AN ABSTRACT OF THE THESIS OF

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Title: THE EFFECTS OF CONCENTRATION AND PARTICLE SIZE OF SUSPENDED MATERIALS ON GROWTH AND CONDITION OF THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*)

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The effects of exposure to suspended silt, kaolin and fuller's earth on growth and condition of Pacific oysters, 16 months of age, were studied. The exposure apparatus maintained a continuous flow of turbid water through chambers containing test oysters. Additional studies measured the filtration rates of oysters exposed to suspended sediments, and microscopic examinations of sediment transport on excised gill tissue were made.

Growth was assessed as total wet weight increase over two-week exposures to sediments. Normal wet weight increase under field conditions averaged 2.30 percent and was similar to that found under laboratory conditions. Laboratory growth, under conditions of flowing, unfiltered seawater, was comparable to growth when test animals were fed laboratory cultured algae in filtered seawater.

Silt particles, 104 to 149 μ and 74 to 104 μ in size, did not inhibit growth. Silt particles 38 to 74 μ in diameter appeared to reduce growth at 0.65 and 1.06 g/liter, with the intensity of effect increasing with an increase in silt concentration. No statistically significant growth reduction occurred after exposure to silt particles <38 μ in size, although visual inspection suggested that some reduction may have occurred at 1.61 g/liter.

Kaolin particles 0.2 μ, 1.5 μ and 9.5 μ in size significantly reduced growth of oysters in the range of 0.36 to 1.81 g/liter, with the level of growth inhibition increasing with kaolin concentration. Many animals appeared to lose weight as a result of exposure to kaolin sediments. Similarly, growth was reduced as the concentration of 5 μ fuller's earth increased from 0.20 to 1.37 g/liter.

None of the sediments tested had a marked effect on condition index, except in tests employing silt particles 74 to 104 μ in size. Although a significant reduction in condition was observed in this test, it was concluded that this may have been an anomalous result.

Filtration rates of oysters exposed to all particle sizes of kaolin and fuller's earth appeared to decrease in response to increase in sediment concentration. Normal filtration rates averaged approximately 1.60 l/hour, and decreased to below 0.40 l/hour at sediment levels of about 0.40 g/liter. A 50% reduction in filtration rate occurred at about 0.02 g/liter of 0.2 μ kaolin and at 0.2 g/liter of

9.5 μ kaolin. The concentration of 50% effect with fuller's earth was intermediate to that of 0.2 μ and 9.5 μ kaolin, and the same as that for 1.5 μ kaolin.

Since apparent clogging of the gills in *in vitro* tests occurred at concentrations of sediments similar to those affecting growth and filtration, overloading of the gills was considered as a possible sensory mechanism regulating the response of oysters to suspended sediments.

The results are discussed in relation to Northwest water quality problems and water resource management.

The Effects of Concentration and Particle Size of Suspended Materials on Growth and Condition of the Pacific Oyster (*Crassostrea gigas*)

by

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INTRODUCTION

Suspended materials occur naturally in estuarine water largely as a result of the transport of sediments during periods of high stream discharge and as a result of high winds. The quantities of suspended sediments may be increased further by the activities of man, including channel dredging, increased stream erosion and the construction of structures which modify current patterns. Because of the impact that man's activities may have on this aspect of estuarine water quality, it is both appropriate and essential to inquire about the consequences of the estuarine biota. Yet this has only been done to a limited extent. The scarcity of information presently available is emphasized by the bibliographies of Hollis, Boone, DeRose and Murphy (1964) and Scherk and Cronin (1970) and by the literature summary of Scherk (1971), all dealing with the problem of suspended sediment effects on aquatic organisms.

The primary purpose of the present study was to examine the effects of exposure to suspended sediments on the growth and condition of the Pacific oyster, *Crassostrea gigas* (Thunberg, 1795), the principal commercial oyster of the North Pacific. Because high levels of suspended solids normally occur during relatively short periods of time associated with storms, spring run-off and periods of dredging activity, the exposure periods employed in this study were limited to two weeks. Emphasis was placed on the effects of suspended sediment concentration and particle size. In separate experiments, filtration rates were estimated from the rate of clearing of turbid water in static systems. Observations of excised gill sections were also made following exposure to sediments.

The Occurrence of Suspended Materials in Natural Waters

Few authors have reported on the occurrence of suspended sediments in marine or estuarine waters, and even fewer have attempted to investigate the effects of these conditions on oysters. Drinkwaard (1959) reported an average annual level of suspended materials of 17.1 mg/liter in the Oosterschelde, Netherlands. However, in winter months, average suspended material concentrations reached 24.0 mg/liter. He noted that wind action raised the levels of suspended matter and turbidity, and he suggested that the growth and fattening of oysters in summer was not favored by these conditions. According to Galtsoff (1956) the Mississippi River annually deposits seven million cubic feet of suspended matter into its delta and adjacent parts of the Gulf, thereby reducing the productivity of oyster reefs in these areas. This suggests that reduced production from oyster grounds may result from either the effects of suspended sediments or the smothering effect of settled sediments.

Hermann (1969) reported that levels of suspended materials in Grays Harbor, Washington, were highest in months of high river run-off, when average suspended solids levels occasionally approached 0.1 g/liter. According to studies of Schubel (1968), maximum levels of suspended sediments in northern Chesapeake Bay exceeded 0.11 g/liter during the period of peak river flow. Sheldon, Loring and Deleu (1969) have stated, however, that most of the annual sedimentary deposition in estuaries is due to reworking of material already in the bay. It seems likely, therefore, that the quantity of suspended materials in an estuary may exceed that introduced by river transport alone.

Added to the natural levels of turbidity found in estuaries are contributions from the activities of dredging operations. During such an operation on the Powder River in Oregon, Wilson (1956) found turbidities of 5 ppm above and 1,700 ppm below the dredge. During dredging of an intracoastal waterway, 22 to 27 cm of sediment composed of a silt-clay mixture was deposited within 0.5 mile of the dredging area (Hellier and Cornicker, 1962). A large portion of dredging in West coast estuaries is done by seagoing hopper dredges. Large quantities of sediments are dredged in this manner annually, ranging up to 30 million cubic yards in Oregon alone (O'Neal and Sceva, 1971). This form of dredging may release greater quantities of fine sediments into the water than by pipeline dredges. In hopper dredge operation, sediments are drawn up and discharged into a hopper on the ship. The liquid overflow from the hopper, which contains most of the fine silt and clay fractions, is returned to the water. This mode of operation leaves a plume of turbid water trailing the ship, which may extend many miles downstream.

Effects of Suspended Materials on Larval Bivalves

Davis (1960) noted that both egg development and larval growth of the clam, *Venus mercenaria*, were retarded at silt concentrations of 0.75 g/liter, and were further retarded or stopped at 3.0 to 4.0 g/liter. However, no appreciable mortality of clam larvae occurred within 12 days even at the higher concentrations. In a later study, Davis and Hidu (1969) found that silt, kaolin, and fuller's earth each affected American oyster egg development with the intensity of effect increasing with concentrations ranging from 0.188 to 4.0 g/liter. They also reported that silicon dioxide particles less than 5 μ in diameter had the greatest effect on the growth and survival of European oyster larvae.

Effects of Suspended Materials on Adult Bivalves

Although many important adult bivalves are inhabitants of estuarine regions where turbid conditions are often found, little effort has been expended in studying the possible biological consequences of suspended materials on this group. Among the earliest studies, Loosanoff and Tommers (1948) reported that the pumping rate of Crassostrea virginica exposed to silt was reduced by 57% at 0.1 g/liter and by 80 to 94% at 1.0 to 4.0 g/liter. They noted similar responses with suspensions of chalk, kaolin and fuller's earth. Although this clearly shows a trend toward a reduction in feeding rate as turbidity increased, these authors noted that the variation between individuals was very great. They also noted that the amplitude of shell movement became greater as turbidity increased. When the turbid water was replaced with regular seawater, pumping rates and shell movement returned to normal. Later, Loosanoff (1962) reported that dead and dying oysters in turbid waters always contained large quantities of silt on the gills, and concluded that lamellibranchs feed most effectively in clear water. Korringa (1952) observed that heavy winter kills of oysters were most often noted during rough weather and turbid waters, and suggested that oysters weakened by prolonged cold temperature could not cleanse their gills of the mud, and, therefore, died.

According to Scherk (1971), Chiba and Ohshima (1957) reported that pumping of Venerupis semidecussata, Mercenaria meretrex, Crassostrea gigas and Mytilus edulis was unaffected by suspensions of charcoal powder, kaolin, bentonite, talc, diatomaceous earth, shale or starch up to 0.01 to 0.02 g/liter. Bentonite presumably represented an unusual situation, in that pumping by these species was not even affected by suspensions up to 0.5 to 1.0 g/liter. In two of the species, V. semidecussata and M. meretrex, the volume of bentonite particles filtered presumably increased proportionally to the concentration of the particles in water, but above 0.01 g/liter, the excess filtered material was cast off as pseudofeces. C. gigas and M. edulis appeared to differ in that the volume of filtered particles at higher concentrations was not proportional to bentonite concentration.

Studies by Johnson (1972) on the slipper limpet, Crepidula fornicata, are of interest. He found that filtration rate of this species decreased as silt concentrations increased up to 1.6 g/liter. The greatest effect was observed between 0.2 and 0.5 g/liter.

Although there is little information, it appears that particle size is important in determining the efficiency of filtration by adult bivalves. In Ostrea spp. and Mytilus spp., particles as small as 1 to 3 microns seemed to represent the lowest limit for efficient filtering (Jorgensen, 1955). In Crassostrea virginica, suspensions of graphite particles 2 to 3 microns in size were much more rapidly cleared than suspensions of particles less than 2 microns (Jorgensen and Goldberg, 1953), indicating that the critical size range for this species is similar to that of Ostrea and Mytilus.

METHODS AND MATERIALS

The approach used in the growth studies involved a 12-day exposure of young adult oysters, Crassostrea gigas, to constant levels of suspended sediments. Three types of suspended materials were tested--fuller's earth, kaolin and bay silt--each at several concentrations. Kaolin and bay silt were studied using three and four particle size ranges, respectively. In these experiments, both wet weight increase and condition index were measured at the end of the 12-day period.

Experimental Apparatus

The laboratory apparatus used in the growth and condition studies (Figure 1) consisted of a 320-liter seawater reservoir, a headbox and four 15-liter exposure chambers. One such structure was used for each concentration of suspended sediment. The reservoir was two 55-gallon drums welded together for a total capacity of 320 liters, and was painted inside with nontoxic fish hatchery white paint (General Paint No. 51311).

The reservoir was refilled twice daily with fresh, filtered seawater and the water adjusted to the appropriate salinity by addition of charcoal filtered tap water. A predetermined quantity of sediment was mixed with the reservoir contents by addition as a

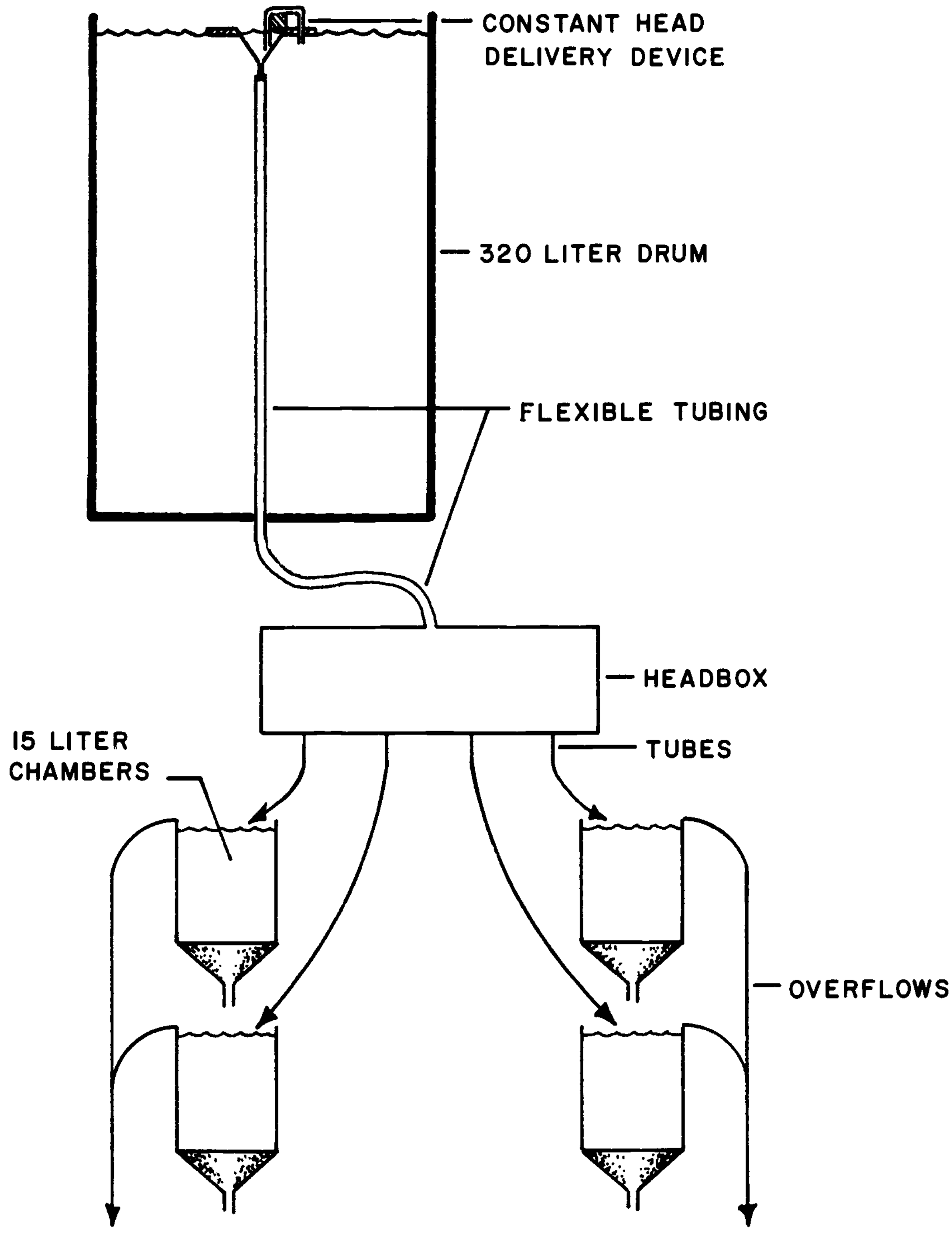

Figure 1. Design of the sediment exposure units used in the growth studies.

slurry in 200 to 300 ml of distilled water. In the first daily filling, algae were also added. Algae and suspended materials were kept in suspension by vigorous aeration of the reservoir contents. The reservoir contents were routed to the headbox by a constant flow, constant head delivery device modified after Freeman (1971) (Figure 2). From the headbox, water was delivered to the four exposure chambers at the rate of 80 ml/minute each. Sediments were kept in suspension in the headboxes by means of recirculating pumps.

The exposure chambers were modified 15-liter plastic containers fitted with a large funnel in the bottom (Figure 3). An airstone at the neck of the funnel provided for resuspension of materials settling to the bottom. Where the funnel was attached to the plastic chamber, additional aeration was provided through a perforated ring to enhance resuspension of settling materials. Oysters were suspended 2 inches below the water surface on a 6 by 8 inch perforated platform which was designed to provide stable support for them, but which allowed minimal surface area for natural settling of the suspended materials.

