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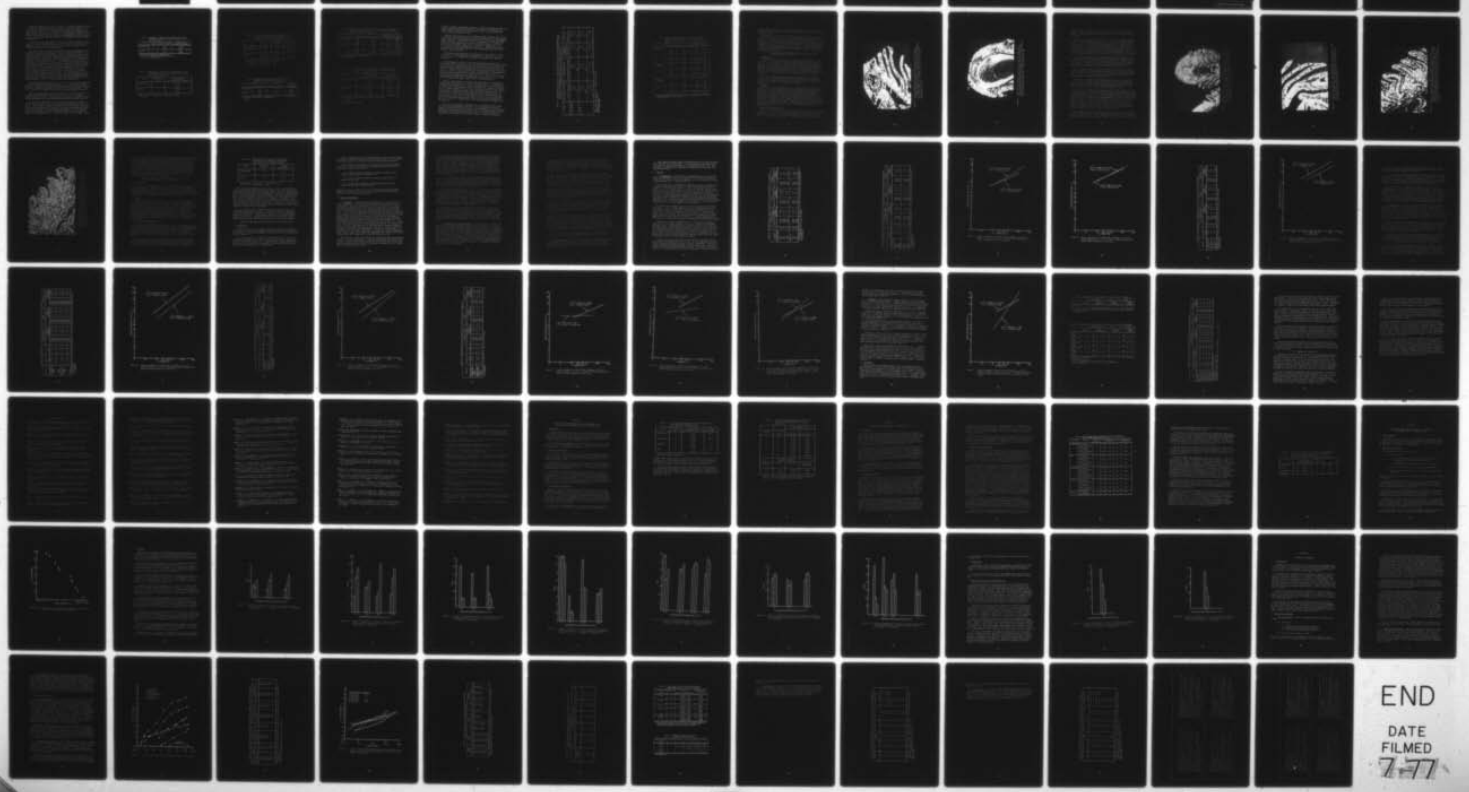

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Sublethal Effects of Suspended Sediments on Estuarine Fish

by

J.M. O'Connor, D.A. Neumann, and J.A. Sherk, Jr.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The objective of this study was to determine the effects, if any, of sublethal concentrations of suspended materials on the fish in estuarine systems. Experimental sediment suspensions reproduced the concentrations frequently found during flooding and at dredging sites and dredged-material disposal sites. The suspensions were of natural sediment, obtained from the Patuxent River estuary, Maryland, or commercially available fuller's earth.

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cont

Fish were collected in the Patuxent River estuary and transported to the laboratory. The selected fish species inhabited ecologically different sections of the estuary; therefore, the overall reactions of each species were unique.

Seven species of estuarine fish were exposed to fuller's earth and natural sediment suspensions for timed periods and hematological changes were noted. The effects of various concentrations of fuller's earth suspensions on white perch gill tissue were determined. Oxygen consumption rates of striped bass, white perch, and toadfish were measured in filtered Patuxent River water and compared to consumption rates in filtered river water suspensions of fuller's earth or Patuxent River sediment.

Fish showed signs of stress in response to suspended sediments in most of the experiments. Results indicate that sublethal concentrations of suspended solids can affect estuarine fish.

Additional experiments are discussed in Appendixes A to D.

PREFACE

This report is published to provide coastal engineers with information on the sublethal effects of suspended sediments on estuarine organisms. The work reported is a part of a continuing program of research on the ecological effects of coastal engineering activities. The report presents the results of part of a 3-year laboratory study on the subject. The work was carried out under a contract originating in the Office, Chief of Engineers, which was monitored under the coastal ecology research program of the U.S. Army Coastal Engineering Research Center (CERC).

The original contract report (CERC Contract No. DACW72-71-C-0003) was prepared by Dr. J.M. O'Connor, D.A. Neumann, and Dr. J.A. Sherk, Jr., while on the staff of the Natural Resources Institute, University of Maryland, College Park, Maryland. Special acknowledgment is given to A.M. Daley for her work, particularly as reported in Appendix C.

A.K. Hurme and A.L. Meyer, CERC, technically reviewed, condensed, and revised that part of the original report pertaining to the sublethal effects of suspended solids on estuarine fish. Robert M. Yancey, Chief, Coastal Ecology Branch, was CERC contract monitor for the report, under the general supervision of R.P. Savage, Chief, Research Division.

Comments on this publication are invited.

Approved for publication in accordance with Public Law 166, 79th Congress, approved 31 July 1945, as supplemented by Public Law 172, 88th Congress, approved 7 November 1963.

JOHN H. COUSINS
Colonel, Corps of Engineers
Commander and Director

CONTENTS

CONTENTS

TABLES

CONTENTS

TABLES-Continued

FIGURES

CONVERSION FACTORS, U. S. CUSTOMARY TO METRIC (SI) UNITS OF MEASUREMENT

U.S. customary units of measurement used in this report can be converted to metric (SI) units as follows:

Multiply	by	To obtain
inches	25.4	millimeters
	2.54	centimeters
square inches	6.452	square centimeters
cubic inches	16.39	cubic centimeters
feet	30.48	centimeters
	0.3048	meters
square feet	0.0929	square meters
cubic feet	0.0283	cubic meters
yards	0.9144	meters
square yards	0.836	square meters
cubic yards	0.7646	cubic meters
miles	1.6093	kilometers
square miles	259.0	hectares
knots	1.8532	kilometers per hour
acres	0.4047	hectares
foot-pounds	1.3558	newton meters
millibars	1.0197×10^{-3}	kilograms per square centimeter
ounces	28.35	grams
pounds	453.6	grams
	0.4536	kilograms
ton, long	1.0160	metric tons
ton, short	0.9072	metric tons
degrees (angle)	0.1745	radians
Fahrenheit degrees	5/9	Celsius degrees or Kelvins[1]

[1] To obtain Celsius (C) temperature readings from Fahrenheit (F) readings, use formula: $C = (5/9)(F - 32)$. To obtain Kelvin (K) readings, use formula: $K = (5/9)(F - 32) + 273.15$.

SUBLETHAL EFFECTS OF SUSPENDED SEDIMENTS ON ESTUARINE FISH

by
J.M. O'Connor, D.A. Neumann, and *J.A. Sherk, Jr.*

I. INTRODUCTION TO SUBLETHAL EFFECTS OF SUSPENDED SOLIDS ON ESTUARINE FISH

The lethal effects of a variety of solids are documented for numerous freshwater fish (Ellis, 1936, 1937; Wallen, 1951; Wilson, 1956; Cordone and Kelley, 1961; Herbert, et al., 1961; Herbert and Merkens, 1961) and for some estuarine species (Rogers, 1969; Sherk and O'Connor, 1971; O'Connor, Neumann, and Sherk, 1976). However, the sublethal effects are only dealt with in histological studies of fish gill tissues (Southgate, 1962; Herbert, et al., 1961; Herbert and Merkens, 1961; Ritchie, 1970). The physiological impact of sublethal concentrations has not been studied previously. This part of a 3-year laboratory study (Sherk, O'Connor, and Neumann, 1976; O'Connor, Neumann, and Sherk, 1976) presents the results of histological and physiological studies of the sublethal effects of suspended solids on estuarine fish.

Seven estuarine fish species (white perch, *Morone americana*; striped bass, *Morone saxatilis*; hogchoker, *Trinectes maculatus*; spot, *Leiostomus xanthurus*; mummichog, *Fundulus heteroclitus*; striped killifish, *Fundulus majalis*; and oyster toadfish, *Opsanus tau*) were placed in fuller's earth and natural sediment suspensions, and hematological changes were noted during timed exposures. The effects of fuller's earth suspensions on gill tissue in white perch and on carbohydrate metabolism in hogchoker were determined at various concentrations. Oxygen consumption rates of striped bass, white perch, and toadfish were measured in filtered water from the Patuxent River, Maryland, and compared to consumption rates in filtered river water suspensions of fuller's earth or Patuxent River sediment.

A knowledge of the sublethal effects of suspended materials is important in evaluating the effects of dredging or of disposal of dredged materials. This report provides base-line data which can be combined with knowledge of local conditions in preproject consideration of the effects of dredging activities.

II. SUBLETHAL EFFECTS OF SUSPENDED SOLIDS ON THE HEMATOLOGY OF ESTUARINE FISH

1. Introduction.

This section presents an assessment of the effects of suspensions of fuller's earth and natural sediments on several basic hematoiogical parameters in fish: Microhematocrit (packed red blood cell volume), red blood cell count, hemoglobin concentration, and osmolality (ionic concentration of the blood).

2. Methods.

Hematological studies of the seven fish species were conducted in both an experimental tank and a control tank. Each species was exposed to a concentration of fuller's earth or natural Patuxent River sediment which had caused less than 10-percent mortality, and was no greater than the previously determined 24-hour lethal concentration for 10-percent mortality (LC_{10}) for each species (O'Connor, Neumann, and Sherk, 1976). A quantity of fuller's earth sufficient to maintain the desired concentration was placed in the experimental tank and mixed by submersible pumps for 24 hours before an experiment. The control tank did not contain fuller's earth. Mineral solids were maintained in suspension throughout the experiment in the two tanks by continuous pumping and aeration.

Twelve or 15 fish were placed in each of the tanks during a test. Blood samples were taken from at least 10 individuals selected at random from the tanks after the exposure period. The samples were obtained from white perch and striped bass by severing the second branchial artery on the right side (McErlean and Brinkley, 1971), and from hogchokers, spot, and killifish by severing the caudal peduncle with a heparinized blade. Blood was collected in heparinized pipets and, when possible, was mixed before samples were removed for analysis.

Microhematocrit was determined according to methods outlined by Hesser (1960). Hemoglobin concentration was estimated by the cyanmethemoglobin method with modifications as suggested by Larsen and Snieszko (1961). Red blood cells were counted at X 100 on an improved Neubauer hemacytometer, using a modified Hayme's solution as the dilution medium (Heinle and Morgan, 1972). Whole blood osmolality was measured with a freezing-point depression osmometer.

3. Results and Interpretation.

Hematological characteristics of white perch, hogchokers, and striped killifish changed in response to sublethal concentrations of suspended solids. The effects of these sublethal concentrations were analyzed extensively for white perch (Table 1). Exposure of white perch to 0.65 gram per liter ($g\ l^{-1}$) fuller's earth for 5 days resulted in significant increases in microhematocrit, hemoglobin concentration, and red blood cell count. The ionic concentration of the blood, estimated by whole blood osmolality, did not change.

There was a relatively greater increase in red blood cell counts than in microhematocrits and hemoglobin concentrations. The increase in red blood cell count for experimental groups was 30 percent greater than the increase for control groups. Hemoglobin concentrations increased by 15 percent; microhematocrit values exceeded those of control fish by 17 percent.

Table 1. Experimental and control values of red blood cell count, microhematocrit, hemoglobin concentration, and osmolality of white perch exposed for 5 days to 0.65 g l^{-1} fuller's earth.[1]

Group	Red blood cells count (cells X 10^6 mm^{-3})	Microhematocrit (pct packed cell volume)	Hemoglobin concentration (g 100 g^{-1})	Osmolality (mOsm kg^{-1})
Experimental	2.53	36.17	8.40	281.61
	±0.25	±4.69	±0.95	±8.87
	(12)[2]	(12)	(12)	(10)
Control	1.96	30.73	7.30	274.01
	±0.23	±2.94	±0.96	±10.31
	(10)	(10)	(12)	(10)
	t = 5.22[3]	t = 3.19	t = 2.830	t = 1.76
	p < 0.01[4]	p < 0.01	p < 0.05	p > 0.05

[1]Mean values are expressed ± standard deviation.
[2]Number of individuals.
[3]t = "Student's t", the deviation of the estimated mean from that of the population.
[4]p = probability.

Hogchokers exposed for 5 days to 1.24 g l^{-1} of fuller's earth increased red blood cell counts from 1.58 to 2.08 cells X 10^6 mm^{-3} (millions of cells per cubic millimeter) and increased microhematocrit from 15.62 to 19.93 percent (Table 2). The red cell count increase for hogchokers was proportionately the same as the increase in microhematocrit (30.4 and 27.6 percent, respectively); for white perch the proportional increase in red cells was much greater than the increase in microhematocrit and hemoglobin concentration.

Striped killifish were exposed to 0.96 g l^{-1} fuller's earth for 5 days (Table 3). Their microhematocrit value rose from 24.99 to 32.29 percent (probability (p) < 0.01), a relative increase of 29.7 percent for the experimental group over the control group.

Experiments with white perch, striped killifish, and hogchokers demonstrated that significant hematological changes occur after exposure to sublethal concentrations of fuller's earth. Although these species show similar responses to sublethal concentrations of suspended solids, they differ markedly in response to lethal concentrations of the same material (O'Connor, Neumann, and Sherk, 1976). The hogchoker and the striped killifish were difficult to kill. An LC-response curve could not be generated for the hogchoker, which may be due to hogchokers' high tolerance for suspended solids. The killifish showed a high 24-hour LC_{50} of 38.18 g l^{-1} fuller's earth, about the same as the mummichog value of 39 g l^{-1} fuller's earth. However, white perch were classified as a sensitive species because their 24-hour LC_{50} values were below 10 g l^{-1} fuller's earth (O'Connor, Neumann, and Sherk, 1976). Low concentrations of suspended solids may induce sublethal effects, such as hematological alteration, even in relatively tolerant species. The highly sediment-tolerant hogchoker showed a significant increase in energy utilization during a 5-day exposure to 1.24 g l^{-1} fuller's earth (see Section IV).

Sublethal hematological effects of 1.6 g l^{-1} fuller's earth suspensions were determined for the common mummichog at 4-, 7-, and 12-day intervals (Table 4). The mean microhematocrit values of experimental fish were significantly different from those of control fish at each interval. There was an increase in the mean value of the experimental group at 12 days.

Spot were studied after a 5-day exposure to 1.27 g l^{-1} fuller's earth, a concentration below the 24-hour LC_{10} value of 13 g l^{-1} (O'Connor, Neumann, and Sherk, 1976). There were no significant differences between the hematological values from experimental and control groups (Table 5).

The data for striped bass were not directly comparable to data for other species because the bass were exposed for 11 and 14 days (Tables 6 and 7). After 11 days' exposure to 0.60 g l^{-1} fuller's earth, there were no detectable differences in red blood cell count, microhematocrit, hemoglobin concentration, or osmolality of experimental and control groups. Striped bass exposed to 1.5 g l^{-1} fuller's earth for 14 days showed an increase in microhematocrit ($p < 0.01$) over control fish. However, these

Table 2. Experimental and control values of red blood cell count and microhematocrit of hogchokers exposed for 5 days to 1.24 g l^{-1} fuller's earth.[1]

Group	Individuals (No.)	Red blood cell count (cells X 10^6 mm^{-3})	Microhematocrit (pct packed cell volume)
Experimental	10	2.08[2] ±0.35	19.93[3] ±4.32
Control	10	1.58 ±0.26	15.62 ±4.06

[1]Mean values are expressed ± standard deviation.
[2]$p < 0.01$ ($t = 3.480$, degrees of freedom (d.f.) = 18).
[3]$p < 0.05$ ($t = 2.299$, d.f. = 18).

Table 3. Experimental and control microhematocrit values of striped killifish exposed for 5 days to 0.96 g l^{-1} fuller's earth.[1]

Group	Individuals (No.)	Microhematocrit (pct packed cell volume)
Experimental	9	32.29[2] ±4.29
Control	9	24.99 ±2.55

[1]Mean values are expressed ± standard deviation.
[2]$p < 0.01$.

Table 4. Experimental and control microhematocrit values of mummichog exposed for 4, 7, and 12 days to 1.62 g l^{-1} fuller's earth.[1]

Group	Exposure		
	4 days	7 days	12 days
Experimental	33.08[2] ±5.74	29.52[3] ±4.43	34.14[4] ±4.28
Control	24.14 ±6.54	23.79 ±4.60	26.52 ±2.24

[1]Mean values are expressed ± standard deviation.
[2]$p < 0.01$ ($t = 3.2488$, d.f. = 18).
[3]$p < 0.02$ ($t = 2.8373$, d.f. = 18).
[4]$p < 0.001$ ($t = 4.9884$, d.f. = 16).

Table 5. Experimental and control values of red blood cell count, microhematocrit, and hemoglobin concentration of spot exposed for 5 days to 1.27 g l^{-1} fuller's earth.[1]

Group	Red blood cell count (cells X 10^6 mm^{-3})	Microhematocrit (pct packed cell volume)	Hemoglobin concentration (g 100 g^{-1})
Experimental	1.54[2] ±0.48	26.19 ±4.87	7.14[3] ±1.37
Control	1.46 ±0.26	28.31 ±7.62	6.69 ±2.21

[1]Mean values are expressed ± standard deviation.
[2]$p > 0.50$.
[3]$p > 0.10$.

Table 6. Experimental and control values of red blood cell count, microhematocrit, hemoglobin concentration, and osmolality of striped bass exposed for 11 days to 0.6 g l^{-1} fuller's earth.[1]

Group	Red blood cell count (cells X 10^6 mm^{-3})	Microhematocrit (pct packed cell volume)	Hemoglobin concentration (g 100 g^{-1})	Osmolality (mOsm kg^{-1})
Experimental	2.48	38.39	7.49	334.06
	±0.48	±5.61	±1.00	±10.88
	(7)[2]	(7)	(7)	(7)
Control	2.35	38.20	8.04	394.4
	±0.32	±6.46	±0.97	±22.50
	(7)	(7)	(7)	(7)
	t = 0.605	t = 0.057	t = 1.058	t = 1.624
	p > 0.5	p > 0.5	p > 0.5	p > 0.01

[1]Mean values are expressed ± standard deviation.
[2]Number of individuals.

Table 7. Experimental and control values of microhematocrit and osmolality of striped bass exposed for 14 days to 1.5 g l^{-1} fuller's earth.[1]

Group	Microhematocrit (pct packed cell volume)	Osmolality (mOsm kg^{-1})
Experimental	30.28[2]	311.22[3]
	±3.88	±10.97
	(10)[4]	(10)
Control	24.17	294.36
	±3.996	±22.35
	(10)	(10)

[1]Mean values are expressed ± standard deviation.
[2]$p < 0.01$.
[3]$p < 0.05$.
[4]Number of individuals.

fish also showed a significant increase in osmolality during the same time period. The increased microhematocrit may reflect a concentration of blood components due to loss of body water (Hall, Gray, and Lepkovsky, 1926; Forster and Berglund, 1956).

Toadfish held in 14.6 g l^{-1} of suspended natural sediment for 72 hours exhibited no significant differences from a control group in hematological values (Table 8). Mean hemoglobin concentrations for control and experimental fish were 3.67 and 3.73 g 100 g^{-1}, respectively. Red blood cell count and mean microhematocrit for experimental fish were 19.90 X 10^6 mm^{-3} and 21.67 percent, respectively. Values for control fish were 17.78 X 10^6 mm^{-3} and 20.10 percent, respectively. Osmolality was 246.63 milliosmoles per kilogram (mOsm kg^{-1}) for experimental fish and 251.69 mOsm kg^{-1} for control fish.

The hematological parameters were measured in spot exposed to 14.68 to 16.96 g l^{-1} resuspended natural sediment over 7 days at 1-, 3-, and 7-day intervals. No significant changes in hematology were observed (Table 9).

A time-dependent study was conducted on white perch exposed to 2 g l^{-1} resuspended natural muds for 4-, 6-, and 14-day intervals. The mean values of red blood cell count, microhematocrit, hemoglobin concentration, and osmolality for experimental fish were greater than for control fish after 4 days of exposure, but the differences were not statistically significant ($0.07 > p > 0.05$). Red blood cell count, microhematocrit, and hemoglobin concentration of experimental fish increased after 6 days ($0.05 > p > 0.01$). Blood osmolality did not change ($p > 0.5$). Red blood cell count, microhematocrit, hemoglobin concentration, and osmolality ($0.5 > p > 0.1$) of the two groups were again similar after 14 days of exposure.

Replicate experiments assessed the sublethal effects of resuspended natural muds on striped bass. Studies were conducted at an arbitrary concentration because LC_{10}, LC_{50}, and LC_{90} responses for this species were not consistent. Hematological analysis revealed that exposure of striped bass to concentrations of 1.5 to 6 g l^{-1} of natural muds for 6 days caused no detectable differences between experimental and control groups. In striped bass, a comparison of the effect of concentrations of 1.5 to 6 g l^{-1} and 6 to 8 g l^{-1} natural mud suggested that a threshold level may exist between 6 and 8 g l^{-1}. Below 6 g l^{-1} survival is essentially 100 percent; no sublethal hematological effects occurred over a period of 6 days at 2 to 6 g l^{-1}. Above 6 g l^{-1}, bass suffer mortality during 6 days of exposure.

