US Environmental Protection Agency

$$C = P + R + U + F$$

where C = total energy consumed
P = production, both somatic and reproductive
R = energy lost through heat production (respiration)
U = energy lost as excreta
F = energy lost as feces

$$C - F = A = P + R + U \quad \text{or}$$

$$P\,(\text{Scope for Growth}) = A - (R + U)$$

US Army Corps of Engineers

FIELD VERIFICATION PROGRAM (AQUATIC DISPOSAL)

TECHNICAL REPORT D-85-6

UTILITY OF THE SCOPE FOR GROWTH INDEX TO ASSESS THE PHYSIOLOGICAL IMPACT OF BLACK ROCK HARBOR SUSPENDED SEDIMENT ON THE BLUE MUSSEL, *MYTILUS EDULIS*: A LABORATORY EVALUATION

by

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September 1985
Final Report

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Prepared for DEPARTMENT OF THE ARMY
US Army Corps of Engineers
Washington, DC 20314-1000

and US Environmental Protection Agency
Washington, DC 20460

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The D-series of reports includes publications of the Environmental Effects of Dredging Programs:

Dredging Operations Technical Support

Long-Term Effects of Dredging Operations

Interagency Field Verification of Methodologies for Evaluating Dredged Material Disposal Alternatives (Field Verification Program)

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Utility of the Scope for Growth Index to Assess the Physiological Impact of Black Rock Harbor Suspended Sediment on the Blue Mussel, Mytilus edulis: A Laboratory Evaluation"

TO: All Report Recipients

1. This is one in a series of scientific reports documenting the findings of studies conducted under the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives (referred to as the Field Verification Program or FVP). This program is a comprehensive evaluation of environmental effects of dredged material disposal under conditions of upland and aquatic disposal and wetland creation.

2. The FVP originated out of the mutual need of both the Corps of Engineers (Corps) and the Environmental Protection Agency (EPA) to continually improve the technical basis for carrying out their shared regulatory missions. The program is an expansion of studies proposed by EPA to the US Army Engineer Division, New England (NED), in support of its regulatory and dredging missions related to dredged material disposal into Long Island Sound. Discussions among the Corps' Waterways Experiment Station (WES), NED, and the EPA Environmental Research Laboratory (ERLN) in Narragansett, RI, made it clear that a dredging project at Black Rock Harbor in Bridgeport, CT, presented a unique opportunity for simultaneous evaluation of aquatic disposal, upland disposal, and wetland creation using the same dredged material. Evaluations were to be based on technology existing within the two agencies or developed during the six-year life of the program.

3. The program is generic in nature and will provide techniques and interpretive approaches applicable to evaluation of many dredging and disposal operations. Consequently, while the studies will provide detailed site-specific information on disposal of material dredged from Black Rock Harbor, they will also have great national significance for the Corps and EPA.

4. The FVP is designed to meet both Agencies' needs to document the effects of disposal under various conditions, provide verification of the predictive accuracy of evaluative techniques now in use, and provide a basis for determining the degree to which biological response is correlated with bioaccumulation of key contaminants in the species under study. The latter is an important aid in interpreting potential biological consequences of bioaccumulation. The program also meets EPA mission needs by providing an opportunity to document the application of a generic predictive hazard-assessment research strategy applicable to all wastes disposed in the aquatic environment. Therefore, the ERLN initiated exposure-assessment studies at the aquatic disposal site. The Corps-sponsored studies on environmental consequences of aquatic disposal will provide the effects assessment necessary to complement the EPA-sponsored exposure assessment, thereby allowing ERLN to develop and apply a hazard-assessment strategy. While not part of the Corps-funded FVP, the EPA exposure assessment studies will complement the Corps' work, and together the Corps and the EPA studies will satisfy the needs of both agencies.

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Utility of the Scope for Growth Index to Assess the Physiological Impact of Black Rock Harbor Suspended Sediment on the Blue Mussel, Mytilus edulis: A Laboratory Evaluation"

5. In recognition of the potential national significance, the Office, Chief of Engineers, approved and funded the studies in January 1982. The work is managed through the Environmental Laboratory's Environmental Effects of Dredging Programs at WES. Studies of the effects of upland disposal and wetland creation are being conducted by WES and studies of aquatic disposal are being carried out by the ERLN, applying techniques worked out at the laboratory for evaluating sublethal effects of contaminants on aquatic organisms. These studies are funded by the Corps while salary, support facilities, etc., are provided by EPA. The EPA funding to support the exposure-assessment studies followed in 1983; the exposure-assessment studies are managed and conducted by ERLN.

6. The Corps and EPA are pleased at the opportunity to conduct cooperative research and believe that the value in practical implementation and improvement of environmental regulations of dredged material disposal will be considerable. The studies conducted under this program are scientific in nature and will be published in the scientific literature as appropriate and in a series of Corps technical reports. The EPA will publish findings of the exposure-assessment studies in the scientific literature and in EPA report series. The FVP will provide the scientific basis upon which regulatory recommendations will be made and upon which changes in regulatory implementation, and perhaps regulations themselves, will be based. However, the documents produced by the program do not in themselves constitute regulatory guidance from either agency. Regulatory guidance will be provided under separate authority after appropriate technical and administrative assessment of the overall findings of the entire program.

James Choromokos, Jr., Ph.D., P.E.
Director, Research and Development
U. S. Army Corps of Engineers

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The sensitivity, variability, and reproducibility of the scope for growth index (SFG) as an indicator of physiological condition was evaluated utilizing the blue mussel, Mytilus edulis, after exposure to highly contaminated dredged material. A preliminary experiment was completed to determine a no-observable-effects-concentration due to suspended reference sediment (REF) alone (50 mg/ℓ). The effect of contaminated dredged material from Black Rock Harbor (BRH) was then tested using three treatments of suspended sediment: (a) 50 mg/ℓ of BRH sediment (100 BRH), (b) 25 mg/ℓ each of BRH and REF sediment (Continued)

20. ABSTRACT (Continued).

(50-50 BRH/REF), and (c) 50 mg/ℓ REF sediment alone (100 REF). This 26-day bioassay demonstrated a significant SFG reduction in mussels exposed to 100 BRH sediment (-3.63 J/hr) and the 50-50 BRH/REF treatment (-2.32 J/hr) compared to mussels exposed to 100 REF sediment (2.53 J/hr). This experiment was replicated to evaluate the reproducibility of the technique. The second experiment produced similar results with the 100 REF treatment mussels having a significantly higher SFG (10.22 J/hr) than both the 50-50 BRH/REF (0.51 J/hr) and 100 BRH (-1.07 J/hr) mussels. The data indicate that in a laboratory exposure the use of the SFG index with M. edulis provides a sensitive and reproducible technique for determining the chronic negative impact due to this dredged material.

This investigation is the first phase in developing field-verified bioassessment evaluations for the Corps of Engineers and the U.S. Environmental Protection Agency regulatory program for dredged material disposal. This report is not suitable for regulatory purposes; however, appropriate assessment methodologies that are field verified will be available at the conclusion of this program.

PREFACE

This report describes work performed by the U.S. Environmental Protection Agency (EPA), Environmental Research Laboratory, Narragansett, R.I. (ERLN), as part of the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives Program (Field Verification Program (FVP)). The program is sponsored by the Office, Chief of Engineers (OCE), and administered by the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss., under the purview of the Environmental Laboratory (EL). The OCE Technical Monitors were Dr. John Hall and Dr. William L. Klesch. The objective of this interagency program is to evaluate the environmental consequences of dredged material disposal under aquatic, wetland, and upland conditions. The aquatic portion of the FVP study is being conducted by ERLN, with the wetland and upland portions conducted by WES.

The principal investigators for this aquatic study were Mr. William Nelson, Ms. Dianne Black, and Dr. Donald Phelps, all of ERLN. Assistance in the design and maintenance of the laboratory system was provided by Ms. Melissa Hughes and Mr. Greg Tracey. Laboratory-cultured algae were also provided by Mr. Tracey. Technical support for the scope for growth measurements was provided by Mr. William Giles. In addition, assistance in statistical analysis was provided by Drs. James Heltshe and Clifford Katz.

The EPA Technical Director for the FVP was Dr. John H. Gentile; Technical Coordinator was Mr. Walter Galloway; and the Project Manager was Mr. Allan Beck.

