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SUBLETHAL PHYSIOLOGICAL STRESS INDUCED BY CADMIUM AND MERCURY IN THE WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS

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SUMMARY

Winter flounder (Pseudopleuronectes americanus) were exposed to 5 and 10 ppb cadmium or mercury for 60 days to determine changes in oxygen consumption rates and hematology, as well as metal uptake. Flounder exposed to cadmium respired at a lower rate than the controls, while those exposed to 10 ppb mercury respired at a higher rate. No significant hematological difference was found between controls and cadmium-exposed fish. In mercury-exposed fish, however, there were differences in plasma protein levels, plasma osmolality, hematocrit, hemoglobin and mean corpuscular hemoglobin. No detectable levels of cadmium were found in blood and gill tissues, but considerable amounts of mercury were accumulated.

INTRODUCTION

Relatively little is known about the effects of low doses of heavy metals on the normal physiological functions of marine fish over extended periods. Until recently, most studies conducted on aquatic organisms have been concerned with determining the concentrations that cause mortality but recent studies have shown deleterious physiological effects of sublethal levels of metals on marine fish [1, 2, 3, 4, 5]. The gradual elimination of a valued marine species by low concentrations of pollutants may be no less serious than their rapid death. In a sense, it is more serious because it is less likely to be obvious and to be traced to its source in time to permit recovery of the environment.

The present study was undertaken to determine any physiological damage caused by low levels of inorganic cadmium and mercury on the commercially valuable winter flounder (Pseudopleuronectes americanus) after 60-day exposures to these metals. The parameters examined were oxygen consumption rate, some aspects of hematology and chemical uptake into the blood and gills.

METHODS AND MATERIALS

Exposure

Winter flounder were collected by otter trawl in Long Island Sound near Milford, Connecticut, and held in the laboratory in flowing, sand-filtered seawater for one to two weeks prior to mercury or cadmium exposure. The fish were fed chopped clams (Spisula solidissima) during holding and throughout the exposure period. Test animals were exposed in 285-liter fiberglass tanks filled to 228 liters with sand-filtered seawater (24-26 ppt salinity) by a proportional-dilution apparatus [6]. This diluter controlled the intermittent delivery of toxicant-containing water and control water at a flow rate of 1.5 liters to each tank every 2.5 minutes throughout the test period. This flow provided approximately 4 complete daily exchanges of water in each tank. Cadmium, as cadmium

chloride ($CdCl_2.2½\ H_2O$), and mercury, as mercuric chloride ($HgCl_2$), were added at concentrations of 5 and 10 ppb. Background concentrations of these two metals in the incoming seawater were less than 1 ppb. Each experiment consisted of 18 fish per concentration per metal (6 flounder in each tank), averaging 219 g in weight (range 98 to 465 g) and 282 mm in total length (range 219-390 mm). Tests were conducted in duplicate, for a total of 108 flounder exposed to each of the two metals. Water temperature ranged from 3 to 6 °C during cadmium exposure and from 7 to 11 °C during mercury exposure. After 60 days' exposure the fish were removed and examined for signs of sublethal stress.

Respiration

A single gill was dissected from each fish, the bony arch removed, and the gill placed in a 15-ml, Warburg-type flask. Each flask contained 5 ml of metal-treated water from the tank in which the fish had been exposed. Oxygen consumption was monitored over a 4-hour period at 20 °C using a Gilson Differential Respirometer. Oxygen consumption rates were calculated as microliters of oxygen consumed per hour per gram dry weight of gill tissue (µl/hr/g) corrected to microliters of dry gas at standard temperature and pressure.

Hematology

Blood was collected by cardiac puncture using a 5-ml plastic syringe and a 20-gauge needle. Red blood corpuscle (RBC) counts were made in a hemocytometer using EDTA treated blood. Blood samples were diluted 1:200 with Hendricks solution using blood diluting pipets. Hemoglobin concentrations were determined on heparinized blood using the cyanmethemoglobin method with Hycel chemicals. Absorbance was read on a Bausch and Lomb Spectronic 20 at 540 nm. Hematocrit was measured using microhematocrit tubes and heparinized blood. Plasma protein was determined in a Bausch and Lomb 3L Refractometer using the plasma obtained in the hematocrit determination. Plasma for osmolality readings was obtained from heparinized blood samples pooled from 2 to 3 fish. These samples were centrifuged at 1700 x g for 20 minutes and read on an Advanced 3L Osmometer. The effect of the heparin on the osmolality of the blood was negligible. The mean corpuscular volume (MCV) was calculated by dividing the hematocrit x 10 by the number of red blood cells in millions per mm^3. The mean corpuscular hemoglobin (MCH) in picograms per cell was calculated by dividing the hemoglobin in grams % by the RBC count. The mean corpuscular hemoglobin concentration (MCHC), or the hemoglobin in grams per 100 ml of packed red cells, was calculated by dividing hemoglobin in grams % x 100 by the hematocrit.