Fish exposed to sublethal concentrations of suspended solids showed the same basic hematological responses as fish deprived of sufficient oxygen--increased red blood cell count, increased hematocrit, and increased hemoglobin concentration in peripheral blood. The hematological responses to sublethal concentrations of suspended solids seen in white perch, hogchokers, and striped killifish were similar to responses observed in

Table 8. Experimental and control values of red blood cell count, microhematocrit, hemoglobin concentration, and osmolality of toadfish exposed for 72 hours to 14.6 g l^{-1} resuspended Patuxent River estuary sediment.[1]

Group	Red blood cell count (cells X 10^6 mm^{-3})	Microhematocrit (pct packed cell volume)	Hemoglobin concentration (g 100 g^{-1})	Osmolality (mOsm kg^{-1})
Experimental	19.90 ±8.02 (9)[2]	21.67 ±8.43 (18)	3.73 ±1.24 (10)	246.63 ±13.66 (9)
Control	17.78 ±8.87 (10)	20.10 ±5.81 (17)	3.67 ±0.72 (10)	251.69 ±11.52 (9)
	t = 0.548 d.f. = 17, N.S.[3]	t = 0.476 d.f. = 16, N.S.	t = 0.147 d.f. = 18, N.S.	t = 0.849 d.f. = 16, N.S.

[1]Mean values are expressed ± standard deviation.
[2]Number of individuals.
[3]Not significant.

Table 9. Experimental and control values of red blood cell count, microhematocrit, hemoglobin concentration, and osmolality of spot exposed for 1-, 3-, and 7-day intervals to a range of 14.68 to 16.96 g l^{-1} resuspended natural sediment.[1]

Exposure	Red blood cell count (cells X 10^6 mm^{-3})	Microhematocrit (pct packed cell volume)	Hemoglobin concentration (g 100 g^{-1})	Osmolality (mOsm kg^{-1})
One day				
Experimental	2.85 ±0.39 (9)[2]	42.10 ±4.16 (10)	8.46 ±0.49 (10)	337.74 ±7.50 (10)
Control	2.94 ±0.40 (7)	40.27 ±3.90 (7)	8.89 ±0.91 (9)	333.02 ±7.60 (10)
	t = 1.47 p > 0.1	t = 0.92 p > 0.2	t = 1.28 p > 0.2	t = 1.40 p > 0.1
Three days				
Experimental	3.12 ±0.40 (9)	39.33 ±4.89 (10)	8.86 ±0.82 (9)	326.95 ±7.76 (10)
Control	3.03 ±0.48 (8)	42.59 ±5.60 (10)	9.13 ±1.06 (10)	323.84 ±6.32 (10)
	t = 0.46 p > 0.5	t = 1.3866 p > 0.1	t = 0.63 p > 0.5	t = 1.02 p > 0.2
Seven days				
Experimental	2.91 ±0.44 (9)	41.58 ±4.86 (10)	8.00 ±0.97 (10)	321.77 ±10.27 (10)
Control	2.90 ±0.31 (9)	41.69 ±4.13 (10)	8.31 ±1.07 (10)	326.79 ±6.58 (10)
	t = 0.08 p > 0.9	t = 0.05 p > 0.9	t = 0.688 p > 0.5	t = 1.30 p > 0.2

[1]Mean values are expressed ± standard deviation.
[2]Number of individuals.

goldfish and trout exposed to extremely low concentrations of dissolved oxygen for periods of 4 to 25 days (Phyllips, 1947; Prosser, et al., 1957; Ostroumova, 1964).

If sublethal concentrations of suspended solids reduce the oxygen available at the gill, then it must be determined if suspended solids can affect gas transport across the respiratory epithelium, inducing a *de facto* hypoxia. Section III presents histological evidence that, in white perch, the primary site of respiratory gas exchange, the secondary lamellae, was damaged by exposure to 0.65 g l^{-1} fuller's earth. It appears that exposure to sublethal concentrations of fuller's earth can reduce a fish's ability to obtain oxygen by disrupting the gill surface and rendering the tissue partially dysfunctional.

III. EFFECTS OF SUBLETHAL CONCENTRATIONS OF FULLER'S EARTH ON WHITE PERCH GILL TISSUE

1. Introduction.

The gills are the primary site of respiratory gas exchange in most fish. The fish's blood is brought in close contact with the surrounding water at the gill surface. A membrane composed of two layers of cells separates the blood from the water; the gas exchange occurs through this membrane. Oxygen is absorbed from the water by the hemoglobin of the red blood cells, while carbon dioxide and other excretory products, such as ammonia, are released into the water. This system provides little barrier to gas transfer, but leaves the gill vulnerable to toxic or abrasive materials.

This section presents the results of a histological study of gill tissue in white perch exposed for 5 days to fuller's earth suspensions. The study was designed to determine the damaging effects, if any, of suspended mineral solids on the gills of white perch.

2. Methods.

White perch were exposed for 5 days to concentrations of 0.65 g l^{-1} fuller's earth. After exposure the fish were removed from the experimental and control tanks and killed. The first gill arch on the right side was removed from each fish and fixed in Bouin's solution. The tissue was embedded in paraffin, and 6-micrometer-thick serial sections were cut. The sectioning plane was dorsoventral, moving serially from the distal to the proximal end of the gill filaments. This made the mucus goblet cells located on the margins of the gill filaments visible; individual secondary lamellae were also clearly visible. Slides containing six to eight serial sections were stained alternately with iron-hematoxylin and Gomori's trichrome technique.

3. Results and Interpretation.

Gill sections from control fish showed the typical structure for teleost fish (Figs. 1 and 2). Control fish had moderate concentrations of mucus goblet cells, particularly on the anterior margin of each gill filament (Fig. 2). There were concentrations of one to several mucus cells in each

Figure 1. Gill section from white perch held 5 days in clean water. Secondary lamellar structure is undisturbed, and epithelium, is applied tightly to the pilar cell structure (ep = epithelium, pc = pilar cells, er = red blood cells, ga = gill artery). Photo taken at X 250.

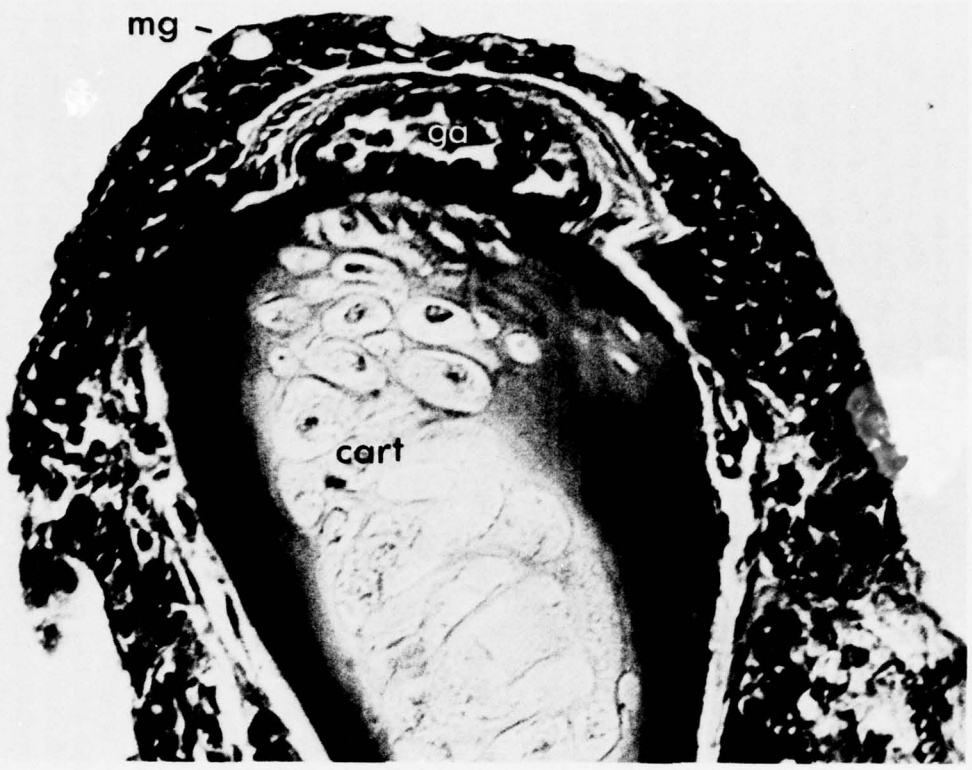

Figure 2. Gill section from white perch held 5 days in clean water. Section includes the anterior margin of the filament; note mucus goblet cells (mg = mucus goblet cells, ga = gill artery, cart = cartilage of gill ray). Photo taken at X 400.

serial section, although a single cell rarely occurred in more than one section. This concentration varied little over the length of a given filament. Individual mucus cells appeared to be less than 6 micrometers in diameter.

Many mucus goblet cells appeared on the gills of white perch exposed to fuller's earth concentrations (Fig. 3). In some cases mucus cells were the only visible cellular component of the tissue at the anterior margin of the filaments. The mucus cells were confined to the margins of the filaments, particularly to the anterior margin which is the first to come in contact with the water. Little, if any, increase in mucus cell concentration was observed elsewhere in the gill. Examination of serial sections revealed no increase in the size of individual mucus cells. High mucus cell concentrations made identification of individual cells difficult.

The secondary lamellae on the gill sections of white perch consisted of a supportive tube of pilar cells with red blood cells present inside the tube. A single, thin layer of epithelium covered the lamellae (Fig. 4). The integrated structure of the secondary lamellae provides for maximum respiratory gas exchange efficiency by maintaining a minimum distance between the hemoglobin-rich red cells and the oxygen-rich water.

The secondary lamellae of white perch exposed to 0.65 g l^{-1} fuller's earth were swollen. The epithelium was separated from the pilar cell tube and the epithelial cells were enlarged, forming a thick covering (compare Figs. 4 and 6). Pilar cell structure usually remained intact (Fig. 5), although it was occasionally disrupted (Fig. 6).

The effects of fuller's earth suspensions on gill tissues of white perch were similar to the effects of diatomaceous earth on rainbow trout gills (Southgate, 1962) and the effects of china-clay mining waste on brown trout gills at high concentrations (Slanina, 1964) and low concentrations (Herbert, et al., 1961; Herbert and Merkens, 1961).

The gills of fish exposed to suspended solids showed separation of the epithelium from the lamellar structure, thickening of the epithelium, and occasional disruption of the pilar cell structure of the lamellae (Herbert and Merkens, 1961; Herbert, et al., 1961; Southgate, 1962; Slanina, 1964). These effects were induced using concentrations of suspended solids between 0.40 and 0.81 g l^{-1}, with a high percentage of particles in the silt-clay range. The effect of particle size on gill tissue has not been fully evaluated. However, based on available data, a definite concentration effect is associated with silt-clay-sized particles. Concentrations of fuller's earth below the 24-hour LC_{10} value may adversely affect the gill tissue structure of the white perch in a 5-day period.

Gill damage caused by suspended solids has not been positively identified as harmful to fish in terms of overall survival. Ritchie (1970) pointed out that the type of gill damage caused by particles in suspension effectively reduces the respiratory surface area. He stated that a reduced

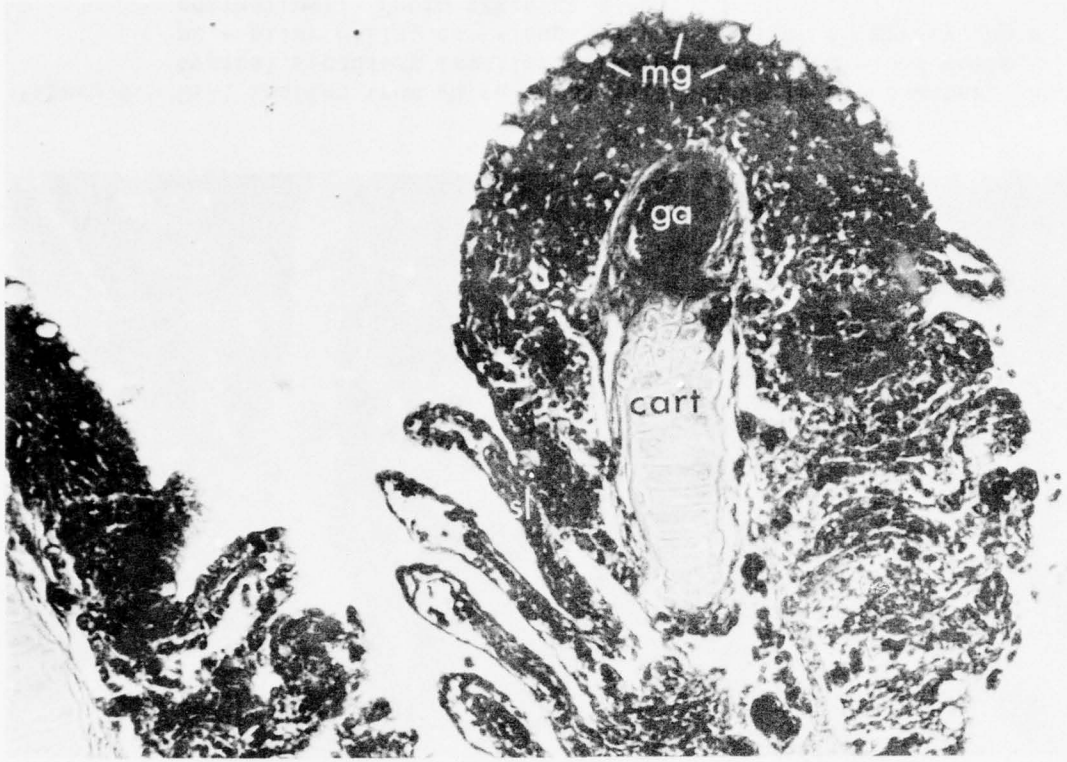

Figure 3. Gill section from white perch exposed for 5 days to 0.65 g l^{-1} fuller's earth. Shows marked proliferation of mucus goblet cells on the anterior margin of the gill filament (ga = gill artery, cart = cartilage of gill ray, mg = mucus goblet cells, sl = secondary lamella). Photo taken at X 160.

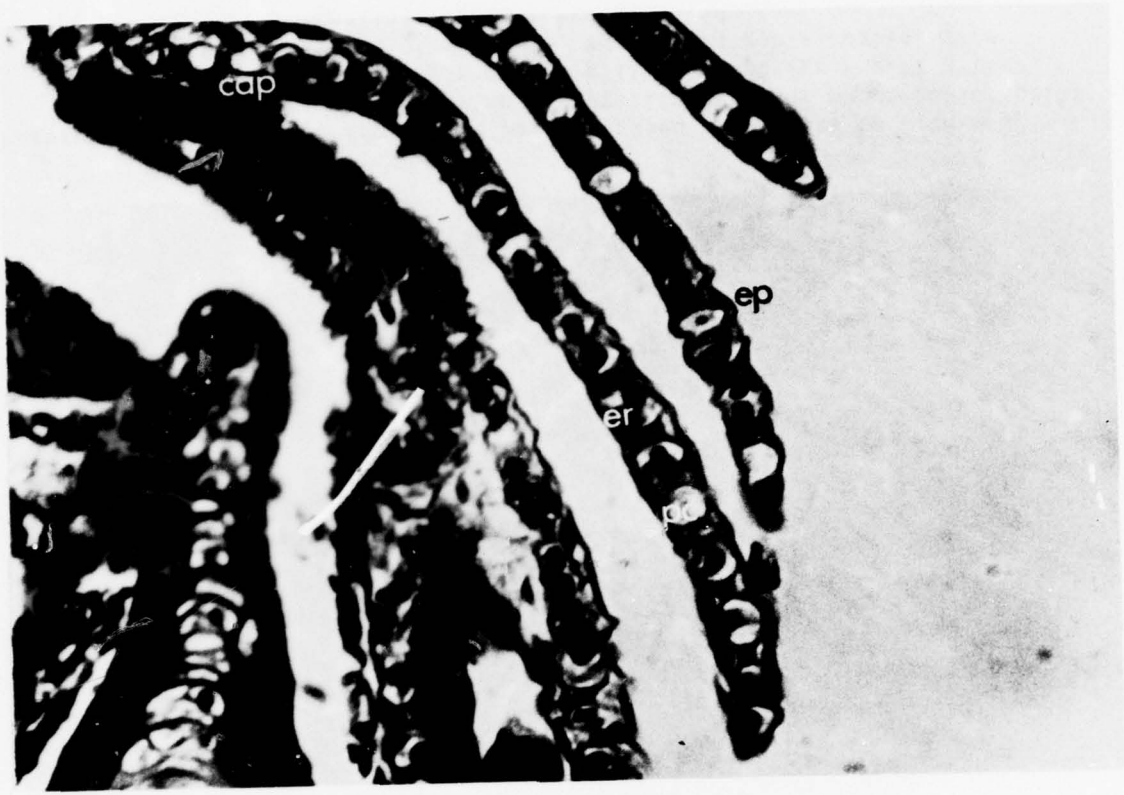

Figure 4. Gill section from white perch held 5 days in clean water. Shows typical secondary lamellar structure (er = red blood cells, pc = pilar cells, cap = capillary connection to artery, ep = epithelium). Photo taken at X 400.

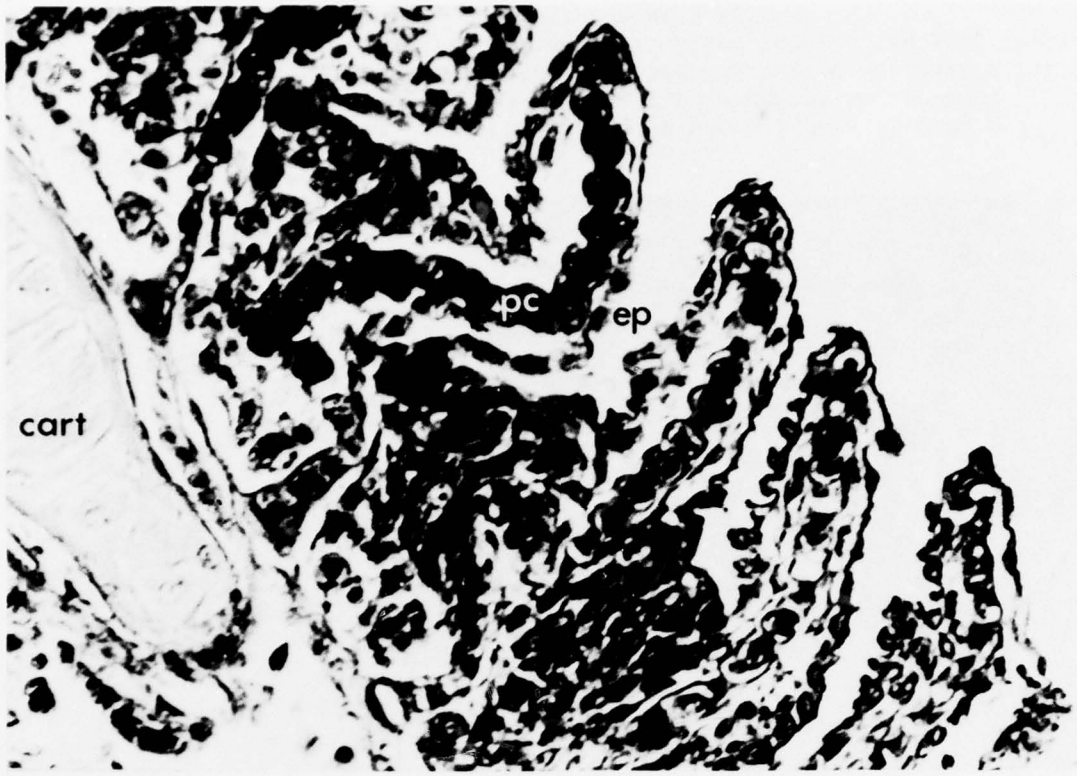

Figure 5. Gill section from white perch exposed for 5 days to 0.65 g l^{-1} fuller's earth. Epithelium of secondary lamellae has separated, leaving space between pilar cells and swollen epithelium. Areas at the base of lamellae appear to be fused (ep = epithelium, pc = pilar cell tube, cart = cartilage of gill ray). Photo taken at X 400.

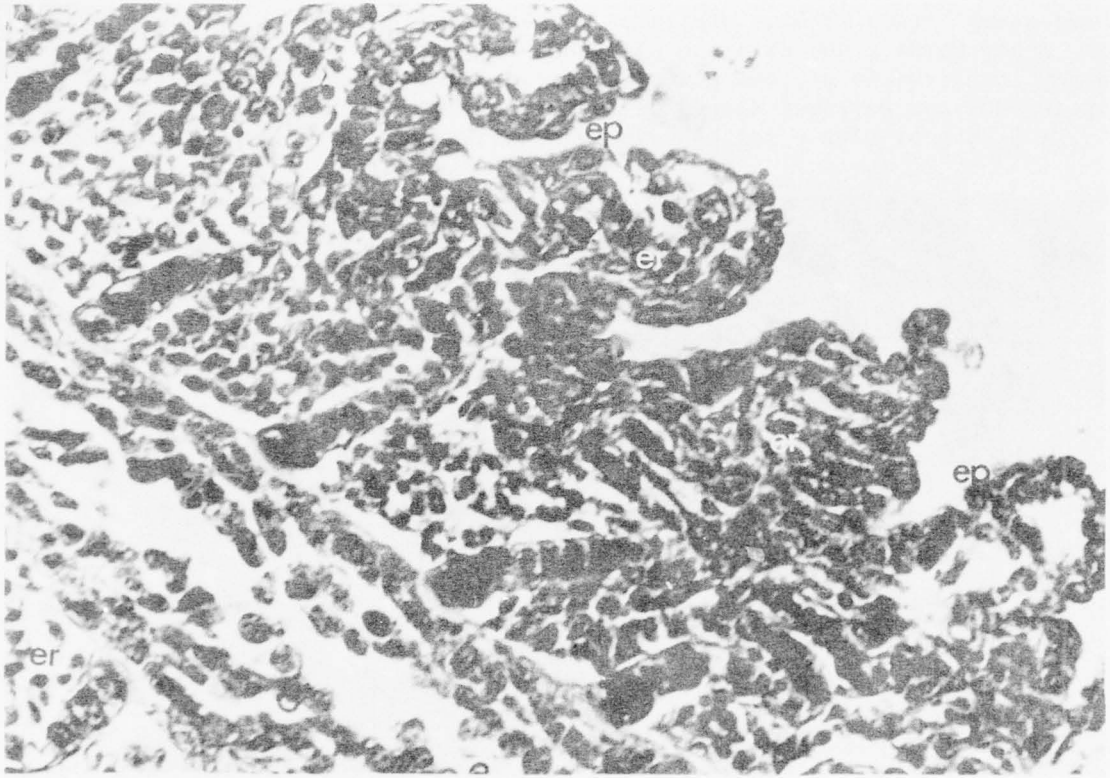

Figure 6. Secondary lamellae from white perch held for 5 days in 0.65 g l^{-1} fuller's earth. Epithelial cells are enlarged on and between lamellae. Pilar cell structure has been disrupted, releasing red blood cells to circulate inside the swollen lamella (ep = epithelium, er = red blood cells). Photo taken at X 400.

gill surface may debilitate fish, but no supporting data were given. Many species of freshwater fish can survive for several weeks in highly turbid conditions (European Inland Fisheries Advisory Commission, 1964), indicating that compensatory reactions may enable fish to survive despite gill damage. Randall (1970) pointed out that shunt mechanisms are commonly used by fish so that not all of the gill surface is used for respiration. By using the "reserve" surface area, fish may have sufficient functional, but damaged, gas exchange surface to survive prolonged exposure to suspended solids. The functional decrease in gill surface area caused by suspended solids also may be offset by compensatory increases in the gas exchange capacity of the blood (Section II).