The study was conducted under the direct management of Dr. Thomas M. Dillon and Dr. Richard K. Peddicord of the Contaminant Mobility and Criteria Group (CMCG), Ecosystem Research and Simulation Division (ERSD), EL; and the general management of Dr. Charles R. Lee, Chief, CMCG, Mr. Donald L. Robey, Chief, ERSD, and Dr. John Harrison, Chief, EL. Mr. Charles C. Calhoun, Jr., and Dr. Robert M. Engler were Program Mangers of the EL Environmental Effects of Dredging Programs.

During the preparation of this report, COL Tilford C. Creel, CE, and COL Robert C. Lee, CE, were Commanders and Directors of WES and Mr. F. R. Brown was Technical Director. At the time of publication, COL Allen F. Grum, USA, was Director and Dr. Robert W. Whalin was Technical Director.

This report should be cited as follows:

> Nelson, W.G., Black, D., and Phelps, D. 1985. "The Utility of the Scope for Growth Index to Assess the Physiological Impact of Black Rock Harbor Suspended Sediment on the Blue Mussel, Mytilus edulis: A Laboratory Evaluation," Technical Report D-85-6, prepared by US Environmental Protection Agency, Narragansett, R.I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

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PART I: INTRODUCTION

Background

1. The U.S. Army Corps of Engineers (CE) and the U.S. Environmental Protection Agency (EPA) are jointly conducting a comprehensive Field Verification Program (FVP). The approach being used in the FVP is to evaluate and field validate assessment methodologies for predicting the environmental impacts of dredged material disposal in aquatic, upland, and wetland environments. The research, evaluation, and field verification of the upland and wetland disposal options will be conducted by the Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The application and field verification of predictive methodologies for the aquatic disposal option will be conducted by the EPA Environmental Research Laboratory (ERLN), Narragansett, R.I.

Purpose

2. There are three major objectives in the aquatic portion of the FVP at ERLN with respect to the scope for growth (SFG) index. The first objective is to evaluate the sensitivity, variability, and reproducibility of the SFG index. The blue mussel, Mytilus edulis, will be exposed to the same level of Black Rock Harbor (BRH) material in two separate laboratory experiments. The mussels will then be physiologically assessed using the SFG index, and the results of each experiment

evaluated to establish the accuracy and reproducibility of the technique. This constitutes the Laboratory Documentation Phase of the FVP and is the subject of this report.

3. Subsequently, a second objective will reproduce field level exposures in the laboratory and observe whether laboratory results accurately predict what is observed in the field. This is termed the Field Verification Phase. A third objective will determine the degree of correlation of tissue residues resulting from the bioaccumulation of contaminants from dredged material and ecologically significant alterations in organism viability as observed in both the laboratory and the field.

Scope

4. The SFG index (Warren and Davis 1967) is a measure of the energy available to an organism for production, both somatic and reproductive, after routine metabolic costs are accounted for. This index has had extensive application with M. edulis ranging from the investigation of the effects of ration levels on mussels (Thompson and Bayne 1974) to the effects of estuarine pollution levels on mussels (Widdows, Phelps, and Galloway 1981). In addition, an ecological relevance of the SFG index has been described by Bayne, Clark, and Moore (1981). They state that a sustained reduction in SFG results in decreased growth efficiencies, subsequently smaller individuals, and ultimately reduced fecundity and fitness.

5. While SFG has been proven useful in other applications, its use in this component of the FVP is to document the usefulness

and reproducibility of the scope for growth index (SFG) as a physiological endpoint in Mytilus edulis for measuring chronic effects of highly contaminated dredged material. In order to fulfill the Laboratory Documentation requirements of the FVP, the sensitivity of this technique was tested using a contaminated sediment (BRH sediment) and a reference sediment as the exposure materials. Reproducibility was assessed by comparison of the results from two separate experimental exposures.

6. A very important point must be emphasized at the begining of this report. SFG may be used to test two different hypotheses. The first hypothesis is that differences in exposure conditions (i.e., levels of suspended particulates, types of sediments, etc.) have no direct and immediate effect on the SFG index. To test this hypothesis the conditions under which SFG is measured replicate the experimental exposure conditions.

7. The second hypothesis is that there is no chronic effect due to differences between experimental exposures. To test this hypothesis the conditions under which SFG is measured are standardized and in no way attempt to replicate the actual experimental exposure conditions. Because standardized conditions are employed, only relative differences between the treatments of an experiment can be compared to evaluate chronic effects. Comparisons of absolute SFG values between experiments are not completely valid and must be made carefully. It is this second application that is being evaluated in the present FVP testing. All statements and comparisons concerning SFG effects and reproducibility must be considered with this fact in mind.

8. This report details two experiments. The first experiment establishes the effect of a 50-mg/ℓ suspended sediment exposure of: (a) 100 percent BRH sediment (100 BRH), (b) 100 percent reference sediment (100 REF), and (c) a 50 percent-50 percent mix of each sediment (50-50 BRH/REF) on the SFG of M. edulis. The second experiment, a replicate of the first, documents the reproducibility of the results obtained. In addition, a preliminary experiment (Appendix A) was completed to establish a "no-observable-effect-concentration" (NOEC) of suspended reference sediment with respect to the SFG index. The laboratory exposure levels were selected strictly as the NOEC. There was no expectation that these levels were in any way representative of suspended sedimentary levels actually occurring in central Long Island Sound.

PART II: MATERIALS AND METHODS

Overview

9. The tests described below generally follow methods prescribed in "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians" (ASTM 1980). Although the ASTM test methods were not specifically designed for sediment tests, they provide guidelines for experimental designs, water quality parameters, statistical analyses, and animal care, handling, and acclimation.

Sediment Collection and Storage

10. Two sediment types were used to conduct the suspended particulate tests in this study. The reference sediment (REF) was collected from the South reference site in Long Island Sound (41°7.95"N and 72°52.7"W) by a Smith-MacIntyre grab (0.2 m^2), press sieved through a 2-mm sieve, and stored at 4°C until used (Figure 1). Black Rock Harbor (BRH) sediment was collected from the highly contaminated and industralized dredge site (41°9"N and 73°13"W) with a gravity box corer (0.1 m^2) to a depth of 1.21 m, thoroughly mixed, press sieved through a 2-mm sieve, and refrigerated (4°C) until used (Figure 2). In all experiments, sediments were allowed to reach test temperature and mixed prior to use.

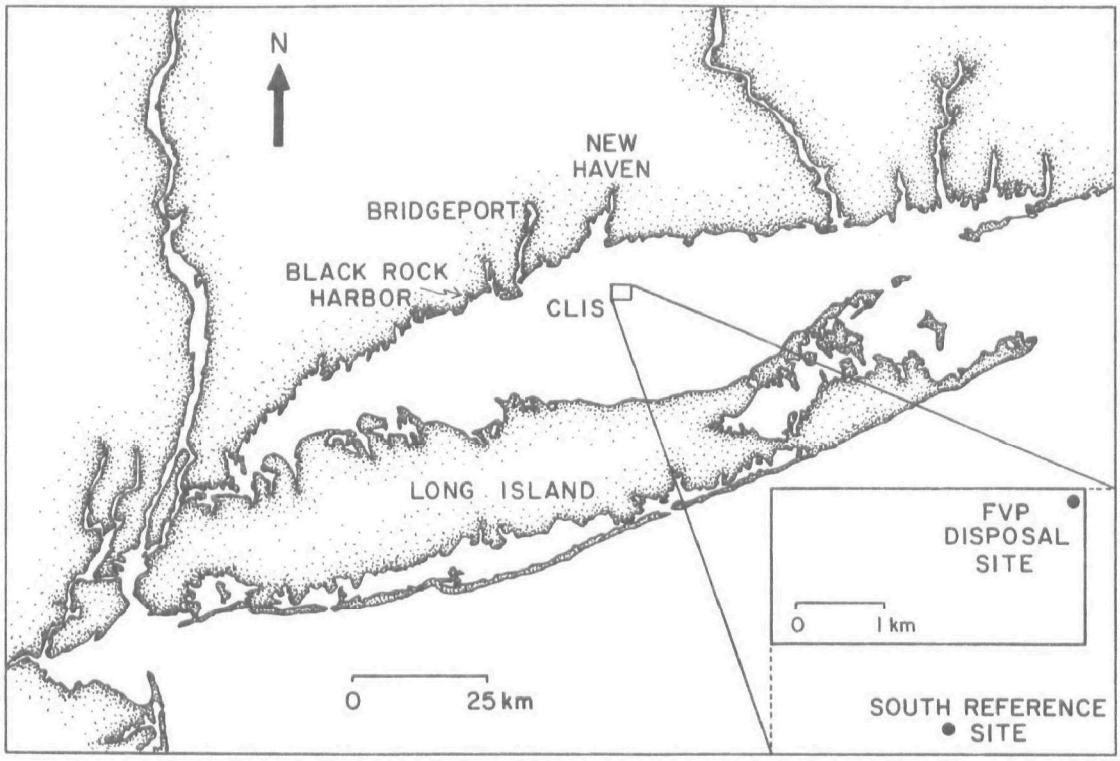

Figure 1. Central Long Island Sound disposal site and South reference site.