The turnover rate of seawater in the chambers was estimated graphically as suggested by Sprague (1969). At the flow rates used, 90% replacement occurred in about 7 hours and 99% in about 14 hours. The test apparatus was kept in a constant temperature room at 12±1° C. The photoperiod was a 12-hour light, 12-hour dark cycle using room

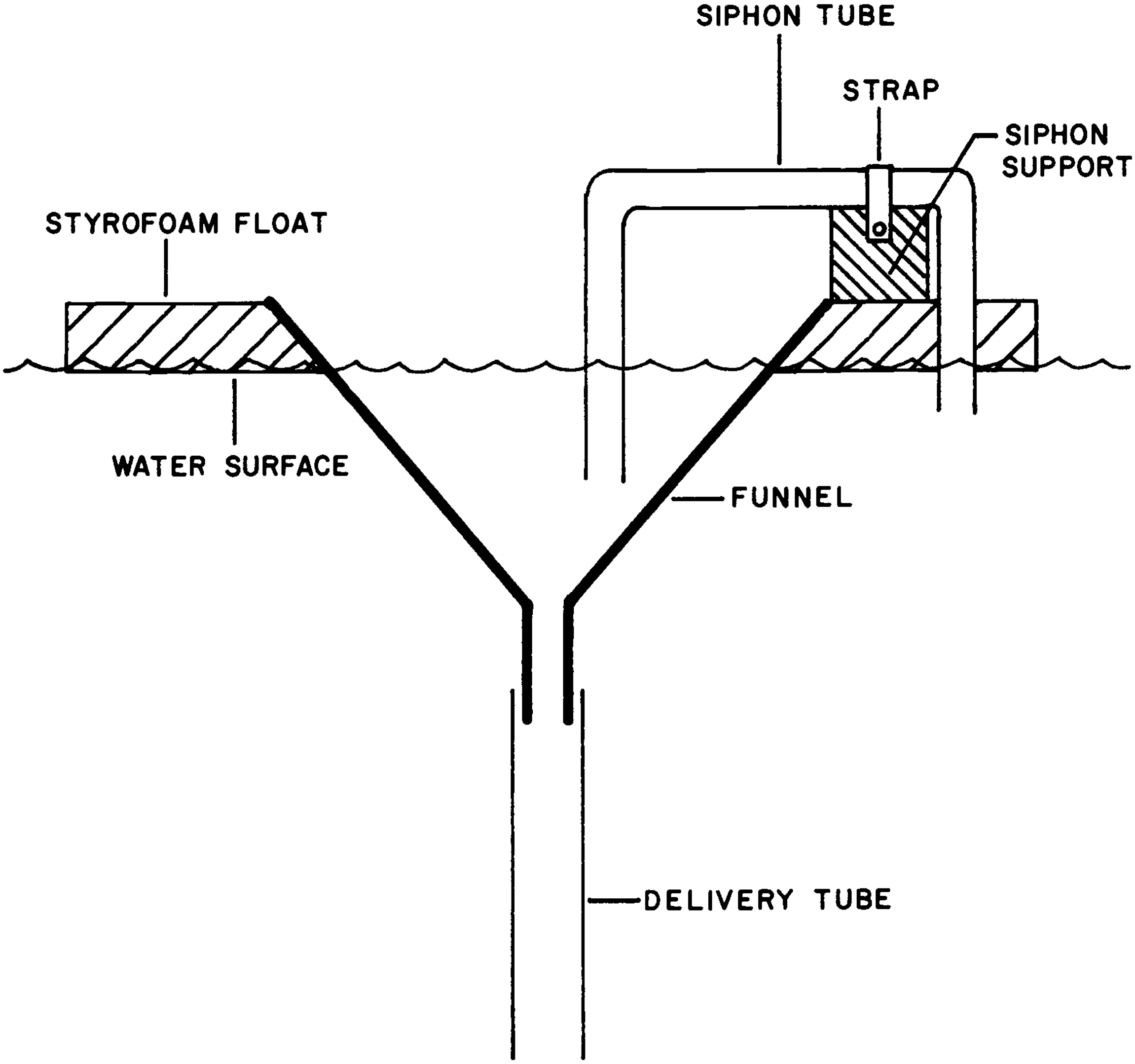

Figure 2. Diagram of the constant flow device for delivery of turbid water to exposure chambers (after Freeman, 1971).

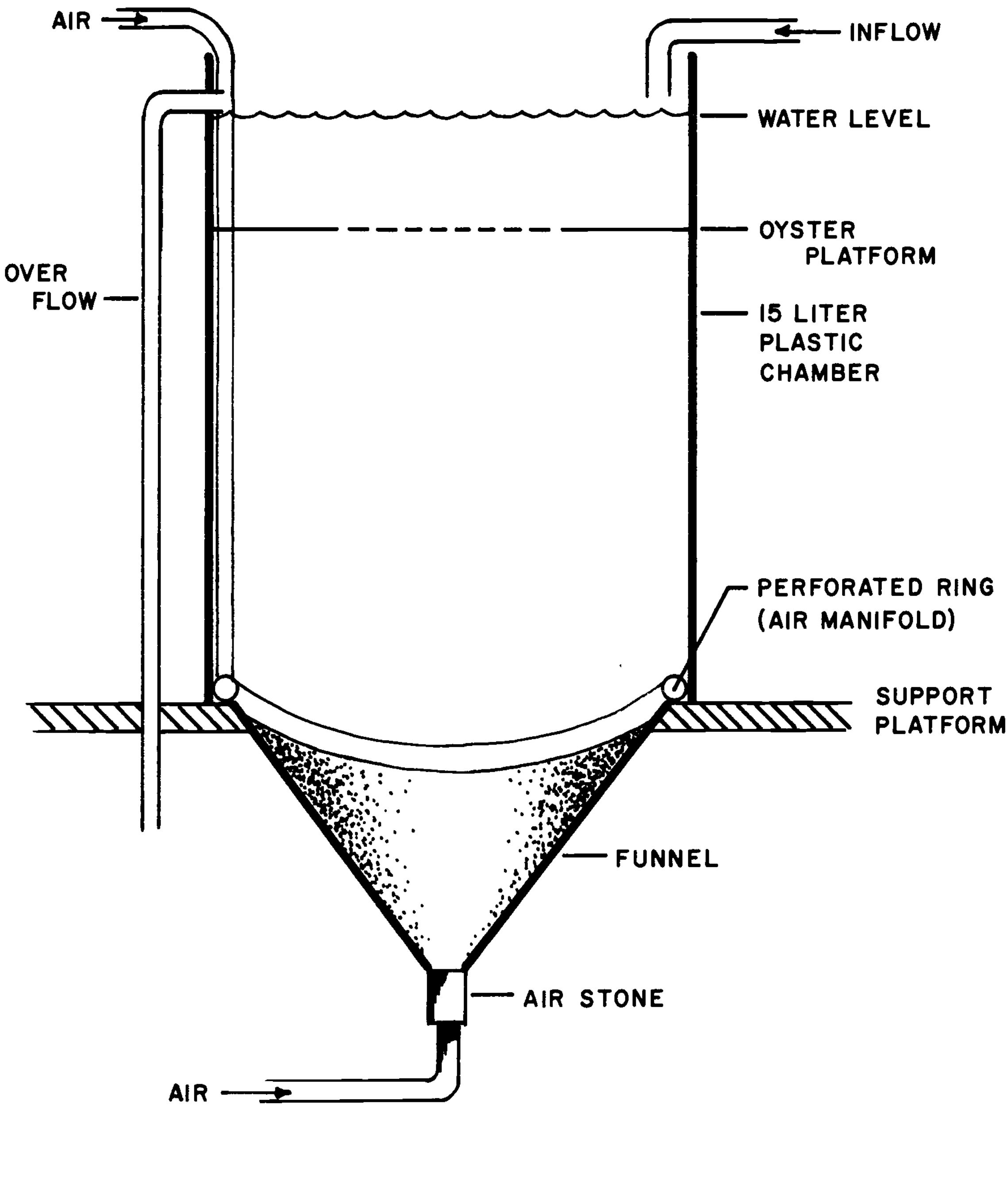

Figure 3. Diagram of an exposure chamber showing details of the aeration system for keeping sediments in suspension.

fluorescent lighting (40 foot candles). Seawater salinities were maintained at 25 to 27‰, except in the fuller's earth experiments, where the salinity was 30.4 ±0.1‰. Dissolved oxygen levels always exceeded 8.5 mg/liter and pH ranged from 8.0 to 8.2.

Experimental Animals

Adult oysters, 16-months-old and 15 to 75 g in weight, were obtained from a commercial stock grown in the middle portion of Yaquina Bay near river mile 5 during the spring of 1971. These oysters were grown from spat, which were produced by the experimental oyster hatchery at the Marine Science Center in Newport, Oregon, after the method of Loosanoff and Davis (1963). Each oyster was scrubbed to clean external surfaces of fouling organisms. Numbered plastic tags were then attached for identification. Monofilament line was passed through a small hole drilled through the shell in the hinge area and the tag attached. Prior to use in experiments, oysters were acclimated in the laboratory for four weeks in flowing unfiltered seawater (26 to 33‰ salinity) under experimental conditions of temperature and photoperiod.

Measurement of oyster growth

In all experiments, growth was determined by measuring the wet weight increase in air after shell closure was induced under water. The oysters were removed from the water, rinsed and the rinse water shaken from the external surfaces. Within 5 minutes, the oysters were weighed to the nearest 0.01 g on a Mettler top-loading balance. Weight loss due to evaporation from the shell exterior was less than 0.2% in this interval (Figure 4). After weighing, the oysters were immediately returned to flowing seawater.

In the fuller's earth experiment, 12 oysters were exposed to each of the three concentrations of suspended sediment, and in the other experiments, 20 oysters were used for each test concentration. At the beginning of an experiment, each oyster was weighed in air and placed in an exposure chamber. The oysters were subsequently reweighed at 3-day intervals, for a total of five weighings each. The animals were kept out of water during weighing for no more than 10 minutes, and other disturbances were kept to a minimum. At the end of the 12-day test period, oysters were routinely placed in flowing, filtered seawater for 12 hours to allow any sediment that was ingested to pass out of the gut. This procedure was not followed at the end of the 104 to 149 μ silt, 75 to 104 μ silt and fuller's earth experiments, however.

Measurement of condition index

Condition index in this study is defined as the ratio of total dry weight of body tissue in grams to the shell cavity volume in milliliters,

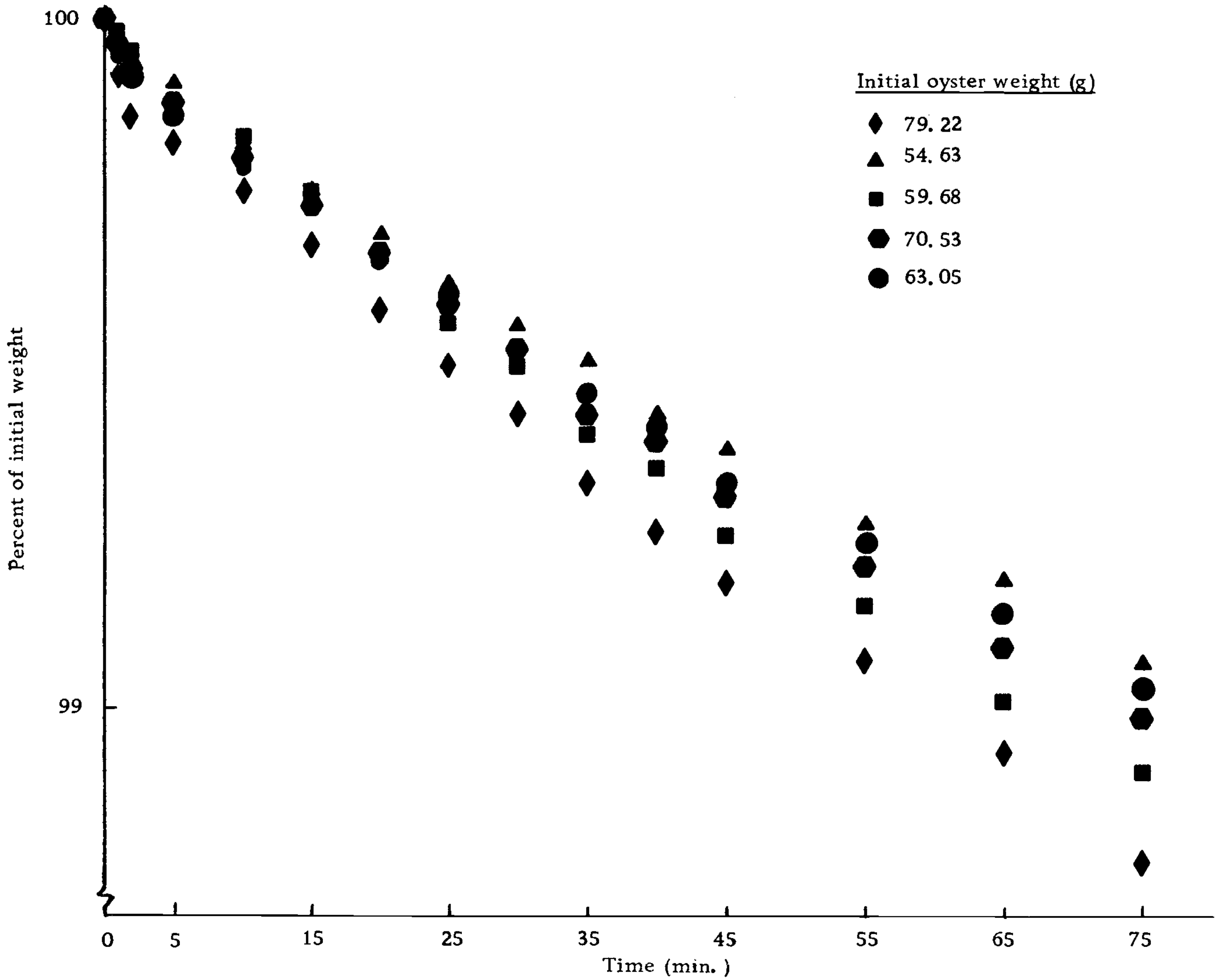

Figure 4. Decrease in weight with time of five oysters due to evaporative water loss from the shell surfaces. The initial weight was determined after shaking the excess water from the exterior shell surfaces.

multiplied by 100 (Medcof and Needler, 1941; Ingle, 1949). Shell cavity volume was the difference between the displacement of the intact live oyster in water and the displacement of the oyster shell minus the body tissue. Displacement in each case was measured by the difference between weight in air and weight under water as proposed by Andrews (1961). For example, to measure a live oyster's displacement, the oyster was weighed in air, then weighed under water, each time to the nearest 0.01 g. Weights under water were obtained by using a specially designed top-loading balance (Figure 5). The specific gravity of this water was adjusted such that a one milliliter sample weighed one gram. Therefore the difference between the weight of the oyster under water and the weight in air is displacement in grams. The weight of the body tissue was determined to the nearest 0.01 g, after drying at 100°C until constant weight was obtained.

The reproductive state of oysters may significantly alter the condition index because of the storage of nutrients prior to gamete development (Loosanoff and Davis, 1963). However, examination of the meats upon opening the oysters for condition index determinations indicated that none of the animals were in spawning condition during the study period and, therefore, any variations of condition index due to reproductive state were considered negligible.