IV. EFFECTS OF SUBLETHAL CONCENTRATIONS OF FULLER'S EARTH ON CARBOHYDRATE METABOLISM IN THE HOGCHOKER

1. Introduction.

Fish livers contain large quantities of carbohydrate stored as animal starch or glycogen. During periods of starvation or stress increased metabolic demands for energy are met by breaking down liver glycogen into glucose and releasing it into the blood. This section presents the results of experiments determining the rate of glycogen utilization in the hogchoker during exposure to sublethal concentrations of fuller's earth.

2. Methods.

The glycogen content in liver samples from hogchokers was determined after the fish had been held for 5 days in either control conditions or in suspensions of 1.24 g l^{-1} fuller's earth. Glycogen was extracted from liver tissue by boiling the tissue in 30-percent potassium hydroxide (KOH), followed by precipitation with 95-percent ethanol (Good, Kramer, and Somogyi, 1933). Quantitative estimates of glycogen concentration were derived colorimetrically using the phenolsulfuric acid technique (Montgomery, 1957). Liver glycogen concentrations were expressed as milligrams per 100 milligrams (mg 100 mg^{-1}) of liver tissue. The results were analyzed statistically using "Student's" t-distribution (Snedecor and Cochran, 1967).

3. Results and Interpretation.

Liver glycogen content from freshly caught hogchokers was about 15 to 17 mg 100 mg^{-1} (Sherk, O'Connor, and Neumann, 1972). Mean glycogen content in hogchoker livers decreased to 15.17 ± 3.6 mg 100 mg^{-1} (Table 10) after 5 days in control conditions. Fish held in a suspension of 1.24 g l^{-1} fuller's earth had a liver glycogen content of 10.77 ± 3.2 mg 100 mg^{-1}, significantly less than the value determined for control fish ($p < 0.01$, Table 10).

Similar studies conducted with white perch and striped bass provided no useful data (App. A). Glycogen mobilization rates in these species were so high that the final liver glycogen concentrations in experimental and control fish were below the limits of the analytical procedure.

Table 10. Experimental and control liver glycogen concentrations of hogchokers exposed for 5 days in 1.24 g l^{-1} fuller's earth.[1]

Group	Individuals (No.)	Glycogen (mg 100 mg^{-1})
Experimental	10	10.77[2] ±3.2
Control	10	15.17 ±3.6

[1]Mean values are expressed ± standard deviation.
[2]$p < 0.01$ ($t = 2.889$, d.f. = 18).

Rates of glycogen mobilization in fish may be used to estimate the energy utilization rate during starvation (Prosser and Brown, 1961; Kamra, 1966; Beamish, 1968; Swallow and Fleming, 1969). Thus, one interpretation of the rapid glycogen utilization in hogchokers exposed to suspended sediments is that the sediment stress resulted in an increased energy requirement. Several observations support this hypothesis. Hogchokers have a daily activity rhythm that persists in the laboratory (O'Connor, 1972). Those exposed to fuller's earth did not restrict their activity to specific parts of the daily cycle as did control fish. Therefore, an increase in locomotor activity may account for an increase in energy utilization during exposure to fuller's earth suspensions.

Fish in fuller's earth suspensions may use more reserve energy for compensatory hematological responses. Hogchokers exposed to suspended solids showed evidence of significant alterations in basic hematological parameters (Section II), indicating an increase in the oxygen exchange capacity of the blood. Compensatory physiological alterations demand energy which must come from existing internal storage during starvation.

V. EFFECTS OF SUSPENDED SOLIDS ON RESPIRATION OF ESTUARINE FISH

1. Introduction.

The gills of fish are in constant contact with water. Water flowing across the gill surface helps supply the oxygen necessary for metabolism. Any materials dissolved or suspended in the water may come in contact with the gill surfaces.

This section assesses the effects of suspended solids on oxygen consumption of estuarine fish. Fuller's earth suspensions were used to test the particle effects of clean clay. Patuxent River sediment suspensions were used to test the effects of naturally occurring particulate matter and associated substances on fish respiration.

Oxygen consumption rates were determined for striped bass, white perch, and toadfish in filtered Patuxent River water (base line) and in filtered river water suspensions of fuller's earth or Patuxent River sediment.

Several methods are commonly used to measure fish oxygen consumption (Fry, 1971). Brett (1962) described the following three levels of fish respiration, in terms of activity:

(a) Standard oxygen consumption, required to support tissue metabolism during periods of inactivity;

(b) routine oxygen consumption, required during periods of random activity; and

(c) active oxygen consumption, required during periods of swimming at moderate to maximum speeds.

Respiration rates of pelagic fish in this report were determined under conditions of moderate activity. Values reported for demersal fish are measures of routine oxygen consumption.

2. Material and Methods.

a. Equipment. A tunnel-type respirometer (Brett, 1964), which maintained suspensions of fine particles and provided the variety of flow rates used to control swimming speeds of fish, was used for this project. A prototype respirometer (72-liter capacity), similar to that described by Farmer and Beamish (1969), was also constructed (Sherk and O'Connor, 1971). The respirometer loop and centrifugal pump were type-316 stainless steel. A cast acrylic chamber with plastic grids at each end was installed in the lower section of the loop. An oval section cut from the top of the chamber permitted insertion and removal of fish from the respirometer. Rubber gaskets and hose clamps sealed the access port during experiments. Straightening vanes upstream from the chamber ensured laminar flow. The centrifugal pump was driven by a variable-speed electric motor. An orifice plate in the upper part of the loop measured flow rates. Two needle valves and a fill pipe on the upper side of the loop and two neoprene stoppered openings in the chamber provided access to the water. These access points were used extensively during experiments to bleed the respirometer of trapped air, and to sample suspensions. At two points on the respirometer loop, 20-meter copper coils controlled temperature via counter-current heat exchange with water pumped from a constant temperature bath.

Four inverted versions of the prototype respirometer (62-liter capacity) were used for these experiments. Flow rates were measured by annular flow sensors. A water jacket around the outside of the lower part of each loop controlled temperature via counter-current heat exchange with water pumped from a constant temperature bath.

All respirometers were wrapped with standard fiberglass insulation to maintain constant temperatures. Plywood enclosures were placed around each chamber during experiments to isolate fish from laboratory activity. Each enclosure contained a 15-watt cool white fluorescent lamp, 42 centimeters above the chamber to provide constant illumination during experiments. Fish were observed through a viewing slit in each enclosure. Surfaces immediately below each chamber were blackened. Changes in dissolved oxygen concentrations were monitored by Yellow Springs Instrument Co., Inc. Model 54RC oxygen meters. Oxygen electrode leads were passed through neoprene stoppers that sealed the fill pipes into which the electrodes were inserted.

b. Fish. Fish were collected by otter trawl from the Patuxent River estuary. Actual collection sites ranged from the Lower Marlboro area to the vicinity of Drum Point, Maryland, depending upon species and time of year. Fish were kept on the collecting vessel in 80-liter plastic trash cans. A constant flow of ambient river water was maintained through the cans until the vessel returned to the laboratory.

The laboratory holding facilities consisted of 208-liter polyethylene tanks immersed in controlled-temperature water baths. Water in the tanks continually passed through an inline protein-skimmer filtration system. Patuxent River water, which passed through a 5-micrometer mesh nylon filter, was usually used to supply the tanks. During the summer months of 1972 a commercial marine salt mix dissolved in laboratory well water was used to supply the holding tanks. Salinity of water used in the laboratory was about 5 parts per thousand. Holding tank and experimental temperatures were adjusted to approximate seasonal changes.

The fish were placed in the laboratory holding tanks, where care was taken to avoid overcrowding. Unhealthy or dead fish were removed immediately. Supplemental aeration was provided when large numbers of fish were held. The fish were under continuous fluorescent illumination. They were not fed following capture because active digestion increases standard and routine oxygen consumption (Beamish, 1964; Glass, 1968). Fish were held a minimum of 3 to 5 days before oxygen consumption rates were determined.

c. Measurement of Oxygen Consumption. Respirometers were filled with water from the holding tanks (Fry, 1971) during experiments. As soon as the water in the apparatus could completely cover the fish, each fish was transferred in a bucket of water from the holding tank to the respirometer. When the respirometers were full, water was circulated at 0.28 to 0.39 foot per second (ft/s) to force out entrained air which was replaced simultaneously by holding tank water. In addition, flow rate was increased by 0.18 ft/s at 4- or 5-minute intervals to drive out trapped air. Maximum flow attained during this procedure was 2.5 to 4 times the minimum experimental rate, depending on species. Flow was reduced to the minimum experimental exposure rate and all access points were closed. The plywood enclosure was placed around the chamber.

Oxygen electrodes were calibrated and inserted through the fill pipes. Time, temperature, dissolved oxygen concentration, and flow rate were recorded for each respirometer as soon as it was set up and at hourly intervals thereafter. Preliminary studies demonstrated that at a constant flow of 0.28 ft/s, the hourly oxygen consumption decreased until the third hour, after which rates were relatively constant. Data from the third hour were used in all analyses.

Several species were tested at various swimming speeds. The above procedure was used until the third-hour data had been recorded. Flow rates were then increased about 0.18 ft/s at 5-minute intervals until the desired speed was achieved. The same parameters were recorded 5 minutes after this speed was attained, and again 1 hour later. If information was required at higher levels of activity this procedure was repeated. All parameters were monitored for 1 hour at each of the increased flow rates.

At the end of each experiment the respirometers were drained and the fish were removed for weighing, length measurement, and sex determination. Respirometers were flushed with tapwater, then refilled with tapwater until used again. Terramycin (oxytetracycline hydrochloride, 15-milligram activity per liter) was added to the water when the respirometers would not be used for longtime periods.

Predetermined volumes of solids were added to about 16 liters of water in 80-liter plastic trash cans. The slurries were aerated continuously, and a submersible electric pump mixed the material. Slurries were prepared 18 hours before use, and were pumped into the respirometers as the units were filled with holding tank water. Respirometers were washed several times with tapwater at the end of experiments to prevent accumulation of materials in the units.

Concentrations of suspended materials were determined by the dry weight difference between three 5-milliliter replicate samples drawn from each respirometer at the beginning of an experiment, and three similar samples drawn from the holding tank (no suspended material added) at the same time. The oxygen demand of natural sediment suspensions was determined by measuring the oxygen uptake of slurries. Slurries were pumped into the respirometers as described above. Respirometers were set up as before but without fish. Mean third-hour oxygen consumption values of the slurries were used to check the oxygen demand of the sediment during experiments.

The equipment used for long-term exposure of fish to suspensions of solids is described in O'Connor, Neumann, and Sherk (1976) for bioassay experiments or for sublethal hematological studies (see Section II).

d. Data Analyses. Oxygen consumption rates were plotted against live weight on double logarithmic grids. Curves were fitted to the data by least squares linear regression analysis (Snedecor and Cochran, 1967). Correlation coefficients were determined for each group of data (Simpson, Roe, and Lewontin, 1960).

Group comparisons were made by covariance analysis of log-transformed data (Snedecor and Cochran, 1967). Sex influence on respiration rates was tested by covariance analysis for each species when possible. Data from males and females were combined for comparisons of base-line and experimental values.

3. Results.

a. Striped Bass. Fish were held at approximately 15° Celsius and 5 parts per thousand salinity for a minimum of 3 days before respiration rates were determined. Oxygen consumption rates were determined at three swimming speeds--0.28, 1.02, and 1.58 ft/s.

In filtered water, a 50-gram fish swimming at 0.28 ft/s consumed 19.0 milligrams oxygen per hour (mg O_2 h^{-1}) and a 150-gram fish used 31.4 mg O_2 h^{-1}. At a speed of 1.02 ft/s, a 50- and a 150-gram fish used 24.3 and 41.3 mg O_2 h^{-1}, respectively. At speeds of 1.58 ft/s, oxygen consumption rates increased to 33.7 mg O_2 h^{-1} for a 50-gram fish, and 63.7 mg O_2 h^{-1} for a 150-gram fish. A significant increase in respiration rates was observed between measurements made at 0.28 and 1.02 ft/s and between 1.02 and 1.58 ft/s (Table 11). Covariance analysis showed that oxygen consumption rates of male and female fish did not differ at either swimming speed (Table 12). Covariance analysis was not made for swimming speeds of 1.58 ft/s due to the small number of females tested.

A 50-gram fish consumed 19.2 mg O_2 h^{-1} and a 150-gram fish consumed 37.8 mg O_2 h^{-1} at a swimming speed of 0.28 ft/s during exposure to 0.79 g l^{-1} fuller's earth. A 50- and a 150-gram fish swimming at 1.02 ft/s under these conditions consumed 24.1 and 41.2 mg O_2 h^{-1}, respectively (Figs. 7 and 8; Table 13). Striped bass swimming at 1.58 ft/s in fuller's earth suspensions consumed less oxygen than fish swimming at that speed under base-line conditions (Fig. 9; Table 13). Oxygen consumption was uniformly depressed by about 25 percent throughout the weight range studied.

A graph of the oxygen consumption rates of striped bass swimming at 0.28 and 1.02 ft/s during exposure to 0.79 g l^{-1} fuller's earth showed different slopes. Respiration rates at swimming speeds of 1.02 and 1.58 ft/s during this exposure were not different. Rates for fish swimming at 0.28 and 1.58 ft/s were different at the 1-percent level (Table 11). Oxygen consumption rates of male and female striped bass swimming at 0.28 and 1.02 ft/s in fuller's earth suspensions were not different (Table 12). Comparisons of male and female oxygen consumption rates at 1.58 ft/s could not be made because too few females were studied.

Striped bass held at 22.5° Celsius and 9 parts per thousand salinity were tested at swimming speeds of 1.05 and 1.58 ft/s under base-line conditions and during exposure to natural sediment suspensions. The experimental procedure was modified because increased temperature, salinity, and sediment-oxygen demand reduced dissolved oxygen. The 3-hour acclimation period at a swimming speed of 0.28 ft/s was changed to 3 hours at 1.05 ft/s

Table 11. Covariance analysis of oxygen consumption and live weight regressions for striped bass[1].

Comparison (ft/s)	No.	Residual variance		Slope		Elevation	
		Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability
Fuller's earth							
0.28	27	0.010	25, 23	0.104	1, 48	0.028	1, 49
1.02	25	0.023	-----[2]	0.017	$p < 0.05$	0.018	-----
1.02	25	0.023	23, 9	0.010	1, 32	0.021	1, 33
1.58	11	0.009	------	0.019	-----	0.019	-----
0.28	27	0.010	25, 9	0.001	1, 34	0.073	1, 35
1.58	11	0.009	------	0.010	-----	0.009	$p < 0.01$
Base line							
0.28	28	0.014	26, 23	0.001	1, 50	0.165	1, 51
1.02	25	0.013	------	0.013	-----	0.013	$p < 0.05$
1.02	25	0.013	23, 9	0.011	1, 32	0.142	1, 33
1.58	11	0.005	------	0.011	-----	0.011	$p < 0.05$

[1]At swimming speeds of 0.28, 1.02, and 1.58 ft/s in 0.70 g l^{-1} fuller's earth and in filtered Patuxent River water (base line) at about 15° Celsius.
[2]Not significant.

Table 12. Covariance analysis of oxygen consumption and live weight regressions of male and female striped bass[1].

Comparison	Swimming speed (ft/s)	No.	Residual variance		Slope		Elevation	
			Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability
Fuller's earth								
Males	0.28	14	0.006	10, 12	0.025	1, 22	0.002	1, 23
Females		12	0.013	-----[2]	0.010	-----	0.011	-----
Males	1.02	13	0.008	9, 11	0.000	1, 20	0.002	1, 21
Females		11	0.004	------	0.006	-----	0.005	-----
Base line								
Males	0.28	14	0.012	12, 7	0.001	1, 19	0.025	1, 20
Females		9	0.010	------	0.011	-----	0.011	-----
Males	1.02	13	0.013	11, 5	0.001	1, 16	0.001	1, 17
Females		7	0.003	------	0.010	-----	0.010	-----

[1]At swimming speeds of 0.28 and 1.02 ft/s during exposure to 0.79 g l^{-1} fuller's earth and under base-line conditions at about 15° Celsius.
[2]Not significant.

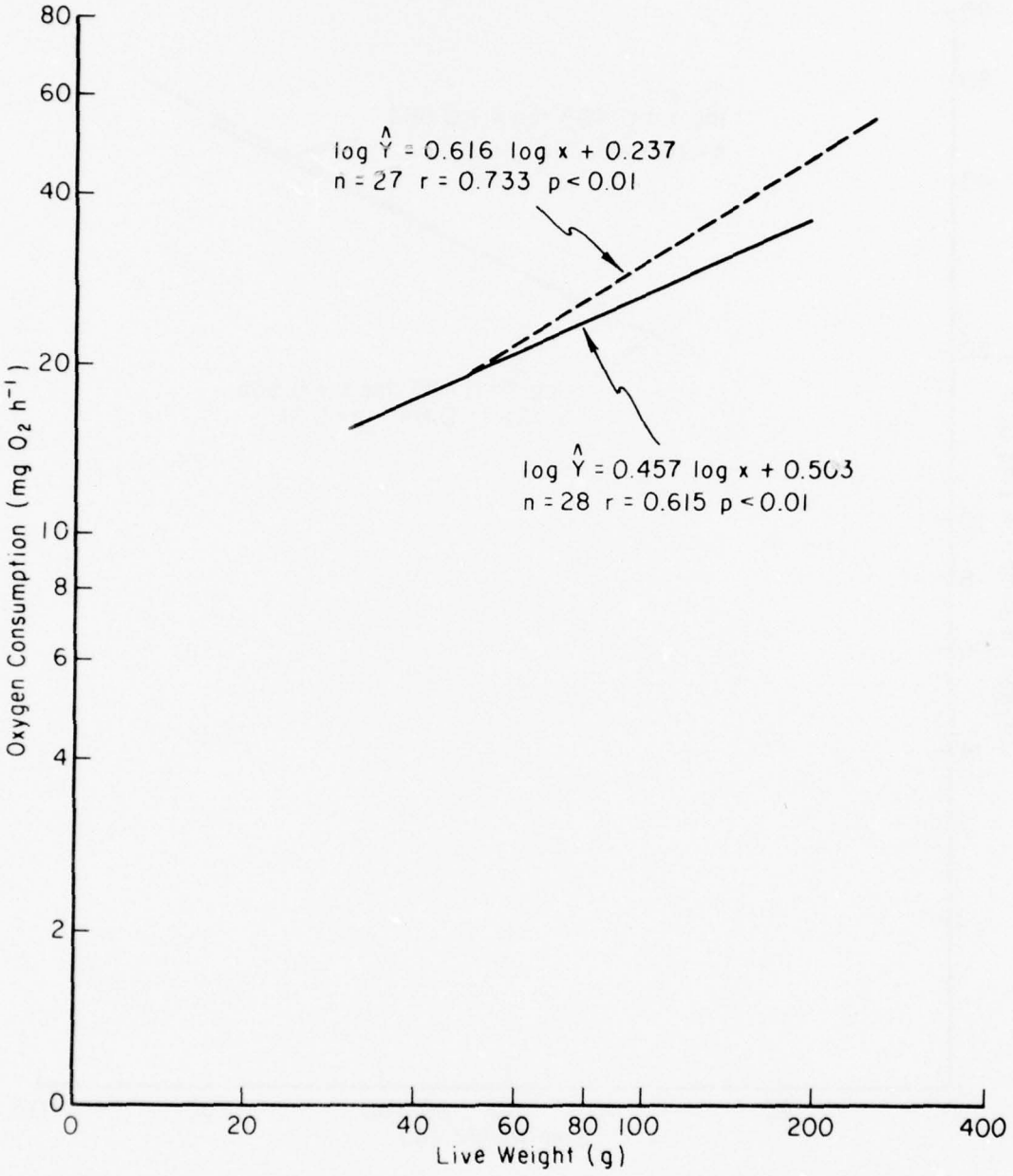

Figure 7. Oxygen consumption of striped bass swimming at 0.28 ft/s during exposure to 0.79 g l^{-1} fuller's earth (dashline) and under control conditions (solid line).

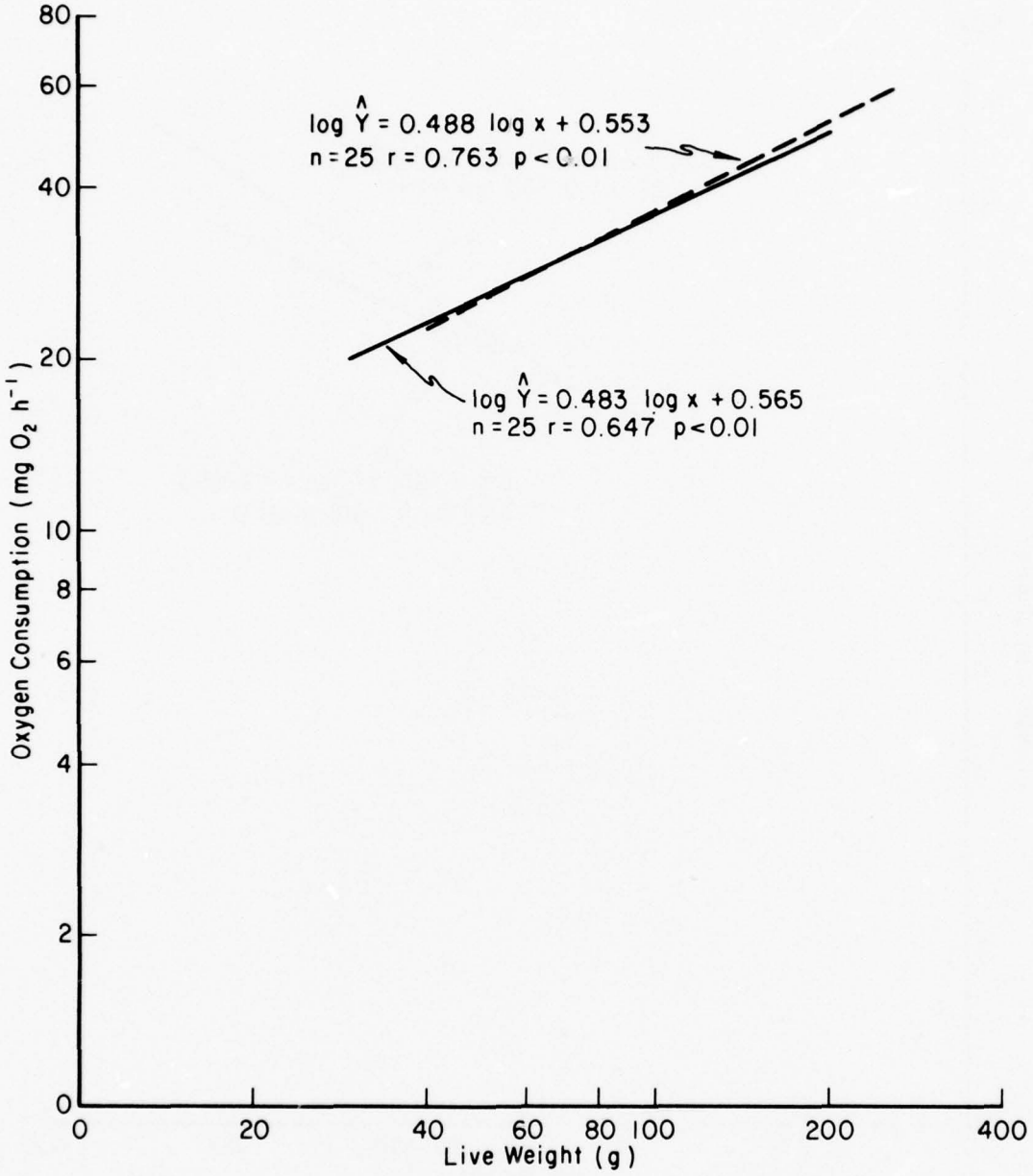

Figure 8. Oxygen consumption of striped bass swimming at 1.02 ft/s during exposure to 0.79 g l^{-1} fuller's earth (dashline) and under control conditions (solid line).