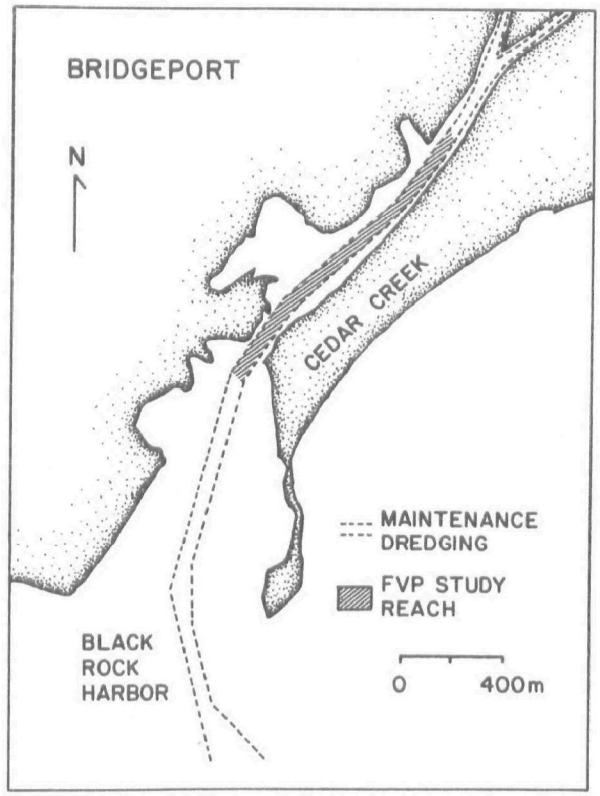

Figure 2. Black Rock Harbor, Connecticut, source of dredged material.

Mussel Collection

11. Mussels for each experiment, the preliminary one (NOEC) and the two BRH exposures (experiments one and two, were collected in a similar manner with a scallop dredge from an uncontaminated site near Dutch Island in the west passage of Narragansett Bay (71°24.0'W by 41°29.4'N) from depths ranging between 5 and 10 m. Collection information for each experiment is listed below:

Experiment	Collection Date	Experiment Begun	Field Temperature °C	Field Salinity °/oo
NOEC	10/7/83	10/11/83	17.5	31.0
Experiment 1	11/10/83	11/16/83	13.0	31.0
Experiment 2	3/8/84	3/19/84	5.0	29.0

The animals were sorted to obtain a size range of 50 to 55 mm shell length and held in a laboratory flow-through system at ambient temperature and in unfiltered seawater until the experiment was initiated. All experiments were run at 15°C. Mussels collected from the field when the temperature was below 15°C were acclimated in running unfiltered seawater at a rate of 1°C per day until 15°C was reached.

Experimental Design

No-observable-effect-concentration experiment

12. In order to obtain an effect with BRH material it was believed that the mussels should be exposed to the highest reasonable level of suspended particulates. The determination of a reasonable

particulate level was termed the no-observable-effect-concentration (NOEC) experiment. This experiment is detailed in Appendix A. Briefly, two criteria were used in selecting the NOEC: (a) maximum exposure to the suspended sediment, and (b) no significant reduction in SFG. The approach taken in the NOEC experiment was to expose mussels to different particulate concentrations (0, 6.25, 12.5, 25, 50, and 100 mg/ℓ) of suspended reference sediment. The results indicated that mussels in the 50-mg/ℓ treatment produced pseudofeces throughout the 28-day experiment, while those in the lower treatment levels did not. This would maximize processing of, and thus exposure to, the suspended material through the gastrointestinal tract of the animal. In addition, mussels from this treatment also exhibited the highest SFG values. For these reasons, a NOEC level of 50 mg/ℓ was selected.

Experiments one and two

13. The first experiment in the laboratory documentation phase of the FVP was designed to establish the sensitivity of the SFG index as a measure of impact, in this case due to possible effects of BRH dredged material on M. edulis. This experiment was repeated a second time to further document the observed sensitivity and thus determine the degree of reproducibility. The following methodology applies to both BRH experiments.

Exposure system

14. Implementation of the experimental design required the construction of two identical sediment dosing systems to simultaneously provide either BRH or REF material as suspended sediment. The dosing system (Figure 3) consisted of conical-shaped slurry reservoirs placed in a chilled fiberglass chamber, a diaphram pump, a 4-ℓ separatory funnel, and several return loops that directed the particulate slurry through the dosing valves. The slurry reservoirs (40 cm diam. x 55 cm high) contained 40 ℓ of slurry composed of 37.7 ℓ of filtered seawater and 2.3 ℓ of either BRH or REF sediment. The fiberglass chamber (94 cm x 61 cm x 79 cm high) was maintained between 4° and 10°C using an externally chilled water source. (The slurry was chilled to minimize microbial degradation during the test.) Polypropylene pipes (3.8 cm diam.) placed at the bottom of the reservoir cones were connected to the diaphragm pumps (16 to 40 ℓ/min capacity) that had Teflon diaphragms. These pumps were used to circulate the slurry but minimize abrasion so that the physical properties and particle sizes of the material remained as unchanged as possible.

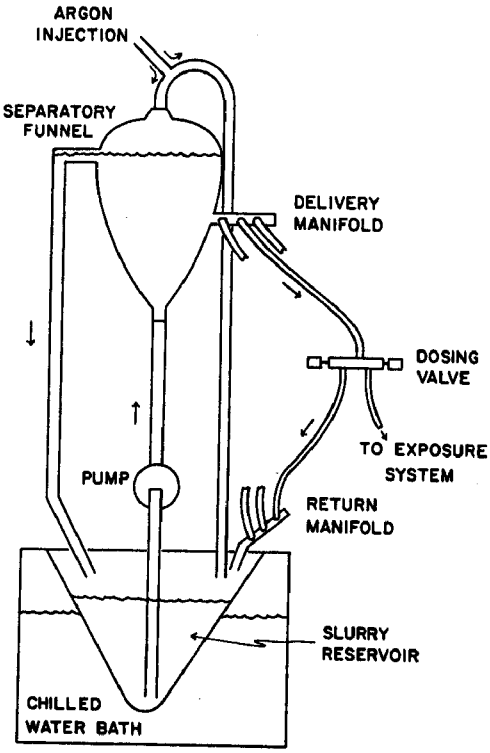

Figure 3. Sediment dosing system with chilled water bath and argon gas supply.

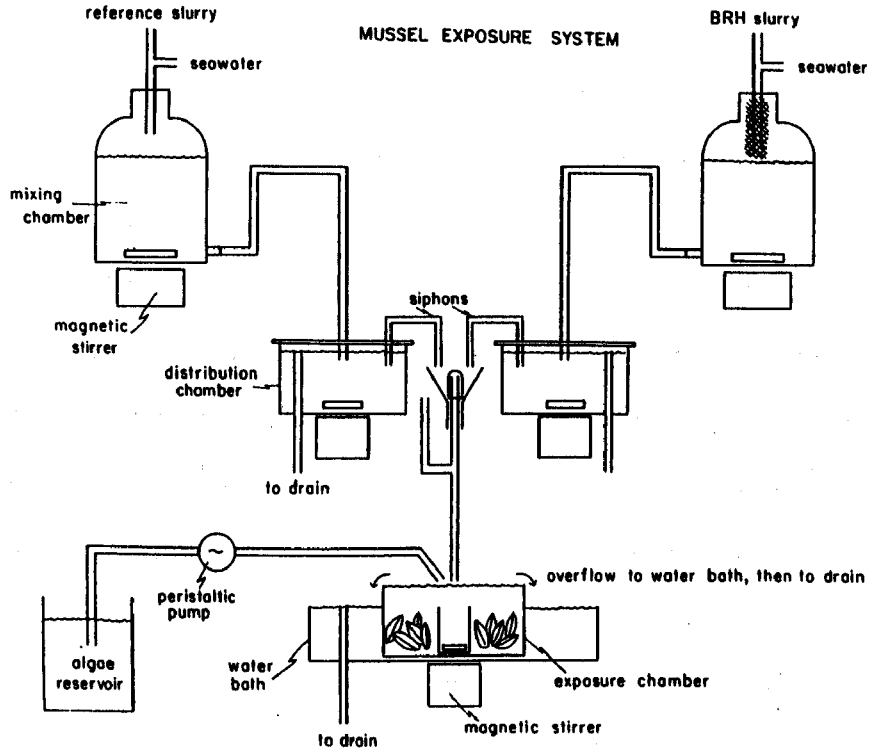

Figure 4. Exposure system used in the Black Rock Harbor experiments.

