 2. Content of copper and cadmium (mean ±SD, μg) in different tissues and organs of male herring at different developmental stages of gonads

Trace metal	Age, years	Developmental stage	Muscles	Liver	Gonads
Copper	2–4	III	2.52±0.55	0.19±0.06	0.45±0.12
	2–4	IV	2.67±1.01	0.28±0.14	0.70±0.19
	2–4	V	2.72±0.75	0.26±0.06	1.34±0.22
	2–4	(VI)II	2.54±0.98	0.17±0.06	0.16±0.08
	5–7	III	5.40±1.07	1.55±0.61	0.84±0.31
	5–7	IV	5.22±0.81	1.34±0.76	2.17±0.42
	5–7	V	5.94±1.84	1.89±1.46	3.19±0.70
	5–7	(VI)II	8.44±1.21	2.23±0.30	0.88±0.29
Cadmium	2–4	III	0.04±0.02	0.03±0.01	0.01±0.00
	2–4	IV	0.05±0.02	0.05±0.03	0.02±0.01
	2–4	V	0.09±0.02	0.04±0.01	0.06±0.01
	2–4	(VI)II	0.06±0.03	0.03±0.02	0.02±0.01
	5–7	III	0.09±0.05	0.20±0.13	0.02±0.01
	5–7	IV	0.10±0.03	0.22±0.13	0.06±0.02
	5–7	V	0.12±0.05	0.21±0.20	0.12±0.04
	5–7	(VI)II	0.09±0.06	0.36±0.21	0.06±0.03

were 1.18 μg for younger and 2.31 μg for older fish (Fig. 1). This corresponded to 33.0% (young fish) or 24.6% (old fish) of the average total content of copper in muscles, liver, and gonads.

For younger fish there was no marked difference in the cadmium content between muscles, liver, and gonads. For older fish the cadmium content was somewhat higher in liver than in muscles or gonads (Table 2). Similarly to copper, the cadmium content in muscles and liver was not affected by the maturity of herring whereas that in gonads was highest at stage V, i.e. during spawning (Table 2). Unlike copper the content of cadmium in gonads did not increase with their development, being similar at stages III, IV, and (VI)II. The total content of cadmium in muscles, liver, and gonads varied between 0.08 and 0.19 μg (average 0.12±0.05) for younger fish and between 0.31 and 0.51 μg (average 0.40±0.19) for older fish. The amounts of cadmium in gonads comprised 12.5–31.6% of that of muscles, liver, and gonads together of younger fish and 6.4–26.7% for older fish (Fig. 2). The amounts excreted during spawning were 0.04 μg for younger and 0.06 μg for older fish. This corresponds respectively to 33.3% or 15.0% of the average total content of cadmium in muscles, liver, and gonads.

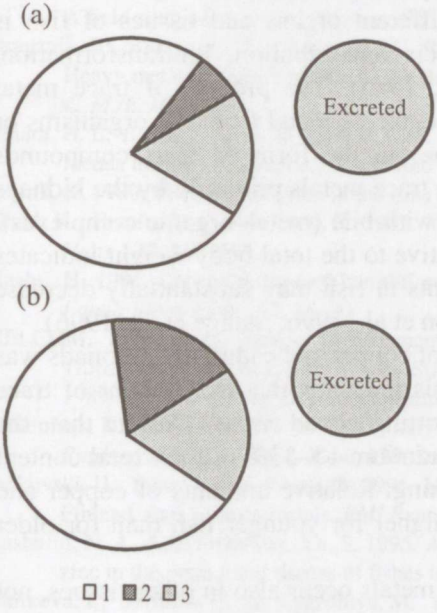

Fig. 1. Average copper content (in percentages) of 2–4- (a) and 5–7-year-old (b) male herring in different tissues and organs and the relative amount excreted during spawning. 1, muscles; 2, liver; 3, gonads.

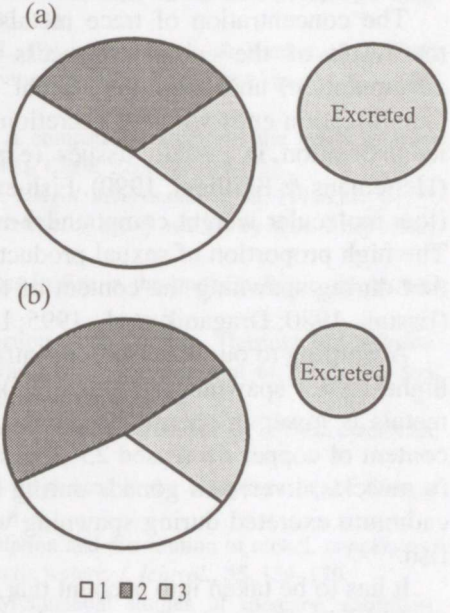

Fig. 2. Average cadmium content (in percentages) of 2–4- (a) and 5–7-year-old (b) male herring in different tissues and organs and the relative amount excreted during spawning. 1, muscles; 2, liver; 3, gonads.

