# EFFECTS OF SUSPENDED AND DEPOSITED SEDIMENTS ON ESTUARINE ORGANISMS. PHASE II.

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A three-year laboratory study identified biological components of selected populations of estuarine organisms which were most sensitive to the effects of particle size and concentration of (1) suspended mineral solids similar in size to sediments likely to be found in, or added to, estuarine systems in concentrations typically found during flooding, dredging, and disposal of dredged material, and (2) natural sediments in idential experiments. Significant mortality of estuarine fishes was demonstrated at these

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## ABSTRACT

A three-year laboratory study identified biological components of selected populations of estuarine organisms which were most sensitive to the effects of particle size and concentration of (1) suspended mineral solids similar in size to sediments likely to be found in, or added to, estuarine systems in concentrations typically found during flooding, dredging, and disposal of dredged material, and (2) natural sediments in identical experiments. Significant mortality of estuarine fishes was demonstrated at these suspended solids concentrations. Estuarine fishes were classified using the results of static bioassays as tolerant (24 hr $LC_{10} > 10$ g $l^{-1}$), sensitive (24 hr $LC_{10} < 10 > 1.0$ g $l^{-1}$), or highly sensitive (24 hr $LC_{10} < 1.0$ g $l^{-1}$) to fuller's earth suspensions. Generally, bottom-dwelling fish species were most tolerant to suspended solids; filter feeders were most sensitive. Early life stages were more sensitive to suspended solids than adults. Bioassays with natural sediments indicated that suspensions of natural muds affect fishes in the same way as fuller's earth, but higher concentrations of natural material were required to produce the same level of response. The effect of finely divided solids on fishes was dependent on several characteristics of suspended particles with different mechanisms operative in producing mortality in fishes, although the cause of death was the same: anoxia. Sublethal solids effects on fishes were identified: hematological compensation for reduction in gas exchange across the gill surface, abrasion of the body epithelium, packing of the gut with large quantities of ingested solids, disruption of gill tissue, increased activity, and reduction in stored metabolic reserves. Oxygen consumption of striped bass and white perch swimming at controlled levels of activity was generally reduced during exposure to suspensions of fuller's earth and natural Patuxent River sediments. Carbon assimilation by four species of phytoplankton was significantly reduced by the light attenuating properties of fine silicon dioxide suspensions. Ingestion of radioactive food cells by two species of calanoid copepods was significantly reduced during exposure to suspensions of fuller's earth, fine silicon dioxide, and natural Patuxent River silt. With adequate knowledge of local conditions (life history stages, sediment types, sediment concentrations, seasonal and resident species, duration of exposure, and habitat preference) at estuarine sites selected for environmental modification, our efforts provide baseline data for pre-project decision making based upon concentration effects of different types of suspended sediments.

# FOREWORD

A three-year laboratory study to determine the effects of suspended artificial and natural (deposited) sediments on estuarine organisms has assessed the effects of particle size and concentration of suspended solids on estuarine organisms independent of, and in addition to, complicating factors associated with natural sediments such as sorbed toxic metals and pesticides, high biochemical oxygen demand and nutrient enrichment. Our approach has been first to identify the biological effects of mineral solids similar in particle size to sediments likely to be found in, or added to, estuarine systems, and second to study the effects of natural sediments in similar experiments.

In the first two chapters we present the results of bioassay tests to establish concentration-response curves predictive of mortality for selected estuarine fishes exposed to suspended particles. The next three chapters present the results of physiological and histological studies of the sublethal effects of suspended solids on estuarine fishes. Chapter 6 presents the effects of suspended solids on fish respiration. The last two chapters present the effects of suspended solids on carbon assimilation and feeding activity of phytoplankton and zooplankton. Four appendices are included in this report.

The project has benefitted from the assistance of numerous investigators associated with several institutions. We wish to express our deepest appreciation to Dr. Kent S. Price, Associate Dean of the College of Marine Studies, University of Delaware, and Director of the Bayside Laboratory, Lewes, Delaware for his kind permission to supplement our studies at

his laboratory. Studies conducted at the Bayside Lab have greatly increased the potential transfer value of results from the entire project. During our studies at Delaware Messrs. Art Handby, Ron Smith and Larry Curtis, and Dr. Robert Biggs provided their time, knowledge and assistance.

Slides for histological studies were prepared by Ms. Elaine Drobeck of the Chesapeake Biological Laboratory. Photographic and photomicrographic equipment and supplies were provided by Dr. Shirley van Valkenburg, Dr. Ray Morgan, and Mr. Michael Reber. Analyses of inorganically bound heavy metals on natural sediments and commercially available mineral solids which we used in our experiments were conducted by Mr. David Boon, Seafood Processing Laboratory, Natural Resources Institute, Crisfield, Maryland. Cultures of the copepods Eurytemora affinis and Acartia tonsa were provided by Mr. Joseph Ustach and Dr. Donald Heinle (Chesapeake Biological Laboratory).

Dr. Dennis Burton of the Academy of Natural Sciences of Philadelphia Field Station, Benedict, Maryland has kindly provided guidance. We thank Dr. Burton not only for his intellectual assistance, but for several hours of boat time when our experimental fish stocks had run dangerously low.

Throughout the course of the study Dr. L. Eugene Cronin, Director of the Natural Resources Institute, and Dr. Joseph Mihursky, Chairman of the Department of Environmental Research had provided valuable advice and helpful criticism. We gratefully acknowledge Drs. Ray Morgan and David Flemer for their technical advice and discussions during the course of the project.

Mr. Gerald Lee Cox of R. V. Anomia provided a great deal of expert assistance in the collection of fish. We also thank Mr. Henry Zion for his assistance in collecting animals and for his help in the laboratory.

Ms. Jackie Groom has labored over many manuscripts through the project. We thank her for her assistance in typing, and her assistance in overseeing matters of budget. We also thank Ms. Cathy Lenda for her assistance in typing this final project report.

To date many summer assistants and interns have provided skilled help. Significant portions of the work completed thus far are due to the efforts of Mr. Mike Chiles, Mr. George Piegols, Mr. James Lynch and Mr. Dunne Fong.

## SUMMARY

Static bioassays conducted with fuller's earth suspensions on white perch, spot, silversides, bay anchovies, mummichogs, striped killifish, and menhaden showed that significant mortality in five of the seven species could be caused by similar suspended solids concentrations typically found in estuarine systems during flooding, dredging, and spoil disposal. Lethal concentrations ranged from 0.58 g $l^{-1}$ fuller's earth (24 hr $LC_{10}$) for silversides to 24.5 g $l^{-1}$ fuller's earth (24 hr $LC_{10}$) for mummichogs. Fishes were classified as either tolerant (24 hr $LC_{10}$ >10 g $l^{-1}$), sensitive (24 hr $LC_{10}$ < 10 >1.0 g $l^{-1}$), or highly sensitive (24 hr $LC_{10}$ < 1.0 g $l^{-1}$) to fuller's earth. The bioassay mortality response of striped bass, croaker, weakfish, bluefish, and menhaden was not sufficiently predictable for calculation of accurate lethal concentration values (see Chapter 1).

Generally, bottom-dwelling species were most tolerant to suspended solids; filter feeders were most sensitive. Early life stages were more sensitive to suspended solids than adults.

Bioassay studies using natural sediments indicated that suspensions of natural mud affect fish in the same way as fuller's earth, but higher concentrations of natural sediments were required to produce the same level of mortality. The effect of finely divided solids was found to be dependent on several characteristics of suspended solids with different mechanisms operative in producing mortality in fishes, although the cause of death was the same: anoxia (see Chapters 1 and 2).

Exposure to sublethal suspended solids concentrations significantly increased hematocrit value, hemoglobin concentration, and erythrocyte

numbers in the blood of white perch, hogchokers, mummichogs, and striped killifish, but not toadfish, spot, and striped bass (Chapter 3). Increases in these hematological parameters indicated increased oxygen exchange capacity of the blood, evidence of interference with $O_2$-$CO_2$ transport. Further evidence of this interference during exposure to sublethal concentrations of fuller's earth was by tissue disruption and increased mucus production of white perch gills (Chapter 4).

Rates of liver glycogen depletion were increased in hogchokers exposed to sublethal concentrations of fuller's earth, indicating an increased carbohydrate utilization and a drain on metabolic reserves during sediment stress (Chapter 5).

Suspensions of fuller's earth or Patuxent River sediments generally reduced oxygen consumption of striped bass and white perch at controlled levels of swimming activity. Oyster toadfish exhibited no signifcant respiratory response to either suspensions of fuller's earth or natural Patuxent River sediments (Chapter 6).

The light attenuation of increasing concentrations of "inert," fine silicon dioxide caused biologically significant reductions (50 to 90%) in carbon assimilation of the phytoplankters Monochrysis lutheri, Chlorella sp., Nannochloris sp., and Stichococcus sp. (Chapter 7).

Suspensions of fuller's earth, fine sand, and Patuxent River silt at concentrations greater than 250 mg $l^{-1}$ caused biologically significant reductions in ingestion of radioactive ($NaHC^{14}O_3$) Monochrysis lutheri by the copepods Eurytemora affinis and Acartia tonsa. Differences in uptake between the two species may have been related to their different life habitats, although both are non selective suspension feeders (Chapter 8).

## ECOLOGICAL IMPLICATIONS

Particulate material can be introduced to or resuspended in the estuarine environment by nature or by man. The complex physical and chemical properties of suspended and resuspended sediments and substratum changes associated with deposition can have both direct and indirect effects on estuarine organisms. The effects of these particles, or substances associated with them, on estuarine organisms are poorly understood. However, mortality, decreased yield, and interference with energy flow have been observed at estuarine areas selected for sediment-producing activities (see Sherk, 1971, 1972). Generally, these geographic sites have inherent physical, chemical and biological limits beyond which adverse effects will occur. The biological limits may be related to growth, survival, or reproductive aspects of various life stages in response to the quantity, quality, and interaction of environmental factors.

We have investigated some of the effects of particle size and concentration of suspended solids on selected estuarine organisms independent of, and in addition to, complicating factors associated with natural sediments. These factors may include sorbed toxic metals, high biochemical oxygen demand, and nutrient content. Our approach was to identify lethal and sublethal biological effects of mineral solids similar in particle size to sediments likely to be found in, or added to, estuarine systems; and second, to study the effects of some natural sediments in similar experiments.

The effects that suspended particles may have on estuarine organisms depend at least upon (1) concentration, (2) composition, (3) sorbed minerals or toxins, and (4) tolerance of those organisms. To determine tolerance limits of fishes to suspended particles, we used known concentrations of minerals of known composition, particle size distribution and organic matter content. Thus, we eliminated the effects of sorbed metals or toxins. The use of commercial mineral solids provided a firm experimental base against which concentration effects, particle size effects and toxic effects of resuspended natural muds could be tested.

The species used in our experiments represented several distinct groups with respect to feeding mechanisms, behavior, habitat preference and trophic level. These organisms perform several of the important energy transfer functions found in estuarine systems: primary producers, primary consumers, detritovores, lower level secondary consumers and upper level secondary consumers. Concentrations of suspended mineral solids which have been recorded during floods or storms and near dredge or spoil disposal sites were found to be potentially lethal to some species (see Chapters 1 and 2).

The lethal effects of suspended mineral solids were most marked on fishes in the lower tropic levels (anchovies, silversides, juvenile white perch). There were differing sensitivities (tolerances) of these estuarine organisms with very different life habits to particle types, size distributions, and concentrations. Differing tolerances were evident even for the same species at different life stages. Concentrations of suspended solids that did not cause mortality, in some cases had sublethal effects such as:

hematological compensation for reduction in gas exchange across the gill surface, abrasion of the body epithelium, packing of the gut with large quantities of ingested solids, disruption of gill tissue, increased activity, and reduction in stored energy reserves. In order to produce given rates of mortality or severity of sublethal physiological change, we found that higher concentrations of natural Patuxent River sediments were required to cause these effects than were required with suspended commercial mineral solids.

The effect of finely divided solids depends upon several factors with different mechanisms to produce mortality in fishes, although the cause of death is the same: anoxia. A coating effect by the fine particles can isolate the respiratory epithelium from contact with the water, a locally anoxic condition is created at the gill surface, and asphyxiation results. Larger particles can become entrapped by the gill lamellae, blocking the narrow channels of water circulation between the secondary lamellae. The blocking produces dead spaces in the gill at the primary site of gas exchange, oxygen exchange decreases and an anoxic condition develops. Oxygen diffusion could occur, to a limited extent, through these dead spaces. However, a coating of clay-sized particulates over the gill surface would be less likely to permit gas exchange. Also, suspended particles could injure the gill which would prevent adequate gas exchange. When the gills are injured, the species with high oxygen requirements would be the first to succumb. Species with very low oxygen requirement would succumb to only very heavy concentrations of suspended solids, or not at all.

The tolerances of various ecological types of fishes can be generalized. The species least likely to be killed by heavy concentrations of suspended solids are forms that either live in, or in association with, the mud-water interface. It is likely that fishes living in or at the mud-water interface have been subject to selective pressures for resistance to the effects of suspended solids, and have developed mechanical and physiological mechanisms to cope successfully with effects of solids in suspension.

The relationship between ecological type and tolerance to suspended solids is less distinct among the pelagic (open water) and littoral (shoal water) fishes. Pelagic and littoral forms were all affected by suspensions of solids, but to widely varying degrees. An interesting example of this variation is the spot, a species which has an intermediate sensitivity to solids in suspension. Spot occupy a feeding niche at the mud-water interface, but are much less tolerant to suspended solids than other bottom dwellers, such as the oyster toadfish and the hogchoker.

The interference of suspended solids with energy flow was demonstrated by biologically significant reductions in carbon uptake by four species of phytoplankters and in cell ingestion by two species of copepods. The major influence of suspended solids in these two studies was the attenuation of light in the case of the phytoplankters, and the dilution of food cells in the case of the primary consumers. The reduction in energy flow from primary to secondary trophic level production could greatly reduce this food supply of larval and juvenile stages of many important estuarine vertebrates and invertebrates. This effect would be in addition to the extreme physical sensitivity of these stages to suspended solids.

Our results show that there are both lethal and sublethal effects of suspended solids on estuarine fishes, and significant sublethal effects on primary producers and consumers at concentrations typically found during flooding and in the vicinity of dredging and disposal operations.

The use of lethal concentration levels causing 10 or 50% mortability over a defined period of exposure (hours or days) to establish suspended solids criteria is customary and useful. However, this procedure ignores the biologically significant sublethal effects of suspended solids on estuarine organisms. Therefore, in the establishment of criteria for the protection of these organisms, the sublethal effects of suspended material on the most sensitive biological components (important species or life stages) must be considered at estuarine sites selected for dredging or disposal of dredged material. Adequate knowledge of local conditions at these sites is absolutely essential. These should include at least life history stages, sediment types, sediment concentrations, species present (seasonal and resident), duration of exposure, and habitat preference.

Our efforts provide baseline data for pre-project decision making based upon concentration effects of different types of suspended solids. We have identified biological components of selected populations of estuarine organisms which are most sensitive to the effects of suspended sediments.

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# EFFECTS OF SUSPENDED AND DEPOSITED SEDIMENTS ON ESTUARINE ORGANISMS PHASE II

## CHAPTER 1

## LETHAL EFFECTS OF SUSPENDED SOLIDS ON ESTUARINE FISHES

### I. INTRODUCTION

Previous reports on the lethal effects of suspended particulate matter on estuarine fishes dealt mostly with either commercial preparations of fine particles such as kaolinite or fuller's earth (Sherk and O'Connor, 1971; Sherk, O'Connor, and Neumann, 1972; Rogers, 1969) or with suspensions of fine particles of a varied and undetermined composition, such as incinerator fly-ash (Rogers, 1969). Studies of the effects of natural suspended solids on fishes have been less quantitative and mostly concerned with growth, yield, or abundance/diversity determinations in natural communities (Ellis, 1936, 1937; Stickney, 1972; review in EIFAC, 1964).

A prime objective of the current study was to differentiate the effects of natural sediments in suspension from the effects of relatively "clean" mineral particles. In this section we present and discuss the results of bioassay tests conducted with five species of estuarine fishes exposed to suspensions of fuller's earth and resuspended natural sediments (see also Sherk and O'Connor, 1971; Sherk et al., 1972).

The fishes tested in suspensions of mineral solids and natural sediments were: white perch (Morone americana), spot (Leiostomus xanthurus), menhaden

(Brevoortia tyrannus), striped killifish (Fundulus majalis), and mummichog (F. heteroclitus). Within this group are represented common littoral or shallow water estuarine forms (F. majalis and F. heteroclitus), the filter-feeding fish (B. tyrannus) which uses the estuary primarily as a nursery ground, and two pelagic (open water) forms of a higher trophic level (M. americana and L. xanthurus). Given this broad ecological range of species types, this study of the lethal effects of suspended and deposited sediments on estuarine fishes should provide the range of tolerance to be expected among estuarine fish exposed to highly turbid waters, and the different effects of fine particles of commercial or industrial origin and natural sediments on different types of estuarine fishes.

## II. MATERIALS AND METHODS

### 1. General

The lethal effects of suspended solids on fishes were determined experimentally using static bioassay test procedures. Five groups of test specimens were exposed to suspended solids at four different concentrations and a control (no added suspended solids) simultaneously. Concentrations of particles varied depending upon the species being tested, and whether the duration of the test was to be 12, 18, 20, 24, or 48 hours.

Sediment was maintained in suspension during the test period by continuous submersible pumping and aeration. Although the control tank contained no added sediment, pumping and aeration procedures were followed to assure consistency of treatment.

The materials used in bioassay experiments were fuller's earth (Fisher F-90, technical grade), kaolinite (Hydrite-10, Georgia Kaolin Co.)

and resuspended bottom sediments from the upper Patuxent River Estuary. Concentrations in the experimental tanks were determined by weight. Replicate 5 ml samples were drawn from test and control tanks and dried. The observed differences between the weight of the dried control sample and the various test tanks represented the added resuspended load in grams/liter ($g\ l^{-1}$).

The fish were exposed to suspended solids in 27 liter polyethylene tanks. Temperatures were maintained within $\pm$ 1.5°C by immersing the test tanks in a circulating water bath. Tanks were checked for mortality, temperature, Ph, and dissolved oxygen. Lethal concentrations (LC) of suspended solids causing 10, 50, and 90% mortality of test fish were determined by normit analysis (Berkson, 1953).

2. Mineral solids

Tests using commercial preparations of mineral solids, kaolinite and fuller's earth, were conducted using 14 species from two locations: six species from the Delaware Bay (bay anchovy, Anchoa mitchilli; Atlantic silverside, Menidia menidia; croaker, Micropogon undulatus; weakfish, Cynoscion regalis; bluefish, Pomatomus saltatrix; cusk eel, Rissola marginata) and eight species from the Patuxent River Estuary, Maryland (Spot, Leiostomus xanthurus; toadfish, Opsanus tau; mummichog, Fundulus heteroclitus; hogchoker, Trinectes maculatus; menhaden, Brevoortia tyrannus; white perch, Morone americana; striped bass, Morone saxatilis; striped killifish, Fundulus majalis). Bioassay tests were conducted at laboratories near the sites of capture. The University of Delaware Bay-side Laboratory, Lewes, Delaware, and the University of Maryland Hallowing

Point Field Station were used for the Delaware and Patuxent studies, respectively (Figs. 1 and 2, Table 1).

Fishes were collected by otter trawl or haul seine and transported to holding facilities in water from the site of capture. Holding facilities at the Bayside Laboratory, Delaware, consisted of 140 liter polyethylene tanks immersed in temperature-controlled water baths. Water quality at Bayside was maintained by a combination of aeration and filtration in a closed system recirculating unit. Hallowing Point holding facilities were 250 liter polyethylene tanks immersed in temperature-controlled water baths. An in-line protein skimmer filtration system was used to maintain water quality in the closed system.

All specimens were held two to five days without being fed before testing.

The mineral solids, Hydrite-10 and Fuller's earth were analyzed for particle size, organic content, and acid-extractable cations (see Appendix D).

Fig. 1. Sampling locations in the lower Delaware River Estuary.

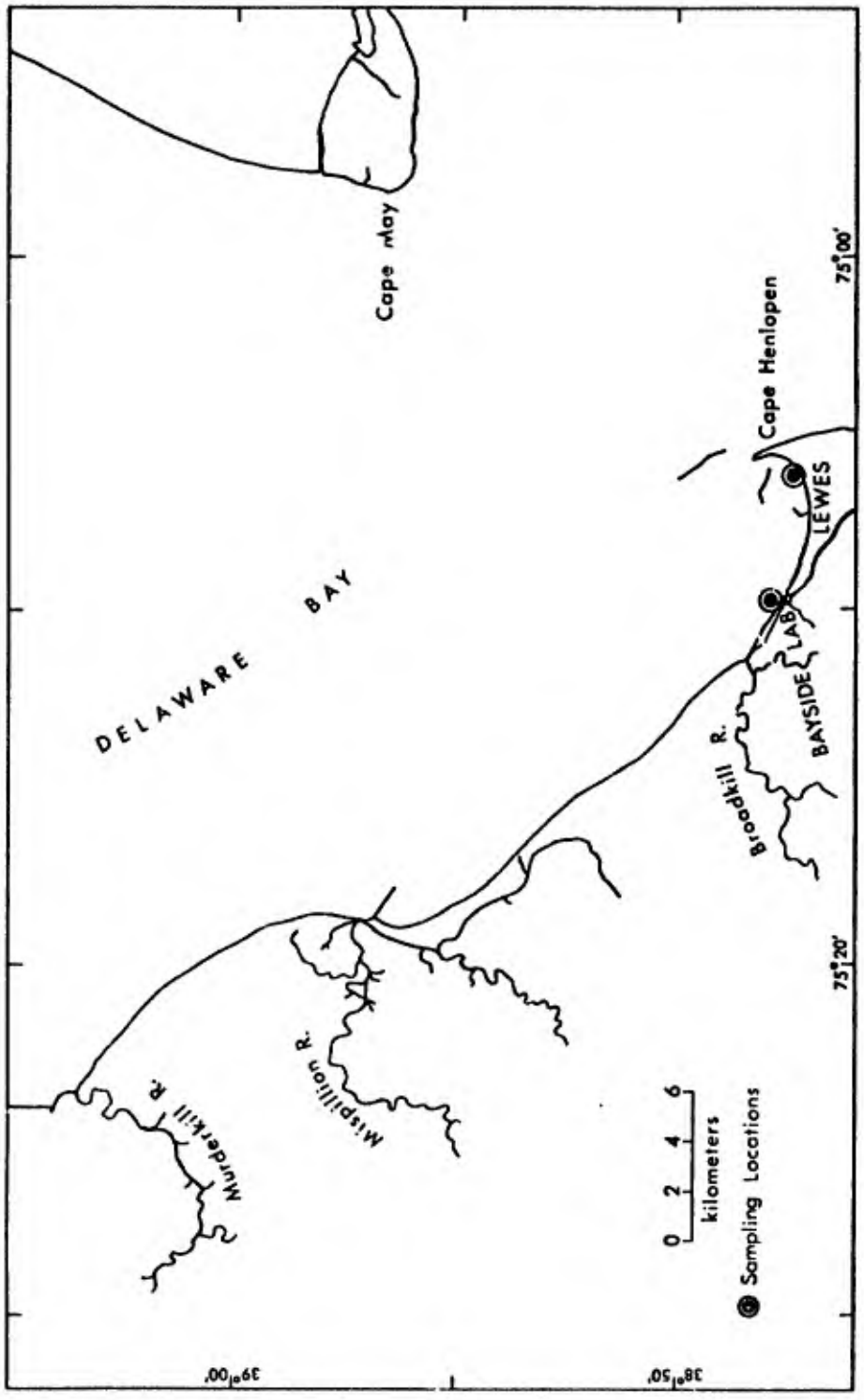

Cape May
Cape Henlopen
LEWES
LAB
BAYSIDE
Broadkill R.
DELAWARE BAY
Mispillion R.
Murderkill R.
0 2 4 6
kilometers
Sampling Locations
75°00'
75°20'
39°00'
38°50'

Fig. 2. Sampling locations in the Patuxent River Estuary, Maryland.

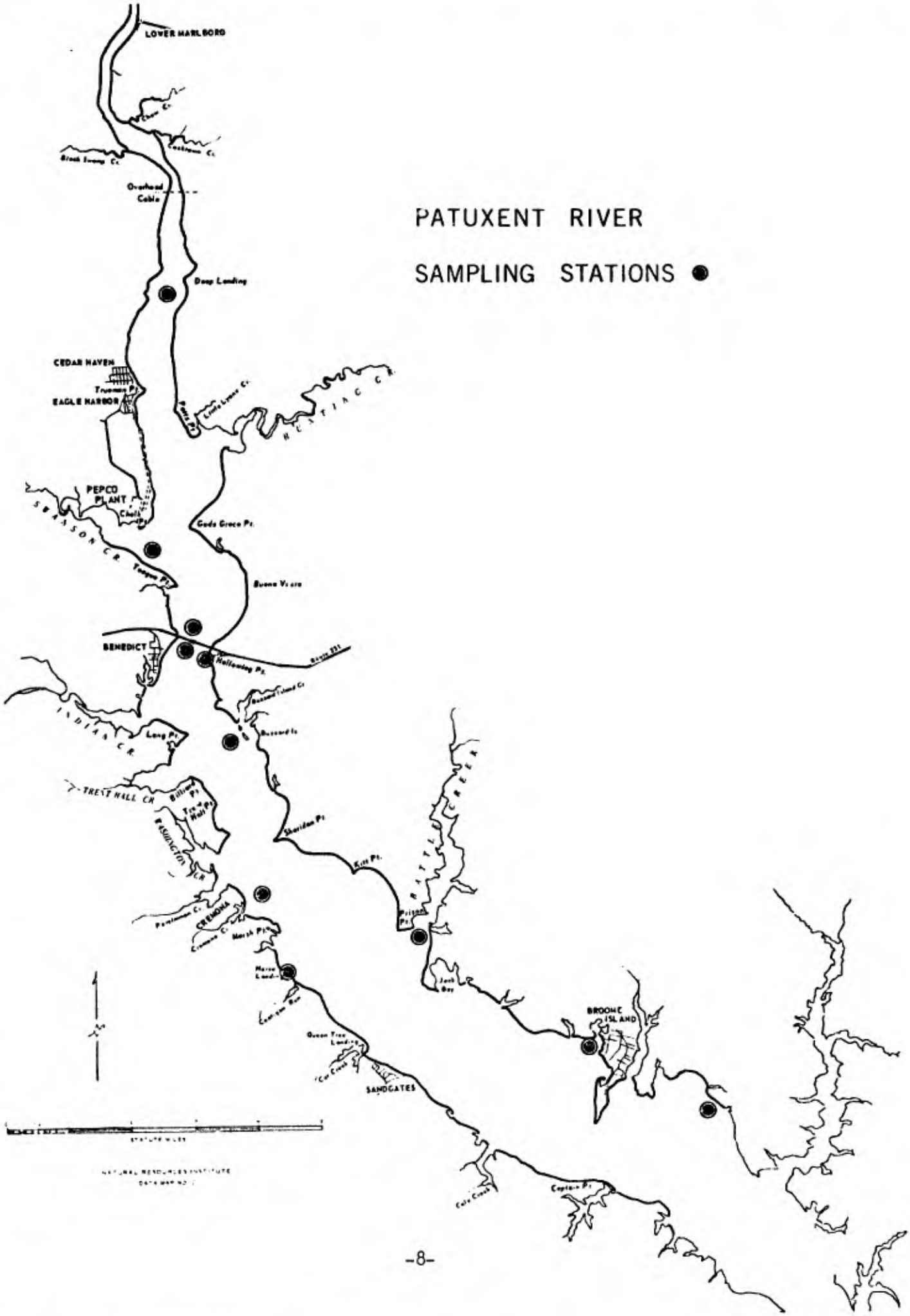

PATUXENT RIVER
SAMPLING STATIONS ●
LOWER MARLBORO
Overhead Cable
Deep Landing
CEDAR HAVEN
EAGLE HARBOR
PEPCO PLANT
Gods Grace Pt.
BENEDICT
Hellen Creek Pt.
Route 231
INDIAN CR.
Long Pt.
TREST HALL CR.
Billings Pt.
Sheridan Pt.
Ellis Pt.
BATTLE CREEK
CREMONA
Marsh Pt.
Jack Bay
BROOME ISLAND
SANDGATES
Cole Creek
Captain Pt.

Table 1. Species used in evaluating the effects of suspended mineral solids on estuarine fishes.

| Species | Common name [1] | Location of capture [2] | Capture method [3] |
|---|---|---|---|
| Brevoortia tyrannus | Menhaden | Del. | H.S. |
| Anchoa mitchilli | Bay anchovy | Del. | H.S. |
| Fundulus majalis | Striped Killifish | P.R. | H.S. |
| F. heteroclitus | Mummichog | P.R. | H.S. |
| Rissola marginata | Cusk eel | Del. | H.S. |
| Menidia menidia | Atlantic silverside | Del. | H.S. |
| Morone saxatilis | Striped bass | P.R. | O.T. |
| M. americana | White perch | P.R. | O.T. |
| Leiostomus xanthurus | Spot | P.R. | O.T. |
| Micropogon undulatus | Croaker | P.R. | O.T. |
| Cynoscion regalis | Weakfish | Del. | H.S. |
| Trinectes maculatus | Hogchoker | P.R. | O.T. |
| Pomatomus saltatrix | Bluefish | Del. | H.S. |
| Opsanus tau | Oyster toadfish | P.R. | O.T. |

1. Amer. Fish. Soc. Spec. Pub. No. 6.
2. Del. = Bayside Laboratory, University of Delaware, Lewes, Delaware.
   P.R. = Patuxent River Estuary, Maryland.
3. H.S. = 30' beach seine.
   O.T. = 20' Otter Trawl pulled at 3 k for 3-5 min.

Tidal activity at the Bayside Laboratory imposed considerable variation on water temperature and salinity. Therefore, the fishes tested at Bayside were tested at a median temperature of 22 ± 2°C, and a salinity which was the same as at the time of capture. Salinity range during testing was 18 to 30 °/oo.

Fishes in the Hallowing Point studies were captured in water of 4 to 6 °/oo salinity over a range of temperatures from 15 to 27°C. Testing was performed at approximately 5.5 °/oo salinity and 25 ± 2°C.

3. Resuspended Natural Sediments.

All bioassay tests with natural sediments were carried out at Hallowing Point. The sediment was obtained in 6.1 m of water near Long Point on the Patuxent River estuary. Several samples were obtained at a time and mixed before use. Although the same batch of sediment was not used in all tests (lethal, sublethal and respiratory studies), the test results using different sediment batches were repeatable.

Natural sediments were maintained in polyethylene containers in the laboratory. Care was taken to keep the samples covered with saline (4 to 6 °/oo) water.

Natural sediments were added to test tanks as a mud/water slurry. Test tanks were partially filled with sediments and concentrations of solids were determined. Desired test concentrations were obtained by diluting the slurry with filtered river water.

## III. RESULTS

### 1. Mineral Solids

Eleven of 14 species used in this study were exposed to suspensions of kaolinite (Hydrite-10). All the fishes exposed to kaolinite survived 24 hour exposures in concentrations up to 140 grams per liter ($g\ l^{-1}$). Several species (white perch, Morone americana; spot, Leiostomus xanthurus; toadfish, Opsanus tau; mummichog, Fundulus heteroclitus; hogchoker, Trinectes maculatus; menhaden, Brevoortia tyrannus) were exposed to 140 $g\ l^{-1}$ kaolinite for 48 hours with the same result; no deaths directly attributable to the mineral solid. In almost all cases the fishes became highly active when placed in suspensions of kaolinite. However, this reaction was short-lived, and activity became normal after 0.5 to 2 hours. Because of the small number of individuals collected, the species not exposed to kaolinite were: bluefish (Pomatomus saltatrix), cusk eel (Rissola marginata) and the bay anchovy (Anchoa mitchilli).

Survival of 14 species was assessed in suspensions of fuller's earth. Three species (toadfish, cusk eel and hogchoker) showed no mortality attributable to the effects of the fuller's earth after 24 hour exposure to concentrations between 96 and 140 $g\ l^{-1}$.

Among the 11 species killed in the fuller's earth suspensions, the response of five species (striped bass, Morone saxatilis, croaker, Micropogon undulatus; weakfish, Cynoscion regalis; bluefish and menhaden) was not consistent enough among replicates to calculate accurate LC values. However, tolerance of these fishes is presented (Table 2) as the lowest concentration at which 100% mortality occurred in any of the replicates.

Exposure of the remaining six species (Atlantic silverside, Menidia menidia; striped killifish, Fundulus majalis; white perch, bay anchovy and mummichog) to fuller's earth resulted in consistent concentration-mortality responses, from which calculations of lethal concentrations for 10, 50, and 90% mortality in 24 hour bioassays could be made. Consistent concentration-mortality responses of white perch were also observed for 12, 18, and 24 hour exposures. Spot provided consistent responses for 12, 20, and 48 hour exposures.

The six species varied widely in sensitivity to suspensions of fuller's earth, as indicated by 24 hour lethal concentrations (Table 3). The $LC_{50}$ response showed a total range of 36.60 g $l^{-1}$, from 39,00 g $l^{-1}$ (mummichog) to 2.40 g $l^{-1}$ for the Atlantic silverside. $LC_{90}$ values for 24 hour assays had a range of 56.57 g $l^{-1}$, ranging from 62.17 g $l^{-1}$ (mummichog) to 9.60 g $l^{-1}$ (bay anchovy). The total range of $LC_{10}$ values was 23.90 g $l^{-1}$, ranging from 24.47 g $l^{-1}$ (mummichog) to 0.57 g $l^{-1}$ (Atlantic silverside).

The range of concentrations from $LC_{10}$ to $LC_{90}$ within each species varied widely. The species with the highest $LC_{50}$ value (mummichog) showed a range of 37.70 g $l^{-1}$ between $LC_{10}$ and $LC_{90}$ (Table 3). The species with the lowest $LC_{50}$ value (Atlantic silverside) showed a range of 9.43 g $l^{-1}$ between $LC_{10}$ and $LC_{90}$. Species with intermediate 24 hour $LC_{50}$ values (white perch and spot) showed a range of 28.76 and 18.54 g $l^{-1}$, respectively, between $LC_{10}$ and $LC_{90}$. However, the range of concentration between $LC_{10}$ and $LC_{90}$ did not necessarily reflect the sensitivity of the species as indicated by the 24 hour $LC_{50}$ value. For

Table 2. Lowest fuller's earth concentration causing 100% mortality in a 24 hr exposure for five estuarine fishes.

| Species | Age class | N | Test conditions | | |
|---|---|---|---|---|---|
| | | | Salinity °/oo | Temperature °C | Concentration g $l^{-1}$ fuller's earth |
| Menhaden | 0+ | 30 | 5.5 | 25±2 | 1.2 |
| Menhaden | 1+ | 60 | 23.6 | 22±2 | 0.8 |
| Bluefish | 1+ | 26 | 20.0 | 22±2 | 0.8 |
| Weakfish | 0+ | 47 | 20.0 | 22±2 | 6.8 |
| Weakfish | 0+ | 20 | 5.5 | 25±2 | 8.2 |
| Striped Bass | 2+ | 31 | 5.5 | 25±2 | 16.6 |
| Croaker | 1+ | 17 | 5.5 | 25±2 | 11.4 |

Table 3. $LC_{10}$, $LC_{50}$ and $LC_{90}$ values determined for 24-h exposure of estuarine fishes. Correlation coefficients (r) and coefficients of determination ($r^2$) derived from regression analyses are presented as statistical estimates of the decimal fraction of mortality accounted for by concentration effects.

| Species | r | $r^2$ | Lethal concentration, g $l^{-1}$ fuller's earth | | |
|---|---|---|---|---|---|
| | | | $LC_{10}$ | $LC_{50}$ | $LC_{90}$ |
| White perch | 0.938 | 0.880 | 3.05 | 9.85 | 31.81 |
| Spot | 1.000 | 1.000 | 13.08 | 20.34 | 31.62 |
| Bay anchovy | 0.760 | 0.577 | 2.31 | 4.71 | 9.60 |
| Atlantic silverside | 0.806 | 0.650 | 0.57 | 2.40 | 10.00 |
| Mummichog | 0.952 | 0.906 | 24.47 | 39.00 | 62.17 |
| Striped killifish | 0.966 | 0.934 | 23.77 | 38.18 | 61.36 |

example, the $LC_{10}$ to $LC_{90}$ range for the white perch was 28.76 g $l^{-1}$, whereas spot, with a 24 hour $LC_{50}$ more than twice that of the white perch (20.34 vs 9.85 g $l^{-1}$) had an $LC_{10}$ to $LC_{90}$ range of only 18.54 g $l^{-1}$. Similarly, bay anchovies ($LC_{50}$ = 4.71 g $l^{-1}$) had an $LC_{10}$ to $LC_{90}$ range of 7.29 g $l^{-1}$, while the more sensitive atlantic silverside ($LC_{50}$ = 2.40 g $l^{-1}$) had an $LC_{10}$ to $LC_{90}$ range of 9.43 g $l^{-1}$ fuller's earth.

White perch and spot exposed to fuller's earth for varying periods of time showed a gradual reduction of $LC_{50}$ with increasing duration of exposure (Table 4). These data were plotted logarithmically (Figs. 3 and 4) in a style similar to that used for presentation of toxicity curves (Sprague, 1969).

2. Resuspended Natural Sediments

White perch, spot, menhaden and striped killifish were tested to determine the lethal concentrations of natural sediments during 24-hr exposures.

$LC_{50}$ values for white perch exposed to resuspended natural sediments were 19.80 g $l^{-1}$, whereas $LC_{50}$ for fuller's earth was 9.85 g $l^{-1}$. A similar range of difference applied to $LC_{90}$ and $LC_{10}$ values in resuspended natural sediments as compared with fuller's earth: 39.40 vs 31.81 g $l^{-1}$ ($LC_{90}$) and 9.97 vs 3.05 g $l^{-1}$ ($LC_{10}$), respectively.

Spot exposed to natural sediment had an $LC_{50}$ of 88.00 g $l^{-1}$, $LC_{10}$ and $LC_{90}$ of 68.75 and 112.63 g $l^{-1}$, respectively, for a 24-hr exposure. These values are in contrast to 24-hr values in fuller's earth of 13.81, 20.34, and 31.62 for $LC_{10}$, $LC_{50}$, and $LC_{90}$, respectively. Spot were pre-

Table 4. $LC_{10}$, $LC_{50}$ and $LC_{90}$ values with increasing duration of exposure determined for white perch and for spot.

| Duration of bioassay in hours | White perch | | | Spot | | |
|---|---|---|---|---|---|---|
| | $LC_{10}$ | $LC_{50}$ | $LC_{90}$ | $LC_{10}$ | $LC_{50}$ | $LC_{90}$ |
| 12 | 32.07 | 41.00 | 52.41 | 27.56 | 42.36 | 65.12 |
| 18 | --- | --- | --- | 21.07 | 33.06 | 51.87 |
| 20 | 7.91 | 14.99 | 28.38 | --- | --- | --- |
| 24 | 3.05 | 9.85 | 31.81 | 13.08 | 20.34 | 31.62 |
| 48 | 0.67 | 2.96 | 13.06 | 1.13 | 1.90 | 3.17 |

Fig. 3. Lethal effect of fuller's earth to white perch at $LC_{90}$ (solid points), $LC_{50}$ (open points), and $LC_{10}$ (x). Dotted line indicates 48 hr exposure, the maximum duration of exposure used in the current study.

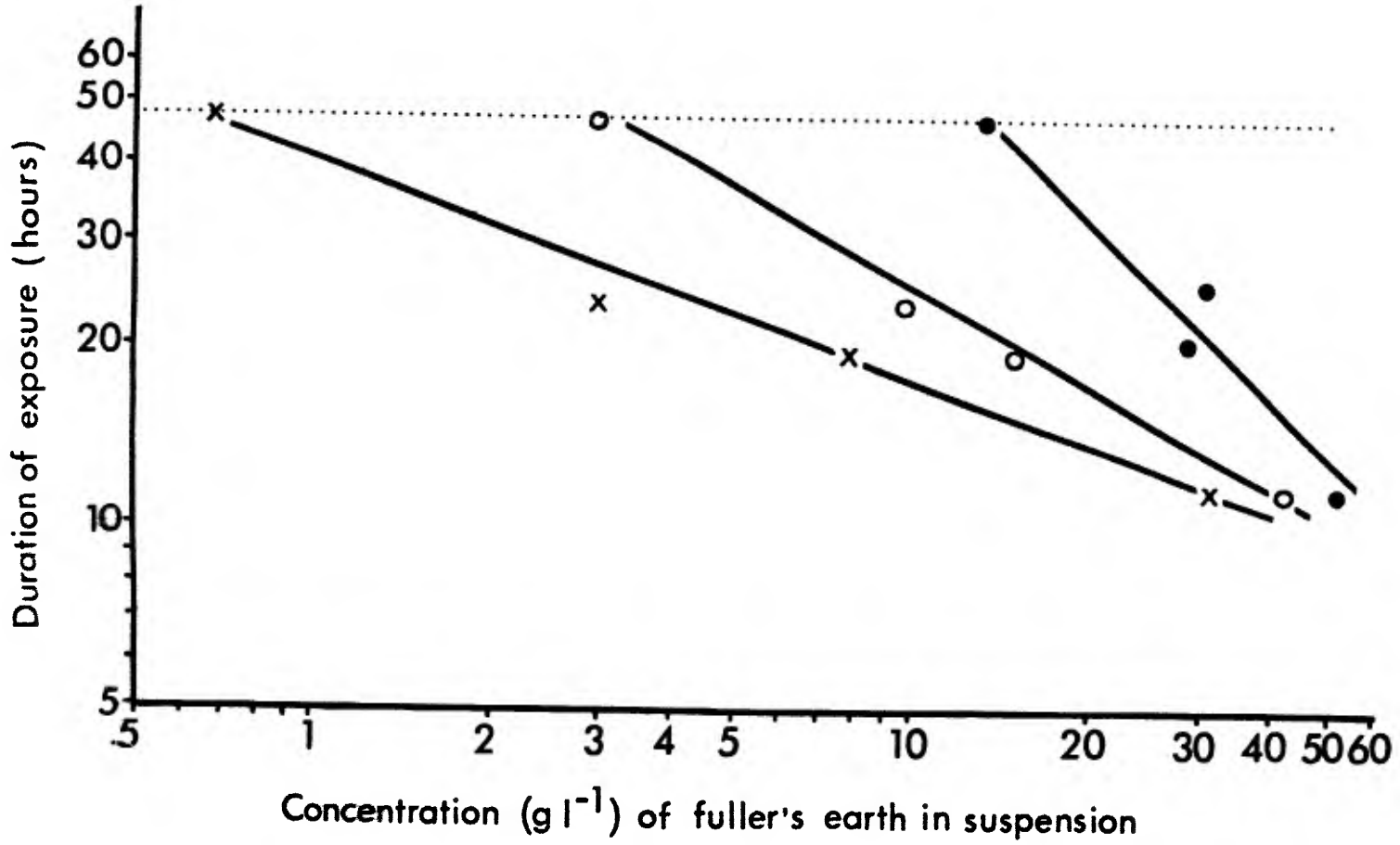
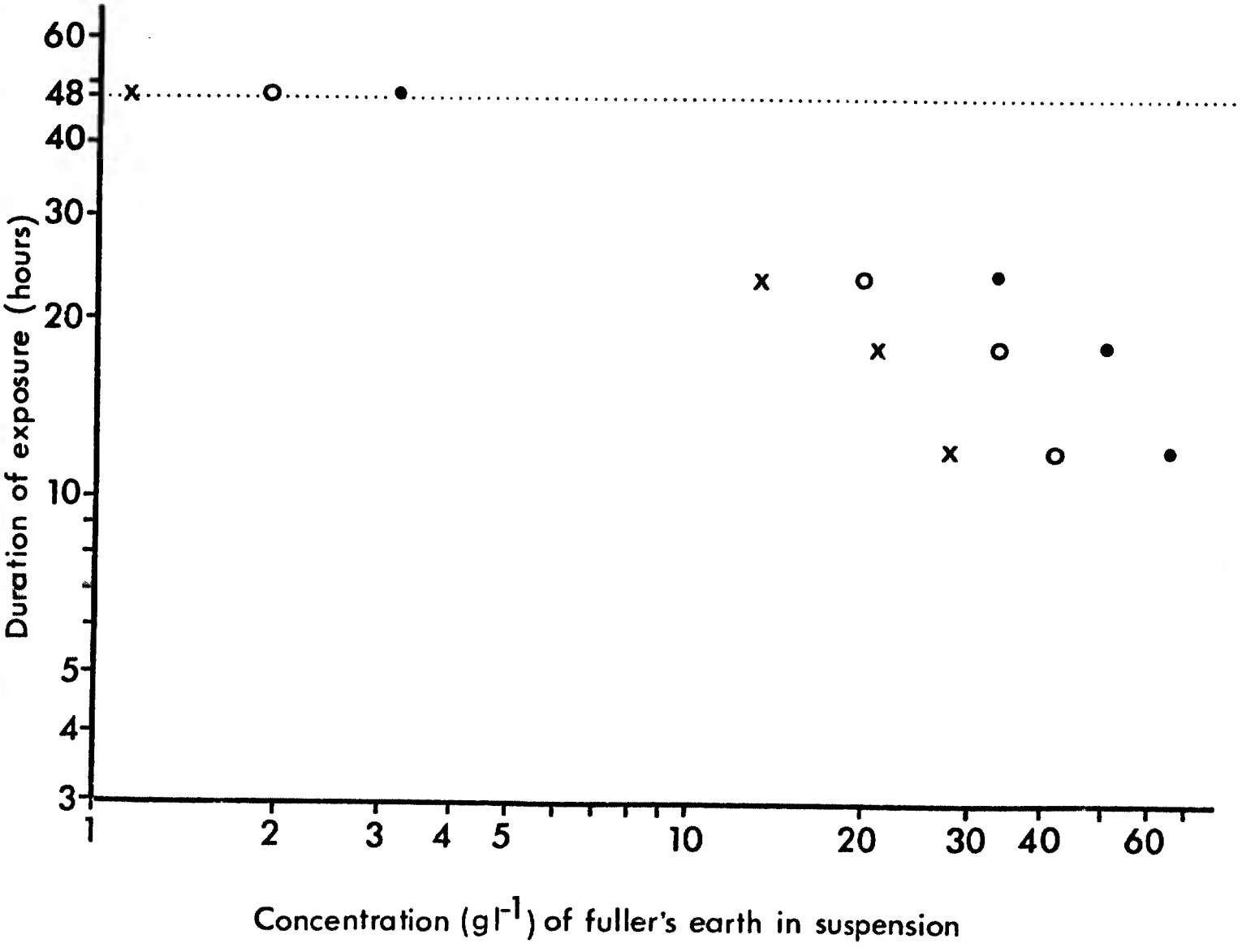

60
50
40
30
20
10
5
Duration of exposure (hours)
.5
1
2
3
4
5
10
20
30
40
50
60
Concentration (g l$^{-1}$) of fuller's earth in suspension

Fig. 4. Lethal effect of fuller's earth to spot at $LC_{90}$ (solid points), $LC_{50}$ (open points) and $LC_{10}$ (x). Dotted line indicates 48 hr exposure, the maximum duration of exposure used in the present study.


60
48
40
30
20
10
5
4
3
Duration of exposure (hours)
1
2
3
4
5
10
20
30
40
60
Concentration (g l⁻¹) of fuller's earth in suspension

viously reported (Annual Report, Year II) to have a relatively low tolerance to resuspended natural sediment (48 hr $LC_{50}$ = approx. 3 g $l^{-1}$). Lethal concentrations for replicated 48 hr exposures were essentially unchanged with respect to the 24 hr values. This evidence suggests that (1) a compensatory mechanism is present in spot which allows them to tolerate high levels of natural suspended material for at least 48 hours, and (2) the preliminary results for this species reported in our Annual Report, Year II (p. 24) were in error. A likely cause of this error was inadequate preparatory oxygenation of the experimental sediment suspensions. These suspensions of natural material had been observed to have a high initial oxygen demand. As a result, the excessively high fish mortality which occurred in experimental tanks probably was caused by low oxygen concentration and not sediment concentrations.

The alimentary canal of fish from these bioassays was packed with sediment. The entire digestive tract was swollen and distorted by this large amount of ingested material.

Separate concentration-dependent mortality determinations (48 hr, 25° C) were conducted on the common mummichog with fuller's earth and natural sediment. For fuller's earth suspensions, 48 hr values were: $LC_{10}$ = 35.86 g $l^{-1}$; $LC_{50}$ = 45.16 g $l^{-1}$; $LC_{90}$ = 56.89 g $l^{-1}$. We could not maintain natural sediment concentrations which were high enough to cause sufficient mortality to determine lethal concentrations. Sixty-two percent of adult mummichogs survived concentrations greater than 125 g $l^{-1}$ for 24 hours and 100% have survived concentrations in excess of 109 g $l^{-1}$ for 72 hours.

Striped killifish exposed to resuspended natural sediments for 24 hours had $LC_{10}$, $LC_{50}$, and $LC_{90}$ values of 97.1, 128.2, and 169.3 g $l^{-1}$, respectively.

## IV. DISCUSSION

The effects of suspended artificial and natural (deposited) sediments upon estuarine fishes are, at least in part, dependent upon several characteristics of the suspended material. None of the fish species tested in suspensions of Hydrite-10, a kaolinite clay (median particle size of 0.55 um), died. Among the species tested were menhaden, which are highly sensitive to suspensions of solids other than kaolinite. However, suspensions of fuller's earth were lethal to the greater number of fish species tested when used in concentrations generally exceeding 0.65 g $l^{-1}$. Comparison of the two mineral solids on the physical level suggests that the differences in the lethal effect of these substances may be due in part to particle size distribution (see APPENDIX D).

Previous work with suspended mineral solids has demonstrated that suspended particles of different composition vary greatly in lethal effect to fishes (EIFAC, 1964; Rogers, 1969). Using a variety of solids, including kaolinite, diatomaceous earth, natural glacial silt, and incinerator fly-ash, Rogers (1969) concluded that the lethal effect of a suspended mineral solid was dependent upon particle shape and angularity (mechanical abrasion?), rather than particle size. However, the mechanism whereby particle angularity enters into a rapid (24 to 96 hr) mortality among test fishes is unclear.

In the observation of dead and moribund test fish, we have noted that common symptoms were extensive hemorrhaging of minute blood vessels over the entire body surface, and packing of the gills with sediment. Microscopic examination of fresh gill preparations from recently dead fish showed no noticeable hemorrhaging associated with exposure to kaolinite or fuller's earth. Thus, from our observations we cannot evaluate Rogers' (1969) claim that angularity increased the lethality of a sediment suspension because particles of kaolinite and fuller's earth are flat and plate-like, not angular. Even the sand grains in the natural sediments used in our experiments were sub-angular at best. The angles at the intersection of crystal faces were relatively smooth and had few sharp edges.*

The data from respiration studies show some of the effects of suspended mineral solids on fishes. Rogers (1969) noted increased survival among test fishes when he bubbled air into his test chanbers, suggesting that exposure to suspended solids leads to anoxia and asphyxiation. The relationship between angularity and lethal effect of mineral solids may be due to the fact that angular particles have a greater likelihood of clinging to the gill surface, covering the respiratory epithelium or abrading the epithelium and causing anoxia.

For every species which we tested, natural sediments were less "toxic" than were suspensions of mineral solids. Previous studies have not attempted to evaluate the differences between mineral solids and resuspensions of natural sediments.

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*This is evidence of reworking by wind and tidal action. Indeed, the largest particles (30 um diam.) were pieces of detritus and shell.