15. The separatory funnel was connected to the pump and returned to the reservoir by polypropylene pipes. The separatory funnel served two functions: (a) to ensure that a constant head pressure was provided by the overflow, and (b) to serve as a connection for the manifold located 4 cm below the constant head level. The manifold served to distribute the slurry by directing a portion of the flow from the funnel (through 6 mm inside diam. polypropylene tubes) through the Teflon dosing valves (Figure 3) and back to the reservoir. At the dosing valves, the slurry was mixed with seawater to provide the desired concentrations for the toxicity tests. Argon gas was provided at the rate of 200 ml/min to the reservoir and the separatory funnel to minimize oxidation of the sediment/seawater slurry. Narragansett Bay seawater filtered (to 15 μm) through sand filters was used for these experiments. The dosing valves were controlled by a microprocessor which was programmed to deliver a pulse with a duration of 0.1 sec up to continuous pulse delivery and at intervals from once every second to once every hour.

16. The exposure system is shown in Figure 4. In these experiments the REF and BRH mixing and distribution chambers (Figure 4) were maintained at 50 mg/ℓ. Exposure conditions were obtained by siphoning suspended sediment from the appropriate distribution chambers to produce a combined flow of 300 ml/min in each exposure chamber. The amount of suspended particulates both entering the exposure chambers (actual incoming concentration) and the concentration surrounding the mussels (actual surrounding concentration) were measured daily using a

spectrophotometer. Prior to the experiment, the relationship between absorbance and dry weight of suspended particulates had been determined by collecting triplicate samples of suspended sediment directly from the diluter or by preparing dilutions from the highest concentration. The dry weight of these samples was measured using the methods reported in Lake et al. (1984) and their absorbance was measured on the spectrophotometer. Linear regression analysis of the data established the relationship between absorbance and dry weight. Analysis of variance and multiple comparison tests were performed on the suspended particulate data collected daily during the experiment.

17. The three exposure treatments consisted of an incoming concentration of 50 mg/ℓ of: (a) 100 percent BRH sediment (100 BRH), (b) 100 percent REF sediment (100 REF), and (c) a 50 percent-50 percent mixture of each sediment (50-50 BRH/REF). Forty mussels were exposed in each treatment and fed Isochrysis aff. galbana (T-Iso) at a rate of 94 mg/mussel/day. On day 26, ten mussels from each treatment were sampled for SFG measurements. The remaining mussels were distributed for other end-point determinations. The first experiment was terminated after 26 days because mortality began to occur in the 100 BRH treatment. Experiment two was stopped after 26 days to replicate Experiment one.

Scope for Growth Methods

18. Calculation of the SFG index for M. edulis required the measurement of four parameters: clearance rate, respiration rate, food absorption efficiency, and ammonia excretion rate. Because the null

hypothesis being tested was that there would be no chronic effect due to exposure to BRH material, the SFG measurements were completed under standardized conditions. All SFG measurements for a given treatment were completed in the order shown below within 28 hrs after termination of the experiment and all measurements were performed at 15°C.

Clearance rate

19. Clearance rate is defined as the volume of water completely cleared of particles >3 microns (μm) in some unit time (Widdows et al. 1979). In the present experiment this was measured by placing mussels into individual chambers through which 1 μm filtered seawater flowed at a rate of 75 ml/min. The unicellular algae, T-Iso, was added to the filtered seawater to deliver an incoming cell concentration of approximately 25,000 cells/ml (about 0.5 mg/ℓ) to each chamber. Each chamber was gently aerated to ensure that complete mixing and no settling of algae occurred. Mussels were allowed to acclimate in the chambers for at least 1 hr prior to any measurements. The incoming and outgoing particle concentrations for each chamber were then measured with a Coulter Counter (Model TAII) and substituted into the following formula to determine clearance rate:

$$\text{Clearance rate} = [(C1 - C2)/C2] \times F \qquad (1)$$

where

C1 and C2 = incoming and outgoing particle concentrations, respectively

F = flow rate in liters/hour through the chamber.

Respiration rate

20. Respiration rates were measured by isolating each mussel in a glass respirometer vessel fitted with an electrode designed to measure the partial pressure of oxygen (PO2). The electrode was connected to a Radiometer oxygen meter (Model PHM71) which was in turn connected to a strip chart recorder. Each mussel was allowed to acclimate for about 10 minutes in the vessel prior to respiration measurements. This short acclimation period was found to be adequate by measuring the respiration rate of several mussels for 1 hr from time of initial placement into the vessel. There was no change in rate after the first 5 minutes. Seawater containing algae was pumped into the vessel during this acclimation period at a rate of 80 ml/min to ensure that food was present in the chamber and that routine metabolic rate was measured. After acclimation the flow of seawater was stopped and the decline in PO2 was recorded on the strip chart recorder for approximately 30 min. The respiration rates were calculated using the following formula:

$$MLO2HR = \frac{MMHG}{160} \times SATO2 \times \frac{RESVOL - MUSVOL}{1000} \times \frac{60}{O2TIME} \quad (2)$$

where

MLO2HR = oxygen consumed per hour by the mussel, ml

MMHG = change in partial pressure of O2 over time, mm mercury

SATO2 = oxygen saturation level of seawater at that temperature, ml/ℓ

RESVOL = respiration vessel volume, ml

MUSVOL = volume of the mussel, ml

O2TIME = time period of the measurement, min

Absorption efficiency

21. After completion of the respiration rate measurements, all fecal material was removed from each feeding chamber. This ensured that only the algae consumed during the SFG procedures were used in the absorption efficiency measurements. At the food concentration used in the SFG measurements, approximately 0.5 mg/ℓ, no pseudofecal production occurred. The mussels were allowed to feed overnight in the chambers. Fecal pellets were collected from each chamber with a Pasteur pipette and filtered onto a 1 μm Nuclepore polycarbonate filter. The filter was removed to a watch glass where a few drops of isotonic ammonium formate were added to facilitate removal of the fecal pellets. The fecal pellets were scraped off with a plastic spatula, deposited onto small aluminum pans (1 cm square), and placed in a drying oven at 100°C for 24 hr. Pellets and pans were weighed using a Perkin Elmer autobalance (Model AD-2Z). Pellets were ashed at 500°C for 4 hr, and reweighed to determine the ash-free dry weight:dry weight ratio for the feces. A similar procedure was completed with the cultured algae to obtain the ash-free dry weight:dry weight ratio of the food. Absorption efficiencies were calculated for each mussel according to the method of Conover (1966) using the following formula:

$$\text{Absorption Efficiency} = \frac{F - E}{(1-E) \times F} \times 100 \qquad (3)$$

where

F = ash-free dry weight:dry weight ratio of the food

E = ash-free dry weight:dry weight ratio of the feces

22. This technique allowed the calculation of an absorption efficiency for each mussel. In the past, at this laboratory and in the literature, fecal material was collected on pre-ashed glass fiber filters which weighed a great deal more than the dried and ashed fecal material. The great differential between the weight of the glass fiber filter and the weight of the dried and ashed fecal material appeared to introduce an artifact into the data. The substitution of the lightweight aluminum pans resulted in a 50 percent reduction in fecal weight variability and the subsequent absorption efficiency differences between individual mussels.

Ammonia excretion rate

23. Mussels were placed individually into HCl-stripped beakers containing 300 ml of 1 μm filtered seawater for a period of 3 hr. Mussels were then removed and a 0.45-μm filtered, 50-ml sample was collected from each beaker, deposited into acid-stripped polyethylene bottles, and stored in a freezer at -20°C until analyzed. Ammonia analyses were completed in duplicate for each sample according to the method of Bower and Holm-Hansen (1980).

Scope for growth calculations

24. After completion of the physiological measurements, the length and volume of each mussel were measured and the tissue excised, dried for 24 hr at 100°C, and weighed. The clearance rates, respiration rates, and ammonia excretion rates were standardized to a 1 g animal by converting the rates and dry weights to log 10, and fitting

the data to the allometric equation to obtain the fitted parameters, a and b, as described by Bayne et al. (1981). The weight-specific rates for each mussel were determined as follows:

$$\text{Weight-Specific Rate} = \frac{\text{Rate}}{\text{Weight}^{b}} \qquad (4)$$

where b = slope in the allometric equations calculated above. Absorption efficiencies were found to be independent of size (slope=0) over this narrow size range so absolute values were used.