DISCUSSION

Earlier data on age-specific concentration of trace metals in fish are controversial. Both lower (Protasowicki & Chodyniecki, 1986; Vas et al., 1993) and higher (Perttilä et al., 1982; Tervo, 1987) trace metal concentrations have been measured in older fish as compared with younger ones. Some authors claim that there exists no correlation between the age (size) and the trace metal concentration (Kulikova et al., 1987; Voigt et al., 1994; Draganik et al., 1995). In most of these works the trace metal concentrations were determined in muscle tissues where the concentrations were usually very low. Hence, it is difficult to generalize the age-specific dynamics of trace metals.

Our data showed that trace metal concentrations in muscles and gonads of male herring were not affected by age. However, copper and cadmium concentrations in liver increased with age. It is known that the deposition of chemical substances, including trace metals, occurs in certain tissues and organs, including liver, of fish (Protasowicki, 1986; Kashulin & Reshetnikov, 1995; Stange et al., 1996). The fact that trace metal concentration in liver increases with age implies that the uptake rate of metals exceeds their excretion rate. In order to minimize the effect of age on the interpretation of maturity-induced changes in trace metal content, copper and cadmium contents were analysed separately in two homogeneous age groups (2–4- vs. 5–7-year-old herring).

The concentration of trace metals in different organs and tissues of fish is the result of the complex process of their redistribution, biotransformation, accumulation, and excretion (Grahl et al., 1985). The process of trace metal detoxification ends with the excretion of the excess metal from the organisms or its deposition in certain tissues (e.g. bone) in the form of inert compounds (Hellemans & Baillieul, 1990). Fish excrete trace metals primarily by the kidneys (low molecular weight compounds) and/or with bile (metal–organic complexes). The high proportion of sexual products relative to the total body weight indicates that during spawning the content of toxicants in fish may substantially decrease (Essink, 1980; Draganik et al., 1995; Larsson et al., 1996; Stange et al., 1996).

According to our data the concentration of copper and cadmium in gonads was highest after spawning, at stage (VI)II. This indicates that the content of trace metals is lower in sperm than in the supporting gonad tissue. Despite that, the content of copper decreased 25–33% and cadmium 15–33% of their total content in muscles, liver, and gonads during spawning. Relative amounts of copper and cadmium excreted during spawning were higher for younger fish than for older fish.

It has to be taken into account that trace metals occur also in other tissues, not analysed in this study. For example, higher copper and cadmium concentrations have been determined in kidneys (Vas et al., 1993; Camusso et al., 1995) or in peripheral organs and tissues, in skin and especially in gills (Seisuma et al., 1984; Emara et al., 1993; Al-Yakoobi et al., 1994). The proportion of these tissues and organs is relatively low in the total body weight of fish. However, for instance

liver, which constitutes a minor part of the body weight, contributed significantly to the trace metal content of male herring. Consequently, the relative amounts of copper and cadmium excreted during spawning are likely to be smaller than presented above. Nevertheless, such short-term single events when large quantities of trace metals are excreted constitute an essential part of the excretion process of trace metals. Hence, while analysing trace metal concentrations in different tissues and organs of fish, especially in environmental monitoring programmes, the changes occurring during breeding should be taken into account.

ACKNOWLEDGEMENT

This study was partly financed by the Estonian Governmental Programme No. 0200792s98.

REFERENCES

Al-Yakoobi, S., Bou-Olayan, A.-H. & Bahloul, M. 1994. Trace metals in gills of fish from the Arabian Gulf. *Bull. Environ. Contam. Toxicol.*, **53**, 718–725.

Camusso, M., Vigano, L. & Balestrini, R. 1995. Bioconcentration of trace metals in rainbow trout: A field study. *Ecotoxicol. Environ. Saf.*, **31**, 133–141.

Draganik, B., Bykowski, P., Domagala, M., Barska, I., Polak-Juszczak, L. & Kuczynski, J. 1995. Heavy metals in adults and fry of the Baltic viviparous eelpout *Zoarces viviparus* (L.). *ICES C. M./E:16*, 1–20.

Emara, H. I., El-Deek, M. S. & Ahmed, N. S. 1993. A comparative study on the levels of trace metals in some Mediterranean and Red Sea fishes. *Chem. Ecol.*, **8**, 119–127.

Essink, K. 1980. Mercury pollution in the Ems estuary. *Helgol. Meeresunters.*, **33**, 111–121.

Grahl, K., Franfe, P. & Hallebach, R. 1985. The excretion of heavy metals by fish. *Symp. Biol. Hung.*, **29**, 357–365.

Haahti, H. 1991. Concentrations of harmful substances in fish in the northern Baltic. *Mem. Soc. Fauna Flora Fenn.*, **67**, 15–20.