The lethal effect of the natural mud was most likely due to a clogging of the interstices of the gills, an effect unlike the coating effect of fuller's earth particles. Ellis (1937) has described several ways whereby particles may cause asphyxiation in fishes. Among these is coating, the effect likely to be produced by very fine particles, and clogging, the probable effect of the larger particles making up the natural mud.

While Ellis' discussion did not include details of fish gill morphology, recent investigations by Muir (1969) and Cameron and Davis (1970) render the following hypothesis as plausible. Suspended particulate matter is lethal to fishes at concentrations well in excess of those observed in nature. The lethal effect of finely divided solids is dependent upon several factors with different mechanisms operative in causing death in fish, although the cause of death is the same: anoxia. Very fine particles generally will not cause death unless the particle is angular. The angularity of particles produces a "sticking" or "coating" effect which removes the respiratory epithelium from contact with the water. Hence, a local anoxic condition exists at the gill surface, and asphyxiation results. Larger particles, capable of being entrapped by the primary and secondary lamellae of the gill, block the minute channels of circulation between the secondary lamellae. The results of this blockage leads to "dead space" at the primary site of gas exchange. Oxygen diffusion may occur, to a certain limited extent, through "dead spaces." A coating of clay-sized particulates over the entire gill surface would be less likely to permit gas exchange. As "dead space" accumulates progressively through clogging of more and more secondary lamellae, oxygen exchange decreases and an anoxic condition develops. The reduced toxicity of natural

sediments to the test fish as compared with the mineral solid used (fuller's earth) is likely due to the less efficient blocking by larger particles or by the diluting effect of the organic matter on the number of inorganic particles available for blocking.

In freshwater systems concentrations of 2.0 to 6.0 g $l^{-1}$ of silt may persist for 15 to 20 days in flood-stage rivers (EIFAC, 1964). Similarly, freshwater streams polluted with china-clay mining waste may carry burdens of 1.0 to 6.0 g $l^{-1}$ continuously (Herbert and Merkins, 1961). More saline waters characteristically carry lower concentrations of suspended particles because of flocculation, dilution and the "salting-out" phenomenon. However, Masch and Espey (1967), in a study of shell-dredging operations in Galveston Bay, Texas have recorded suspended solids concentrations of 4.15 g $l^{-1}$ in the immediate vicinity of a dredge discharge. At a distance of 2750 feet from the discharge, concentrations were 0.3 g $l^{-1}$ suspended solids. Suspended solids concentrations may reach 1.2 g $l^{-1}$ during flood conditions (Tropical Depression Agnes, 1972) in the upper Patuxent River, Maryland. Values recorded during summer, 1972, were generally in the range 0.08 to 0.14 g $l^{-1}$, dependent upon local weather conditions and tidal scouring. Suspended solids concentrations capable of causing significant mortality of estuarine fish species, at the 10% and 50% levels, can be maintained by natural estuarine systems, near dredging operations, or during times of excessively high run-off.

Assuming that the species used in these experiments were representative of adult estuarine fishes, they may be placed in three groups according to their capacity to tolerate suspended solids concentrations. The classification is subjective, and is based upon $LC_{10}$ values of fuller's earth as we consider 10% mortality in addition to natural mortality rates a more realistic maximum than the 50 % mortality limit (see discussion in Ricker, 1954).

CLASS I. SUSPENSION TOLERANT SPECIES:

Concentration of fuller's earth required to attain the 24 hour $LC_{10}$ value is in excess of 10 g $l^{-1}$. Tolerant species were the mummichog, striped killifish, and spot. Other species were tested for suspension tolerance, but concentration-dependent mortality curves were not determined. These were the toadfish, hogchoker, and cusk eel. A common feature of these tolerant species is that their habitat preference is the mud-water interface where suspended solids concentrations in natural systems tend to be higher than elsewhere in the water column (Masch and Espey, 1967). For example, the killifish, the hogchoker, and the cusk eel frequently burrow into the substrate and remain covered for extended periods of time (Hildebrand and Schroeder, 1928). The toadfish is a bottom dweller and a relatively inactive organism (Neumann, pers. obs.).

CLASS II. SUSPENSION SENSITIVE SPECIES:

$LC_{10}$ values for 24 hour exposure to fuller's earth were between 1.0 and 9.9 g $l^{-1}$. Sensitive species were the white perch, the

bay anchovy and the juvenile menhaden tested at Hallowing Point (see Sherk, O'Connor, and Neumann, 1973). Common biological characteristics were difficult to ascertain for these species. Habitat preferences were quite similar to those of spot, a species already classified as a tolerant form. Our data would place three important commercial species, the striped bass, the croaker, and the weakfish in this class.

CLASS III. HIGHLY SENSITIVE SPECIES:

Twenty-four hour $LC_{10}$ values were less than 0.9 g $l^{-1}$ fuller's earth. The Atlantic silverside with 24 hr $LC_{10}$ of 0.58 g $l^{-1}$ was classified as a highly sensitive species. Other highly sensitive forms, particularly juvenile forms and young-of-the-year life stages (Sherk and O'Connor, 1971), are juvenile bluefish, juvenile menhaden, and young-of-the-year white perch. Juvenile bluefish and juvenile menhaden tested at the University of Delaware Bayside Lab. failed to survive in concentrations of 0.8 g $l^{-1}$ for more than 18 hours. Likewise, young-of-the-year white perch suffered 100% mortality in 0.75 g $l^{-1}$ fuller's earth in 20 hours.

The lethal effects of suspended solids on fishes differ at different stages in the life history of a given species. Juvenile white perch are much more likely to be killed by lower concentrations of suspended solids than are adults. The basis for such age-specific differences in tolerance is unknown. When fishes are exposed to lethal concentrations of fuller's earth, the gill filaments and the secondary lamellae act as a sieve to

entrap particles (O'Connor, pers. obser.), which clog the gill, and result in asphyxiation (Ellis, 1937). The physical dimensions of the fish gill increase with increased size of the fish (Muir, 1969). As the fish grows and the gill dimensions increase, it follows that the size of the openings in the gill filter also increase. Thus, for the larger fish, fewer particles may become entrapped, thereby decreasing the lethal effect of a given concentration of suspended solids.

Another factor which may explain the increased tolerance of fishes with size is that the smaller fish have a greater metabolic rate than larger fish (see Respiration Studies, Chapter 6). Smaller fishes, which demand more oxygen per unit body weight than larger fishes, may not be able to tolerate the same relative amount of gill clogging as larger fish. The combined effect of the higher metabolic rate and a finer, more efficient, filter would render juveniles of a species highly sensitive to suspended solids, regardless of the tolerance shown by the adult.

EFFECTS OF SUSPENDED AND DEPOSITED SEDIMENTS

ON ESTUARINE ORGANISMS PHASE II

CHAPTER 2

TIME-CONCENTRATION-MORTALITY STUDIES OF WHITE PERCH AND SPOT

EXPOSED TO FULLER'S EARTH

## I. INTRODUCTION

Sherk and O'Connor (1971) (O'Connor, Prince and Sherk, unpub. data) showed that increased exposure time decreased the quantity of suspended solids needed to cause 50% and 10% mortality among white perch and spot. In Chapter 1 we showed that doubling the duration of exposure to fuller's earth suspensions from 24 to 48 hours caused a drop in $LC_{50}$ values in these two species.

In this section we present a synthesis of 12, 18, 20, 24 and 48-hour concentration-response studies of white perch and spot to relate the probability of survival to the time of exposure to suspended solids.

## II. METHODS

Techniques utilized in this section were essentially the same used in determination of concentration-response curves in Chapter 1.

## III. RESULTS AND INTERPRETATION

Time-concentration-mortality studies were conducted (Table 5) and concentration-dependent response curves for increasing lengths of exposure were determined for white perch (Fig. 5) and for spot (Fig. 6).

Note that in the white perch (Fig. 5) the slopes of the 12- and 20-hr response curves were essentially the same. However, the slopes of the 24- and the 48-hr response curves differ. The effect of this change in slope was that with increased exposure time, the concentration of suspended solids causing 10% mortality decreased much more rapidly over time than the concentration causing 90% mortality. The concentration needed to cause 90% mortality for a 48-hr exposure was 25%, by weight, of the 12-hr $LC_{90}$ value. However, the $LC_{10}$ value for white perch exposed to fuller's earth for 48 hours was only 2.2% of the 12-hr $LC_{10}$ value (Table 5). Thus, very low concentrations of suspended solids caused obvious low, yet important levels of mortality during longer exposure periods.

When $LC_{10}$ and $LC_{50}$ data for 12, 20, 24, and 48 hour exposures of white perch were plotted (Fig. 7), hyperbolic curves were generated. approximated by $C = KT^a$, where C is the $LC_{10}$ or $LC_{50}$ concentration, T is the duration of the exposure period, and K equals the intercept of the hyperbola with the Y-axis. For $LC_{10}$ data

$$C = 64.3 \cdot T^{-0.043}$$

and for $LC_{50}$ data

$$C = 68.3 \cdot T^{-0.030}$$

Table 5. Decreasing $LC_{50}$ and $LC_{10}$ values in g $l^{-1}$ fuller's earth with increasing duration of exposure determined for white perch and spot.

| Duration of bioassay in hours | White perch | | Spot | |
|---|---|---|---|---|
| | $LC_{50}$ | $LC_{10}$ | $LC_{50}$ | $LC_{10}$ |
| 12 | 41.00 | 32.07 | 42.36 | 27.56 |
| 18 | --- | --- | 33.06 | 21.07 |
| 20 | 14.99 | 7.91 | --- | --- |
| 24 | 9.85 | 3.05 | 20.34 | 13.08 |
| 48 | 2.96 | 0.67 | 1.90 | 1.13 |

Fig. 5. Concentration-mortality curves for white perch exposed to suspensions of fuller's earth for 12 hr (1) 20 hr (2) 24 hr (3) and 48 hr (4).

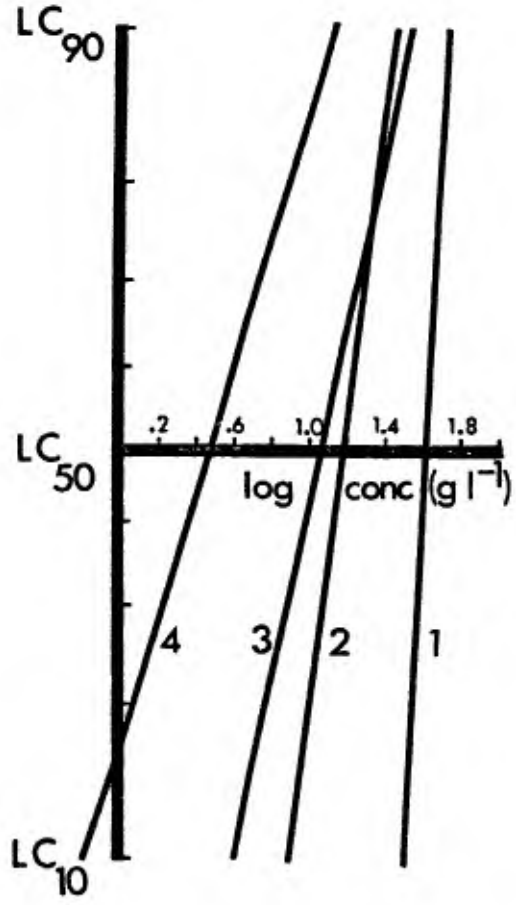

LC90
LC50
LC10
.2
.6
1.0
1.4
1.8
log conc (g l⁻¹)
4
3
2
1

Fig. 6. Concentration-mortality curves for spot exposed to suspensions of fuller's earth for (1) 12 hr (2) 18 hr (3) 24 hr and (4) 48 hr.

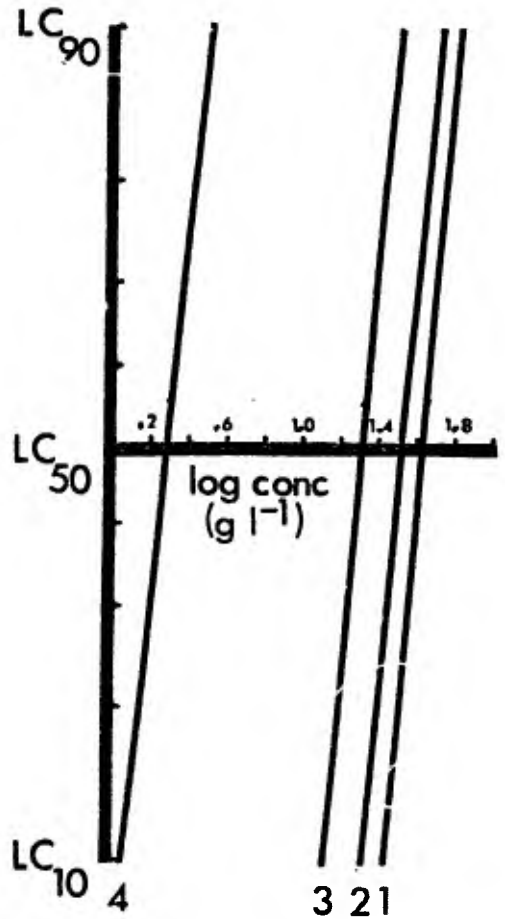
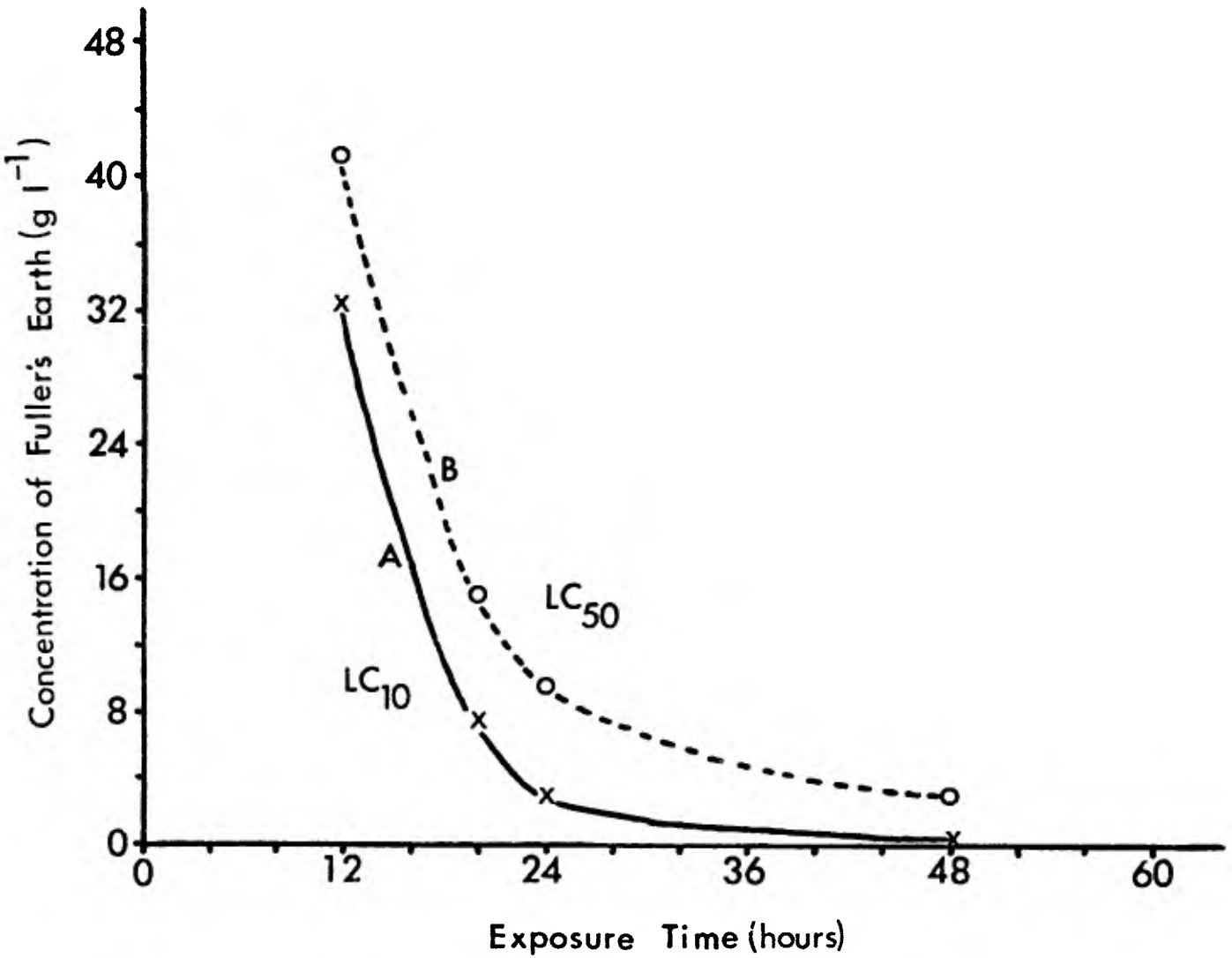

LC90
LC50
LC10
.2
.6
1.0
1.4
1.8
log conc
(g l-1)
4
3 2 1

Fig. 7. $LC_{10}$ (A) and $LC_{50}$ (B) values at exposure times of 12, 20, 24 and 48 hr for white perch exposed to suspensions of fuller's earth.


Concentration of Fuller's Earth (g l$^{-1}$)
48
40
32
24
16
8
0
0
12
24
36
48
60
Exposure Time (hours)
A
B
LC$_{50}$
LC$_{10}$

The vertex of the hyperbola for $LC_{50}$ was defined to be at 16.35 hours ($\frac{dx'}{Dt} = 0$) and for the $LC_{10}$ plot the vertex was defined to be at 23.78 hours. In comparing the plot of decreasing $LC_{10}$ and $LC_{50}$ with duration of exposure (Fig. 7), and the concentration-response curves of four separate exposures of white perch for varying periods of time (Fig. 5), the calculated vertexes agree closely with the apparent point at which the slopes of the concentration-response curves start to deviate one from the other. This estimated point may be clearer when considered from the $LC_{10}$ data because the duration of exposure had its effect primarily on the lower levels of mortality. Thus, using $LC_{10}$ data for determination of the hyperbolic vertex, we found that the visual approximation afforded an excellent estimate of inflection between 20 and 24 hours. As mentioned above, the calculated value was 23.78 hours.

The relationship of $LC_{10}$ concentration to duration of exposure was interesting in that it predicted almost instantaneous 10% mortality of white perch exposed to concentrations at or greater than 64 g $l^{-1}$ fuller's earth. This was in good agreement with preliminary laboratory data from the initial stages of this work, where we found that white perch placed in concentrations of 58 g $l^{-1}$ and higher died almost instantaneously.

The 10% mortality concentration for more than a 48-hr exposure approached zero, or, conversely, the time necessary for 10% mortality at very low concentrations approached infinity. $LC_{10}$ values for 72-hr and 96-hr exposures were 0.06 g $l^{-1}$ and 0.0045 g $l^{-1}$, respectively, or, well within the range of suspended material carried by "undisturbed" natural systems.

An appropriate conclusion would be that fishes exposed to concentrations of suspended solids normally found in natural waters would not be adversely affected by concentrations below a certain threshold value. The concentration of 0.0045 g $l^{-1}$ (or 4.5 mg $l^{-1}$ - 4.5 parts per million) would be well below that threshold. The concentration threshold for a given species of fish may be determined by the ability of the fish to cleanse the gills by the coughing reflex, or by continuous secretion and sloughing of a protective mucus sheet.

Concentration-response curves for spot exposed to fuller's earth for 12, 18, 24 and 48-hours diverged slowly, suggesting that the response for this species remained the same for longer exposures as for brief exposure. $LC_{50}$ and $LC_{10}$ concentrations were plotted (Fig. 8) and found to be hyperbolic functions similar to those for the white perch, although the slopes were somewhat different, and concentrations were greater than for white perch. The function was defined, for $LC_{10}$ values, as

$$C = 100 \cdot T^{-0.40}$$

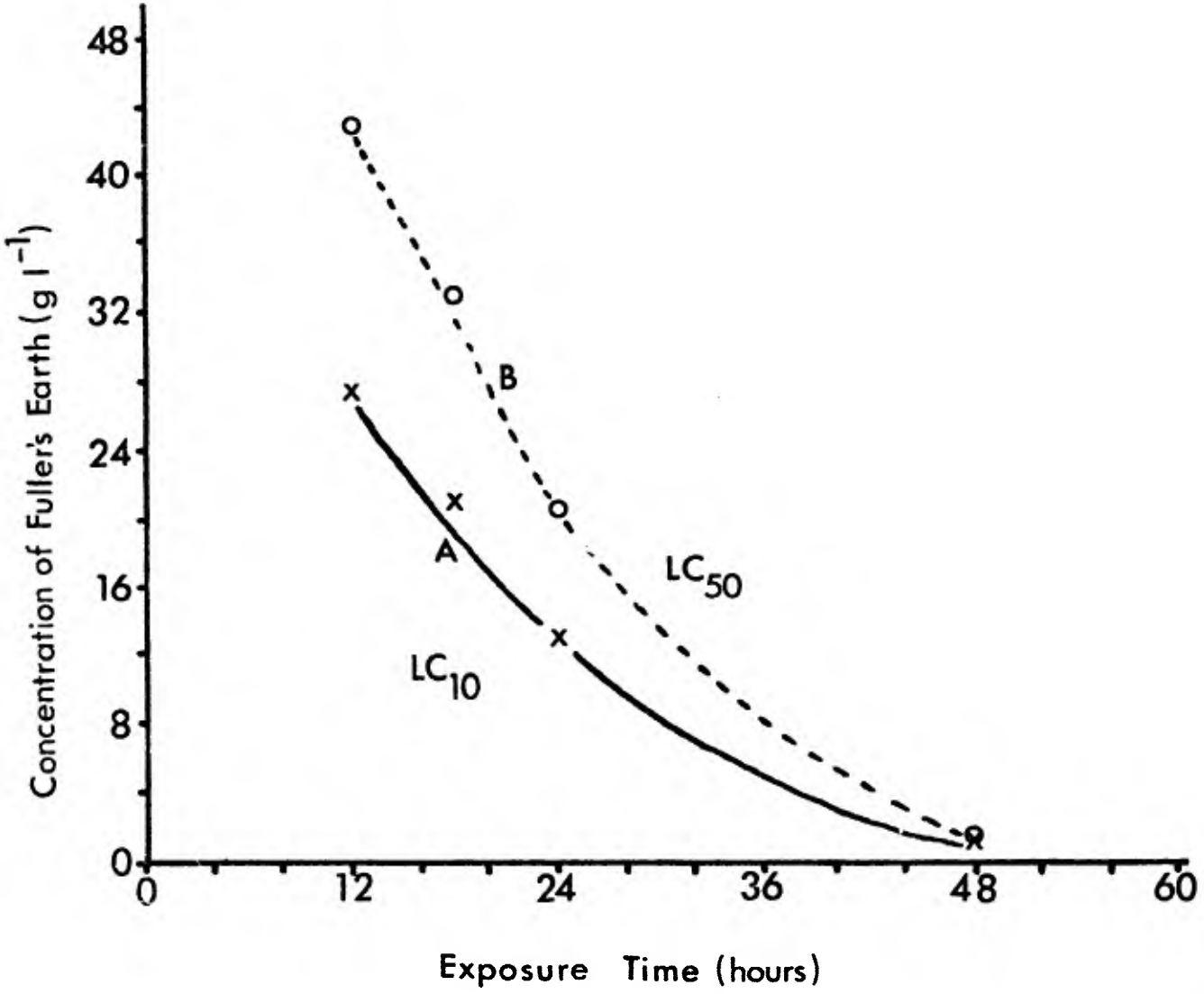
and for $LC_{50}$ values as

$$C = 149.6 \cdot T^{-0.39}$$

Thus, a predicted concentration of 100 g $l^{-1}$ would cause instantaneous 10% mortality in spot; 150 g $l^{-1}$ would instantly kill 50%.

Spot were, in general, more tolerant of suspended solids than white perch (Chapter 1). $LC_{10}$ values after 48-hr exposure predicted by the mathematical approximation were (1.20 g $l^{-1}$) in close agreement with observed values (1.13 g $l^{-1}$) (Table 5). Seventy-two-hr $LC_{10}$ values were

Fig. 8. $LC_{10}$ (A) and $LC_{50}$ (B) values at exposure times of 12, 18, 24 and 48 hr for spot exposed to suspensions of fuller's earth.


Concentration of Fuller's Earth (g l⁻¹)
48
40
32
24
16
8
0
0
12
24
36
48
60
Exposure Time (hours)
B
A
LC50
LC10

predicted to be approximately 0.135 g $l^{-1}$ fuller's earth. Ninety-six-hr $LC_{10}$ values were calculated to be about 0.017 g $l^{-1}$. The 72-hr and 96-hr $LC_{10}$ values for spot, as well as those for white perch, were within the range of concentrations found in natural waters.

White perch exposures to suspended solids were carried out at 0.65 g $l^{-1}$ for periods as long as five days (Chapter 3). From the mathematical approximation of $LC_{50}$ and $LC_{10}$ relating exposure duration to concentration, one would expect concentrations of 0.65 g $l^{-1}$ to cause 10% mortality in less than five days. A threshold concentration, between 0.67 and 0.65 g $l^{-1}$ appears to exist, above which suspended solids may cause death (at least at the 10% level) during exposure of 48 hours, and below which mortality is unlikely. However, the fish exposed for five days to 0.65 g $l^{-1}$ showed sublethal physiological changes in blood characteristics and also showed evidence of damage to gill tissues (Chapters 3 and 4).

EFFECTS OF SUSPENDED AND DEPOSITED SEDIMENTS

ON ESTUARINE ORGANISMS PHASE II

CHAPTER 3

# SUBLETHAL EFFECTS OF SUSPENDED SOLIDS ON THE HEMATOLOGY OF ESTUARINE FISHES

## I. INTRODUCTION

The lethal effects of a variety of solids are documented for numerous freshwater fishes (Ellis, 1936, 1937; Wallen, 1951; Wilson, 1956; Cordone and Kelley, 1961; Herbert, Alabaster, Dart and Lloyd, 1961; Herbert and Merkens, 1961) and for some estuarine species (Rogers, 1969; Sherk and O'Connor, 1971). However, to a great extent, the subtle, sublethal effects of suspended solids have been ignored, except for histological studies of gill tissues of fishes (Southgate, 1962; Herbert, Alabaster, Dart and Lloyd, 1961; Herbert and Merkens, 1961; Ritchie, 1970). The physiological effects of exposure to suspended solids have not been studied previously.

In this section we present the results of experiments carried out to assess the effects of suspensions of fuller's earth earth and natural sediments on several basic hematological parameters in fishes: blood cell volume (hematocrit), erythrocyte (red blood cell) count, hemoglobin concentration, and ionic concentration of the blood (osmolality).

## II. METHODS

Hematological studies were conducted with seven species: white

perch, striped bass, hogchokers, spot, mummichog, striped killifish, and the oyster toadfish. Each species was exposed to a concentration of fuller's earth or natural Patuxent River sediment which previously had been shown to cause less than 10% mortality. Ideally, this was no greater than the previously determined 24-hr $LC_{10}$ concentrations for each species (Chapter 1).

Twenty-four hours before the start of an experiment, a quantity of fuller's earth sufficient to maintain the desired concentration was placed in the experimental tank and mixed by submersible pumps. The control tank contained no fuller's earth. Mineral solids were maintained in suspension throughout the experiment by continuous pumping and aeration; similar conditions were provided in the control tank.

During a test, 12 or 15 fish were placed in each of the control and experimental tanks. After the exposure period, blood was sampled from each of at least 10 individuals selected at random from the tanks. Blood was collected from white perch and striped bass by severing the second branchial artery on the right side (McLean and Brinkley, 1971). In experiments with hogchokers, spot and killifish, blood was collected from the caudal artery after severing the caudal peduncle with a heparinized blade. The blood was collected in heparinized pipets and, when possible, mixed before samples were removed for analysis.

Microhematocrit, or determination of packed blood cell volume, was determined according to methods outlined by Hesser (1960). Hemoglobin

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

## III. RESULTS AND INTERPRETATION

White perch, hogchokers and striped killifish showed alteration in hematological characteristics in response to sublethal concentrations of suspended solids. The effects of sublethal concentrations of suspended solids have been analyzed more extensively for the white perch (Table 6) than for the other species studied. Exposure of white perch to 0.65 g $l^{-1}$ fuller's earth for five days resulted in significant increases in three hematological parameters: hematocrit, hemoglobin and red blood cell count. The ionic concentration of blood, estimated by whole blood osmolality, did not change in response to the suspended solid.

Red blood cell (RBC) counts increased more, on a relative basis than did hematocrit and hemoglobin concentration. For example, RBC increase in experimental groups was 30% over the values for control groups. Hemoglobin concentrations also increased, but only 15%, while hematocrit values exceeded those of control fish by 17%.

In the hogchoker, a five day exposure to 1.24 g $l^{-1}$ fuller's earth increased red cell counts from 1.58 to 2.08 x $10^{6}$ cells $mm^{-3}$; hematocrit increased from 15.62% to 19.93% (Table 7). Unlike the white perch, where

Table 6. Hematocrit, hemoglobin, erythrocyte count and osmolality of white perch exposed for 5 days to 0.65 g $l^{-1}$ fuller's earth suspension, and for control fish. Mean values are expressed ± standard deviation. Number of individuals is in parentheses.

| | Hematocrit (% packed cell volume) | Hemoglobin (g 100 $g^{-1}$) | Erythrocyte count ($10^6$ cells $mm^{-3}$) | Osmolality (mOsm $kg^{-1}$) |
|---|---|---|---|---|
| Experimental | 36.17 | 8.40 | 2.53 | 281.61 |
| | ±4.69 | ±0.95 | ±0.25 | ±8.87 |
| | (12) | (12) | (12) | (10) |
| Control | 30.73 | 7.30 | 1.96 | 274.01 |
| | ±2.94 | ±0.96 | ±0.23 | ±10.31 |
| | (10) | (12) | (10) | (10) |
| | t = 3.19 | t = 2.830 | t = 5.22 | t = 1.76 |
| | p < 0.01 | p < 0.05 | p < 0.01 | p > 0.05 |

Table 7. Control and experimental values of hematocrit and red blood cell counts from hogchokers exposed to 1.24 g $l^{-1}$ fuller's earth for five days. Values are expressed as mean ± s.d.

| | n | Hematocrit (% packed cell volume) | Red blood cells (cells mm X $10^6$) |
|---|---|---|---|
| Experimental | 10 | 19.93 ±4.32 | 2.08** ±0.35 |
| Control | 10 | 15.62 ±4.06 | 1.58 ±0.26 |

*$p < 0.05$ ($t = 2.299$ d.f. = 18).

**$p < 0.01$ ($t = 3.480$ d.f. = 18).

the proportional increase in red cells was much greater than the increase in hemoglobin and hematocrit, the hematocrit increase observed in the hogchoker was proportionately the same as the increase in red cell count (27.6% and 30.4%, respectively).

Striped killifish were exposed to 0.96 g $l^{-1}$ fuller's earth for five days (Table 8). In this species as in the white perch and the hogchoker, exposure to suspended solids resulted in an increased hematocrit value, from 24.99% to 32.29% ($p < 0.01$), a relative increase of 29.7% for the experimental group over controls.

In the three species considered thus far, we have demonstrated significant hematological changes accompanying exposure to sublethal concentrations of fuller's earth. Although similarity in response to sublethal concentrations of the suspended solid has been shown for these species, they differ markedly in their response to lethal concentrations of the same material. As noted in Chapter 1, the hogchoker and also the striped killifish were extremely difficult to kill. We have been unable to generate an LC response curve for the hogchoker. This may be due to the very high tolerance of this species for suspended solids. The killifish showed the highest $LC_{50}$ value of the six species tested (24-hour $LC_{50}$ = 38.19 approximately the same as mummichog value of 39.00 g $l^{-1}$ fuller's earth). White perch, on the other hand, have been classified as a sensitive species, with 24-hour $LC_{50}$ values below 10 g $l^{-1}$ (see Chapter 1). It would appear, then, that sublethal effects, such as hematological alteration may be induced by low concentrations of suspended solids even though the species exposed to the suspension is

Table 8. Control and experimental hematocrit values from striped killifish exposed for five days to a suspension of 0.96 g $l^{-1}$ fuller's earth. Results expressed as mean ± s.d.

| | N | Hematocrit (% packed cell volume) |
|---|---|---|
| Experimental | 9 | 32.29* ±4.29 |
| Control | 9 | 24.99 ±2.55 |

*$p < 0.01$.

relatively tolerant of this material. As further evidence supporting this point, note that the highly suspension-tolerant hogchoker showed a significant increase in energy utilization during a five-day exposure to 1.24 g $l^{-1}$ fuller's earth (Chapter 5).

Sublethal hematological effects of fuller's earth suspensions (1.6 g $l^{-1}$) were determined for the common mummichog (Table 9) at 4, 7, and 12 day intervals. Mean hematocrit values of experimental fish were significantly different from control fish at 4, 7, and 12 days. There was an increase in the mean value of the experimental group at 12 days.

Spot were studied after a five day exposure to 1.27 g $l^{-1}$, a concentration below the 24-hr $LC_{10}$ value of 13 g $l^{-1}$ (Chapter 1). There were no significant differences between values derived from experimental and control groups (Table 10).

Striped bass data were not directly comparable to data derived from other species because the striped bass were exposed for longer periods, 11 and 14 days (Tables 11 and 12). After 11 days experosure to 0.60 g $l^{-1}$ fuller's earth, no differences were detectable in hematocrit, hemoglobin, RBC or blood osmolality between control and experimental groups. Striped bass exposed to 1.5 g $l^{-1}$ fuller's earth for 14 days showed an increase in hematocrit ($p<0.01$) over control fish. However, these fish also showed a significant increase in blood osmolal concentrations during the same period of time. The increased hematocrit may reflect simple concentration of blood components due to loss of body water (Hall, Gray and Lepkovsky, 1926; Forster and Berglund, 1956).

Table 9. Control and experimental hematocrit values from the mummichog exposed for four, seven, and twelve days to a suspension of 1.62 g $l^{-1}$ fuller's earth. Results expressed as mean ± s.d.

| | 4 Days | 7 Days | 12 Days |
|---|---|---|---|
| Experimental | 33.08* | 29.52** | 34.14*** |
| | ±5.74 | ±4.43 | ±4.28 |
| Control | 24.14 | 23.79 | 26.52 |
| | ±6.54 | ±4.60 | ±2.24 |

*$p < 0.01$ ($t = 3.2488$, d.f. = 18).

**$p < 0.02$ ($t = 2.8373$, d.f. = 18).

***$p < 0.001$ ($t = 4.9884$, d.f. = 16).

Table 10. Control and experimental values of hematocrit, hemoglobin and red blood cell counts for spot exposed to a 1.27 g $l^{-1}$ suspension of fuller's earth for five days. Values expressed as mean ± s.d.

| | Hematocrit (% packed cell volume) | Hemoglobin (g 100 $g^{-1}$ blood) | Red cell count (cells $mm^{-3}$ x $10^6$) |
|---|---|---|---|
| Experimental | 26.19 ±4.87 | 7.14* ±1.37 | 1.54** ±0.48 |
| Control | 28.31 ±7.62 | 6.69 ±2.21 | 1.46 ±0.26 |

*$p > 0.10$

**$p > 0.50$

Toadfish held in 14.6 g $l^{-1}$ of suspended natural sediment for 72 hr exhibited no significant differences in hematological characteristics when compared to a control group (Table 13). Mean hemoglobin concentration for control and experimental fish was 3.67 g 100 $g^{-1}$ and 3.73 g 100 $g^{-1}$, respectively. Mean hematocrit and erythrocyte count for control fish were 20.10% and 17.78 x $10^6$ cells $mm^{-3}$, respectively. Values for experimental fish were 21.67% and 19.90 x $10^6$ cells $mm^{-3}$. Blood osmolal concentration for control fish was 251.69 mOsm $kg^{-1}$ and was 246.63 mOsm $kg^{-1}$ for experimental fish.

Changes in hematological parameters were measured in spot exposed to resuspended natural sediment (14.75 to 16.96 g $l^{-1}$) over seven days at one, three, and seven day intervals. No significant changes in hematology were observed within any sampling interval or between sampling intervals (Table 14).

A time-dependent study was carried out on white perch exposed to resuspended natural muds (2.0 g $l^{-1}$) for four, six and 14 days. Blood parameters (hematocrit, hemoglobin, RBC, and osmolality) remained the same in control and experimental groups through four days of exposure. Mean values for experimental fish were greater than control fish, but the differences were not statistically significant ($0.07<p>0.05$). Blood osmolality did not change ($p>0.5$). After six days hematocrit, hemoglobin, and RBC of experimental fish increased ($0.05<p>0.01$). Continuing the exposure to 14 days resulted in another change: hematological parameters of the two groups were, once again, similar

Table 11. Effects of an 11-day exposure to an 0.6 g $l^{-1}$ suspension of fuller's earth on striped bass hematology. Values are mean ± s.d. Number of individuals in parentheses.

| | Hematocrit (% packed cell volume) | Hemoglobin (g 100 $g^{-1}$) | Erythrocyte count ($10^6$ cells $mm^{-3}$) | Osmolality (mOsm kg-1) |
|---|---|---|---|---|
| Experimental | 38.39 | 7.49 | 2.48 | 334.06 |
| | ±5.61 | ±1.00 | ±0.48 | ±10.88 |
| | (7) | (7) | (7) | (7) |
| Control | 38.20 | 8.04 | 2.35 | 349.4 |
| | ±6.46 | ±0.97 | ±0.32 | ±22.50 |
| | (7) | (7) | (7) | (7) |
| t | 0.057 | 1.058 | 0.605 | 1.624 |
| p | >1.0 | >0.5 | >0.5 | >0.01 |

Table 12. Hematocrit and plasma osmolality of striped bass in control conditions and exposed to 1.5 g $l^{-1}$ fuller's earth for 14 days. Values are mean ± s.d. Numbers of individuals are in parentheses.

| | Hematocrit (% packed cell volume) | Osmolality (mOsm $kg^{-1}$) |
|---|---|---|
| Experimental | 30.28 | 311.22 |
| | ±3.88 | ±10.97 |
| | (10) | (10) |
| Control | 24.17** | 294.36* |
| | ±3.996 | ±22.35 |
| | (10) | (10) |

*$p < 0.05$.

**$p < 0.01$.

Table 13. Microhematocrit, hemoglobin concentration, erythrocyte count, and blood osmolal concentration of toadfish exposed to 14.6 g $l^{-1}$ resuspended Patuxent River Estuary sediment for 72 hr.

| | Microhematocrit (% packed cell volume) $\bar{X} \pm S$ | Hemoglobin (g 100 $g^{-1}$) $\bar{X} \pm S$ | Erythrocyte Count ($10^6$ cells $mm^{-3}$) $\bar{X} \pm S$ | Blood Osmolal Concentration (mOsm $kg^{-1}$) $\bar{X} \pm S$ |
|---|---|---|---|---|
| Experimental | 21.67 ± 8.43 | 3.73 ± 1.24 | 19.90 ± 8.02 | 246.63 ± 13.66 |
| (Number of Individuals) | (18) | (10) | (9) | (9) |
| Control | 20.10 ± 5.81 | 3.67 ± 0.72 | 17.78 ± 8.87 | 251.69 ± 11.52 |
| (Number of Individuals) | (17) | (10) | (10) | (9) |
| | t = 0.476 | t = 0.147 | t = 0.548 | t = 0.849 |
| | d.f. = 16 | d.f. = 18 | d.f. = 17 | d.f. = 16 |
| | N.S. | N.S. | N.S. | N.S. |

Table 14. Hematocrit, hemoglobin, erythrocyte count and osmolality of spot exposed for 1, 3 and 7 day intervals to a range of 14.68 - 16.96 g $l^{-1}$ resuspended natural sediment. Mean values are expressed ± standard deviation. Number of individuals is in parentheses.

| | | Hematocrit (% packed cell volume) | Hemoglobin (g 100 $g^{-1}$) | Erythrocyte count ($10^6$ cells $mm^{-3}$) | Osmolality (mOsm $kg^{-1}$) |
|---|---|---|---|---|---|
| Day 1* | Experimental | 42.10 ±4.16 (10) | 8.46 ±.49 (10) | 2.85 ±.39 (9) | 337.74 ±7.50 (10) |
| | Control | 40.27 ±3.90 (7) | 8.89 ±.91 (9) | 2.94 ±. 0 (7) | 333.02 ±7.60 (10) |
| Day 3** | Experimental | 39.33 ±4.89 (10) | 8.86 ±.82 (9) | 3.12 ±.40 (9) | 326.95 ±7.76 (10) |
| | Control | 42.59 ±5.60 (10) | 9.13 ±1.06 (10) | 3.03 ±.48 (8) | 323.84 ±6.32 (10) |
| Day 7*** | Experimental | 41.58 ±4.86 (10) | 8.00 ±.97 (10) | 2.91 ±.44 (9) | 321.77 ±10.27 (10) |
| | Control | 41.69 ±4.13 (10) | 8.31 ±1.07 (10) | 2.90 ±.31 (9) | 326.79 ±6.58 (10) |
| * | | t = 0.92 p > 0.2 | t = 1.28 p > 0.2 | t = 1.47 p > 0.1 | t = 1.40 p > 0.1 |
| ** | | t = 1.3866 p > 0.1 | t = 0.63 p > 0.5 | t = 0.46 p > 0.5 | t = 1.02 p > 0.2 |
| *** | | t = 0.05 p > 0.9 | t = 0.688 p > 0.5 | t = 0.08 p > 0.9 | t = 1.30 p > 0.2 |

(hematocrit, hemoglobin, RBC, osmolality; $0.5 < p > 0.1$).

Replicate experiments were conducted to assess the sublethal effects of resuspended natural muds on striped bass. These studies were conducted at an arbitrary concentration because $LC_{10}$, $LC_{50}$, and $LC_{90}$ responses for this species have not been consistent. Hematological analyses showed that exposure of striped bass to concentrations of 1.5 to 6.0 g $l^{-1}$ for six days resulted in no detectable differences between control and experimental groups. A comparison of survival tolerance of striped bass to concentrations of 6.0 to 8.0 g $l^{-1}$ natural mud and sublethal effects at 1.5 to 6.0 g $l^{-1}$ suggests that a threshold effect exists between 6.0 and 8.0 g $l^{-1}$. Above 6.0 g $l^{-1}$ (natural muds), bass suffer mortality during six days exposure. Below 6.0 g $l^{-1}$ survival is essentially 100%. No sublethal hematological effects occurred in a period of six days at 2.0 to 6.0 g $l^{-1}$.

The hematological response to sublethal concentrations of suspended solids seem in white perch, hogchokers and striped killifish was similar to hematological responses observed in goldfish and trout exposed to extremely low concentrations of dissolved oxygen for periods of four to 25 days (Phyllips, 1946; Prosser, Barr, Ping and Lauer, 1957; Ostroumova, 1964). That is to say, fishes exposed to sublethal concentrations of suspended solids show the same basic hematological responses as fishes deprived of sufficient oxygen: increased red cell counts, increased hematocrit, and increased concentrations of hemoglobin in the peripheral blood.

In order to justify the contention that exposure of fishes to sublethal concentrations of suspended solids reduced the available oxygen at the gill,

evidence is needed to demonstrate that suspended solids can affect gas transport across the respiratory epithelium, inducing a de facto hypoxia. In Chapter 4 we present histological evidence that the prime site of respiratory gas exchange, the secondary lamella, was damaged by exposure to 0.65 g $l^{-1}$ fuller's earth. Since function is largely dependent upon structure in living systems, we believe that the effect of exposure to sublethal concentrations of fuller's earth can reduce oxygen availability to the fish at the gill surface by disruption of that surface, rendering the tissue partially dysfunctional.

EFFECTS OF SUSPENDED AND DEPOSITED SEDIMENTS

ON ESTUARINE ORGANISMS PHASE II

CHAPTER 4

# THE EFFECTS OF SUBLETHAL CONCENTRATIONS OF FULLER'S EARTH ON WHITE PERCH GILL TISSUE

## I. INTRODUCTION

In most fishes the gill is the primary site for respiratory gas exchange. The main functions of the gill are the transfer of oxygen from the surrounding medium to the blood and the transfer of carbon dioxide and other excretory products, such as ammonia, from the blood to the water. The structure of fish gill tissue is such that only two layers of cells separate the internal fluids of the organism from the environment. This condition exists out of necessity for maintaining the least possible barrier to the transfer of life-giving oxygen. However, the same condition makes the gill the most vulnerable area of the fish's body to the lethal and sublethal effects of toxic materials and physically abrasive materials.

In this section we present the results of an histological study of gill tissue in white perch exposed for periods of five days to suspensions of fuller's earth. This portion of our project was designed to determine whether suspended mineral solids have any damaging effects on the respiratory surface of this species.

## II. METHODS

White perch were exposed for five days to concentrations of 0.65 g $l^{-1}$

fuller's earth. At the end of the exposure period the fish were removed from the experimental and control tanks and immobilized by a sharp blow to the head. 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 serial sections were cut (6 μm thick). The plane of sectioning was dorso-ventral, proceeding serially from the distal to the proximal end of the gill filaments. This technique made visible the mucus goblet cells located on the margins of the gill filaments. Individual secondary lamellae stood out clearly. Slides containing six to eight serial sections were stained alternately with either iron-hematoxylin, or by Gomori's trichrome technique.

## III. RESULTS AND INTERPRETATION

Gill sections from control fish showed typical gill structure for teleost fishes (Figs. 9 and 10). Control fish had moderate concentrations of mucus goblet cells, particularly on the anterior margin of each gill filament (Fig. 10). Examination of serial sections showed concentrations of one to several mucus cells in each section. This concentration varied little over the length of a given filament. Individual mucus cells appeared to be less than 6 μm in diameter; rarely did a single cell appear in more than one section.

More mucus goblet cells appeared on the gills of white perch which had been exposed to the fuller's earth concentration (Fig. 11). In some cases mucus cells were the only visible cellular component of the tissue at the anterior margin of the filaments. The proliferation of mucus

Fig. 9. Section of gill from white perch held five days in clean water. Secondary lamellar structure is undisturbed, and epithelium is applied tightly to the pilar cell structure (EP = epithelium, PC = pilar cells, CART = cartilage of gill ray, ER = red blood cells). Photo taken at 250X, Bouin's, Iron hematoxylin-triosin.

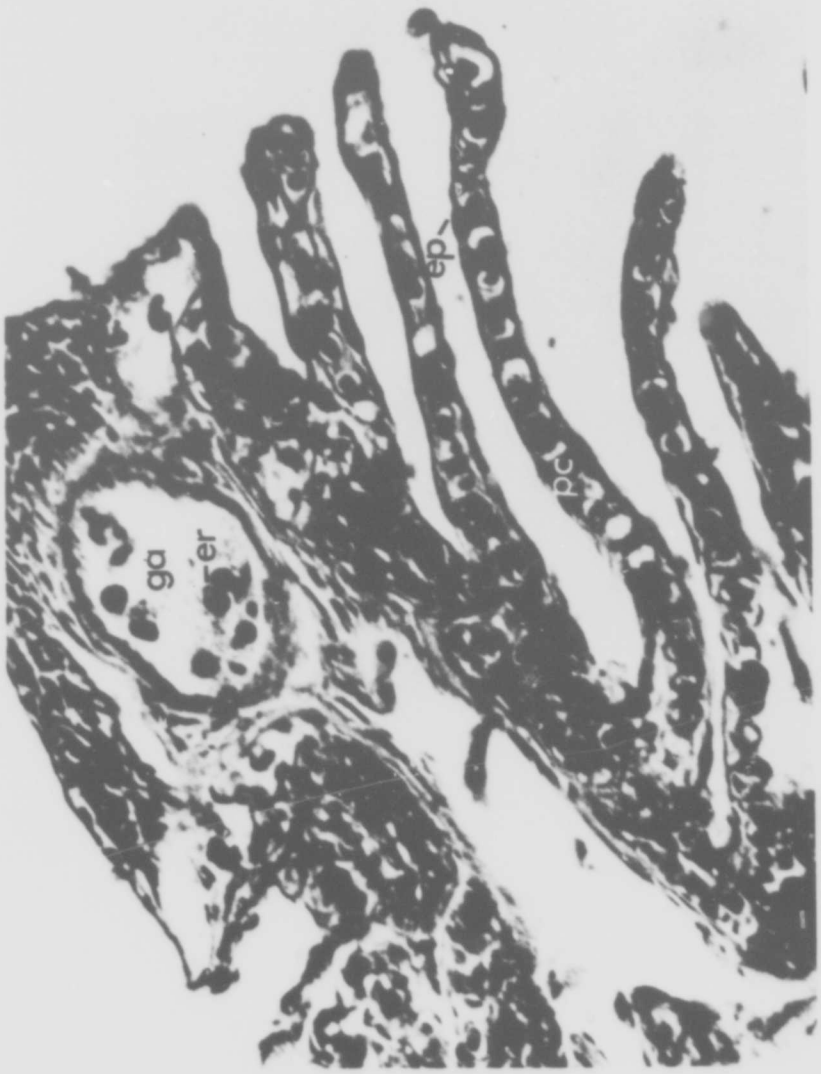

ga
er
ep
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Fig. 10. Section of gill from white perch held five days in clean water. Section includes the anterior margin of the filament; note mucus goblet cells (MG = mucus goblet cells, GA = gill artery, CART = cartilage of gill ray). Photo taken at 400X, Bouin's, Iron hematoxylin-triosin.

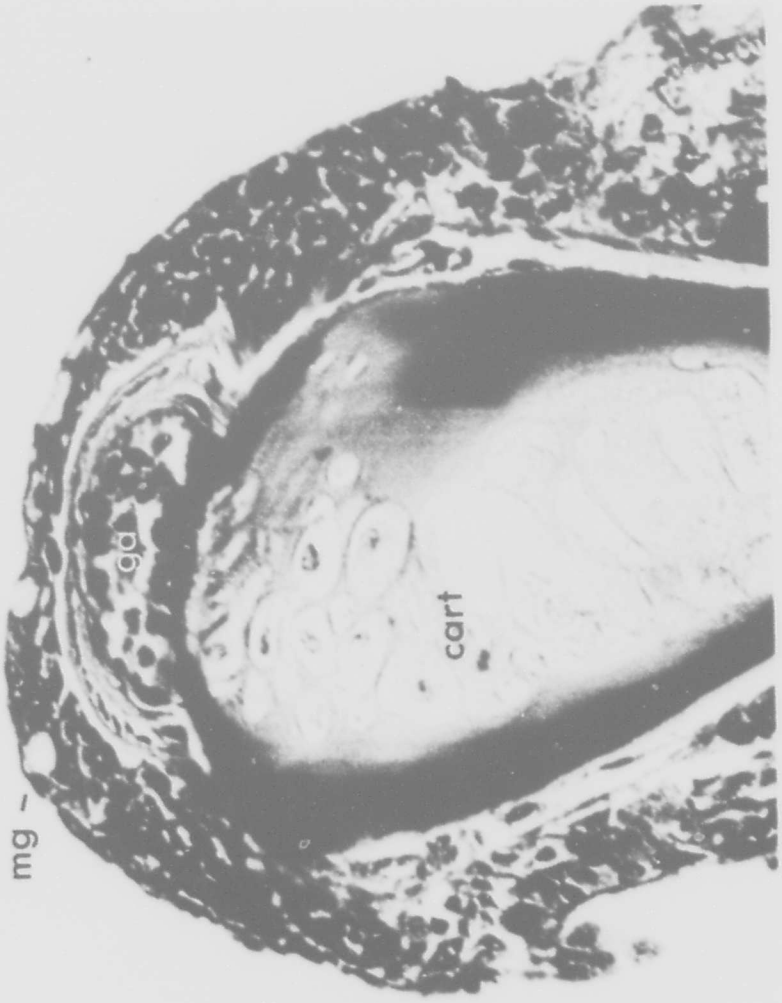

mg
ga
cart

Fig. 11. Gill section from white perch exposed to 650 mg $l^{-1}$ fuller's earth for five days showing 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). Photo taken at 160X, Bouin's, Gomori's trichrome.


mg
ga
cart
sl

goblet cells was apparently confined to the margins of the filaments, particularly the anterior margin which is the first to come in contact with the stream of water which irrigates the gills. Little, if any, increase in concentration of mucus cells was observed elsewhere in the gill. Examination of serial sections did not reveal any increase in the size of the mucus cells in the fishes exposed to fuller's earth; the heavy concentrations of overlapping cells made identification of individual cells in serial sections difficult.