Table 13. Covariance analysis of oxygen consumption and live weight regressions of striped bass[1].

Comparison	Swimming speed (ft/s)	No.	Residual variance		Slope		Elevation	
			Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability
Fuller's earth	0.28	27	0.010	26, 25	0.012	1, 51	0.012	1, 52
Base line		28	0.014	-----[2]	0.009	-----	0.020	-----
Fuller's earth	1.02	25	0.023	23, 23	0.018	1, 46	0.019	1, 47
Base line		25	0.013	------	0.042	-----	0.028	-----
Fuller's earth	1.58	11	0.009	9, 9	0.007	1, 18	0.007	1, 19
Base line		11	0.005	------	0.001	-----	0.072	$p < 0.01$

[1]Exposed to 0.79 g l^{-1} fuller's earth and under base-line conditions at about 15° Celsius.
[2]Not significant.

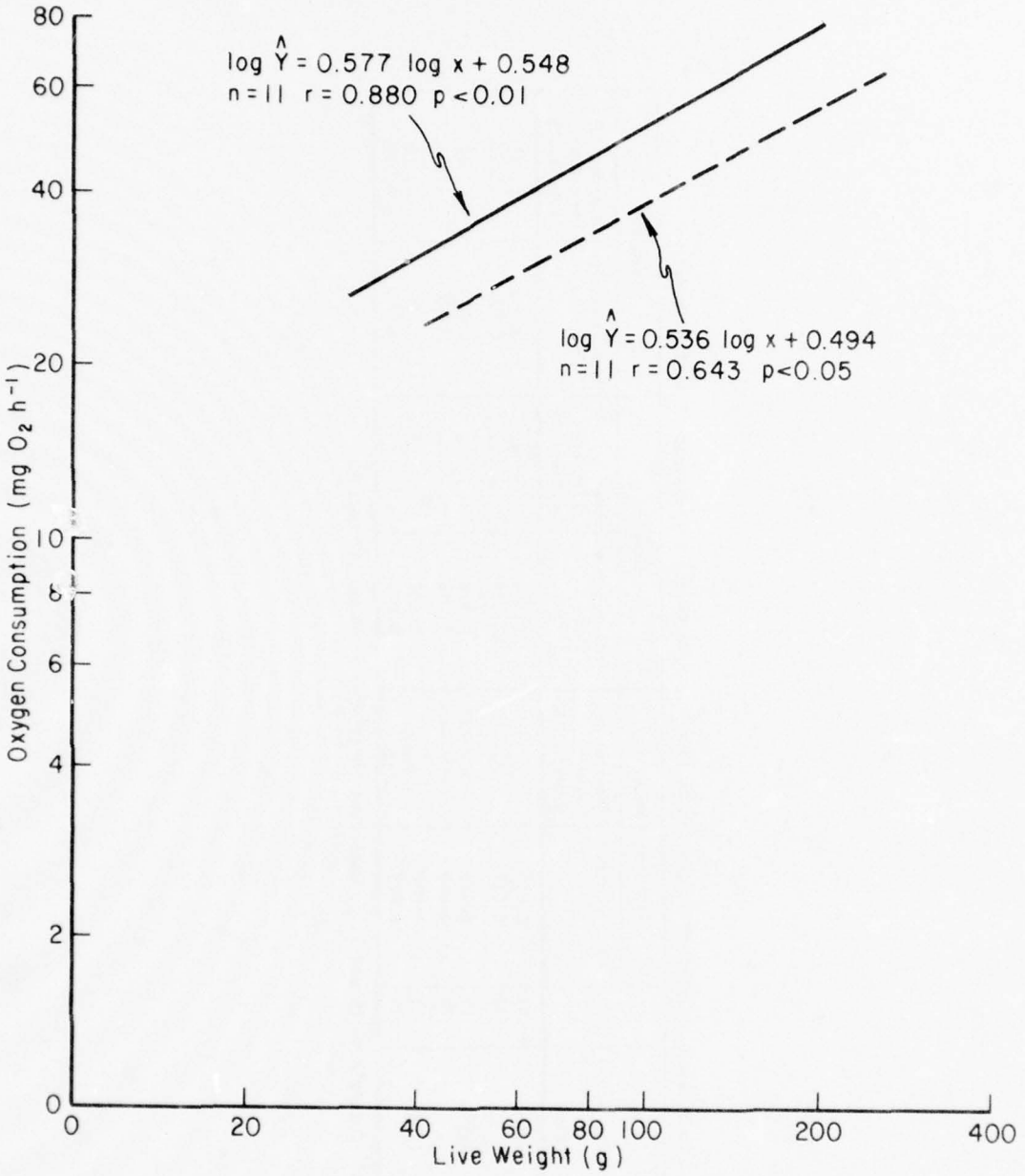

Figure 9. Oxygen consumption of striped bass swimming at 1.58 ft/s during exposure to 0.79 g l^{-1} fuller's earth (dashline) and under control conditions (solid line).

with continuous aeration. Respirometers were closed at the end of the third hour and oxygen concentrations were monitored for 1 hour. Flow rates were gradually increased to 1.58 ft/s.

Base-line oxygen consumption rates of a 50- and a 150-gram fish swimming at 1.05 ft/s were 23.2 and 55.0 mg O_2 h^{-1}, respectively. At a swimming speed of 1.58 ft/s, a 50-gram fish consumed 23.2 mg O_2 h^{-1}; a 150-gram fish consumed 48.1 mg O_2 h^{-1}. Respiration rates at the two swimming speeds were not significantly different, and sex influence on oxygen consumption rates was not apparent at either speed (Table 14).

Respiration rates of striped bass swimming at 1.05 and 1.58 ft/s during exposure to 1.31 and 1.33 g l^{-1} natural sediment, respectively, were reduced by 30 to 40 percent of the base-line values. At a swimming speed of 1.05 ft/s, a 50-gram fish used 14.2 mg O_2 h^{-1}; a 150-gram fish used 34.4 mg O_2 h^{-1} (Fig. 10; Table 15). Similar weight fish swimming at 1.58 ft/s consumed 14.1 and 34.9 mg O_2 h^{-1} (Fig. 11; Table 15). Respiration rates at the two swimming speeds were not significantly different, and oxygen consumption rates of males and females during exposure to natural sediment were not different at either swimming speed (Table 14).

b. White Perch. Fish were maintained at about 15° Celsius and 5 parts per thousand salinity for a minimum of 3 days. Oxygen consumption rates were determined at swimming speeds of 0.28 and 1.02 ft/s under base-line conditions. At a swimming speed of 0.28 ft/s, a 50-gram fish used 13.3 mg O_2 h^{-1}; a 150-gram fish used 27.1 mg O_2 h^{-1}. Fish of the same weights swimming at 1.02 ft/s consumed 24.4 and 44.6 mg O_2 h^{-1}, respectively. Respiration rates were greater at swimming speeds of 1.02 ft/s than 0.28 ft/s (Table 16); male and female respiration rates did not differ at either speed (Table 16).

Oxygen consumption rates were determined for white perch during exposure to 1.09 g l^{-1} fuller's earth suspension at swimming speeds of 0.28 (Fig. 12) and 1.02 (Fig. 13) ft/s. The data were dispersed at both swimming speeds--correlation coefficient, $r = 0.017$ (not significant) at 0.28 ft/s and $r = 0.201$ (not significant) at 1.02 ft/s. Covariance analyses were not attempted because of poor data correlation.

Similar results were observed when oxygen consumption was determined for white perch swimming at 0.39 and 1.05 ft/s during exposure to 2.12 g l^{-1} natural sediment suspensions. Low correlation coefficients, $r = 0.143$ (not significant) at 0.39 ft/s and $r = 0.017$ (not significant) at 1.02 ft/s, prevented further statistical treatment of these data.

White perch were held in suspensions of 2.58 g l^{-1} natural sediment for 72 hours. Oxygen consumption rates were measured in filtered river water at swimming speeds of 0.39 and 1.05 ft/s. Data for fish swimming at 0.39 ft/s after a 72-hour exposure were too scattered, $r = 0.508$ (not significant), to permit further analysis (Fig. 14). After a 72-hour exposure to natural sediment the oxygen consumption rates of a 50- and a

Table 14. Covariance analysis of oxygen consumption and live weight regressions of striped bass[1].

Comparison	Swimming speed (ft/s)	Individuals (No.)	Residual variance		Slope		Elevation	
			Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability
Experimental	1.05	22	0.0322	20, 18	0.0003	1, 38	0.0002	1, 39
Males		9	0.0182	7, 11	0.0599	1, 18	0.1093	1, 19
Females		13	0.0316	-----[2]	0.0264	-----	0.0282	-----
	1.58	20	0.0334	------	0.0328	-----	0.0319	-----
Males		7	0.0351	5, 11	0.0405	1, 16	0.1316	1, 17
Females		13	0.0231	------	0.0268	-----	0.0276	-----
Base line	1.05	21	0.0193	19, 15	0.0099	1, 34	0.0084	1, 35
Males		12	0.0262	10, 7	0.0154	1, 17	0.0045	1, 18
Females		9	0.0120	------	0.0204	-----	0.0201	-----
	1.58	17	0.0084	------	0.0145	-----	0.0144	-----
Males		11	0.0092	9, 4	0.0176	1, 13	0.0061	1, 14
Females		6	0.0049	------	0.0079	-----	0.0086	-----

[1]At swimming speeds of 1.05 and 1.58 ft/s during exposure to 1.32 g l^{-1} natural sediment and under base-line conditions at 22.5° Celsius.
[2]Not significant.

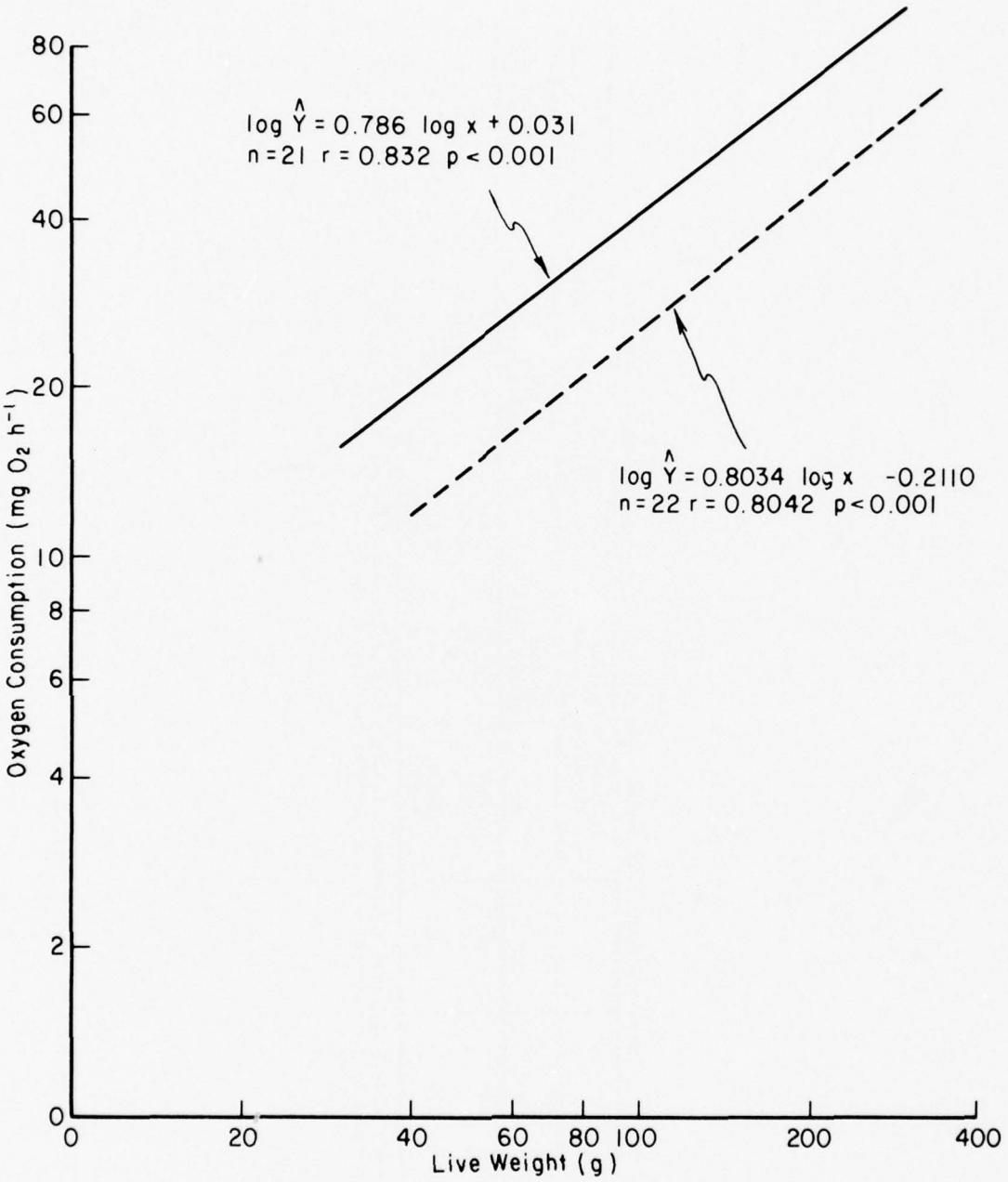

Figure 10. Oxygen consumption of striped bass swimming at 1.05 ft/s during exposure to 1.31 g l^{-1} natural sediment (dashline) and under base-line conditions (solid line).

Table 15. Covariance analysis of oxygen consumption and live weight regressions of striped bass[1].

Comparison	Swimming speed (ft/s)	Individuals (No.)	Residual variance		Slope		Elevation	
			Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability
Experimental	1.05	22	0.0322	19, 20	0.0259	1, 39	0.0253	1, 40
Base line		21	0.0193	-----[2]	0.0005	-----	0.3666	$p < 0.005$
Experimental	1.58	20	0.0322	15, 18	0.0221	1, 33	0.0219	1, 34
Base line		17	0.0084	------	0.0184	-----	0.2360	$p < 0.001$

[1]At swimming speeds of 1.05 ft/s during exposure to 1.31 g l^{-1} natural sediment and under base-line conditions and at 1.58 ft/s during exposure to 1.33 g l^{-1} natural sediment and under base-line conditions at 22.5° Celsius.
[2]Not significant.

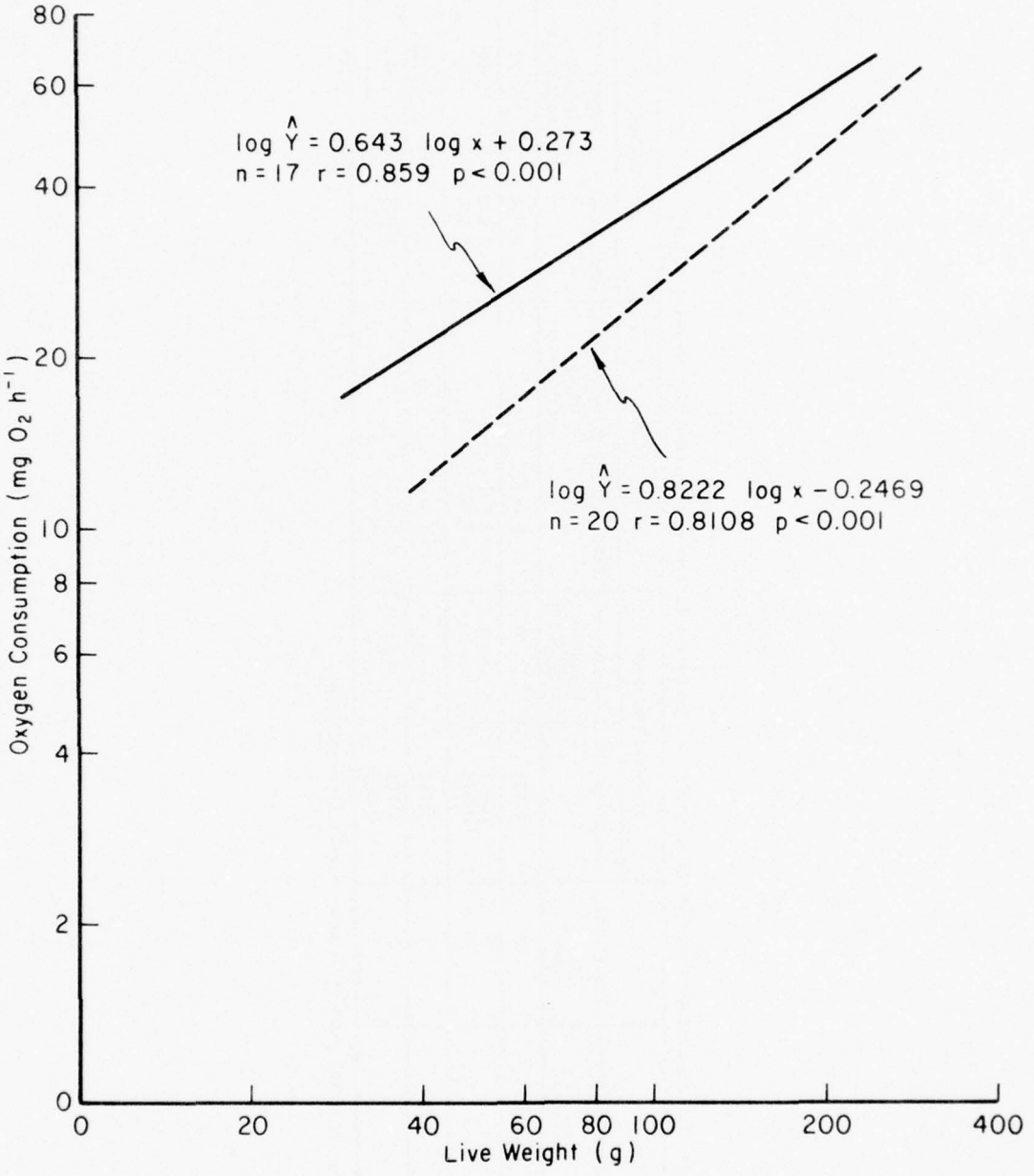

Figure 11. Oxygen consumption of striped bass swimming at 1.58 ft/s during exposure to 1.33 g l^{-1} natural sediment (dashline) and under base-line conditions (solid line).

Table 16. Covariance analysis of oxygen consumption and live weight regressions of white perch[1].

Comparison	Swimming speed (ft/s)	Individuals (No.)	Residual variance		Slope		Elevation	
			Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability
Random selection Combined sexes	0.28	13	0.023	11, 9	0.002	1, 21	0.208	1, 22
Males		5	0.025	3, 6	0.001	1, 9	0.078	1, 10
Females		8	0.015	----[2]	0.019	-----	0.018	-----
Random selection Combined sexes	1.02	11	0.012	-----	0.018	-----	0.017	$p < 0.05$
Males		5	0.001	3, 4	0.011	1, 7	0.028	1, 8
Females		6	0.016	-----	0.010	-----	0.010	-----

[1]At swimming speeds of 0.28 and 1.02 ft/s under base-line conditions.
[2]Not significant.