Metal Uptake

Blood and gill samples were analyzed for metal content. Blood was drawn by heart puncture and analyzed as whole blood. Gills were dissected from each fish, the bony arch discarded, and the gills rinsed in seawater and frozen prior to analysis. All samples were wet ashed with 70% nitric acid on a hot plate, taken up in 25 ml of 10% nitric acid, then read directly on an atomic absorption spectrophotometer (Perkin-Elmer Model 403).

RESULTS

Respiration

Gill-tissue oxygen consumption in winter flounder was significantly reduced ($P < .05$) after 60 days' exposure to cadmium at 5 and 10 ppb. Flounder exposed to 10 ppb mercury, however, respired at a significantly higher rate ($P < .05$) than the controls, while fish exposed to 5 ppb respired at the same rate as control fish (Fig. 1).

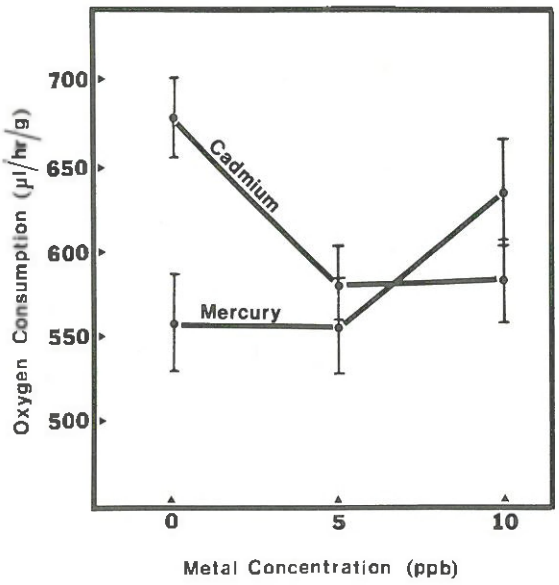

Fig. 1. Effects of mercury and cadmium on oxygen consumption of winter flounder. Each point represents mean gill-tissue oxygen consumption value of 32 fish. Bars represent standard errors.

The difference in oxygen consumption rate between the two control groups is attributed to seasonal reproductive condition. The cadmium group was tested in February when the fish were gravid, a condition requiring a high metabolic rate and a consequent increase in oxygen consumption. The mercury group was tested in May after the fish had spawned and no longer possessed the metabolic burden of reproduction. Beamish [7] reported similarly high rates of oxygen consumption in brook trout (Salvelinus fontinalis) and other fishes during spawning season.

Hematology

There was no significant difference between controls and cadmium-exposed fish for any hematological test, but significant differences were noted in mercury-exposed fish (Table 1). Plasma protein rose from 5.4 to

Table 1

Summary of hematological data from winter flounder exposed to mercury or cadmium for 60 days. These data were analyzed by the Student's t-test.

CADMIUM CONCENTRATION

Test	Controls		5 ppb		10 ppb	
	Mean ± S.E.	n	Mean ± S.E.	n	Mean ± S.E.	n
Hematocrit (% packed red cells)	35 ±1	35	34 ±1	32	33 ±1	32
Hemoglobin (g/100 ml whole blood)	7.4 ±0.3	32	8.1 ±0.3	31	7.4 ±0.3	32
RBC (10^6 cells/mm^3)	2.90±1.7	17	2.69±0.12	10	2.96±0.18	14
Plasma protein (g %)	6.7 ±0.2	34	6.7 ±0.2	30	6.8 ±0.2	31
Plasma osmolality (mOsm/Kg_{H_2O})	401 ±7	22	380 ±7	22	401 ±5	19
Mean Corpuscular Volume (μ^3/cell)	121 ±7.0	17	114 ±6.1	9	114 ±5.0	14
Mean Corpuscular Hb (picograms/cell)	26.2 ±1.6	14	27.9 ±3.7	9	26.2 ±1.6	14
Mean Corpuscular Hb Conc. (g/100 ml packed red cells)	22.7 ±0.7	31	23.8 ±1.0	31	22.7 ±0.7	31