25. The weight specific values for each mussel were then used to calculate the SFG of each individual by substitution into the following equation:

$$\text{Scope for Growth} = (C \times A) - (R + E) \qquad (5)$$

where

C = energy consumed (clearance rate X surrounding food concentration X energy of food)

A = absorption efficiency

R and E = energy lost through respiration and nitrogen excretion, respectively

The following energy conversions were used to calculate SFG:

One mg of T-Iso = 4.5×10^7 cells (this experiment)
One mg of T-Iso = 19.24 J (this experiment)
One ml O2 respired = 20.08 J (Crisp 1971)
One mg NH4-N = 24.56 J (Elliot and Davidson 1975)

The energy content of T-Iso was determined by filtering a volume of the algae onto preweighed glass fiber filters, drying them at 100°C for 24 hr, and reweighing them to determine algal dry weight. They were then

analyzed using the dichromate wet oxidation method of Maciolek (1962) to determine oxygen consumed and the resultant energy content.

26. Another index, the oxygen:nitrogen (O:N) ratio (the atomic ratio of oxygen consumed to ammonia-nitrogen excreted) can also be derived from the data obtained for the SFG calculations. This index was calculated for comparison with the SFG index.

Statistical analysis

27. Differences in physiological data and the resultant SFG values between each treatment in the NOEC and BRH exposure experiments were tested using one-way analysis of variance (Snedecor and Cochran 1978). All statistical tests were completed at the 0.05 level of significance. Tukey's studentized range test was applied to determine between-treatment differences. Comparison of results between laboratory experiments were completed using the Student's t-test, also at the 0.05 significance level. This distinction was made because laboratory exposure experiments were completed at different points in time.

PART III: RESULTS

Exposure System Monitoring

28. Data from the daily monitoring of exposure conditions for BRH sediment experiments one and two are presented in Table 1.

Table 1
Daily monitoring data for Black Rock Harbor Experiments One and Two.*

	Treatment		
	100 REF	50-50 BRH/REF	100 BRH
Experiment One			
Actual incoming concentration (mg particulates/ℓ)	56.2 (8.2)	59.4 (5.5)	62.8 (9.9)
Actual surrounding concentration (mg particulates/ℓ)	11.6 (5.1)	24.5(15.4)	30.2(17.5)
Experiment Two			
Actual incoming concentration (mg particulates/ℓ)	49.4 (6.1)	52.9 (5.7)	56.2 (8.6)
Actual surrounding concentration (mg particulates/ℓ)	14.1 (6.4)	23.5(10.1)	29.0(11.4)

* Values are means with standard deviation in parentheses.

29. The data presented in Table 1 indicate that comparable levels of sediments were delivered to each treatment, and that those levels were reasonably consistent throughout the course of the experiments.

Physiological Parameters

30. The physiological results of the two experiments are summarized in Tables 2 through 9. In the first experiment, two mussels died during the SFG measurements, one from each of 100 REF and 50-50 BRH/REF treatments. As a result the mean values from these treatments included only nine individuals, while the 100 BRH treatment means represent ten mussels. No mortality occurred in the second experiment; thus all mean values included ten individuals.

31. The weight-specific clearance rates are listed in Table 2. In the first experiment, the 100 REF and 50-50 BRH/REF treatments were similar while those mussels exposed to 100 BRH were significantly lower. The results of the second BRH experiment indicate that the mussels from the 100 REF treatment exhibited a significantly higher clearance rate than the mussels from either of the other two treatments.

Table 2
The mean (SE) weight-specific clearance rates of mussels from the two BRH experiments.

Treatment	Clearance Rate (ℓ/hr)	Group*
	Experiment One	
100 REF	3.81(0.23)	A
50-50 BRH/REF	3.59(0.35)	A
100 BRH	2.25(0.44)	B
	Experiment Two	
100 REF	3.99(0.37)	A
50-50 BRH/REF	1.54(0.26)	B
100 BRH	1.40(0.34)	B

* Means with the same group letter are not significantly different.

32. The absorption efficiencies (Table 3) were all relatively high in the first experiment with the 100 BRH and 100 REF treatments significantly higher than the 50-50 BRH/REF group. In the second experiment the absorption efficiencies were even higher; however, there were no differences between any of the treatments.

Table 3
The mean (SE) absorption efficiencies of mussels exposed to three exposure treatments in the two BRH experiments.

Treatment	Absorption Efficiency (percent)	Group*
	Experiment One	
100 BRH	92(0.9)	A
100 REF	89(0.8)	A
50-50 BRH/REF	82(2.1)	B
	Experiment Two	
100 REF	96(0.3)	A
50-50 BRH/REF	96(0.3)	A
100 BRH	96(0.3)	A

* Means with the same group letter are not significantly different.

33. The respiration rates for each treatment are listed in Table 4. There were no significant differences between treatments in either experiment. The actual rates were lower in the second experiment than in the first possibly due to seasonal differences.

Table 4
Mean (SE) weight-specific respiration rates of mussels exposed to three types of suspended sediments.

Treatment	Respiration Rate (ml-02/hr)	Group*
	Experiment One	
50-50 BRH/REF	1.00(0.02)	A
100 REF	0.91(0.04)	A
100 BRH	0.87(0.06)	A
	Experiment Two	
100 REF	0.61(0.03)	A
100 BRH	0.53(0.04)	A
50-50 BRH/REF	0.51(0.03)	A

* Means with the same group letter are not significantly different.

34. The data listed in Table 5 indicate that the ammonia excretion rates of the mussels from the 50-50 BRH/REF treatment were significantly elevated as compared with those from the 100 REF group in the first experiment, and the same differences were evident in the second experiment.

Table 5
The mean (SE) ammonia excretion rates for mussels from the two BRH exposure experiments.

Treatment	Ammonia Excretion Rate (µg NH4-N/hr/g)	Group*
	Experiment One	
50-50 BRH/REF	37.11(3.14)	A
100 BRH	26.88(2.81)	A B
100 REF	20.50(3.93)	B
	Experiment Two	
50-50 BRH/REF	20.93(1.09)	A
100 BRH	19.42(2.48)	A B
100 REF	14.13(1.47)	B

* Means with the same group letter are not significantly different.

Scope for Growth Index

35. While the inter-experimental comparisons of the individual physiological parameters are important, it is the comparison of the integrative SFG index that is of prime interest. The weight-specific SFG values for each treatment are listed in Table 6. In Experiment One, the 100 REF group exhibited a significantly higher SFG than those mussels exposed to 100 BRH and the 50-50 BRH/REF treatment. The same relative treatment differences were observed in the second BRH experiment, indicating the reproducibility of the technique. The actual mean SFG value of the 100 REF treatment in the second experiment (10.22 J/hr) was significantly higher than the first (2.53 J/hr), possibly due to seasonal differences; however, the relative differences were the same in both experiments. This will be further detailed in the discussion section.

Table 6
The mean (SE) weight-specific scope for growth values for the three exposure treatments in the two BRH experiments.

Treatment	Scope for Growth (J/hr/g)	Group*
	Experiment One	
100 REF	2.53(0.78)	A
50-50 BRH/REF	-2.32(1.17)	B
100 BRH	-3.63(1.58)	B
	Experiment Two	
100 REF	10.22(1.71)	A
50-50 BRH/REF	0.51(2.01)	B
100 BRH	-1.07(1.80)	B

* Means with the same group letter are not significantly different.

36. Another index, the O:N ratio, was calculated using the respiration rate and ammonia-nitrogen excretion rate data. The results, Table 7, indicate that there were no differences in these values between treatments in the first experiment. In the second experiment, the mean O:N ratio of the 100 REF mussels was significantly higher than 100 BRH mussels. The differences and inconsistencies between the results of this index and the SFG index will be discussed later in the report.

Table 7
The mean O:N ratios of each treatment in Experiments One and Two.

Treatment	O:N ratio	Group*
	Experiment One	
100 REF	77	A
50-50 BRH/REF	65	A
100 BRH	50	A
	Experiment Two	
100 REF	63	A
50-50 BRH/REF	49	A B
100 BRH	34	B

* Means with the same group letter are not significantly different.

37. One objective of the laboratory documentation phase of the FVP is to investigate the variability of the SFG index. One way to do this is to look at the coefficient of variation (CV) which is the standard deviation divided by the mean. The absolute value of the CV has been calculated for each physiological parameter and the SFG index and is presented in Table 8.