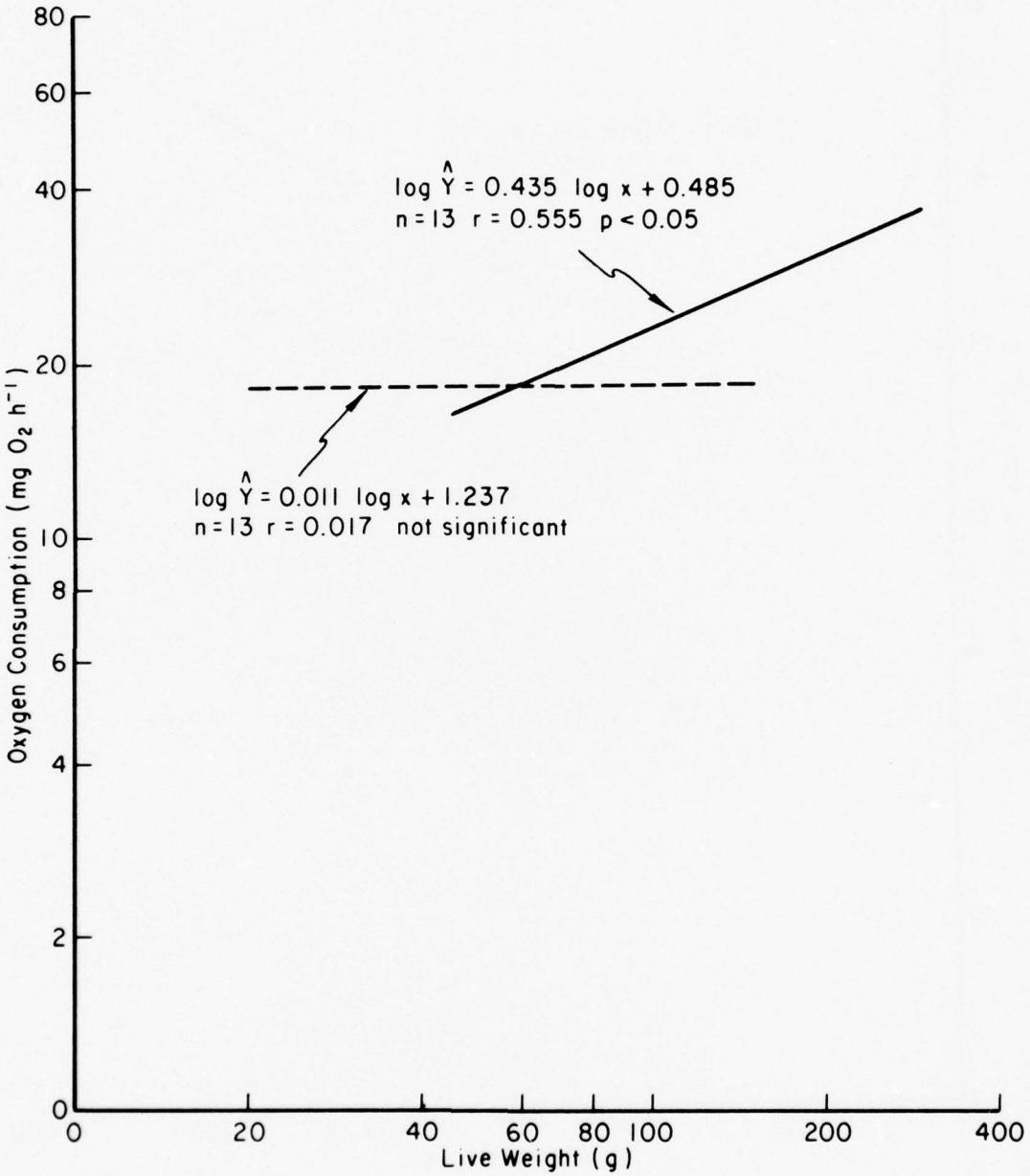

Figure 12. Oxygen consumption of white perch swimming at 0.28 ft/s during exposure to 1.09 g l^{-1} fuller's earth (dashline) and under base-line conditions (solid line).

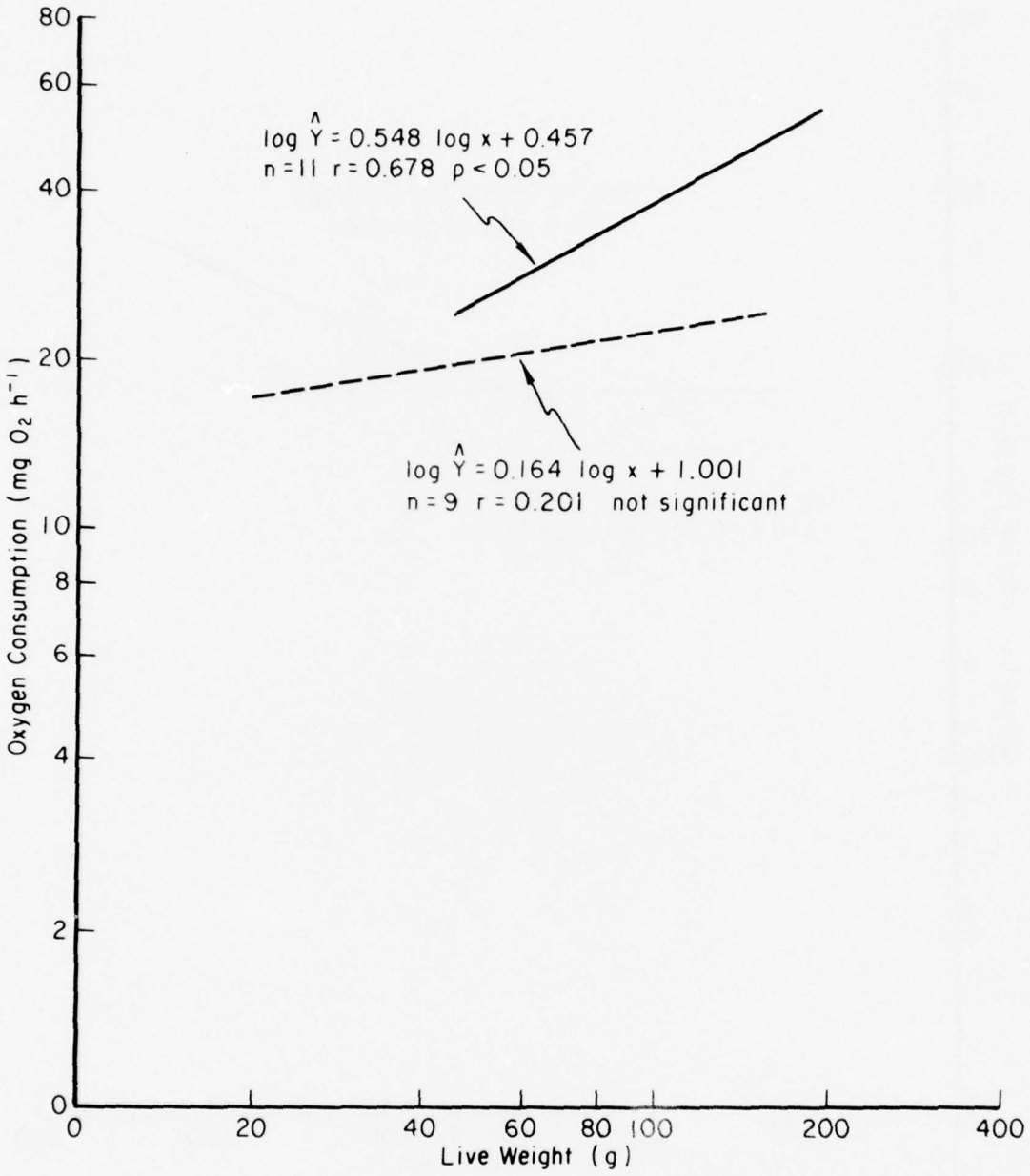

Figure 13. Oxygen consumption of white perch swimming at 1.02 ft/s during exposure to 1.09 g l^{-1} fuller's earth (dashline) and under base-line conditions (solid line).

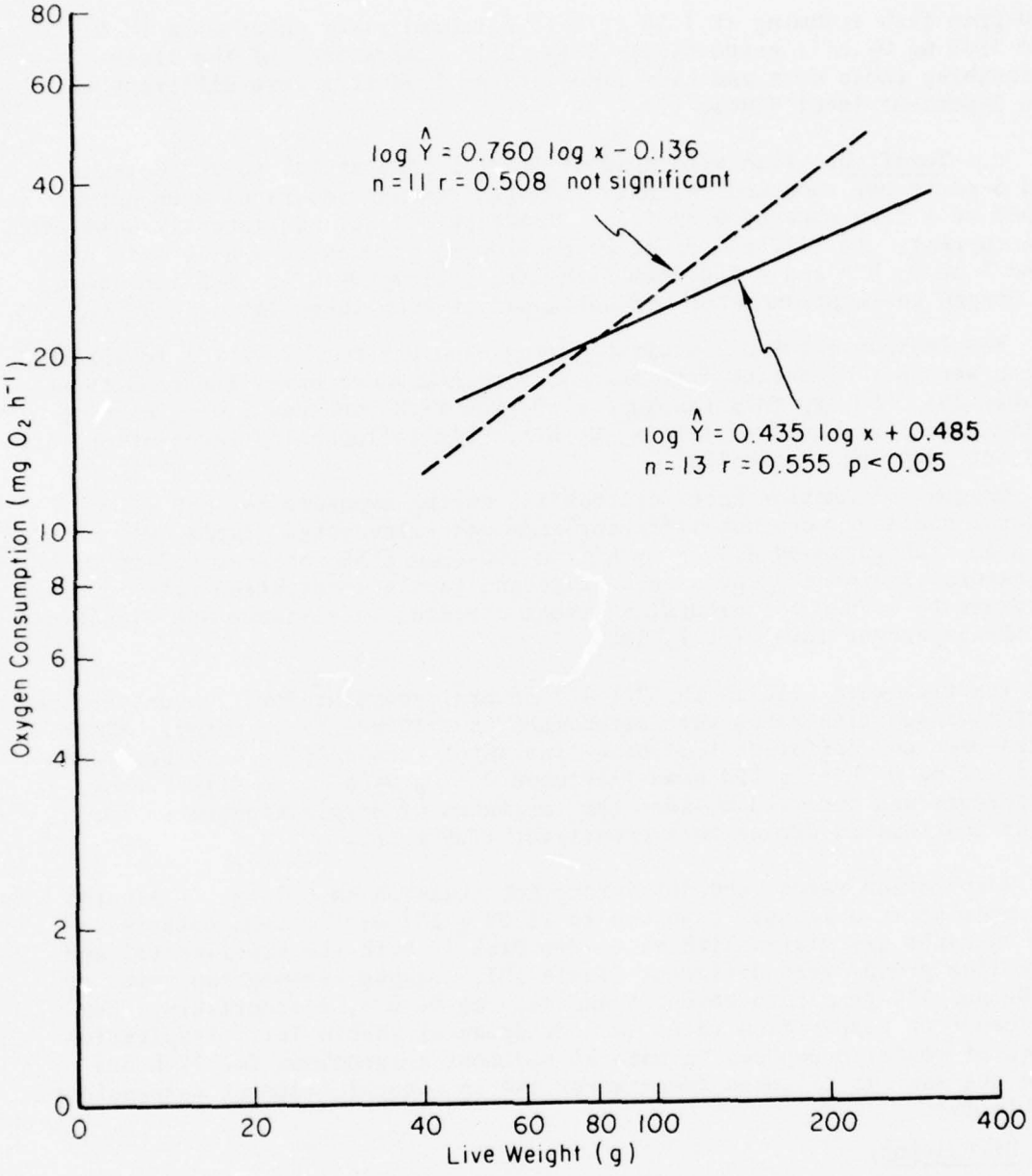

Figure 14. Oxygen consumption of white perch swimming at 0.39 ft/s in clean water following 72-hour exposure to 2.58 g l^{-1} resuspended natural sediment (dashline) and at 0.28 ft/s under base-line conditions (solid line).

150-gram fish swimming at 1.58 ft/s in filtered river water were 10.8 and 55.3 mg O_2 h^{-1}, respectively (Fig. 15). Elevations of the lines describing these data and base-line data at 1.58 ft/s were different at the 5-percent level (Table 17).

c. Toadfish. Fish were held at least 5 days at 18° to 20° Celsius and 5 parts per thousand salinity. Oxygen consumption rates were determined at a flow rate of 0.39 ft/s. Toadfish did not consistently swim into the current. In filtered river water flowing at 0.39 ft/s, a 50-gram fish used 5 mg O_2 h^{-1} and a 150-gram fish used 10.1 mg O_2 h^{-1}. Sex influence on oxygen consumption rates was not apparent from these data (Table 18).

Respiration rates of toadfish during exposure to 2.20 g l^{-1} fuller's earth were not different from rates determined under base-line conditions (Table 19). During this exposure a 50-gram fish consumed 5.6 mg O_2 h^{-1}; a 150-gram fish consumed 12.4 mg O_2 h^{-1}. Sex influence on respiration rates was not apparent (Table 18).

Oxygen consumption rates of toadfish during exposure to 1.58 g l^{-1} natural sediment were not different from base-line rates (Table 19). A 50-gram fish consumed 4.8 mg O_2 h^{-1}; a 150-gram fish consumed 9.7 mg O_2 h^{-1} in natural sediment suspensions. Male and female respiration rates during exposure to 1.58 g l^{-1} natural sediment differed in variance and elevation at the 5-percent level (Table 18).

Toadfish were held in 10.37 g l^{-1} natural sediment for 72 hours before oxygen consumption rates were determined in filtered river water. These rates were not different from base-line rates (Table 19). A 50-gram fish used 2.2 mg O_2 h^{-1}; a 150-gram fish used 7.3 mg O_2 h^{-1}. A significant difference was observed between the variances of respiration rates for males and females during this experiment (Table 18).

Respiration rates were determined for toadfish in 3.36 g l^{-1} natural sediment after a 72-hour exposure to 11.09 g l^{-1} of the same material. The variance associated with rates for fish in both the experimental and base-line groups were different (Table 19). Oxygen consumption rates of a 50- and 150-gram fish were 2.5 and 11.5 mg O_2 h^{-1}, respectively. Sex influence on respiration rates was not apparent (Table 18). Respiration rates of toadfish exposed to natural sediment suspensions for 72 hours were the same in filtered river water and in natural sediment suspensions (Table 19).

4. Discussion.

Concentrations of suspended materials in an estuarine system are highly variable. Storms, floods, tidal scour, or engineering activities may increase concentrations of suspended particles. Naturally occurring suspended loads exceeding 1 g l^{-1} are uncommon (Sherk, 1972). Masch and Espey (1967) reported concentrations exceeding 10 g l^{-1} in dredge discharge plumes and 100 g l^{-1} in dredge-generated density flows. Suspended solids concentrations used in this study typify those found near dredging operations.

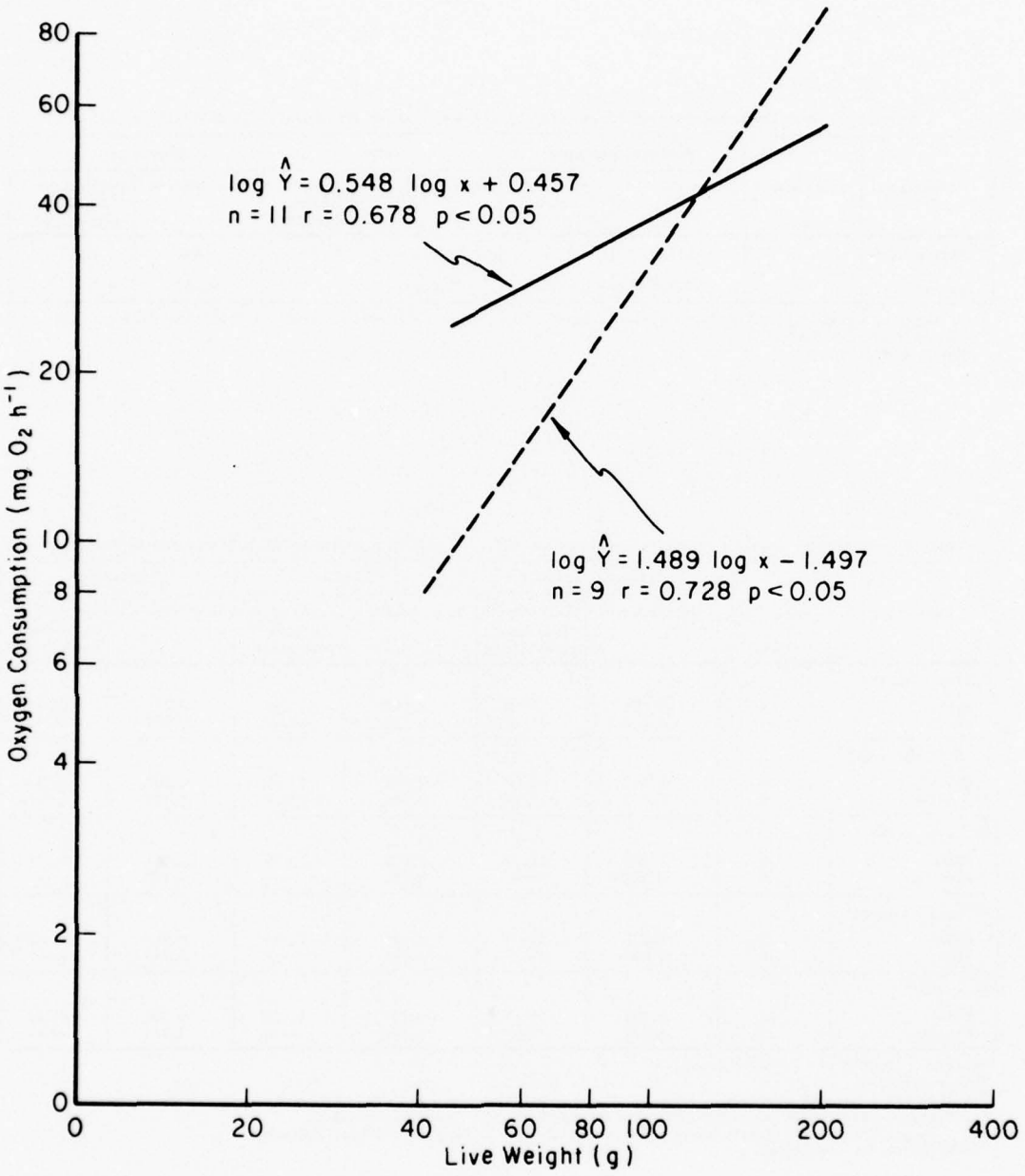

Figure 15. Oxygen consumption of white perch swimming at 1.02 ft/s following 72-hour exposure to 2.58 g l^{-1} resuspended natural sediment (dashline) and under base-line conditions (solid line).

Table 17. Covariance analysis of oxygen consumption and live weight regressions of white perch[1].

Comparison	Individuals (No.)	Residual variance		Slope		Elevation	
		Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability
Experimental	9	0.349	9, 7	0.028	1, 16	0.033	1, 17
Base line	11	0.107	$p < 0.05$	0.098	----[2]	0.164	$p < 0.05$

[1]At swimming speeds of 1.58 ft/s in filtered water after 72-hour exposure to 2.58 g l^{-1} natural sediment and under base-line conditions.
[2]Not significant.

Table 18. Covariance analysis of oxygen consumption and live weight regressions of male and female toadfish.

Comparison	Individuals (No.)	Residual variance		Slope		Elevation	
		Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability
Fuller's earth[1]							
Males	8	0.033	6, 9	0.043	1, 15	0.011	1, 6
Females	11	0.020	----[2]	0.025	-----	0.026	-----
Natural sediment[3]							
Males	14	0.005	12, 4	0.052	1, 16	0.099	1, 17
Females	6	0.011	-----	0.006	$p < 0.01$	0.009	$p < 0.01$
Filtered water[4]							
Males	14	0.042	12, 4	0.001	1, 16	0.061	1, 17
Females	6	0.014	-----	0.035	-----	0.033	-----
Natural sediment[5]							
Males	14	0.028	12, 4	0.014	1, 16	0.013	1, 17
Females	6	0.092	-----	0.044	-----	0.043	-----
Base line[6]							
Males	11	0.032	9, 7	0.008	1, 16	0.068	1, 17
Females	9	0.014	-----	0.024	-----	0.023	-----

[1]Tested in 2.20 g l^{-1} fuller's earth.
[2]Not significant.
[3]In 1.58 g l^{-1} natural sediment.
[4]In filtered river water after 72-hour exposure to 10.37 g l^{-1} natural sediment.
[5]In 3.36 g l^{-1} natural sediment after 72-hour exposure to 11.09 g l^{-1} natural sediment.
[6]Under base-line conditions.

Table 19. Covariance analysis of oxygen consumption and live weight regressions of toadfish.

Comparison	Laboratory particle exposure history ($g\ l^{-1}$)	Experimental concentration ($g\ l^{-1}$)	No.	Residual variance		Slope		Elevation	
				Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability	Mean squared	Degrees of freedom-probability
Fuller's earth[1]	0	2.20	19	0.025	18, 17	0.025	1, 35	0.025	1, 36
Base line	0	0	20	0.025	-----[2]	0.003	-----	0.051	-----
Natural sediment[3]	0	1.58	20	0.025	18, 18	0.033	1, 36	0.032	1, 37
Base line	0	0	20	0.025	------	0.00002	-----	0.004	-----
Natural sediment[4]	10.37	0	20	0.025	18, 18	0.056	1, 36	0.057	1, 37
Base line	0	0	20	0.086	$p < 0.05$	0.113	-----	0.182	-----
Natural sediment[5]	11.09	3.36	20	0.025	18, 18	0.047	1, 36	0.050	1, 37
Base line	0	0	20	0.068	$p < 0.05$	0.187	-----	0.042	-----
Natural sediment [4, 5]	10.37	3.36	20	0.068	18, 18	0.051	1, 36	0.050	1, 37
	11.09	0	20	0.034	------	0.020	-----	0.167	-----

[1]During exposure to 2.20 $g\ l^{-1}$ fuller's earth.
[2]Not significant.
[3]During exposure to 1.58 $g\ l^{-1}$ natural sediment.
[4]In filtered water after 72-hour exposure to 10.37 $g\ l^{-1}$ natural sediment.
[5]During exposure to 3.36 $g\ l^{-1}$ natural sediment after 72-hour exposure to 11.09 $g\ l^{-1}$ of the same material.

The fish studied are from three ecologically distinct estuarine niches. Striped bass, a major sport and commercial fish, are anadromous and use the estuary as a spawning and nursery area (Talbot, 1966). White perch make semianadromous migrations (Mansueti, 1964) and are usually restricted to a certain segment of the estuary throughout their lifespan. Toadfish are sedentary, demersal fish and inhabit the sediment-water interface.

The swimming abilities of striped bass and white perch suggest that during periods of high turbidity these fish can move to more favorable areas. However, in laboratory experiments that prevented escape, suspensions of fuller's earth or Patuxent River sediments generally reduced oxygen consumption at controlled levels of swimming activity. Respiratory responses of striped bass and white perch to suspended solids were observed in the laboratory at concentrations exceeding those which occur naturally in estuaries. These concentrations may occur temporarily near dredge discharges.

Toadfish exhibited no significant respiratory responses to suspensions of fuller's earth or natural sediment. The sediment-water interface is characterized by periods of low oxygen concentration, high turbidity, or both. Hall (1929, 1930) reported that oxygen consumption of toadfish is almost directly proportional to the oxygen tension of the water, and that the fish are able to remove all the oxygen from a limited volume of water before respiratory movement ceases. This may explain the absence of response.

Suspension concentrations produced by dredging operations probably have a limited effect on striped bass and white perch respiration because of the mobility of these species. Toadfish are sedentary, but their high tolerance to suspended solids may minimize the effects of suspended solids on their respiration.

VI. SUMMARY AND CONCLUSIONS

Suspensions of particulate matter deposited in estuarine systems by nature or man can affect estuarine fish. Stress from suspended sediments may cause changes in growth, survival, and reproduction of fish. The effects of suspended particles on fish depend on the concentration and composition of the particles and the stress tolerance of the fish. Suspensions of commercial mineral solids were tested to determine the effects of suspended sediments of known composition, particle-size distribution, and organic matter content. Additional tests were run with resuspended Patuxent River estuary muds to test the effects of a natural sediment.

Exposure to sublethal suspended solids concentrations increased microhematocrit, hemoglobin concentration, and red blood cell count in white perch, hogchoker, mummichog, and striped killifish. Increases in these hematological parameters raise the blood's oxygen exchange capacity. Hematological values probably change in response to suspended solids' interference with oxygen-carbon dioxide transport at the gill. No significant increases occurred in striped bass, spot, or toadfish.