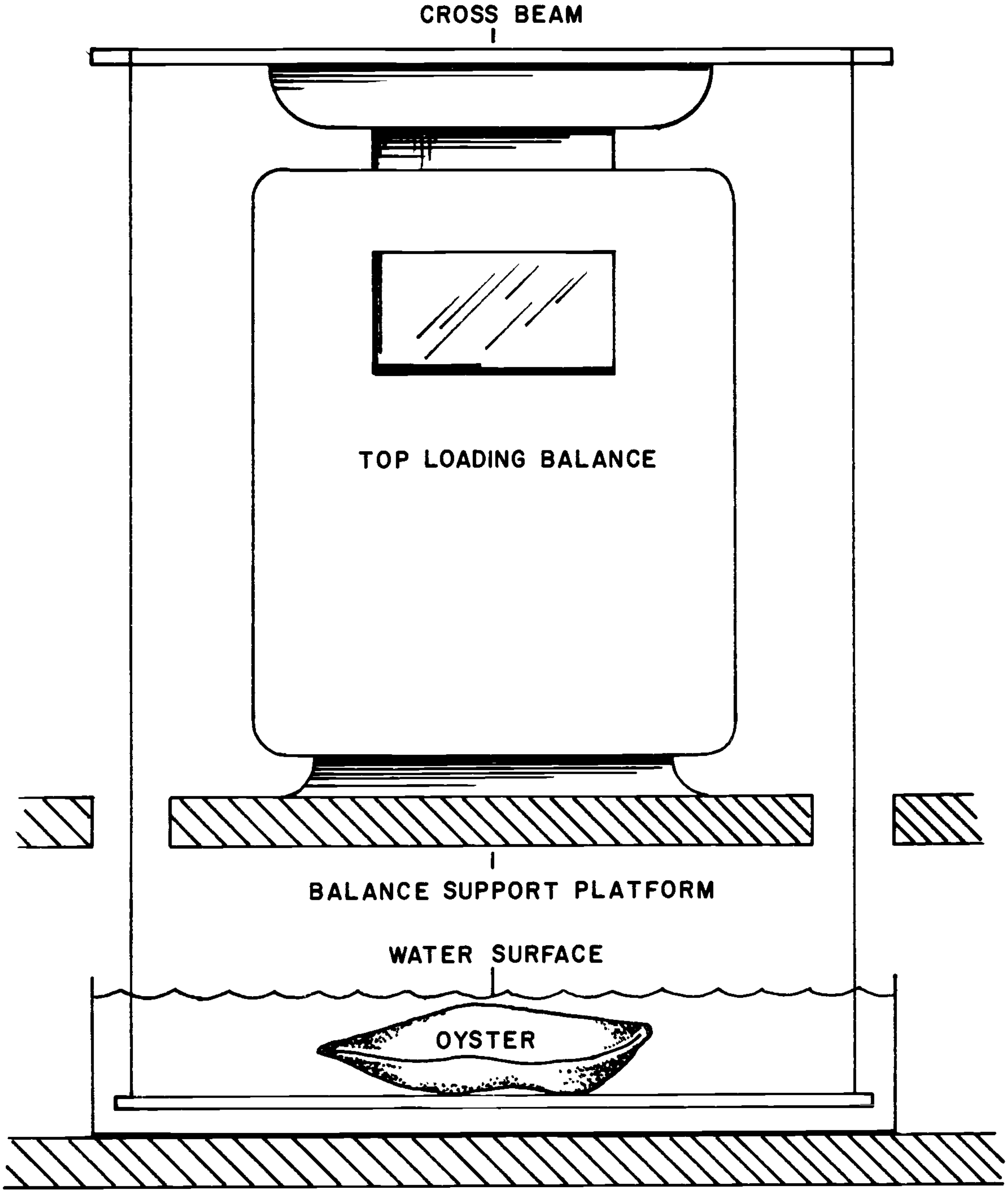

Figure 5. Modified top-loading balance used for weighing oysters under water.

Observations on excised gill tissue

Sections of gill, approximately 1/2 in^2 in size and including the edge, were removed from healthy oysters and placed in dishes of filtered seawater at 30‰ salinity. Microscopic observations were made, both in the presence and absence of suspended sediments, on ciliary movements, current patterns and, where sediments were used, on particle transport on the gill surface.

Filtration rate measurement

Water filtration rates for oysters were determined in the presence of several types and concentrations of sediments by measuring the difference between the decrease in sediment concentration in control beakers and experimental beakers containing oysters. Filtration rates were calculated using the formula introduced by Jorgensen (1943) as modified by Willensen (1952):

$$m = V \frac{\ln C_o - \ln C_t}{t} - a$$

where m is the volume of water in liters transported per hour, C_o and C_t are the initial and final concentrations of suspended sediments after t hours, V is the volume of suspension in liters and a is a correction factor for the settling of particles due to gravity. The correction factor, a, was determined from observations of particle settling in control beakers (i. e. in the absence of oysters) and was calculated using the formula

$$a = \frac{\ln C_o - \ln C_t}{t}$$

Suspensions of particles were made by placing a given weight of silt, kaolin or fuller's earth, as a slurry in 100 ml of filtered seawater, in glass beakers containing 3 liters of filtered seawater. The final concentrations ranged from 0.01 to 1.35 g/liter. Control beakers contained the same concentrations of sediments. The suspensions were maintained by aeration, sufficient to ensure homogenous mixing without disturbing the oysters, yet allowing feces and pseudofeces to settle out immediately. Each experimental beaker held three oysters with an average weight of about 65 g each. Dissolved oxygen remained at saturation, salinity was 30.0 ± 1.0‰ and temperature was 18° C.

At the beginning and after a 1 to 5 hour interval, a 25 to 100 ml aliquot was taken from a uniform position in the solution. The concentration of sediment particles in each beaker was determined by measuring the dry weight of the aliquot after passing through a Whatman GF/C 2.1 cm filter placed in a Gooch crucible.

Preparation and Analysis of Experimental Sediments

In late spring of 1971, approximately 1,500 pounds of sediment were collected from the intertidal region of a Yaquina Bay mudflat adjacent to the Marine Science Center Laboratory (Figure 6). After air drying in large shallow containers in the laboratory, the sediments were ground in a mortar to break up clumps, and then sieved through Tyler screens to isolate specific size ranges. The sizes obtained were: less than 38 μ, 38 to 74 μ, 74 to 104 μ and 104 to 149 μ. The sediments were screened in 300 g batches by shaking for 5 minutes per batch with the W. S. Tyler Ro-tap Testing Sieve Shaker (W. S. Tyler Company, 1970).

Other materials used in these experiments were obtained commercially. Fuller's earth, obtained from the Matheson, Coleman and Bell Chemical Company, had a mean particle size diameter of 5 μ. Three sizes of kaolin, 0.2 μ, 1.5 μ and 9.5 μ in mean particle size diameters, were obtained from the Georgia Kaolin Company. These represented the range from finest to largest particle sizes available. Curves showing percent variation of the spherical diameter range about the median particle diameter are shown in Figure 7.

Volatile and fixed solids were expressed as percent by weight after one hour ignition of the dry samples at 600° C in an electric muffle furnace (APHA, et al., 1965).

Water Quality Determinations

Salinity was determined in either of two ways. In the initial experiments, it was measured with a Hytech Inductive Salinometer,

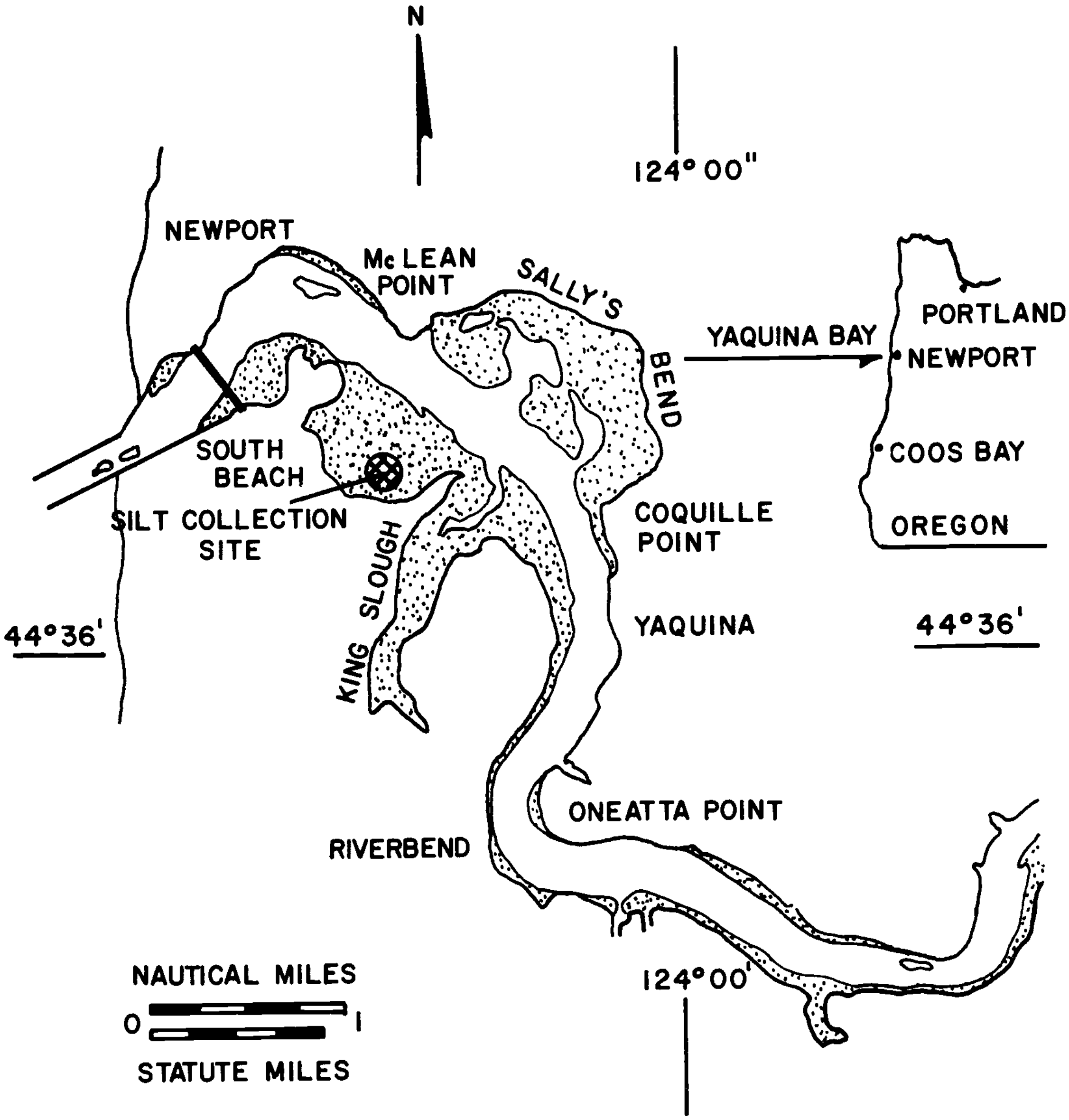

Figure 6. Map of Yaquina Bay, Oregon, showing site of sediment collection.

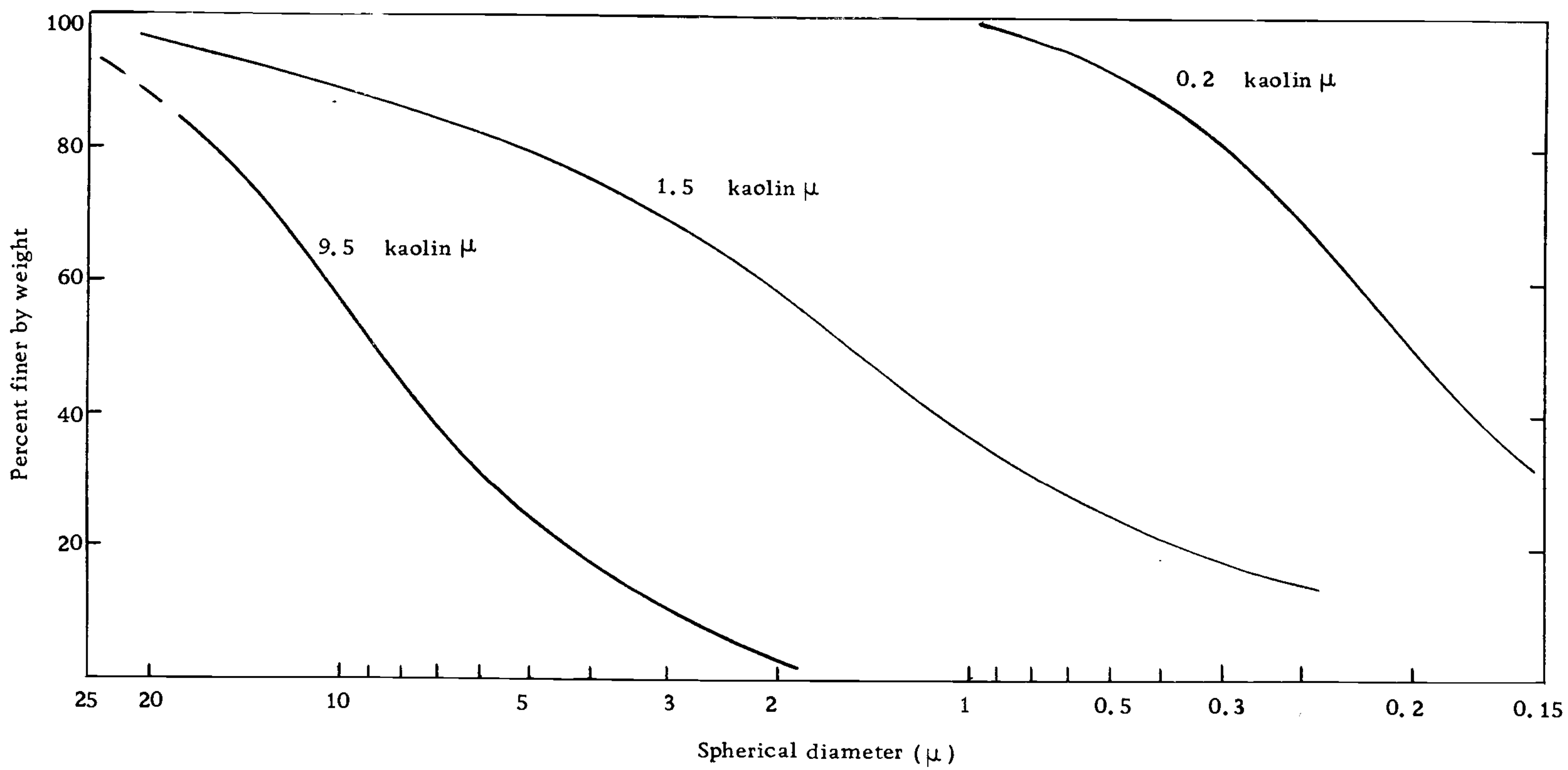

Figure 7. Particle size distributions of the three kaolins (taken from data sheet TSB-2, Georgia Kaolin Company).

Model 6220, standardized against Copenhagen water. In later experiments, specific gravity techniques were used. Dissolved oxygen was determined using the azide modification of the iodometric Winkler method (APHA, *et al.*, 1965). Chlorine levels were monitored in the freshwater which was used to dilute the seawater, by a modified orthotolidine method (APHA, *et al.*, 1965).

Statistical Procedures

Analysis of variance was used to test for significance of differences between a series of sediment concentrations. Sequential LSD tests were also used for comparison between two means (Snedecor and Cochran, 1968).

RESULTS

Growth under Field Conditions

It was necessary to determine the normal growth in the field, in order to provide a basis for evaluating growth measured in the laboratory. An initial group of 20 oysters was selected at random, cleaned, tagged and weighed to the nearest 0.01 g in air. The oysters were placed in Yaquina Bay at the Marine Science Center on August 7, 1972. After 2 weeks, they were brought to the laboratory and each oyster reweighed. During this period temperatures remained at 12.6 ± 2.9 °C, dissolved oxygen values were 8.24 ± 0.52 mg/liter and salinity was 33.0 ± 1.0‰. Under these conditions the mean weight increase during the 2-week period was equivalent to 2.30% of the initial weight (Figure 8, Group 1). A second sample of 20 oysters was treated similarly and placed in Yaquina Bay for 2 weeks beginning on August 28, 1972. During this time the temperature was 12.5±3.0° C, dissolved oxygen 8.20± 0.60 mg/liter and salinity 33.0 ± 1.0‰. Weight gain in this group averaged 2.32% of the initial weight in 2 weeks (Figure 8, Group 2).