MERCURY CONCENTRATION

Test	Controls		5 ppb		10 ppb	
	Mean ± S.E.	n	Mean ± S.E.	n	Mean ± S.E.	n
Hematocrit	28 ± 1.6	25	29 ± 1.5	25	23 ±1.4*	27
Hemoglobin	5.3 ± 0.3	27	5.4 ± 0.2	22	4.2 ±0.2**	27
RBC	1.87± 0.16	12	2.10± 0.18	12	1.78±0.16	11
Plasma protein	5.4 ± 0.2	26	6.3 ± 0.2**	25	6.0 ±0.2*	27
Plasma osmolality	362 ± 2.6	12	348 ± 4**	11	360 ±4	15
MCV	151 ±10.5	11	145 ±12	12	127 ±6	10
MCH	30.7 ±2	12	26.5 ± 1.5	11	23.5 ±1.9*	11
MCHC	19.4 ± 0.8	25	18.9 ± 0.4	22	17.8 ±0.6	26

*Significantly different from controls at 0.05 levels.
**Different at 0.01 level.

6.3% in fish exposed to 5 ppb mercury with a decrease in plasma osmolality. The normally hyposmotic blood became even more so. Exposure to 10 ppb mercury also resulted in a number of changes: Plasma protein rose significantly while hematocrit, hemoglobin and MCH decreased. Hematological data from control fish were similar to those observed in winter flounder by Umminger and Mahoney [8] and Bridges et al. [9].

Metal Uptake

Significant amounts of mercury but no detectable amounts (<0.2-0.3 ppm) of cadmium were found in blood and gill tissues of flounder exposed to these metals for 60 days. Mean levels of 20.6 and 42.8 ppm mercury, wet weight basis, were found in gills of fish exposed to 5 and 10 ppb mercury, respectively. Although blood accumulated considerably less mercury than gills, the levels of 2.9 and 3.8 ppm found in the exposed fish were significantly higher than the controls (Table 2).

Table 2

Levels of cadmium and mercury in gills and blood of winter flounder exposed to these metals for 60 days. Values given are the mean of 4 samples. Each sample was a pool from 3-6 fish.

Exposure Levels	MEAN CONCENTRATION (ppm, wet wt.)			
	Cadmium		Mercury	
	Gill	Blood	Gill	Blood
0	<0.3	<0.3	<0.14	<0.04
5	<0.3	<0.2	20.6(S.E., 0.72)	2.9(0.55)
10	<0.3	<0.2	42.8(2.53)	3.8(0.77)

DISCUSSION

Oxygen consumption is a valuable indication of sublethal stress. Any significant variation from the normal or control value might reflect an alteration in the metabolic demand of the fish or damage to the respiratory system. In this study, cadmium depressed oxygen consumption of winter flounder at a concentration as low as 5 ppb, a level found in certain polluted estuarine waters [10]. Thurberg and Dawson [11] noted a similar depression in the cunner (Tautogolabrus adspersus), an estuarine fish, exposed to sublethal levels of cadmium; Newman and MacLean [12] noted gill tissue abnormalities in these same fish. Ledgerwood and Brown [13] reported cadmium-induced aneurysms in the gill lamellae of sticklebacks (Gasterosteus aculeatus). The fact that no gill pathology was observed [14] and no detectable amounts of cadmium were found in gill tissues of flounder exposed to this metal suggests cadmium-induced disruption elsewhere in the fish.

Mercury-exposed flounder exhibited elevated gill-tissue respiration rates, a possible indication of increased metabolic demand due to mercury-induced physiological or biochemical alterations within the fish.

Striped bass (Morone saxatilus) exposed to the same levels of mercury exhibited slightly depressed oxygen consumption after a 60-day exposure, but elevated rates after a 120-day exposure [unpublished data]. The high metabolic rate of flounder exposed to mercury may have contributed to the high levels of mercury found in the gill tissues. MacLeod and Pessah [15] reported high mercury residues in the gills of rainbow trout (Salmo gairdneri) held in mercury-contaminated water at high temperatures and attributed this to the increased rate at which mercury-contaminated water was pumped over the gills.

Hematological tests have been an important diagnostic tool in medicine for many years, and recent speculation has indicated that they may be equally valuable as indicators of disease or stress in fish [16]. Hematological changes in fish have been related to temperature and season [9], diet [17], pesticide stress [18] and metal stress [19]. In this study, cadmium-exposed flounder showed no hematological changes and no detectable uptake of cadmium. Mercury-exposed fish, however, showed high tissue levels of mercury and several hematological alterations.

The results of this study are valid only under the conditions of the experiment. These data would almost certainly change if such factors as temperature, season, reproductive state or salinity were changed. Interaction of several pollutants and/or natural factors can also alter the character or degree of a pollutant effect [20, 21]. Such findings emphasize the need for a broader research effort to obtain a comprehensive understanding of the role of heavy metal pollutants in the marine environment.

Note: The use of trade names is to facilitate description and does not imply endorsement by the National Marine Fisheries Service.

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