In section the secondary lamella of white perch consisted of a supportive tube of pilar cells, with a single, thin layer of epithelium covering the lamella (Fig. 12). Red blood cells were present inside the tube of pilar cells; no endothelium was seen. The integrated structure of the secondary lamella provided for maximum efficiency of respiratory gas exchange in that the total distance from the hemoglobin-rich red cells to the oxygen-rich water was maintained at a minimum.

Secondary lamellae from perch exposed to 0.65 g $l^{-1}$ fuller's earth showed pronounced differences in structure (Figs. 13 and 14). Several abnormalities of lamellar structure were observed. Typically, the secondary lamellae showed a swollen condition when compared to the lamellae of control fish (cf. Fig. 9 and Fig. 13). The epithelium had become separated from the pilar cell tube which, upon closer examination could be seen to be intact in most instances (Fig. 13). In some cases the pilar cell structure had been disrupted (Fig. 14). Epithelial cells in fishes exposed to the fuller's earth suspension were enlarged, and formed a thicker covering than in control fish (cf. Figs. 12 and 14).

Fig. 12. Typical secondary lamellar structure in gill section from white perch held five days in clean water (ER = red blood cells, PC = pilar cells, CAP = capillary connection to artery, EP = epithelium). Photo taken at 400X, Bouin's, Gomori's trichrome.


ep
er
cap

Fig. 13. Gill from white perch exposed to 650 mg $l^{-1}$ fuller's earth for five days. 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 400X, Bouin's, Gomori's trichrome.

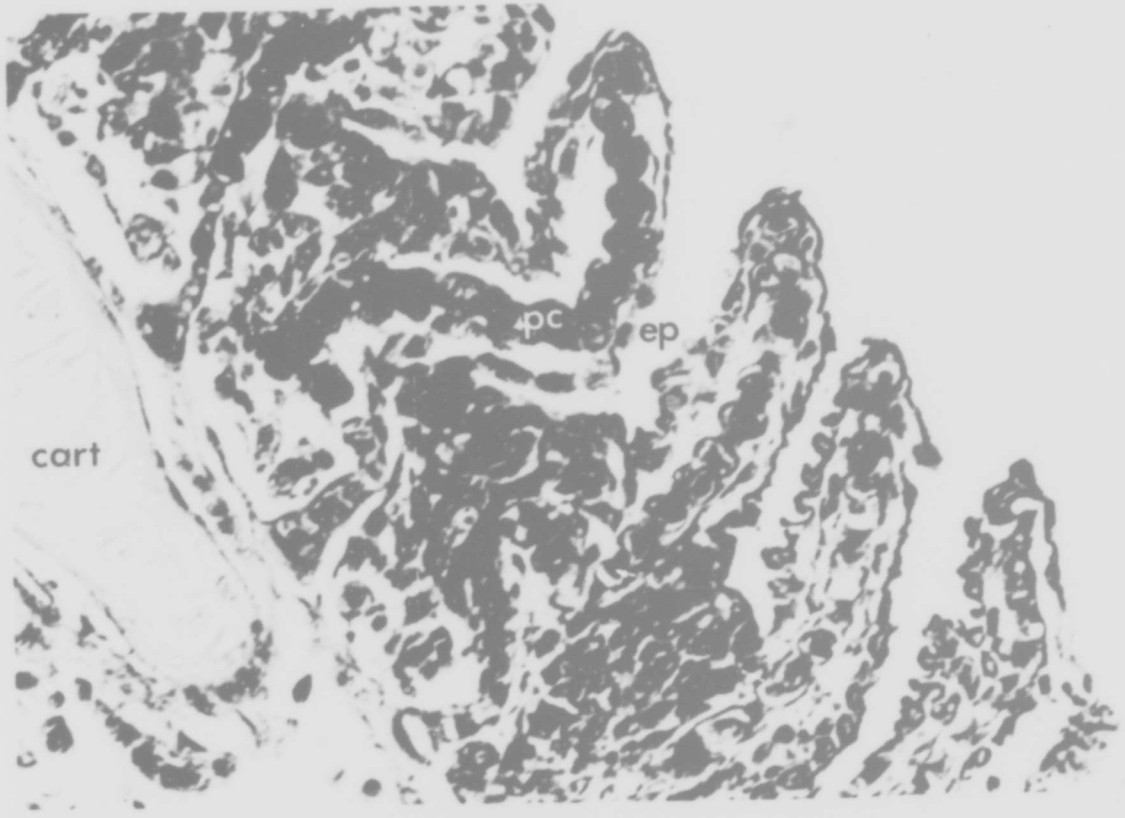
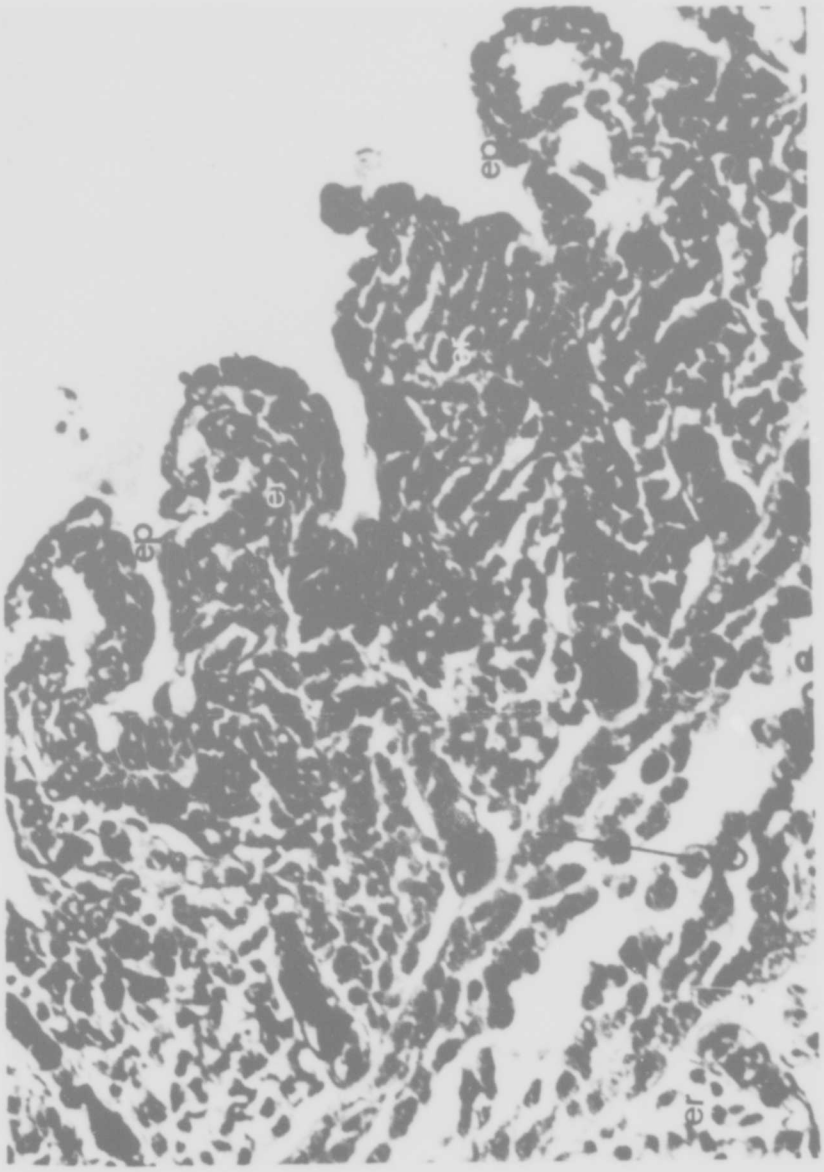

pc
ep
cart

Fig. 14. Secondary lamellae from white perch exposed to 650 mg $l^{-1}$ fuller's earth for five days. Epithelial cells are enlarged, both on lamellae 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 400X, Bouin's, Gomori's trichrome.


ep
er
ep
er

Exposure to suspensions of fuller's earth caused an increase in concentration of mucus goblet cells on the margins of the gill filaments. This result had not been reported previously. Previous reports (review in EIFAC, 1964) made little mention of mucus cell proliferation in response to either lethal or sublethal concentrations of suspended solids. In the present study, sections cut in the dorso-ventral plane made the mucus goblet cells on the margins of the gills highly visible. In sections cut parallel to the long axis of the gill filament, it may be possible to overlook the mucus goblet cells. Although sectioning in the plane parallel to the filament enables one to examine all the secondary lamellae on a gill filament simultaneously, the major sites of mucus cell concentration would appear in only one or two sections in a series across a filament. Since these sections would contain no secondary lamellae, they might be discarded.

The effects of suspensions of fuller's earth 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 the gills of brown trout at high concentrations (Salanina, cited in EIFAC, 1964) and low concentrations (Herbert, Alabaster, Dart and Lloyd, 1961; Herbert and Merkens, 1961).

In the present study and in those cited above, the gills of fishes 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 lamella. These effects were induced using roughly similar concentrations, between 400 mg $l^{-1}$ and 810 mg $l^{-1}$

of suspended solids with a high percentage of particles in the silt-clay range. The effects of different particle sizes on gill tissue has not been evaluated. However, based on the available data, a definite concentration effect associated with the size range of silt-clay sized particles is apparent. In the white perch, concentrations of fuller's earth well below the $LC_{10}$ value (24-hour exposure) may adversely effect the structure of the gill tissue in a period of five days.

Gill damage caused by suspended solids has not been positively identified as being harmful to fishes in terms of overall survival rates. Ritchie (1970) has pointed out that the type of gill damage which may be caused by particles in suspension effectively reduces the respiratory surface area. He stated that this reduced gill surface may be debilitating to fishes, although no data were given to support this statement. Many species of freshwater fishes have been shown to survive for several weeks in highly turbid conditions (EIFAC, 1964) which indicates that compensatory reactions may occur which enable fishes to survive despite the damage to the gill. Randall (1970) has pointed out that shunt mechanisms are commonly employed by fishes such that, under normal conditions, not all the gill surface is utilized for respiration. A certain portion of the gill surface may be envisioned as being held in reserve. By employing the "reserve" surface area a fish may possess sufficient functional, albeit damaged, gas exchange surface to survive prolonged exposure to suspended solids. Also, the functional decrease in gill surface area caused by suspended solids may be offset by compensatory increases in the gas exchange capacity of the blood (Chapter 3).

EFFECTS OF SUSPENDED AND DEPOSITED SEDIMENTS

ON ESTUARINE ORGANISMS PHASE II

CHAPTER 5

# EFFECTS OF SUBLETHAL CONCENTRATIONS OF FULLER'S EARTH ON CARBOHYDRATE METABOLISM IN THE HOGCHOKER, AS MEASURED BY DEPLETION OF LIVER GLYCOGEN STORES

## I. INTRODUCTION

Fish livers contain considerable quantities of carbohydrate stored as animal starch, or glycogen. During periods of starvation or stress, increased metabolic demands for energy are met by mobilization of glycogen reserves; liver glycogen is broken down into glucose, and released to the blood.

In this section we present the results of experiments to determine glycogen utlization in an estuarine fish, the hogchoker, during exposure to sublethal concentrations of fuller's earth.

## II. METHODS

The glycogen content of liver samples from hogchokers was determined after the fish had been held for five days in either control conditions or in a suspension of 1.24 g $l^{-1}$ fuller's earth. Glycogen was extracted from the liver tissue by boiling in 30% KOH, followed by precipitation with 95% ethanol (Good, Kramer and Somogyi, 1933). Quantitative estimates of glycogen concentration were derived colorimetrically using the phenol-

sulfuric acid technique (Montgomery, 1957). Liver glycogen concentrations were expressed as milligrams glycogen per 100 milligrams of liver tissue (mg 100 $mg^{-1}$). The results were analyzed statistically using Student's test of "t" (Snedecor and Cochran, 1967).

## III. RESULTS AND INTERPRETATION

Liver glycogen content from freshly caught hogchokers was determined to be approximately 15 to 17 mg 100 $mg^{-1}$ (Sherk, O'Connor and Neumann, 1972). After five days in control conditions, the mean glycogen content of hogchoker livers had decreased to 15.17 $\pm$ 3.6 mg 100 $mg^{-1}$ (Table 15). However, in fishes held in a suspension of 1.24 mg $l^{-1}$ fuller's earth, liver glycogen content was 10.8 $\pm$ 3.2 mg 100 $mg^{-1}$, significantly less than the value determined for control fish ($p < 0.01$, Table 15).

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

Rates of glycogen mobilization in fishes may be used as an estimate of the rate of energy utilization during starvation (Prosser and Brown, 1961; Kamra, 1965; Beamish, 1968; Swallow and Fleming, 1971). Thus, one possible interpretation of the more rapid utilization of glycogen in hogchokers exposed to suspended sediments is that the sediment stress resulted in an increased energy requirement.

Several observations support this hypothesis. Hogchokers have been shown to possess a daily rhythm of activity which persists under laboratory

Table 15. Liver glycogen concentrations in hogchokers held for 5 days in clean water (control) and in water containing 1.24 g $l^{-1}$ fuller's earth.

| | number | Glycogen (mg 100 $mg^{-1}$) ± s.d. |
|---|---|---|
| Control | 10 | 15.17 ±3.6 |
| Experimental | 10 | 10.77* ±3.2 |

*t = 2.889 (d.f. = 18) $p < 0.01$

conditions (O'Connor, 1972). It was apparent, however, that hogchokers exposed to fuller's earth did not restrict their activity to specific portions of the daily cycle, whereas the fish in the control tanks did. An increase in locomotor activity thus may account for the increased energy utilization during exposure to suspensions of fuller's earth.

Another possible interpretation is that the fish exposed to the suspension of fuller's earth used more energy reserve in compensatory hematological responses. As we have shown in Chapter 3, hogchokers exposed to suspended solids showed evidence of significant alterations in basic hematological parameters indicative of an increase in oxygen exchange capacity of the blood. Compensatory alterations of physiology characteristically demand energy in excess of the normal maintenance requirements of fish. Additional energy demanded for compensatory response to the effects of suspended solids could only have come from existing internal energy stores, since the organisms used in the experiments were starved during the exposure period.

EFFECTS OF SUSPENDED AND DEPOSITED SEDIMENTS

ON ESTUARINE ORGANISMS, PHASE II

CHAPTER 6

# THE EFFECTS OF SUSPENDED SOLIDS ON RESPIRATION OF ESTUARINE FISHES

## I. INTRODUCTION

The gills of fish are in constant contact with water. Flow of water across the gill surface aids gas exchange which supplies the oxygen necessary to support tissue metabolism. Because of the contact of the gill with water, any dissolved or suspended materials affecting water quality also have direct contact with the respiratory surfaces. The action of these materials on gills may be sufficient to affect fish respiration.

This portion of the project has been directed toward assessing possible effects of suspended solids on fish oxygen consumption. Suspensions of fuller's earth were used to treat 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 toad fish in filtered Patuxent River water (baseline) and during exposure to suspensions of fuller's earth or Patuxent River (natural) sediment in river water.

Several methods are available to measure oxygen consumption of fishes (Fry, 1971). Brett (1962) described three levels of fish respiration in terms of activity as follows:

1. Oxygen required to support tissue metabolishm of inactive fishes is standard oxygen consumption.
2. Oxygen consumed during periods of random activity is routine oxygen consumption.
3. Oxygen consumed by fishes swimming at moderate to maximum speeds is active oxygen consumption.

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

## II. MATERIALS AND METHODS

### 1. Equipment

A tunnel-type respirometer (Brett, 1964) was most suited to the needs of this project. This type of respirometer could maintain suspensions of fine particles and was able to provide a variety of flow rates which controlled activity levels (swimming sppeds) of fishes.

A prototype respirometer (72 liter capacity) similar in design to that described by Farmer and Beamish (1969), was constructed (see 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 or removal

of fishes from the respirometer. Rubber gaskets and hose clamps sealed the access port during experiments. Straightening vanes upstream from the chamber provided laminar flow. The centrifugal pump was driven by a variable speed electric motor. An orifice plate in the upper part of the loop was used to measure 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 in the apparatus. These access points were used extensively during experiments to bleed off air trapped in the respirometer and to sample suspensions. Copper coils (20 m each) at two points on the respirometer loop controlled temperature via counter current heat exchange with water pumped through them from a constant temperature bath.

Four slightly smaller (62 liter capacity), inverted versions of the prototype respirometer were placed into operation during this project year. Flow rates were measured by Annubar flow sensors. A water jacket around the outside of the lower portion 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 aid in maintaining constant temperatures. Plywood enclosures were placed around each chamber during experiments to isolate fishes from outside activity in the laboratory. Each enclosure contained a 15 w cool white fluorescent lamp (42 cm above the chamber) which provided constant illumination during experiments. A viewing slit in each enclosure permitted observation of the fishes. Surfaces immediately below each chamber were blackened. Changes in dissolved

oxygen concentrations were monitored by Y.S.I. Model 54 RC oxygen meters. Oxygen electrode leads were passed through neoprene stoppers which sealed the fill pipes into which the electrodes were inserted.

2. Fishes

All fishes were collected by otter trawl from the Patuxent River Estuary, Maryland. Depending upon species and time of year, actual collection sites ranged from the Lower Marlboro area to the vicinity of Drum Point. Fishes were held aboard the collecting vessel in plastic trash cans (80 liter capacity) supplied with a constant flow of ambient river water. Constant flow was maintained until the vessel returned to the laboratory.

Holding facilities at the laboratory consisted of polyethylene tanks (208 liter capacity) immersed in controlled temperature water baths. Water in the tanks was continually passed through an in-line protein-skimmer filtration system. Patuxent River water, passed through a nylon filter (5 $\mu$m mesh), 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 ppt. Holding tank and experimental temperatures were adjusted to approximate seasonal changes.

Fishes returned to the laboratory were placed in the holding tanks. Care was taken not to overcrowd the facilities, and unhealthy or dead fishes were removed immediately. If large numbers of fishes were being held, supplemental aeration was provided. Fishes were held under continuous fluorescent illumination. They were not fed following capture

because active digestion due to recent feeding can increase standard and routine oxygen consumption (Beamish, 1964; Glass, 1968). Fishes were held a minimum of three to five days before oxygen consumption rates were determined.

3. Measurement of Oxygen Consumption

Respirometers were filled with water from the holding tanks (Fry, 1971) during experiments. Each fish was transferred from the holding tanks to the respirometers in a bucket of water as soon as the water level in the apparatus was sufficient to completely cover the fish. When the respirometers were full, the water was circulated at 0.28 to 0.39 ft $sec^{-1}$ to force out entrained air which was replaced simultaneously by holding tank water. In addition, flow rate was gradually increased by 0.18 ft $sec^{-1}$ at four or five minute intervals to drive out the trapped air. Maximum flow attained during this procedure was 2.5 to 4 times the minimum depending on species. Then, flow was reduced to the minimum rate to which the fish would be exposed during the experiment, 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 each was set up and at hourly intervals thereafter. Preliminary studies of all species used demonstrated that at a constant flow of 0.28 ft $sec^{-1}$ 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 more than one swimming speed. The above procedure was used until the third hour data had been recorded. Flow rates were then increased at a rate of about 0.18 ft $sec^{-1}$ at five minute intervals until the desired higher speed was achieved. The same parameters were recorded five minutes after this speed was attained and again one hour later. This procedure was repeated if information was required at even higher levels of activity. All parameters were monitored for one hour at each of the increased flow rates.

At the end of each experiment the respirometers were drained and the fishes removed for weighing, length measurement, and sex determination. Respirometers were filled with tap water which was circulated through the apparatus and drained, then refilled with tap water until used again. Terramycin (oxytetracycline hydrochloride, 15 mg activity per liter) was added to the water when the respirometers were not expected to be used for relatively long periods of time.

Procedures for measuring oxygen consumption of fishes during exposure to suspended solids were as described above. Separate slurries were prepared 18 hours before use in each respirometer. Predetermined volumes of solids were added to about 16 liters of water in plastic trash cans (80 liter capacity). A submersible, electric pump was placed in each container to mix the material and constant aeration was provided. Slurries were pumped into the respirometers as the units were being filled with holding tank water. At the end of experiments with suspended solids, respirometers were washed several times with tap water to prevent accumulation of the materials in the units.

Concentrations of suspended materials were determined by the dry weight difference between three 5 ml replicate samples drawn from each respirometer at the beginning of an experiment and three similar samples drawn from the fish holding tank (no added suspended material) at the same time.

Oxygen demand of natural sediment suspensions was determined by measuring oxygen uptake of slurries. Slurries were pumped into the respirometers as described above. Respirometers were set up as usual, but without placing fish into the chamber. Mean third hour oxygen consumption values of the slurries were used to correct for the oxygen demand of the sediment during experiments with fish.

Long-term exposure of fishes to suspensions of suspended solids was provided by using equipment described in Chapters 1 and 2 for bioassay experiments or as described in Chapter 3 for sublethal hematological studies.

4. 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). Between group comparisons were made by covariance analysis of log-transformed data (Snedecor and Cochran, 1967). Sex influence on respiration rates was tested for by covariance analysis for each species when possible. Male and female data were pooled for all comparisons of baseline and experimental values.

## III. RESULTS

### 1. Striped Bass

Fish were held at about 15°C and 5 ppt salinity for a minimum of three days before respiration rates were determined. Oxygen consumption rates were determined at three swimming speeds: 0.28, 1.02, and 1.58 ft $sec^{-1}$.

In filtered water a 50 g fish consumed 19.0 mg $O_2$ $hr^{-1}$ and a 150 g fish used 31.4 mg $O_2$ $hr^{-1}$ at swimming speeds of 0.28 ft $sec^{-1}$. At a speed of 1.02 ft $sec^{-1}$ 50 and 150 g fish used 24.3 and 41.3 mg $O_2$ $hr^{-1}$, respectively. Oxygen consumption rates increased to 33.7 mg $O_2$ $hr^{-1}$ for a 50 g fish and 63.7 mg $O_2$ $hr^{-1}$ for a 150 g fish swimming at speeds of 1.58 ft $sec^{-1}$. A sigmificant increase in respiration rates was observed betwyen measurements made at 0.28 and 1.02 ft $sec^{-1}$ and between 1.02 and 1.58 ft $sec^{-1}$ (Table 16). Covariance analysis showed that oxygen consumption rates of male and female fish did not differ at either swimming speed (Table 17). This test was not made for rates determined at swimming speeds of 1.58 ft $sec^{-1}$ due to the small number of females tested.

At a swimming speed of 0.28 ft $sec^{-1}$ during exposure to fuller's earth (0.79 g $l^{-1}$), a 50 g fish consumed 19.2 mg $O_2$ $hr^{-1}$ and a 150 g fish consumed 37.8 mg $O_2$ $hr^{-1}$. Swimming at 1.02 ft $sec^{-1}$ under these conditions a 50 g and a 150 g fish consumed 24.1 and 41.2 mg $O_2$ $hr^{-1}$, respectively (Figs. 15 and 16; Table 18). Striped bass swimming at

Table 16. Covariance analysis of oxygen consumption - live weight regressions for striped bass swimming at speeds of 0.28, 1.02, and 1.58 ft $sec^{-1}$ in filtered Patuxent River water (baeline), or in suspensions of 0.70 g $l^{-1}$ fuller's earth at about 15°C.

| Comparison | N | Residual Variance | | Slope | | Elevation | |
|---|---|---|---|---|---|---|---|
| | | M.S. | d.f. | M.S. | d.f. | M.S. | d.f. |
| Baseline | | | | | | | |
| 0.28 ft. $sec.^{-1}$ | 28 | 0.014 | 26, 23 | 0.001 | 1, 50 | 0.165 | 1, 51 |
| 1.02 ft. $sec.^{-1}$ | 25 | 0.013 | n.s. | 0.013 | n.s. | 0.013 | * |
| 1.02 ft. $sec.^{-1}$ | 25 | 0.013 | 23, 9 | 0.011 | 1, 32 | 0.142 | 1, 33 |
| 1.58 ft. $sec.^{-1}$ | 11 | 0.005 | n.s. | 0.011 | n.s. | 0.011 | * |
| Fuller's Earth | | | | | | | |
| 0.28 ft. $sec.^{-1}$ | 27 | 0.010 | 25, 23 | 0.104 | 1, 48 | 0.028 | 1, 49 |
| 1.02 ft. $sec.^{-1}$ | 25 | 0.023 | n.s. | 0.017 | * | 0.018 | n.s. |
| 1.02 ft. $sec.^{-1}$ | 25 | 0.023 | 23, 9 | 0.010 | 1, 32 | 0.021 | 1, 33 |
| 1.58 ft. $sec.^{-1}$ | 11 | 0.009 | n.s. | 0.019 | n.s. | 0.019 | n.s. |
| 0.28 ft. $sec.^{-1}$ | 27 | 0.010 | 25, 9 | 0.001 | 1, 34 | 0.073 | 1, 35 |
| 1.58 ft. $sec.^{-1}$ | 11 | 0.009 | n.s. | 0.010 | n.s. | 0.009 | ** |

* $p < 0.05$, ** $p < 0.01$

Table 17. Covariance analysis of oxygen consumption - live weight regressions of male and female striped bass swimming at 0.28 and 1.02 ft sec-1 under baseline conditions and during exposure to 0.79 g $l^{-1}$ Fuller's earth at about 15 C.

| Comparison | Swimming Speed ft sec-1 | N | Residual Variance M.S. | Residual Variance d.f. | Slope M.S. | Slope d.f. | Elevation M.S. | Elevation d.f. |
|---|---|---|---|---|---|---|---|---|
| Baseline | | | | | | | | |
| Males | 0.28 | 14 | 0.012 | 12, 7 | 0.001 | 1, 19 | 0.025 | 1, 20 |
| Females | | 9 | 0.010 | N.S. | 0.011 | N.S. | 0.011 | N.S. |
| Males | 1.02 | 13 | 0.013 | 11, 5 | 0.001 | 1, 16 | 0.001 | 1, 17 |
| Females | | 7 | 0.003 | N.S. | 0.010 | N.S. | 0.010 | N.S. |
| Fuller's earth | | | | | | | | |
| Males | 0.28 | 14 | 0.006 | 10, 12 | 0.025 | 1, 22 | 0.002 | 1, 23 |
| Females | | 12 | 0.013 | N.S. | 0.010 | N.S. | 0.011 | N.S. |
| Males | 1.02 | 13 | 0.008 | 9, 11 | 0.000 | 1, 20 | 0.002 | 1, 21 |
| Females | | 11 | 0.004 | N.S. | 0.006 | N.S. | 0.005 | N.S. |

Fig. 15. Oxygen consumption of striped bass swimming at 0.28 ft $sec^{-1}$ under control conditions (solid line) and during exposure to 0.79 g $l^{-1}$ fuller's earth suspensions (broken line).

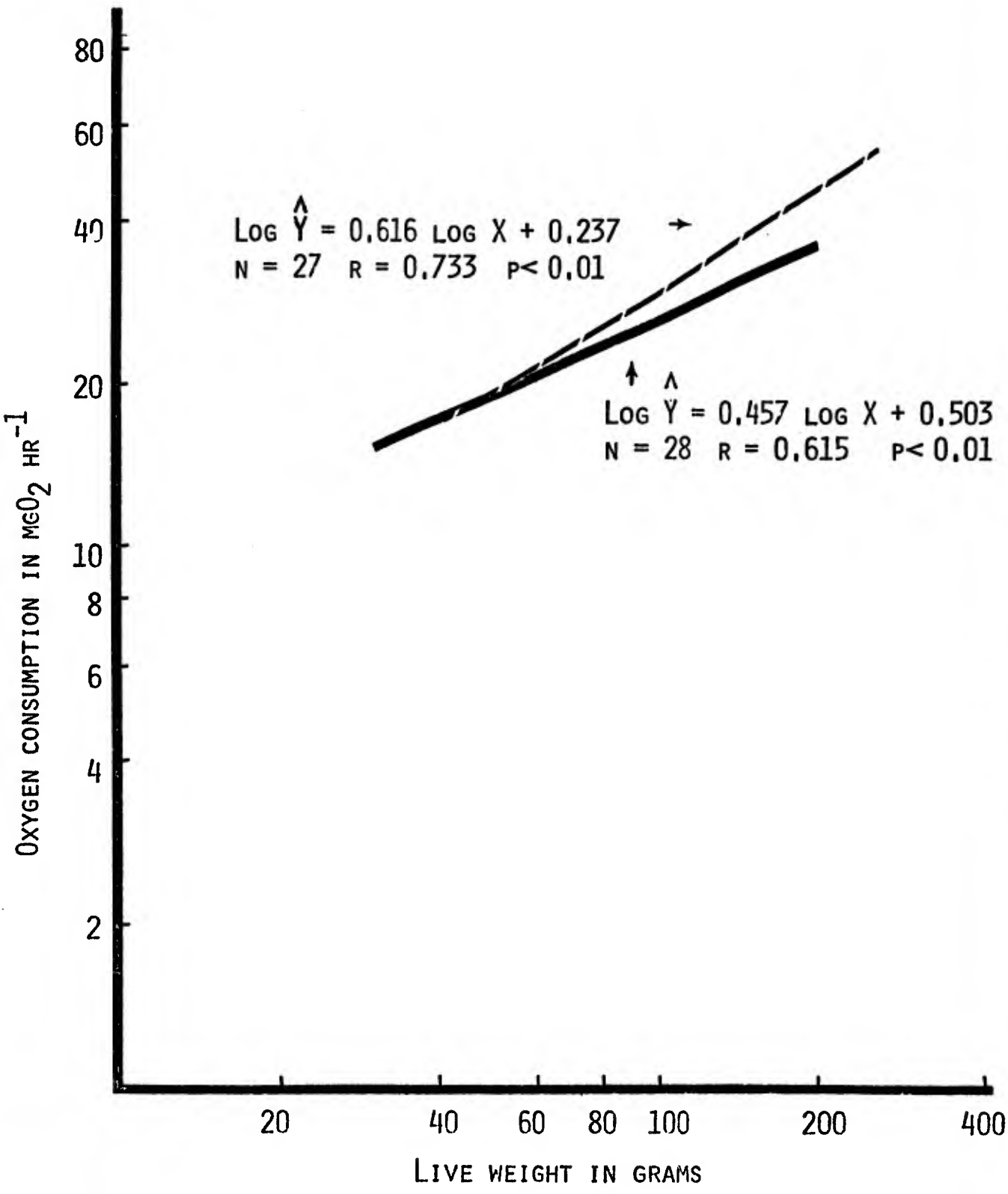

Log $\hat{Y}$ = 0.616 Log X + 0.237
N = 27 R = 0.733 P< 0.01
Log $\hat{Y}$ = 0.457 Log X + 0.503
N = 28 R = 0.615 P< 0.01
Oxygen consumption in $mlO_2$ $hr^{-1}$
80
60
40
20
10
8
6
4
2
20
40
60
80
100
200
400
Live weight in grams

Fig. 16. Oxygen consumption of striped bass swimming at 1.02 ft $sec^{-1}$ under control conditions (solid line) and during exposure to 0.79 g $l^{-1}$ fuller's earth suspensions (broken line).

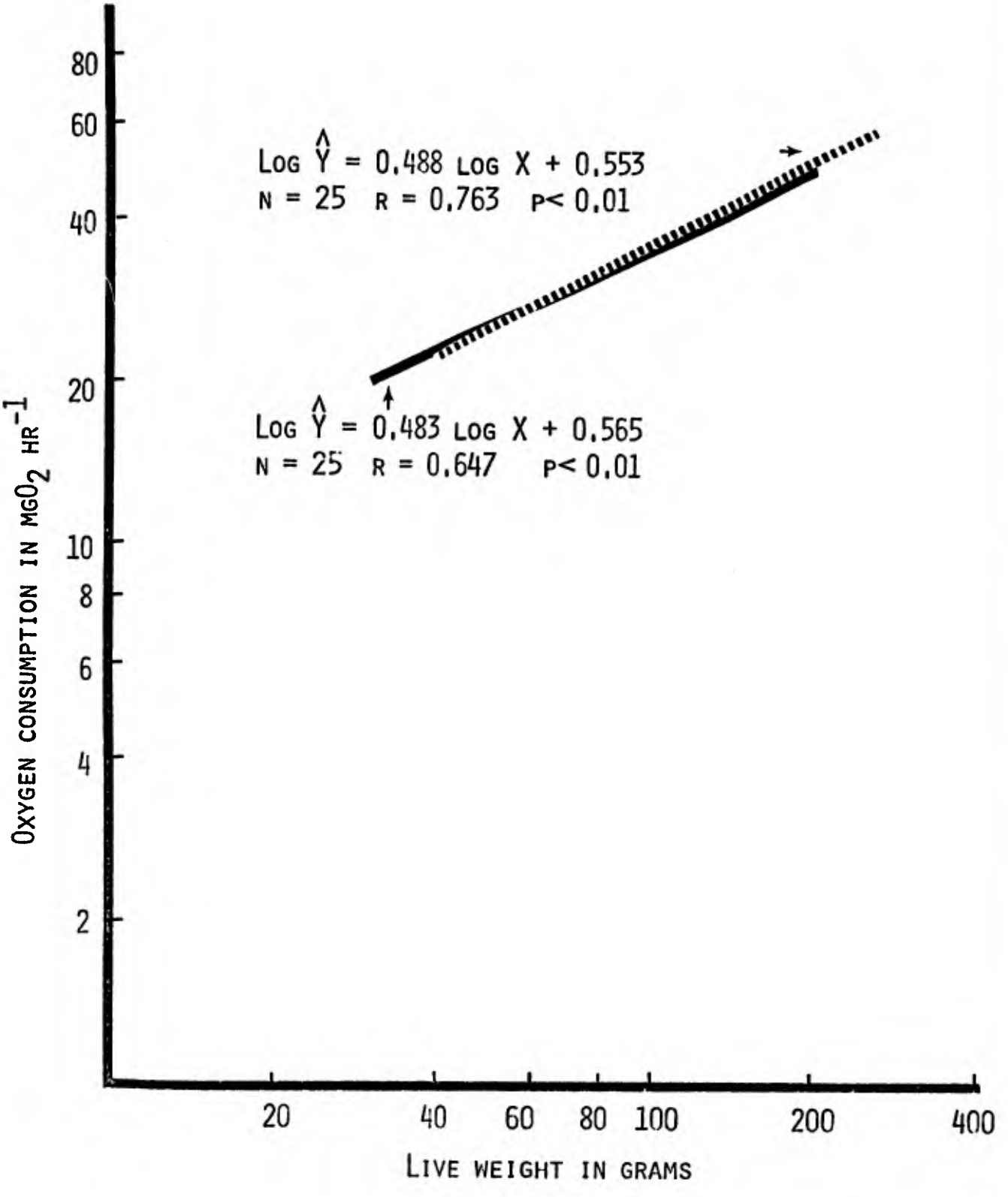

Log Ŷ = 0.488 log X + 0.553
N = 25 R = 0.763 P< 0.01
Log Ŷ = 0.483 log X + 0.565
N = 25 R = 0.647 P< 0.01
Oxygen consumption in mgO2 hr-1
80
60
40
20
10
8
6
4
2
20
40
60
80
100
200
400
Live weight in grams

Table 18. Covariance analysis of oxygen consumption - live weight regressions of striped bass under baseline conditions and in 0.79 g $l^{-1}$ suspensions of Fuller's earth at about 15 C.

| Comparison | Swimming Speed ft $sec^{-1}$ | N | Residual Variance M.S. | Residual Variance d.f. | Slope M.S. | Slope d.f. | Elevation M.S. | Elevation d.f. |
|---|---|---|---|---|---|---|---|---|
| Baseline | 0.28 | 28 | 0.014 | 26, 25 | 0.009 | 1, 51 | 0.020 | 1, 52 |
| Fuller's earth | | 27 | 0.010 | N.S. | 0.012 | N.S. | 0.012 | N.S. |
| Baseline | 1.02 | 25 | 0.013 | 23, 23 | 0.042 | 1, 46 | 0.028 | 1, 47 |
| Fuller's earth | | 25 | 0.023 | N.S. | 0.018 | N.S. | 0.019 | N.S. |
| Baseline | 1.58 | 11 | 0.005 | 9, 9 | 0.001 | 1, 18 | 0.072 | 1, 19 |
| Fuller's earth | | 11 | 0.009 | N.S. | 0.007 | N.S. | 0.007 | * |

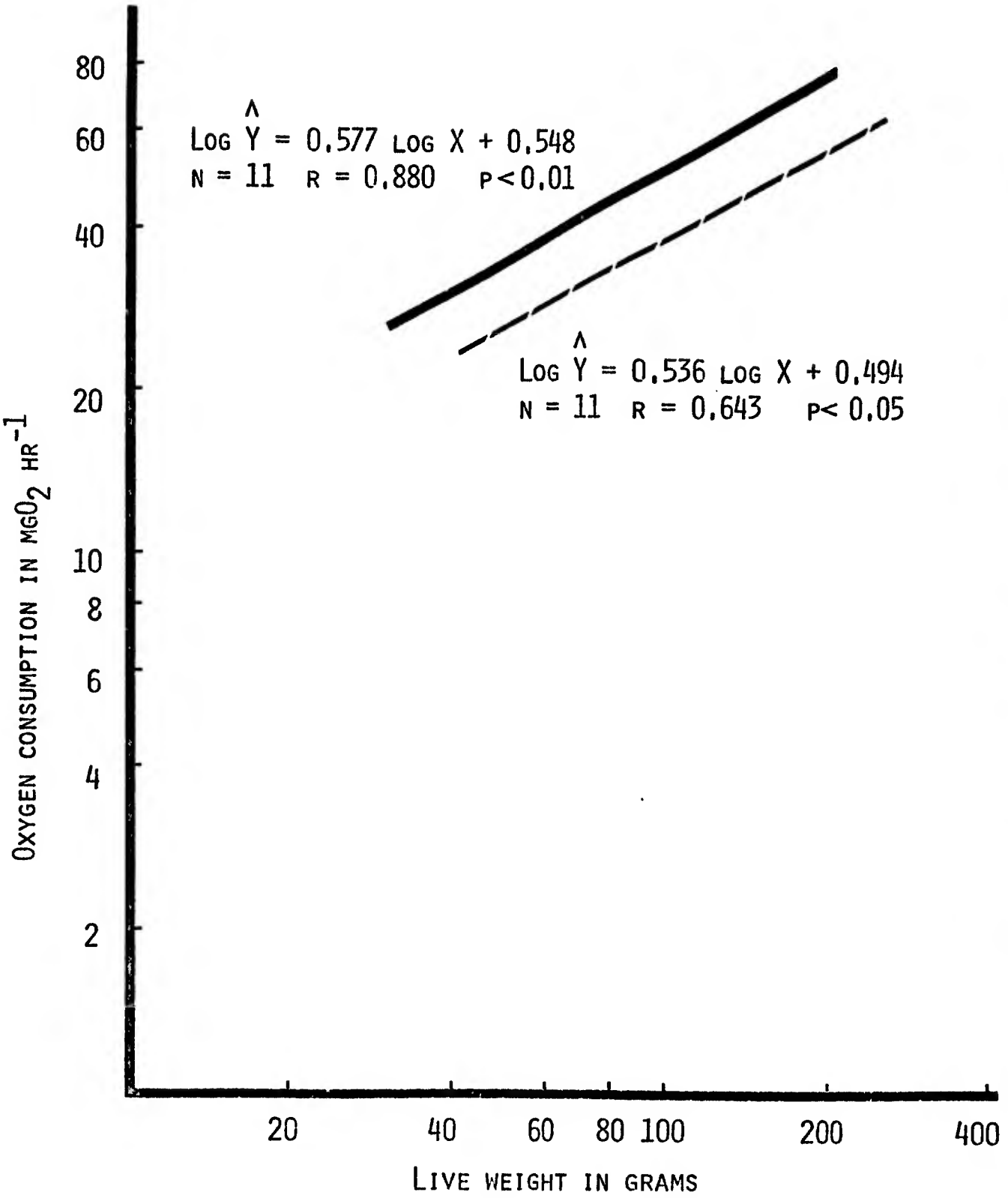
* $p < 0.01$

1.58 ft $sec^{-1}$ in fuller's earth suspensions consumed significantly less oxygen than fish swimming at that speed under baseline conditions (Fig. 17; Table 18). Oxygen consumption was uniformly depressed by about 25% throughout the weight range studied.

Oxygen consumption rates of striped bass swimming at 0.28 and 1.02 ft $sec^{-1}$ during exposure to 0.79 g $l^{-1}$ fuller's earth had different slopes. Respiration rates at swimming speeds of 1.02 and 1.58 ft $sec^{-1}$ during this exposure were not different. Rates for fish swimming at 0.28 and 1.58 ft $sec^{-1}$ were different at the 1% level (Table 16). Oxygen consumption rates of male and female striped bass swimming at 0.28 and 1.02 ft $sec^{-1}$ in fuller's earth suspensions were not different at either speed (Table 17). Sex comparisons of respiration rates of fish swimming at 1.58 ft $sec^{-1}$ could not be made due to the small number of females studied.

Striped bass held at 22.5°C and 9.0 ppt salinity were tested at swimming speeds of 1.05 and 1.58 ft $sec^{-1}$ under baseline conditions and during exposure to natural sediment suspensions. The experimental procedure was modified in consideration of reduced dissolved oxygen concentrations due to the increased temperature and salinity and to the oxygen demand of the sediment. The three hour acclimation period at a swimming speed of 0.28 ft $sec^{-1}$ was changed to three hours at 1.05 ft $sec^{-1}$ with continual aeration. Respirometers were closed at the end of the third hour and oxygen concentrations were monitored for one hour. Flow rates were then gradually increased to 1.58 ft $sec^{-1}$.

Fig. 17. Oxygen consumption of striped bass swimming at 1.58 ft $sec^{-1}$ under control conditions (solid line) and during exposure to 0.79 g $l^{-1}$ fuller's earth suspensions (broken line).


Log Ŷ = 0.577 Log X + 0.548
N = 11 R = 0.880 P<0.01
Log Ŷ = 0.536 Log X + 0.494
N = 11 R = 0.643 P< 0.05
Oxygen consumption in mgO2 hr-1
80
60
40
20
10
8
6
4
2
20
40
60
80 100
200
400
Live weight in grams

Oxygen consumption rates of a 50 g and a 150 g fish swimming at 1.05 ft $sec^{-1}$ under baseline conditions were 23.2 and 55.0 mg $O_2$ $hr^{-1}$, respectively. At a swimming speed of 1.58 ft $sec^{-1}$ a 50 g fish consumed 23.2 mg $O_2$ $hr^{-1}$ and a 150 g fish consumed 48.1 mg $O_2$ $hr^{-1}$. Respiration rates at the two swimming speeds were not significantly different (Table 19). Sex influence on oxygen consumption rates was not apparent at either speed (Table 20).

Respiration rates of striped bass swimming at 1.05 and 1.58 ft $sec^{-1}$ during exposure to 1.31 and 1.33 g $l^{-1}$ natural sediment, respectively, were reduced by 30-40% of the baseline values. At a swimming speed of 1.05 ft $sec^{-1}$ a 50 g fish used 14.2 mg $O_2$ $hr^{-1}$ and a 150 g fish used 34.4 mg $O_2$ $hr^{-1}$ (Fig. 18; Table 21). Similar weight fish swimming at 1.58 ft $sec^{-1}$ consumed 14.1 and 34.9 mg $O_2$ $hr^{-1}$ (Fig. 19; Table 21). Respiration rates at the two swimming speeds were not different (Table 19). Oxygen consumption rates of males and females during exposure to natural sediment were not different (Table 20) at either swimming speed.

2. White Perch

Fish were maintained at about 15°C and 5 ppt salinity for a minimum of three days before oxygen consumption rates were determined at swimming speeds of 0.28 and 1.02 ft $sec^{-1}$ under baseline conditions. At a swimming speed of 0.28 ft $sec^{-1}$ a 50 g fish used 13.3 mg $O_2$ $hr^{-1}$ and a 150 g fish used 27.1 mg $O_2$ $hr^{-1}$. Fish of the same weights swimming at 1.02 ft $sec^{-1}$ consumed 24.4 and 44.6 mg $O_2$ $hr^{-1}$, respectively. Respiration rates were significantly greater at swimming speeds of

Table 19. Covariance analysis of oxygen consumption - live weight regressions of striped bass swimming at 1.05 and 1.58 ft $sec^{-1}$ under baseline conditions and at the same speeds during exposure to natural sediment suspensions of 1.32 g $l^{-1}$ at 22.5 C.

| Comparison | Swimming Speed ft $sec^{-1}$ | N | Residual Variance | | Slope | | Elevation | |
|---|---|---|---|---|---|---|---|---|
| | | | M.S. | d.f. | M.S. | d.f. | M.S. | d.f. |
| Baseline | 1.05 | 21 | 0.0193 | 19, 15 | 0.0099 | 1, 34 | 0.0084 | 1, 35 |
| | 1.58 | 17 | 0.0084 | N.S. | 0.0145 | N.S. | 0.0144 | N.S. |
| Experimental | 1.05 | 22 | 0.0322 | 20, 18 | 0.0003 | 1, 38 | 0.0002 | 1, 39 |
| | 1.58 | 20 | 0.0334 | N.S. | 0.0328 | N.S. | 0.0319 | N.S. |

Table 20. Covariance analysis of oxygen consumption - live weight regressions of male and female striped bass at swimming speeds of 1.05 and 1.58 ft $sec^{-1}$ under baseline conditions and during exposure to 1.32 g $l^{-1}$ natural sediment at 22.5 C.

| Comparison | Swimming Speed ft $sec^{-1}$ | N | Residual Variance M.S. | Residual Variance d.f. | Slope M.S. | Slope d.f. | Elevation M.S. | Elevation d.f. |
|---|---|---|---|---|---|---|---|---|
| Baseline | | | | | | | | |
| Males | 1.05 | 12 | 0.0262 | 10, 7 | 0.0154 | 1, 17 | 0.0045 | 1, 18 |
| Females | | 9 | 0.0120 | N.S. | 0.0204 | N.S. | 0.0201 | N.S. |
| Males | 1.58 | 11 | 0.0092 | 9, 4 | 0.0176 | 1, 13 | 0.0061 | 1, 14 |
| Females | | 6 | 0.0049 | N.S. | 0.0079 | N.S. | 0.0086 | N.S. |
| Experimental | | | | | | | | |
| Males | 1.05 | 9 | 0.0182 | 7, 11 | 0.0599 | 1, 18 | 0.1093 | 1, 19 |
| Females | | 13 | 0.0316 | N.S. | 0.0264 | N.S. | 0.0282 | N.S. |
| Males | 1.58 | 7 | 0.0351 | 5, 11 | 0.0405 | 1, 16 | 0.1316 | 1, 17 |
| Females | | 13 | 0.0231 | N.S. | 0.0268 | N.S. | 0.0276 | N.S. |

Fig. 18. Oxygen consumption of striped bass under baseline conditions (solid line) and during exposure to natural sediment suspensions (1.31 g $l^{-1}$, broken line) at 1.05 ft $sec^{-1}$.

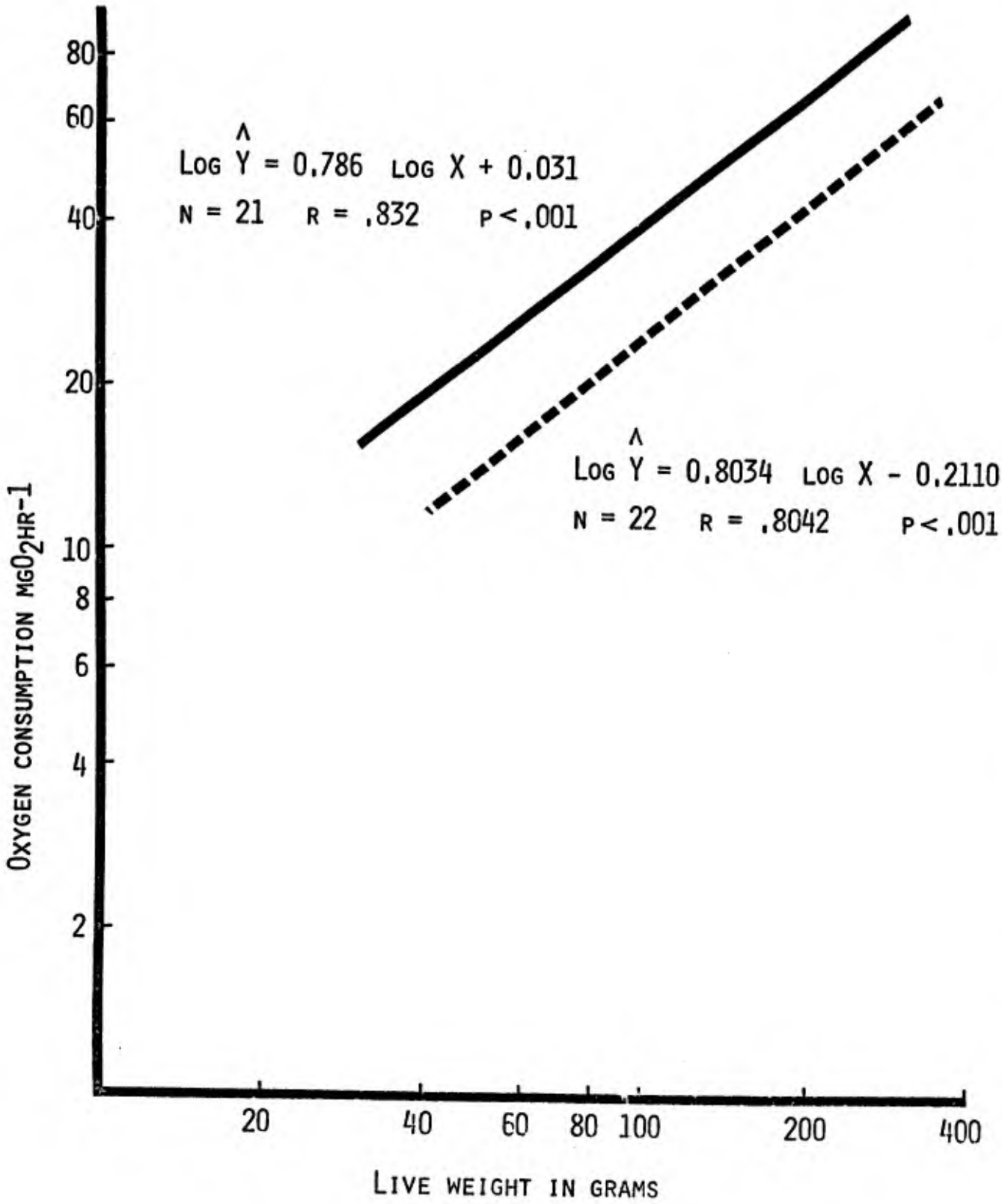

Log Ŷ = 0.786 Log X + 0.031
N = 21 R = .832 P < .001
Log Ŷ = 0.8034 Log X - 0.2110
N = 22 R = .8042 P < .001
Oxygen consumption mg$O_2$hr-1
80
60
40
20
10
8
6
4
2
20
40
60
80
100
200
400
Live weight in grams

Fig. 19. Oxygen consumption of striped bass under baseline conditions (solid line) and during exposure to natural sediment suspensions (1.33 g $l^{-1}$, broken line) at 1.58 ft $sec^{-1}$.

