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Preliminary studies on selected elements in organs of dab *Limanda limanda* and their relation to fish disease state

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ABSTRACT: During the Bremerhaven Workshop, concentrations of Hg, Cd, Pb, Cu and Zn were determined in the liver, kidney, gills and muscle of healthy and diseased dab *Limanda limanda*. The results of this preliminary study indicate relationships between metal concentrations and the state of health of the fish.

INTRODUCTION

Increasing environmental pollution is frequently regarded as a factor reducing natural immunity of organisms, including aquatic species (Mearns & Sherwood 1974). Metabolic processes in the body depend to a large extent on an organism's condition (Newell et al. 1979). Chronic heavy metal intoxication may cause disturbances in fish embryonic development (Westernhagen et al. 1979, Ojaveer et al. 1980) and in the metabolism of essential elements in fish (Protasowicki 1989). The present work attempts to determine differences between contents of selected elements in organs of healthy and diseased fish.

METHODS AND MATERIALS

Assays were made on females of dab *Limanda limanda* measuring (total length) 20 to 25 cm. The fish were collected during cruises of RV 'Solea' (14 to 17 March 1990) and RV 'Walther Herwig' (22 to 25 March 1990) at 6 stations on the German Bight transect (Stebbing & Dethlefsen 1992).

The fish collected were divided into 2 groups:

(1) healthy individuals; and

(2) diseased individuals showing ulcers, symptoms of lymphocytosis or papillomas.

Numbers of individuals in each group by area of capture are presented in Table 1.

The following organs were dissected out: muscles, liver, kidneys, and gills. Samples taken from individual fish were pooled separately for each kind of organ, and particular fishing ground. Before the assays, each sample was homogenized. All assays for sample prepared in this way were run in triplicate.

Mercury content was determined using cold vapour technique (CV AAS) after digestion in HNO_3-$HClO_4$ mixture as in Adrian (1971). The remaining elements (Cd, Pb, Cu, Zn, and Mg) were determined with FAAS after wet combustion (Protasowicki 1985). The following mean recoveries (and SD) of the methods were obtained: 96.90 (4.11), 92.66 (8.89), 89.51 (11.09), 95.50

Table 1. Numbers of dab *Limanda limanda* examined

State of fish	Station 3	5	6	7	8	9	Total no.
Healthy	31	33	36	31	36	39	206
Diseased[a]	8	13	11	7	8	16	63
Total no.	39	46	47	38	44	55	269

[a] All diseased females measuring 20 to 25 cm caught in the area were taken for study

Table 2. *Limanda limanda*. Metal contents in organs of dab, µg g^{-1} dry weight taken from stations on the German Bight transect. Each value is the mean calculated on the basis of results from 3 parallel analyses of homogenized samples of dab organs; for nos. of fish see Table 1. M: muscles; L: liver; K: kidney; G: gills; ND: not detected

Stn	Health state	Organ	Hg	Cd	Pb	Cu	Zn	Mg
3	Healthy	M	0.386	0.041	2.28	0.41	38.47	1919
		L	0.208	0.142	1.92	10.17	153.57	1377
		K	0.664	ND	2.54	2.47	159.10	1401
		G	0.277	0.561	8.43	3.40	138.30	2525
	Diseased	M	0.613	0.069	2.21	0.61	36.61	1788
		L	0.373	0.329	1.91	7.34	131.54	1355
		K	0.467	0.205	2.51	4.04	93.45	847
		G	0.310	0.484	7.48	3.07	172.45	2933
5	Healthy	M	0.153	0.013	1.97	0.33	24.18	1354
		L	0.425	0.220	2.09	24.43	175.70	1465
		K	0.902	0.023	2.08	2.02	52.26	199
		G	0.304	0.464	8.61	3.43	130.58	2656
	Diseased	M	0.522	0.025	1.94	0.56	18.67	983
		L	0.410	0.306	1.82	15.51	150.08	1517
		K	0.820	ND	2.83	2.30	61.77	462
		G	0.329	0.506	8.40	3.67	131.88	2417
6	Healthy	M	0.161	0.162	2.50	0.92	21.89	1157
		L	0.307	0.509	2.57	17.85	241.45	2008
		K	0.924	0.045	3.49	2.67	52.57	168
		G	0.195	0.731	7.96	3.43	151.31	2289
	Diseased	M	0.291	0.079	1.70	0.47	10.82	1310
		L	0.433	0.793	1.98	13.89	144.75	1106
		K	0.936	0.111	3.34	2.94	95.64	1283
		G	0.214	0.845	7.63	3.91	102.85	1764
7	Healthy	M	0.272	0.051	2.00	0.43	21.10	1152
		L	0.229	0.417	2.01	24.57	172.64	1377
		K	0.692	0.018	1.66	1.85	66.60	475
		G	0.205	0.382	7.91	3.50	147.64	2489
	Diseased	M	0.282	0.067	1.58	0.46	11.82	627
		L	0.211	0.155	2.10	10.16	97.87	757
		K	0.584	ND	1.63	0.85	135.94	880
		G	0.172	0.455	8.59	3.57	134.27	2496
8	Healthy	M	0.086	0.059	1.93	0.65	31.98	2081
		L	0.401	0.505	2.06	16.21	273.40	2731
		K	0.843	0.139	2.75	2.62	71.28	667
		G	0.220	0.527	6.91	3.54	186.39	3357
	Diseased	M	0.317	0.115	1.91	1.06	45.84	1766
		L	0.389	0.397	2.53	9.69	209.92	2619
		K	1.258	0.012	3.85	3.21	116.86	1446
		G	0.197	0.652	8.91	3.32	164.06	3124
9	Healthy	M	0.142	0.050	0.98	0.63	46.09	2126
		L	0.184	0.311	1.29	9.44	266.66	2498
		K	0.948	0.050	2.00	3.10	169.23	1224
		G	0.110	0.623	6.56	3.89	138.46	2417
	Diseased	M	0.233	0.052	1.18	0.68	34.76	1557
		L	0.270	0.751	1.36	7.68	172.19	1846
		K	0.840	0.399	3.78	3.14	147.61	1118
		G	0.128	0.654	6.94	3.56	155.06	2664