Table 8
The coefficients of variation (percent) for each of the physiological parameters, the SFG index, and the O:N ratio.

Parameter	Treatment		
	100 REF	50-50 BRH/REF	100 BRH
	Experiment One		
Clearance rate	18	29	63
Absorption efficiency	3	8	3
Respiration rate	14	7	21
Ammonia excretion rate	58	25	33
Scope for growth	92	152	138
O:N ratio	51	43	25
	Experiment Two		
Clearance rate	29	53	76
Absorption efficiency	1	1	1
Respiration rate	18	18	24
Ammonia excretion rate	33	16	40
Scope for growth	53	1250	530
O:N ratio	36	22	47

38. The data in Table 8 would indicate that exposure to BRH material causes a great increase in the variability associated with the clearance rate measurement and the SFG index in both experiments.

39. The means of the dry weights and lengths of the mussels from each treatment are listed in Table 9. The results from Experiment One indicate that the lengths of the mussels from each treatment were not significantly different, with coefficients of variation, CV, of 2-3 percent. The dry weights of those same animals indicated that the mussels from the 100 REF treatment were significantly higher than the other two treatments, with the CV increasing directly with increased exposure to BRH material. The results of the second experiment also indicated that the lengths of the mussels used were very similar.

Unlike the first experiment, however, there were no differences between the dry weights of the animals from each treatment. There was no evidence of spawning in any of the treatments.

Table 9
Mean dry weight and length of mussels from each treatment in the BRH experiments.

Treatment	Dry Weight (g)	Group*	CV** (%)	Length (cm)	Group*	CV (%)
		Experiment One				
100 REF	0.86(0.04)	A	15	5.32(0.05)	A	3
50-50 BRH/REF	0.59(0.04)	B	21	5.32(0.04)	A	2
100 BRH	0.59(0.06)	B	32	5.41(0.05)	A	3
		Experiment Two				
100 REF	0.91(0.05)	A	24	5.45(0.04)	A	4
50-50 BRH/REF	0.88(0.04)	A	15	5.46(0.01)	A	2
100 BRH	0.73(0.05)	A	24	5.40(0.01)	A	2

* Means with the same group letter are not significantly different.
** CV = coefficient of variation.

PART IV: DISCUSSION

40. The objective of the studies reported here is to evaluate the sensitivity, variability, and reproducibility of the SFG index as a method to assess the effects of BRH dredged material on M. edulis in a laboratory exposure. As shown in Table 6, a significant reduction in the SFG index was observed in mussels exposed to BRH dredged material when compared to mussels exposed to REF sediment, thus demonstrating the sensitivity of this index.

41. The BRH dredged material contains polychlorinated biphenyls (PCB) (6800 ng/g), polynuclear hydrocarbons (PAH) (9800 ng/g), and trace metals (Cu, Cr, and Pb, at 2380, 1430, and 380 µg/g, respectively) (Lake et al. 1984). In the same paper M. edulis was reported to accumulate 44% of the sediment PCB's, 28% of the sediment polynuclear hydrocarbons, and various amounts of the Cu, Cr, and Pb trace metals during a 28-day exposure to the BRH material. It is likely that the same materials were accumulated by the mussels during this experiment. A correlation is indicated between the significant differences in the physiological parameters and SFG values and the contaminants in the BRH material.

42. The data in Experiment One indicated that the mussels from the 100 BRH treatment exhibited a significantly lower clearance rate when compared to the other two treatments (Table 2). Stickle et al. (1983) reported a decreased clearance rate in M. edulis after a 28-day exposure to the water-soluble fraction of crude oil. Abel (1976)

investigated the effects of several metals on filtration rates in Mytilus and found that Cu, Zn, and Hg caused a reduction in this physiological parameter. Gilfillan et al. (1976) reported a reduced carbon flux in the soft-shelled clam, Mya arenaria, from areas that had been exposed to an oil spill. They attributed this to decreased filtration rates and higher respiration rates. Gilfillan (1975) showed that exposure to crude oil extracts also caused a reduction in filtration rates in M. edulis. Thus, sufficient evidence exists to support the hypothesis that individual exposures to heavy metals or oils can cause reduced filtration rates.

43. The results of the second experiment were similar with differences again between the clearance rates in the 100 REF and 100 BRH treatments. However, in the second experiment the mussels from the 50-50 BRH/REF treatment also exhibited a significantly reduced clearance rate from those mussels in the 100 REF treatment. While the reason for this is not immediately obvious, one possible explanation may be forwarded. The concentration of BRH material in the 50-50 BRH/REF treatment, 25 mg/ℓ of BRH material, may be near the threshold level that produces this effect. If the mussels filtered slightly more in the second experiment than the first, their exposure to the the BRH material would have been increased. A significant decrease in the clearance rates of the 100 BRH mussels was observed in both experiments. Therefore, a slight difference in exposure level (i.e., clearance rate) in the 50-50 BRH/REF treatment may have produced the reduced clearance rate in the second experiment.

44. In addition to affecting the absolute clearance rate, BRH dredged material also resulted in an increase in the variability of the clearance rate measurement (Table 8). In both experiments the response was consistent: an increased exposure to BRH material resulted in a corresponding increase in the CV of this measurement.

45. In contrast to the lower clearance rates, no differences were observed in respiration rates between the treatments in either BRH experiment. Stickle et al. (1983) found no differences in respiration rates between treatments in the exposures to crude oil previously mentioned. During acute tests, Brown and Newell (1972) observed a decrease in whole animal respiration rates in M. edulis during exposure to Cu. Scott and Major (1972) reported a similar decrease in the same species. In contrast, Gilfillan et al. (1976) stated that reduced carbon flow in M. arenaria was partially due to increased respiration rates. Engel and Fowler (1979) found that Cu caused an increase in respiration rate in excised oyster gill tissue. It is evident from the literature that no one respiration rate response is elicited consistently after exposure to a pollutant. While no differences in respiration rates were evident in the present study, it is important, for the purposes of this report, to note that the same response was obtained in both experiments.

46. The absorption efficiencies in both experiments were quite high. In the first experiment the 50-50 BRH/REF treatment was significantly lower than the 100 REF and 100 BRH treatments (Table 3), while in the second experiment there were no differences between any treatment. The algae used in this study were T. Iso, a naked flagellate.

This fact, along with the low food concentrations, 0.5 mg/ℓ used during the SFG measurements, can account for these high efficiencies. Widdows, Phelps, and Galloway (1981) fed Tetraselmis suecica to mussels at a concentration of 0.35 mg/ℓ and found similarly high absorption efficiencies of 93 percent. Other reported absorption efficiencies that use such species as the diatom Phaeodactylum tricornutum or natural seston would be expected to be lower than those reported here.

47. While each individual physiological parameter measured is of interest, the advantage of the SFG index is that it integrates the whole animal response of each individual. In the first experiment, the SFG data indicate that the mussels exposed to the 50-50 BRH/REF and 100 BRH treatments exhibited significantly lower SFG values, -2.32 and -3.63 J/hr, respectively, than those mussels from the 100 REF treatment (2.53 J/hr). The results of the second BRH experiment were the same as the first one, demonstrating the reproducibility of the technique. Those mussels from the 100 REF treatment displayed a significantly higher SFG (10.22 J/hr) than mussels from the 50-50 BRH/REF (0.51 J/hr) or 100 BRH (-1.07 J/hr) treatments. The SFG results are typical of a dose-response effect. A similar decrease in SFG was reported for M. edulis by Stickle et al. (1983) and for M. arenaria by Gilfillan et al. (1976) following exposure to oil. Widdows et al. (1981) have also reported a dose response type effect between SFG and aromatic petroleum hydrocarbon exposure concentrations in M. edulis.

48. The results of another index, O:N ratio, are listed in Table 7. With this index, a lower O:N ratio is indicative of a more stressed condition. In Experiment One there were no differences between treatments using this index. The results of the second experiment, however, indicated that the 100 REF treatment mussels exhibited a significantly higher O:N ratio than those mussels from the 100 BRH treatment. The results of this index are inconsistent with respect to the SFG index and they are also different between the two experiments. In addition, the O:N ratio results do not follow the dry weight data listed in Table 9. The dry weight data from Experiment One indicated that the 100 REF treatment mussels weighed significantly more than the mussels from the other two treatments. This is the same pattern that the SFG index showed. In the second experiment, there were no significant differences between treatments; however, the order was the same as in the first experiment, as were the SFG results. The dry weight measurements listed in Table 9 were made initially to calculate weight-specific physiological rates, not to infer changes due to treatment differences. One may infer, however, that an index used to measure stress, such as SFG and O:N ratio, might parallel changes in actual tissue weight. In these experiments the SFG index corresponds more closely to these changes than do the O:N ratio.