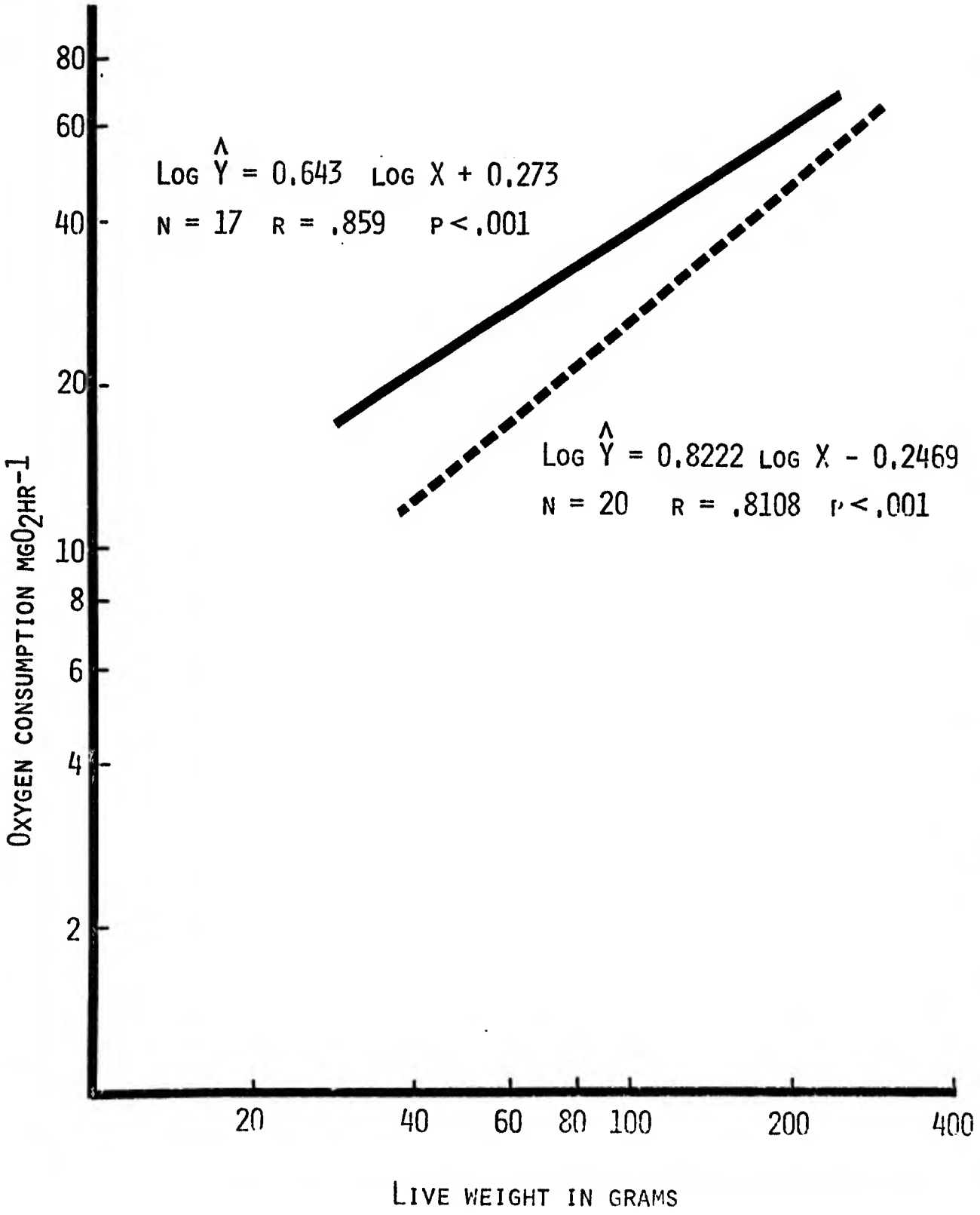

Log $\hat{Y}$ = 0.643 Log X + 0.273
N = 17 R = .859 P < .001
Log $\hat{Y}$ = 0.8222 Log X - 0.2469
N = 20 R = .8108 P < .001
Oxygen consumption $mgO_2HR^{-1}$
80
60
40
20
10
8
6
4
2
20
40
60
80
100
200
400
Live weight in grams

Table 21. Covariance analysis of oxygen consumption - live weight regressions of striped bass swimming at 1.05 ft $sec^{-1}$ under baseline conditions and during exposure to 1.31 g $l^{-1}$ natural sediment, and swimming at 1.58 ft $sec^{-1}$ under baseline conditons and during exposure to 1.33 g $l^{-1}$ natural sediment at 22.5 C.

| Comparison | Swimming Speed ft $sec^{-1}$ | N | Residual Variance M.S. | Residual Variance d.f. | Slope M.S. | Slope d.f. | Elevation M.S. | Elevation d.f. |
|---|---|---|---|---|---|---|---|---|
| Baseline | 1.05 | 21 | 0.0193 | 19, 20 | 0.0005 | 1, 39 | 0.3666 | 1, 40 |
| Experimental | | 22 | 0.0322 | N.S. | 0.0259 | N.S. | 0.0253 | ** |
| Baseline | 1.58 | 17 | 0.0084 | 15, 18 | 0.0184 | 1, 33 | 0.2360 | 1, 34 |
| Experimental | | 20 | 0.0322 | N.S. | 0.0221 | N.S. | 0.0219 | * |

* $p < 0.005$
** $p < 0.001$

1.02 ft $sec^{-1}$ than at 0.28 ft $sec^{-1}$ (Table 22). Respiration rates of males and females were found not to differ (Table 23) at either speed.

Oxygen consumption rates were determined for white perch during exposure to fuller's earth suspensions of 1.09 g $l^{-1}$ at swimming speeds of 0.28 (Fig. 20) and 1.02 (Fig. 21) ft $sec^{-1}$. At both swimming speeds the data were dispersed: $r = 0.017$ (n.s.) at 0.28 ft $sec^{-1}$ and $r = 0.201$ (n.s.) at 1.02 ft $sec^{-1}$. Due to the poor correlation of these data covariance analyses were not attempted since this test assumes a linear relationship of the data.

Similar results were observed when oxygen consumption was determined for white perch swimming at 0.39 and 1.05 ft $sec^{-1}$ during exposure to natural sediment suspensions of 2.12 g $l^{-1}$ (Figs. 22 and 23). Low correlations, $r = 0.143$ (n.s.) at 0.39 ft $sec^{-1}$ and $r = 0.017$ (n.s.) at 1.02 ft $sec^{-1}$, prevented further statistical treatment of these data.

White perch were held in suspensions of 2.58 g $l^{-1}$ natural sediment for 72 hr before measurement of oxygen consumption rates in filtered river water at swimming speeds of 0.39 and 1.05 ft $sec^{-1}$. Data for fish swimming at 0.39 ft $sec^{-1}$ after 72 hr exposure were too scattered, $r = 0.508$ (n.s.), to permit further analysis (Fig. 24). Oxygen consumption rates of a 50 g and a 150 g fish swimming at 1.58 ft $sec^{-1}$ in filtered river water after 72 hr exposure to natural sediment were 10.8 and 55.3 mg $O_2$ $hr^{-1}$, respectively (Fig. 25). Elevations of the lines describing these data and baseline data at 1.58 ft $sec^{-1}$ were different at the 5% level (Table 24).

Table 22. Covariance analysis of oxygen consumption - live weight regressions of white perch swimming at 0.28 and 1.02 ft. sec.$^{-1}$ under baseline conditions.

| Comparison | N | Residual Variance | | Slope | | Elevation | |
|---|---|---|---|---|---|---|---|
| | | M.S. | d.f. | M.S. | d.f. | M.S. | d.f. |
| 0.28 ft. sec.$^{-1}$ | 13 | 0.023 | 11, 9 | 0.002 | 1, 21 | 0.208 | 1, 22 |
| 1.02 ft. sec.$^{-1}$ | 11 | 0.012 | n.s. | 0.018 | n.s. | 0.017 | * |

* $p < 0.05$

Table 23. Covariance analysis of oxygen consumption - live weight regressions of male and female white perch swimming at 0.28 and 1.02 ft $sec^{-1}$ under baseline conditions.

| Comparison | Swimming Speed ft $sec^{-1}$ | N | Residual Variance | | Slope | | Elevation | |
|---|---|---|---|---|---|---|---|---|
| | | | M.S. | d.f. | M.S. | d.f. | M.S. | d.f. |
| Males | 0.28 | 5 | 0.025 | 3, 6 | 0.001 | 1, 9 | 0.078 | 1, 10 |
| Females | | 8 | 0.015 | N.S. | 0.019 | N.S. | 0.018 | N.S. |
| Males | 1.02 | 5 | 0.001 | 3, 4 | 0.011 | 1, 7 | 0.028 | 1, 8 |
| Females | | 6 | 0.016 | N.S. | 0.010 | N.S. | 0.010 | N.S. |

Fig. 20. Oxygen consumption of white perch swimming at 0.28 ft $sec^{-1}$ under control conditions (solid line) and during exposure to 1.09 g $l^{-1}$ fuller's earth suspensions (broken line).

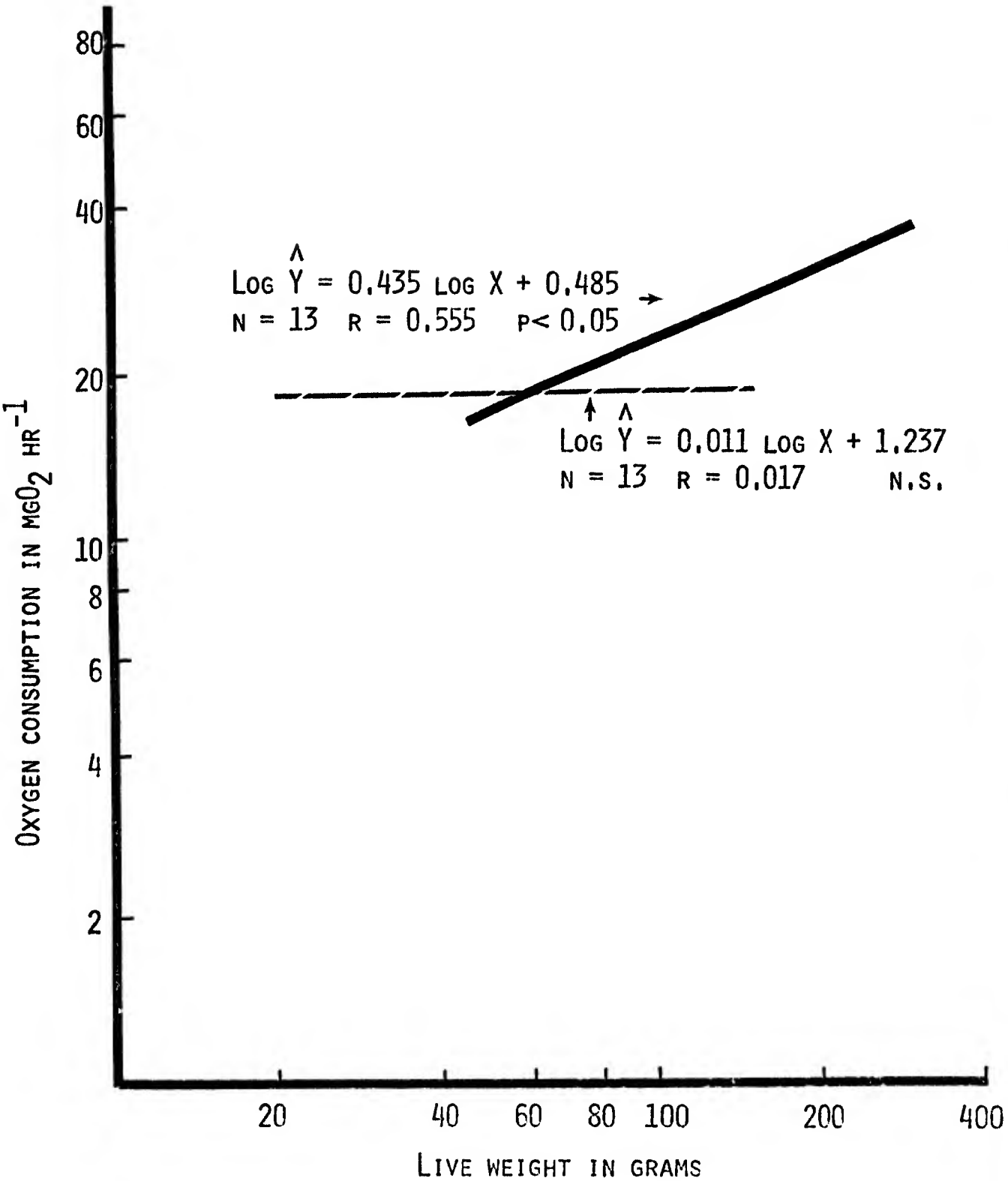

80
60
40
20
10
8
6
4
2
Oxygen consumption in $MgO_2$ $HR^{-1}$
$\hat{Y}$ = 0.435 log X + 0.485
N = 13 R = 0.555 P< 0.05
$\hat{Y}$ = 0.011 log X + 1.237
N = 13 R = 0.017 N.S.
20
40
60
80
100
200
400
Live weight in grams

Fig. 21. Oxygen consumption of white perch swimming at 1.02 ft $sec^{-1}$ under control conditions (solid line) and during exposure to 1.09 g $l^{-1}$ fuller's earth suspensions (broken line).

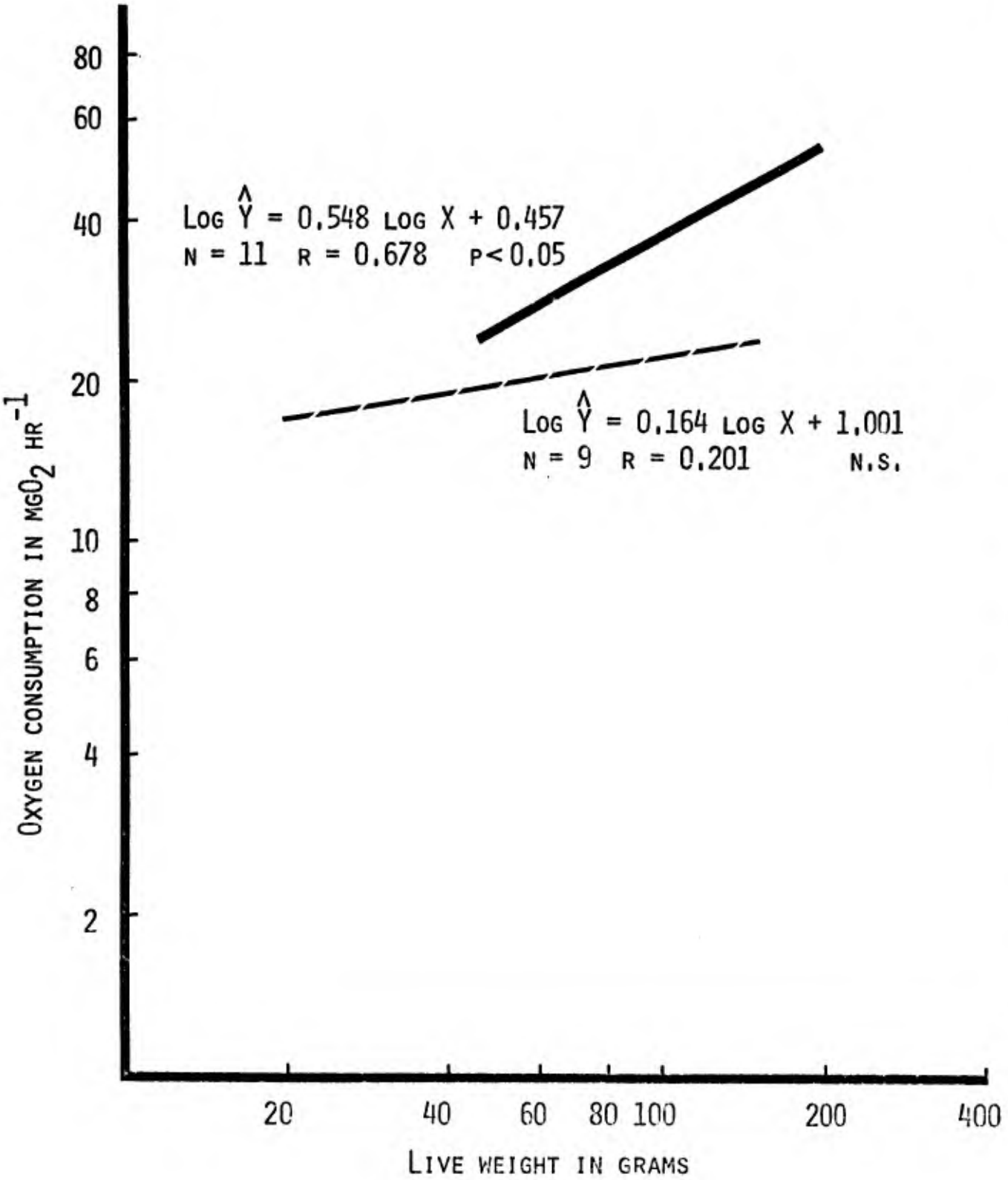

80
60
40
20
10
8
6
4
2
Log $\hat{Y}$ = 0.548 Log X + 0.457
N = 11 R = 0.678 P< 0.05
Log $\hat{Y}$ = 0.164 Log X + 1.001
N = 9 R = 0.201 N.S.
Oxygen consumption in mg$O_2$ $hr^{-1}$
20
40
60
80
100
200
400
Live weight in grams

Fig. 22. Oxygen consumption of white perch swimming at 0.39 ft $sec^{-1}$ during exposure to 2.12 g $l^{-1}$ natural sediment suspensions.

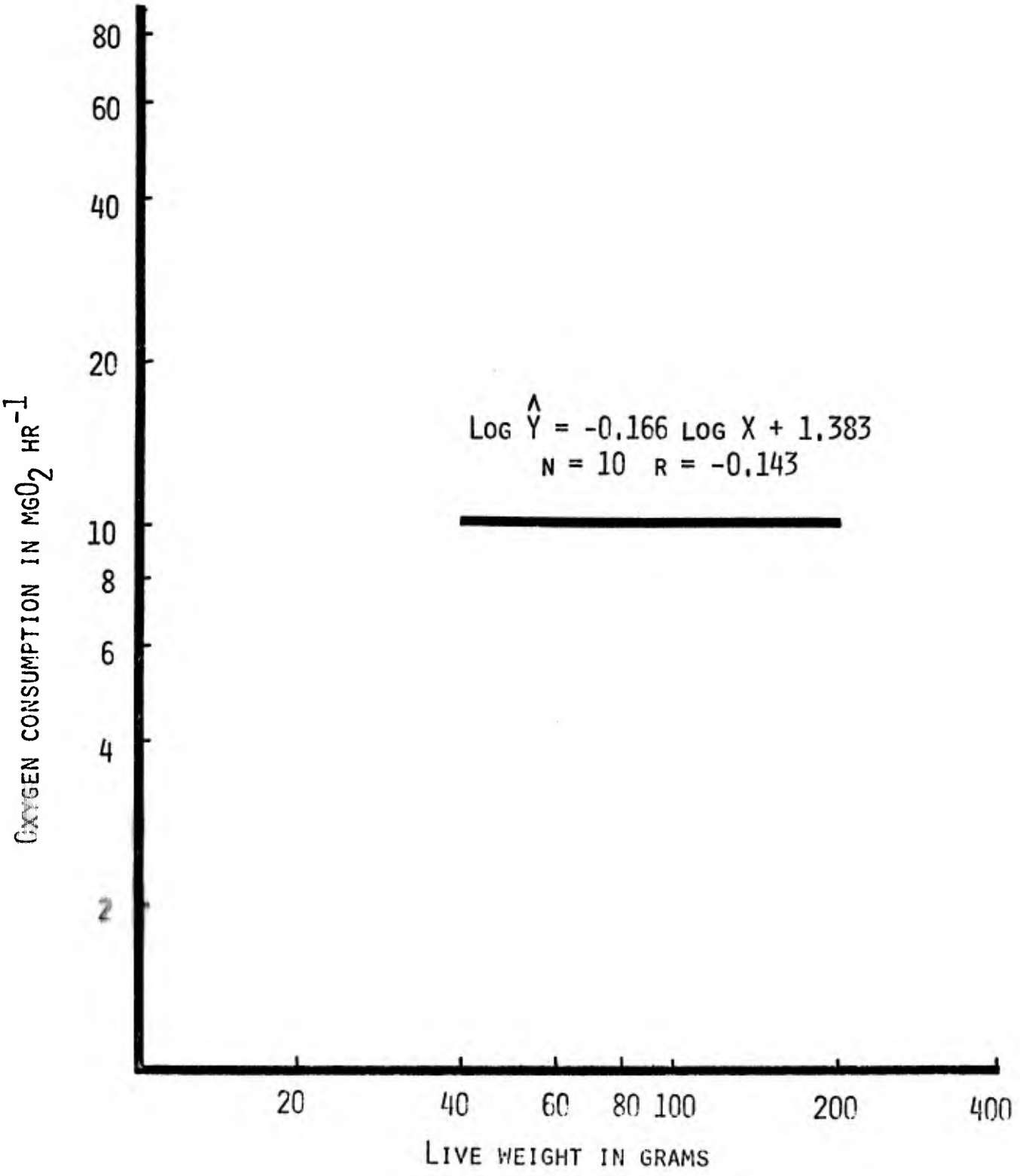

80
60
40
20
10
8
6
4
2
OXYGEN CONSUMPTION IN MG$O_2$ $HR^{-1}$
Log $\hat{Y}$ = -0.166 Log X + 1.383
N = 10 R = -0.143
20
40
60
80
100
200
400
LIVE WEIGHT IN GRAMS

Fig. 23. Oxygen consumption of white perch swimming at 1.05 ft $sec^{-1}$ during exposure to 2.12 g $l^{-1}$ natural sediment suspensions.

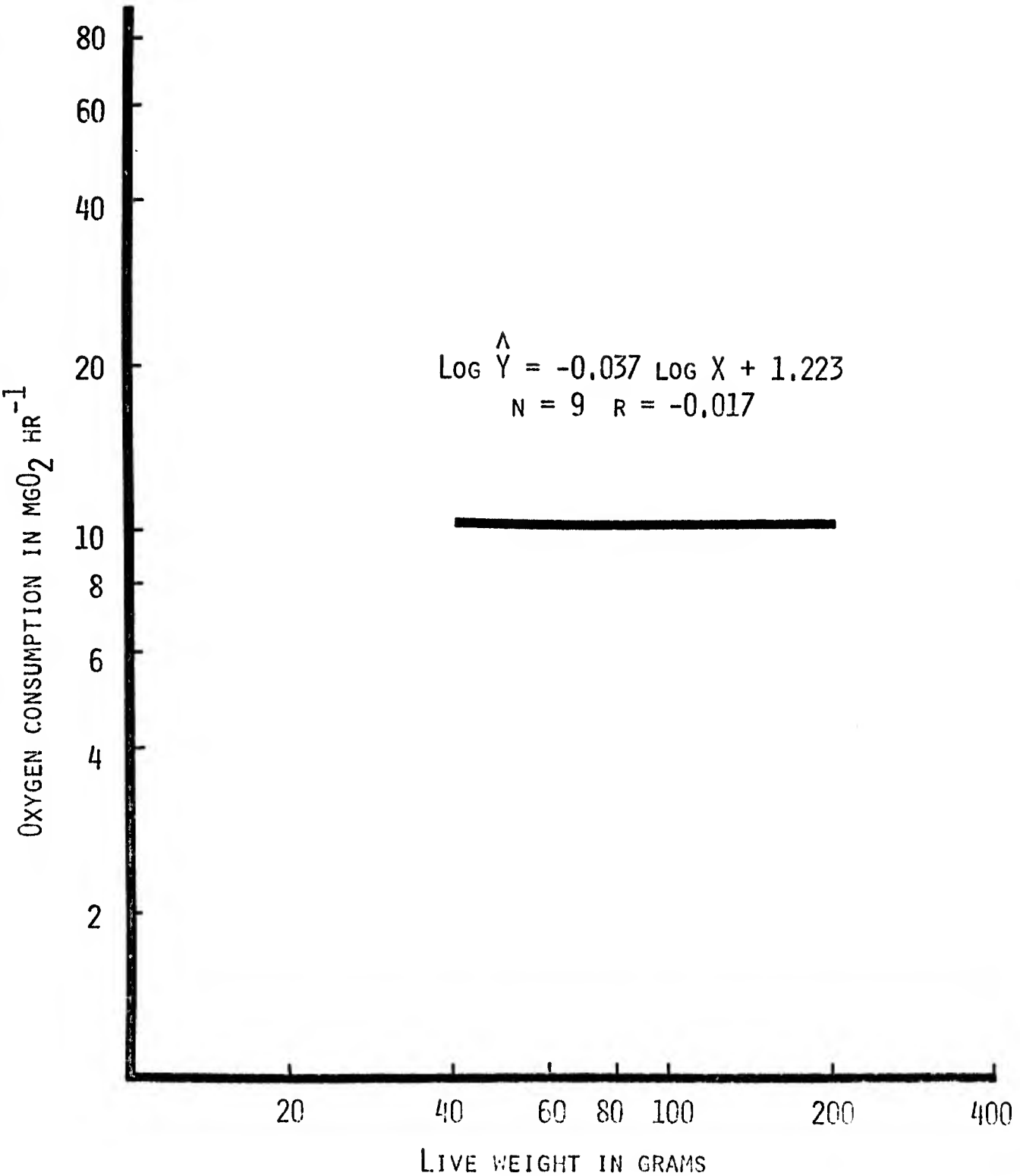

80
60
40
20
10
8
6
4
2
Oxygen consumption in mg$O_2$ $hr^{-1}$
Log $\hat{Y}$ = -0.037 Log X + 1.223
N = 9 R = -0.017
20
40
60
80
100
200
400
Live weight in grams

Fig. 24. Oxygen consumption of white perch swimming at 0.28 ft $sec^{-1}$ under control conditions (solid line) and at 0.39 ft $sec^{-1}$ in clean water following 72 hr exposure to 2.58 g $l^{-1}$ resuspended natural sediment (broken line).

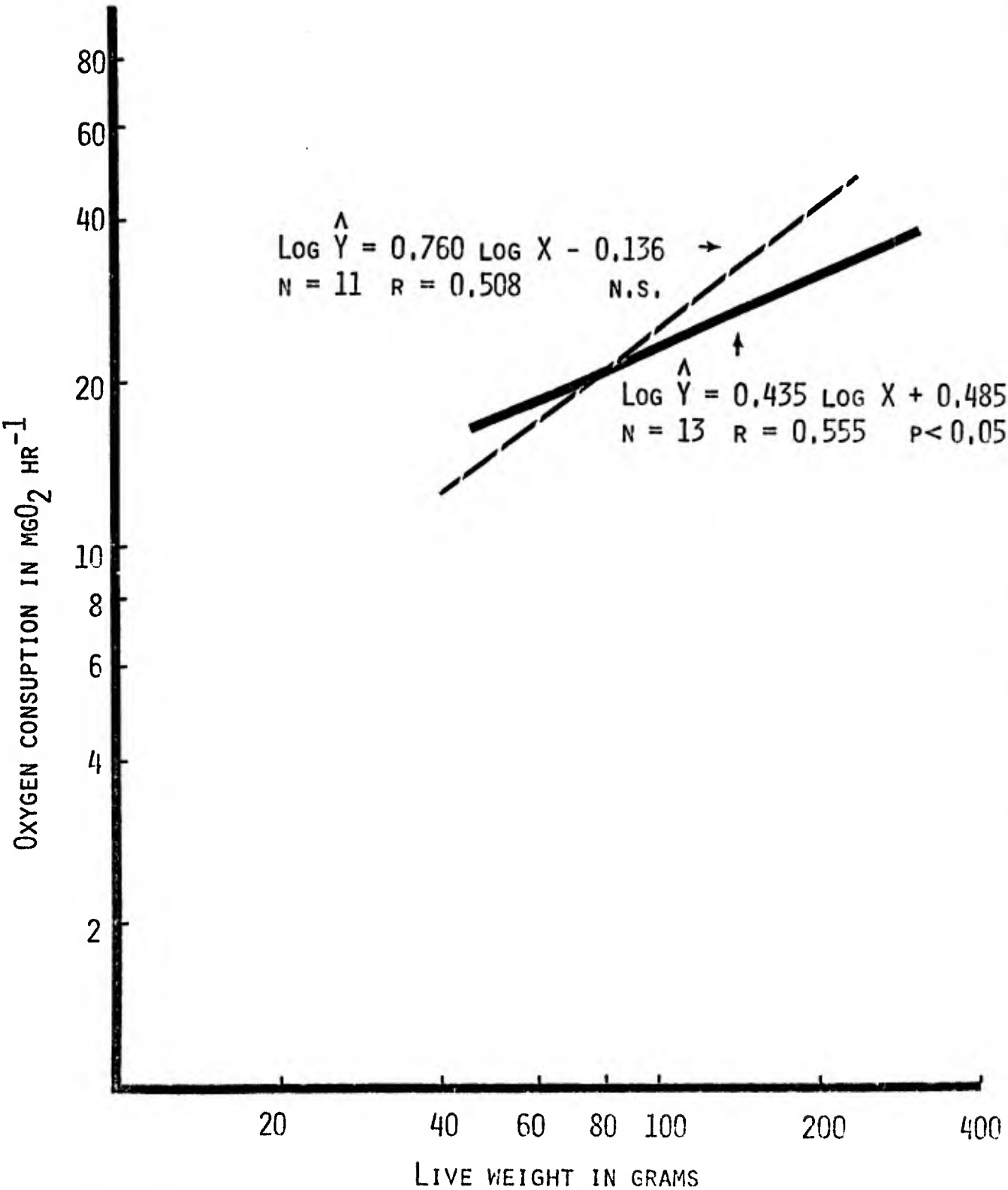

Log $\hat{Y}$ = 0.760 Log X - 0.136
N = 11 R = 0.508 N.S.
Log $\hat{Y}$ = 0.435 Log X + 0.485
N = 13 R = 0.555 P< 0.05
Oxygen consuption in mg$O_2$ $hr^{-1}$
80
60
40
20
10
8
6
4
2
20
40
60
80
100
200
400
Live weight in grams

Fig. 25. Oxygen consumption of white perch swimming at 1.02 ft $sec^{-1}$ under control conditions (solid line) and following 72 hr exposure to 2.58 g $l^{-1}$ resuspended natural sediment (broken line).

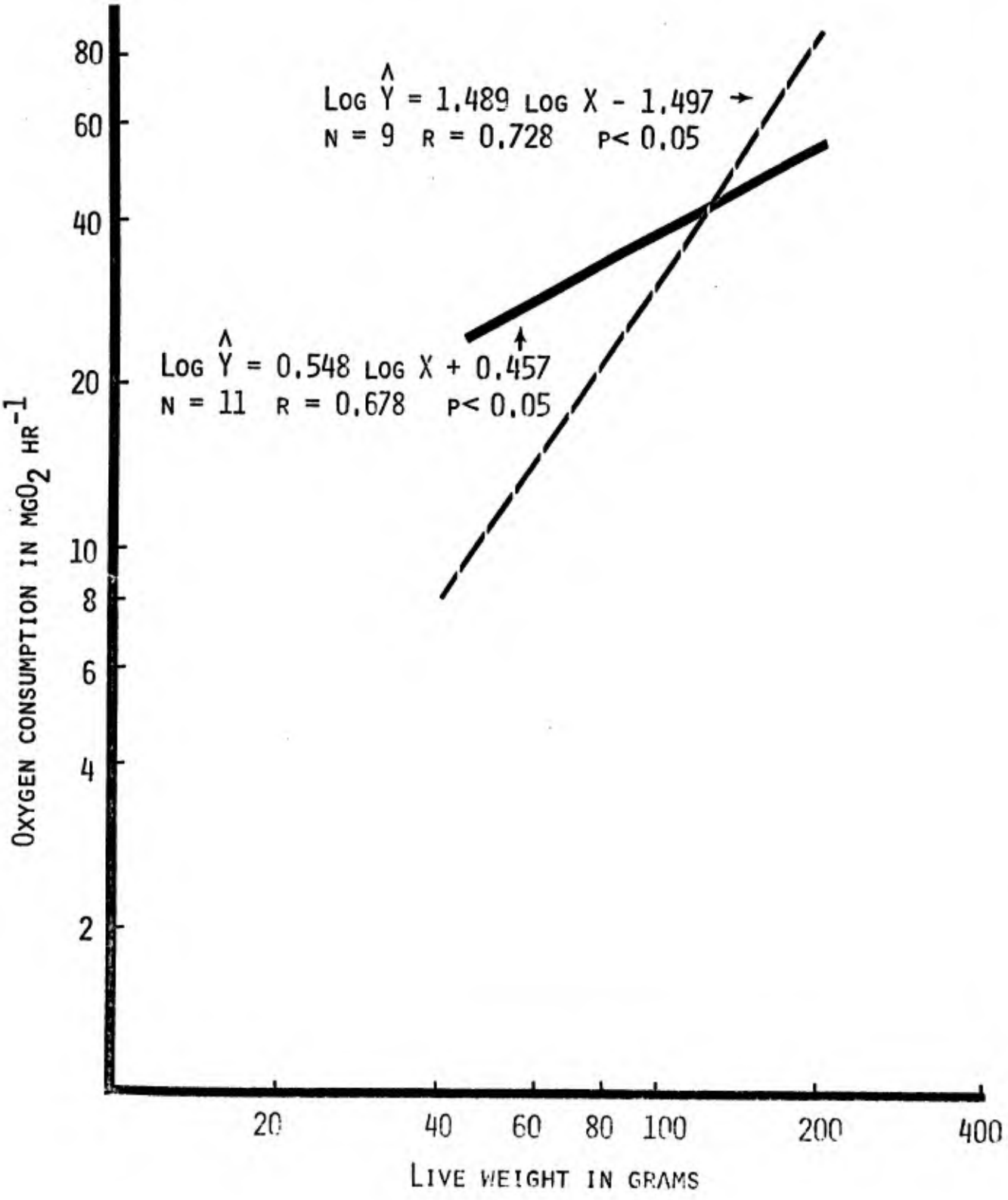

Log Ŷ = 1.489 Log X - 1.497
N = 9 R = 0.728 P< 0.05
Log Ŷ = 0.548 Log X + 0.457
N = 11 R = 0.678 P< 0.05
Oxygen consumption in mgO2 hr-1
80
60
40
20
10
8
6
4
2
20
40
60
80
100
200
400
Live weight in grams

Table 24. Covariance analysis of oxygen consumption - live weight regressions of white perch swimming at 1.58 ft $sec^{-1}$ under baseline conditions and in filtered water after 72 hr exposure to 2.58 g $l^{-1}$ natural sediment.

| Comparison | N | Residual Variance | | Slope | | Elevation | |
|---|---|---|---|---|---|---|---|
| | | M.S. | d.f. | M.S. | d.f. | M.S. | d.f. |
| Baseline | 11 | 0.107 | 9, 7 | 0.098 | 1, 16 | 0.164 | 1, 17 |
| Experimental | 9 | 0.349 | * | 0.028 | n.s. | 0.033 | * |

*p < 0.05

3. Toadfish

Fish were held at least five days at 18-20°C and 5 ppt salinity before oxygen consumption rates were determined at flow rates of 0.39 ft $sec^{-1}$. Toadfish did not consistently swim into the current. In filtered river water flowing at 0.39 ft $sec^{-1}$ a 50 g fish used 5.0 mg $O_2$ $hr^{-1}$ and a 150 g fish used 10.1 mg $O_2$ $hr^{-1}$. Sex influence on oxygen consumption rates were not apparent from these data (Table 25).

Respiration rates of toadfish during exposure to 2.20 g $l^{-1}$ fuller's earth were not different from rates determined under baseline conditions (Fig. 26; Table 26). During this exposure a 50 g fish consumed 5.6 mg $O_2$ $hr^{-1}$ and a 150 g fish consumed 12.4 mg $O_2$ $hr^{-1}$. Male and female fish did not exhibit differences in respiration rates (Table 25).

Oxygen consumption rates of toadfish during exposure to 1.58 g $l^{-1}$ natural sediment were not different from baseline rates (Fig. 27; Table 26). In natural sediment suspensions a 50 g fish consumed 4.8 mg $O_2$ $hr^{-1}$ and a 150 g fish consumed 9.7 mg $O_2$ $hr^{-1}$. Male and female respiration rates during exposure to 1.58 g $l^{-1}$ natural sediment differed in variance and elevation at the 5% level (Table 25).

Toadfish were held in 10.37 g $l^{-1}$ natural sediment for 72 hr before oxygen consumption rates were determined in filtered river water. These rates were not different from baseline rates (Fig. 28; Table 26). A 50 g fish used 2.2 mg $O_2$ $hr^{-1}$ and a 150 g fish used 7.3 mg $O_2$ $hr^{-1}$. A significant difference was observed between the variances of respiration rates for males and females during this experiment (Table 25).

Table 25. Covariance analysis of oxygen consumption - live weight regressions of male and female toadfish tested under baseline conditions (A), in 2.20 g $l^{-1}$ fuller's earth (B), in 1.58 g $l^{-1}$ natural sediment (C), in filtered river water after 72 hr. exposure to 10.37 g $l^{-1}$ natural sediment (D), and in 3.36 g $l^{-1}$ natural sediment after 72 hr. exposure to 11.09 g $l^{-1}$ natural sediment (E).

| Comparison | N | Residual Variance | | Slope | | Elevation | |
|---|---|---|---|---|---|---|---|
| | | M.S. | d.f. | M.S. | d.f. | M.S. | d.f. |
| Baseline (A) | | | | | | | |
| Males | 11 | 0.032 | 9, 7 | 0.008 | 1, 16 | 0.068 | 1, 17 |
| Females | 9 | 0.014 | n.s. | 0.024 | n.s. | 0.023 | n.s. |
| Fuller's Earth (B) | | | | | | | |
| Males | 8 | 0.033 | 6, 9 | 0.043 | 1, 15 | 0.011 | 1, 6 |
| Females | 11 | 0.020 | n.s. | 0.025 | n.s. | 0.026 | n.s. |
| Natural Sediment (C) | | | | | | | |
| Males | 14 | 0.005 | 12, 4 | 0.052 | 1, 16 | 0.099 | 1, 17 |
| Females | 6 | 0.011 | n.s. | 0.006 | * | 0.009 | * |
| Filtered Water (D) | | | | | | | |
| Males | 14 | 0.042 | 12, 4 | 0.001 | 1, 16 | 0.061 | 1, 17 |
| Females | 6 | 0.014 | n.s. | 0.035 | n.s. | 0.033 | n.s. |
| Natural Sediment (E) | | | | | | | |
| Males | 14 | 0.028 | 12, 4 | 0.014 | 1, 16 | 0.013 | 1, 17 |
| Females | 6 | 0.092 | n.s. | 0.044 | n.s. | 0.043 | n.s. |

* $p < 0.01$

Table 26. Covariance analysis of oxygen consumption - live weight regressions of toadfish baseline values and (1) during exposure to 2.20 g $l^{-1}$ fuller's earth, (2) during exposure to 1.58 g $l^{-1}$ natural sediment, (3) in filtered water after 72 hr. exposure to 10.37 g $l^{-1}$ natural sediment, and (4) during exposure to 3.36 g $l^{-1}$ natural sediment after 72 hr. exposure to 11.09 g $l^{-1}$ of the same material. Comparison of response to respiration measurement conditions after 72 hr. exposure to natural sediment suspensions is made in (5).

| | Comparison | Lab. Particle Exposure History g $l^{-1}$ | Experimental Concentration g $l^{-1}$ | N | Residual Variance M.S. | Residual Variance d.f. | Slope M.S. | Slope d.f. | Elevation M.S. | Elevation d.f. |
|---|---|---|---|---|---|---|---|---|---|---|
| (1) | Baseline | 0 | 0 | 20 | 0.025 | 18, 17 | 0.003 | 1, 35 | 0.051 | 1, 36 |
| | Fuller's Earth | 0 | 2.20 | 19 | 0.025 | n.s. | 0.025 | n.s. | 0.025 | n.s. |
| (2) | Baseline | 0 | 0 | 20 | 0.025 | 18, 18 | 0.00002 | 1, 36 | 0.004 | 1, 37 |
| | Natural Sediment | 0 | 1.58 | 20 | 0.025 | n.s. | 0.033 | n.s. | 0.032 | n.s. |
| (3) | Baseline | 0 | 0 | 20 | 0.086 | 18, 18 | 0.113 | 1, 36 | 0.182 | 1, 37 |
| | Natural Sediment | 10.37 | | 20 | 0.025 | * | 0.056 | n.s. | 0.057 | n.s. |
| (4) | Baseline | 0 | 0 | 20 | 0.068 | 18, 18 | 0.187 | 1, 36 | 0.042 | 1, 37 |
| | Natural Sediment | 11.09 | 3.36 | 20 | 0.025 | * | 0.047 | n.s. | 0.050 | n.s. |
| (5) | Natural Sediment | 10.37 | 0 | 20 | 0.034 | 18, 18 | 0.020 | 1, 36 | 0.167 | 1, 37 |
| | Natural Sediment | 11.09 | 3.36 | 20 | 0.068 | n.s. | 0.051 | n.s. | 0.050 | n.s. |

* $p < 0.05$

Fig. 26. Oxygen consumption of toadfish, O. tau, under baseline conditions (solid line) and during exposure to fuller's earth suspensions of 2.20 g $l^{-1}$ (broken line).

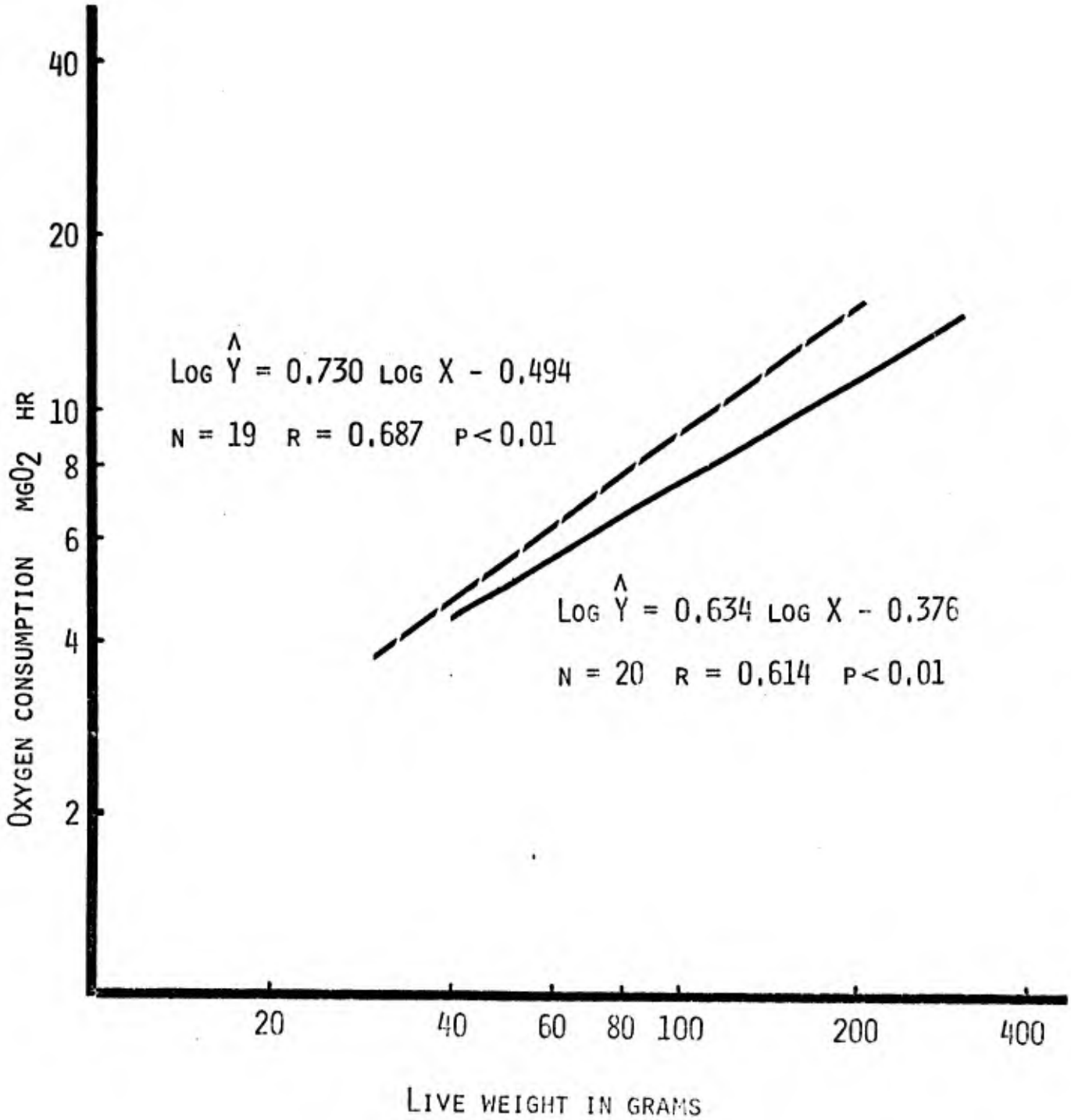

40
20
10
8
6
4
2
OXYGEN CONSUMPTION MGO2 HR
LOG Ŷ = 0.730 LOG X - 0.494
N = 19 R = 0.687 P < 0.01
LOG Ŷ = 0.634 LOG X - 0.376
N = 20 R = 0.614 P < 0.01
20
40
60
80
100
200
400
LIVE WEIGHT IN GRAMS

Fig. 27. Oxygen consumption of toadfish, O. tau, under baseline conditions (solid line) and during exposure to 1.58 g $l^{-1}$ resuspended Patuxent River sediment (broken line).

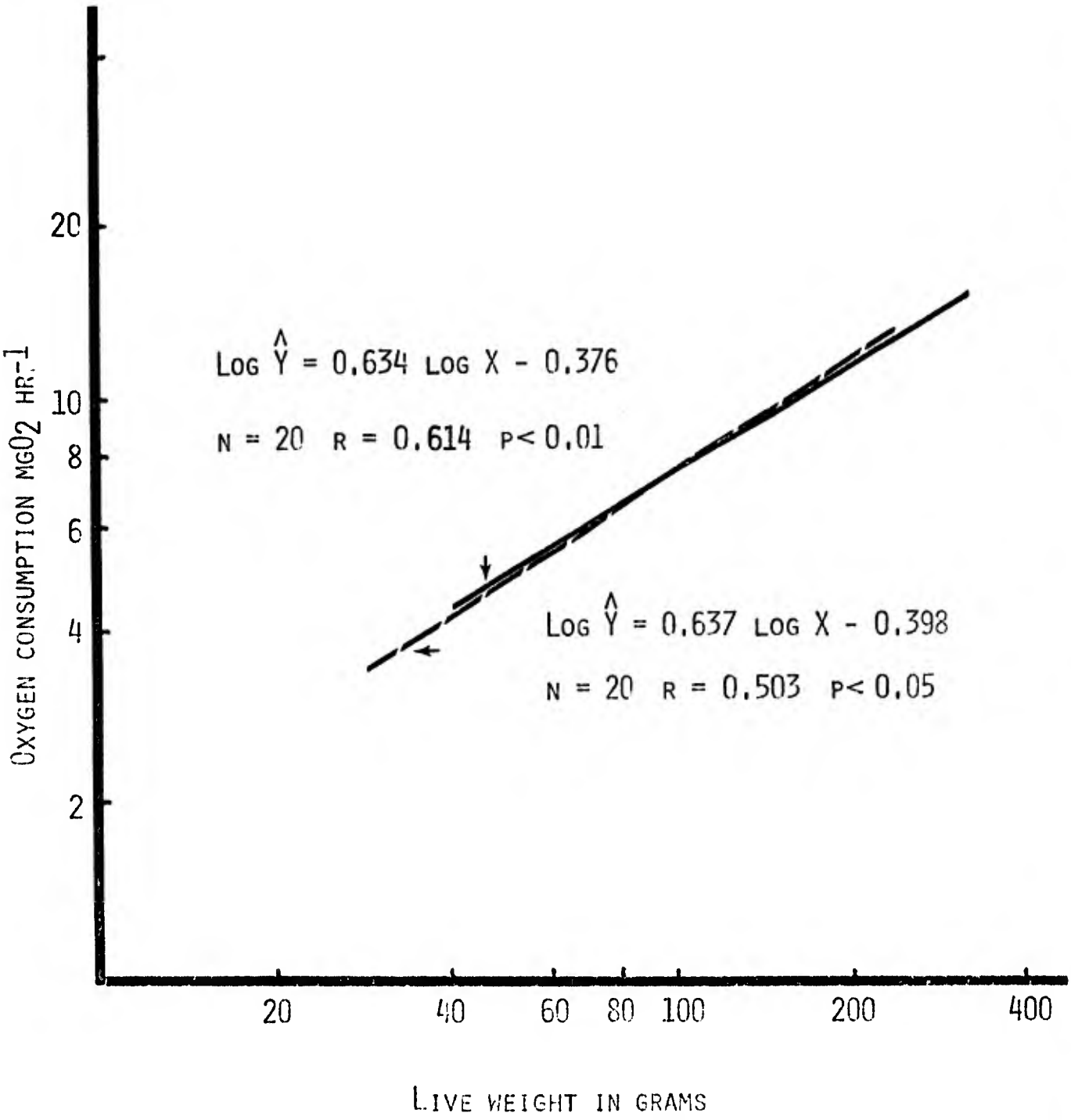

Log $\hat{Y}$ = 0.634 Log X - 0.376
N = 20 R = 0.614 P< 0.01
Log $\hat{Y}$ = 0.637 Log X - 0.398
N = 20 R = 0.503 P< 0.05
Oxygen consumption $MgO_2$ $HR.^{-1}$
20
10
8
6
4
2
20
40
60
80
100
200
400
Live weight in grams

Fig. 28. Oxygen consumption of toadfish, O. tau, under baseline conditions (solid line) and in filtered Patuxent River water after 72 hour exposure to 10.37 g $l^{-1}$ resuspended Patuxent River sediment (broken line).