HELCOM, 1996. Baltic Marine Environment Protection Commission – Helsinki Commission. Third Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1989–93; Background Document. *Baltic Sea Environ. Proc.*, **64B**.

Hellemans, K. & Baillieul, M. 1990. Toxicity and accumulation of copper by a freshwater fish, *Rutilus rutilus* L. *Belg. J. Zool.*, **120**, Suppl. 1, 1–37.

Jankovski, H., Simm, M. & Roots, O. 1996. Harmful substances in the ecosystem of the Gulf of Finland. Part I. Trace metals. *EMI Report Ser.*, **4**.

Kashulin, N. A. & Reshetnikov, Yu. S. 1995. Accumulation and distribution of nickel, copper, and zinc in the organs and tissues of fishes in subarctic waters. *J. Ichtyol.*, **35**, 154–170.

Kulikova, I., Seisuma, Z. & Legzdinya, M. 1987. Multiannual studies of mercury, cadmium, chromium, lead, zinc and manganese accumulation and distribution in biotic components of the Riga Gulf ecosystem. *Problemy fonovogo monitoringa sostoyaniya prirodnoj sredy*, **5**, 159–168 (in Russian).

Larsson, P., Backe, C., Bremle, G., Eklöv, A. & Okla, L. 1996. Persistent pollutants in a salmon population (*Salmo salar*) of the southern Baltic Sea. *Can. J. Fish. Aquat. Sci.*, **53**, 62–69.

Leivuori, M., Tikkanen, P., Puhakainen, T. & Riikonen, J. 1998. Heavy metals in Baltic herring. In *ICES Symposium on Brackish Water Ecosystems, 25–28 August 1998, Helsinki. Book of Abstracts*, p. 30.

Perttilä, M., Tervo, V. & Parmanne, R. 1982. Age dependence of the concentrations of harmful substances in Baltic herring (*Clupea harengus*). *Chemosphere*, **11**, 1019–1026.

Polak-Juszczak, L. 1997. The assessment of the hazard of contamination with heavy metals to consumers' health on monitoring research on the quality of Baltic fish and their products. *Bull. Sea Fish. Inst.*, **1**, 49–57.

Protasowicki, M. 1986. Sex effects on Cd, Pb, Cu and Zn contents in selected fish organs. *Baltic Sea Environ. Proc.*, **19**, 433–441.

Protasowicki, M. & Chodyniecki, A. 1986. The heavy metal content of Baltic ichthyofauna against their background levels in fish from other areas of the world ocean. In *Proc. of Symposium on Ecological Investigations of the Baltic Sea Environment*, pp. 131–147. Riga.

Seisuma, Z., Kulikova, I., Vadzis, D. & Legzdinya, M. 1984. *Heavy Metals in Water Organisms of the Gulf of Riga.* Zinatne, Riga (in Russian).

Stange, K., Maage, A. & Klungsoyr, J. 1996. Contaminants in fish and sediments in the North Atlantic Ocean. *TemaNord*, **522**, 1–79.

Tervo, V. 1987. Concentrations of metals in fish and benthic invertebrates in the Gulf of Finland and in the Gulf of Bothnia during 1982–1986. *ICES C. M. 1987/E:20. Marine Environ. Qual. Com.*, 1–14.

Vas, P., Gordon, J. D. M., Fielden, P. R. & Overnell, J. 1993. The trace metal ecology of ichthyofauna in the Rockall Trough, Northeastern Atlantic. *Mar. Poll. Bull.*, **26**, 607–612.

Voigt, H.-R., Wiklund, T. & Bylund, G. 1994. Mercury in flounder *Platichthys flesus* (L.) from Finnish coastal waters. *Diseases and Parasites of Flounder in the Baltic Sea. BMB Publ.*, **15**, 117–119.

VASE JA KAADMIUMI SISALDUSE MUUTUMINE ISASTES RÄIMEDES KUDEMISE AJAL

Mart SIMM ja Jonne KOTTA

On määratud vase ja kaadmiumi kontsentratsioon Soome lahe keskosast püütud isaste räimede lihastes, maksas ja gonaadides. Raskmetallide kontsentratsioon lihastes ja gonaadides ei sõltu kalade vanusest. Maksas on vase ja kaadmiumi kontsentratsioon vanematel kaladel (5–7-aastased) oluliselt kõrgem kui nooremat el (2–4-aastased). Raskmetallide kontsentratsioon gonaadides sõltub kalade suguküpsuse astmest ja suurimad muutused toimuvad kudemisel. Niisaga organismist eritatav raskmetallide hulk moodustab 15–33% vase ja kaadmiumi sisaldusest lihastes, maksas ja gonaadides kokku. Kudemisel eraldub noorte kalade organismist rohkem vaske ja kaadmiumi kui vanade kalade organismist.