Growth under Laboratory Conditions

Since the method used to maintain sediments in suspension in the laboratory experiments involved vigorous aeration, an experiment

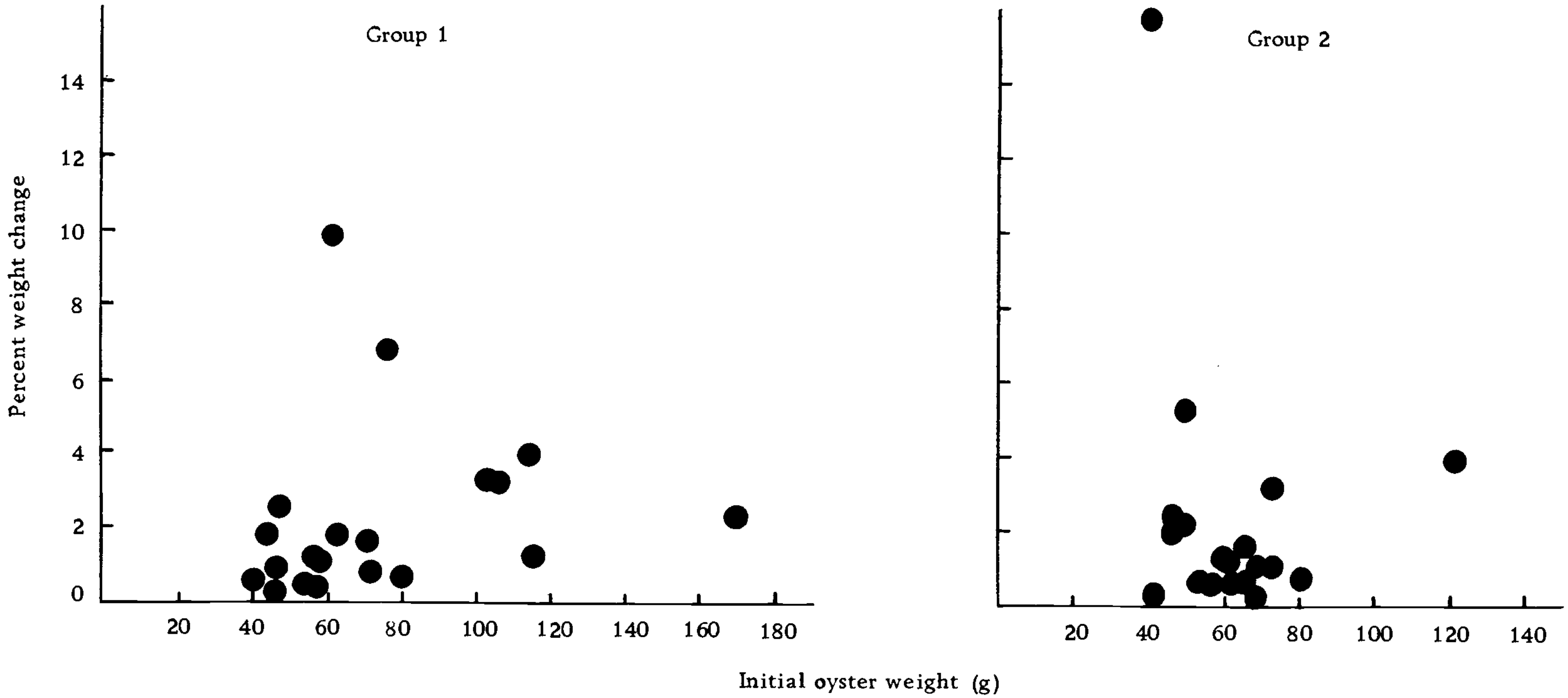

Figure 8. Growth of oysters during two-week exposures to field conditions.

was conducted to test the effect of various levels of aeration on oyster growth. Four 15-liter plastic containers of seawater were provided with platforms suspended below the water surface. Fifteen oysters were weighed as previously described and placed on the platform in each container; two airstones with flexible air hoses attached were fitted beneath the platforms. The aeration rate was adjusted to create several levels of turbulence in three of the containers in which the highest exceeded that required for sediment suspension--1.2, 1.7 and 2.2 pounds per square inch gauge (PSIG). A control group of oysters was kept in nonturbulent water. During the two-week exposure period, dissolved oxygen remained near saturation, temperatures were 14.0 ± 1.3° C and salinities ranged from 32 to 34‰. Seawater replacement in the vessels was at the rate of 99% in 2.5 hours.

Mean percentages of wet weight increase of the control and the three turbulence-exposed groups after 2 weeks were 3.23, 3.90, 3.02 and 3.59, respectively (Figure 9). These results indicated that turbulence does not affect oyster growth, since weight increase in treatment groups was equivalent to that in the controls. Subsequently, air pressure used for keeping sediments in suspension in the turbidity experiments was approximately 1.7 PSIG, and the amount of induced turbulence approximated that of the moderate group.

Although the preceding experiment demonstrated that weight

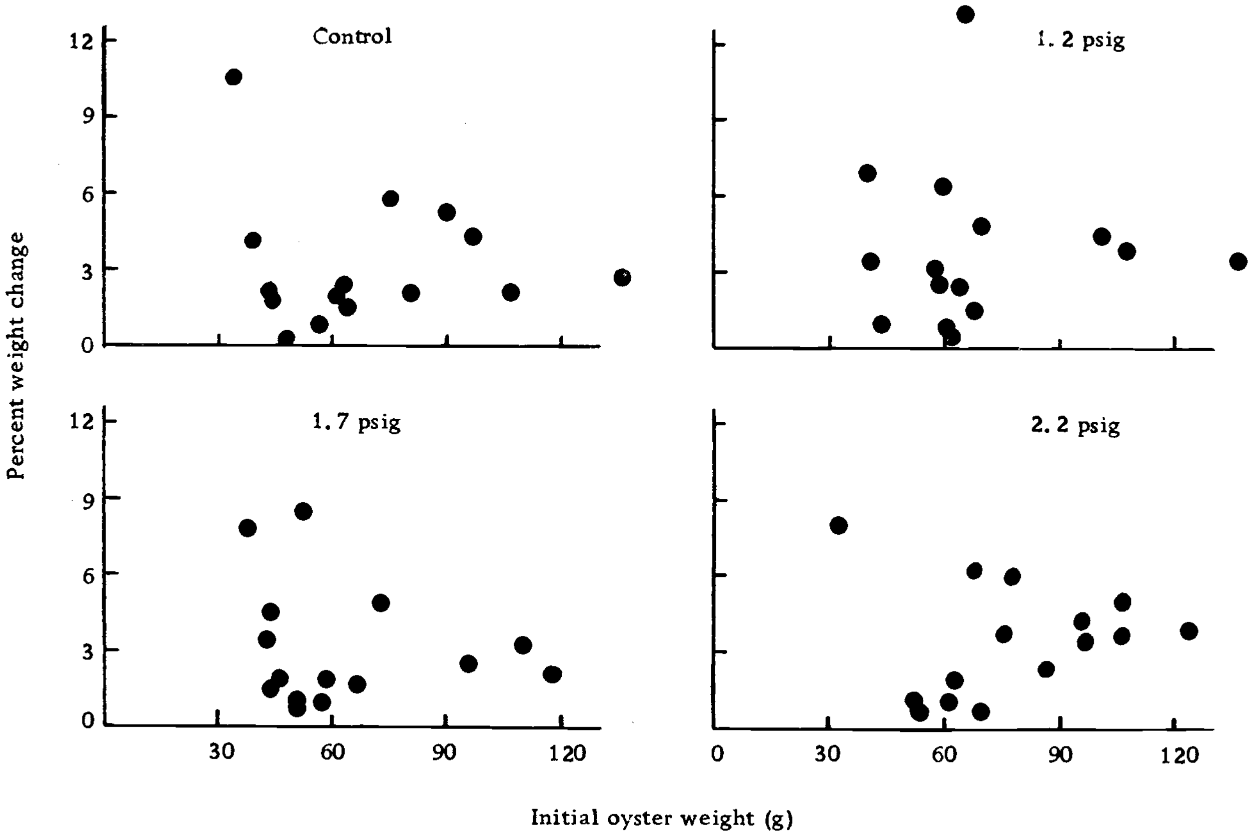

Figure 9. Growth of oysters during two-week exposures to three levels of air induced turbulence. Turbulence was induced by 1.2, 1.7 and 2.2 pounds per square inch air pressure.

gain in the laboratory was equivalent to that found in the field, this growth occurred under conditions where food availability might be considered normal. However, in the sediment-exposure experiments, very low flow rates were required to minimize the quantity of sediments needed to obtain a given suspended solids level. Under these conditions, supplemental feeding seemed necessary to insure that growth comparable to that in the field would occur.

The results of seven tests, in which oysters were maintained at low flow rates of unfiltered seawater and fed a supplementary diet identical to those in the sediment exposure experiments, are shown in Table 1. The two algal species fed were the naked flagellates *Isochrysis galbana* and *Monochrysis lutheri* (Class Chrysophyceae). The choice of algal species tested was dictated by the availability of each from the OSU pilot oyster hatchery. The results showed no correlation of growth response to algal density, but there was an indication that weight gain may have been proportional to *Monochrysis* levels. However, measurable growth was observed after feeding algae in each test, and the level of growth was essentially similar to that found in the field and laboratory tests employing high flow rates of unfiltered seawater.

Table 1. Effect of algal composition and density on growth of oysters during a two-week period.[1]

Number of oysters fed	Initial oyster weight (g)	Algal composition		Algal density (No. of cells x 10^9)	Percent change in oyster weight
12	48.22 ± 14.08[2]	Isochrysis Monochrysis	(75%) (25%)	16.96	2.16 ± 1.09[2]
20	42.05 ± 18.74	Isochrysis Monochrysis	(50%) (50%)	16.28	3.98 ± 2.17
20	57.40 ± 26.54	Isochrysis Monochrysis	(60%) (40%)	21.32	2.59 ± 2.22
20	33.69 ± 20.09	Isochrysis	(100%)	15.48	1.74 ± 0.72
20	43.96 ± 16.79	Isochrysis	(100%)	20.47	2.06 ± 1.25
20	50.21 ± 21.60	Isochrysis	(100%)	21.62	1.68 ± 0.76
20	44.06 ± 27.70	Isochrysis	(100%)	28.95	0.81 ± 0.68

[1] Growth conditions are described in the text.

[2] Mean ± one standard deviation.

Effects of Sediments on Growth

Bay silt

The first series of experiments on sediments dealt with the effect of natural silt on oyster growth. Equipment limitations in these and later experiments restricted testing all combinations of particle size and concentration of silt simultaneously; therefore, two-week experiments were run, testing three concentrations of one silt particle size range per trial. In the first two experiments using silt particles 104 to 149 μ and 74 to 104 μ in diameter, it appeared that growth was not affected by concentrations up to 0.22 and 0.66 g/liter, respectively (Figures 10 and 11). These were the highest levels that could be kept in suspension with the apparatus used. In these first experiments the oysters were not placed in filtered water after the final weighing. As a result the final weights, and therefore growth, may have been overestimated because of the presence of silt on the gill surfaces and in the gut.

In tests with two smaller particle size ranges of silt in which a "sediment cleansing period" was allowed, growth appeared to be reduced with 38 to 74 μ particles at the lowest concentration exposed, 0.65 g/liter, and was significantly reduced in two replicate exposures at the highest concentration tested, 1.06 g/liter ($p < 0.05$) (Figure 12). No significant growth reduction was observed after exposure to silt

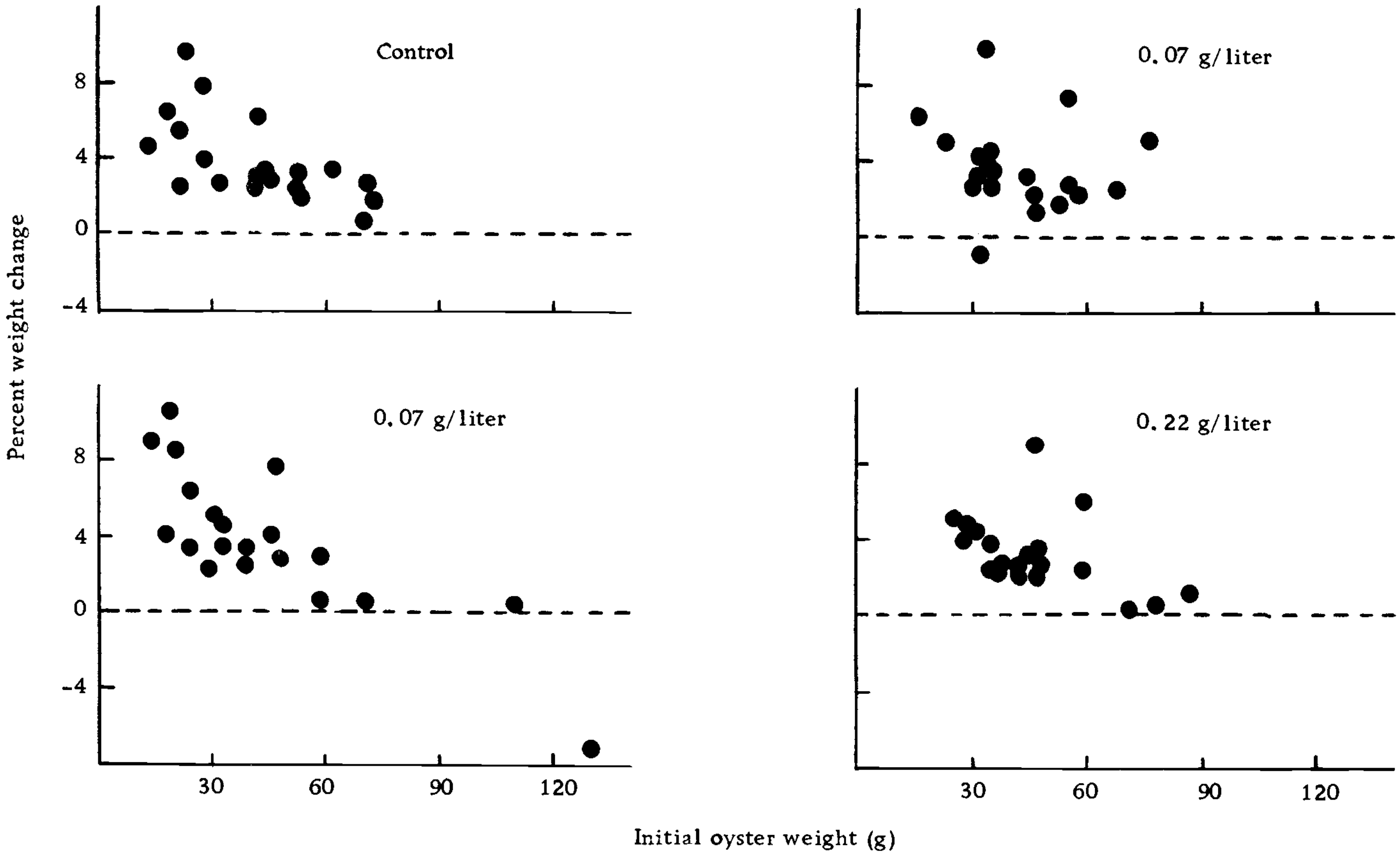

Figure 10. Growth of oysters during two-week exposures to three levels of 104 to 149 μ bay silt particles.