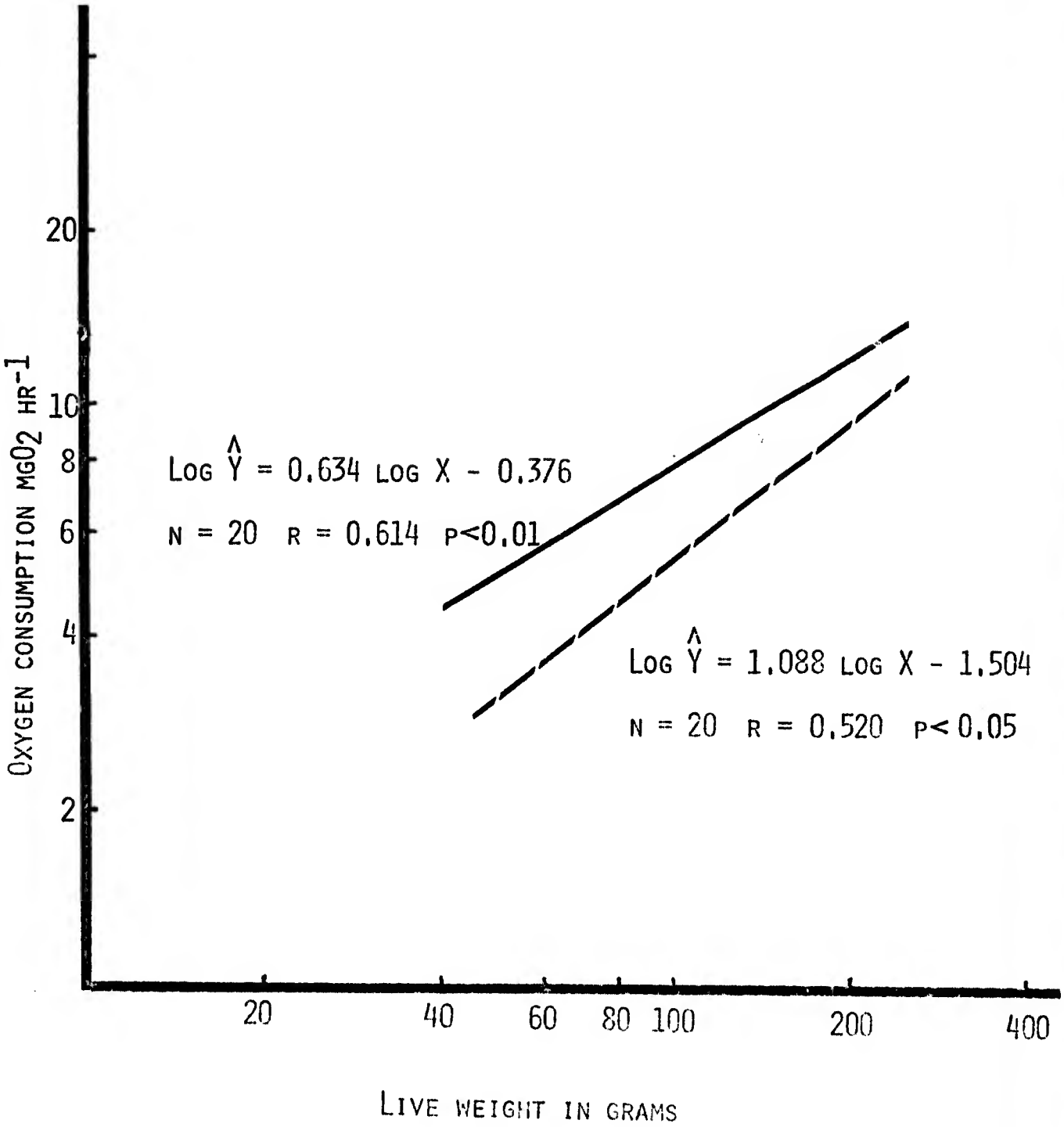
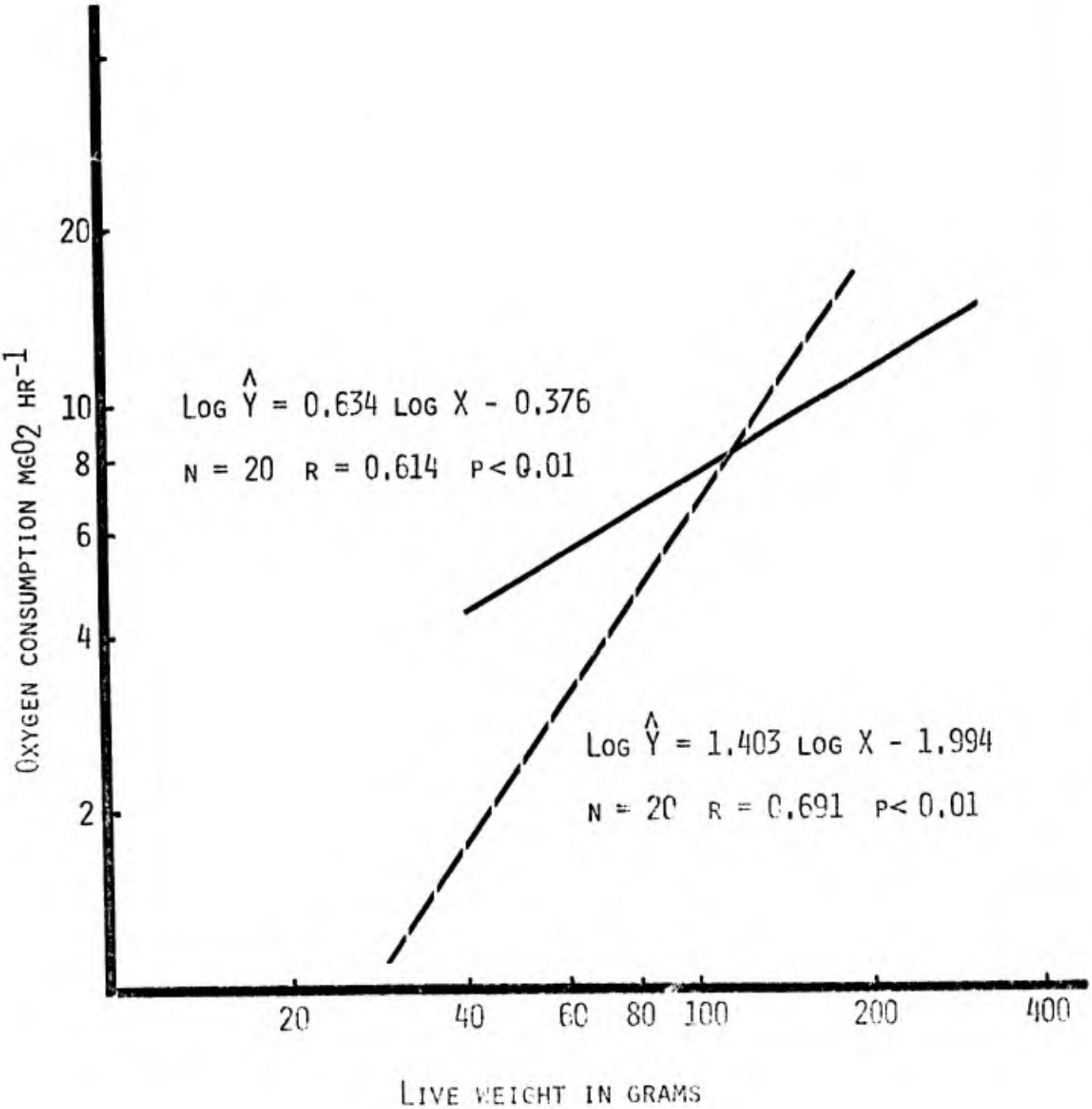

Oxygen consumption $MgO_2$ $HR^{-1}$
20
10
8
6
4
2
Log $\hat{Y}$ = 0.634 Log X - 0.376
N = 20 R = 0.614 P<0.01
Log $\hat{Y}$ = 1.088 Log X - 1.504
N = 20 R = 0.520 P< 0.05
20
40
60
80
100
200
400
Live weight in grams

Fig. 29. Oxygen consumption of toadfish, O. tau, under baseline conditions (solid line) and during exposure to 3.36 g $l^{-1}$ resuspended Patuxent River sediment after 72 hour exposure to 11.09 g $l^{-1}$ of the same material (broken line).


Log $\hat{Y}$ = 0.634 Log X - 0.376
N = 20 R = 0.614 P < 0.01
Log $\hat{Y}$ = 1.403 Log X - 1.994
N = 20 R = 0.691 P < 0.01
Oxygen consumption mgO2 hr-1
20
10
8
6
4
2
20
40
60
80
100
200
400
Live weight in grams

Respiration rates were determined for toadfish in 3.36 g $l^{-1}$ natural sediment after 72 hr exposure to 11.09 g $l^{-1}$ of the same material. Variance associated with rates for fish in the experimental group and for fish in the baseline group were different (Fig. 29; Table 26). Oxygen consumption rates of a 50 g and 150 g fish were 2.5 and 11.5 mg $O_2$ $hr^{-1}$, respectively. Sex influence on respiration rates was not apparent (Table 25). Respiration rates of toadfish exposed to natural sediment suspensions for 72 hr were not different regardless of whether measurements were made in filtered river water or in natural sediment suspensions (Table 26).

## IV. 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. Suspended loads exceeding one g $l^{-1}$ are uncommon in terms of naturally occurring phenomena which influence concentrations (see Sherk, 1972). Masch and Espey (1967) reported concentrations which exceeded 10 g $l^{-1}$ in dredge discharge plumes and in excess of 100 g $l^{-1}$ in dredge-generated density flows. Suspended solids concentrations used in the present study were representative concentrations likely to occur in the vicinity of dredging operations.

The fishes studied most thoroughly are representative of three ecologically distinct species. Striped bass, major components of estuarine sport and commercial fisheries, are anadromous fish which use the estuary as a spawning and nursery area (Talbot, 1966). White perch were described by Mansueti (1964) as an estuarine species

exhibiting semi-anadromous migrations. These fish are usually restricted to a certain segment of the estuary throughout their life spans. Toadfish are sedentary, demersal fish inhabiting the sediment-water interface of estuarine systems.

Striped bass and white perch swimming abilities suggest that during periods of high turbidity these fish have the potential to move to areas of more favorable conditions. In laboratory experiments which prevented fishes from moving from areas of high turbidity, suspensions of fuller's earth or Patuxent River sediments generally reduced oxygen consumption of these fishes at controlled levels of swimming activity.

Toadfish exhibited no significant respiratory responses to suspensions of fuller's earth or natural sediment. Adaptation of these fish to life at the sediment-water interface may explain this absence of response. The sediment-water interface is characterized by periods of low oxygen availability, or high turbidity conditions, 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 movements ceased.

Respiratory responses of striped bass and white perch to suspended solids were observed in the laboratory at concentrations exceeding those which naturally occur in estuaries. These concentrations may occur temporarily in the vicinity of dredge discharges. Toadfish exhibited no respiratory response to similarly high concentrations of suspended solids. Suspension concentrations produced by dredging operations probably have limited influence on striped bass and white perch respiration due to the mobility of these species. Toadfish are sedentary, but highly tolerant to suspended solids which may minimize the effects of dredging operations on the oxygen consumption rates of these fish.

EFFECTS OF SUSPENDED AND DEPOSITED SEDIMENTS

ON ESTUARINE ORGANISMS PHASE II

CHAPTER 7

# THE EFFECTS OF SUSPENDED SOLIDS ON CARBON ASSIMILATION OF SELECTED SPECIES OF ESTUARINE PHYTOPLANKTON

## I. INTRODUCTION

Phytoplankton growth is usually measured in terms of photosynthesis (carbon-14 uptake and oxygen evolution). This growth responds in a more or less complex way to various factors singly or in combination, not the least of which are nutritive factors, temperature, salinity, and intensity of illumination (Jitts, McAllister, Stephens and Strickland, 1964).

The release of silt, clay, and fine sand-sized particles (organic and inorganic) into the water column is a dominant feature of bay and estuarine areas (see review in Sherk, 1971). These releases may be caused by beach erosion, river contribution, storm reagitation, dredging and disposal of dredged material. One of the major effects of the resulting suspended loads is the impairment of light penetration (by absorption, Raleigh small particle scatter, large particle scatter), thus limiting the column of water in which light intensity is sufficient for the rate of phytoplanktonic photosynthesis to exceed the respiration rate.

In this chapter we present the results of experimental work which investigated the effects of increasing concentrations of extremely fine silicon dioxide particles on carbon assimilation of various monospecific cultures of phytoplankton grown at estuarine salinities.

## II. MATERIALS AND METHODS

### 1. Cultures

Cultures of phytoplankton were obtained from the Woods Hole Oceanographic Institution (WHOI) as follows:

| Name | WHOI Clone Symbol |
|---|---|
| *Monochrysis lutheri* | Mono |
| *Nannochloris* sp. | GSB Nanno |
| *Stichococcus* sp. | GSB Sticho |
| *Chlorella* sp. | 0-10 |

These cultures were grown in Guillard's f/2 medium (Guillard and Ryther, 1962) made with autoclaved (15 psi, 30 min) Patuxent water which had been filtered previously through sintered Whatman GFC glass fiber filters.

Stock cultures of each species were maintained in 50 ml of the f/2 medium (salinity range 5.5 to 7.5 °/oo) contained in 125 ml Erlenmeyer flasks either plugged with sterile cotton wads or tightly covered with aluminum foil. Transfers (one ml of stock) into fresh sterile (autoclaved) medium were made at approximately biweekly intervals. No attempt was made to maintain the cultures in an axenic (bacteria-free) condition. However, the cultures were checked periodically (1) to ascertain whether contamination by other species of phytoplankton had occurred, and (2) to monitor growth rate of the stock by increase in cell numbers which were counted at 100X on an improved Neubauer hemacytometer. Motile forms were fixed before counting with 6.5% glutaraldehyde (pH = 7.4) buffered with sodium cacodylate (0.14 M).

Cultures were grown at 20 $\pm$ 0.5°C under continuous cool white fluor-

escent illumination ($0.8 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$) in a constant temperature incubator. Under these culture conditions, an exponential growth rate (Sorokin, 1969) range of 0.35 to 0.40 $day^{-1}$ could be maintained for these species at cell concentrations between $5 \times 10^5$ and $5 \times 10^6$ cells $ml^{-1}$.

Prior to the start of an experiment, approximately 10 ml (depending on cell concentration) of stock culture in exponential growth phase was transferred into 1.5 liters of fresh, sterile (autoclaved) f/2 medium (enriched, filtered Patuxent River water) in sterile Erlenmeyer flasks (two liter capacity). The increase in cell numbers under culture light intensity was monitored until a cell concentration of approximately 50,000 cells $ml^{-1}$ was attained, after which the experimental run was conducted with minimum dilution of these cells by fresh medium. This cell concentration, 50,000 cells $ml^{-1}$, may be representative of natural concentrations in certain areas of the Chesapeake Bay system (D. A. Flemer, personal communication). Carbon-12 concentration during experimental work and in stock cultures was monitored by the method of Karlgren (1962), reported in Sherk (1969). Dissolved inorganic carbon concentrations were maintained at 15 to 20 mg C/l in both cultures and experimental media.

2. Sediments

Graded series of concentrations of silicon dioxide (Fisher No. S-153, see Appendix D) were used for all assimilation experiments.

3. Determination of the Saturation Curves.

Saturation curves for phytoplanktonic photosynthesis were determined under the illumination from six 40 watt cool white fluorescent lamps. Light from these lamps was passed through Pyrex infrared reflecting glass

(Kaufmann Glass Co., Wilmington, Delaware) before reaching the plexiglass top of a constant temperature shaking-type incubator. The light intensity for experimental work recorded inside the incubator at the level normally occupied by incubation bottles was $1.45 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$. All measurements of light intensity were made with a Y.S.I. Model 65A radiometer.

Two sets of six ground glass-stoppered bottles (130 $\pm$ 1.5 ml capacity) were used for each determination. These bottles had been previously washed in 0.01 N $H_2SO_4$, rinsed twice with deionized glass-distilled water, and rinsed twice with filtered, sterile (autoclaved) Patuxent River water enriched with f/2 medium. Each bottle was filled with the same number of cells (differences between bottles could not be distinguished from counting error, $p > 0.1$), as close to $5 \times 10^4$ cells $ml^{-1}$ as possible.

Replicate pairs of bottles were wrapped in 0, 1, 2, 3, 4, and 8 layers of black plastic window screen to attenuate incident light. The black plastic screens were apparently an effective (neutral density?) filter. The attenuation of light by successive layers of this screen was determined with the radiometer in the incubator, as above, by placing the instrument inside the various layers of screen which were in the position normally occupied by incubation bottles during experimental runs (Fig. 30).

After the bottles were filled with cells and placed within the screen layers, 1.0 µCi $NaHC^{14}O_3$ (one ml solution, pH = 9.5, adjusted with NaOH in distilled water) was injected into each bottle with a Hamilton gas-tight syringe. For work with Monochrysis lutheri, 10.0 µCi $NaHC^{14}O_3$ was injected into each bottle. The syringe was rinsed once with water from each bottle after initial injection. The bottles were stoppered and incubated in the

constant temperature (20°C) shaking (2 cycles/sec) incubator for 4 hours under a light intensity of $1.45 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$.

After incubation, replicate pairs of bottles were removed from the incubator. Carbon assimilation of the phytoplankton was determined immediately by the acidification and bubbling technique of Schindler, Schmidt, and Reid (1972). Two 20 ml samples were withdrawn from each incubation bottle and placed into two short Allihn tubes (30 ml capacity) which contained 0.5 ml 0.1 N HCl to ensure complete stripping of dissolved inorganic carbon. Air was bubbled through the tubes for 20 min. to remove the dissolved inorganic carbon. After bubbling, two 2 ml samples were withdrawn by pipet from each tube and placed into liquid scintillation vials. Fifteen milliliters of Bray's (1960) dioxane-base scintillation fluid were added to each vial.

All samples were counted at 9°C using a Packard Tricarb 3320 liquid scintillation spectrometer. After background subtraction, sample counts were corrected for quenching by an external standard quench curve constructed from counts of standard toluene C-14 with increasing amounts (0 to 400 µl) of chloroform in 15 ml of Bray's solution. Counting efficiency of most of the samples was approximately 85%. Sample counts were averaged for each set of screens. Results are reported as gross counts per minute assimilated per milliliter per hour (cpm $ml^{-1}$ $hr^{-1}$).

4. Determination of Suspended Solids Effects

Two sets of assimilation experiments were conducted with each of the four phytoplankton species exposed to graded concentrations of silicon

dioxide. These effects were determined for each species on the same day as the light saturation curves were determined (see above). Monochrysis lutheri was exposed to increasing concentrations (100, 250, 500, 1,000, 2,250 mg $l^{-1}$) of the $SiO_2$ particles with a median size of approximately 17 μm. The other three species were exposed to graded concentrations (50, 100, 500, and 1,000 mg $l^{-1}$) of the $SiO_2$ particles with a median size of 6.2 μm (see Appendix D).

A slurry of $SiO_2$ particles was made up with filtered, sterile (autoclaved), enriched (f/2 medium) Patuxent River water prior to the start of each experiment. A magnetic stirring device was used to maintain the suspension. Concentration of particles by weight (mg $l^{-1}$) was determined by the dry weight difference between the average of three oven-dried aliquots (5 ml each) of the slurry and the average of three aliquots (5 ml) of the enriched river water (salt correction).

Fifteen glass-stoppered bottles, rinsed and washed as in the saturation curve determinations, were used during each experiment. Of these, five replicate pairs were light bottles. Each of these pairs had a corresponding dark bottle which had been wrapped with two layers of heavy gauge aluminum foil. All bottles were filled with cells (approximately 50,000 cells $ml^{-1}$) from the same culture flask. In addition, small amounts of the $SiO_2$ slurry were measured by volume (Oxford precision micropipet) and injected into the bottles (two light and one dark bottle received the same concentration) to make up the series of increasing $SiO_2$ concentrations. The maximum amount of slurry injected into the bottles for the highest concentration

never exceeded 0.5% of bottle volume (130 ml). Then, approximately 1.0 μCi $NaHC^{14}O_3$ was injected into each bottle with a gas-tight syringe, the bottles were stoppered and wrapping completed, in the case of the dark bottles.

The rest of the procedure (incubation and isotope counting) was identical with that of the saturation curve determinations, except for the following:

1. Dark bottle counts (dark carbon fixation) were subtracted from the average of the light bottle samples after background subtraction and quench correction.
2. Results are reported as net counts per minute assimilated per ml per hour (cpm $ml^{-1}$ $hr^{-1}$) during exposure to increasing concentrations of $SiO_2$.
3. In addition, the reduction in carbon uptake (expressed as % of control) is reported at each concentration of $SiO_2$.

## III. RESULTS AND INTERPRETATION

### 1. Saturation Curves

While it is clearly evident that the spectral quality of light is significant in studies of photosynthesis (see action spectra in Jitts et al., 1964), there is no typical submarine spectral distribution because of the interactions of depth, light attenuating properties of sea water, and the conditions of the sky. Light of wavelengths greater than 6500 Å or less than 4000 Å may be completely missing in even moderately clear coastal waters (Jitts et al., 1964). No form of submarine light resembles

the spectral distribution of unfiltered sunlight or the illumination from cool white fluorescent lamps used in this study.

The attenuation of light intensity by the use of successive layers of black plastic screen provided a rough geometrical decrease (Fig. 30). With respect to the experimental incident radiation (0 layers of screen), one screen layer reduced this intensity to about 59%, two screen layers attenuated this intensity to about 40%, three screen layers to about 28%, four screen layers to about 17%, and eight screen layers to about 7%.

The low intensities of light used in culturing the phytoplankton and in conduction of the experiments were thought to be typical of upper to middle turbid areas of tributaries to the Chesapeake Bay at depths greater than 0.5 m. These intensities were calculated very roughly from our own water surface insolation measurements with the Y.S.I. radiometer and Flemer's extinction coefficients; conversion factors were according to Strickland (1958).

At 20°C photosynthesis of Monochrysis lutheri was apparently light saturated, or very close to not being light-limited, at $1.45 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$ as evidenced by the leveling off of the curve (Fig. 31). This value is approximately 41% of that reported by Craigie (1969) for this species. Our value is approximately 14% of that reported by Jitts et al. (1964) for optimal growth of this species at 19°C, although their preconditioning intensity was roughly $4.55 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$ vs $0.8 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$ in our project. They also used enriched synthetic sea water with a salinity of about 29 °/oo compared to our filtered, natural Patuxent River water enriched with f/2 medium

Fig. 30. Extinction effect of layers of black plastic screens used to cover incubation bottles during photosynthetic saturation curve determinations.

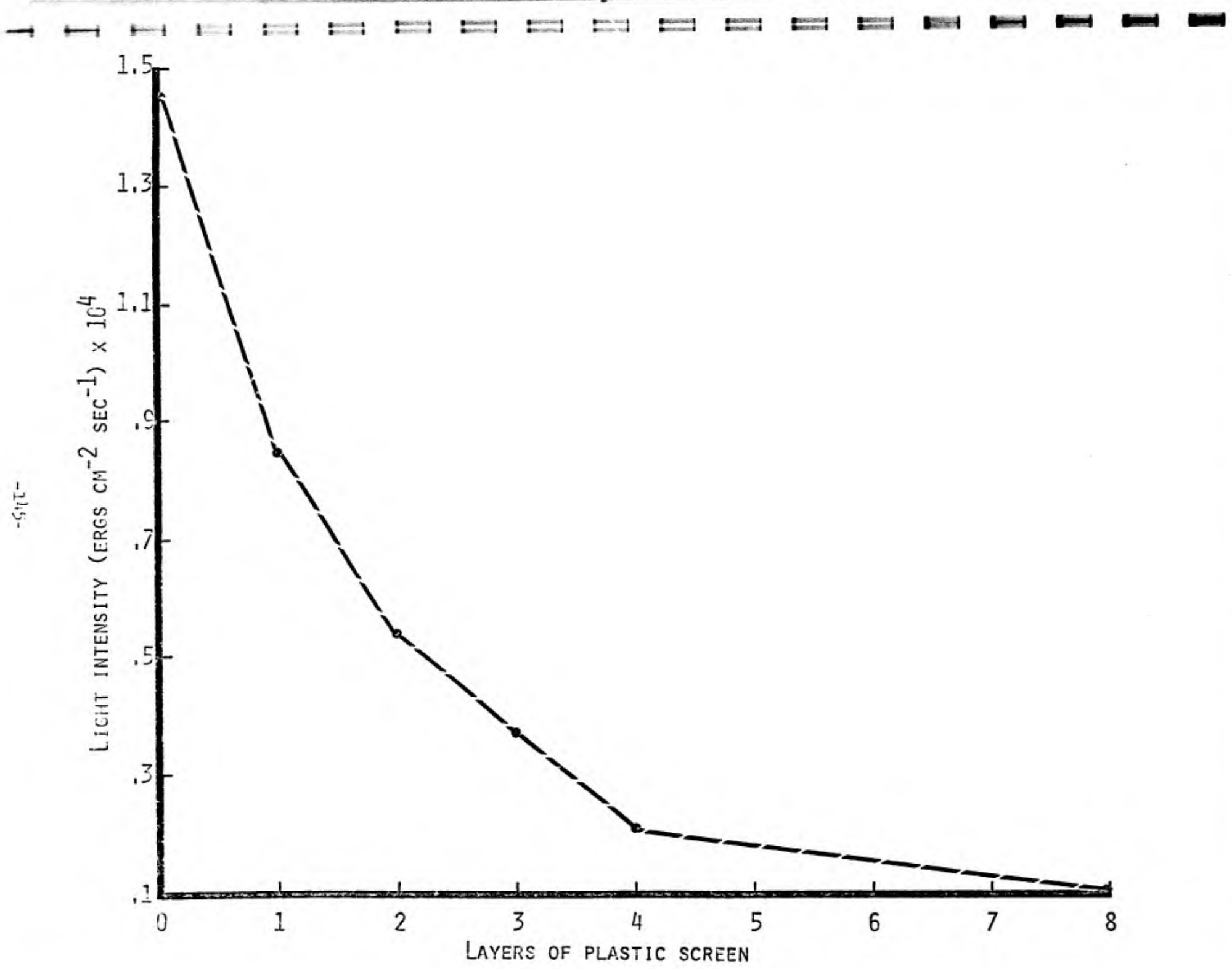
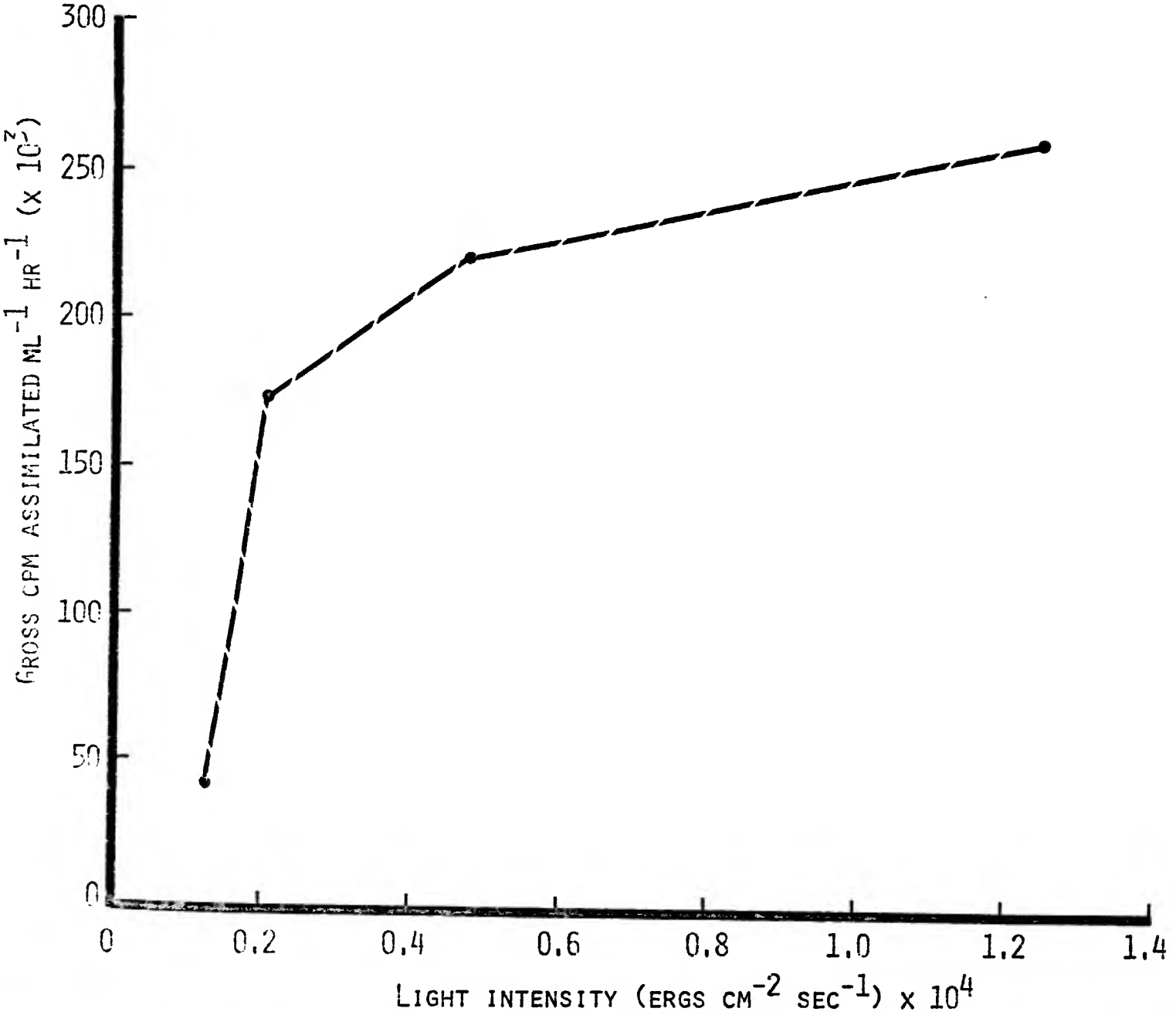

1.5
1.3
1.1
.9
.7
.5
.3
.1
LIGHT INTENSITY (ERGS CM$^{-2}$ SEC$^{-1}$) x $10^4$
0
1
2
3
4
5
6
7
8
LAYERS OF PLASTIC SCREEN

Fig. 31. Saturation curve of photosynthesis for *Monochrysis lutheri* (cultured under $0.8 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$) exposed to cool white fluorescent illumination.


300
250
200
150
100
50
0
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
GROSS CPM ASSIMILATED ML$^{-1}$ HR$^{-1}$ (x 10$^2$)
LIGHT INTENSITY (ERGS CM$^{-2}$ SEC$^{-1}$) x 10$^4$

(salinity 5.5 to 7.5 °/oo). These data show that M. lutheri can be acclimated to grow optimally under strikingly different laboratory conditions.

The saturation curves for Chlorella sp. (Fig. 32) and Nannochloris sp. (Fig. 33) indicate that these species were close to light saturation, also. The curve for Stichococcus sp. (Fig. 34) seems to indicate that this species was further away from the light saturation plateau than any of the other three.

2. Suspended Solids Effects

With increasing concentrations of $SiO_2$, the carbon uptake of M. lutheri (Fig. 35) progressively decreased. Close to an 80% reduction in uptake occurred at 2,250 mg $l^{-1}$ $SiO_2$ (median size = 17 µm). Chlorella sp. (Fig. 36) showed a decrease of 90% in carbon uptake at concentrations of 1,000 mg $l^{-1}$ <15 µm $SiO_2$ (median size = 6.2 µm). Practically the same magnitude of reduction (approx. 90%) in carbon uptake occurred at 1,000 mg $l^{-1}$ of <15 µm $SiO_2$ for the other two species (Figs. 37 and 38). The decrease curves for M. lutheri, Chlorella sp., and Nannochloris sp. were practically the same shape, and were not linear. The peculiar shape of the Stichococcus sp. curve may have been supporting evidence that photosynthesis for this species had been light limited even before exposure to concentrations of solids.

These curves mirrored the relationships reported for increasing seston concentration and decreasing transmission of light in water determined by Biggs (1970) for increasing concentrations of kaolinite (median size = 4.0 µm) which attenuated less light than identical increasing concentrations of particles in a natural water sample (median size = 1.9 µm).

Fig. 32. Saturation curve of photosynthesis for Chlorella sp. (cultured under $0.8 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$) exposed to cool white fluorescent illumination.

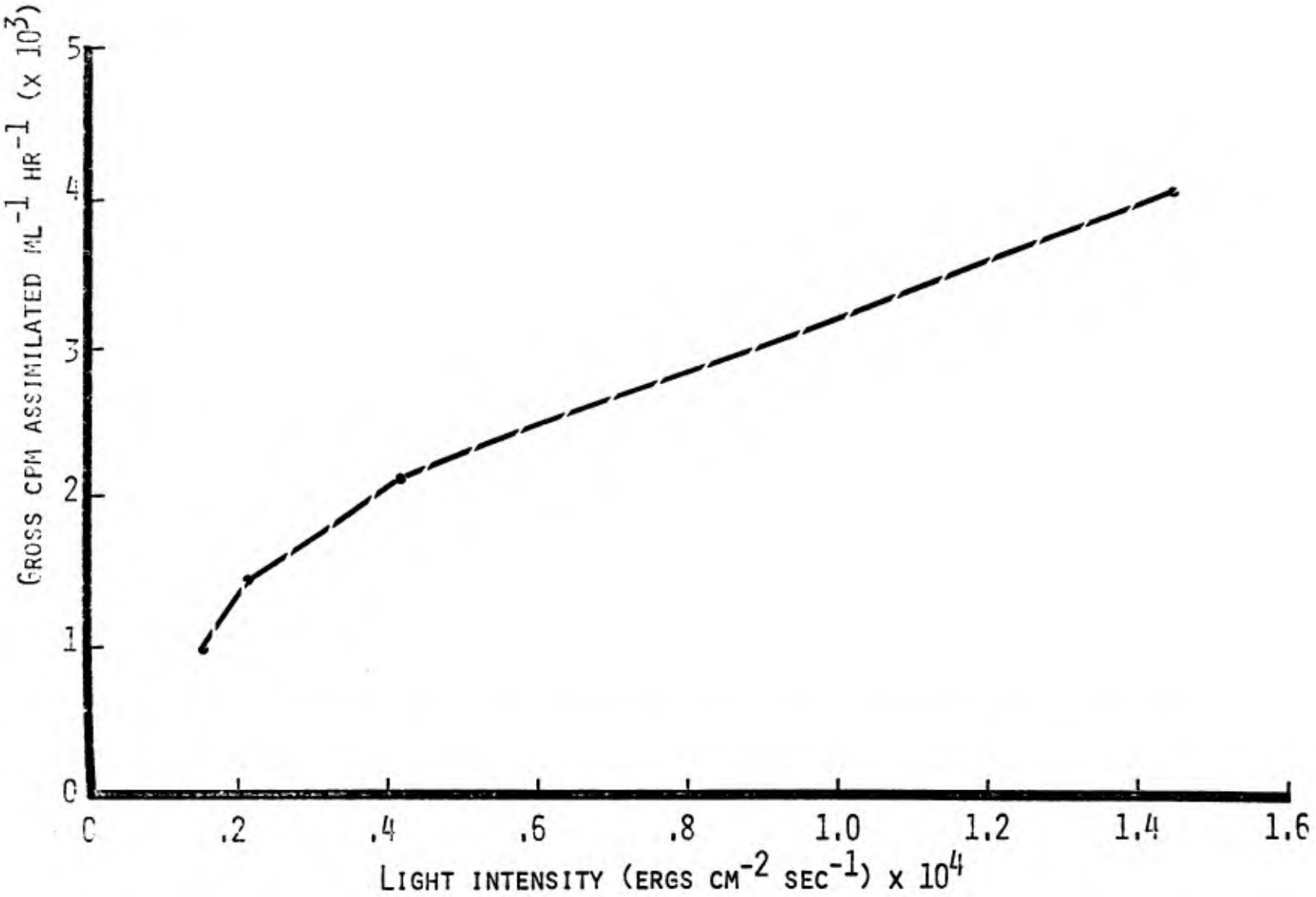

GROSS CPM ASSIMILATED ML$^{-1}$ HR$^{-1}$ (X $10^3$)
0
1
2
3
4
5
0
.2
.4
.6
.8
1.0
1.2
1.4
1.6
LIGHT INTENSITY (ERGS CM$^{-2}$ SEC$^{-1}$) X $10^4$

Fig. 33. Saturation curve of photosynthesis for Nannochloris sp. (cultured under $0.8 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$) exposed to cool white fluorescent illumination.

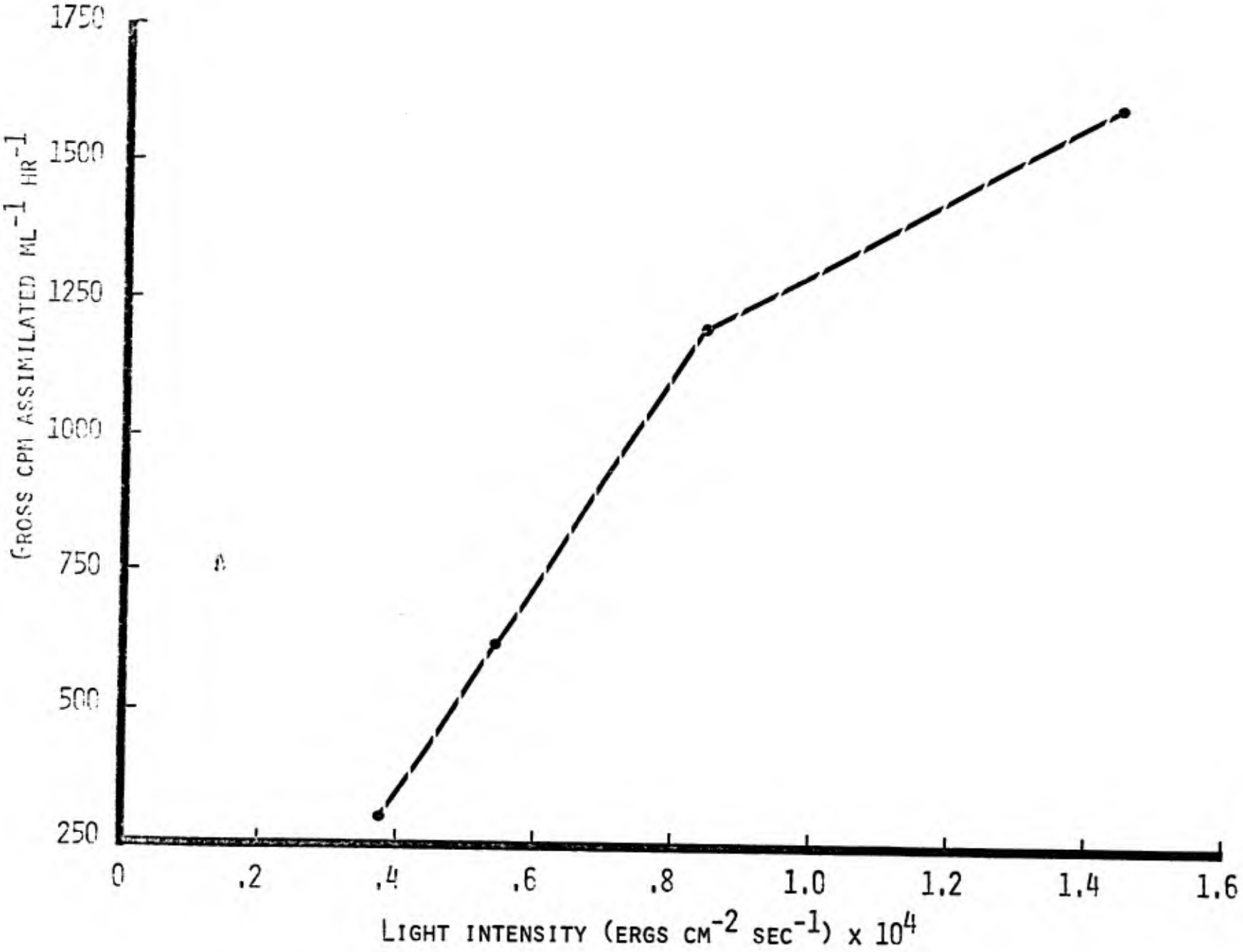

1750
1500
1250
1000
750
500
250
GROSS CPM ASSIMILATED ML$^{-1}$ HR$^{-1}$
0
.2
.4
.6
.8
1.0
1.2
1.4
1.6
LIGHT INTENSITY (ERGS CM$^{-2}$ SEC$^{-1}$) x $10^4$

Fig. 34. Saturation curve of photosynthesis for Stichococcus sp. (cultured at $0.8 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$) exposed to cool white fluorescent illumination.

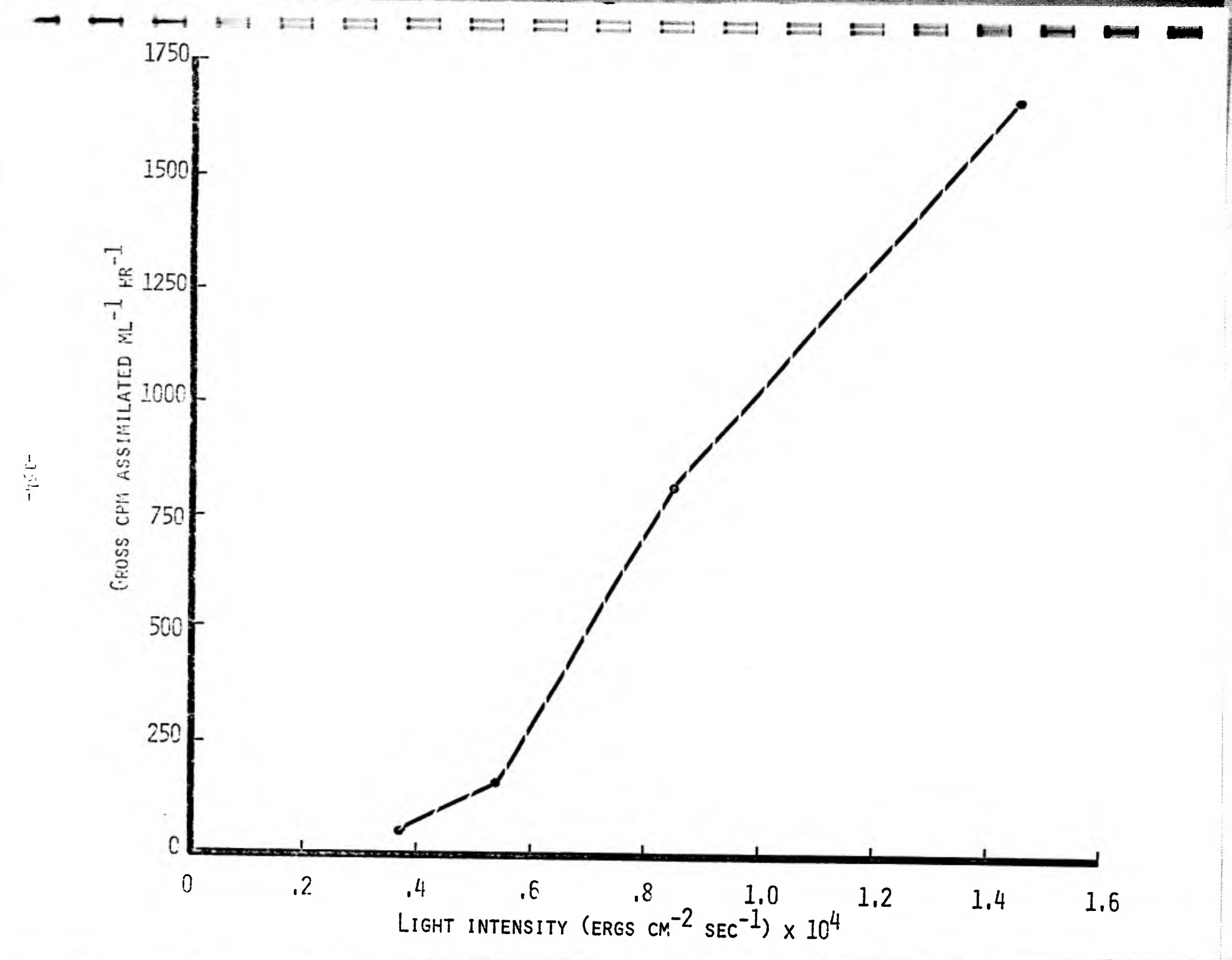

1750
1500
1250
1000
750
500
250
0
GROSS CPM ASSIMILATED ML$^{-1}$ HR$^{-1}$
0
.2
.4
.6
.8
1.0
1.2
1.4
1.6
LIGHT INTENSITY (ERGS CM$^{-2}$ SEC$^{-1}$) x $10^4$

Fig. 35. The effect of increasing concentrations of silicon dioxide on carbon assimilation of Monochrysis lutheri.

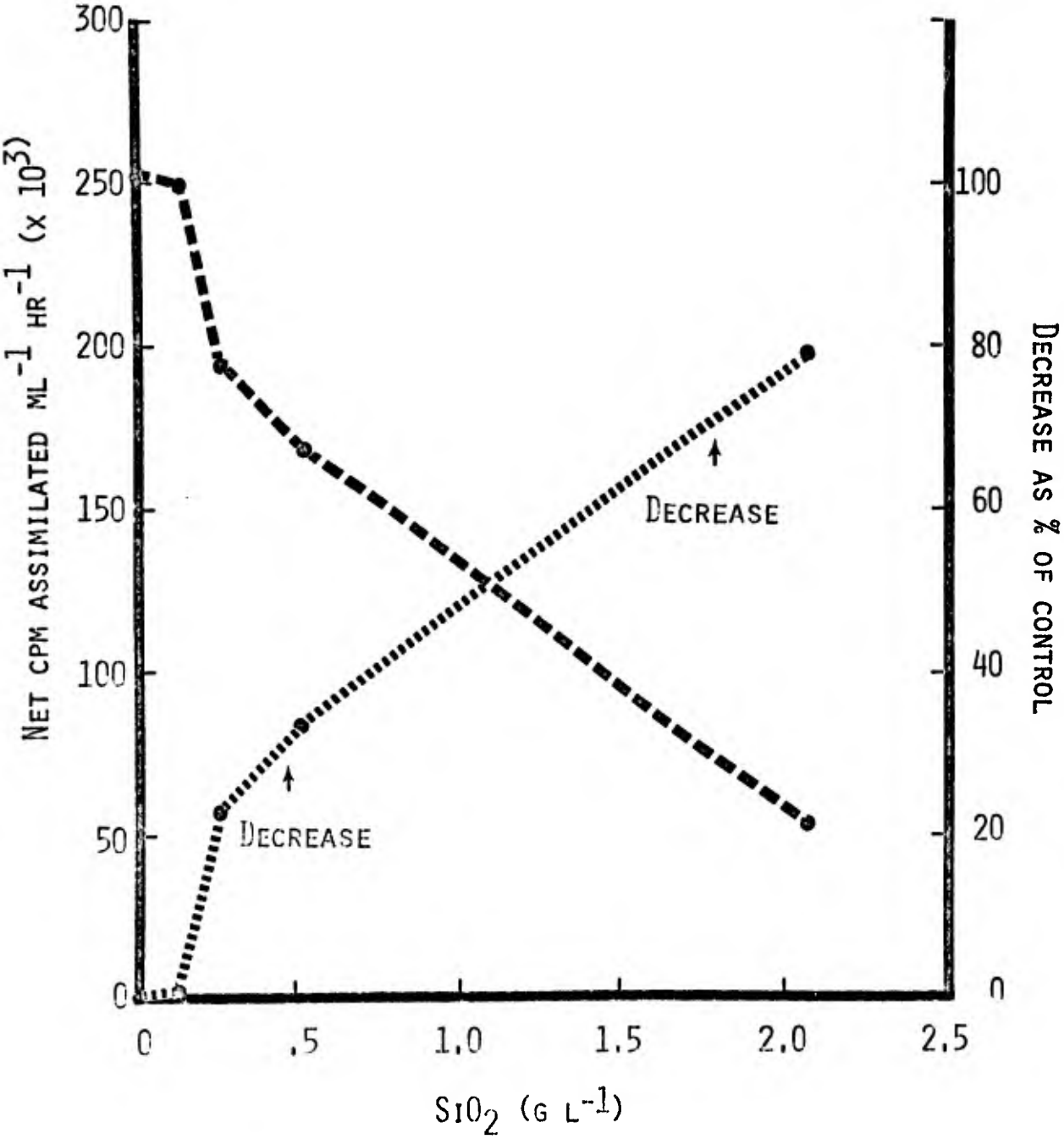

300
250
200
150
100
50
0
NET CPM ASSIMILATED ML$^{-1}$ HR$^{-1}$ (x 10$^3$)
100
80
60
40
20
0
DECREASE AS % OF CONTROL
DECREASE
DECREASE
0
.5
1.0
1.5
2.0
2.5
SiO$_2$ (G L$^{-1}$)

Fig. 36. The effect of increasing concentrations of silicon dioxide (all particles <15 $\mu$m) on carbon assimilation of Chlorella sp.

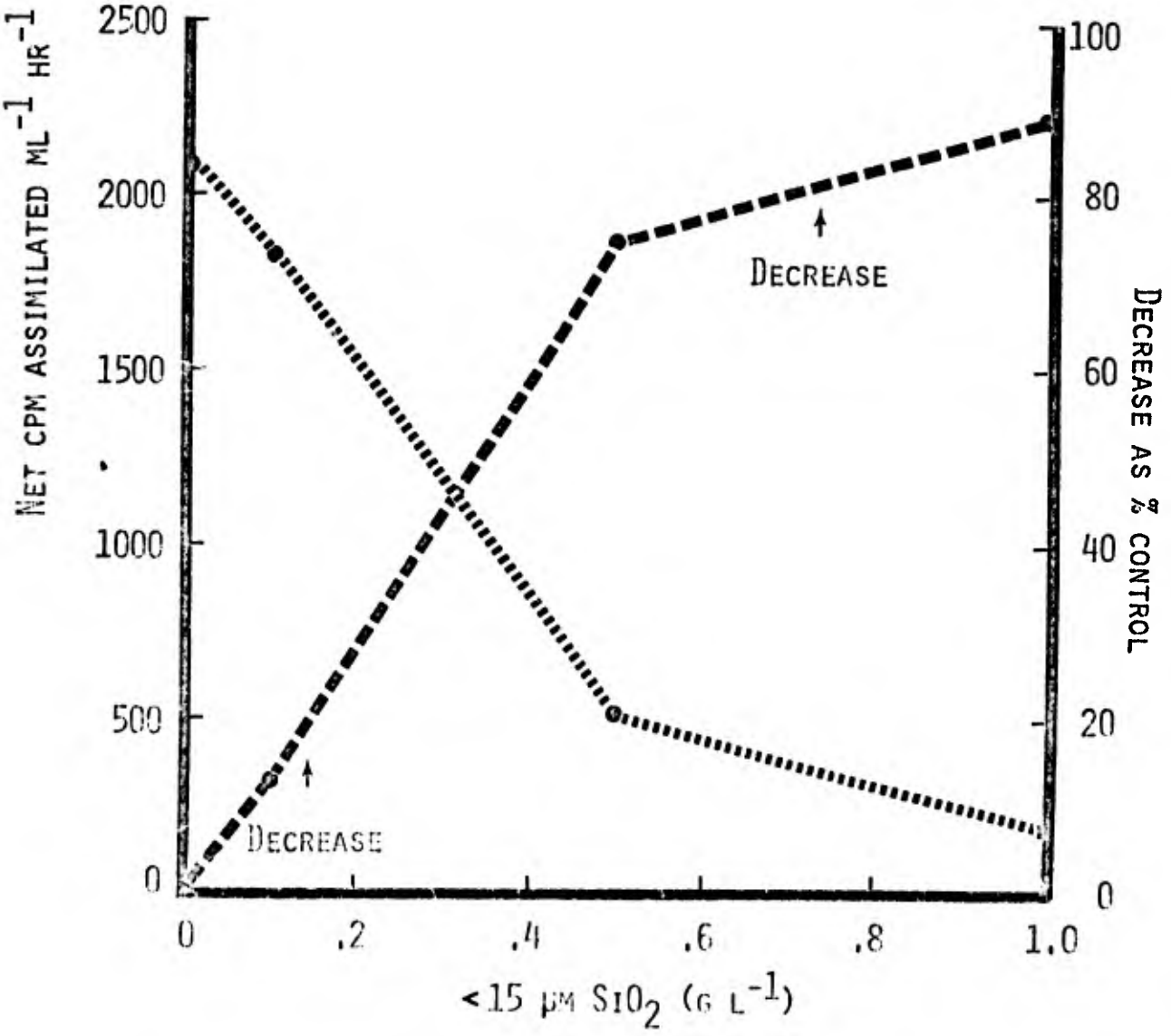

NET CPM ASSIMILATED ML$^{-1}$ HR$^{-1}$
2500
2000
1500
1000
500
0
100
80
60
40
20
0
DECREASE AS % CONTROL
DECREASE
DECREASE
0
.2
.4
.6
.8
1.0
<15 µM SiO$_2$ (G L$^{-1}$)

Fig. 37. The effect of increasing concentrations of silicon dioxide (all particles <15$\mu$m) on carbon assimilation of Nannochloris sp.