White perch experienced gill tissue disruption and intensified mucus production on the gills during exposure to fuller's earth suspensions. Although fuller's earth concentrations below the 24-hour LC_{10} value were used, the gill tissue structure of white perch was adversely effected and the respiratory surface area was reduced in a 5-day period.

High rates of liver glycogen depletion were recorded in hogchokers exposed to sublethal fuller's earth concentrations. This indicates carbohydrate utilization and drainage of metabolic reserves during periods of sediment stress. Hogchokers live at the sediment-water interface, but still demand extra energy for compensatory alterations of their physiology while exposed to suspended sediments.

Oxygen consumption of striped bass and white perch increased with swimming speed in control tanks containing filtered river water. However, suspensions of fuller's earth or Patuxent River sediments generally reduced fish oxygen consumption rates at high levels of swimming activity. This indicates that sediment suspensions interfered with the fish's respiratory ability. Both striped bass and white perch are common to the open waters of the estuary. However, toadfish, which inhabit the turbid sediment-water interface, showed no significant respiratory responses to fuller's earth or natural sediment suspensions.

It is customary and useful to establish suspended solids criteria by applying the lethal concentration levels causing 10- to 50-percent mortality over a defined period of exposure. However, this procedure ignores the biologically significant sublethal effects of suspended solids on estuarine fish. Concentrations of suspended sediments found in estuarine systems during storms, flooding, dredging, and dredged-material disposal are within the range of sublethal concentrations used in these experiments. Since the experimental suspensions induced stress responses in several fish species, preproject evaluations of the effects of dredging and related activities should include consideration of this effect.

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APPENDIX A

LIVER GLYCOGEN CONCENTRATIONS IN FOUR ESTUARINE FISH
AND LIVER GLYCOGEN DEPLETION RATES IN WHITE PERCH

1. Introduction.

To derive useful data from studies of glycogen utilization in response to suspended solids, several species of estuarine fish were screened and "natural" liver glycogen values were established under field conditions. Glycogen depletion studies were conducted to establish the expected rates of glycogen mobilization in the selected species.

Liver glycogen determinations were performed on four species: White perch, striped bass, hogchokers, and spot. A glycogen depletion study was conducted at two temperatures with white perch.

2. Materials and Methods.

Liver glycogen was determined for each of the four species within 8 hours of capture. Glycogen was extracted according to the method of Good, Kramer, and Somogyi (1933) and quantified by the phenolsulfuric acid method (Montgomery, 1957).

Glycogen mobilization of white perch is dependent on the breakdown of glycogen (a long-chain polymer of glucose) to glucose 6-phosphate (Black, Robertson, and Parker, 1961; Ingram, 1970). This enzymatic reaction is highly dependent on temperature.

Glycogen depletion in white perch was determined at 10 ± 2° and 20 ± 2° Celsius. On the day of capture, white perch were divided into two groups of 60 fish and placed in separate holding tanks maintained at either 10° or 20° Celsius by immersion in a water bath. Ten fish were removed at random from each group at the initiation of the experiment and after 2, 3, 7, and 8 days in the holding tanks. Liver glycogen was determined according to the methods previously described.

3. Results and Interpretation.

Mean liver glycogen values for white perch, striped bass, and spot resembled one another on the day of capture (Table A-1). Liver glycogen values for hogchokers were significantly greater than those for the other three species. The precision of liver glycogen determinations was satisfactory in perch, bass, and hogchokers (Table A-1). In spot the variation was greater; however, the standard error of the mean was only 15.5 percent of the mean. These data show that levels of glycogen reserves were relatively constant in a population.

The rate of glycogen mobilization in starved white perch increased with temperature. At 10° Celsius, glycogen stores decreased by 59.7

Table A-1. Liver glycogen determined on day of capture for four species of estuarine fish.

Species	Individuals (No.)	Liver glycogen (mg 100 mg^{-1} ± S.E.$\bar{x}^{-1}$)	S.E.$\bar{x}$ as pct of mean
White perch	10	5.407 ±0.572	10.5
Striped bass	10	4.780 ±0.508	10.5
Hogchoker	10	16.304 ±0.868	5.3
Spot	10	5.277 ±0.816	15.5

[1]Standard error of the mean.

percent, from a mean of 5.41 ± 0.57 mg 100 mg^{-1} liver at the time of capture to 2.22 ± 0.39 mg 100 mg^{-1} (Table A-2). At 20° Celsius, glycogen decreased from 5.41 ± 0.57 mg 100 mg^{-1} to 0.098 ± 0.015 mg 100 mg^{-1}, a 98.2-percent reduction over the 8-day holding period (Table A-2).

The glycogen mobilization curve for white perch at both temperatures was changed to linear form by straight-line regression. Based on this regression line, mobilization at 10° Celsius occurred at the rate of 0.39 mg 100 mg^{-1} per day. At 20° Celsius, mobilization almost doubled to 0.67 mg 100 mg^{-1} per day.

Table A-2. Liver glycogen depletion and regression analysis of white perch at 10° and 20° Celsius[1].

Days after start	Individuals (No.)	Liver glycogen value	
		10 ± 2°C (mg 100 mg^{-1})	20 ± 2°C (mg 100 mg^{-1})
0	20	5.407 ±0.572	5.407 ±0.572
2	10	5.205 ±0.570	5.561 ±0.436
3	10	5.422 ±0.699	3.768 ±0.345
7	10	3.588 ±0.875	0.590 ±0.128
8	10	2.223 ±0.390	0.098 ±0.015
Regression analysis			
Temperature (°C)	Slope	Intercept	Correlation coefficient
10°	-2.301	13.853	-0.919
20°	-1.266	7.905	-0.972

[1]Values of mean liver glycogen are given for each sampling day plus or minus standard error of the mean.

APPENDIX B

HEMATOLOGICAL CORRELATIONS IN ESTUARINE FISH

1. Introduction.

Red blood cell count, microhematocrit (packed blood cell volume), and hemoglobin concentration in fish blood have been estimated by several analytical techniques (Hesser, 1960; Anthony, 1961; Larsen and Snieszko, 1961; Larsen, 1964; Summerfelt, Lewis, and Ulrich, 1967; Berinati and Crowley, 1972). From these parameters other useful hematological indexes may be calculated, such as mean microhematocrit and hemoglobin content of individual red blood cells (Wintrobe, 1956; Holton and Randall, 1967).

Several attempts have been made to establish predictive correlations between microhematocrit and red blood cell count or hemoglobin concentration in both marine and freshwater teleosts (Eisler, 1965; Summerfelt, Lewis, and Ulrich, 1967; Houston and DeWilde, 1968). A highly predictive regression of microhematocrit with red blood cell count and hemoglobin concentration may enable workers to derive useful hematological data from a single microhematocrit measurement without red blood cell enumeration and hemoglobin determination.

Houston and DeWilde (1968) showed that an estimate of microhematocrit for the rainbow trout, *Salmo gairdneri*, may be used to predict red blood cell counts and hemoglobin concentration in routine assessments of hematological status. However, the prediction was not sufficiently exact for research purposes.

2. Materials and Methods.

Hematological data were taken from five species common to the Patuxent River estuary: White perch, *Morone americana*; striped bass, *M. saxatilis*; spot, *Leiostomus xanthurus*; hogchoker, *Trinectes maculatus*; and menhaden, *Brevoortia tyrannus*. Male and female fish from each species were used to study hematological response to suspensions of mineral solids (Sherk and O'Connor, 1971). Fish were captured by otter trawl from the Patuxent River estuary. Blood samples were taken from both the control fish and the fish exposed to sublethal concentrations of fuller's earth. The data represent hematology of fish under normal laboratory conditions (18 ± 1° Celsius, salinity 5.5 parts per thousand), and fish under stress from suspended sediment.

Blood was collected in heparinized pipets and mixed before analysis. Microhematocrit was determined according to methods outlined by Hesser (1960), and was read on an International Equipment Company microcapillary reader. Hemoglobin was determined by the cyanmethemoglobin method. Samples were centrifuged at 11,500 revolutions per minute for 20 minutes to remove red cell nuclei from suspension before taking a reading (Larsen, 1964). Optical density of the hemoglobin samples was determined at 540

nanometers, using a Coleman Junior II spectrophotometer. Concentration was plotted against a Hycel (mammalian) standard curve. Red blood cell counts were made at X 100, using an improved Newbauer hemacytometer. A modified Hayme's solution (Heinle and Morgan, 1972) was the diluting medium for red blood cell enumeration.

Regression and correlation analyses were done according to Snedecor and Cochran (1967). Simple and partial correlation coefficients were calculated for each combination of parameters. Microhematocrit data were first transformed to log to the base 10 ($\log_{10}$) to permit the use of parametric statistical procedures throughout the analyses.

3. Results and Interpretation.

Regression analyses between independent pairs of hematological parameters in the five fish species showed significant correlation between microhematocrit and hemoglobin concentration in white perch, spot, and striped bass ($p < 0.01$, Table B-1). Correlation between microhematocrit values and red blood cell counts were also found to be significant in white perch, spot, and hogchokers. Correlation of hemoglobin concentration and red cell counts was significant in white perch and spot ($p < 0.01$).

The significance of correlation is important in estimating a parameter from a statistical relationship between it and another parameter. The predictive capacity of the mathematical model is also important; e.g., the correlation data for white perch. All three paired comparisons are significant at the 0.01 level; i.e., chances are 1 in 100 (or less) that the relationship established between any two of the blood parameters could be due to chance alone. However, the correlation coefficients (r) differ by as much as 0.21 between the microhematocrit with hemoglobin concentration correlation (0.885) and the red blood cell count with hemoglobin concentration correlation (0.676). These correlations may be significant at the same probability levels, but the predictive capacity of the relationship differs. This is shown by the coefficient of determination (r^2), a measure of correlation which estimates the proportion of variance accounted for by the correlation. Given a microhematocrit value with the hemoglobin concentration correlation $r^2 = 0.784$ from a white perch, the hemoglobin content (y) is estimated by regression, knowing that the microhematocrit value accounts for 78.4 percent of the variance in the hemoglobin concentration estimate.

In the estimation of red blood cell count from microhematocrit, $r^2 = 0.464$; only 46.4 percent of the variance of the predicted red blood cell count can be accounted for by the microhematocrit. The correlation coefficients were highly significant when estimating two blood parameters from the same microhematocrit value, but the predictive capacity of the former relationship was almost 80 percent; the latter was below 50 percent.

The coefficients of determination must exceed 0.75 for a paired relationship before estimated parameters may be used for research purposes.

Table B-1. Regression, significance of correlation, and coefficients of determination of paired hematological parameters from estuarine fish.

Species	Comparison	Individuals (No.)	Slope	Correlation coefficient	Probability	Coefficient of determination
White perch	Microhematocrit versus hemoglobin concentration	23	3.8808	0.8854	<0.01	0.7839
	Microhematocrit versus red blood cell count	23	3.9572	0.6813	<0.01	0.4642
	Hemoglobin concentration versus red blood cell count	23	2.0275	0.6760	<0.01	0.4570
Spot	Microhematocrit versus hemoglobin concentration	18	3.009	0.8750	<0.01	0.7656
	Microhematocirt versus red blood cell count	17	8.910	0.6024	<0.01	0.3629
	Hemoglobin concentration versus red blood cell count	18	2.389	0.5869	<0.05	0.3445
Striped bass	Microhematocrit versus hemoglobin concentration	14	4.9203	0.8373	<0.01	0.7011
	Microhematocrit versus red blood cell count	14	4.1040	0.2828	>0.05	0.0800
	Hemoglobin concentration versus red blood cell count	14	1.0206	0.4133	>0.05	0.1708
Menhaden	Microhematocrit versus hemoglobin concentration	9	2.1403	0.5547	>0.05	0.3077
	Microhematocrit versus red blood cell count	9	7.2995	0.6554	>0.05	0.4295
	Hemoglobin concentration versus red blood cell count	9	1.5556	0.5389	>0.05	0.2904
Hogchoker	Microhematocrit versus red blood cell count	39	9.9183	0.8632	<0.01	0.7451

Coefficients of determination between 0.60 and 0.74 were sufficient in routine estimates of hematological status.

Coefficients of determination sufficient for research purposes were found in the correlation of microhematocrit with hemoglobin concentration for white perch and spot (Table B-1). The correlation of microhematocrit with red blood cell count in hogchokers was of no predictive value in parametric estimates for research purposes ($r^2 = 0.7451$). The microhematocrit with hemoglobin concentration correlation in striped bass was sufficient for routine hematological work ($r^2 = 0.7011$).

The correlation of microhematocrit with red blood cell count and the correlation of hemoglobin concentration with red blood cell count, did not account for sufficient variance to use regression methods in predictive estimates of hematological parameters in perch, spot, striped bass, and menhaden (Table B-1).

The three hematological parameters are closely related in a physical and biological sense. Microhematocrit measures the percent volume of red blood cells in a sample. Red blood cells in most vertebrates transport the respiratory pigment, hemoglobin. Therefore, the predictive correlations between microhematocrit and hemoglobin concentration in white perch and spot are largely dependent on the quantity of red blood cells present. To establish whether the correlation of microhematocrit and hemoglobin concentration was significant and independent of red blood cell count, partial correlation coefficients were determined for microhematocrit-hemoglobin concentration-red blood cell count interrelationships. This statistic estimated the correlation of two variables, microhematocrit and hemoglobin concentration; the red blood cell count variable was held constant.

Partial correlation coefficients were determined for all species in which the three variables were studied (Table B-2). Partial correlation coefficients showed the relationship of microhematocrit and hemoglobin concentration in white perch and spot statistically independent of red blood cell count (Table B-2).

The relationships established for these species have significant value. The ability to estimate blood parameters in estuarine fish from a simply determined value, such as microhematocrit, may facilitate physiological studies of estuarine fish in the field and in the laboratory. These hematological studies can estimate stress responses in fish (Hesser, 1960; Summerfelt, Lewis, and Ulrich 1967). Physiological and hematological field studies could increase the value of onsite environmental disturbance studies. Estimates of sublethal effects of various pollutants on fish populations should prove useful to estuarine biologists.

Table B-2. Analysis of partial correlation of microhematocrit with hemoglobin concentration eliminating the effect of red blood cell count.

Species	No.	Partial correlation coefficient[1]	Degrees of freedom	Probability
White perch	23	0.7876	20	<0.01
Spot	18	0.8069	15	<0.01
Striped bass	14	0.8248	11	<0.01
Menhaden	9	0.3167	6	>0.05

[1] $\gamma_{12.3}$

APPENDIX C

PRELIMINARY OBSERVATION ON THROUGH-GUT TRANSPORT OF SUSPENDED SOLIDS BY ESTUARINE FISH

1. Introduction.

The objective of this study was to determine the accumulation of particulate matter on the gills and in the alimentary canal of fish exposed to sublethal concentrations of suspended solids. Observations were made on white perch, striped bass, and hogchokers.

2. Materials and Methods.

The following suspended solids were used:

(a) Kaolinite clays:

(1) Hydrite MP, median particle size 9 micrometers.
(2) Hydrite Flat-D, median particle size 4.5 micrometers.
(3) Hydrite-10, median particle size 0.55 micrometer.

(b) Fuller's earth, median particle size 0.50 micrometer.

(c) Natural bottom muds taken from Long Point, Patuxent River, Maryland.

The particle-size distribution of the natural Patuxent River mud is shown in Figure C-1.

Fish were captured by otter trawl in the Patuxent River estuary and transported to the laboratory in a flow-through system of river water. All specimens were starved 72 to 96 hours before exposure to suspended solids, and were not fed during an exposure.

Groups of 6 to 10 fish, dependent upon size and species, were exposed to graded concentrations of suspended solids for 24 hours. The gills, stomach, and intestine of each individual were examined to determine the accumulation of suspended solid following an exposure.

Accumulation of solids on the gills, in the stomach, and in the intestine was scored on a scale from 0 (no accumulation) to 4 (continuous coating of particulate matter). Scoring was based on visual observation by a single, trained observer. Mean accumulation for each group of fish exposed to each concentration was plotted as a histogram.

Replicate exposures of each species to graded concentrations of each solid were not done. Data are from the preliminary observations.

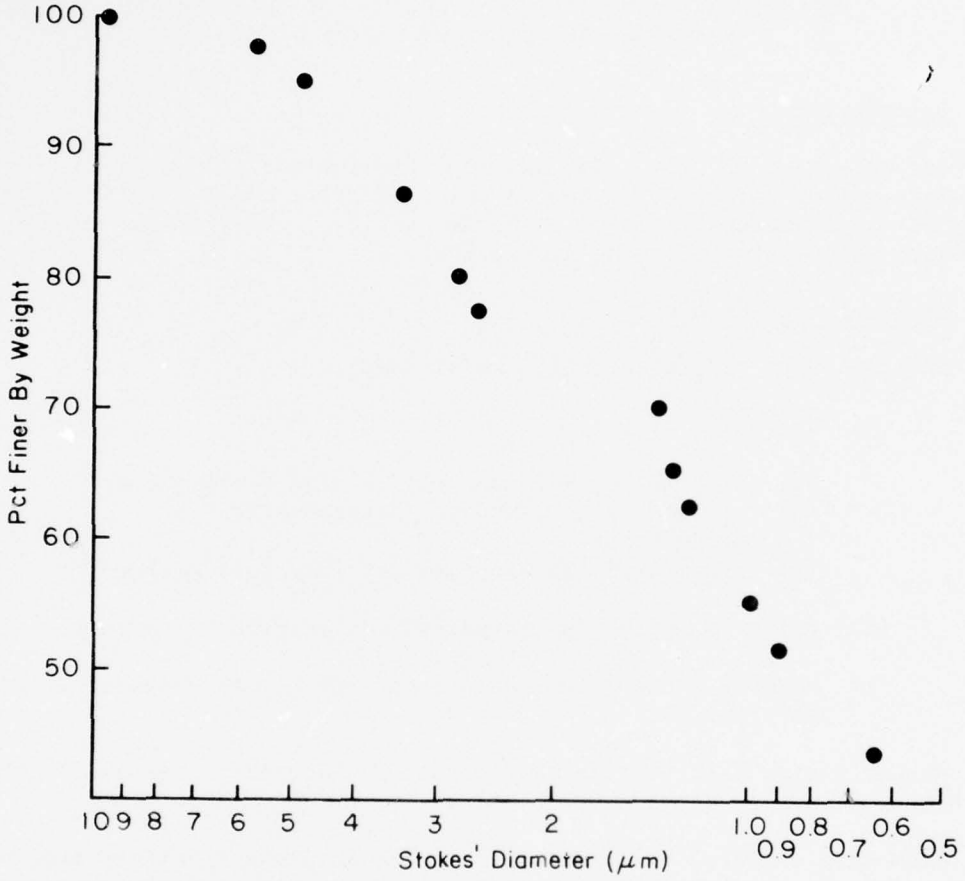

Figure C-1. Particle-size distribution of natural mud collected at Long Point, Patuxent River, Maryland.

3. Results.

White perch accumulated little Hydrite MP clay (mean particle size 9 micrometers) in a 24-hour period. Accumulation from concentrations of 6 to 13 g l^{-1} was greatest in the intestine and least on the gills. Accumulation in the intestine at 9 and 13 g l^{-1} Hydrite MP clay was approximately double the accumulation at 6 g l^{-1} (Fig. C-2).

More Hydrite Flat-D (mean particle size 4.5 micrometers) than Hydrite MP clay particles were accumulated by white perch (Fig. C-3). At 6.7 g l^{-1}, Flat-D accumulation in the stomach and the intestine was greater than MP accumulation by a factor of three. Less accumulation occurred at 16.3 g l^{-1} than at 6.7 g l^{-1}. Particle accumulation in the intestine exceeded 2 at concentrations of 7, 24, and 37 g l^{-1}.

Fuller's earth accumulation on the gills of white perch was greater than either of the kaolinite clays, regardless of concentration (Fig. C-4). Accumulation of fuller's earth on the gills of fish exposed to 6.7, 8.3, and 10.7 g l^{-1} ranged between 1.5 and 2; little or no fuller's earth was detected in the stomachs or the intestines.

White perch exposed to suspensions of 6.7 to 36.2 g l^{-1} natural muds for 24 hours, showed greatest accumulation on the gill, in the stomach, and in the intestine at 6.7 g l^{-1} (Fig. C-5). Least accumulation was noted at 14.1 g l^{-1}. Gill accumulation was high at 23.9 g l^{-1}. Accumulation in stomachs and intestines was approximately the same (between 1.5 and 2) at 23.9 and 36.2 g l^{-1}.

White perch exposed to lower concentrations of natural muds for 72 hours had approximately the same accumulation scores, from 5.6 to 17.8 g l^{-1} (Fig. C-6). Intestinal accumulation scores remained between 2 and 2.5 over the range of concentrations. Particle accumulation in stomachs and on gills was slightly more variable. Gill accumulation was greatest for fish exposed to 5.6 g l^{-1} and least accumulation occurred at 17.8 g l^{-1}.

Striped bass accumulation scores for Hydrite MP particles ranged from 0.75 to 1.5 which was relatively low (Fig. C-7). Accumulation on the gills, in the stomach, and in the intestines was similar over concentrations ranging from 6 to 13 g l^{-1}. Accumulation of Hydrite MP by striped bass was greater than accumulation by white perch by a factor of three or more (Fig. C-2).

Hydrite Flat-D accumulation by striped bass was greatest on the gill (Fig. C-8). Particle accumulation in stomachs and intestines ranged from 0 to 1.2 in concentrations of 3.3 to 17.1 g l^{-1}, except for a value of 2 in the stomachs of bass exposed to 5 g l^{-1} Flat-D.

Single exposure of striped bass and hogchokers to natural muds suspensions resulted in large accumulation on the gills, moderate accumulation

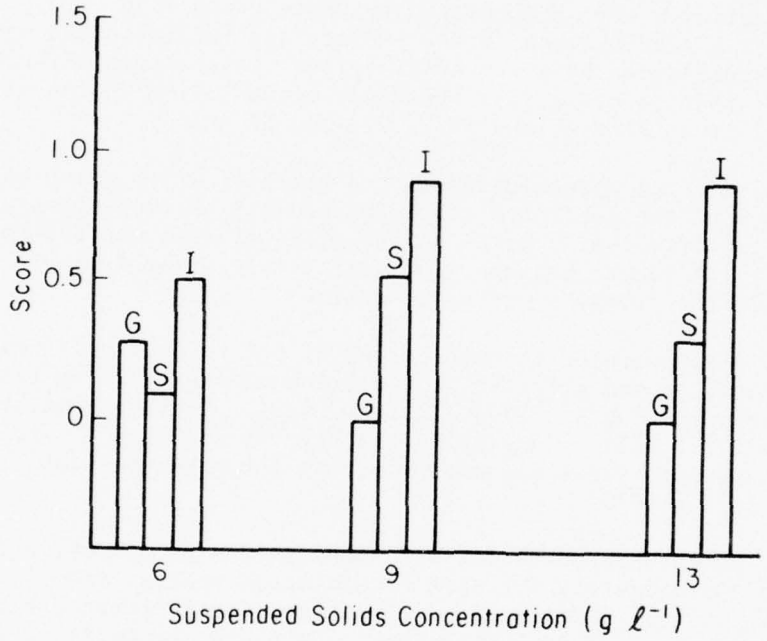

Figure C-2. Relative accumulation of solids in white perch exposed to graded concentrations of Hydrite MP (G = gill, S = stomach, I = intestine).