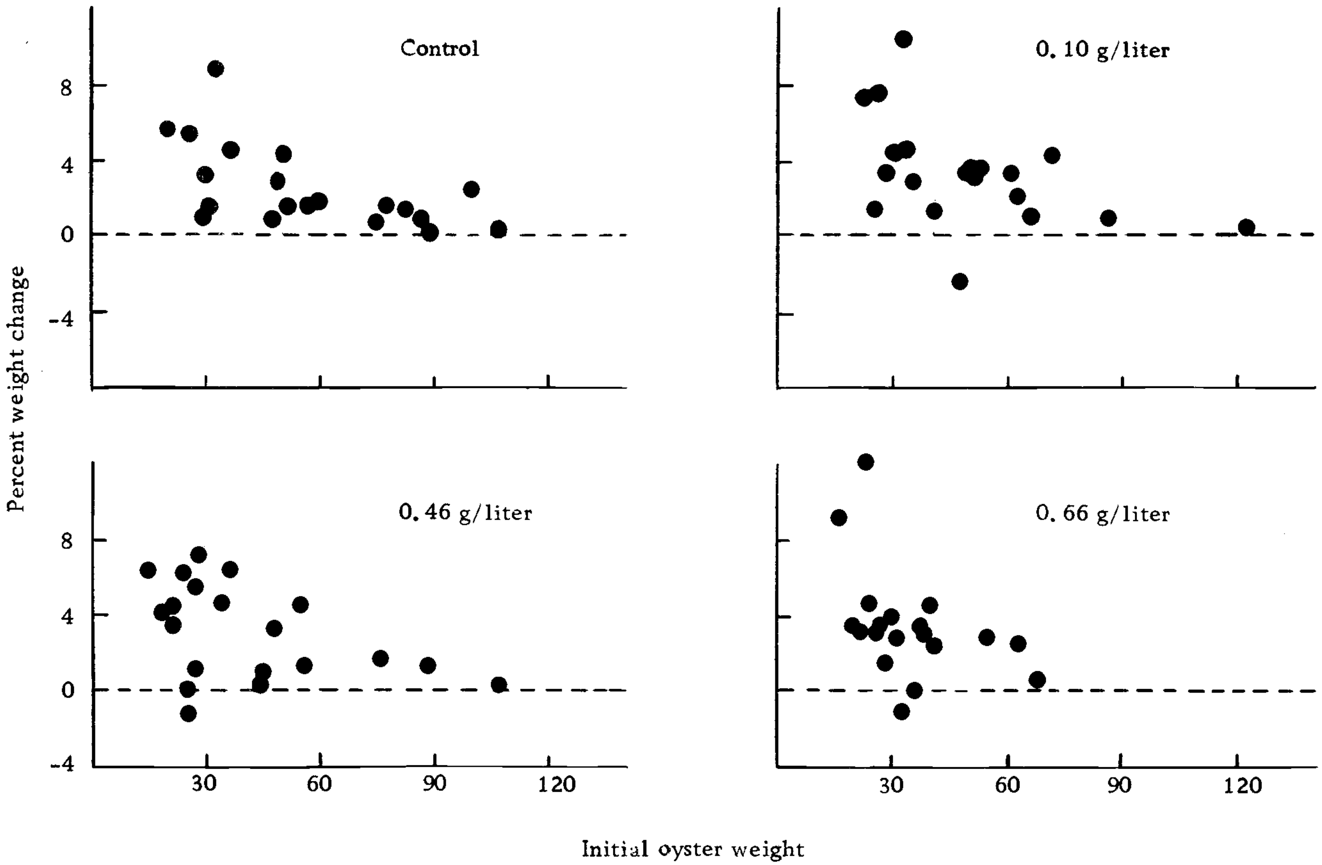

Figure 11. Growth of oysters during two-week exposures to three levels of 74 to 104 μ bay silt particles.

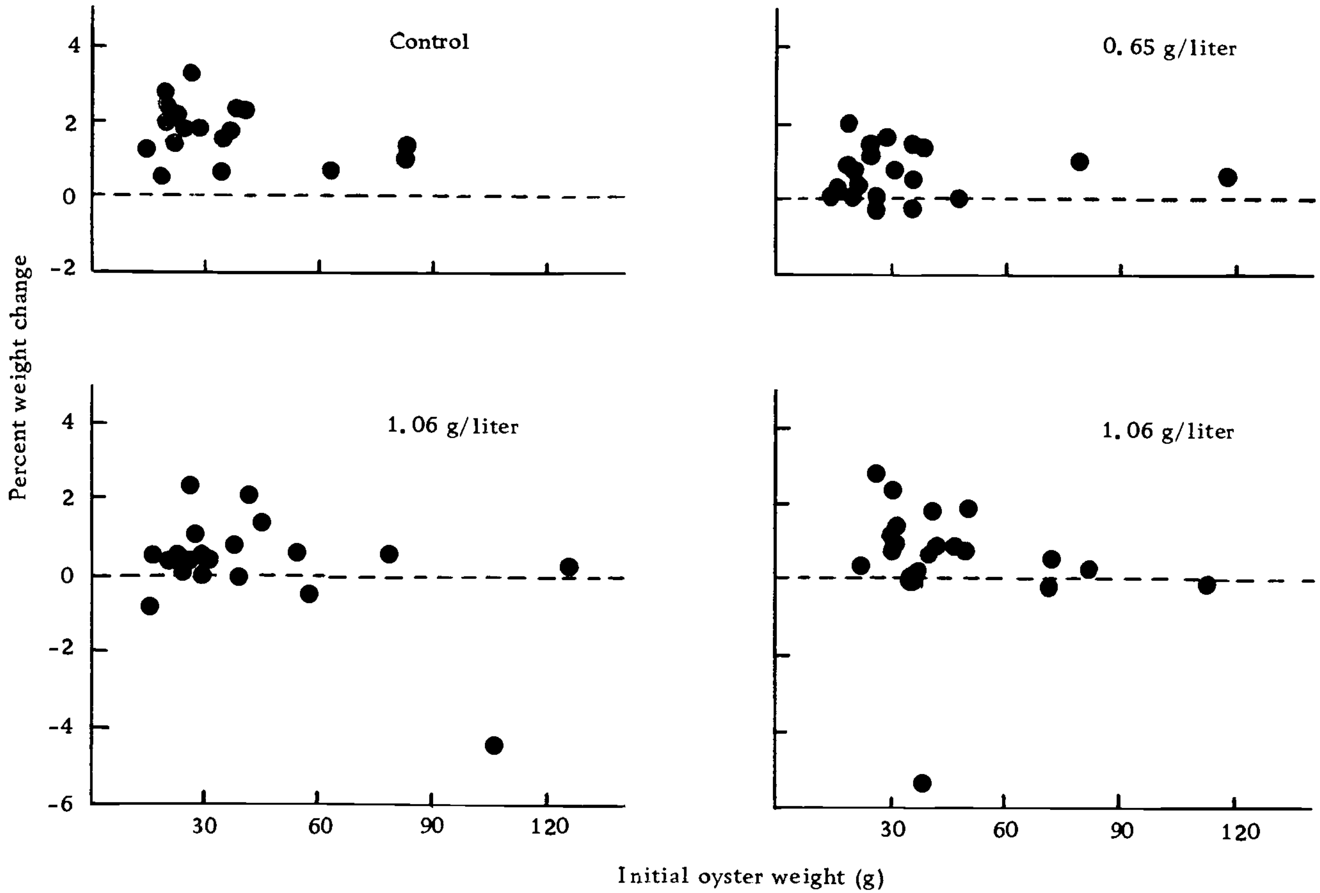

Figure 12. Growth of oysters during two-week exposures to three levels of 38 to 74 μ bay silt particles.

particles less than 38 μ in size at 1.61 g/liter, the only concentration tested (Figure 13). The analysis of variance statistics for the silt data are listed in Table 2. Figure 14 summarizes the data obtained from the silt experiments by plotting percent weight change (±S. E.) against silt concentrations.

Kaolinite sediments

A further series of experiments dealt with the effect of commercial grades of clay sediments on oyster growth. Three particle sizes of clay materials were tested in three separate experiments. In the first experiment using kaolin particles with a 0.2 μ mean diameter, it appeared that growth was significantly affected by all concentrations that were tested (Figure 15). Many oysters lost weight as a result of exposure to the sediments. Growth at the lower concentrations, 0.36 and 0.85 g/liter, was significantly reduced ($p < 0.05$). The significance of growth reduction was even greater at 1.44 g/liter ($p < 0.01$), the highest concentration tested.

In tests with larger clay particles, growth of oysters was significantly reduced from control levels with 1.5 μ particles at each of three concentrations, 0.54, 1.11 and 1.81 g/liter ($p < 0.01$) (Figure 16). Growth reduction was also significant ($p < 0.01$) in oysters exposed to 1.69 g/liter of the 9.5 μ kaolin particles (Figure 17). Many of the kaolin exposed animals lost weight as a result of exposure to these sediments, similar to the response noted with the smallest

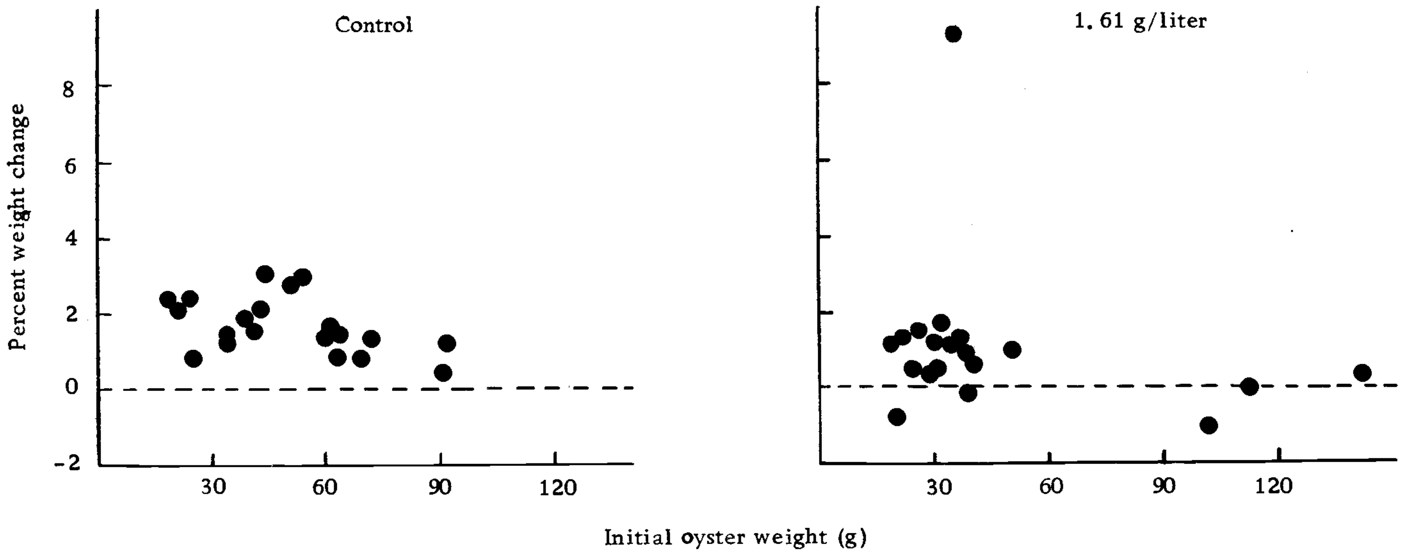

Figure 13. Growth of oysters during two-week exposures to 0 to 38 μ bay silt particles.

Table 2. Analysis of variance tests for the growth rates of oysters exposed to different sediment concentrations.

Sediment	d.f.	S.S.	M.S.	F
104 to 149 μ Silt	3	5.65	1.88	0.26
74 to 104 μ Silt	3	3.23	1.08	1.04
38 to 74 μ Silt	3	2.15	7.16	2.90[1]
0 to 38 μ Silt	1	2.89	2.89	1.21
0.2 μ Kaolin	3	1.64	5.47	4.24[2]
1.5 μ Kaolin	3	6.05	2.02	9.94[2]
9.5 μ Kaolin	1	20.10	20.10	22.58[2]
5 μ Fuller's Earth	3	8.13	2.71	5.71[2]

[1] Significance between concentrations observed at 0.05 level as determined by sequential LSD tests.

[2] Significance between concentrations observed at 0.01 level as determined by sequential LSD tests.

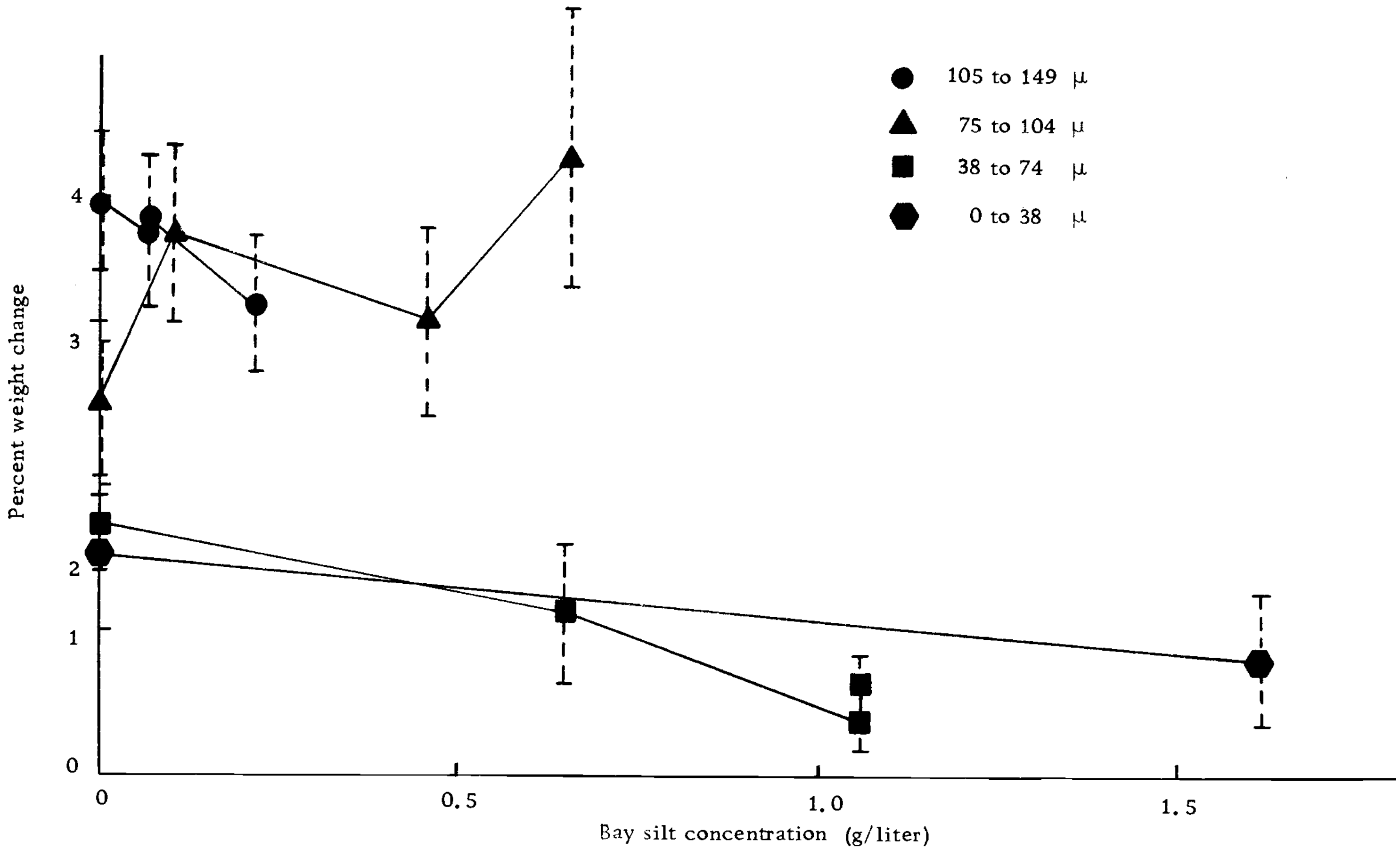

Figure 14. Comparison of growth of oysters during two-week exposures to four sizes of bay silt particles as a function of silt concentration. Points represent the mean ± one standard error of 20 oysters (the highest concentration of 74 to 104 μ silt is a mean of only 19 oysters).