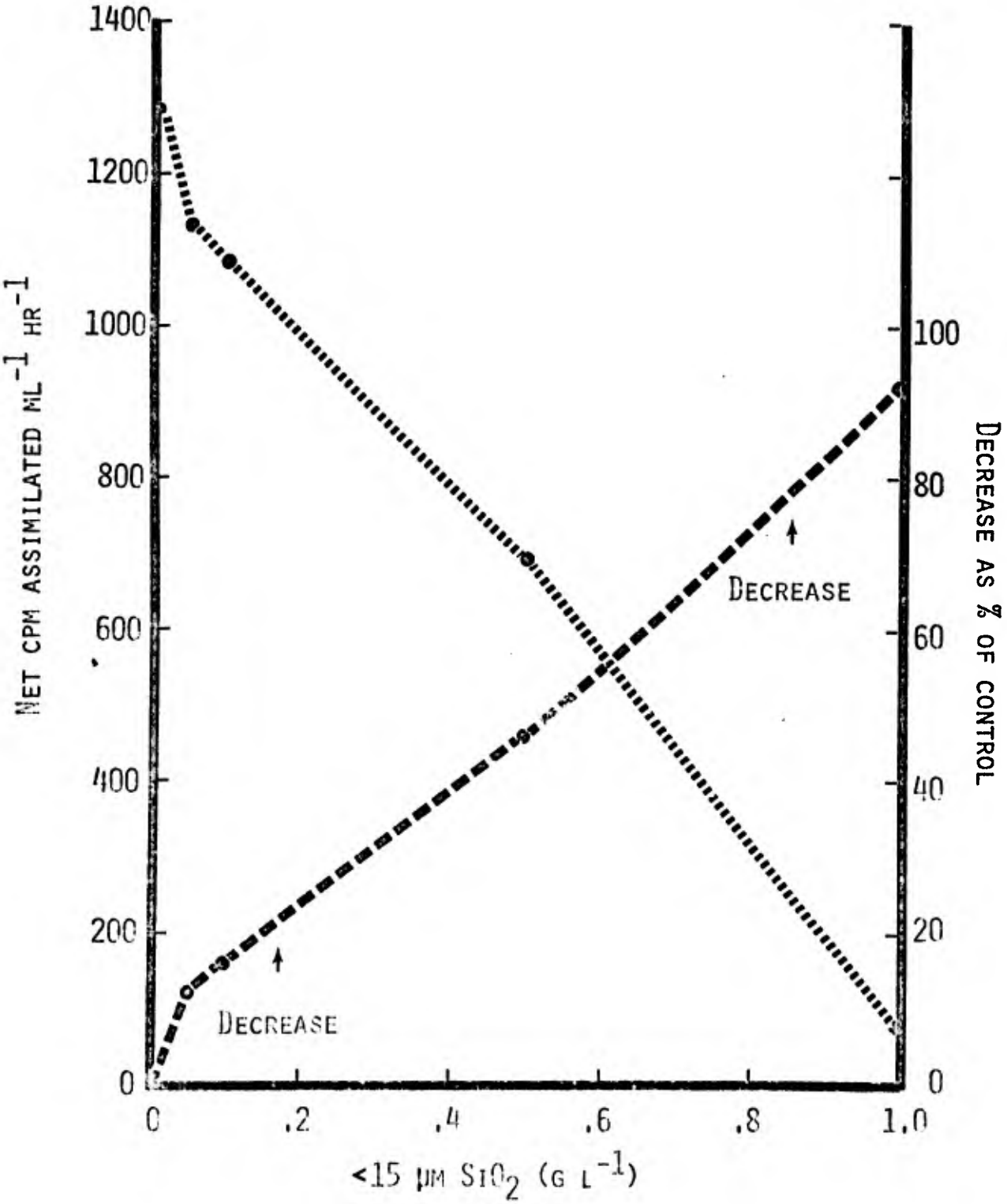
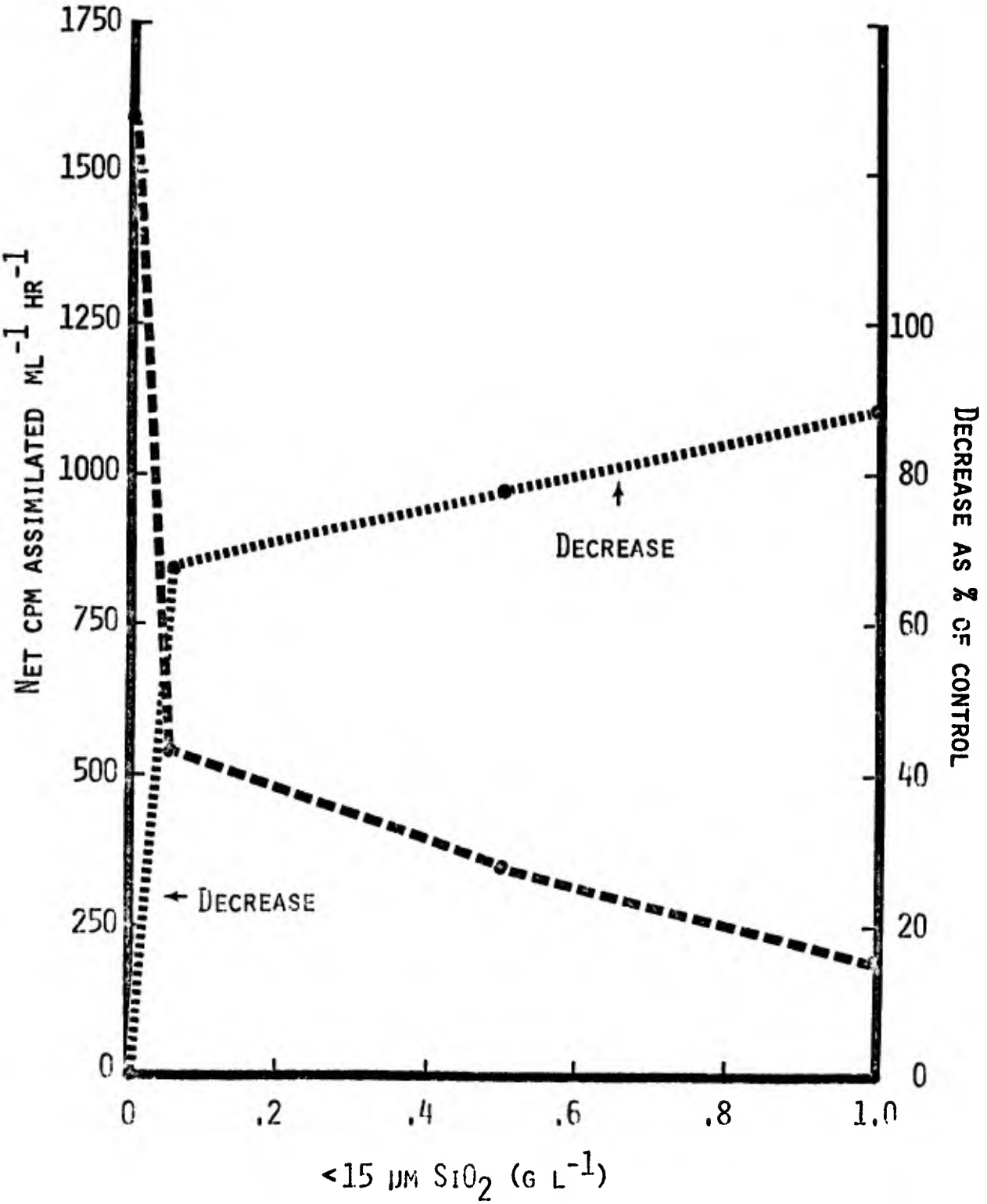

1400
1200
1000
800
600
400
200
0
NET CPM ASSIMILATED ML$^{-1}$ HR$^{-1}$
100
80
60
40
20
0
DECREASE AS % OF CONTROL
DECREASE
DECREASE
0
.2
.4
.6
.8
1.0
<15 µM $SiO_2$ (G L$^{-1}$)

Fig. 38. The effect of increasing concentrations of silicon dioxide (all particles <15 μm) on carbon assimilation of Stichoccoccus sp.


1750
1500
1250
1000
750
500
250
0
NET CPM ASSIMILATED ML$^{-1}$ HR$^{-1}$
100
80
60
40
20
0
DECREASE AS % OF CONTROL
DECREASE
DECREASE
0
.2
.4
.6
.8
1.0
<15 µM $SiO_2$ (G L$^{-1}$)

The magnitude of the effects of $SiO_2$ on M. lutheri was apparently less than for either Nannochloris sp. or Chlorella sp. (cf. Figs. 35, 36, and 37). However, the median size of particles to which M. lutheri was exposed was about 17 µm. The median size of particles to which the other two species were exposed was 6.2 µm. Evidently, the lesser effect on carbon uptake observed with M. lutheri was due in part to the lesser attenuation of light by the larger particles at concentrations identical with those for the other two species. A concentration of 2,250 mg $l^{-1}$ $SiO_2$ was required to cause an 80% reduction in carbon uptake with M. lutheri. At a concentration of 1,000 mg $l^{-1}$ this reduction was approximately 50%. For the other three species tested in the finer $SiO_2$ size distribution, the effect at 1,000 mg $l^{-1}$ was almost 90% reduction. Over twice the concentration of particles with a median size of 17 µm was required to produce an identical reduction in carbon uptake as those particles with a median size (6.2 µm) which was slightly greater than one third as large for different species.

In addition to this lesser attenuation effect of the distribution with the larger median size, the motility of M. lutheri must be taken into account. As light intensity was progressively attenuated, the cells of this species could have moved towards the sides of the incubation bottles, i.e., nearer to the source of incident light. The result of this movement could have contributed substantially to the improved carbon uptake, although still a drastic reduction, at the higher concentrations of $SiO_2$.

Regardless of the size distribution relationships, the reductions in carbon uptake which have been demonstrated for concentrations between 100 and 500 mg $l^{-1}$ are biologically significant with respect to reduced

energy available to filter-feeding organisms (see Chapter 8). Concentrations of particles with similar size distributions are typical of those which occur during dredging and disposal operations (May, 1973, see Table D-3, APPENDIX D; Masch and Espey, 1967) during storm reagitation (e.g., Tropical Depression Agnes 1972, see Chapter 1), or as normal "high" background values in Galveston Bay (Masch and Espey, 1967) and Louisiana marshes (Mackin, 1961).

Additional factors associated with the resuspension of solids in the water column during disposal activities may be the release of organic matter and nutrients which can stimulate growth (Flemer, 1970; Odum, 1963), although the magnitude of release is questionable (May, 1973) or dilution could be very rapid. Additional releases of detrimental substances during resuspension, sorbed heavy metals and pesticides, could occur for incorporation by various biological pathways and biological magnification in the trophic structure of estuarine systems. No apparent difficulty was evident in our carbon uptake studies from these additional factors, since we used the relatively "inert" $SiO_2$ particles (see APPENDIX D). Nevertheless, future work should be directed toward illucidation of the effects of these factors in addition to those of light attenuation on the productivity of other phytoplankton species, groups of species, and natural populations. Necessarily, this work will have to be conducted at a more sophisticated level than was attempted here, especially when studies are conducted with more than one species or with natural sediment effects on carbon assimilation of phytoplankton.

EFFECTS OF SUSPENDED AND DEPOSITED SEDIMENTS

ON ESTUARINE ORGANISMS PHASE II

CHAPTER 8

# EFFECTS OF SUSPENDED SEDIMENTS ON FEEDING ACTIVITY OF THE COPEPODS EURYTEMORA AFFINIS AND ACARTIA TONSA

## I. INTRODUCTION

Suspension feeding organisms that live in estuaries can be exposed to suspended sediment concentrations which tend to be quite high at times. Mechanical or abrasive action of suspended particles is important to these suspension feeders with respect to gill clogging, impairment of respiratory and excretory functioning, and feeding activity. In strongly agitated water, as during storms or dredging and disposal activities, a substantial portion of material suspended in the water column can consist of fine sand, silt, and clay-sized particles which have been resuspended from the bottom. Considerable quantities of these inorganic (mineral) solids together with particulate food can enter the gut of filter-feeding organisms which inhabit the water column.

In this chapter we present the results of studies which have investigated the interference of suspended mineral solids and resuspended natural sediments with the feeding activity of two major zooplankton species of the Chesapeake Bay system: the calanoid copepods Eurytemora affinis and Acartia tonsa.

## II. MATERIALS AND METHODS

### 1. Zooplankton Cultures

Cultures of *E. affinis* and *A. tonsa* were made available to this project by Dr. Don Heinle and Mr. Joseph Ustach, Chesapeake Biological Laboratory, Solomons, Maryland. Cultures were held in a constant temperature incubator at 20°C under constant cool white fluorescent illumination ($0.8 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$) in Erlenmeyer flasks (2 liter capacity) containing 2 liters of Patuxent River water (salinity range 5.5 to 7.5 °/oo which had been filtered previously through sintered Whatman GFC glass fiber filter pads.

The zooplankters were fed initially a mixed diet of the motile chrysophyte *Monochrysis lutheri* and the bacillariophyte *Phaeodactylum tricornutum*. Cell concentrations of these phytoplankters were maintained within the range $5 \times 10^4$ and $1 \times 10^5$ cells $ml^{-1}$ in the zooplankton cultures. However, spurious feeding rate values during initial feeding experiments coupled with the "blooming" tendency of the *P. tricornutum* in the zooplankton cultures, caused us to limit the diet of these cultures to *M. lutheri*, especially during the days preceding a feeding rate experiment.

Every third or fourth day, one third to one half of the water in each culture flask was replaced with new glass-fiber filtered Patuxent River water of the same salinity. At the same time additional phytoplankton cells were added to the zooplankton culture flasks to make up for (1) the amounts removed and dilution of the remaining cells with new filtered Patuxent water, and (2) the amounts grazed by the growing culture.

Efforts were made to keep each culture in a state of rapid growth so that a constant supply of adults of both species (both sexes) would be available for experimental work. However, A. tonsa tended towards cannibalism when the adult population in each flask exceeded between 20 and 30 individuals. As a result, the culture population would boom and crash unexpectedly. This necessitated maintenance of this species in many culture flasks, and required constant monitoring of available algal food supply and water quality as well as adult populations in the flasks.

2. Phytoplankton Cultures

Cultures of the phytoplankters Monochrysis lutheri and Phaeodactylum tricornutum were maintained in exponential growth phase in filtered, sterile (autoclaved) Patuxent River water enriched with f/2 medium as described in Chapter 7.

3. Sediments

Some physical and chemical characteristics of sediment types used for experimental work in this chapter are presented in Appendix D. The sediment types and concentrations used for each zooplankton species were as follows:

Eurytemora affinis

1. $SiO_2$ (50, 100, 1,000, 10,000 mg $l^{-1}$).
2. <15 $\mu$m $SiO_2$ (50, 100, 500, 1,000 mg $l^{-1}$).
3. Fuller's earth (50, 100, 500, 1,000 mg $l^{-1}$).
4. Natural sediment (50, 100, 250, 500, 1,000 mg $l^{-1}$).

Acartia tonsa

1. <15 μm $SiO_2$ (50, 100, 500, 1,000 mg $l^{-1}$).
2. Fuller's earth (50, 100, 500, 1,000 mg $l^{-1}$).
3. Natural sediment (50, 100, 500, 1,000 mg $l^{-1}$).

4. Determination of Time to Maximum Ingestion

Feeding studies were conducted using motile food cells (M. lutheri) labeled with carbon-14. Cell motility greatly reduced the need for shaking the incubation bottles during experiments. These studies measured the uptake of radioactivity of zooplankton that had not fed on radioactive food for a time sufficient to produce radioactive fecal material. The method had been outlined in Rigler (1971) and was introduced by Nauwerck (1959) from a slightly modified technique originally used by Marshal and Orr (1955). This method eliminates three significant problems usually associated with feeding rate determinations as follows:

1. It is suitable for measurements of feeding rates of organisms that do not produce coherent fecal pellets.
2. It eliminates the need for estimates of the leaching of tracer carbon out of fecal pellets.
3. It reduces the error associated with excretion of tracer carbon because some ingested food cells will remain undigested at the end of the short feeding period.

Approximately 10.0 μCi $NaHC^{14}O_3$ was injected into each of two ground glass-stoppered bottles (130 ml capacity) containing high cell concentrations of actively growing Monochrysis lutheri. These cells were incubated under

cool white fluorescent illumination at 20°C for 4 hr. After incubation, the cells were centrifuged at 6,000 rpm for 10 min. Supernatant water was decanted and the pellet was resuspended in enriched (f/2 medium), sterile (autoclaved) Patuxent River water which had been previously filtered through sintered Whatman GFC glass fiber filters (see Chapter 7). The cells were then recentrifuged, the supernatant decanted and the pellet resuspended a second time. This procedure effectively removed most of the unassimilated dissolved inorganic carbon-14 and excreted fixed carbon compounds which had accumulated in the bottles during the incubation period. After the second resuspension with filtered, sterile, enriched Patuxent River water, cell concentration was determined by counting at 100X on an improved Neubauer hemacytometer. The radioactivity of a 10 ml aliquot of this cell suspension was determined by the acidification and bubbling procedure of Schindler, Schmidt, and Reid (1972) outlined in Chapter 7.

During the phytoplankton incubation period, replicate pairs of ground glass-stoppered bottles (130 ml capacity) were prepared for containing the zooplankton during the feeding experiment. Each pair of bottles represented a specific interval of a time series of feeding activity for zooplankton exposed to radioactive phytoplankton cells (food). These intervals were 5, 10, 15, 20, 30, 45, and 60 min. or longer depending upon the number of adults of either species which was available for experimental use. Each bottle was rinsed twice with 0.01 N $H_2SO_4$ to remove any carbon-14 from previous experimental work, rinsed twice with deionized glass distilled water, rinsed twice with filtered (Whatman CFC glass fiber filters)

Patuxent River water, and filled with 50 ml of the filtered river water (salinity range 5.5 to 7.5 °/oo).

Adult zooplankton individuals (both sexes) of whichever species was being investigated were "captured" individually from the culture flasks in a minimum amount of water (usually less than 5 ml per individual) with a glass tube fitted with a rubber bulb at one end which provided suction for drawing the copepod into the tube. Ten copepods were transferred into each bottle. The water volume of each bottle was adjusted to 125 ml with water from the zooplankton culture flasks containing M. lutheri. In this way, phytoplankton cell concentrations (not radioactive) in the bottles generally ranged between 5,000 and 10,000 cells $ml^{-1}$. Zooplankters were allowed to feed on these cells for 0.5 to 1.0 hr before the start of the experiment. During this time they also became somewhat acclimated to the environment of the bottle.

The feeding experiment was initiated by injecting a volume of radioactive M. lutheri cell suspension into one bottle of a replicate pair of bottles which contained 10 zooplankters each. The other bottle in this pair received an identical volume of the radioactive cells five minutes later. The reason for this procedure will become evident in the following paragraphs. The volume of water containing these cells never exceeded 1% of bottle volume (125 ml), but was sufficient to provide a final radioactive cell concentration of as close to 50,000 cells $ml^{-1}$ as possible. Experiments were conducted at 20 $\pm$ 2°C under laboratory cool white fluorescent illumination (approximate intensity = $0.2 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$).

Feeding activity was terminated by pouring the contents of each bottle onto a stainless steel (type 304) wire sieve (325 mesh). The bottles were rinsed with 50 ml of Whatman GFC filtered Patuxent River water. This water was then poured through the sieve. Preliminary studies showed that this 50 ml rinse effectively washed away sorbed carbon-14 and phytoplankton cells adhering to the zooplankters which were trapped on the stainless steel mesh. Immediately after rinsing, the sieve was placed under a dissecting microscope, and the live zooplankters were removed individually by grasping one of the antennae with a fine-pointed forceps. Each organism was placed into a separate liquid scintillation vial containing one ml of Soluene-100 (Packard Instrument Co., Downers Grove, Ill.), a rapid acting tissue solubilizer (quarternary amine). The vials were capped and digestion was allowed to proceed for at least 2 hr at room temperature. With practice all zooplankters (10 under optimal experimental conditions) could be efficiently removed from the sieve and placed into liquid scintillation vials within 2 min. of termination of feeding.

The zooplankters in successive replicate pairs of bottles representing longer time intervals of feeding were handled in exactly the same way (5 min. lag between bottles of a replicate pair).

At the end of the digestion period, 15 ml of acidified Instagel were added to each vial. Acidified Instagel was prepared as a 1:9 (v/v) solution of 0.5 N HCl and Instagel (Packard Instrument Co., Downers Grove, Ill.), a complete liquid scintillation counting fluid. The vials were placed into the Packard Tricarb (Model 3320) liquid scintillation spectrometer and counted at 9°C after allowing 24 hr for reduction of chemiluminescence, an

undesirable feature of this counting fluid formulation. After background subtraction, counts were corrected for quenching by an external standard quench curve constructed from counts of standard toluene C-14 with increasing amounts (0 to 400 µl) of chloroform in a solution of 15 ml of acidified Instagel and one ml of Soluene-100. Counting efficiency of most samples was approximately 88%.

Results are reported as average carbon uptake (counts $min^{-1}$ $hr^{-1}$) of twenty zooplankters in the paired replicate bottles (10 from each bottle) plotted against the time interval of feeding.

5. Determination of the Effects of Increasing Concentrations of Different Sediment Types on Zooplankton Feeding Activity at Time of Maximum Ingestion

The times to maximum ingestion (maximum uptake of carbon-14) of radioactive Monochrysis lutheri cells (50,000 cells $ml^{-1}$) at 20 ± 2°C under approximately 0.2 x $10^4$ ergs $cm^{-2}$ $sec^{-1}$ light intensity in a salinity range of 5.5 to 7.5 °/oo were determined to be 5 min. for adult Acartia tonsa and 10 min. for adult Eurytemora affinis (see Figs. 39 and 40).

Slurries of different sediment types (see 3. Sediments, this chapter) were prepared and maintained as outlined in Chapter 7, except that the filtered Patuxent River was was not enriched with f/2 medium. Slurries of natural sediment were well aerated, to a light tan color, before use in these experiments.

Phytoplankton, zooplankton, and scintillation vials were prepared as in the preceding section with slight procedural changes as follows:

a. For E. affinis, replicated experimental sets with series of increasing concentrations were conducted with the two different size distributions of $SiO_2$ (see APPENDIX D). For fuller's earth only one experimental run was made, but all bottles were replicated. For natural sediment two experimental runs were made with controls replicated only.

b. For A. tonsa, two experimental runs with replicated controls were made with fuller's earth and $SiO_2$ (<15 μm). Only one experimental run was made with natural sediment for this species due to a shortage of adults.

c. The time course of these feeding experiments with increasing concentrations of each sediment type was 5 min. for A. tonsa and 10 min. for E. affinis.

d. After the zooplankters were placed into the bottles and acclimated for 0.5 to 1.0 hr as before, appropriate amounts (by volume) of sediment slurry were added by precision micropipet to successive bottles (pairs in some cases) to create an increasing series of sediment concentrations. The amount added for the maximum sediment concentration did not exceed 1% of bottle volume. Then, an appropriate volume of radioactive M. lutheri cells was added by micropipet, as before, to each bottle to provide a cell concentration of 50,000 cells $ml^{-1}$.

The natural sediments and mineral solids used in these experiments were so fine-grained that no settling of particulate material occurred during

the short time of these experiments. The bottles containing the zooplankton were not shaken to maintain these solids in suspension.

Preliminary work showed that the natural sediments and mineral solids used in these experiments did not sorb carbon-14 counts that were significantly higher than background.

Results are reported as reduction in carbon uptake. The reduction is the difference between the uptake in control and sediment treatments, and is expressed as a percent of the control value. The differences between carbon uptake in controls and sediment treatments were tested for significance by Student's "t" test (Snedecor and Cochran, 1967). Values for individual zooplankters were coded and logarithmically transformed before testing was done.

6. Determination of the Effects of Suspended Patuxent River Sediment (500 mg $l^{-1}$) on Zooplankton Feeding Activity with Increasing Time of Exposure

The procedure for this determination was identical with that used for the determination of time to maximum ingestion except for the following:

a. Replicate pairs of bottles were not used because of the extensive nature of the time series (5, 10, 15, 30, 45, and 60 min., 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 3.75, and 4.0 hr). Instead, two sets of bottles (one control and one sediment treatment), 10 adult zooplankters per bottle, were used at each interval in this time series.

b. An amount of natural sediment slurry to make a concentration of 500 mg $l^{-1}$ was added to each bottle in the sediment treatment set.

Then, radioactive M. lutheri cells were pipetted simultaneously into a control bottle and its corresponding sediment treatment bottle for a specific feeding time interval. This procedure was continued throughout the time series of control and treatment bottle pairs. Bottle pairs for the long time intervals were inverted every 15 minutes to maintain the particles in suspension.

Results are reported as cells ingested per organism per day (cells $org^{-1}$ $day^{-1}$). This daily rate (f) was calculated from the formula

$$f = C(R_2 \times 24)/(R_f \times t)$$

where

$R_2$ = average activity (counts per minute) for zooplankters exposed to the sediment concentration for a given time.

$R_f$ = average activity (counts per minute) of food cells (M. lutheri) in one milliliter of suspension.

t = feeding time in hours.

C = average concentration of food cells per milliliter over the feeding periods.

These values were plotted against feeding time. The differences between sediment treatment and control values at each feeding time were tested for significance with Student's "t" test after logarithmic transformation. Before sample counts per minute were used in any calculations, they were corrected by background subtraction and quench corrected from a curve constructed as before. This curve related machine counting efficiency of standard toluene C-14 to counts of an external standard during addition

of increasing amounts of a severe quenching agent (chloroform) to acidified Instagel-Soluene-100 liquid scintillation counting fluid.

## III. RESULTS AND INTERPRETATION

### 1. Time to Maximum Ingestion

The shortest time in which a food particle could pass through the gut of Eurytemora affinis was demonstrated by a decrease in uptake of radioactivity for feeding periods in excess of ten minutes (Fig. 39). These results are in good agreement with those of Heinle (unpublished) who has noted formation of fecal pellets at ten minutes after start of feeding for this species. Filtering rates for this species are lower than those reported for similar organisms by Anraku (1964). This may have been caused by decrease in size of adults as the age of our cultures increased.

The shortest time in which a food particle could pass through the gut was determined to be five minutes for adult individuals of Acartia tonsa (Fig. 40).

In Fig. 39 there are two inflection points in the first hour of feeding. In Fig. 40 preliminary work showed that the first inflection point occurred between 0 and 5 min. These inflections are similar to those reported by Bourne (1959) for Daphnia magna feeding on 60,000 to 250,000 cells $ml^{-1}$ of Chlorella vulgaris at 10 to 20 min. and again at 40 to 55 min. (cf. Figs. 39 and 40), depending upon the cell concentration. Bourne's data demonstrated that the inflection of the carbon uptake curve at 40 to 55 min. was associated with the onset of defecation following initial filling of the gut.

Fig. 39. Ingestion of radiocarbon-labeled *Monochrysis lutheri* by adult *Eurytemora affinis* in water previously filtered through sintered Whatman GFC glass fiber filters.

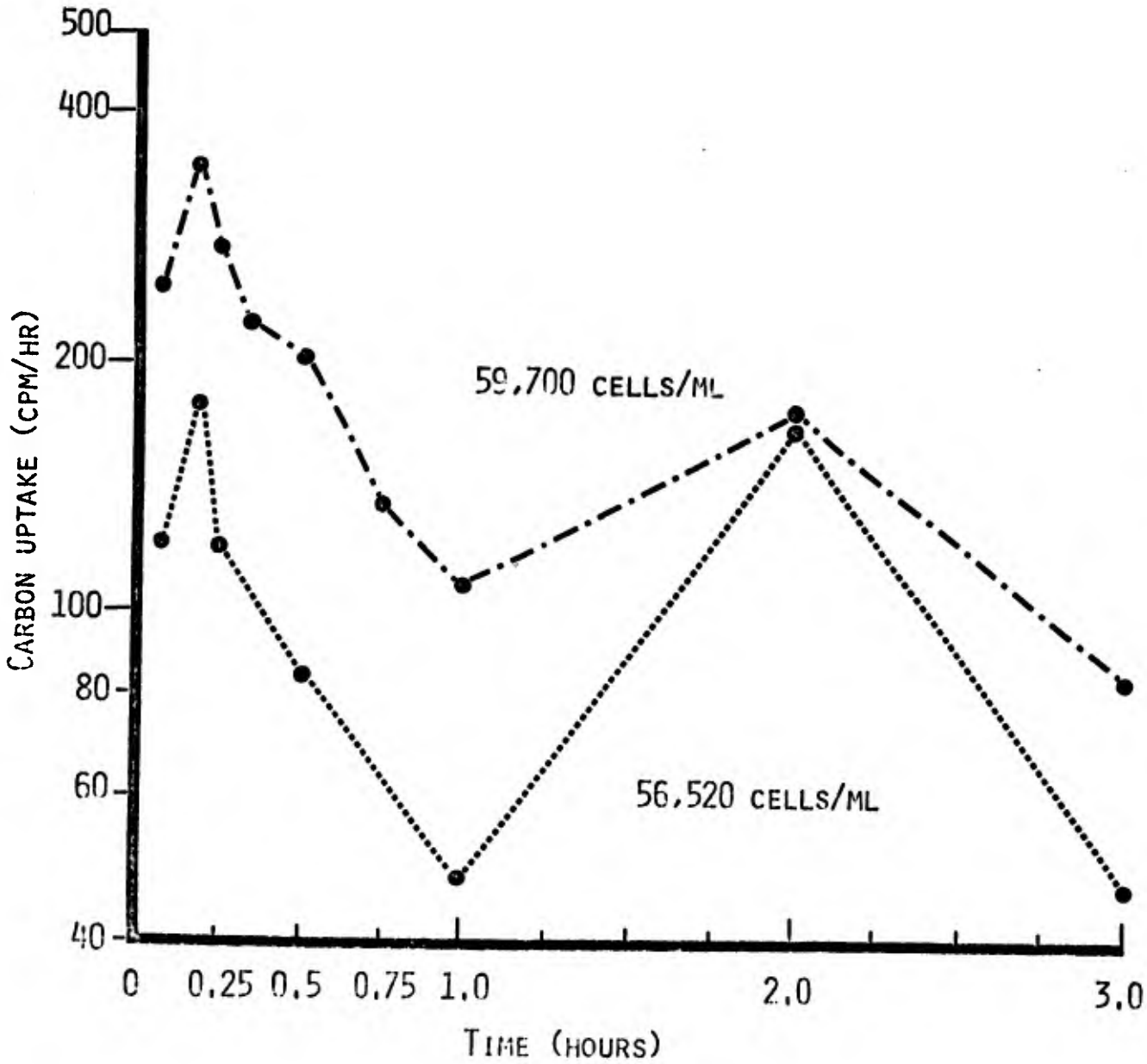
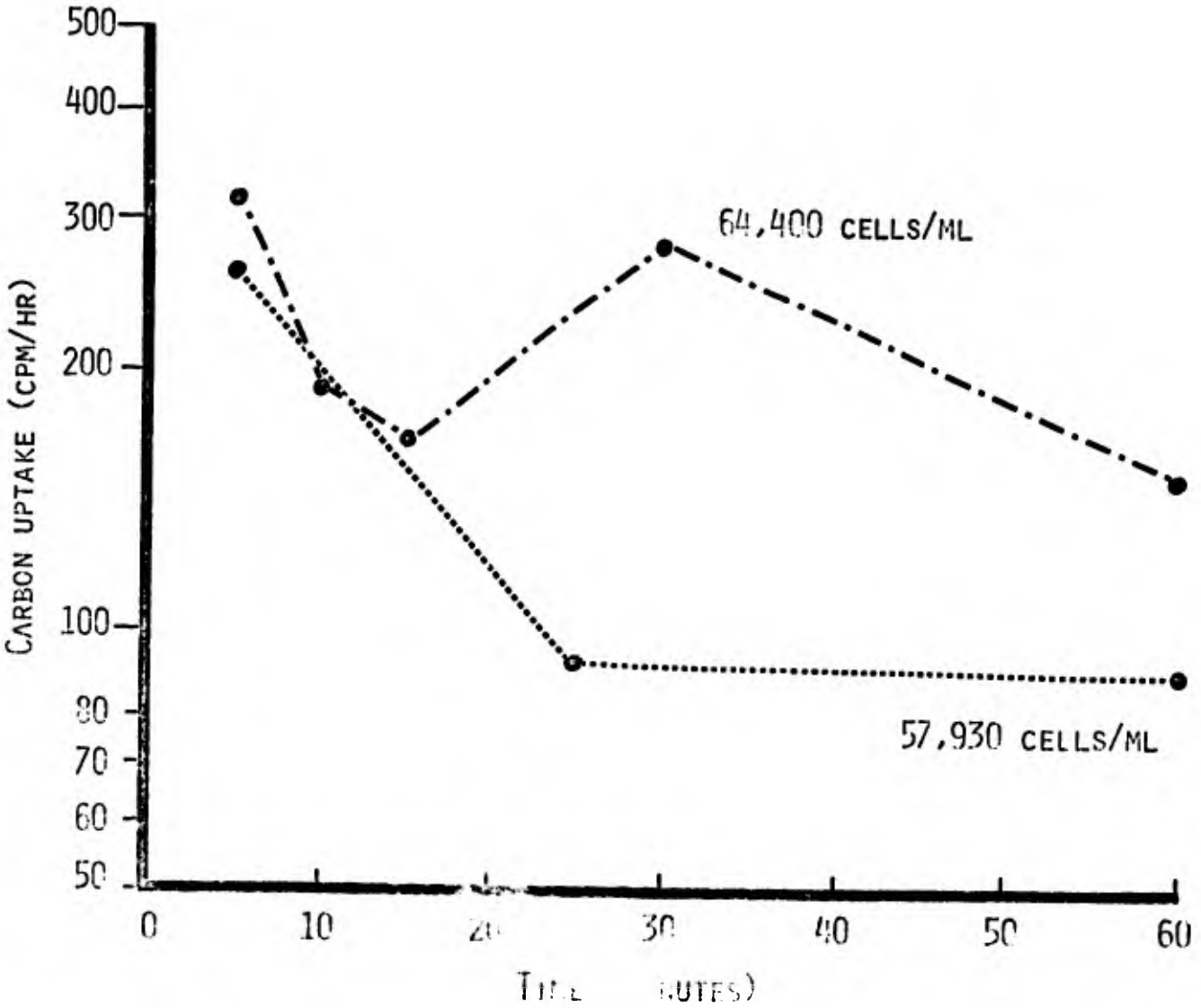

500
400
200
100
80
60
40
CARBON UPTAKE (CPM/HR)
59,700 CELLS/ML
56,520 CELLS/ML
0
0.25
0.5
0.75
1.0
2.0
3.0
TIME (HOURS)

Fig. 40. Ingestion of radiocarbon-labeled Monochrysis lutheri by Acartia tonsa (adults, mixed sexes) at 20°C in water previously filtered through sintered Whatman GFC glass fiber filters.


500
400
300
200
100
80
70
60
50
CARBON UPTAKE (CPM/HR)
64,400 CELLS/ML
57,930 CELLS/ML
0
10
30
40
50
60

McMahon and Rigler (1963) found that the initial peak represented filling of the oral groove of this species. This collecting rate was higher by about 5% than the actual ingestion rate. However, the total time of these types of experiments, i.e., the time between initial exposure of zooplankton to radioactive food and their removal from the water for counting, must be less than the time for gut passage as sizable errors are introduced at longer intervals due to excretion and respiration of carbon-14 (Rigler, 1971). We chose the 5 and 10 min. experiment time intervals for A. tonsa and E. affinis to coincide with Heinle's observations of fecal formation in these two species.

2. Effects of Suspended Solids on Feeding Activity at Maximum Ingestion Time

Our results demonstrate that considerable quantities of inorganic material along with particulate food can interfere with ingestion of food by the copepods Eurytemora affinis and Acartia tonsa. Additions of two different particle size distributions of fine sand, fuller's earth, and Patuxent River silt to a cell suspension of heavily labeled ($NaHC^{14}O_3$) Monochrysis lutheri (50,000 cells $ml^{-1}$) significantly reduced the maximum ingestion rate of these cells by E. affinis at solids concentrations in excess of 250 mg $l^{-1}$ (Fig. 41, Table 27). At 100 mg $l^{-1}$ a significant enhancement of uptake was evident in fuller's earth. Some non-significant reductions at this concentration occurred for other particle types (Fig. 41, Table 27).

The effects of Patuxent River silt caused a larger reduction in ingestion of radiocarbon-labeled cells than other particle types over all concentrations

Fig. 41. Reduction in maximum ingestion rate of adult Eurytemora affinis feeding on radiocarbon-labeled Monochrysis lutheri at increasing concentrations of Patuxent River silt, fuller's earth, $SiO_2$, and <15 $\mu$m $SiO_2$.

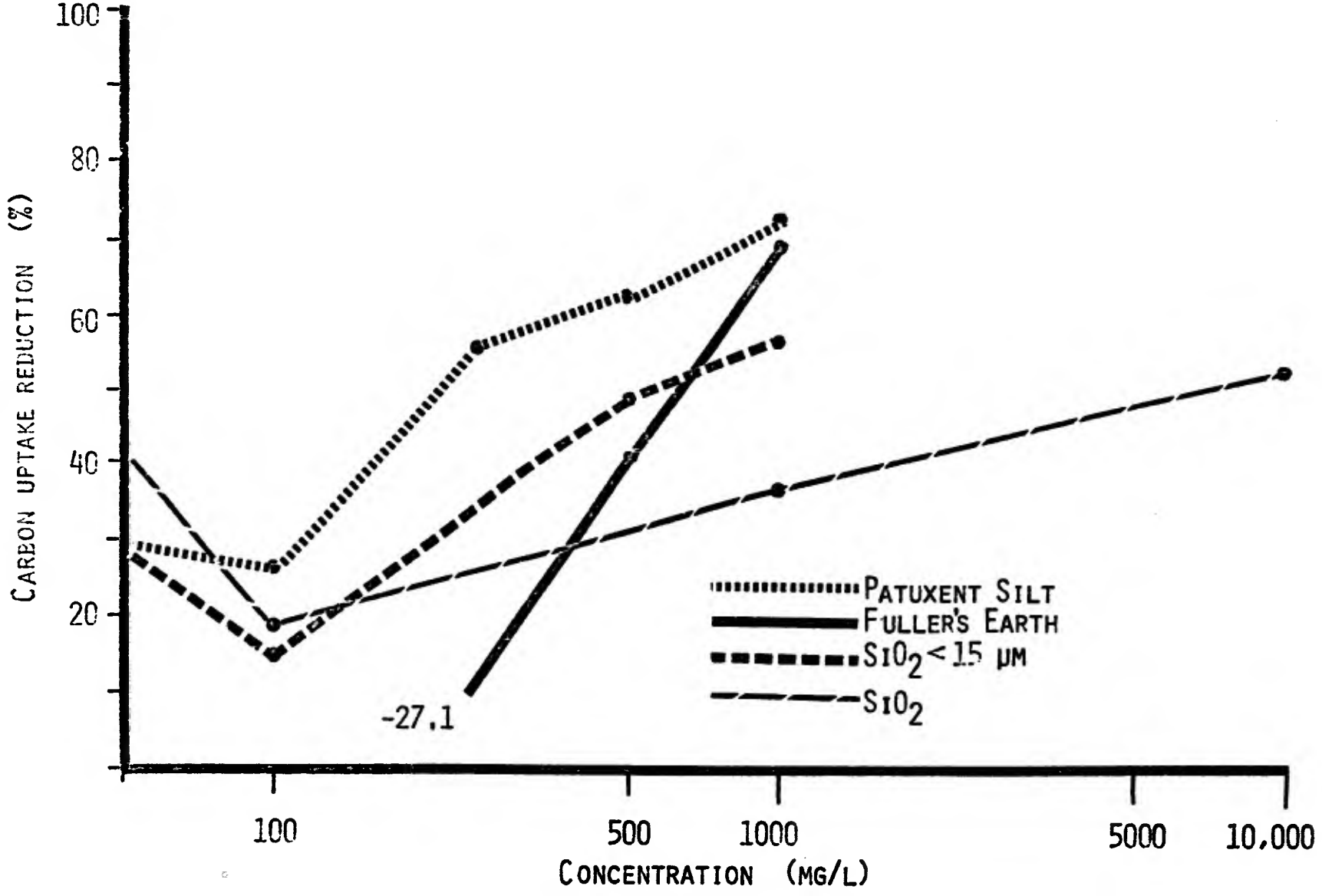

100
80
60
40
20
CARBON UPTAKE REDUCTION (%)
-27.1
PATUXENT SILT
FULLER'S EARTH
$SiO_2$ < 15 µM
$SiO_2$
100
500
1000
5000
10,000
CONCENTRATION (MG/L)

Table 27. Effect of increasing concentrations of different sediment types on uptake of radioactively labeled *Monochrysis lutheri* by *Eurytemora affinis*. Mean values are cpm taken up in 10 minutes.

| Concentration (mg/l) | $SiO_2$ $\bar{X} \pm S$ (N) | Reduction (% of control) | <15 µm $SiO_2$ $\bar{X} \pm S$ (N) | Reduction (% of control) | Fuller's Earth $\bar{X} \pm S$ (N) | Reduction (% of control) | Natural Sediment $\bar{X} \pm S$ (N) | Reduction (% of control) |
|---|---|---|---|---|---|---|---|---|
| | Run 1 | | Run 1 | | | | Run 1 | |
| 0 | 2.55 ± 1.51 (20) | 0 | 46.24 ± 17.13 (18) | 0 | 8.14 ± 2.55 (19) | 0 | 47.03 ± 17.07 (20) | 0 |
| 50 | 1.29 ± 0.59 (20) | 49.4 ** | 32.77 ± 11.69 (19) | 29.1 ** | 10.41 ± 2.01 (15) | -27.9 ** | 31.00 ± 9.93 (10) | 34.1 ** |
| 100 | 2.07 ± 1.52 (19) | 18.8 N.S. | 33.84 ± 10. 4 (19) | 26.8 * | 10.35 ± 2.69 (17) | -27.1 ** | 36.50 ± 16.50 (8) | 22.4 N.S. |
| 250 | | | | | | | 13.57 ± 8.47 (8) | 71.1 ** |
| 500 | | | 22.25 ± 11.84 (7) | 51.9 ** | 4.75 ± 2.49 (20) | 41.6 ** | 24.40 ± 10.02 (9) | 48.1 ** |
| 1,000 | 1.52 ± 0.92 (19) | 40.4 * | 19.50 ± 6.87 (10) | 57.8 ** | 2.54 ± 1.25 (20) | 68.8 ** | 9.55 ± 8.94 (10) | 79.7 ** |
| 10,000 | 1.34 ± 0.77 (17) | 47.5 ** | | | | | | |
| | Run 2 | | Run 2 | | | | Run 2 | |
| 0 | 4.10 ± 1.73 (20) | 0 | 73.15 ± 32.09 (18) | 0 | | | 92.57 ± 48.33 (19) | 0 |
| 50 | 2.60 ± 0.97 (20) | 36.5 ** | 50.73 ± 16.16 (18) | 30.7 * | | | 59.43 ± 23.93 (10) | 25.0 |
| 100 | 3.30 ± 1.98 (19) | 19.4 N.S. | 71.23 ± 18.46 (20) | 2.6 N.S. | | | 62.62 ± 21.72 (9) | 32.4 * |
| 250 | | | | | | | 54.68 ± 19.30 (9) | 40.9 * |
| 500 | | | 38.71 ± 13.16 (20) | 47.1 ** | | | 21.24 ± 14.34 (9) | 77.1 ** |
| 1,000 | 2.77 ± 1.25 (19) | 32.4 ** | 33.24 ± 10.91 (18) | 54.6 ** | | | 33.09 ± 11.81 (10) | 64.3 ** |
| 10,000 | 1.90 ± 1.55 (17) | 53.7 ** | | | | | | |

* $p < 0.05$
** $p < 0.01$

tested except at 50 mg $l^{-1}$ (Fig. 41). Interestingly enough, the apparent enhancement effect of the silt at 100 mg $l^{-1}$ was smaller than for all other particle types.

Acartia tonsa exhibited drastic biologically significant reductions in maximum ingestion at all concentrations of all particle types tested (Fig. 42, Table 28). The effects increased with increasing concentration. However, with this species the effect of Patuxent River silt was lower than fuller's earth or $SiO_2$ particles ( <15 µm) except at the highest concentration (1,000 mg $l^{-1}$).

The differences in the shapes of the uptake curves may be related to the habitats in which these species are usually found. E. affinis is typically found in upper, more turbid estuarine areas (Herman, Mihursky, and McErlean, 1968), and may be stimulated to increase ingestion by low concentrations of suspended solids which are found normally in these areas. Low concentrations of suspended solids may be indicative of the presence of food for this species, and in turn the organism is stimulated to begin feeding or to increase its rate of ingestion. The survival value in this case is evident.

Stimulation of pumping activity in the American oyster at low particle concentrations (Loosanoff, 1961), increased activity of Doliolida and Salpida by the presence of suspended particles (Jørgensen, 1966), and tripling of the ingestion rate of algal cells by Artemia sp. upon the addition of fine sand to the cell suspension (Reeve, 1963) lend support to our observation with E. affinis.

Fig. 42. Reduction in maximum ingestion rate of adult Acartia tonsa feeding on radiocarbon-labeled Monochrysis lutheri at increasing concentrations of Patuxent River silt, <15 $\mu$m $SiO_2$, and fuller's earth.

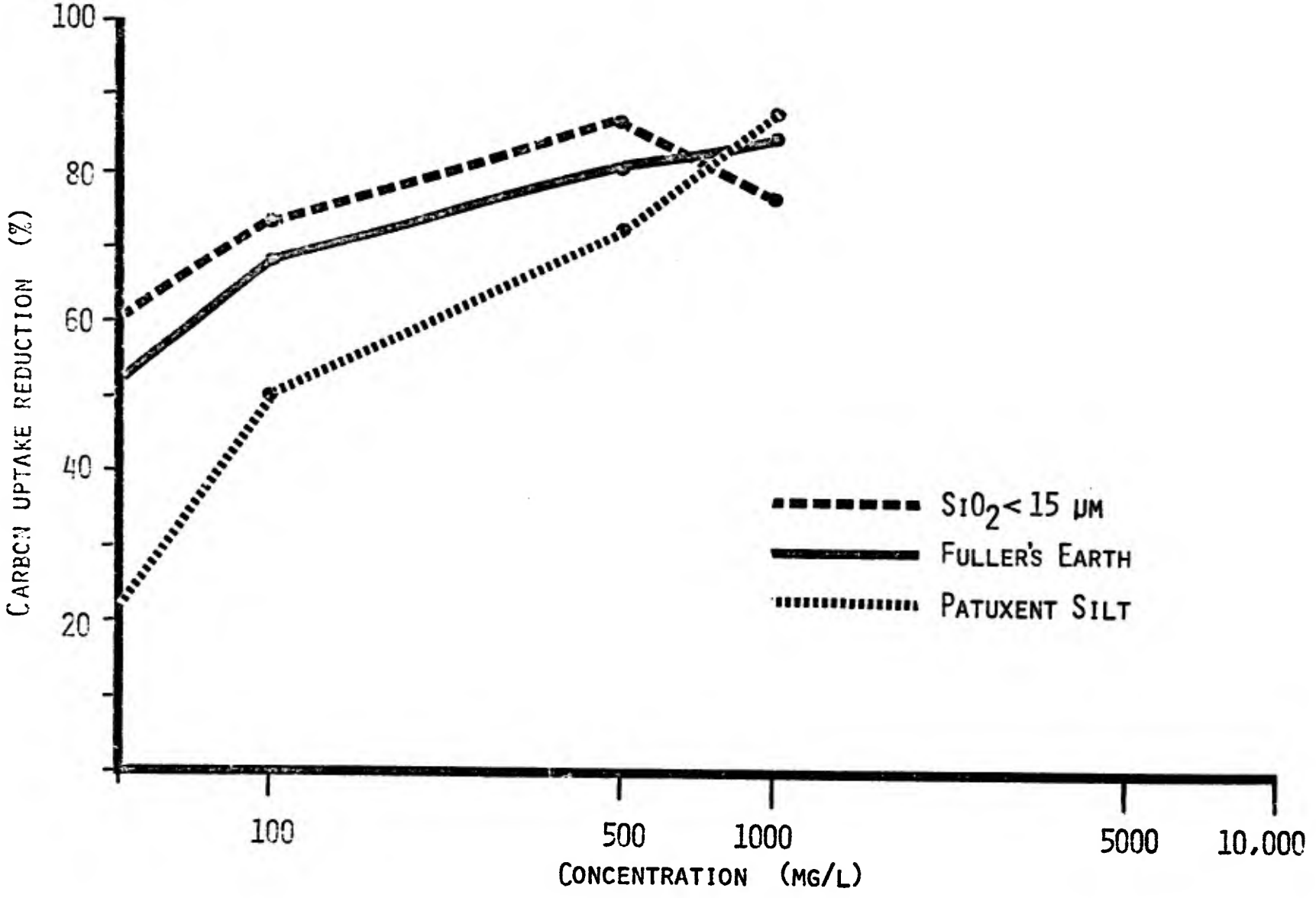

100
80
60
40
20
CARBON UPTAKE REDUCTION (%)
100
500
1000
5000
10,000
CONCENTRATION (MG/L)
$SiO_2$ < 15 µM
FULLER'S EARTH
PATUXENT SILT

Table 28. Effect of increasing concentrations of different sediment types on uptake of radioactively labeled Monochrysis lutheri by Acartia tonsa. Mean values are cpm taken up in 5 minutes.

| Concentration (mg/l) | Natural Sediment $\bar{X} \pm S$ (N) | Reduction (% of control) | Fuller's Earth $\bar{X} \pm S$ (N) | Reduction (% of control) | <15 µm $SiO_2$ $\bar{X} \pm S$ (N) | Reduction (% of control) |
|---|---|---|---|---|---|---|
| | | | Run 1 | | Run 1 | |
| 0 | 12.50 ± 5.85 (7) | 0 | 21.64 ± 9.28 (17) | 0 | 21.64 ± 9.28 (17) | 0 |
| 50 | 9.68 ± 8.00 (8) | 22.6 N.S. | 10.03 ± 7.25 (10) | 53.6 ** | 8.40 ± 4.63 (8) | 61.2 ** |
| 100 | 6.15 ± 3.92 (6) | 50.8 N.S. | 6.74 ± 2.23 (9) | 68.9 ** | 5.56 ± 2.52 (8) | 74.3 ** |
| 500 | 3.39 ± 1.75 (7) | 72.9 * | 4.02 ± 1.05 (7) | 81.4 ** | 2.84 ± 1.21 (8) | 86.9 ** |
| 1000 | 1.49 ± 1.22 (6) | 88.1 ** | 3.28 ± 2.04 (10) | 84.8 ** | 4.97 ± 2.11 (8) | 77.0 ** |
| | | | Run 2 | | Run 2 | |
| 0 | | | 69.72 ± 47.53 (20) | 0 | 69.72 ± 47.53 (20) | 0 |
| 50 | | | 30.08 ± 15.83 (10) | 56.9 ** | 49.96 ± 31.45 (9) | 28.3 N.S. |
| 100 | | | 23.75 ± 8.82 (8) | 66.0 ** | 28.72 ± 30.59 (8) | 58.8 ** |
| 500 | | | 11.76 ± 6.75 (8) | 83.1 ** | 30.24 ± 19.35 (8) | 56.6 ** |
| 1000 | | | 13.80 ± 9.32 (8) | 80.2 ** | 18.07 ± 16.75 (9) | 74.1 ** |

* $p < 0.05$
** $p < 0.01$

On the other hand, A. tonsa is typically found in open, less turbid waters of the Chesapeake Bay system. Apparently, this species is not stimulated to increase feeding activity by low particle concentrations which may be uncharacteristic of its natural habitat (Herman et al., 1968). Because these species are non-selective suspension feeders, the much reduced uptake of radioactive phytoplankton observed with increasing concentrations of suspended solids can be accounted for simply by the ingestion of increasing numbers of unlabeled particles, since the gut of both species was full during all experimental treatments.