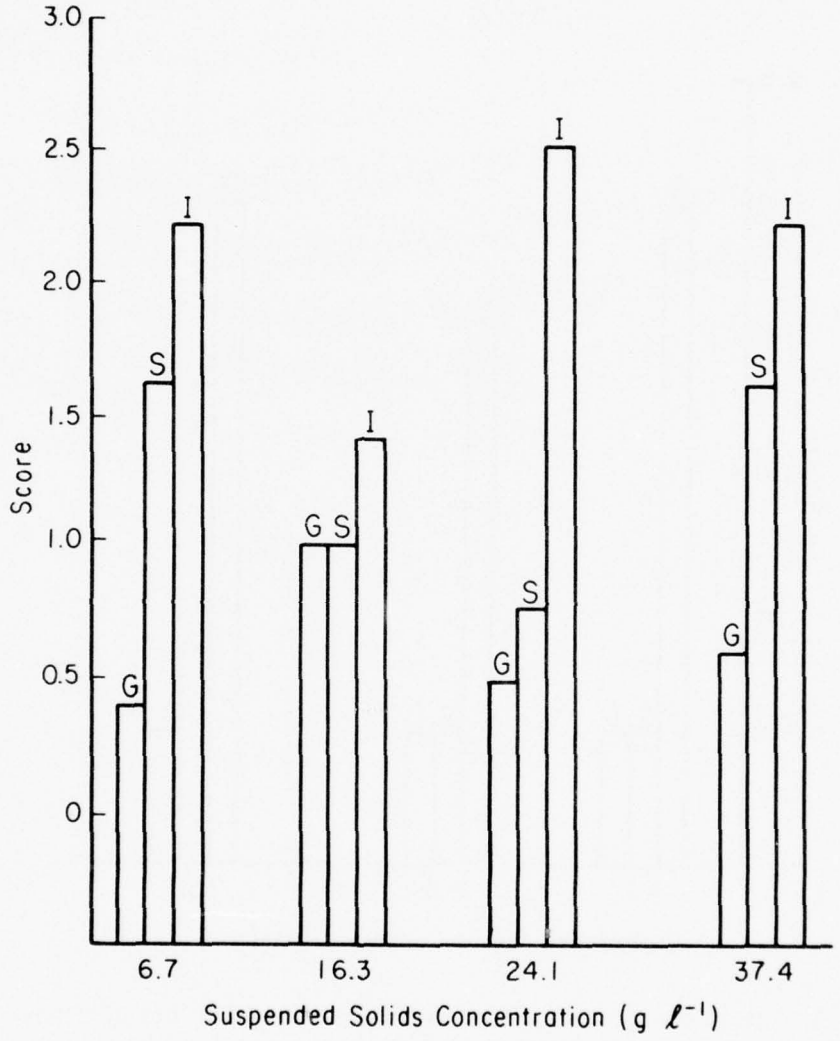

Figure C-3. Relative accumulation of solids in white perch exposed to graded concentrations of Hydrite Flat-D (G = gill, S = stomach, I = intestine).

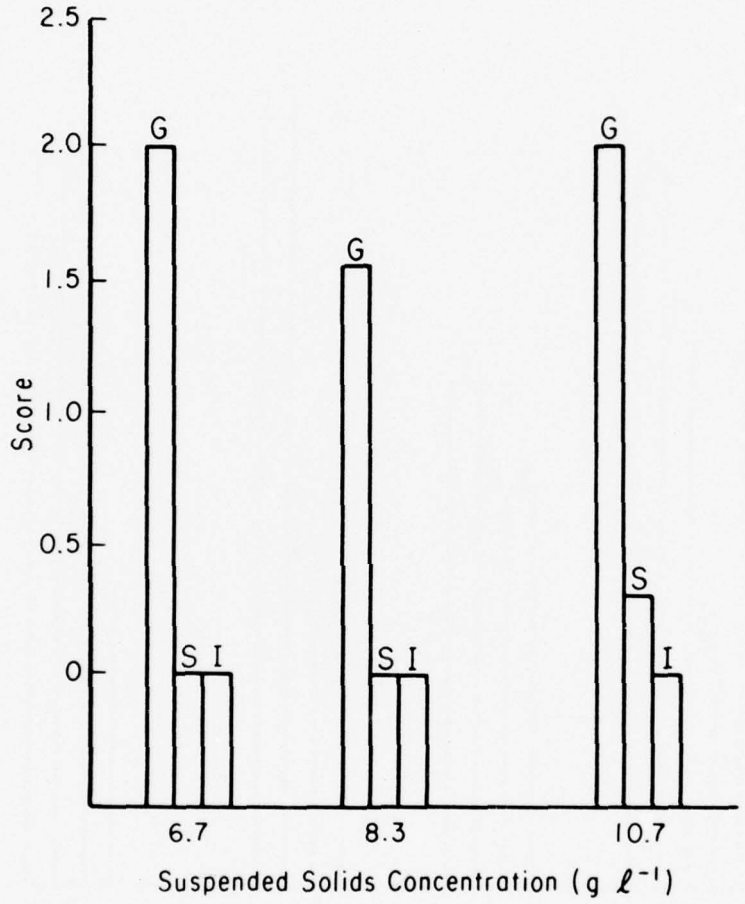

Figure C-4. Relative accumulation of solids in white perch exposed to graded concentrations of fuller's earth (G = gill, S = stomach, I = intestine).

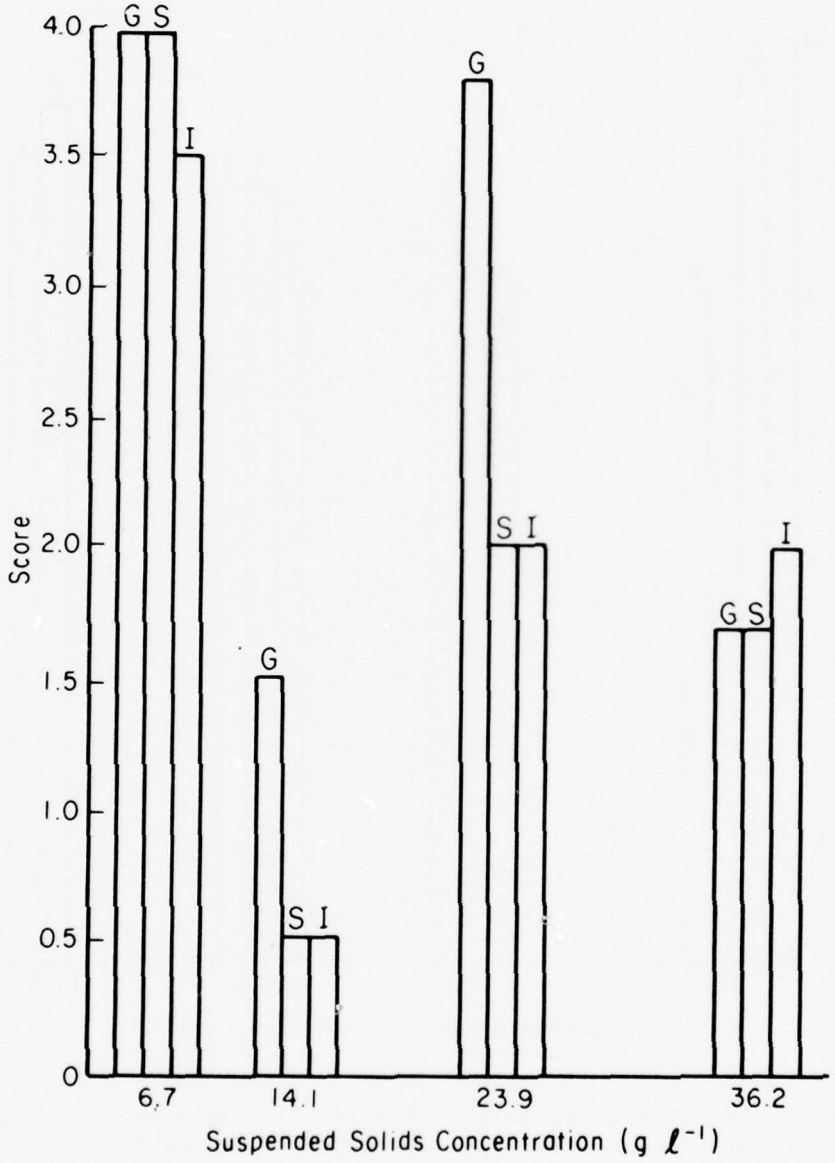

Figure C-5. Relative accumulation of solids in white perch exposed to graded concentrations of natural Patuxent River mud for 24 hours (G = gill, S = stomach, I = intestine).

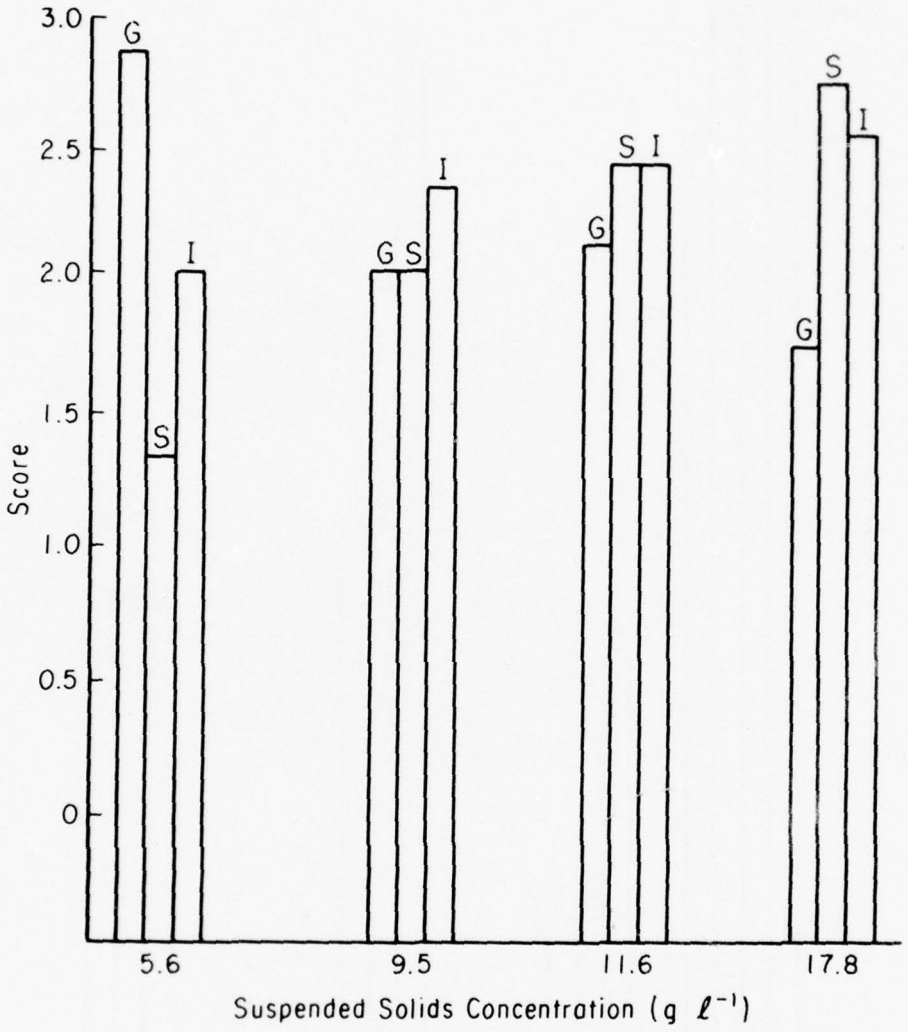

Figure C-6. Relative accumulation of solids in white perch exposed to graded concentrations of natural Patuxent River mud for 72 hours (G = gill, S = stomach, I = intestine).

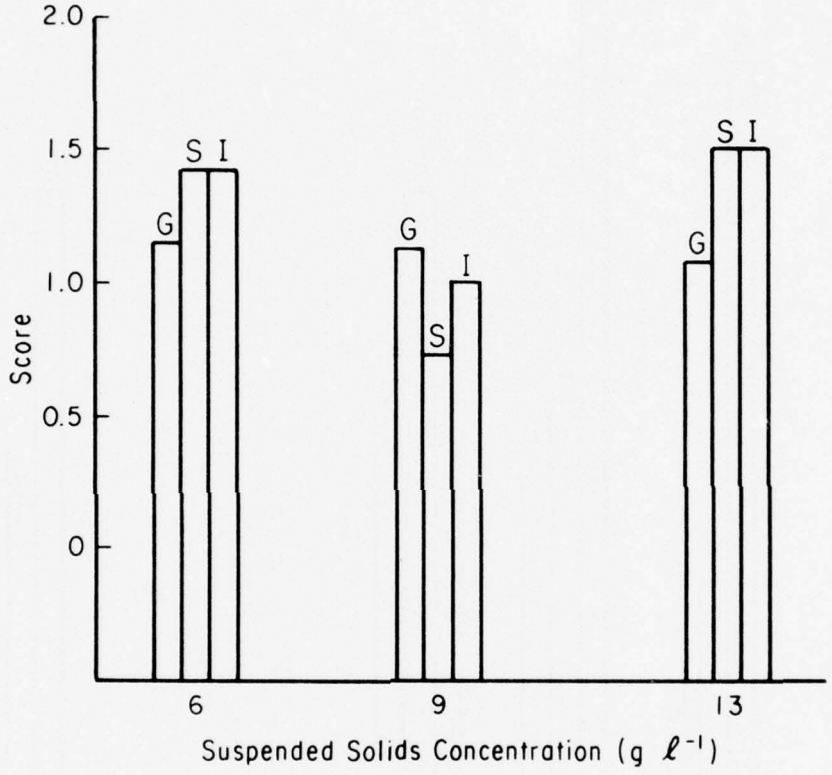

Figure C-7. Relative accumulation of solids in striped bass exposed to graded concentrations of Hydrite MP (G = gill, S = stomach, I = intestine).

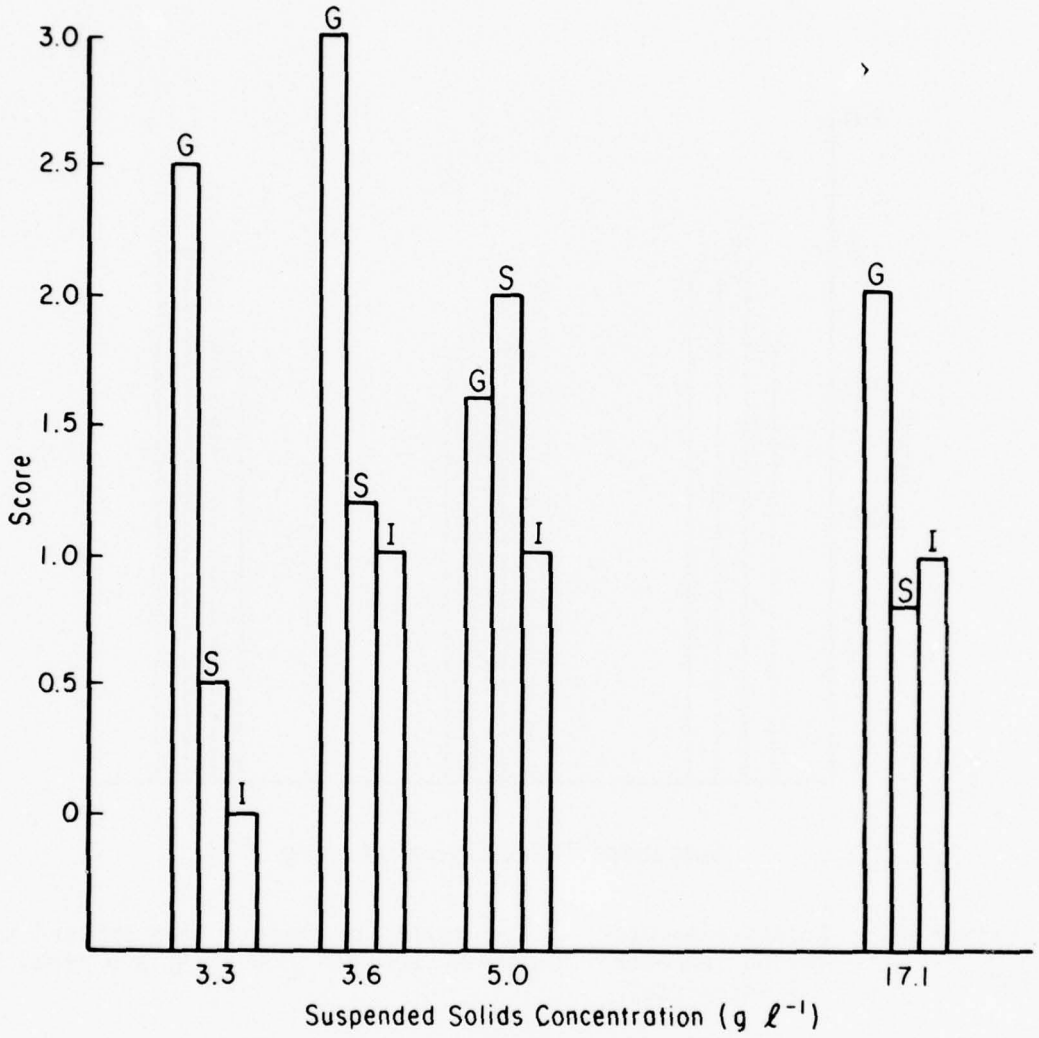

Figure C-8. Relative accumulation of solids in striped bass exposed to graded concentrations of Hydrite Flat-D (G = gill, S = stomach, I = intestine).

in the stomachs, and little or no accumulation in the intestines (Figs. C-9 and C-10).

4. Conclusions.

A mechanism for gill cleansing in white perch, striped bass, and hogchokers in highly turbid water was entrapment of particulate matter on the gills and transport of entrapped particles through the alimentary canal.

Extremely fine particles, such as resuspended bottom muds, fuller's earth, and Hydrite Flat-D, accumulate more than larger particles (Hydrite MP).

5. Additional Observations and Discussion.

Microscopic examination of gills revealed a possible mechanism for through-gut transport of suspended particles. Gills examined at X 30 showed that fine particles become entrapped between gill filaments and between the secondary lamellae. Fish exposed to the suspended solids had streams of particle-laden mucus on the gill and attached to the pharyngeal teeth on the inner margin of the gill arch. A function of the pharyngeal teeth is to assist in passing food from the mouth to the esophagus, and it is likely that the mucus streams on the gill and on the pharyngeal teeth were being ingested. However, the hogchoker, a demersal fish, had a reduced accumulation of particulate matter in the gut when exposed to similar concentrations of the solids used with white perch and bass.

Few of these data may be directly compared because of the wide range of suspended solids concentrations. However, it is evident that accumulation of particles was much the same in several instances, regardless of concentration. Finer solids accumulated the most; i.e., fuller's earth (75 percent <2 micrometers, median size 0.5 micrometer), Hydrite Flat-D (40 percent <2 micrometers, median size 4.5 micrometers), and resuspended natural muds (70 percent <2 micrometers, median size 0.87 micrometer).

Mucus stream transport of particles exposes the entrapped material to normal digestive processes and, thus, to a wide range of chemical environments. Particles are exposed for varying periods of time to acid conditions in the stomach. The pH ranges from 2 to 3 and material undergoes a strong acid hydrolysis. The pH environment changes from acid to moderately basic as material passes from the stomach to the intestine (pH 7 to 8). Hydrolysis of food material in the intestine is carried out by enzymatic processes.

Particles in the stomach are exposed to approximately the same conditions as absorbed materials stripped from particulate matter for chemical analysis. Potentially toxic materials such as heavy metal ions, pesticide residues, petrochemical residues, and various biocides of organic origin, may become available to the organism. Through-gut transport of particles removed from suspension provides an avenue for accumulation of noxious material in the tissue of fish.

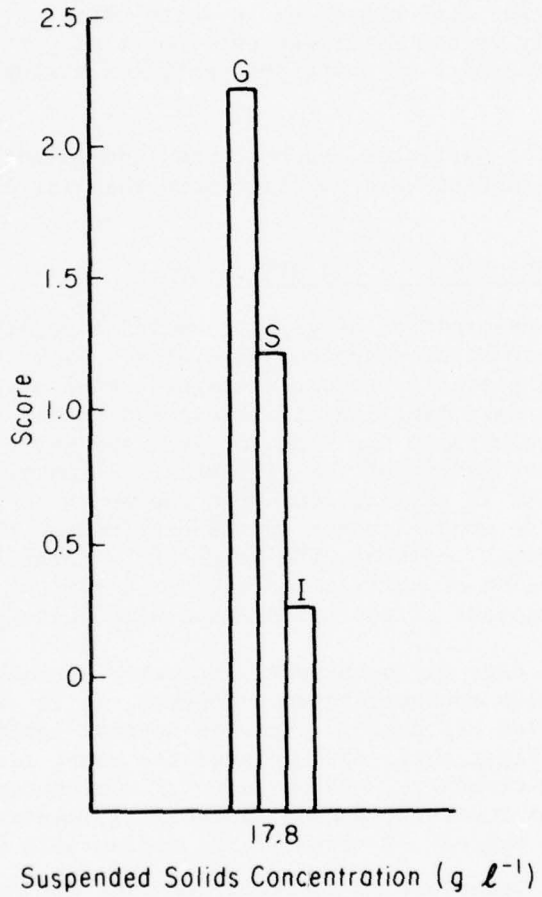

Figure C-9. Relative accumulation of solids in striped bass exposed to graded concentrations of natural Patuxent River mud (G = gill, S = stomach, I = intestine).