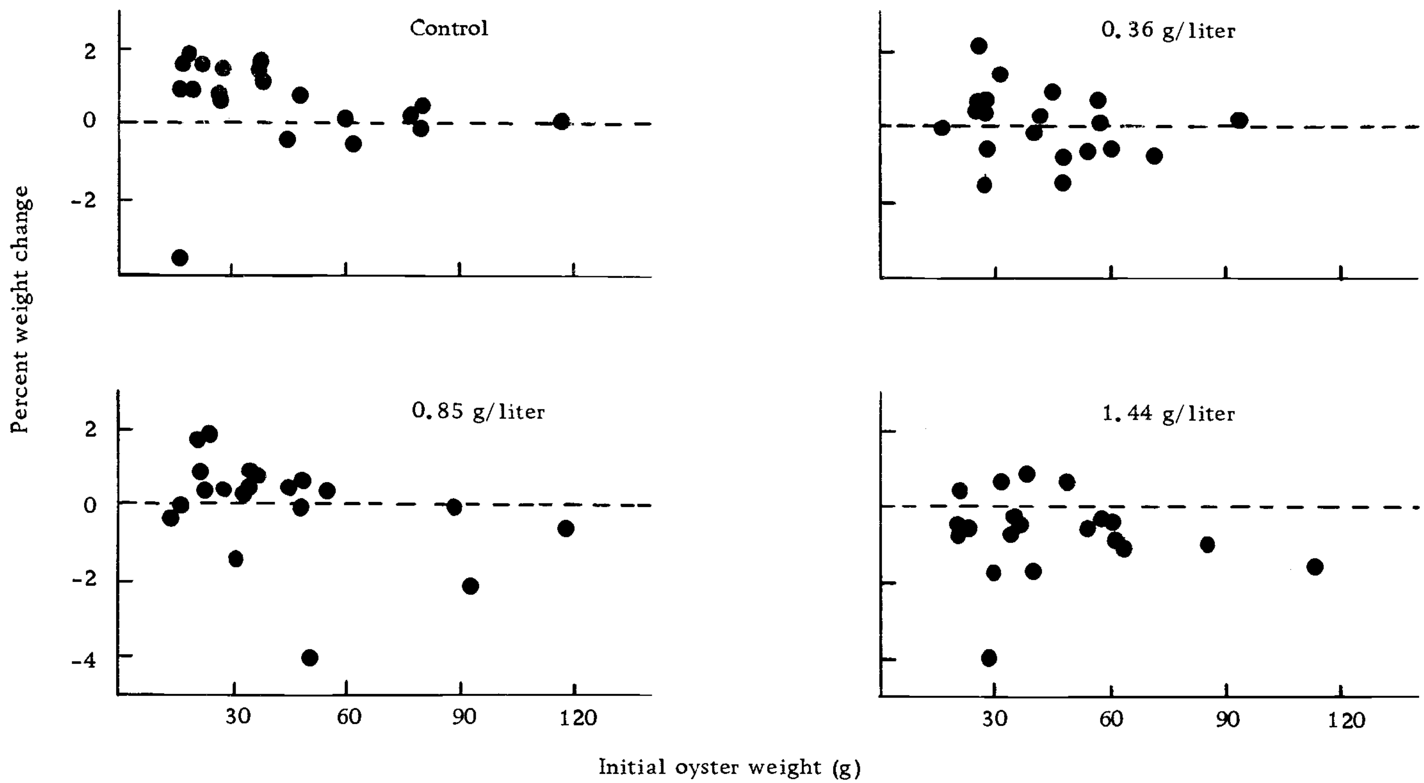

Figure 15. Growth of oysters during two-week exposures to three levels of 0.2 μ kaolin particles.

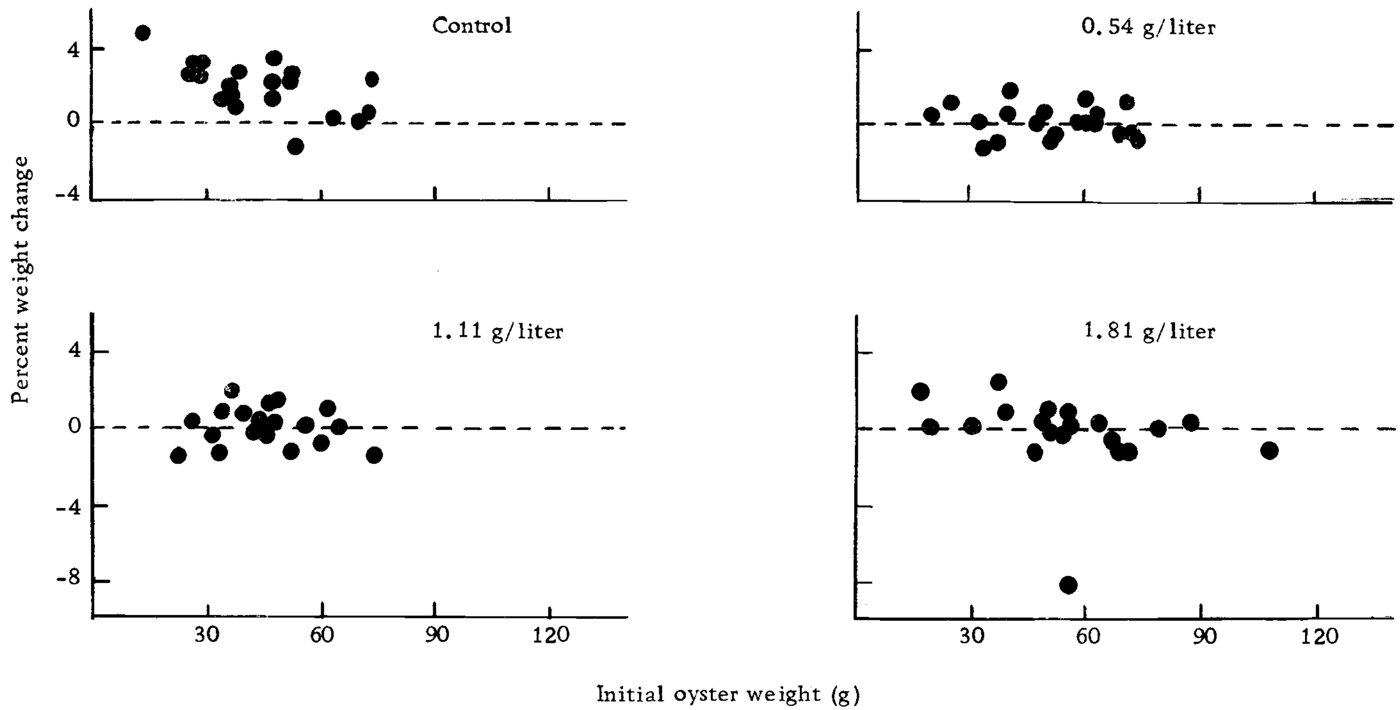

Figure 16. Growth of oysters during two-week exposures to three levels of 1.5 μ kaolin particles.

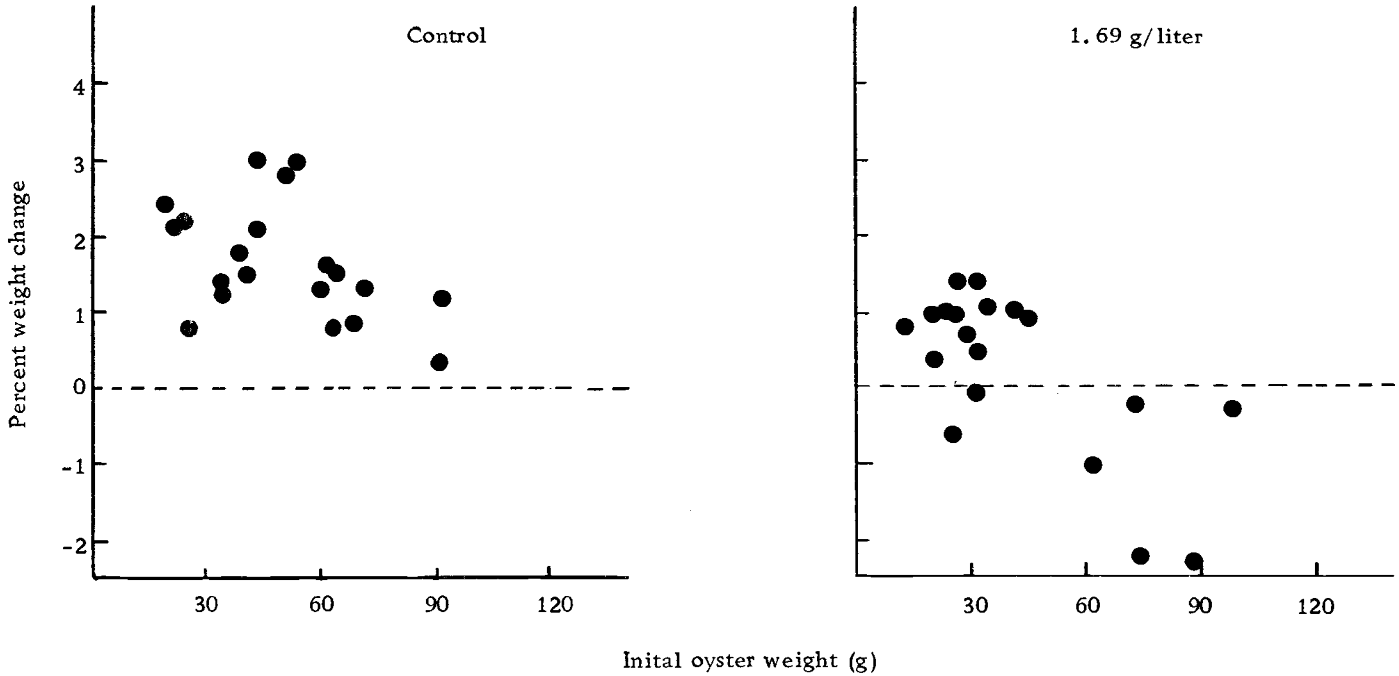

Figure 17. Growth of oysters during two-week exposures to 9.5 μ kaolin particles.

kaolin particles. The analysis of variance statistics for the kaolin tests are listed in Table 2. Figure 18 summarizes the data obtained from the kaolin experiments as percent weight change (± S. E.) against clay concentration, and shows that mean growth rate in all three tests diminished to negative values as the highest concentrations were reached.

Fuller's earth

A final sediment experiment was run to test the effects of fuller's earth particles, 5 μ in diameter, on oyster growth. It appeared that growth was slightly affected with increase in sediment concentration (Figure 19). Growth reduction at the lower concentrations of fuller's earth, 0.20 and 0.70 g/liter was significant at the 5% level and at the highest concentration, 1.37 g/liter, at the 1% level (Table 2). Figure 20 summarizes data obtained from the fuller's earth experiment by plotting percent weight change (± S. E.) against sediment concentration.

Condition Index Results

The condition index of control groups did not differ from that of groups of oysters exposed to several sizes and concentrations of bay silt, kaolin and fuller's earth, except in the experiment involving 74 to 10 μ bay silt (Tables 3 and 4). In the latter experiment,

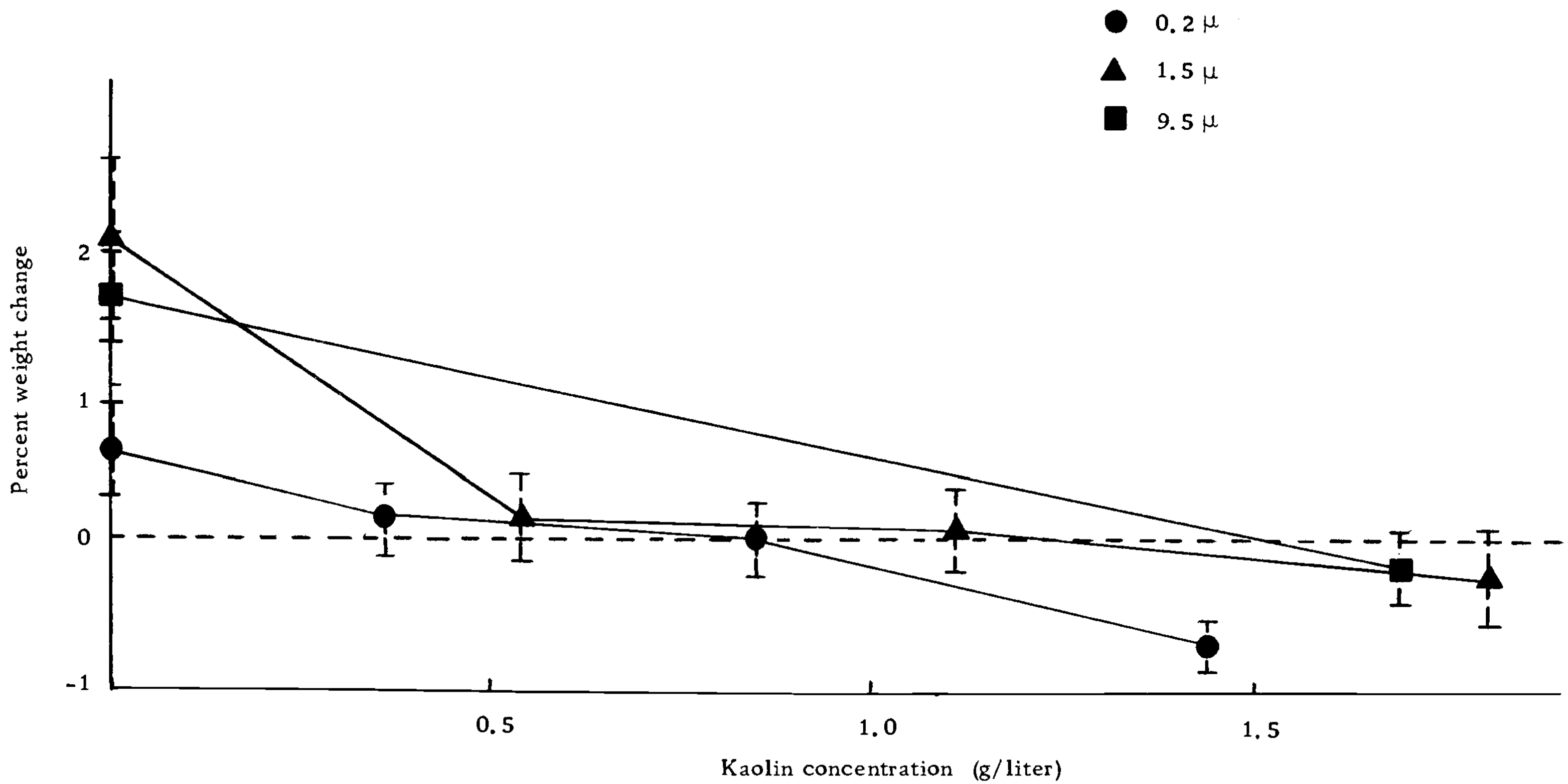

Figure 18. Comparison of growth of oysters during two-week exposures to three sizes of kaolin particles as a function of kaolin concentration. Points represent the mean ± one standard error of 20 oysters.