Our results are not directly comparable with those of Wilson (1973). The plastic beads he used to feed A. tonsa were optically sized, and he measured uptake of particle populations according to size frequency distribution. The median size (diameter) of food (M. lutheri) particles used in our experiments was 8 to 10 μm (optically measured). Interestingly enough, this size is below the minimum bead size (13.9 μm) which Wilson found that A. tonsa ingested. However, Wilson noted the artificiality of his feeding study with plastic beads which may have eliminated the variables of particle shape and palatability.

Wilson (1973) demonstrated that passive filtering behavior of A. tonsa could not account for the selection of a narrow, variable size range of plastic beads. However, passive filtering did exist because the minimum selected food size did not change with body size. This is typical of a process in which particles are not grasped individually, but are concentrated against closely spaced setae and are handled collectively. Bead sizes outside the selected range were ingested in the same proportion as they were

present in the water column. Therefore, both selective grasping and non-selective filtering, taking all particles indiscriminately, were operating at the same time.

The median sizes (Stokes' diameters) of our sediment treatments are reported by weight and not by optical size frequency distributions. These were: $SiO_2$ (17 μm), <15 μm $SiO_2$ (6.2 μm), natural sediment (<0.8 μm), and fuller's earth ( <0.5 μm). These diameters are equivalent to those of a sphere with the same specific gravity which has settled through a column of water at some specified temperature. Since the fuller's earth particles and those of the natural sediment were not spherical, there may have been a significant departure between values given as equivalent diameter (Stokes') and actual dimensions. In fact, the natural particle size distribution of the Patuxent River sediment was destroyed by our analytical procedure in order to remove the large quantity of organic matter (approx. sp. gr. 1.1) from the inorganic mineral particles (approx. sp. gr. 2.6) before size analysis because of the differences in settling velocities of particles with different densities. In addition, both the natural sediments and the fuller's earth formed fairly large agglomerates when suspended in saline water (microscopical observation).

The equivalent median diameters of the natural sediment (minerals) and the fuller's earth could not be verified by the light microscope, since many of the particles were beyond the detectable size limits of that instrument. However, the particles of $SiO_2$ and fuller's earth were subjected to particle population analyses using a Coulter Model T counter equipped with a 100 μm orifice. This counter verified our observations with the light microscope.

By far, the larger proportion of numbers of particles was present for both of these materials at Coulter volume sizes less than about 8 μm, even though their median equivalent spherical diameter sizes (by weight) were different by a factor of more than 30 (< 0.5 μm for fuller's earth vs 17 μm for $SiO_2$).

These data do not support Wilson's (1973) hypothesis (next section). We have no evidence to show that selective grasping of particles was or was not occurring. However, the reductions in ingestion of food cells with increasing sediment concentrations, which we have demonstrated, lead us to believe that non-selective filtering, taking all particles indiscriminately, was operating in our experiments.

The increase in cell ingestion (decreased reduction) at 100 mg $l^{-1}$ for E. affinis (Fig. 41, Table 27) may have been due merely to a stimulated increase in water filtering rate for this species which inhabits typically turbid estuarine areas.

3. Effects of Patuxent Silt (500 mg $l^{-1}$) on Feeding Activity with Increasing Time

The short term feeding experiments for A. tonsa and E. affinis (5 min. and 10 min., respectively) were extended to 125 min. (Fig. 43, Table 29) and 240 min. (Fig. 44, Table 30) for exposure to 500 mg $l^{-1}$ Patuxent River silt.

The shapes of the curves for A. tonsa (Fig. 43) and the depression in the number of radioactive cells ingested during exposure to the sediment treatment stayed remarkably constant for as long as 125 min. The reduction (77.5%) is almost the same as the reduction in carbon uptake in Table 28 for 500 mg $l^{-1}$ natural sediment (72%). The decrease in cell numbers filtered

Fig. 43. Effect of Patuxent River silt (500 mg $l^{-1}$) on feeding rates of adult Acartia tonsa (org.) feeding on radiocarbon-labeled Monochrysis lutheri cells with increasing time of exposure.

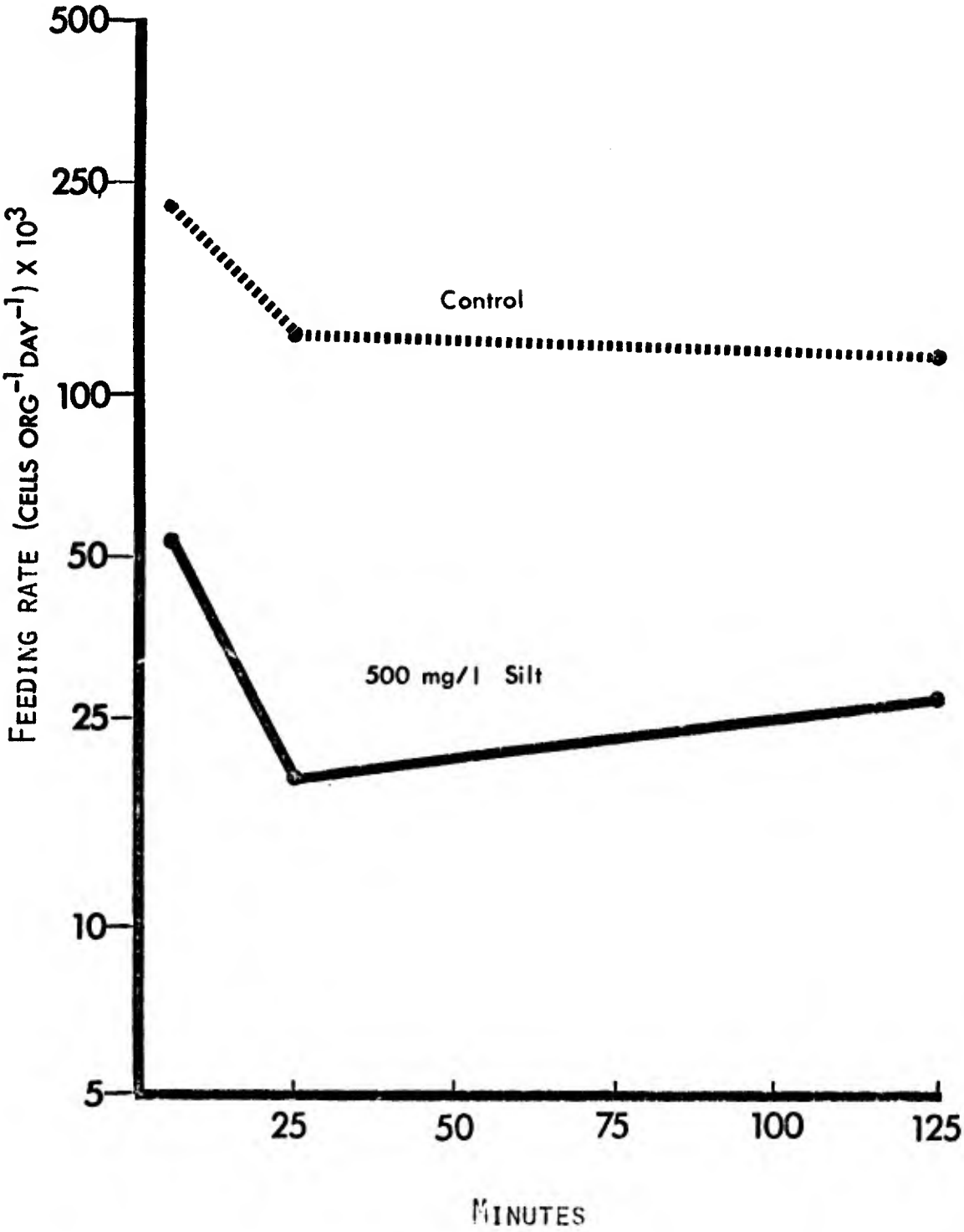

500
250
100
50
25
10
5
FEEDING RATE (CELLS ORG$^{-1}$DAY$^{-1}$) X $10^3$
Control
500 mg/l Silt
25
50
75
100
125
MINUTES

Table 29. Comparisons of feeding rates for Acartia tonsa (adults, mixed sexes) feeding on labeled Monochrysis lutheri cells in clean water (control) and 500 mg $l^{-1}$ Patuxent River silt at increasing time intervals.

| Time (minutes) | Reduction (% of control) | Feeding Rate (cells $org^{-1}$ $day^{-1}$) Control $\bar{X} \pm S.E._{\bar{X}}$ N | Patuxent Silt $\bar{X} \pm S.E._{\bar{X}}$ N |
|---|---|---|---|
| 5 | 76.8* | 262,801 ± 71,939 (9) | 60,906 ± 9,580 (9) |
| 25 | 85.2** | 152,200 ± 25,263 (9) | 22,522 ± 4,635 (10) |
| 125 | 77.5** | 140,400 ± 24,741 (9) | 31,644 ± 8,176 (10) |

*$p < 0.05$.
**$p < 0.01$.

Fig. 44. The effect of Patuxent River silt (500 mg $l^{-1}$) on feeding rates of adult Eurytemora affinis (org.) feeding on radiocarbon-labeled Monochrysis lutheri cells with increasing time of exposure.

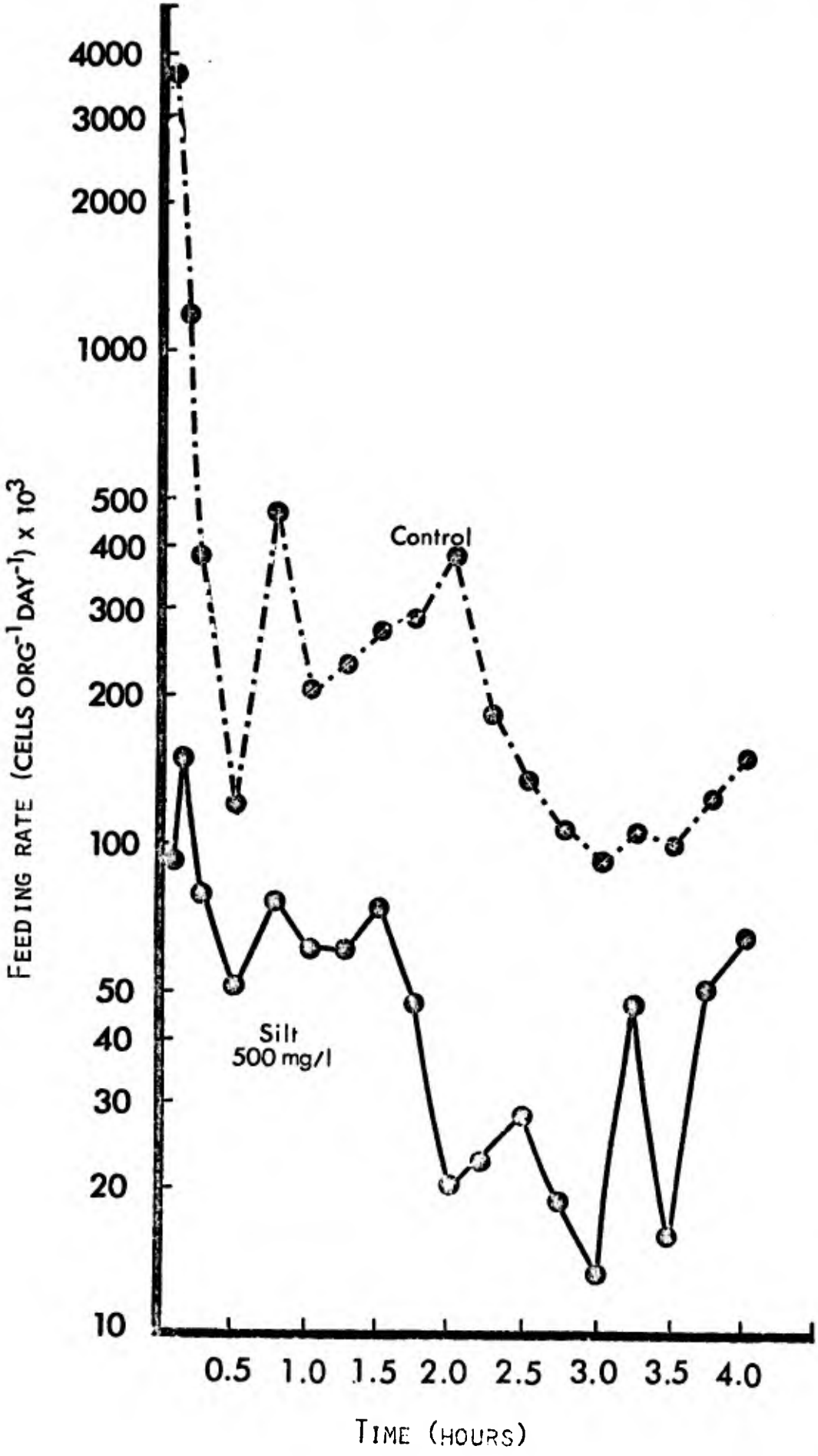

4000
3000
2000
1000
500
400
300
200
100
50
40
30
20
10
FEEDING RATE (CELLS ORG$^{-1}$DAY$^{-1}$) x $10^3$
Control
Silt
500 mg/l
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
TIME (HOURS)

Table 30. Comparisons of feeding rates for Eurytemora affinis (adults, mixed sexes) feeding on labeled Monochrysis lutheri cells in clean water (control) and 500 mg $l^{-1}$ Patuxent River silt at increasing time intervals.

| Time (hours) | Reduction (% of control) | Feeding Rate (cells $org^{-1}$ $day^{-1}$) Control $\bar{X}$ ± S.E.$_{\bar{X}}$ | N | Patuxent Silt $\bar{X}$ ± S.E.$_{\bar{X}}$ | N |
|---|---|---|---|---|---|
| 0.083 | 97.5*** | 3,510,179 ± 456,520 | (10) | 87,896 ± 13,654 | (8) |
| 0.167 | 87.3* | 1,125,181 ± 350,339 | (8) | 143,447 ± 30,666 | (8) |
| 0.250 | 80.2** | 388,312 ± 104,289 | (9) | 76,840 ± 16,854 | (9) |
| 0.50 | 56.7** | 115,480 ± 16,815 | (9) | 50,007 ± 7,445 | (9) |
| 0.75 | 84.5*** | 473,169 ± 51,393 | (9) | 73,561 ± 23,681 | (6) |
| 1.00 | 70.9* | 203,288 ± 55,791 | (9) | 59,242 ± 15,523 | (8) |
| 1.25 | 73.7*** | 224,669 ± 33,631 | (10) | 59,004 ± 9,488 | (8) |
| 1.50 | 73.2** | 268,712 ± 52,840 | (10) | 72,072 ± 7,572 | (10) |
| 1.75 | 83.6** | 281,963 ± 50,613 | (10) | 46,269 ± 15,460 | (8) |
| 2.00 | 94.9*** | 391,987 ± 54,845 | (9) | 20,117 ± 5,037 | (8) |
| 2.25 | 86.3*** | 177,508 ± 20,268 | (8) | 24,346 ± 3,802 | (8) |
| 2.50 | 77.5*** | 131,906 ± 21,771 | (8) | 29,621 ± 5,845 | (10) |
| 2.75 | 81.4*** | 104,567 ± 13,363 | (10) | 19,404 ± 3,643 | (8) |
| 3.00 | 85.2*** | 91,260 ± 17,539 | (10) | 13,464 ± 2,820 | (10) |
| 3.25 | 54.1** | 103,008 ± 12,695 | (10) | 47,282 ± 8,649 | (7) |
| 3.50 | 83.7** | 98,442 ± 21,270 | (10) | 16,014 ± 3,564 | (9) |
| 3.75 | 59.0 N.S. | 126,616 ± 34,299 | (9) | 51,924 ± 4,910 | (6) |
| 4.00 | 57.3** | 149,891 ± 19,933 | (8) | 64,041 ± 6,283 | (10) |

*$p<0.05$.

**$p<0.01$.

***$p<0.001$.

in both control and sediment treatment over the time of this experiment may be indicative that cell concentration was becoming low enough to be a limiting factor.

The feeding activity of E. affinis (Fig. 44 and Table 30) under the same 500 mg $l^{-1}$ sediment treatment is more clearly defined over the four hour feeding period, excluding the serious errors of excretion and respiration of the carbon tracer (see Conover and Francis, 1973 for extensive discussion of these problems). The control curve has three peaks (5 min., 45 min., and 120 min.) apparently indicative of times of maximum ingestion. The rise between 3.5 and 4.0 hr was unexpected, but may be indicative of a leveling off of feeding activity (time interval between peaks is increasing) as cell concentration was beginning to be low enough to become a limiting factor. The valleys in the curve are indicative of defecation.

The curve for 500 mg $l^{-1}$ silt (Fig. 44) is more erratic than the control curve (valleys and peaks are not as distinct), but generally follows the control curve and remains significantly depressed except for 3.75 hr. Lack of significance at this time was due to the low number of degrees of freedom (13) for the comparison.

These data for sediment effects over long exposure time lend support to our contention that these two species are not able to distinguish food from non-food. The sediment treatment curves may be indicative of simply the diluting effect that sediment particles had on radioactive cell concentrations. Since the guts of these organisms were full even during the 4.0 hr feeding interval for E. affinis and the 125 min. interval for A. tonsa, we believe that the reduced ingestion of cells (reduced cell filtering rate) was

caused simply by the high numbers of sediment particles which filled the gut and not any reduction in filtering rate of water.

These reductions (approx. 50 to 60 %) in numbers of cells ingested by zooplankton in the presence of suspended non-food particles at concentrations which are typical of "high" normal background level in Galveston Bay (Masch and Espey, 1967), storm agitation in the Chesapeake Bay, or during dredging and disposal activities have drastic biological implications. The effects of a 50 to 80% decrease in energy flow from primary production to the secondary production level would have disastrous effects on nutrition and reproduction of zooplankton standing stock--the food supply of larval and juvenile stages of many important estuarine vertebrates and invertebrates. This effect would be in addition to the extreme sensitivity of these juvenile stages to suspended solids which we have already demonstrated (see Chapter 1).

Supporting evidence for these implications is provided by the work of Paffenhöfer (1972) who has reported serious problems of nutrition, survival, and reproduction of Calanus helgolandicus exposed to suspensions of "red mud," the residue after extraction of aluminum from bauxite. Mortality of C. helgolandicus juveniles was not excessively high (9.6 to 16%) even though they ingested these particles. However, the copepods were weakened and were not able to make their characteristic sudden escape movements because of reduced ingestion of phytoplankton cells when "red mud" was present. They ingested large quantities of particles, but did not obtain sufficient nutrient material to develop as well as the control organisms. There was reduced growth rate and lower dry weight in copepodid stages

which had been exposed to "red mud."

Due to the small amount of phytoplankton ingested, ovary development in females was much reduced because of insufficient available energy reserves stored as fat droplets in this sex. This energy reserve is used at times of food shortage. None of the females exposed to "red mud" had fat droplets which were present in most of the control females (Paffenhöfer, 1972).

Our results presented here with those of Paffenhöfer may not lend support to Wilson's (1973) contention that non-selective filtering of A. tonsa at high particle densities is non-existent and that selective feeding predominates. While these organisms may select particles by their size, selection is not necessarily by food or non-food quality. However, it definitely appears that our results and those of Paffenhöfer show that non-selective feeding, i.e., taking all particles indiscriminately, does exist at high particle densities. We have no evidence to show that selective grasping was or was not being employed at the same time.

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# APPENDIX A

## LIVER GLYCOGEN CONCENTRATIONS IN FOUR ESTUARINE FISHES, AND RATES OF LIVER GLYCOGEN DEPLETION IN WHITE PERCH

### INTRODUCTION

In order to derive useful data from our studies of glycogen utilization in response to the effects of suspended solids, it was necessary to screen several species of estuarine fishes to establish "natural" values of liver glycogen under field conditions. It was also necessary to conduct glycogen depletion studies to establish what the expected rates of glycogen mobilization might be in the species selected for use in critical experiments.

In this appendix we present the results of liver glycogen determinations performed on four species: white perch, striped bass, hogchokers and spot. In addition, we present the results of a glycogen depletion study conducted at two temperatures with white perch.

### METHODOLOGY

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

Glycogen depletion in white perch was determined at two temperatures, 10 $\pm$ 2°C and 20 $\pm$ 2°C. On the day of capture white perch were divided into two groups of 60 fish each, and placed in separate holding tanks maintained at either 10°C or 20°C by immersion in a water bath. Ten fish were removed

at random from each group at the time of initiation of the experiment, and after two, three, seven and eight days in the holding tanks. Liver glycogen was determined according to the methods described previously.

## RESULTS AND INTERPRETATION

Mean values for liver glycogen at the day of capture for white perch, striped bass and spot resemble one another strongly (Table A-1). Liver glycogen values for the hogchoker were significantly greater than the values for the other three species. Precision of liver glycogen determinations was satisfactory in perch, bass and hogchokers (Table A-1). In the spot, however, standard error of the mean comprised more than 15% of the mean. We believe that these data show that levels of glycogen reserves were relatively constant in a population.

The rate of glycogen mobilization in starved white perch increased at higher temperatures. At 10°C glycogen stores decreased by 59.7% from a mean of 5.41 $\pm$ 7 mg 100 $mg^{-1}$ liver at the time of capture to 2.22 $\pm$ 0.39 mg 100 $mg^{-1}$ (Table A-2). At 20°C glycogen mobilization was more rapid, decreasing from 5.41 $\pm$ 0.57 mg 100 $mg^{-1}$ to 0.098 $\pm$ 0.015 mg 100 $mg^{-1}$, a total decrease of 98.2% over the eight-day holding period (Table A-2).

The glycogen mobilization curve for white perch at both temperatures was linear. The rate of glycogen mobilization was constant over the experimental period. Mobilization at 10°C occurred at the rate of 0.39 mg 100 $mg^{-1}$ per day. At 20°C mobilization was almost doubled, 0.67 mg 100 $mg^{-1}$ per day.

Rate function comparison ($Q_{10}$) is the factor by which a reaction velocity

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

| Species | n | liver glycogen (mg 100 mg$^{-1}$) $\pm$ s.e.$_{\bar{x}}$ | s.e.$_{\bar{x}}$ as % of $\bar{x}$ |
|---|---|---|---|
| White perch | 10 | 5.407 $\pm$0.572 | 10.5 |
| Striped bass | 10 | 4.780 $\pm$0.508 | 10.5 |
| Hogchoker | 10 | 16.304 $\pm$0.868 | 5.3 |
| Spot | 10 | 5.277 $\pm$0.816 | 15.5 |

TABLE A-2. Liver glycogen depletion in white perch at 10 C and 20 C. Values of mean liver glycogen are given for each sampling day ± $s.e._{\bar{x}}$. Results of regression analysis are presented for each group.

| Days after start | n | 10±2 C Liver glycogen as mg 100 $mg^{-1}$ liver | 20± 2 C Liver glycogen as mg 100 $mg^{-1}$ liver |
|---|---|---|---|
| 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 | b (slope) | m (intercept) | r |
|---|---|---|---|
| 10 | -2.301 | 13.853 | -0.919 |
| 20 | -1.266 | 7.905 | -0.972 |

is increased for a 10°C rise in temperature (Prosser and Brown, 1961; Rose, 1967; Zeuthen, 1967). In the case of glycogen mobilization of white perch, the critical reaction is dependent upon the breakdown of glycogen (a long-chain polymer of glucose) to glucose 6-phosphate (Ingram, 1970; Black, Robertson and Parker, 1961). This enzymatic reaction was shown to be highly temperature dependent, increasing by a factor of 1.825 in response to the 10°C temperature increase.

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APPENDIX B

# HEMATOLOGICAL CORRELATIONS IN ESTUARINE FISHES

## INTRODUCTION

Erthrocyte abundance, packed blood cell volume (hematocrit) and hemoglobin concentration in fish blood may be estimated directly by several suitable analytical techniques (Hesser, 1960; Anthony, 1961; Larsen and Snieszko, 1961; Larsen, 1964; Summerfelt, Lewis and Urich, 1967; Berinati and Crowley, 1972). From these parameters other useful hematological indices may be calculated, such as mean erythrocytic volume, and hemoglobin content of individual erthrocytes (Wintrobe, 1956; Holeton and Randall, 1967).

Several attempts have been made to establish predictive correlations between hematocrit and erythrocyte count or hemoglobin concentration in both marine and freshwater teleosts (Eisler, 1965; Summerfelt et al., 1967; Houston and DeWilde, 1968). Highly predictive regression of the more readily determined hematocrit or micro-hematocrit with erythrocyte count and hemoglobin concentration may enable workers to derive potentially useful hematological data from a single micro-hematocrit measurement without the specialized facilities needed for erythrocyte enumeration and hemoglobin determination.

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

## MATERIALS AND METHODS

The hematological data for this study was taken from five species common to the Patuxent River Estuary, Maryland; the white perch, Morone americana, the striped bass, M. saxatilis, spot, Leiostomus xanthurus, the hogchoker, Trinectes maculatus and the menhaden, Brevoortia tyrannus. Male and female fish from each species were used in a study of hematological response to suspension of mineral solids (Chapter 3; Sherk and O'Connor, 1971). The fish were captured by otter trawl from the Patuxent River Estuary. Blood sampling included both control fish and specimens which had been exposed to sublethal concentrations of fuller's earth. Consequently the data represent stress response hematology as well as data from fish in normal laboratory conditions (18 ± 1°C, salinity 5.5 °/oo).

Blood was collected in heparinized pipets and mixed before analysis. Microhematocrit was determined according to methods outlined by Hesser (1960). Packed blood cell volume was read on an I.E.C. micro-capillary reader. Hemoglobin was determined by the cyanmethemoglobin method. Samples were centrifuged at 11,500 rpm for 20 minutes to remove red cell nuclei from suspension before reading (Larsen, 1964). Optical density of the hemoglobin samples was determined at 540 nm using a Coleman Junior II spectrophotometer. Concentration was determined graphically against a Hycel (mammalian) standard curve. Red blood cell counts were made at 100 X using an improved Neubauer hemacytometer. A modified Hayme's solution (Heinle and Morgan, 1972) was used as the diluting medium for erythrocyte enumeration.

Regression and correlation analyses were carried out according to Snedecor and Cochran (1967). Both simple and partial correlation coefficients were calculated for each combination of parameters. Microhematocrit data were first transformed to $\log_{10}$ thereby permitting the use of parametric statistical procedures throughout the analysis.

## RESULTS AND INTERPRETATION

The results of regression analyses between independent pairs of hematological parameters in the five species studied showed significant correlation between hematocrit and hemoglobin concentration in spot, striped bass and white perch ($p<0.01$, Table B-1). Likewise, correlation between hematocrit values and erythrocyte counts (RBC) were found to be significant in spot, white perch and hogchokers. Correlation of hemoglobin and red cell counts were significant in spot and white perch ($p<0.01$).

Precisely what is meant by the significance of correlation is important in the estimation of a parameter from a statistical relationship between itself and another parameter. Attention must be paid to the predictive capacity of the mathematical model by which the estimation is carried out. Examine, for example, the correlation data for white perch. All three paired comparisons are adjudged to be significant at the 0.01 level, i.e. chances are one in 100 (or less) that the relationship established between any two of the blood parameters could be due to chance alone. On the other hand, note that the correlation coefficient (r) differ by as much as 0.21 between, for example, the hematocrit with hemoglobin correlation (0.885) and the hemoglobin with red blood

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

| Species | Comparison | N | Slope | r | p | $r^2$ |
|---|---|---|---|---|---|---|
| White perch | Hematocrit vs Hemoglobin | 23 | 3.8808 | 0.8854 | <0.01 | 0.7839 |
| | Hematocrit vs RBC | 23 | 3.9572 | 0.6813 | <0.01 | 0.4642 |
| | Hemoglobin vs RBC | 23 | 2.0275 | 0.6760 | <0.01 | 0.4570 |
| Spot | Hematocrit vs Hemoglobin | 18 | 3.009 | 0.8750 | <0.01 | 0.7656 |
| | Hematocrit vs RBC | 17 | 8.910 | 0.6024 | <0.01 | 0.3629 |
| | Hemoglobin vs RBC | 18 | 2.389 | 0.5869 | <0.05 | 0.3445 |
| Striped Bass | Hematocrit vs Hemoglobin | 14 | 4.9203 | 0.8373 | <0.01 | 0.7011 |
| | Hematocrit vs RBC | 14 | 4.1040 | 0.2828 | >0.05 | 0.0800 |
| | Hemoglobin vs RBC | 14 | 1.0206 | 0.4133 | >0.05 | 0.1708 |
| Menhaden | Hematocrit vs Hemoglobin | 9 | 2.1403 | 0.5547 | >0.05 | 0.3077 |
| | Hematocrit vs RBC | 9 | 7.2995 | 0.6554 | >0.05 | 0.4295 |
| | Hemoglobin vs RBC | 9 | 1.5556 | 0.5389 | >0.05 | 0.2904 |
| Hogchoker | Hematocrit vs RBC | 39 | 9.9183 | 0.8632 | <0.01 | 0.7451 |

cell correlation (0.676). While these correlations may be significant at the same probability levels, 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. Thus, in the hematocrit with hemoglobin correlation $r^2 = 0.7839$. That is to say, given a hematocrit value (x) from a white perch, one may estimate the hemoglobin content (y) by regression for that individual, knowing that 78.4% of the variance in the hemoglobin estimate is accounted for by the hematocrit value.

Similarly, in the estimation of RBC from hematocrit, $r^2 = 0.4642$. Only 46.4% of the variance of the predicted RBC can be accounted for by the hematocrit. Thus, in the estimation of two blood parameters from the same hematocrit value, though the correlation coeffieients were highly significant, the predictive capacity of the former relationship was almost 80%, while in the latter the predictive capacity was below 50%.

We have established the criterion that the coefficients of determination must exceed 0.75 for a paired relationship before estimated parameters may be used for research purposes. Coefficients of determination between 0.60 and 0.74 were considered sufficient for use in routine estimates of hematological status.

Among the species studied, coefficients of determination sufficient for research purposes were found in the correlation of hematocrit with hemoglobin for spot and for white perch (Table B-1). The correlation of hematocrit with RBC in hogchokers was not found to be of predictive value for use in parametric estimates for research purposes ($r^2=0.7451$). The hematocrit with hemoglobin correlation in striped bass was found to be sufficient for routine hematological work ($r^2=0.7011$).

In perch, spot, striped bass and menhaden the correlation of hematocrit with RBC, and the correlation of hemoglobin with RBC did not account for sufficient variance to justify use of regression methods for predictive estimates of hematological parameters (Table B-1).

The three parameters discussed in this section are very closely related in a physical sense, as well as in a biological sense. Hematocrit determinations are a measure of the percent volume of red blood cells in a sample of blood. Red blood cells in most vertebrates are vessels for the transport of the respiratory pigment, hemoglobin. Thus, there is a basis for assuming that the highly predictive correlations seen between hematocrit and hemoglobin in spot and white perch are, in great part, dependent upon the quantity of red blood cells present. To establish whether the correlation of hematocrit and hemoglobin was significant and independent of red cell count, partial correlation coefficients were determined for hematocrit-hemoglobin-red cell count inter-relationships. In effect, this statistic estimated the correlation of two variables, hematocrit and hemoglobin, both of which were related to a third, RBC, while the third variable was held constant.

Partial correlation coefficients were determined for all species in which the three aforementioned variables were studied (Table B-2). In the white perch and in the spot, partial correlation coefficients showed the relationship of hematocrit and hemoglobin to be significant, i.e. not statistically dependent upon RBC (Table B-2).

The relationships established for these species have proved to be of significant value in our studies. We feel that the ability to estimate blood parameters in estuarine fishes from a simply determined value, such as

TABLE B-2. Analysis of partial correlation of hematocrit with hemoglobin eliminating the effect of RBC.

| Species | n | $r_{12 \cdot 3}$ | d.f. | p |
|---|---|---|---|---|
| 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 |

hematocrit, may make it possible for physiological studies of estuarine fishes to proceed in the field as well as in the laboratory. As we have shown above (Chapter 3), these hematological studies can serve as estimates of stress response in fish (Hesser, 1960; Summerfelt, et al., 1967.) Physiological studies and hematological studies carried out in the field could increase the power of on-site studies of environmental disturbance, and estimates of sublethal effects of various pollutants on fish populations should prove to be a powerful tool in the hands of estuarine biologists.

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# APPENDIX C

PRELIMINARY RESULTS:

THROUGH GUT TRANSPORT OF SUSPENDED SOLIDS BY ESTUARINE FISHES

Joseph M. O'Connor

J. Albert Sherk, Jr.

Ann Marie Daley

A subproject deriving from U. S. Army Corps of Engineers Contract No. DACW72-71-C-0003 to the Natural Resources Institute, University of Maryland, Department of Environmental Research, Hallowing Point Field Station, Prince Frederick, Maryland 20678.

N.B.: This material has not been published and is the property of the University of Maryland and the U. S. Army Corps of Engineers. The data contained herein are not to be considered as conclusive pending completion of the project, and are furnished in confidence. Use of these data for public distribution or in litigation without the consent of the investigators shall be considered a breach of confidence. We thank you for this professional courtesy.

# THROUGH GUT TRANSPORT OF SUSPENDED SOLIDS BY ESTUARINE FISHES

Joseph M. O'Connor

J. Albert Sherk Jr.

Ann Marie Daley

Objective:

To determine accumulation of particulate matter on the gills and in the alimentary canal of fishes exposed to sublethal concentrations of suspended solids.

Materials:

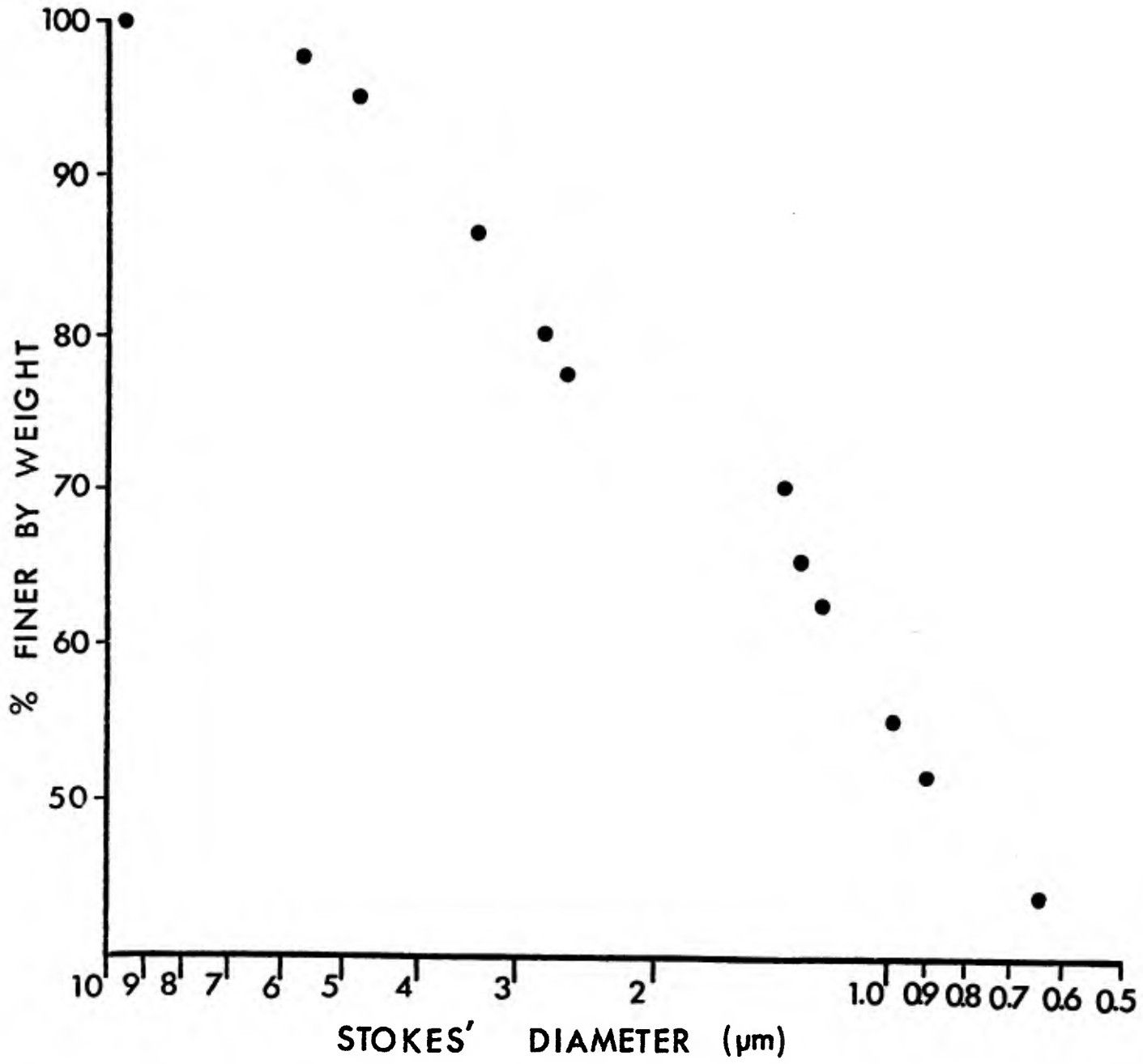
A. Solids

1. Kaolinite clays
   a. Hydrite MP, median particle size 9.0 μm.
   b. Hydrite Flat-D, median particle size 4.5 μm.
   C. Hydrite-10, median particle size 0.55 μm.
2. Fuller's earth, median particle size 0.50 μm.
3. Natural bottom muds derived from Long Point, Patuxent River Maryland. Particle size distribution as shown in Fig. C-1.

B. Organisms

1. White perch, Morone americana (Gmelin) Serranidae.
2. Striped bass, M. saxatilis (Walbaum) Serranidae.
3. Hogchoker, Trinectes maculatus (Bloch and Schneider) Soleidae.

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


100
90
80
70
60
50
% FINER BY WEIGHT
10 9 8 7 6 5 4 3 2 1.0 0.9 0.8 0.7 0.6 0.5
STOKES' DIAMETER (μm)

Methods

Fishes were captured by otter trawl in the Patuxent River estuary, Maryland, and transported to the lab in a flow-through system using running river water. All specimens were starved 72-96 hr before exposure to suspended solids. The fish were not fed during an exposure.

Groups of six to 10 fish, dependent upon size and species were exposed for 24-hr to graded concentrations of suspended solids. Following exposure, the gills, stomach, and intestine of each individual were examined to determine accumulation of material identifiable as the suspended solid to which the fish had been exposed.

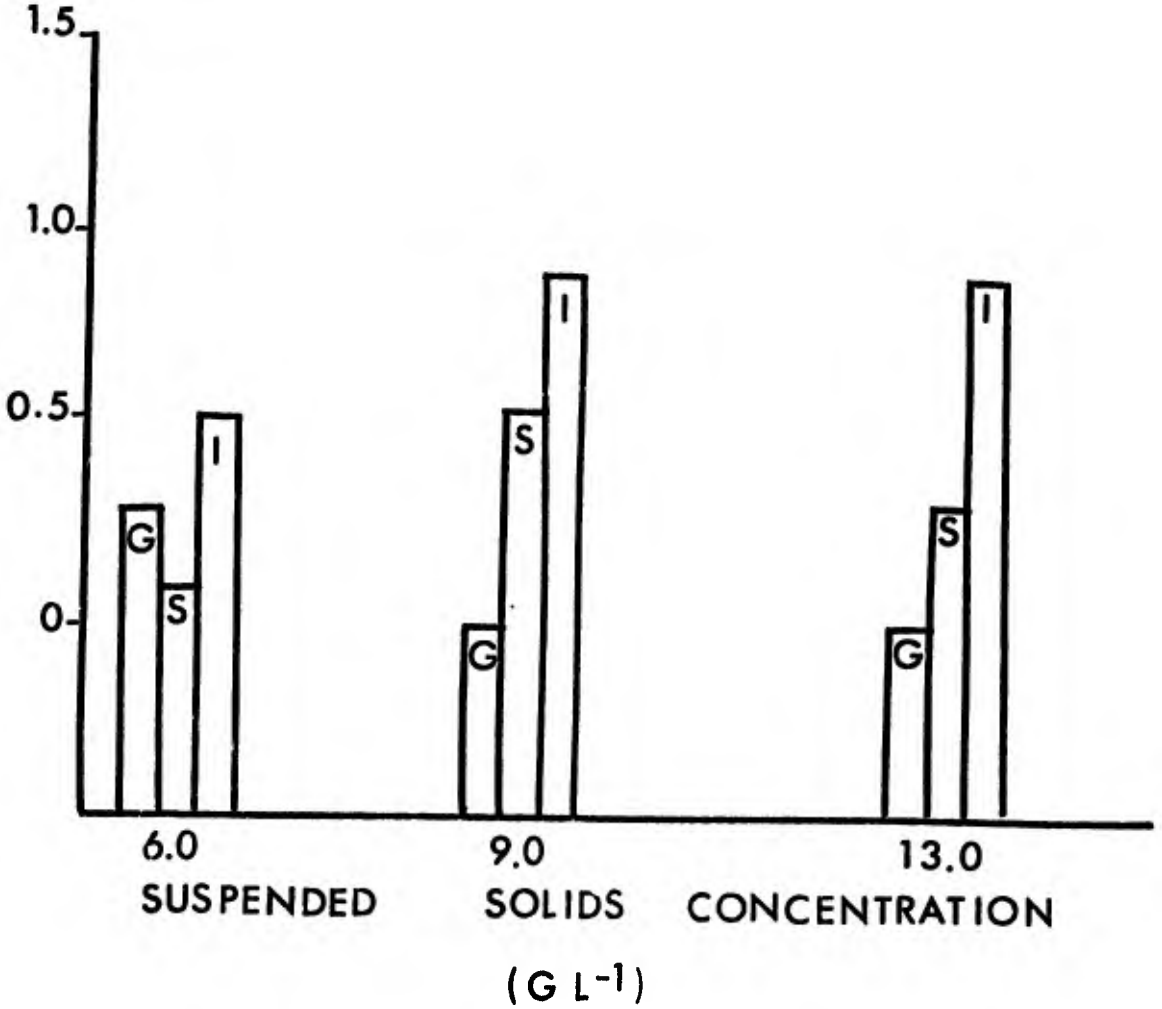
Accumulation of solids on the gills, in the stomach and in the intestine was scored on a scale, from 0 (no accumulation) to 4 (continuous coating of particulate matter). Scoring was based on visual observation by a single, trained observer. "Mean" accumulation for each group of fish exposed to each concentration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

The complete design (replicate exposure of each species to graded concentrations of each solid) has not yet been fulfilled. Data for the completed exposures are reported here.

Results

White perch accumulated little MP clay (m.p.s. 9.0 $\mu$m) in a 24-hr period. Overall, accumulation of this solid from concentrations of 6.0 to 13.0 g/l (6,000 to 13,000 ppm) was greatest in the intestine, and least on the gills. Accumulation in the intestine at 9.0 and 13.0 g/l MP clay was approximately double the accumulation at 6.0 g/l (Fig. C-2).

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


1.5
1.0
0.5
0
G
S
I
6.0
SUSPENDED
9.0
SOLIDS
13.0
CONCENTRATION
(G L-1)

Accumulation of Hydrite Flat-D particles (m.p.s. 4.5 μm) by white perch was greater than MP clay (Fig. C-3). At 6.0 g/l Flat-D accumulation in the stomach and in the intestine was greater than MP accumulation by a factor of three. Less accumulation was noted at 16.0 g/l than at 6.0 g/l. Particle accumulation in the intestine exceeded a value of 2.0 concentrations of 24 and 37 g/l. Accumulation of Flat-D in stomachs was greater at 6.0 g/l than at 16, 24 and 37 g/l.

White perch exposed to concentrations of fuller's earth showed accumulation on the gills greater than either of the kaolinite clays, regardless of concentration (Fig. C-4). Accumulation scores for fish exposed to 6.7, 8.3 and 10.7 g/l ranged between 1.5 and 2.0. Little or no fuller's earth was detected in the stomachs or in the intestines.

Exposure of white perch to suspensions of natural muds (6.6 to 36 g/l) for 24-hr showed greatest accumulation in gill, stomach, and intestine at the lowest concentration, 6.6 g/l (Fig. C-5). Least accumulation was noted at 14 g/l. While gill accumulation was quite high at 23.9 g/l, accumulation of mud in stomachs and intestines was approximately the same at 23.9 and 36.2 g/l, i.e. between 1.5 and 2.0 on a scale of 0 to 4.

Exposure of white perch to lower concentrations of natural muds for 72-hr resulted in approximately the same accumulation scores across the range of concentrations, from 5.6 to 17.8 g/l (Fig. C-6). Intestinal scores remained between 2.0 and 2.5 over the range of concentrations, while particle accumulation in stomachs and on the gills was slightly more variable. Greatest gill accumulation was recorded for fish exposed to 5.6 g/l; least gill accumulation occurred at 17.8 g/l.