PART V: CONCLUSIONS AND RECOMMENDATIONS

49. The major objectives of the laboratory documentation portion of the Field Verification Program were to assess the sensitivity, reproducibility, and variability associated with various biological indices using BRH dredged material. The results of this experiment indicate that the Scope For Growth index, with M. edulis as the test organism, is a sensitive enough technique for measuring the chronic effects of this dredged material.

50. The primary purpose of this report, however, was to document the reproducibility of this index. The data in Table 6 indicate that the results of one experiment can be reproduced in a second replicate study. The SFG value of the 100 REF treatment from experiment two (10.22 J/hr) was significantly higher than the 100 REF SFG value from the first experiment (2.53 J/hr). This is not unexpected because SFG values do change normally during the year with changes in the reproductive cycle (Bayne et al. 1976). The important point is that the SFG values indicated the same relative response in both BRH experiments. This was the hypothesis being tested in this experiment and the rationale for using standardized conditions in the SFG measurements. The results of this experiment indicate that the SFG index, when used with M. edulis, satisfies the question of reproducibility for the Laboratory Documentation phase of the FVP.

51. In addition to measuring the sensitivity and reproducibility of the SFG index, the effect this material has on the variability of the measurements was also of interest. The data presented in

Table 8 would indicate that this material increases the variability observed in several of the measurements. The effect of BRH material on the variability of the clearance rates has already been mentioned. Clearance rate differences have a dramatic effect on the total amount of energy consumed, and, therefore, on the subsequent calculation of the SFG index. This can also be seen from Table 8, where large variation was also observed in the SFG index. The exact nature of how BRH causes more variability in this measurement is not clear. However, it does point out one advantage of using an index such as SFG; the physiological system that is negatively impacted may be isolated. In these two experiments it was apparent that BRH material had an effect on clearance rate. The actual mechanistic basis for this effect can now be studied further.

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APPENDIX A

NO-OBSERVABLE-EFFECT-CONCENTRATION EXPERIMENT

Introduction

1. As stated in the main text it was determined that in order to obtain an effect with BRH material it was believed that the mussels should be exposed to the highest reasonable level of suspended particulates. The determination of a reasonable particulate level was termed the no-observable-effect-concentration (NOEC) experiment. Two criteria were used in selecting the NOEC: (a) maximum exposure to the suspended sediment, and (b) no significant reduction in SFG. The null hypothesis was that there was no particulate level of suspended reference sediment that would have a chronic effect on the mussels. As described in Part I of the main text, SFG was measured under standardized conditions to determine whether some chronic effect had been incurred during the 28-day exposure.

Material and Methods

2. To test this hypothesis, reference sediment (REF) from Long Island Sound (Lake et al. 1984) was used and mussels were exposed in groups of twelve each to the following nominal dilution series: 100, 50, 25, 12.5, 6.25, and 0 mg/ℓ. Figure 4 shows the mussel exposure system. Sediment was delivered to the mixing chamber from the composite dosing system through a microprocessor-controlled valve as previously reported by Lake et al. (1984). For this experiment, the mixing and distribution chambers labeled BRH in Figure 4 contained filtered seawater only, while total suspended particulates (100 mg/ℓ) were maintained in the REF mixing and distribution chambers. The various dilutions were achieved by blending suspended

particulates from the REF distribution chamber with the filtered seawater. Each exposure chamber contained 12 mussels and received the appropriate mixture at a rate of 100 ml/min. The duration of the experiment was 28 days with samples of mussels taken on day 28 for SFG determinations. The procedures used for measuring SFG were the same as those descibed in the main text. The highest particulate level that did not produce a negative lasting effect would be chosen as the NOEC for the two BRH exposure experiments.

3. Measurements of the amount of suspended particulates entering the exposure chambers were made daily using a spectrophotometer, as previously detailed in Part II. The actual incoming and surrounding suspended particulate concentrations are shown in Table A1.

Table A1
Results of daily monitoring of exposure system in the NOEC experiment.

	Treatment					
	Control	6.25	12.5	25	50	100
Actual incoming conc. (mg/ℓ)	2(0.5)	7(2.3)	12(4.5)	26(6.2)	53(13.8)	106(27.6)
Actual surrounding concentration (mg/ℓ)	7(8.0)	10(7.1)	12(6.5)	15(6.9)	21(9.3)	49(15.6)
Algae (g/mussel/day)	0.094	0.094	0.094	0.094	0.094	0.094
Ref sediment (g/mussel/day)	0.0	0.075	0.150	0.300	0.600	1.200
Percent Food	100	55.6	38.5	23.9	13.5	7.3

4. The mussels were continuously fed laboratory cultured T-Iso at a rate of 94 mg (dry weight) per mussel per day (Table A1). Conditions and techniques of algal culture were modified after Guillard (1975). Guillard's "f/2" nutrient media was used, except that all trace metals but iron were eliminated and the concentration of the vitamins thiamine and B12 were doubled.

Results

Physiological parameters

5. Results of the physiological measurements are summarized in Tables A2 through A5. They indicate no consistent pattern between each parameter and the final SFG values (Table A6). The most probable reason for this is that some mussels from all treatments, except 100 mg/ℓ, were observed spawning during the physiological measurements (Table A7). This point will be discussed more fully in the following Discussion Section but should be kept in mind as the results in Tables A2 through A5 are presented.

6. The results of the reference sediment experiment are summarized in Tables A2 through A7. Table A2 lists the weight-specific mean clearance rates for the mussels from the six treatment levels. Although there was a wide range in the mean values, there were no significant differences between any of the treatments.

Table A2
Comparison of mean (standard error) weight-specific clearance rates of ten mussels per treatment in the NOEC test with reference sediment.

Treatment mg/ℓ	Clearance Rate (ℓ/hr)	Group*
100	4.22(0.45)	A
50	4.13(0.42)	A
Control	3.43(0.40)	A
12	2.95(0.52)	A
25	2.93(0.19)	A
6	2.64(0.39)	A

* Means with the same group letter are not significantly different.

7. The absorption efficiency results are listed in Table A3. Again there is a large variation in the means. The mussels from the 25-mg/ℓ treatment were significantly lower than the mussels from the 50-, control, and 100-mg/ℓ treatments.

Table A3
The mean (SE) absorption efficiencies of ten mussels per treatment in the NOEC reference sediment experiment.

Treatment mg/ℓ	Absorption Efficiency (percent)	Group*
50	76(0.04)	A
CONTROL	73(0.03)	A
100	72(0.03)	A
12	66(0.05)	A B
6	56(0.06)	A B
25	51(0.07)	B

* Means with the same group letter are not significantly different.

8. The mean weight-specific respiration rate for each treatment is listed in Table A4. The control group was significantly higher than all other groups.

Table A4
The mean (SE) weight-specific respiration rates for the NOEC experiment.

Treatment mg/ℓ	Respiration Rate (ml O2/hr/g)	Group*
Control	1.20(0.07)	A
25	0.95(0.05)	B
100	0.95(0.05)	B
50	0.84(0.06)	B
6	0.79(0.03)	B
12	0.78(0.02)	B

* Means with the same group letter are not significantly different.

9. Ammonia excretion rate means for each treatment are listed in Table A5. The mussels from the 6-mg/ℓ treatment had a significantly higher mean excretion rate than those mussels from the 12-mg/ℓ and control treatments. No other differences were observed.

Table A5
Mean (SE) ammonia excretion rates for ten mussels from each treatment from the NOEC experiment.

Treatment mg/ℓ	Excretion Rate (µg NH4-N/hr/g)	Group*
6	29.07(4.64)	A
25	21.48(4.59)	A B
100	20.33(5.91)	A B
50	15.05(3.12)	A B
12	11.80(2.19)	B
Control	7.99(1.99)	B

* Means with the same group letter are not significantly different.

Scope for growth index

10. The mean SFG values for each treatment in the NOEC experiment are listed in Table A6. The 50-, 100-, and 12-mg/ℓ treatments were statistically similar as were the 12-, 6-, control, and 25-mg/ℓ groups.

In addition, the 100-, 12-, and 6-mg/ℓ treatments were not statistically different. There was a large amount of variability in this data set which is reflected in the 95% confidence limits.

Table A6
The mean(SE) weight-specific scope for growth values for each treatment in the NOEC experiment. Also listed are the 95% confidence limits for each mean.