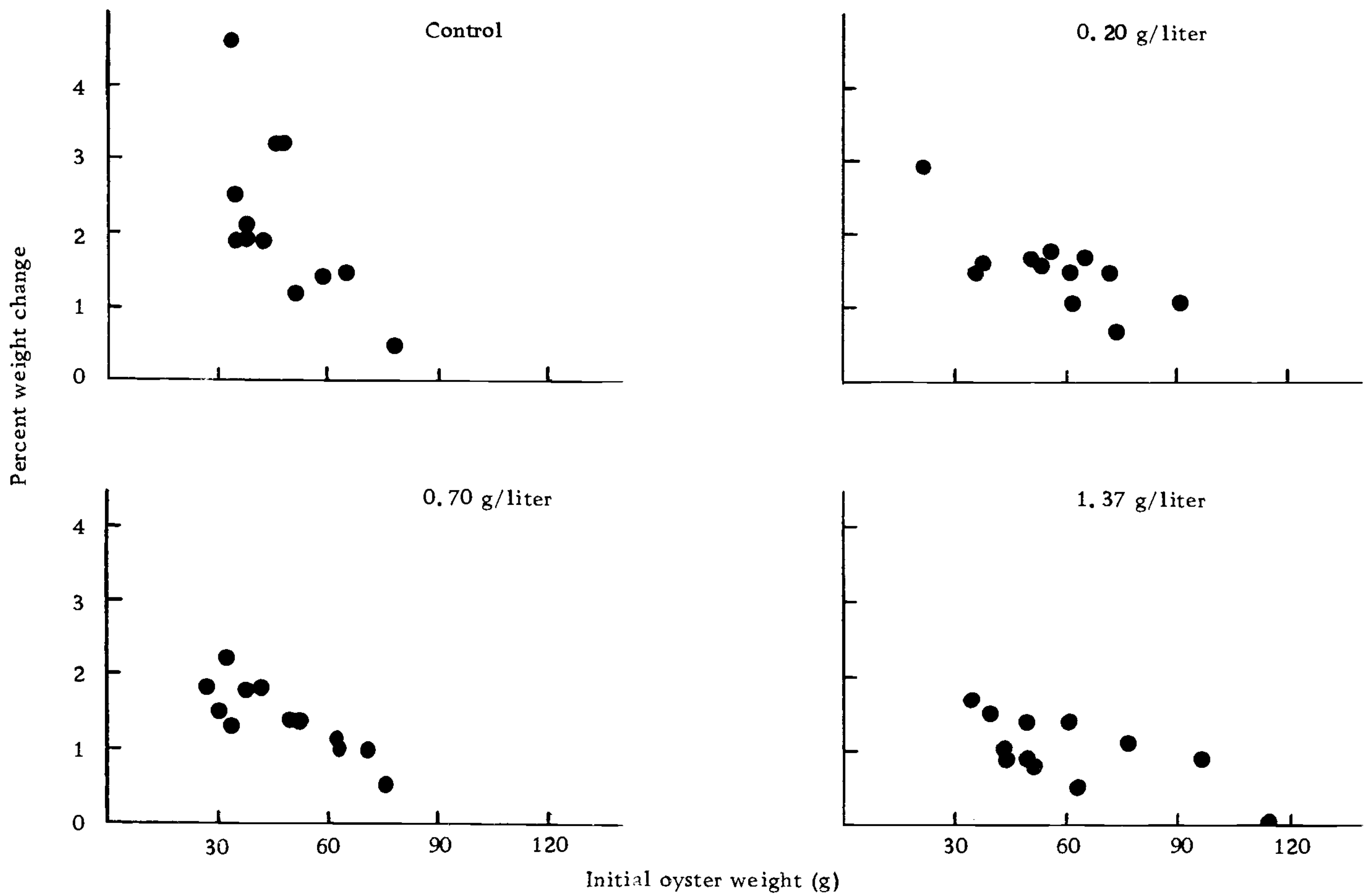

Figure 19. Growth of oysters during two-week exposures to three levels of 5 μ fuller's earth particles.

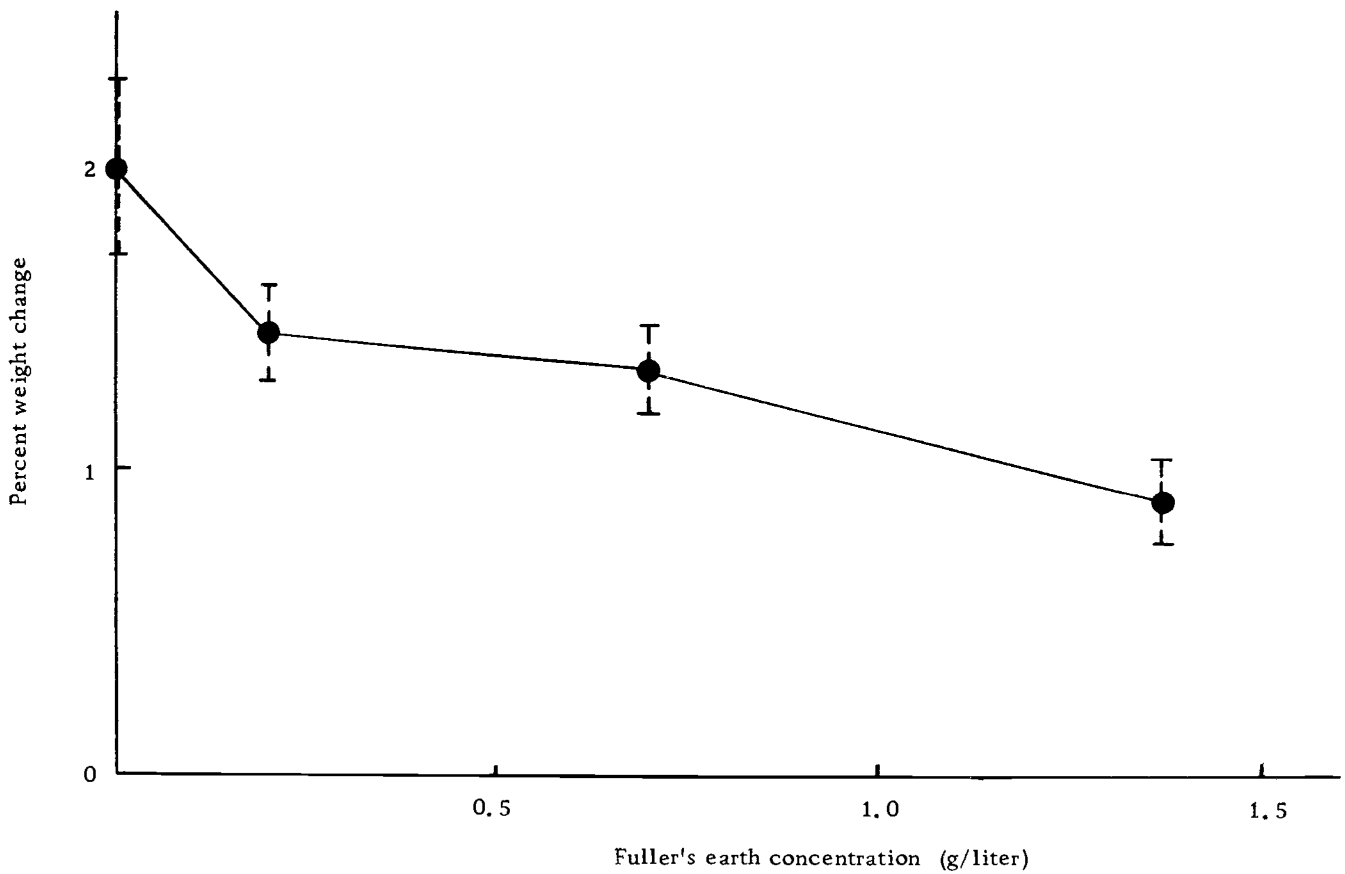

Figure 20. Growth of oysters during two-week exposures to fuller's earth particles as a function of fuller's earth concentration. Points represent the mean ± one standard error of 12 oysters.

Table 3. Condition indices of oysters exposed for two weeks to bay silt.

Bay silt particle diameter (μ)	Concentration (g/liter)	Condition index[1]
104 to 149	0	6.69 ± 1.82
	0.07	8.28 ± 1.68
	0.07	7.14 ± 2.63
	0.22	8.05 ± 1.28
74 to 104	0	6.46 ± 1.47
	0.10	4.42 ± 1.31
	0.46	4.71 ± 1.14
	0.66	5.42 ± 1.17
38 to 74	0	8.02 ± 1.95
	0.65	9.00 ± 2.10
	1.06	7.43 ± 2.15
	1.06	7.43 ± 2.16
0 to 38	0	8.71 ± 2.73
	1.61	6.87 ± 2.05

[1] Mean ± one standard deviation of 20 oysters (for the 704-104 μ, 0.66 g/liter silt, only 19 oysters were used).

Table 4. Condition indices of oysters exposed for two weeks to kaolin and fuller's earth.

Sediment particle diameter (μ)	Concentration (g/liter)	Condition index[1]
Kaolin 0.2	0	7.74 ± 2.83
	0.36	9.16 ± 2.39
	0.85	10.03 ± 2.65
	1.44	7.67 ± 3.56
Kaolin 1.5	0	11.74 ± 2.75
	0.54	13.05 ± 2.31
	1.11	13.13 ± 2.40
	1.81	12.78 ± 4.23
Kaolin 9.5	0	8.71 ± 2.73
	1.69	8.08 ± 2.62
Fuller's earth 5	0	5.83 ± 1.83[1]
	0.20	6.37 ± 1.87[1]
	0.70	7.48 ± 1.78[1]
	1.37	6.54 ± 1.75[1]

[1] Mean ± one standard deviation of 20 oysters (only 12 oysters were used in each of the fuller's earth tests).

condition indices of the silt groups were lower than those of the controls with $p < 0.01$ for 0.10 and 0.46 g/liter groups, and $p < 0.05$ for the 0.66 g/liter group. The condition indices in the kaolin tests were higher than for other groups. Since these tests were run in mid to late summer, the higher values may reflect improved conditions for fattening associated with warmer water and more abundant food supply.

Effects of Sediments on Filtration Rate

The filtration rates of oysters exposed to all three kaolins and to fuller's earth appeared to decrease in response to increases in suspended sediment concentrations (Figure 21). In these experiments the normal filtration rates were 1.60 l/hour or slightly higher, and in all cases decreased to below 0.40 l/hour at sediment levels of about 0.40 g/liter. The smaller kaolin particles inhibited filtration at lower concentrations than did the larger particles. A 50% reduction of filtration in the presence of 9.5 μ kaolin occurred at levels of about 0.20 g/liter, while that with 0.2 μ and 1.5 μ kaolin occurred at 0.02 and 0.10 g/liter, respectively. The concentration of 50% effect with fuller's earth (0.10 g/liter) was intermediate to that of 0.2 μ and 9.5 μ kaolin and essentially the same as that for 1.5 μ kaolin.

A similar set of experiments conducted on bay silt were inconclusive because of rapid and nearly complete sedimentation in the

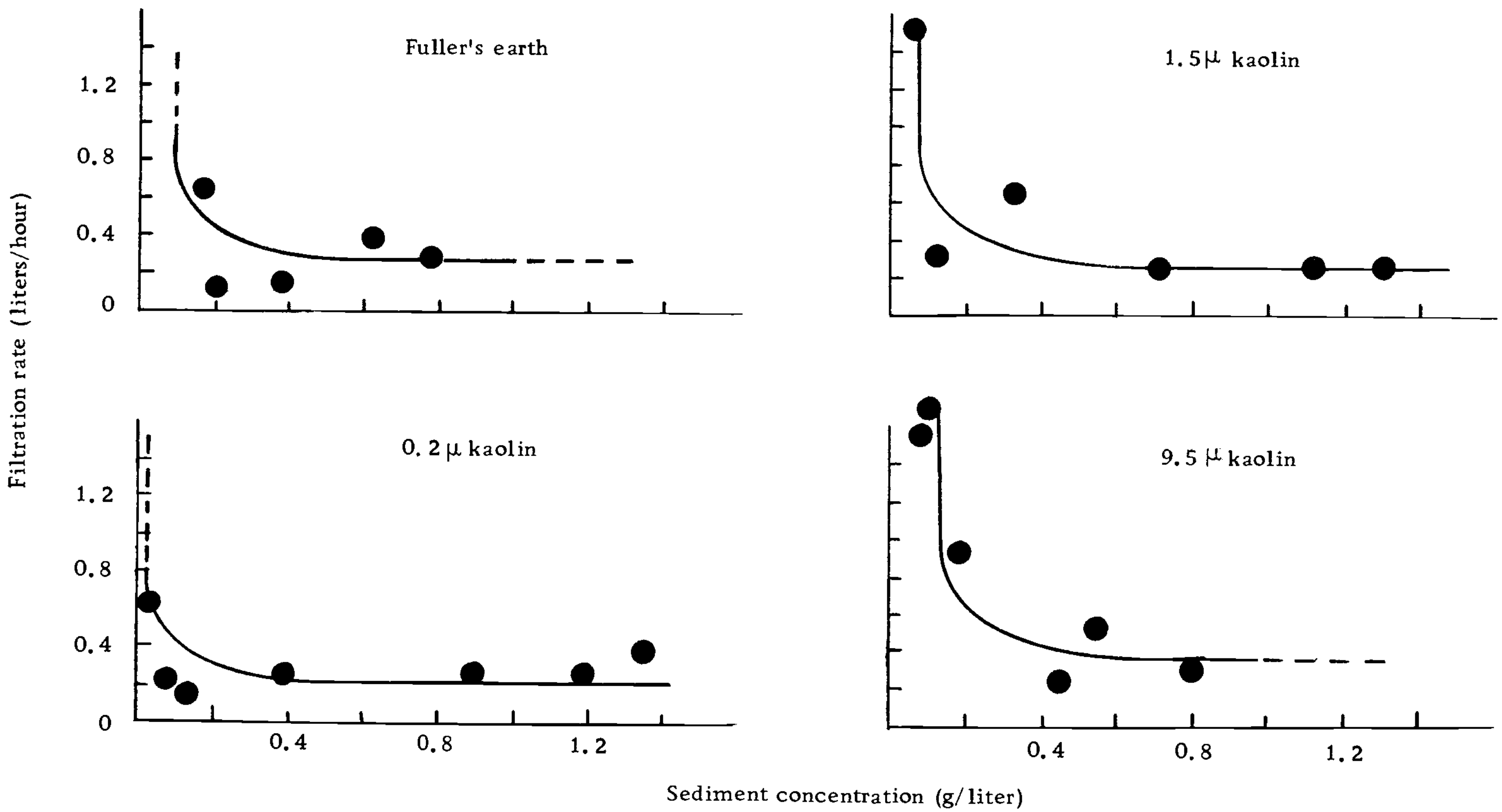

Figure 21. Filtration rates of oysters exposed to fuller's earth and 0.2μ, 1.5μ and 9.5 μ kaolin as a function of sediment concentration. Each point represents a single determination of the combined filtration rate of three oysters of approximately 65 g each.

test vessels.