(3.70), 97.50 (3.80), and 96.31 (7.58) % for Hg, Cd, Pb, Cu, Zn, and Mg, respectively.

RESULTS AND DISCUSSION

The present study follows contents of 5 trace metals (Hg, Cd, Pb, Cu, and Zn) and 1 macroelement (Mg) in organs of dab in relation to disease condition. Three of the heavy metals studied, mercury, cadmium, and lead, are – in the light of present knowledge – regarded as toxic for animals. The 2 remaining metals, copper and zinc, are elements indispensable for life, and both their excess and deficit are harmful. Magnesium is also an indispensable element.

A comparison is made between metal concentrations in muscles, liver, kidney, and gills of the healthy and diseased fish from 6 stations on the German Bight transect (Table 2). The data indicate considerable variation in levels of different metals in fish. A 2-way analysis of variance without replicates was carried out on these data (Table 2). In only about 20 % of the cases (Table 3) were the differences, between healthy and diseased fish, statistically significant (source of variation B: $F_{calc.} > F_{0.05 \text{ or } 0.01}$).

Mercury contents were higher in muscles of the diseased fish, while the levels in liver, kidneys, and gills remained statistically the same. Differences between cadmium and lead contents in organs of healthy and diseased fish were statistically non-significant. Similarly the copper levels found in muscles, kidney, and gills were found to be independent of the health state of fish. Higher concentrations of copper were found in livers of healthy fish. Livers of the healthy fish contained more zinc than those of diseased fish. On the other hand, most of the healthy individuals showed lower levels of zinc in the kidney. Contents in the remaining organs were similar in the 2 groups. Muscles

Table 3. Results of the 2-way analysis of variance without replicates. Source of variation: A, between capture sites; B, between healthy and diseased groups. Degrees of freedom: for A= 5,5, and for B = 1,5. Tabular values: $F_{A;\,0.05} = 5.05$; $F_{A,\,0.01} = 10.97$; $F_{B;\,0.05} = 5.79$; $F_{B,\,0.01} = 13.27$. Differences between mean values: =: values statistically equal; # and ##: values statistically different

Element Material examined & source of variation		F_{calc}	Conclusion	Element Material examined & source of variation		$F_{calc.}$	Conclusion
Mercury				Copper			
Muscles	A	3.46	=	Muscles	A	1.24	=
	B	11.78	#		B	0.43	=
Liver	A	4.61	=	Liver	A	3.91	=
	B	2.94	=		B	11.07	#
Kidney	A	2.83	=	Kidney	A	3.01	=
	B	0.02	=		B	0.74	=
Gills	A	25.36	##	Gills	A	1.11	=
	B	0.69	=		B	0.01	=
Cadmium				Zinc			
Muscles	A	2.18	=	Muscles	A	1.05	=
	B	0.07	=		B	0.34	=
Liver	A	3.15	=	Liver	A	6.88	#
	B	1.52	=		B	22.19	##
Kidney	A	0.82		Kidney	A	9.22	##
	B	1.13	=		B	18.62	##
Gills	A	12.28	##	Gills	A	1.44	=
	B	2.95	=		B	0.20	=
Lead				Magnesium			
Muscles	A	5.14	#	Muscles	A	10.76	#
	B	1.68	=		B	7.12	#
Liver	A	3.87	=	Liver	A	8.84	#
	B	0.07	=		B	5.36	=
Kidney	A	2.80	=	Kidney	A	1.19	=
	B	3.23	=		B	1.68	=
Gills	A	1.38	=	Gills	A	5.20	#
	B	0.39	=		B	0.16	=

of the diseased individuals were found to contain less magnesium (as total mean) than the respective organs of healthy fish. Magnesium contents in liver, kidney, and gills were statistically the same.

The results obtained and their analysis allow the conclusion that some relationships exist between element contents in dab organs and their disease state. This conclusion should be, however, confirmed on the basis of more detailed studies. Moreover, in the present study it was found that contents of elements in fish differ between various stations along a pollution gradient in the German Bight (Table 3; source of variation A: $F_{calc.} > F_{0.05 \text{ or } 0.01}$) and between organs.

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