Treatment mg/ℓ	Scope For Growth (Joules/hr/g)	Group*	95% Confidence Limits
50	1.73(1.85)	A	5.85 to -2.29
100	-0.96(1.13)	A B	1.56 to -3.48
12	-3.86(1.42)	A B C	-0.70 to -7.02
6	-7.07(0.98)	B C	-4.89 to -9.25
Control	-8.48(1.48)	C	-6.30 to -10.66
25	-9.40(1.58)	C	-5.88 to -12.92

* Means with the same group letter are not significantly different.

11. Several other measurements were completed on these mussels and are listed in Table A7. There was no difference between the mean length of the mussels in each treatment; however, the dry weight of the mussels from the 12- and 50-mg/ℓ treatments were statistically higher than those from the 100-mg/ℓ group. In addition, several mussels were observed spawning during and/or after the clearance rate measurements. While these numbers are listed in Table A7, there is no assurance that these were the only mussels to have spawned. Other mussels may have spawned either at night or prior to any of the physiological measurements.

Table A7

Mean (SE) lengths and dry weights of ten mussels from each treatment in the NOEC experiment. Also listed are the number of mussels observed spawning during physiological measurements from each treatment.

Treatment mg/ℓ	Length(SE) * (cm)	Dry Weight(SE)* (g)	Mussels Spawning
12	5.33(0.06) A	0.66(0.07) A	1
50	5.40(0.06) A	0.65(0.04) A	1
25	5.35(0.04) A	0.62(0.03) A B	2
6	5.33(0.05) A	0.60(0.03) A B	1
Control	5.41(0.04) A	0.55(0.02) A B	8
100	5.35(0.05) A	0.47(0.04) B	0

* Means with the same group letter are not significantly different.

Discussion

12. The purpose this preliminary experiment was to determine a suspended sediment load that would have no observeable effect on the SFG of Mytilus edulis. This concentration was determined to be a nominal incoming concentration of 50 mg/ℓ, based on two facts: (a) psuedofeces were continuously produced in this treatment, thus giving maximum exposure to the suspended sediment; and (b) the mean SFG value was highest for this treatment. This decision may seem arbitrary after inspection of the results of this experiment. A great deal of variability and inconsistency occurred in the data from this experiment, most probably associated with the spawning activity during the measurements. However, for the purpose of this report, which is to test the accuracy and reproducibility of the SFG index, the results are quite important and are included as this appendix.

13. The most obvious result of this experiment was the lack of a consistent pattern in the response of the individual physiological parameters used to calculate the SFG index, relative to the exposure level. SFG is an integrative index; however, the data from individual physiological measures can be important to the interpretation of results. In previous studies the effects of particular pollutants have been documented with M. edulis (Phelps et al. 1983, Stickle et al. 1983, Widdows at al. 1981, Bayne et al. 1979). In these studies one of the measured SFG parameters contributed overwhelmingly to the observed change in SFG. The reference sediment used in the present experiment was from the relatively clean reference site in

Long Island Sound. The effects observed in the present NOEC experiment, therefore, are probably due to physical action of the suspended material rather than to a toxic chemical compound. The results of the NOEC experiment showed no clear pattern between the individual physiological paramerers in response to the sediment load. Three possible explanations are considered. First, the exposure system itself may have introduced variability due to some unidentified artifact. Second, the physiological measurements may have introduced some unknown error into the data set. Third, a factor other than sediment concentration, i.e., differences in the reproductive condition of mussels between treatments, may have been influencing the results.

14. After inspection of the system monitoring data, it would appear that the first possibility is not likely. Table A1 shows that the system was working properly throughout the experiment.

15. The second alternative would appear to be unsubstantiated as well. For example, the clearance rate data indicated that the mussels with the highest suspended levels during the experiment (50 and 100 mg/ℓ) also had the highest clearance rates (Table A2) during the SFG measurements. If this were an artifact from the experiment, one would expect just the opposite. Winter (1978) stated that mussels try to maintain a constant ingestion rate by decreasing clearance rate with increasing particle concentration. This same effect was described by Widdows et al. (1979). Therefore, the observed clearance rates do not seem to be due to some factor such as improper acclimation time before the clearance rate measurement was initiated.

16. The absorption efficiency data likewise follow no distinct pattern. While variable, the only significant differences were between the 50- and 25-mg/ℓ treatments (Table A3). Thompson and Bayne (1972) showed that absorption efficiency was inversely proportional to the suspended particulate load. If something from the sediment experiment were affecting this measurement, one would expect differences between the highest and lowest particulate concentrations. In addition, the respiration rates were highest in the control and 100-mg/ℓ treatments, which had the greatest difference in particle levels during the test.

17. The important conclusion is that no individual physiological factor was apparently responsible for the SFG differences between treatments. In this experiment the factor most closely correlated to the order of SFG values was the total suspended particulate levels, and consequently food levels. The amount of food supplied to each treatment was the same; however, the particulate levels were different (Table A1). In effect, the reference sediment caused a decrease in the percentage of algae ingested with increasing particle concentration (Table A1). This had the effect of supplying more algae to the control group and proportionally less to each higher particulate level group.

18. Differences in food levels between treatments lead to the third alternative, which offers the most probable explanation for the observed variability and inconsistencies in the NOEC experiment. Sastry (1975), working with another bivalve, Argopectin irradians, found that both an adequate food supply and temperature regime were necessary to initiate the gametogenic cycle in the bay scallop. Bayne (1976)

postulated that the same process was necessary with M. edulis. In the present experiment, it is possible that the combination of temperature (constant 15°C) and adequate food supply in the control and lower particle level treatments may have been sufficient to initiate the gametogenic cycle. The higher concentrations may not have had a sufficient food supply for gametogenesis to occur at the same rate as the lower concentrations. The number of mussels spawning during the SFG measurements may add some credence to this assumption (Table A7). The control treatment had eight out of ten mussels at least partially spawn, while the 25-mg/ℓ treatment had two spawn, and one mussel out of ten in each of the 6-, 12-, and 50-mg/ℓ treatments. No spawning occurred in the 100-mg/ℓ treatment mussels. Bayne et al. (1976) stated that SFG values are lower during gametogenesis, and this may be a possible explanation for the variable results. While this hypothesis is speculative at the present time, inspection of the reproductive cycle of the field population from which these mussels were collected may help to clarify it.

19. In another study, Nelson (in prep) followed the gametogenic cycle of this population during the past year. Using sterology and mantle dry weight as reproductive indices, he found that mussels from this area exhibited a large spawning peak in late March and a smaller peak in early December. This second spawning period coincides with the collection period for the NOEC experiment. The mussels used in this experiment, therefore, were probably at different stages of the gametogenic cycle. This fact, in addition to the possible

treatment food differences mentioned above, may help to explain the spawning differences observed in this experiment. The important point is that differences in the reproductive condition of experimental organisms can lead to serious problems when the data are interpreted. This is one factor that must be considered in any experimental design.

20. With this problem in mind, the SFG values from this experiment will be discussed briefly. These values can only be compared in a relative sense because the physiological measurements were completed under standardized conditions. A mussel with an SFG value of zero is, by definition, at its maintenance ration. Inspection of the 95 percent confidence limits about the means indicate that the 50-, 100-, and 12-mg/ℓ treatments were around that maintenance ration. The other three treatments were below this ration level for this test only. During the physiological measurements, cultured algae were supplied at approximately 0.5 mg/ℓ of seawater. This ration was chosen based on the metabolic rates of mussels collected at this time of the year in the field (Nelson in prep). Because this test was completed in less than 24 hr, it is believed that any sub-maintenance ration did not adversely affect the animals. In a relative sense, therefore, it is believed that the statistical differences observed between means are valid. Long-term holding under these conditions would not be recommended. However, in the exposure system, the food levels were considerably higher.

21. The final SFG values (Table A6) indicate that the mussels in the 50-, 100-, and 12-mg/ℓ treatments were not different statistically.

Of these three, only the 50-mg/ℓ treatment did not overlap any other group. In light of this fact and the previous information concerning pseudofecal production, a nominal incoming concentration of 50 mg/ℓ was chosen as the NOEC for the BRH experiments.

22. One problem encountered during the NOEC test was the reproductive condition of different groups of mussels. This problem was not encountered during the BRH experiment. Differences in reproductive condition can cause complications when interpreting SFG data measured at different times of the year. One possible solution would be to use juvenile mussels in place of adults. The use of adult mussels to measure the effects of pollutants, including SFG, is well documented. With the proper experimental design a hypothesis could be tested to determine if the same results could be achieved using juvenile mussels instead of adults. This would alleviate one source of variability and possibly make the comparison of SFG results more straightforward.