Observation of Sediment Transport by Excised Gill Sections

Microscopic examination of sections of gill excised from five oysters demonstrated that all sizes of silt particles that were tested in the growth studies were easily passed across the gill to the food groove at the gill edge. In passing to the food groove, the particles were entangled in mucous threads. Infrequently some particles of silt in the 0 to 38 μ diameter range were observed within the water tubes. In this situation these particles seemed to pass through the tubes with difficulty, possibly due to their irregular shape. At high concentrations of silt approximate to those tested in the growth experiments, the smaller silt particles stacked up near the food groove, which appeared to be filled to capacity. As more sediment particles were added to the gill, they continued to back up, and the gill surface became partially clogged with sediment. In the heavy suspensions of large particles, the mucous-sediment matrix occasionally fell off the gill edge and was not passed along the food groove.

Observations were also made on gill sections exposed to kaolin sediments. It appeared that many of the 0.2 μ particles were moved across the gill in a similar manner to silt particles. However, some of the particles were presumed to pass through the ostia, since they were observed flowing in an opposite direction, within the water tubes.

This movement was observed at both high and low concentrations; but at the higher concentrations (approximately 1.5 g/liter) a greater percentage of the sediments was moved toward the food groove. Kaolin and fuller's earth particles 1.5 to 9.5 μ in size seemed to flocculate on the gill, and when these mucous-sediment masses passed over gill filaments, the ostia and interfilamentary spaces were observed to contract slightly. No particles 1.5 to 9.5 μ in size were observed within water tubes.

Physical and Chemical Properties of Sediments

X-ray diffraction and emission spectroscopy tests were run at the U.S. Bureau of Mines in Albany, Oregon, to determine the mineral and elemental composition of sediments. The results of both tests are reported in Appendices I and II, respectively.

The organic content of 0.2 μ kaolin particles was highest at 12.3% by weight and that of the 38 to 74 μ silt particles was lowest at 2.8%. The organic content of the other sediments ranged from 4.5 to 9.5%.

DISCUSSION

Previous work by other authors indicates that suspended sediments may cause a variety of responses in marine filter-feeding animals. These responses include alterations of embryonic development (Davis, 1960; Davis and Hidu, 1969), growth rate (Davis, 1960; Loosanoff, 1962), pumping and filtration rates (Loosanoff, 1962; Johnson, 1972) and feeding activity (Jorgensen, 1949 and 1960). Additional responses may include effects on respiratory gas exchange and excretory functions (Cairns, 1968).

Growth studies with larval bivalves have shown that sediment levels as low as 0.50 to 2.00 g/liter are detrimental to filter-feeding organisms (Davis, 1960; Loosanoff, 1962 and 1965; Davis and Hidu, 1969). In these studies, kaolin and fuller's earth have been used to represent clay sized particles (approximately 1 to 10 μ). Larger particles (> 10 μ) usually have been obtained by screening natural sediments, and have been collectively referred to as silts.

The levels of kaolin and fuller's earth affecting growth of larval clams, Mercenaria mercenaria, were found by Davis (1960) and Davis and Hidu (1969) to be about 0.50 g/liter. In studies with larvae of Crassostrea virginica, kaolin affected growth at levels of 1.00 to 2.00 g/liter (Loosanoff, 1962; Davis and Hidu, 1969). Growth studies have also shown that the levels of silt which affected larval bivalves were about 0.75 to 2.00 g/liter for larvae of M. mercenaria (Davis, 1960; Davis and Hidu, 1969) and for larvae of C. virginica (Loosanoff, 1962 and 1965; Davis and Hidu, 1969).

Although there are no corresponding studies reported in the literature with adult bivalves, the results of my study suggest that similar sediment levels inhibit growth of adult C. gigas. Thus 0.36, 0.54, 1.69 and 0.20 g/liter of 0.2 μ, 1.5 μ, 9.5 μ kaolin and 5 μ fuller's earth, respectively, significantly reduced growth in this species (Figures 15, 16, 17 and 19). Since these were the lowest levels of each material tested, the possibility remains that significant growth inhibition may have been detected at even lower levels. However, with 0.2 μ and 1.5 μ kaolin and 5 μ fuller's earth, additional inhibition of growth occurred at higher levels of sediments, suggesting that the threshold of effect may not have been greatly below the lowest levels tested.

The results with the silt materials were less clear. With the two largest particle ranges (74 to 104 μ and 104 to 149 μ), there were clearly no effects on growth up to the highest concentrations tested (Figure 15). However, because of greater settling of these larger particles, the highest concentrations tested were less than 0.70 and 0.25 g/liter respectively. Certainly higher concentrations of these particles would have eventually caused growth inhibition. Additionally, the fewer particles per unit weight of these sediments, as well as their larger physical size, may have facilitated their handling by the gills so that feeding rates were unaffected. Growth inhibition by the two smaller size ranges of silt appeared similar to one another, although slight (Figure 15). Growth rates were halved at silt concentrations of approximately 1.60 and 0.80 g/liter, respectively, for 0 to 38 μ and 38 to 74 μ silts. However, comparison of treatment groups by statistical tests indicated that none of the differences was significant, with the exception of the highest concentration of 38 to 74 μ silt, and this was significant only at the 5% level. These results suggest that effects on growth by finer silts are intermediate to those obtained with approximately equal concentrations by weight of the larger silt particles and the smaller clays.

Thus, it can be conclusively stated that adult oysters respond to suspended sediments at levels in the vicinity of 1.0 g/liter, by exhibiting a decrease in growth rate, and that the effect is most severe with the smallest particles. As noted earlier, larval growth is also affected at similar concentrations. In the literature there is evidence showing that sediments also reduce the success of development of bivalve embryos to the shelled veliger stage. In both clams and oysters, a reduced survival was observed during egg development at kaolin and fuller's earth levels of 1.00 to 4.00 g/liter (Davis, 1960; Loosanoff, 1962). A similar effect was also noted with natural silt at levels of 0.25 to 4.00 g/liter (Davis, 1960; Loosanoff, 1962; Davis and Hidu, 1969). Therefore, development and larval growth are both adversely affected by suspended sediments in the ranges of those found detrimental to adult oyster growth in the present study.

It is conceivable that growth rates in the presence of suspended particles may be influenced in several ways. These include toxicity of sediments due to adsorbed toxins, production of a hyperexcited state resulting in increased food requirements and impairment of gas exchange because of mechanical damage to the gills or excessive mucous production. In the absence of gill damage, a reduction in the rate of gas exchange, and also food availability, might occur because overloaded gills may be less capable of filtering normal volumes of water or because pumping and filtration rates may be voluntarily reduced. Of these several possibilities, impairment of pumping and filtration rates, either voluntarily or because of gill clogging, seemed most likely to affect growth. In order to test this hypothesis, experiments were conducted to determine the sediment concentrations that affected filtration rates, an indirect measure of pumping rates, of C. gigas. A 50% reduction in filtration rate occurred at a fuller's earth concentration of about 0.10 g/liter. With exposure to 0.2 μ, 1.5 μ and 9.5 μ kaolin, a 50% inhibition of filtration rate was observed at 0.02, 0.10 and 0.20 g/liter, respectively (Figure 21).

Johnson (1972) reported a similar sharp reduction in the filtration rate of Crepidula fornicata at silt, kaolin and fuller's earth levels of approximately 0.17, 0.22 and 0.25 g/liter, respectively. In C. virginica, silt and kaolin levels of 0.10 g/liter reduced pumping by 57%, while 0.50 g/liter of fuller's earth reduced pumping by 60% (Loosanoff and Tommers, 1948). At 1.0 to 4.0 g/liter of silt and kaolin, pumping was reduced up to 94% in the same species. Bentonite (clay) levels of 0.50 to 1.00 g/liter slightly reduced pumping of C. gigas (Chiba and Ohshima, 1957), while 0.10 g/liter of mixed sediment significantly reduced pumping rates.

My data and that of Chiba and Ohshima, on C. gigas and that of others on different species thus show a fairly good correlation between the levels of suspended particles which result in reduced filtration or pumping rates, and the levels reducing growth (Jorgensen, 1966; Owen, 1966; Ali, 1970). This provides strong circumstantial evidence that growth inhibition by suspended sediments is directly related to either a reduced availability of food materials or a reduction in gas exchange as a result of inhibition of pumping.

The cause of reduced pumping rates in the presence of suspended sediments is not clear. One possibility is that pumping is controlled by a mechanism involving sensory perception of the concentration of particles in the water. This is conceivable, since Hopkins (1932) has shown that C. virginica is capable of responding to chemicals in the water by a sensory mechanism. However, there is presently no data to support or reject this hypothesis for suspended sediments.

Alternatively, pumping rates may be regulated by some mechanism relating to the degree of overloading of the gill by sediments. This would also be a sensory response to particle concentration, but the sensing mechanism would be indirect, since it would occur after filtration had occurred and would be related to the build-up of particles on some part of the gill surface. To test this possibility, I conducted observations with excised gill exposed to concentrations of sediments similar to those used in the growth and filtration studies. Considerable loading of the gill with sediments was observed at the highest concentrations tested. At 0.50 g/liter, passage of particles along the gill surface appeared to be unhindered. However, in experiments where the concentrations were approximately 1.50 g/liter, the sediments clumped together as a mucous-sediment block, and passage of these particles along the gill surfaces appeared to be slower. The concentrations causing build-up on the excised gills can only be approximately compared with those causing inhibition of filtration, however, since in the tissue studies, water was not actively pumped across the gill. With gill tissue *in situ*, clogging may occur at lower sediment levels, since the quantity of suspended sediments contacting the gill might be considerably greater. Because of the similarity between the concentration of sediments which caused a reduction in filtration rate and the concentration which appeared to clog the gill, the possibility of overloading being the regulating mechanism may have merit.

Of the three responses to suspended sediments which were examined in intact oysters in this study--growth, filtration rate and condition index--condition index was the only measurement not routinely affected by the levels of sediments tested. Only in the 74 to 104 μ silt test was the condition index significantly reduced during the two-week exposure period. This response is difficult to rationalize, however, because none of the other sediment groups showed a similar response even though growth inhibition may have been more pronounced. The possibility exists that this may have been an anomalous result caused by a chance selection of oysters in the control group having an initially higher condition index than those in the treatment groups.

A reduction in condition index would occur either because of an enlargement of the shell cavity due to deposition around the shell margin, or because of a reduction in the quantity of soft tissues, or both. In starved oysters, it would seem unlikely that shell deposition would continue. The most likely possibility would be that a reduction in soft tissues would occur because of the mobilization of tissue reserves for respiratory needs. It is reasonable to assume that this must have occurred to some extent in the sediment-exposed oysters, but because of the considerable variation in condition index of individual oysters, a small decrease in tissue was not evident.

The results of this study allow inferences to be drawn concerning the significance of suspended materials to water resources. Bays and estuaries in the Northwest are frequently made turbid when large amounts of sediments are discharged into rivers because of heavy coastal rainfall and storm activity. In addition, man's activities, such as forest clearcutting and poor farmland management, enhance turbid conditions in estuaries. Heavy shipping in deep water, as well as small boat traffic in shallow areas, may suspend benthic sediments and periodically subject oyster grounds to elevated levels of suspended silts and clays. Dredging operations, such as hopper dredging, allow the escape of fine silts and clays into the water (O'Neal and Sceva, 1971). Man's contribution to the turbidity of the receiving water may be especially significant during a season when turbid conditions do not normally prevail.

This study shows that during short periods, fine clay sediments reduce the growth of adult oysters in the laboratory. If such sediments are maintained in the water column in the vicinity of oyster grounds for prolonged periods, a reduction in oyster production may result. The concentrations of fuller's earth and kaolin that may seriously affect oyster growth are 0.36 to 1.81 g/liter. These particles, which range from 0.2 μ to 9.5 μ in size, are easily suspended in the water column, and tend to remain suspended for long periods, the length of time depending on the electrolytic balance in the water, the strength of the water current, the presence of eddies, the degree of flocculation or the presence of organic pollutants (Postma, 1967). The concentrations of bay silt affecting oyster growth, although not as clearly demonstrated in this study, are about the same.

Since under natural conditions the levels of suspended sediments may be as high as 0.11 g/liter (Schubel, 1968), there is clearly little margin of safety under some conditions for additional turbidity due to dredging. There is little published information on the levels of suspended solids produced by dredges. Wilson (1956) reported up to 1.7 g/liter downstream from a dredging operation on the Powder River in Oregon. If similar levels occurred in estuaries, it is clear that oyster growth would be affected, the magnitude of effect being related to the period of high sediment exposure. Clearly, more information is needed on the levels and persistence of suspended solids in estuaries around dredges. If these levels reach the 1.0 g/liter range, some control of dredging operations in oyster growing areas may be warranted. Since the levels of sediments affecting other species, for example that found for <u>Crepidula fornicata</u> (Johnson, 1972), may be similar, control of dredging may be indicated in other than oyster growing areas as well.

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APPENDICES

Appendix I. Mineral composition of sediments as determined by X-ray diffraction.[1]

Mineral components	Sediment		
	Silt	Kaolin	Fuller's earth
α quartz	P[2]	N	P
NaCl	T	N	N
Montmorillonite	BDT	N	N
Illite	BDT	N	N
Kaolinite	BDT	P	N
Plagioclase	BDT	N	N
$Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$	N	P	N
Al	N	T	T
$Mg_5Si_8O_{20}(OH)_2 \cdot 8H_2O$	N	N	P
Unidentified	N	BDT	N

[1] The mineral composition of the sediments was examined by the X-ray diffraction powder technique (Cullity, 1956). A small (approximately 0.1 g), finely ground sample of sediment was irradiated with nickel filtered copper X-rays in a Norelco X-ray diffractometer at 45 kilovolts and 35 milliamperes. The diffractometer scanned the sample from 4 degrees two-theta to 76 degrees two-theta. The diffracted X-rays were detected with a scintillation detector with pulse height discrimination, and the resulting signal recorded on a strip chart recorder synchronized with the diffractometer. The resulting diffraction patterns are characteristic of the compounds present. The observed patterns of the sediments were compared with published patterns from samples of known composition (ASTM, 1971).

[2] Estimates from analysis: P = present (30 to 35%)
T = trace (1 to 10%)
BDT = barely detectable trace
N = not detected

Appendix II. Elemental composition of sediments as determined by optical emission spectroscopy.[1]

Element components	Sediment				
	Silt	0.2 μ kaolin	1.5 μ kaolin	9.5 μ kaolin	Fuller's earth
Al	A+[2]	A+	A+	A+	A
Ca	A	N	N	N	A+
Cr	D	D+	D+	D+	D
Cu	N	N	D	N	N
Fe	B	C+	C+	C	B
Mg	B+	C	C	D+	A
Mn	C	N	D	N	C
Na	B+	N	N	N	C
Ni	D+	D+	D+	D+	N
Si	A+	A+	A+	A+	A+
Ti	B	B+	B	B	C
V	D+	D+	D+	D+	D+

[1] Qualitative estimates of elemental composition of sediment materials were obtained by optical emission spectroscopy (Waring and Annell, 1953). A 10 mg sample was intimately mixed with 10 mg of high purity graphite and weighed into a cratered, graphite electrode. This sample was excited using a 10 ampere DC arc and was burned for 3 minutes with the sample being totally consumed (complete burn). The average temperature of the arc plasma was approximately 6,000°Kelvin. Using a Baird 3-meter, Eagle mount, optical spectrograph, the spectrum for the sample was recorded on photographic plates and visually compared to synthetic standards. To relate to the samples, the synthetic standards were made up in a graphite matrix. An Applied Research Laboratory densitometer-comparator was used to determine the elemental composition of the experimental sample.

[2] Estimate from analysis:

A+	=	10-100%	C	=	0.03-0.3%
A	=	3-30%	D+	=	0.1-0.1%
B+	=	1-10%	D	=	0.003-0.03%
B	=	0.3-3%	N	=	Not detected
C+	=	0.1-1%			