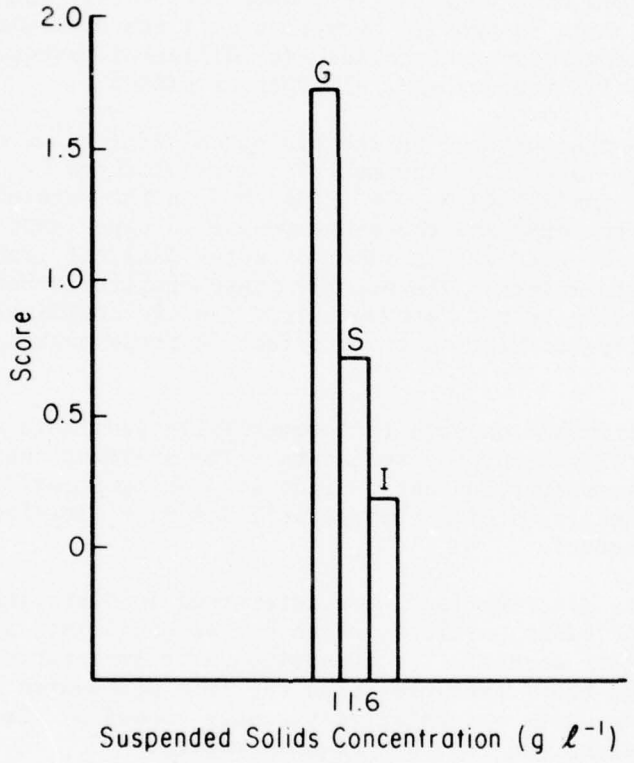

Figure C-10. Relative accumulation of solids in hogchokers exposed to graded concentrations of natural Patuxent River mud (G = gill, S = stomach, I = intestine).

APPENDIX D

ANALYSIS OF SEDIMENTS

1. Introduction.

Experimental work during 1971 and 1972 used artificial (commercially available) mineral solids to provide base-line data for biological effects of (a) different concentrations of solids, (b) different particle-size distributions, and (c) different mineral types of solids.

Work during 1973 concentrated on the biological effects of naturally occurring sedimentary material. The material was collected by anchor dredge at Long Point (38°29'30" N., 76°39'45" W.) in the Patuxent River and stored in large polyethylene tanks before use in experiments. The sediment surface was covered with a layer of water (salinity range 4 to 6 parts per thousand) to maintain the natural ionic equilibria between sediment and water occurring in the Patuxent River. A microoxidized sediment layer developed at the sediment-water interface in these tanks after a few days of storage.

Analyses were performed on both the commercially available mineral solids and the naturally occurring sediments. The sediment characteristics measured were organic matter content (weight loss on ignition), inorganically bound heavy metals (atomic absorption), and particle-size distributions (settling diameter).

The particle-size distributions were determined in distilled water, and may represent the basic particles which can be bound into aggregates by atomic and molecular forces. The composite units are stable under dispersion methods. The basic particles also may form aggregates in saline water; however, these units are relatively weakly bonded by electrostatic forces, surface tension, and "sticky" organic matter.

2. Materials and Methods.

a. Size Distribution. Artificial sediments (mineral solids) were as follows:

(a) Kaolinite

(1) Hydrite-10 (Georgia Kaolin Company)
(2) Hydrite Flat-D (Georgia Kaolin Company)
(3) Hydrite MP (Georgia Kaolin Company)

(b) Fuller's earth (Fisher No. F-90)

Particle-size distributions were determined by the sedimentation method (American Society for Testing and Materials, 1968) for paper-coating clays.

The natural sediments collected from the Patuxent River were analyzed as follows: Preliminary work showed this material was approximately 75- to 80-percent salt and water by weight. Appropriate triplicate volumes of natural sediments were removed from the holding tanks. The volumes were calculated to contain between 5- and 10-gram inorganic dry solids, and were corrected upward for the weight of organic matter present (see method of analysis below). Measured quantities of solids were placed in 1-liter-capacity Pyrex beakers and an appropriate amount of 30-percent hydrogen peroxide (H_2O_2) was added. The volume of H_2O_2 needed to oxidize the organic matter present in the sediment produced a final 5-percent concentration of H_2O_2 in the sediment volume. The oxidation reaction was initially violent. It proceeded overnight in a hood with air bubbling slowly through the sediment-H_2O_2 mixture to remove the excess H_2O_2.

When gas evolution had ceased, 750 milliliters of deionized, glass-distilled water were added to each beaker. The sediment was resuspended by stirring with a glass rod and allowed to settle. The supernatant was carefully decanted, and another 750 milliliters rinse of deionized, glass-distilled water was added to each beaker.

A 0.2-milliliter sample of supernatant water was taken from each beaker and the dissolved ion concentration of each solution was determined with the freezing-point depression osmometer. Salt concentration was read from a standard curve relating freezing-point depression and osmolal concentration to sodium chloride (NaCl) concentration in milligram per kilogram (mg kg^{-1}) water. If the salt concentration was greater than 300 mg NaCl kg^{-1} water, the suspension was allowed to settle, the clear supernatant was decanted, and an additional rinse of 750-milliliter deionized distilled water was added to each beaker. The sediment was resuspended and allowed to settle. The clear supernatant was decanted and the beaker containing the washed sediment was filled to 500 milliliters with fresh, deionized, glass-distilled water and placed into an ultrasonic bath (45 kilohertz) for 30 minutes. The suspension was placed in a glass cylinder, made up to volume with deionized distilled water, and analyzed as described in American Society for Testing and Materials (1968), but the dispersing agent sodium pyrophosphate ($Na_4P_2O_7$), was not added.

Values are reported as percent by weight remaining in suspension (percent finer than) plotted against equivalent spherical diameters according to Stokes' law.

b. Organic Matter Content. Samples of the natural sediment collected from the Patuxent River at Long Point were ovendried for 24 hours at 100° Celsius, ground fine with a porcelain mortar and pestle, and ashed for 3 hours at 500° Celsius. Organic matter values are reported as percent of dry weight lost on ignition. No appreciable loss of inorganic carbonate occurred during the ashing procedure, as evidenced by nonsignificant weight losses of calcium carbonate ($CaCO_3$) samples ashed along with the ovendried natural sediments.

c. Heavy Metals. Amounts of extractable cations in the mineral solids and in the natural sediment samples were determined through mild acid extraction and atomic absorption analysis by Mr. David Boon, Seafood Processing Laboratory, Crisfield, Maryland. Tests for inorganically bound cations as described by Soil Testing and Plant Analysis Laboratory (1970) and Perkin-Elmer Corporation (1971) were conducted for zinc, copper, iron, manganese, lead, cobalt, nickel, chromium, and cadmium. Total mercury values are reported from sediments digested for 1 minute in boiling aqua regia (Dow Method, CAS-AM-70.13, 22 June 1970 revised, Chlorine Institute, Madison Avenue, New York, New York). Metal values are mg kg^{-1} dry weight of solids.

3. Results and Discussion.

a. Size Distributions. Particle-size distributions of the extremely fine mineral solids and the natural sediment are listed in Figure D-1 and Table D-1. Materials are ranked coarsest to finest by median size as follows: Hydrite MP, kaolinite (Georgia Kaolin Company), median size = 9.5 micrometers, <2 micrometers = 12 percent; Hydrite Flat-D, kaolinite (Georgia Kaolin Company), medium size = 4.5 micrometers, <2 micrometers = 34 percent; Patuxent River silt (composite less organic matter fraction, 11.5 percent of dry weight), median size = <0.8 micrometer, <2 micrometers = 72 percent; fuller's earth, montmorillonite, and attapulgite (Fisher No. F-90), median size = <0.5 micrometer, <2 micrometers = 82 percent; Hydrite-10, kaolinite (Georgia Kaolin Company), median size = <0.5 micrometer, <2 micrometers = 92 percent. Graphic solutions (Folk, 1968) and mathematical calculations (Trask, 1968) can be used to determine the second, third, and fourth moments of these distributions.

Additional size-distribution analyses for the natural sediments (by date of collection) are presented in Figure D-2 and Table D-2. Median sizes ranged from a high of approximately 1.1 to a low of <0.5 micrometer (August collection). Fraction by weight finer than 2 micrometers ranged from a high of approximately 82 percent to a low of 65 percent (August collection). These particle-size distributions of solids (Tables D-1 and D-2, Figs. D-1 and D-2) are comparable with those reported by May (1973) in the mudflow from a shell dredge (Table D-3).

b. Organic Matter Content. Organic matter content of natural sediment samples tended to increase throughout the summer of 1973 from 8.9 percent in June to over 11 percent in August and September (Table D-4). A comparison of mean organic matter values (Table D-5) showed the differences between early and late samples were significant. Organic matter, which has settled out at Long Point, may come from marshes which line the shores of the Patuxent watershed.

Organic matter analyses were also conducted on the mineral solids. Ashing caused no significant weight loss in fuller's earth solids. Substantial weight losses in the kaolinites (about 11 percent of dry weight) were attributed to the bound water lost (at temperatures of 500° Celsius)

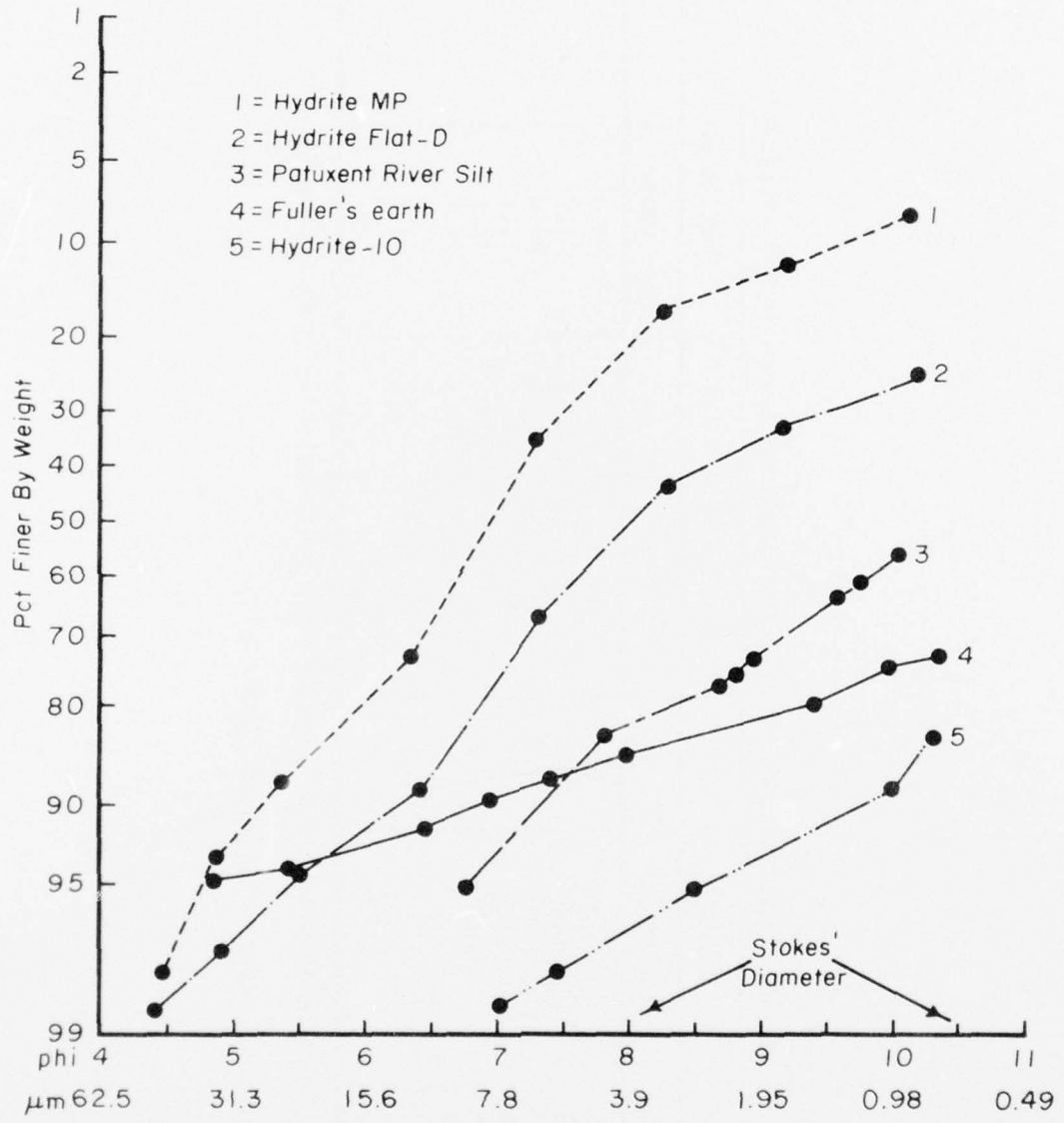

Figure D-1. Particle-size distribution of sediments used in this project.

Table D-1. Particle-size distributions of artificial sediments used in this project[1].

Fuller's earth (4)[2]		Hydrite-10 (5)		Hydrite Flat-D (2)		Hydrite MP (1)	
pct finer	µm	pct finer	µm	pct finer	µm	pct finer	µm
95.0	35.8	98.6	7.7	98.6	49.3	97.9	49.3
94.2	25.3	97.9	6.1	97.2	34.9	93.7	35.1
92.0	12.7	95.0	2.9	94.4	24.8	88.0	25.1
90.0	8.1	88.4	1.0	88.8	12.5	71.1	12.8
88.3	6.4	83.6	0.8	65.7	6.5	34.5	6.7
85.8	4.0			43.4	3.3	16.9	3.4
80.0	1.6			33.6	1.7	12.0	1.7
75.0	1.0			24.5	0.9	7.7	0.9
73.3	0.8						

[1]Percent finer = fraction (expressed as percent) finer by weight than Stokes' diameter in micrometer.

[2]Numbers in parentheses refer to lines in Figure D-1.

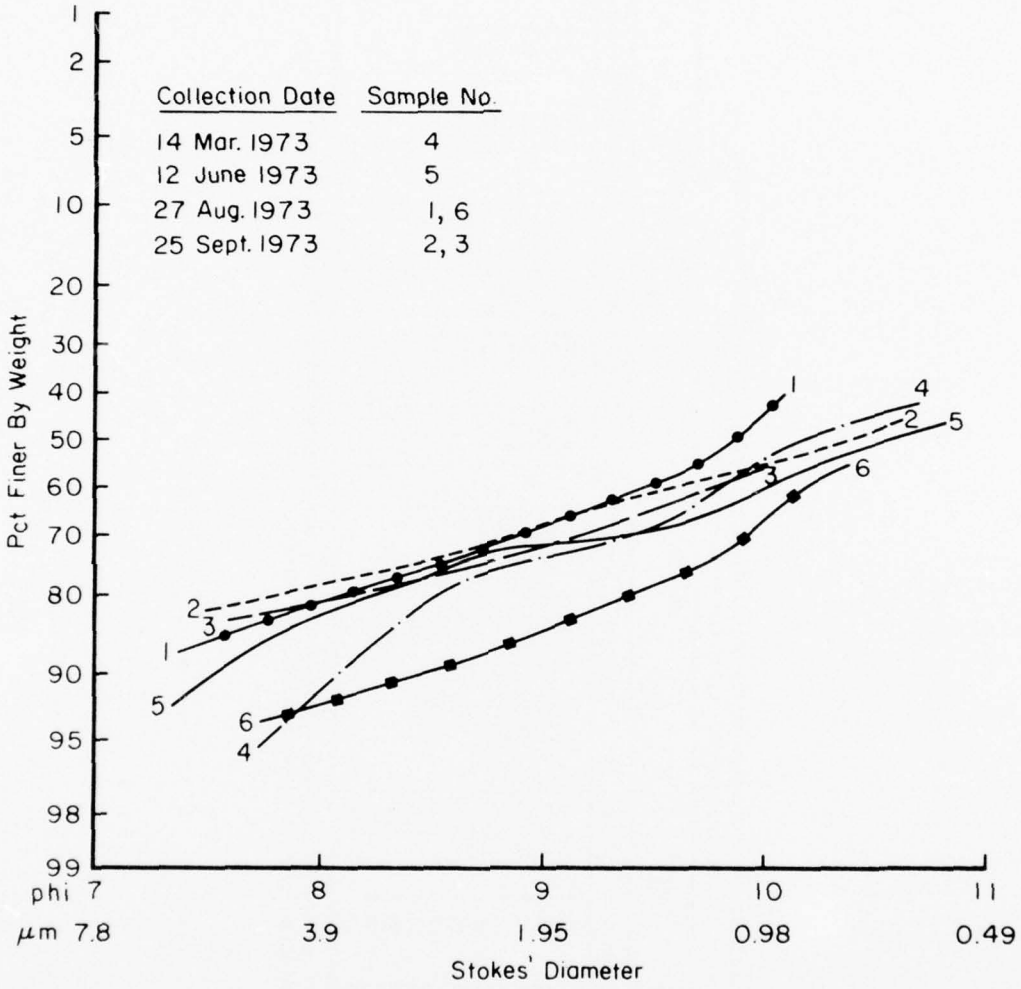

Figure D-2. Particle-size distributions of natural Patuxent River silt samples (two replicate determinations) collected by anchor dredge at Long Point.

Table D-2. Particle-size distributions of representative natural Patuxent River sediment samples collected at Long Point during 1973[1].

14 March (4)[2]		12 June (5)		27 August (6)		27 August (1)		28 September (3)		28 September (2)	
pct finer	µm	pct finer	µm	pct finer	µm	pct finer	µm	pct finer	µm	pct finer	µm
95.0	4.8	92.5	6.5	93.9	4.9	87.2	6.5	83.8	4.4	80.4	4.5
86.3	3.4	84.0	4.6	89.8	2.9	74.3	2.8	77.5	2.4	75.3	3.1
80.0	2.8	79.5	3.3	77.6	1.5	58.5	1.4	74.9	2.1	71.5	2.6
77.5	2.6	74.3	2.5	75.5	1.3	54.3	1.2	73.0	2.0	68.2	2.2
70.0	1.5	69.0	1.4	73.5	1.2	45.9	1.0	63.0	1.4	51.0	0.8
65.0	1.3	67.0	1.3	67.4	1.0			60.1	1.2	47.9	0.7
62.5	1.2	64.5	1.2	61.2	0.9			55.5	1.0		
55.0	1.0	61.3	1.0	57.1	0.8						
51.3	0.9	59.3	0.9								
43.8	0.7	54.8	0.8								
		47.4	0.6								

[1]Percent finer = fraction (expressed as percent) finer by weight than Stokes' diameter in micrometer.
[2]Numbers in parentheses refer to sample numbers in Figure D-2.

Table D-3. Particle-size percentages by weight of suspended solids in the mudflow from a shell dredge (May, 1973).

Distance from discharge (m)	Size range (pct by weight)				
	62 to 39 µm	38 to 19 µm	18 to 10 µm	9 to 5 µm	4 to 2 µm
0	10.5	20.3	25.5	27.7	16.1
15.25	11.7	26.5	25.1	25.6	11.3
30.5	8.6	21.2	30.1	32.0	7.9
122.0	4.7	17.6	24.9	29.3	23.5
244.0	0.9	14.5	27.2	34.2	23.0

Table D-4. Organic matter content of natural mud collected by anchor dredge from the Patuxent River (Long Point)[1].

Collection date (1973)	Sample No.	Organic content (mean ± standard deviation)	Standard error of the mean
17 June	1	9.4120 ± 1.0038	0.3174
	2	10.3580 ± 1.0272	0.4594
	3	10.9400 ± 0.3270	0.1463
28 June	1	9.8740 ± 0.3808	0.1703
	2	9.4720 ± 0.4127	0.1846
	3	8.9120 ± 0.6079	0.2719
14 July	1	11.2467 ± 0.5555	0.2268
	2	10.0983 ± 0.8682	0.3545
27 Aug.	1	11.4483 ± 0.8321	0.3397
	2	11.4617 ± 0.7849	0.3205
	3	11.9700 ± 0.6712	0.2740
	4	12.6483 ± 0.4317	0.1762
18 Sept.	1	11.8567 ± 0.3038	0.1240
25 Sept.	1	11.4217 ± 0.4881	0.1993
	2	11.8750 ± 0.5131	0.2095
	3	11.2200 ± 0.4626	0.1889

[1]Samples were dried for 24 hours at 100° Celsius, ground fine with a mortar and pestle, then ashed for 3 hours at 500° Celsius. Organic matter values reported are percent loss of dry weight on ignition.

Table D-5. Comparison of means of organic matter determinations by collection date.

Sample collection dates (1973)	28 June	14 July	27 Aug.	18 Sept.	25 Sept.
17 June	---------[1]	---------	$p < 0.001$	$p < 0.001$	$p < 0.001$
28 June		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
14 July			$p < 0.001$	---------	---------
27 Aug.				---------	---------
18 Sept.					---------

[1]Not significant.

from these clays (Michael Taranto, Georgia Kaolin Company, personal communication, 1973).

c. Heavy Metals. The mineral solids contained biologically insignificant amounts of metal (Table D-6). The values reported for Patuxent silt (Long Point) are in the "natural" range of metal found in similar estuarine salinity ranges by Huggett (Virginia Institute of Marine Science, personal communication, 1973) in the York, the James, and the Elizabeth Rivers, which drain into the Virginia part of the Chesapeake Bay system.

Table D-6. Inorganically bound cations in artificial and natural sediment[1].

Element	Hydrite-10	Hydrite Flat-D	Hydrite MP	Fuller's earth	Patuxent silt[2] ($\bar{n}$ = 13)
Zn	0.12	0.08	0.10	0.05	36.0 ± 2.0
Cu	0.14	0.05	0.02	<0.01	4.4 ± 0.4
Fe	4.5	6.2	3.8	<0.3	2,100.0 ± 94.0
Mn	<0.06	<0.06	<0.06	0.14	2,300.0 ± 260.0
Pb	<0.5	<0.5	<0.5	<0.5	<10.0
Co	<0.1	<0.1	<0.1	<0.1	<4.0
Ni	<0.3	<0.3	<0.3	<0.3	<4.0
Cd	----[3]	-----	-----	-----	1.0
Cr	<0.3	<0.3	<0.3	<0.3	<3.0
Hg	<0.01	<0.01	<0.01	<0.01	<0.2

[1]Extraction by 0.075 normal hydrochloric-sulfuric acid and analysis by atomic absorption spectroscopy. Values are mg kg^{-1}.
[2]Mean ± standard error of the mean.
[3]Not tested.

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Cu	0.14	0.05	0.02	<0.01	4.4 ± 0.4
Fe	4.5	6.2	3.8	<0.3	2,100.0 ± 94.0
Mn	<0.06	<0.06	<0.06	0.14	2,300.0 ± 260.0
Pb	<0.5	<0.5	<0.5	<0.5	<10.0
Co	<0.1	<0.1	<0.1	<0.1	<4.0
Ni	<0.3	<0.3	<0.3	<0.3	<4.0
Cd	----[3]	-----	-----	-----	1.0
Cr	<0.3	<0.3	<0.3	<0.3	<3.0
Hg	<0.01	<0.01	<0.01	<0.01	<0.2

[1]Extraction by 0.075 normal hydrochloric-sulfuric acid and analysis by atomic absorption spectroscopy. Values are mg kg^{-1}.
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