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

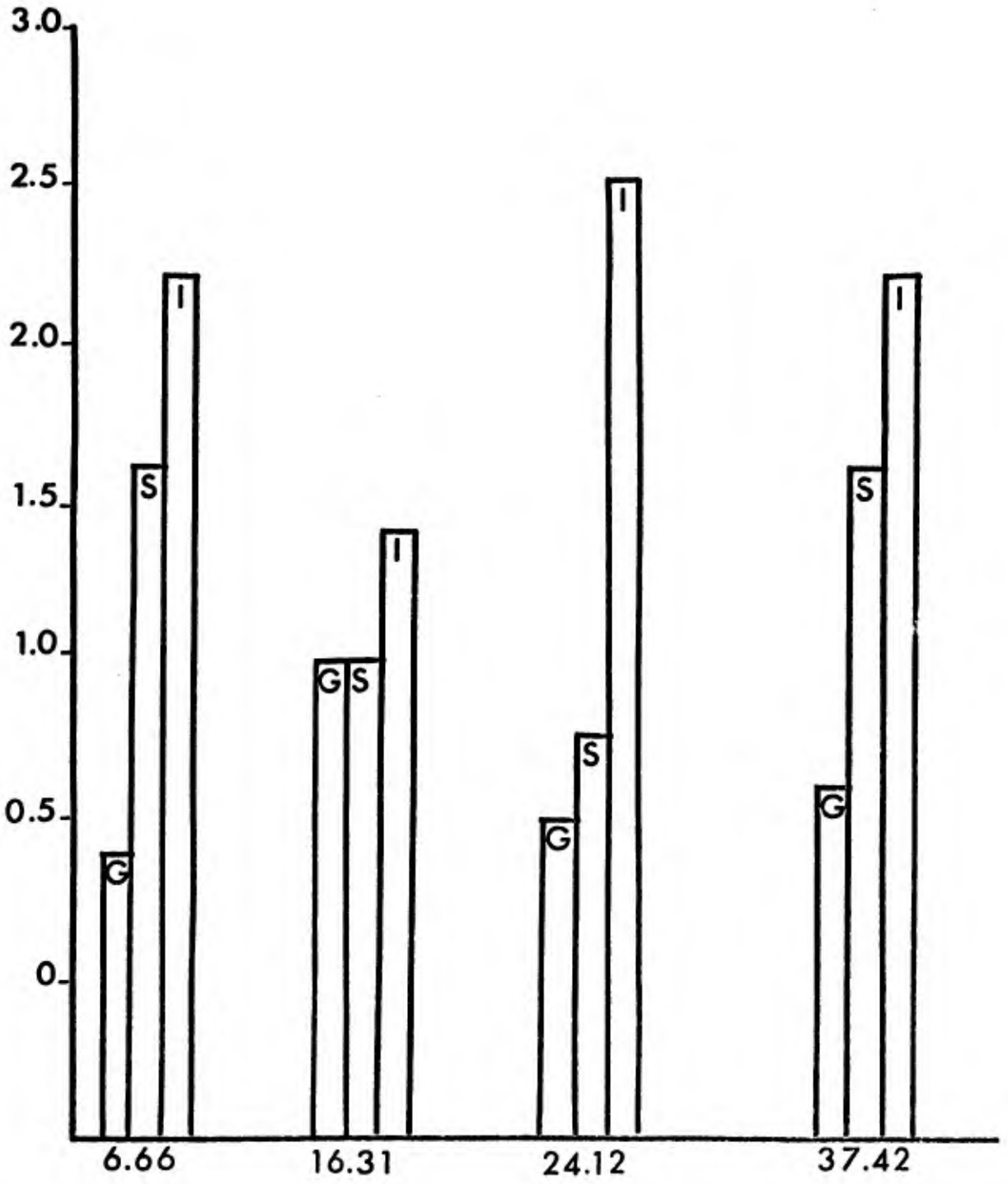

3.0
2.5
2.0
1.5
1.0
0.5
0
G
S
I
6.66
16.31
24.12
37.42
SUSPENDED SOLIDS CONCENTRATION
(G L-1)

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

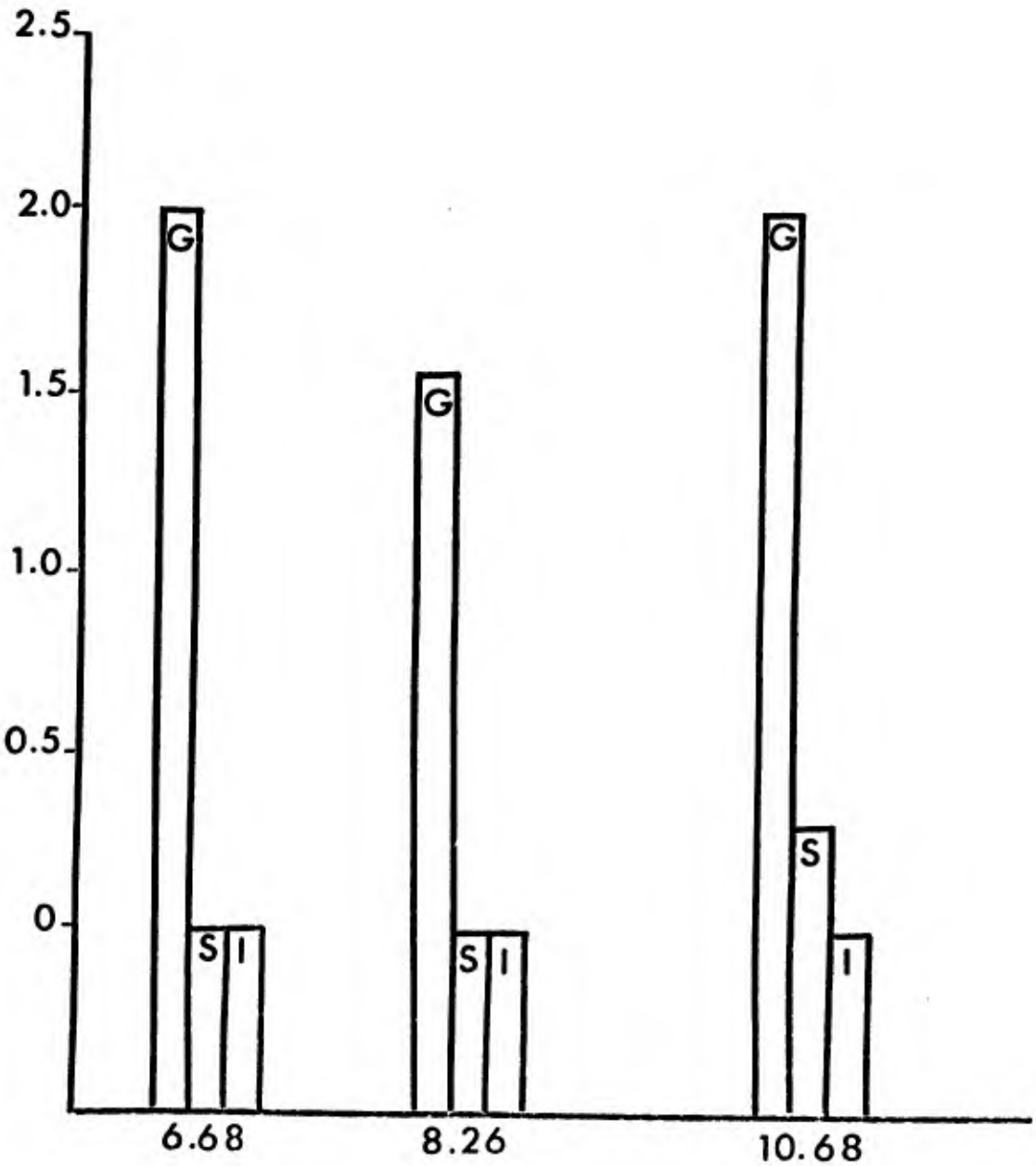

2.5
2.0
1.5
1.0
0.5
0
G
S
I
6.68
8.26
10.68
SUSPENDED SOLIDS CONCENTRATION
(G L-1)

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

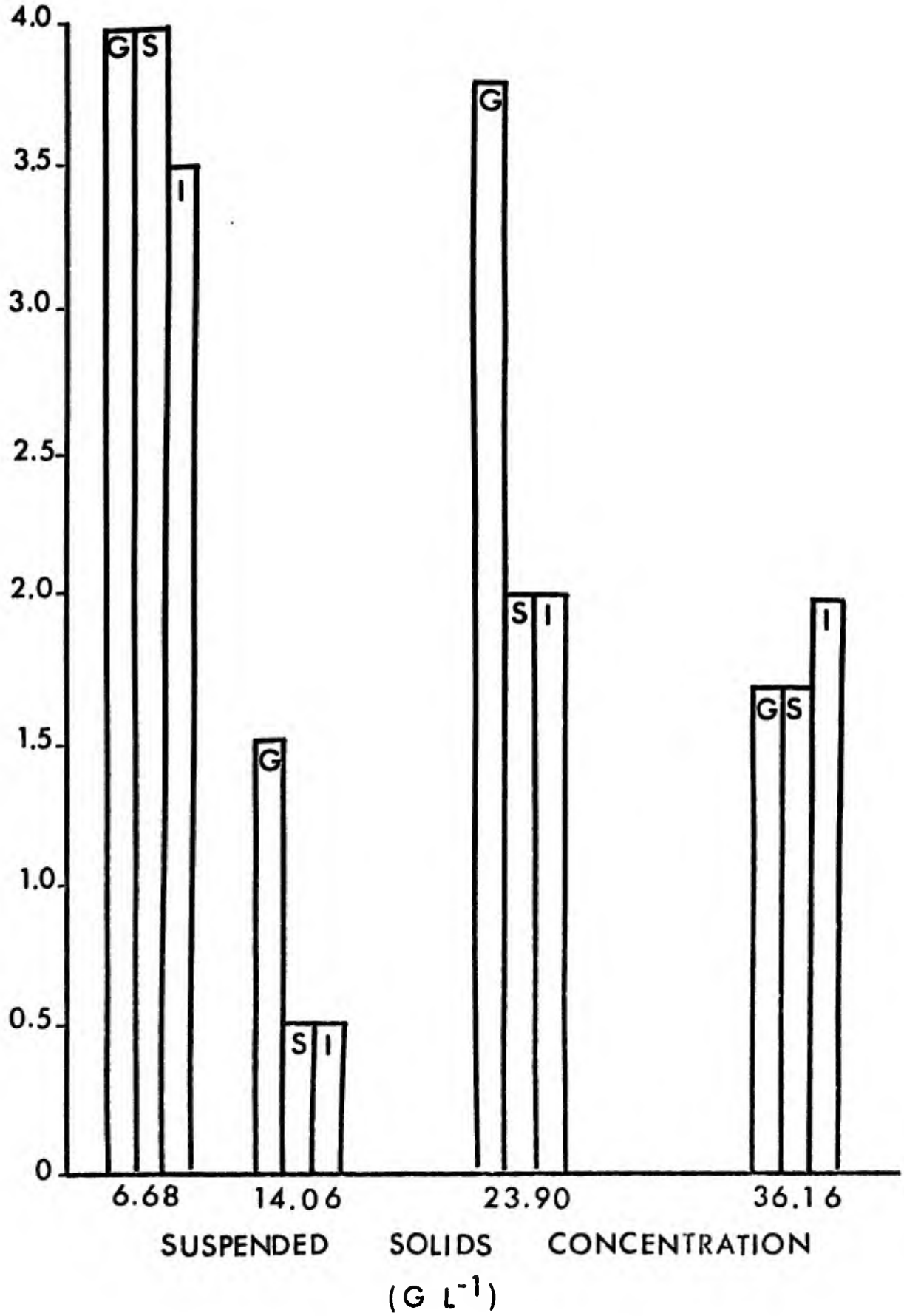
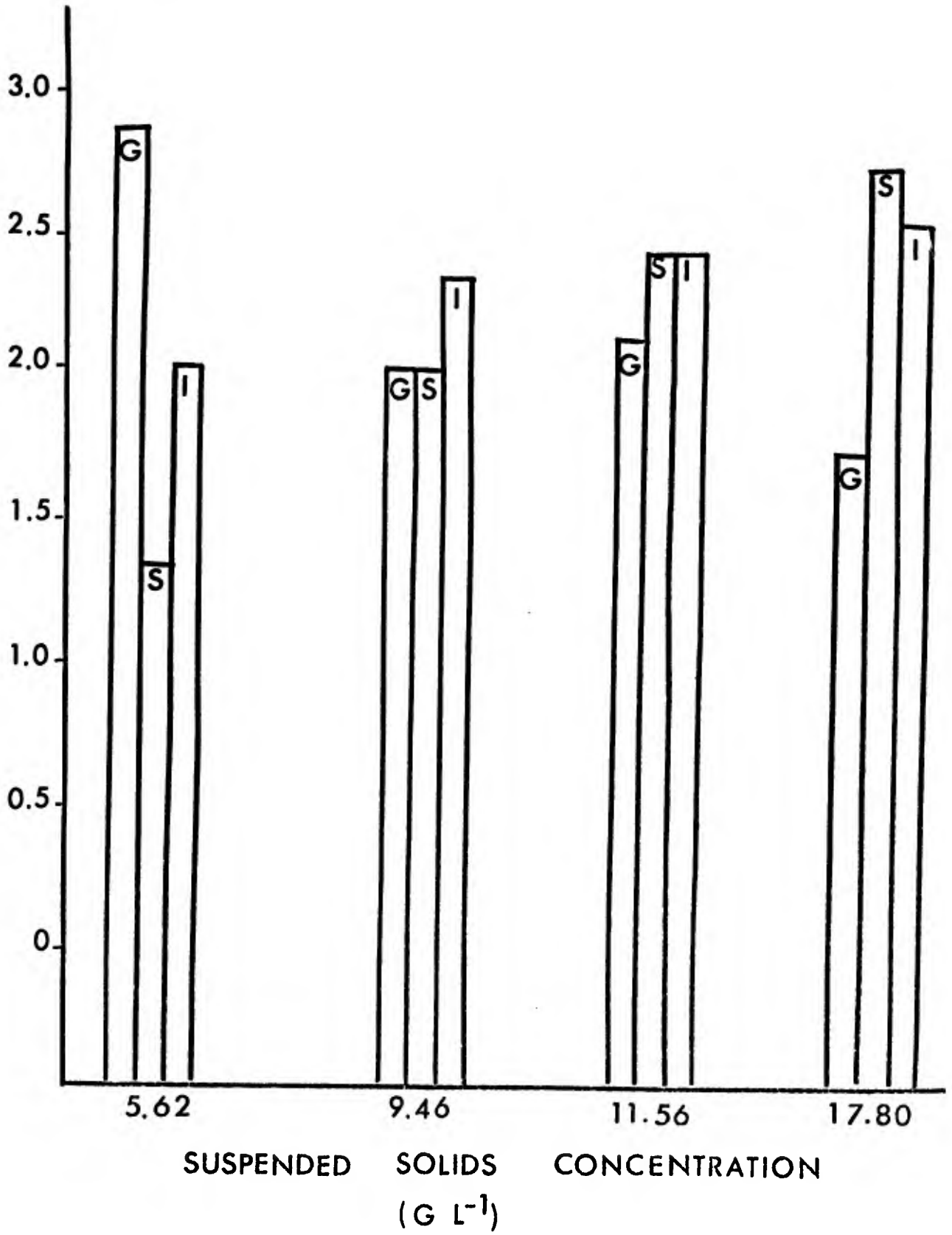

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
G
S
I
6.68
14.06
23.90
36.16
SUSPENDED SOLIDS CONCENTRATION
(G L$^{-1}$)

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


3.0
2.5
2.0
1.5
1.0
0.5
0
G
S
I
5.62
9.46
11.56
17.80
SUSPENDED SOLIDS CONCENTRATION
(G L-1)

Striped Bass

Accumulation of Hydrite MP particles by striped bass was relatively low, with accumulation scores ranging from 0.75 to 1.5 (Fig. C-7). However, accumulation in gills, stomachs, and intestines was much the same over the range of concentrations employed (6.0 to 13.0 g/l). In comparison to white perch (Fig. C-2) accumulation of Hydrite MP by striped bass was greater by a factor of three or more.

Accumulation of Hydrite Flat-D by striped bass was greatest on the gill (Fig. C-8). Particle accumulation in stomachs and intestines ranged from 0 to 1.2 over the range of concentrations employed (3.3 to 17.1 g/l) except for a value of 2.0 in the stomachs of bass exposed to 5 g/l Flat-D.

Single exposure of striped bass and hogchokers to suspensions of natural muds (Figs. C-9, C-10) showed greatest accumulation on the gills of both species, moderate accumulation in the stomachs, and little or no accumulation in the intestines.

Conclusions

Among three species of common estuarine fishes, mechanisms for gill cleansing in highly turbid water included 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 are accumulated to a greater extent than larger particles (Hydrite MP).

Additional observations

Microscopic examination of gills revealed a possible mechanism for through gut transport of suspended particles. Examination of gills at

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

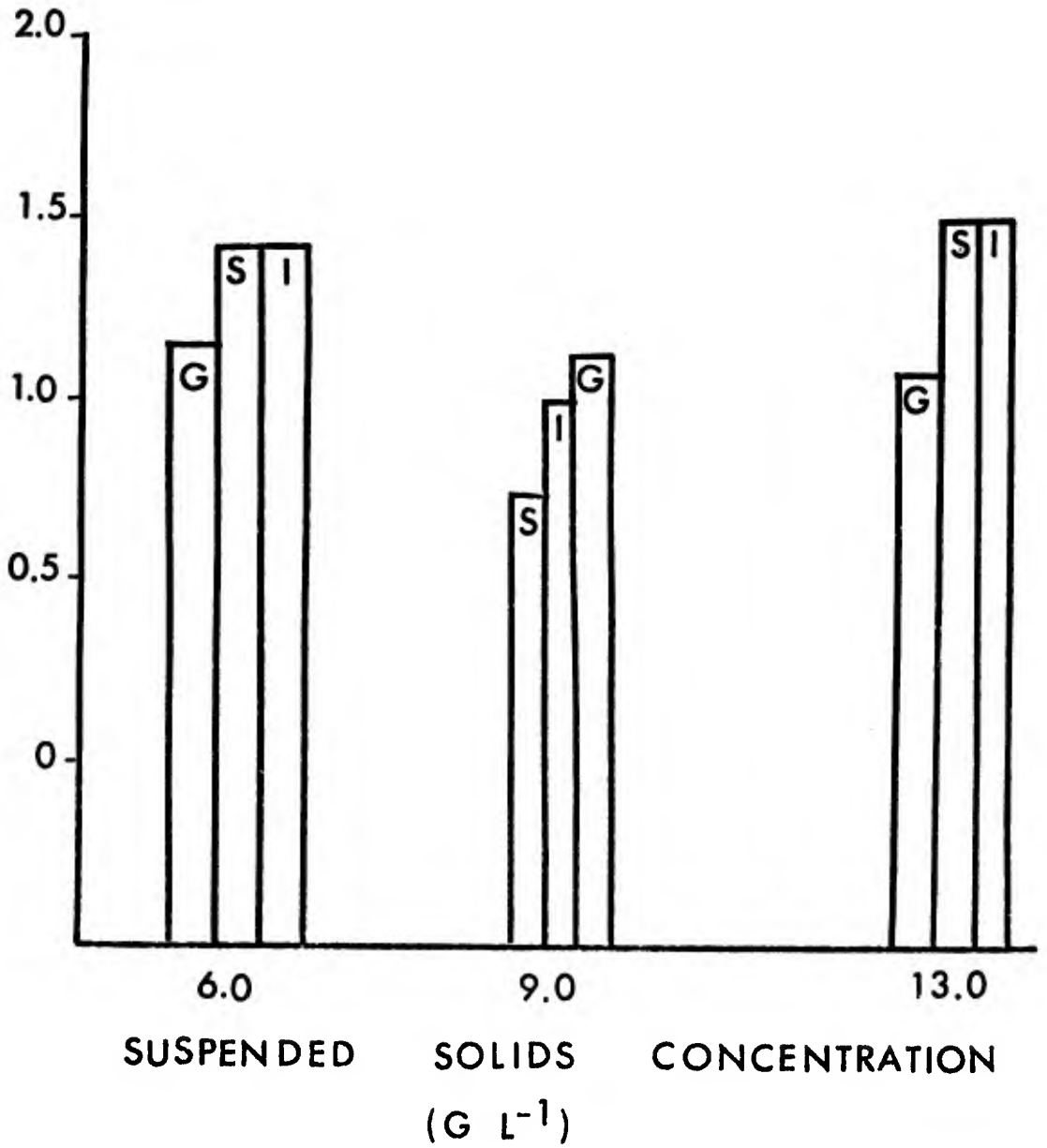

2.0
1.5
1.0
0.5
0
G
S
I
6.0
9.0
13.0
SUSPENDED SOLIDS CONCENTRATION
(G L$^{-1}$)

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

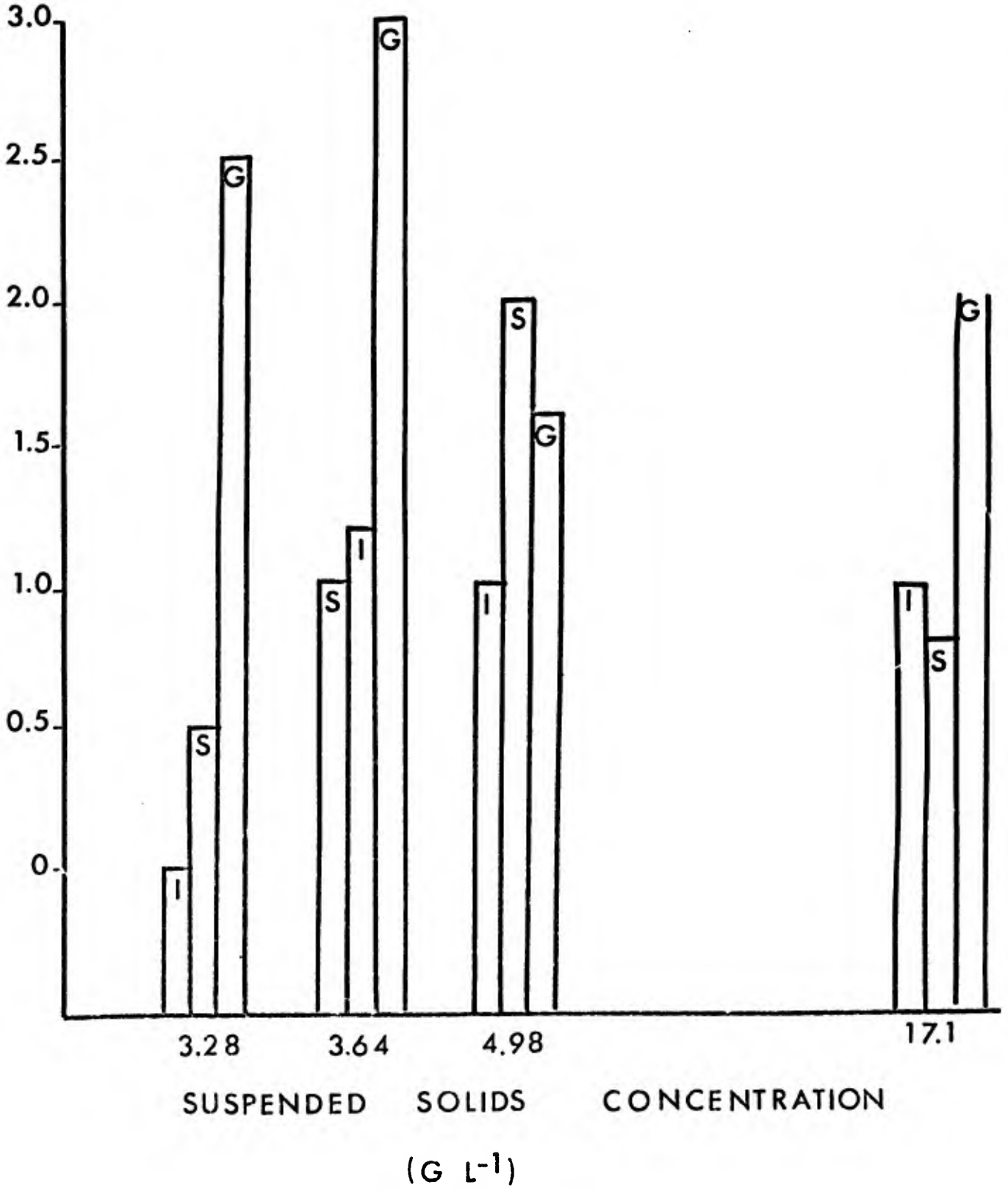

3.0
2.5
2.0
1.5
1.0
0.5
0
I
S
G
3.28
3.64
4.98
17.1
SUSPENDED SOLIDS CONCENTRATION
(G L-1)

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

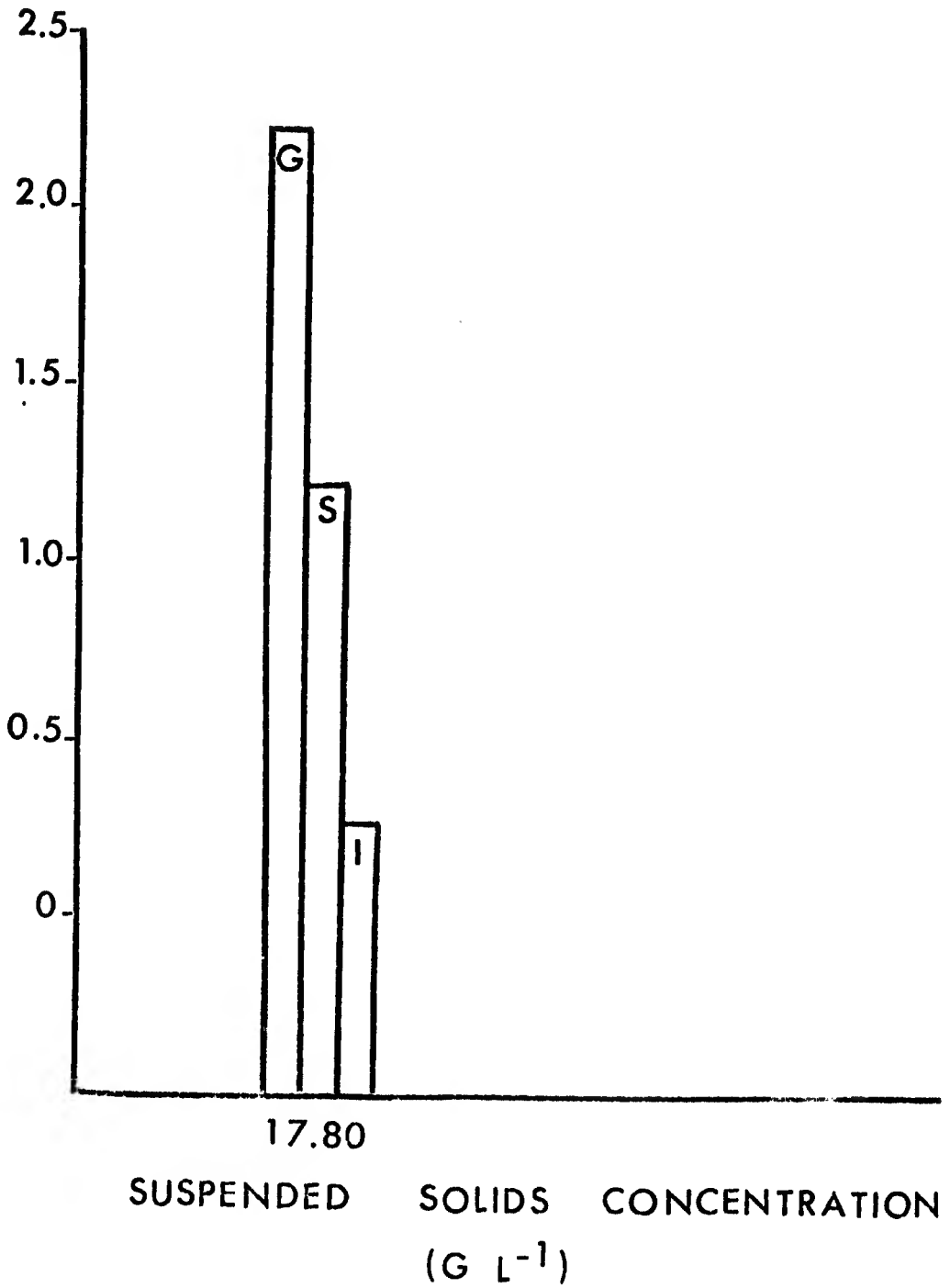
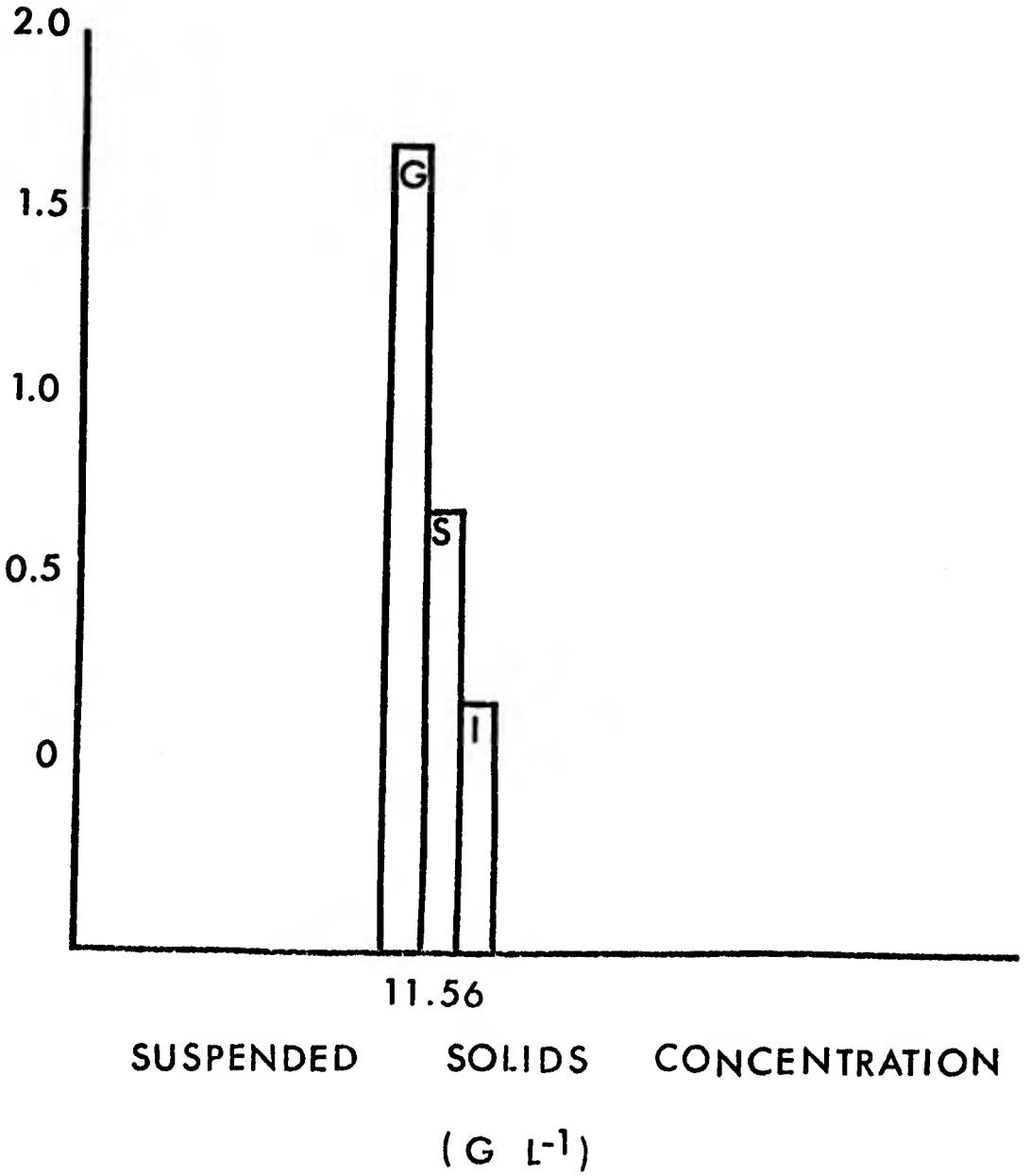

2.5
2.0
1.5
1.0
0.5
0
G
S
I
17.80
SUSPENDED SOLIDS CONCENTRATION
(G L$^{-1}$)

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


2.0
1.5
1.0
0.5
0
G
S
I
11.56
SUSPENDED SOLIDS CONCENTRATION
(G L-1)

30X showed that in fishes exposed to suspended solids, fine particles become entrapped between gill filaments and between the secondary lamellae. Fishes exposed to the suspensions showed streams of particle-laden mucus on the gill and attached to the pharyngeal teeth on the inner margin of the gill arch. Since a function of the pharyngeal teeth in fishes is to assist in the passage of food from the mouth to the esophagus, it is likely that the mucus streams on the gill and the pharyngeal teeth were in the process of being ingested.

The expansion of the study to include the demersal fish Trinectes maculatus brought to our attention a species difference in accumulation and transport of particles. When exposed to similar concentrations of the same types of solids used with white perch and bass, hogchokers showed a reduced accumulation of particulate matter in the gut.

Few of these data may be used in direct comparison due to the wide range of suspended solids concentrations employed. However, in several instances it is evident that accumulation of particles from suspension was much the same, regardless of the concentration. The greatest accumulation occurred in the finer solids, i.e., fuller's earth (75% < 2.0 $\mu$m, median size 0.5 $\mu$m), Hydrite Flat D (median size 4.5 $\mu$m, 40% < 2.0 $\mu$m), and resuspended natural muds (median size 0.87 $\mu$m, 70% < 2.0 $\mu$m).

At first glance gut transport of entrapped particles seem analogous to the ctenidial clearance-food selection mechanism of the lamellibranch molluscs. However, in the lamellibranchs, particles exceeding a certain size are eliminated as pseudofeces, and do not pass through the digestive tract. Mucus stream transport of particles in fishes exposes the entrapped

material to normal digestive processes. Thus, the particles are exposed to a wide range of chemical environments. For example, in the stomach, the particles are exposed for varying periods of time to acid conditions, with pH in the range of 1.0 to 2.0. Under these conditions material is exposed to a strong acid hydrolysis. Passage from the stomach to the intestine entails a marked change of pH environment, from acid to moderately basic (pH 8.0 to 9.0). In the intestine hydrolysis of food material is carried out by enzymatic processes rather than by chemical hydrolysis.

It is perhaps significant to note that in the stomach, entrapped particles are exposed to approximately the same conditions used in stripping sorbed materials from particulate matter for chemical analysis. Thus potentially toxic materials such as heavy metal ions, pesticide residues, petro-chemical residues and various biocides of organic origin, may be freed, and made available to the organism. Through gut transport of particles removed from suspension may provide an avenue for accumulation of noxious material into the tissue of fishes exposed to suspended solids concentrations likely to induce function of the gut-transport mechanism for gill cleansing.

The data suggested that greater quantities of particles may be present in the stomach or intestine than on the gill. Future experiments should be designed to determine a precise relationship between mucus-stream gill clearance and the coughing reflex in an effort to explain the apparent contradiction in these data. Similarly, work towards establishing rates of gut evacuation will allow more accurate sampling of fishes for estimates of particle accumulation.

# APPENDIX D

# ANALYSES OF SEDIMENTS

## I. INTRODUCTION

Experimental work during Project Years I and II utilized artificial (commercially available), mineral solids to provide baseline data for biological effects of (1) concentrations of solids, (2) different particle size distributions, and (3) different mineral types of solids.

Work during Year III concentrated heavily on the biological effects of naturally occurring sedimentary material which was collected by anchor dredge at Long Point (38° 29' 30" N, 76° 39' 45" W) in the Patuxent River, Maryland. This material was stored in large polyethylene tanks prior to use in our experiments. The sediment surface was covered with a layer of water (salinity range 4 to 6 °/oo) to maintain natural ionic equilibria between the sediment and water, as would naturally occur in the Patuxent River. A micro-oxidized sediment layer developed at the sediment-water interface in these tanks after a few days of storage.

This appendix contains the results of analyses which were performed on both the mineral (commercially available) solids and the naturally occurring sediments. Sediment characteristics measured were organic matter content (weight loss on ignition), inorganically bound heavy metals (atomic absorption analysis), and particle size distributions (settling diameter analysis).

The particle size distributions presented in this appendix were determined in distilled water, and may represent the basic or fundamental

unit particles which can form aggregates with other units, and be strongly bound by molecular and atomic forces. These composite units are stable under dispersion methods. Also, the basic particles may form agglomerates in saline water. These composites are relatively weakly bonded by electrostatic forces, surface tension, and "sticky" organic matter.

## II. MATERIALS AND METHODS

### 1. Size Distribution

Artificial sediments (mineral solids) used in this study were as follows:

1. Kaolinite
   a. Hydrite-10 (Georgia Kaolin Co.)
   b. Hydrite Flat D (Georgia Kaolin Co.)
   c. Hydrite MP (Georgia Kaolin Co.)
2. Fuller's earth (Fisher No. F-90)
3. Silica ($SiO_2$, Fisher No. S-135)

Particle size distributions of these materials were determined by the sedimentation method (ASTM, 1968) for paper coating clays. In addition, a finer particle size distribution of $SiO_2$ was generated from the commercially available Fisher No. S-135 by allowing these solids to settle for 25 min. through a specified distance in a column of distilled water at 20°C. The solids finer by weight than 15 μm were calculated to be remaining in suspension in this column of water from tables presented by Trask (1968) and from Casagrande's (1934) nomographic solution of Stokes' Law given in ASTM (1968). The suspended particles were decanted, oven-dried for 24 hr at 100°C, ground fine with a porcelain mortar and pestle, and analyzed for size distribution by the ASTM (1968) method performed for the other mineral

solids. This particular size distribution of $SiO_2$ particles is referred to in this report as $<15\ \mu m$ $SiO_2$.

The natural sediments collected from the Patuxent River were analyzed by a slightly modified procedure from the above method. Preliminary work showed that this material was approximately 75 to 80% salt and water by weight. Appropriate triplicate volumes of this natural material were removed from the holding tanks. These volumes were calculated to contain between 5 and 10 g dry solids (inorganic). Also, these volumes were corrected upward for the amount (weight) of organic matter present (see method of analysis below). These quantities of solids were placed into large Pyrex beakers (one liter capacity) and an appropriate amount of 30% $H_2O_2$ was added to each beaker. The amount (volume) of $H_2O_2$ (30%) needed to oxidize the organic matter present in the sediment was found to be a volume which would produce a final concentration of $H_2O_2$ in the sediment volume of approximately 5%. The oxidation reaction was quite violent initially. The reaction was allowed to proceed overnight in a hood with air bubbling slowly through the sediment-$H_2O_2$ mixture to remove the excess $H_2O_2$.

The next day, when gas evolution had ceased, 750 ml 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 decanted very carefully, and another 750 ml of deionized glass-distilled water rinse were added to each beaker.

A 0.2 ml sample of supernatant water was then taken from each beaker and the dissolved ion concentration of each solution was determined with

the freezing point depression osmometer normally used in our hematological analyses. Salt concentration was read from a standard curve relating freezing point depression and osmolal concentration to NaCl concentration in 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 ml 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 made up to 500 ml with fresh, deionized glass-distilled water was placed into an ultrasonic bath (45 KHz) for 30 min. Then, the suspension was placed into a glass cylinder, made up to volume with deionized distilled water, and the analysis followed as described in ASTM (1968), except that the dispersing agent ($Na_4P_2O_7$) was not added.

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

2. Organic Matter Content

Samples of the natural sediment collected from the Patuxent River at Long Point were oven-dried for 24 hr at 100°C, ground fine with a porcelain mortar and pestle, and ashed for 3 hr at 500°C. Organic matter values are reported as percent loss of dry weight on ignition. There was no appreciable loss of inorganic carbonate during the ashing procedure as evidenced by non-significant weight losses of $CaCO_3$ samples which were ashed along with the oven-dried natural sediments.

3. Heavy Metals

Amounts of extractable cations in the mineral solids and the natural sediment samples were determined with mild acid extraction and atomic absorption analysis by Mr. David Bcon, Seafood Processing Laboratory, Crisfield, Maryland. Routine procedures for inorganically bound cations as described by Anon. (1971) and Anon. (1970) were conducted for Zn, Cu, Fe, Mn, Pb, Co, Ni, Cr, and Cd. Mercury values reported are for total mercury from sediments digested for one minute in boiling aqua regia (Dow Method, CAS-AM-70.13, June 22, 1970 revised, Chlorine Institute, Madison Avenue, New York, New York). Metal values are reported as mg $kg^{-1}$ dry weight of solids.

## III. RESULTS AND DISCUSSION

1. Size Distributions

Particle size distributions of the extremely fine mineral solids and natural sediment used in this project are presented in Fig. D-1 and Table D-1. Useful descriptions of these materials ranked coarsest to finest by median size are as follows: Silicon dioxide (Fisher No. S-153), median size = 17 $\mu$m, <2 $\mu$m = 6%; Hydrite MP, kaolinite (Georgia Kaolin Co.), median size = 9.5 $\mu$m, <2 $\mu$m = 12%; Silicon dioxide (all particles <15 $\mu$m), median size = 6.2 $\mu$m, <2 $\mu$m = 13%; Hydrite Flat D, kaolinite (Georgia Kaolin Co.), median size = 4.5 $\mu$m, <2 $\mu$m = 34%; Patuxent River silt (composite less organic matter fraction 11.5% of dry weight), median size = <0.8 $\mu$m, <2 $\mu$m = 72%; Fuller's earth, montmorillonite and attapulgite (Fisher No. F-90), median size = <0.5 $\mu$m, <2 $\mu$m = 82%; Hydrite 10, kaolinite (Georgia Kaolin Co.), median size = <0.5 $\mu$m, <2 $\mu$m = 92%.

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

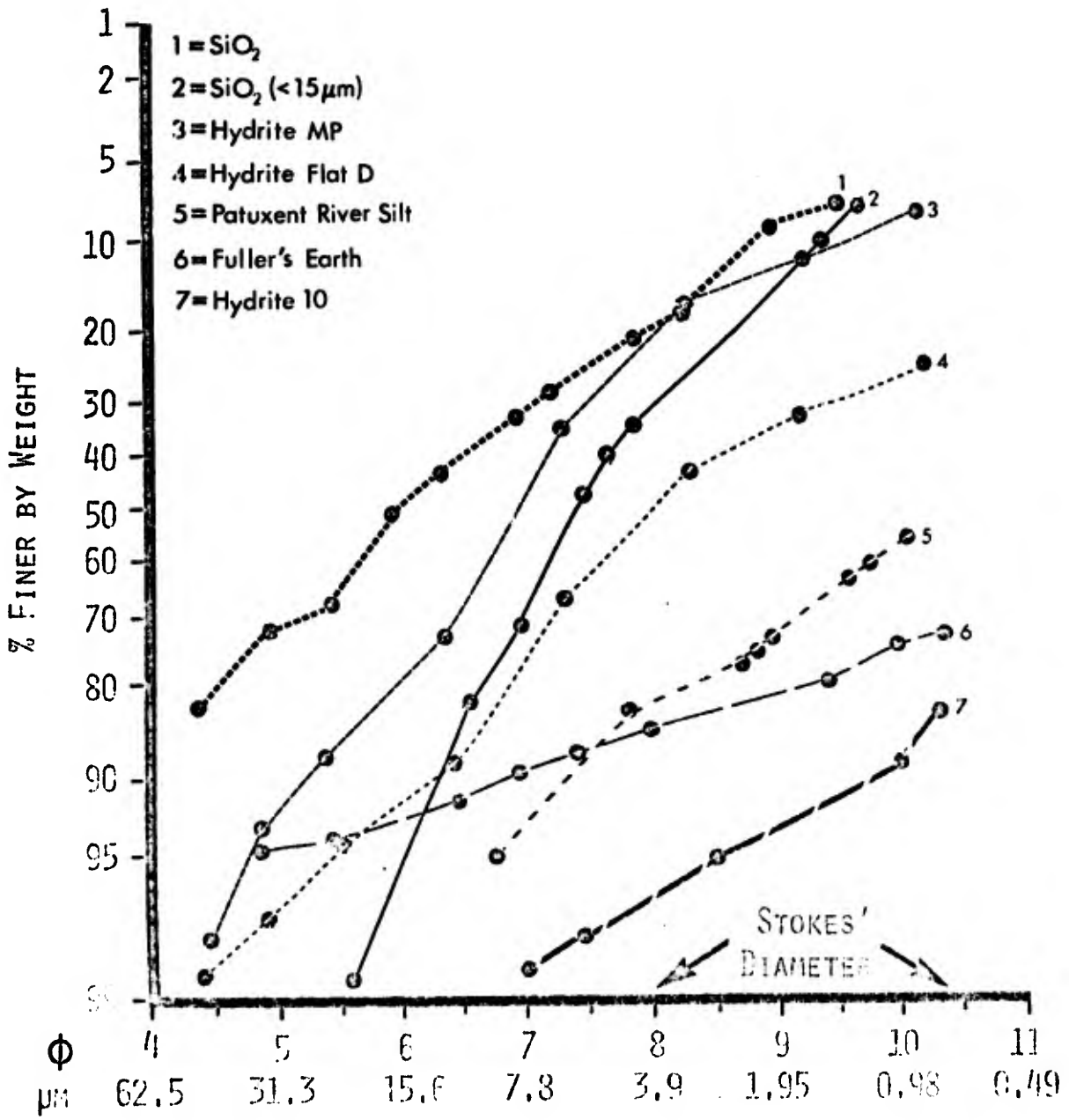

1 = SiO2
2 = SiO2 (<15μm)
3 = Hydrite MP
4 = Hydrite Flat D
5 = Patuxent River Silt
6 = Fuller's Earth
7 = Hydrite 10
% Finer by Weight
1
2
5
10
20
30
40
50
60
70
80
90
95
Stokes' Diameter
Φ
4
5
6
7
8
9
10
11
μm
62.5
31.3
15.6
7.8
3.9
1.95
0.98
0.49

Table D-1. Particle size distributions of artificial sediments used in this project. % finer = fraction (expressed as per cent) finer by weight than Stoke's diameter D in μm. Numbers in parentheses refer to lines in Fig. L-1.

| Fuller's Earth (6) | | Hydrite 10 (7) | | Hydrite Flat D (4) | | Hydrite MP (3) | | Silicon Dioxide (1) | | <15 μm | $SiO_2$ (2) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| % Finer | D | % Finer | D | % Finer | D | % Finer | D | % Finer | D | % Finer | D |
| 95.0 | 35.8 | 98.6 | 7.7 | 98.6 | 49.3 | 97.9 | 49.3 | 82.2 | 51.5 | 100 | 31.8 |
| 94.2 | 25.3 | 97.9 | 6.1 | 97.2 | 34.9 | 93.7 | 35.1 | 69.9 | 36.9 | 98.7 | 22.6 |
| 92.0 | 12.7 | 95.0 | 2.9 | 94.4 | 24.8 | 88.0 | 25.1 | 66.1 | 26.2 | 82.0 | 11.5 |
| 90.2 | 9.1 | 88.4 | 1.0 | 88.8 | 12.5 | 71.2 | 12.8 | 50.9 | 16.9 | 72.0 | 8.5 |
| 88.3 | 6.4 | 83.6 | 0.8 | 65.7 | 6.5 | 34.5 | 6.7 | 42.4 | 13.4 | 48.0 | 6.0 |
| 85.3 | 4.0 | | | 43.4 | 3.3 | 16.9 | 3.4 | 32.2 | 8.6 | 40.0 | 5.2 |
| 80.0 | 1.6 | | | 33.6 | 1.7 | 12.0 | 1.7 | 26.1 | 6.8 | 34.7 | 4.5 |
| 75.0 | 1.0 | | | 24.5 | 0.9 | 7.7 | 0.9 | 19.1 | 4.3 | 10.7 | 1.6 |
| 72.3 | 0.8 | | | | | | | 15.3 | 3.5 | 8.0 | 1.4 |
| | | | | | | | | 7.2 | 2.2 | | |
| | | | | | | | | 5.1 | 1.5 | | |

These data have been presented in such a way that graphic solutions (Folk, 1968) and mathematical calculations (Trask, 1968) can be used by the interested reader 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 Fig. D-2 and Table D-2. Median sizes ranged from a high of approximately 1.1 to a low of <0.5 μm (August collection). Fraction by weight finer than 2.0 μm ranged from a high of approximately 82% to a low of 65% (August collection). These particle size distributions of solids used in our work (Tables D-1 and D-2, Figs. D-1 and D-2) are comparable with those reported by May (1973) in the mud flow from a shell dredge (presented here as Table D-3).

2. Organic Matter Content

Organic matter content of natural sediment samples tended to increase throughout the summer of 1973 from a low of 8.9% in June to values in excess of 11% in August and September (Table D-4). A comparison of mean organic matter values (Table D-5) showed that these differences between earlier and later samples were significant. These differences may indicate significant importation of organic matter, which has settled out at Long Point, from marshes which line the shores of the Patuxent watershed These organic matter values are as high as those reported by Masch and Espey (1967) for Galveston Bay.

Organic matter analyses were conducted on the mineral solids, also. No significant weight loss from ashing was detectable in the $SiO_2$ and

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

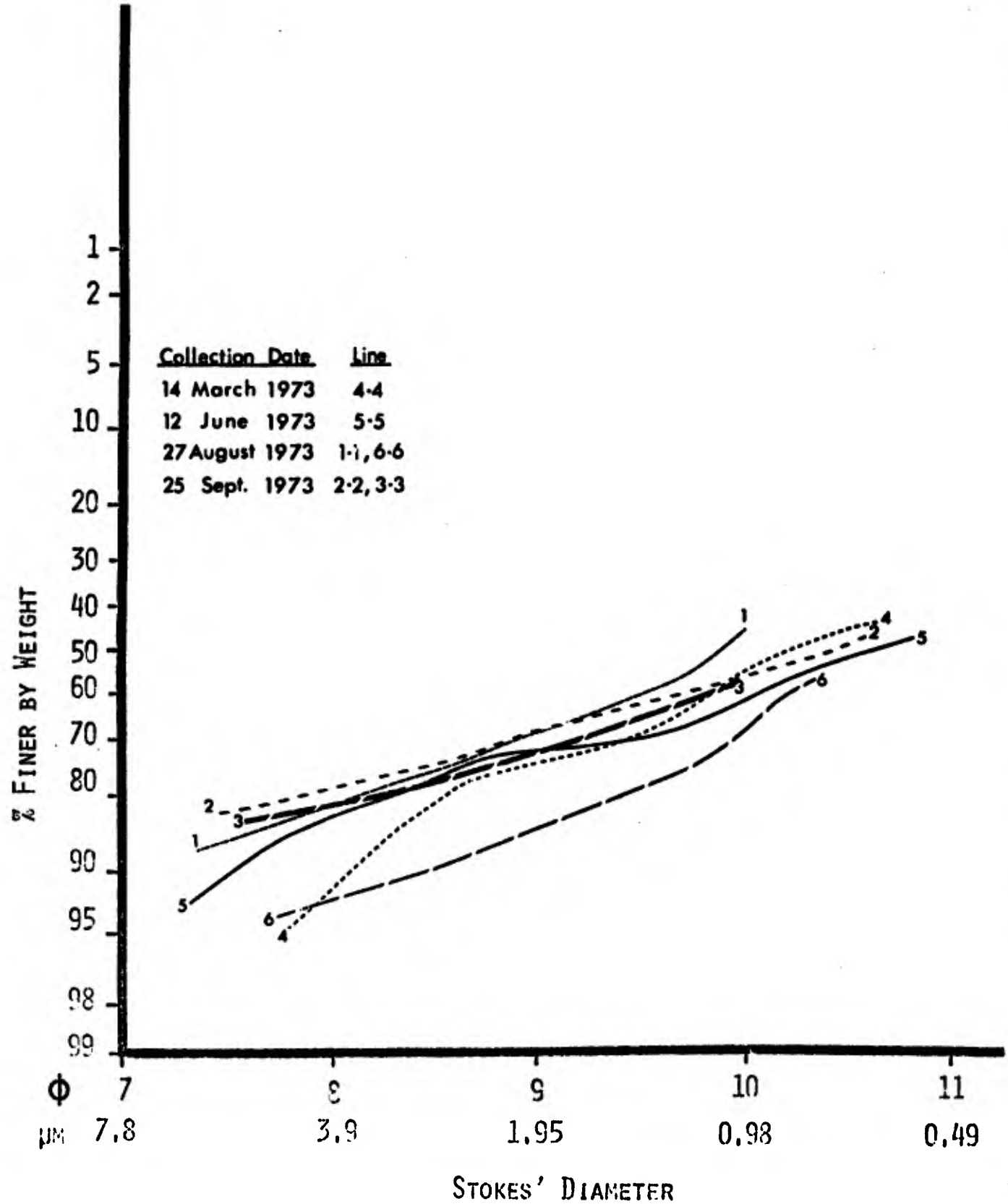

Collection Date Line
14 March 1973 4-4
12 June 1973 5-5
27 August 1973 1-1, 6-6
25 Sept. 1973 2-2, 3-3
% Finer by Weight
1
2
5
10
20
30
40
50
60
70
80
90
95
98
99
Φ 7 8 9 10 11
μm 7.8 3.9 1.95 0.98 0.49
Stokes' Diameter

Table D-2. Particle size distributions of representative natural Patuxent River sediment samples collected at Long Point during 1973. % finer = fraction (expressed as per cent) finer by weight than Stoke's diameter D in μm. Numbers in parentheses refer to lines in Fig.L-2.

| 14 March (4-4) | | 12 June (5-5) | | 27 August (6-6) | | (1-1) | | 28 September (3-3) | | (2-2) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| % Finer | D | % Finer | D | % Finer | D | % Finer | D | % Finer | D | % Finer | D |
| 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 | | | | | | | | |

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

| Meters from Discharge | SIZE RANGE | | | | |
|---|---|---|---|---|---|
| | 62-39 μm | 38-19 μm | 18-10 μm | 9-5 μm | 4-2 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). Samples were dried for 24 hours at 100°C, ground fine with a mortar and pestle, then ashed for 3 hours at 500°C. Organic matter values reported are percent loss of dry weight on ignition.

| Collection Date | Sample No. | Organic Content $\bar{X} \pm s$ | S.E. $\bar{x}$ |
|---|---|---|---|
| 6-17-73 | 1 | 9.4120 ± 1.0038 | .3174 |
| | 2 | 10.3580 ± 1.0272 | .4594 |
| | 3 | 10.9400 ± .3270 | .1463 |
| 6-28-73 | 1 | 9.8740 ± .3808 | .1703 |
| | 2 | 9.4720 ± .4127 | .1846 |
| | 3 | 8.9120 ± .6079 | .2719 |
| 7-14-73 | 1 | 11.2467 ± .5555 | .2268 |
| | 2 | 10.0983 ± .8682 | .3545 |
| 8-27-73 | 1 | 11.4483 ± .8321 | .3397 |
| | 2 | 11.4617 ± .7849 | .3205 |
| | 3 | 11.9700 ± .6712 | .2740 |
| | 4 | 12.6483 ± .4317 | .1762 |
| 9-18-73 | 1 | 11.8567 ± .3038 | .1240 |
| 9-25-73 | 1 | 11.4217 ± .4881 | .1993 |
| | 2 | 11.8750 ± .5131 | .2095 |
| | 3 | 11.2200 ± .4626 | .1889 |

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

| Sample Collection Dates | 6-28-73 | 7-14-73 | 8-27-73 | 9-18-73 | 9-25-73 |
|---|---|---|---|---|---|
| 6-17-73 | N.S. | N.S. | p<.001 | p<.001 | p<.001 |
| 6-28-73 | --- | p<.001 | p<.001 | p<.001 | p<.001 |
| 7-14-73 | --- | --- | p<.001 | N.S. | N.S. |
| 8-27-73 | --- | --- | --- | N.S. | N.S. |
| 9-18-73 | --- | --- | --- | --- | N.S. |

fuller's earth solids. Substantial weight losses in the kaolinites (about 11% of dry weight) were attributed to loss of bound water (at temperatures of 500°C) associated with these paper coating and pigment extending clays (Michael Taranto, Georgia Kaolin Co., personal communication).

3. Heavy Metals

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

Table D-6. Inorganically bound cations in artificial and natural sediment. Extraction by 0.075 N $HCl-H_2SO_4$ and analysis by atomic absorption spectroscopy. Values are mg $kg^{-1}$.

| Element | Hydrite 10 | Hydrite Flat D | Hydrite MP | Silicon Dioxide | Fullers Earth | Patuxent Silt $\bar{X} \pm S.E._{\bar{x}}$ (N=13) |
|---|---|---|---|---|---|---|
| Zn | 0.12 | 0.08 | 0.10 | 0.08 | 0.05 | 36. ± 2. |
| Cu | 0.14 | 0.05 | 0.02 | 0.04 | <0.01 | 4.4 ± 0.4 |
| Fe | 4.5 | 6.2 | 3.8 | 2.6 | <0.3 | 2100. ± 94. |
| Mn | <0.06 | <0.06 | <0.06 | <0.06 | 0.14 | 2300. ± 260. |
| Pb | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 | <10. |
| Co | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <4. |
| Ni | <0.3 | <0.3 | <0.3 | <0.3 | <0.3 | <4. |
| Cd | --- | --- | --- | --- | --- | 1. |
| Cr | <0.3 | <0.3 | <0.3 | <0.3 | <0.3 | <3. |
| Hg | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.2 |

## IV. LITERATURE CITED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