AN ABSTRACT OF THE THESIS OF

William L. Shapeero for the degree of Doctor of Philosophy in Fisheries and Wildlife presented on April 30, 1976.

Title: The Effects of Sewage on the Distribution and Abundance of Benthic Animals in a Puget Sound Bay.

Abstract approved: Redacted for Privacy
Charles E. Warren

The effects of discharged sewage on physico-chemical conditions and the distribution and abundance of marine benthic animals were studied in Shilshole Bay, a part of Puget Sound, off Seattle, Washington. For over 50 years prior to 1965, when this study was initiated, 87,300 pounds (32,400 pounds BOD) per day of mostly raw, domestic sewage with some undefined industrial waste and storm drainage had entered the Bay via a submarine outfall. As a result of the prolonged period of discharge, different habitat types were established. These ranged from relative clean sediment to the sludge bed itself. Four areas representing the two extremes and two intermediate conditions were selected and sampled, first in 1965 when sewage was being discharged and then in 1968, three years after discharge ceased.

In the water at the sediment-water interface, salinity, temperature, pH, dissolved oxygen, hydrogen sulfide, chromium, and dissolved organics were not found to be responsible for the observed differences of animal distribution and abundances. Current velocity and amount of inorganic material were interrelated in that the former prevented or

allowed the latter to settle and seal off the sediment from the water column, thus helping to drive some sediments anoxic. Although relatively low in concentration, copper and lead may have had some subtle effects on the animals, especially larvae or sensitive species.

In the interstitial water, salinity, temperature, pH, and ammonia were similar at the four sampling stations and were not important factors in determining the distribution of the animals. Very low oxygen levels in the sediments may not have been of much direct importance, but resultant increases in hydrogen sulfide at the two most polluted stations probably affected many species. The amount of organic material in the water column did not appear to be of direct importance in determining the distribution and abundance of animals. Upon settling, however, it was probably the greatest factor in determining different habitat types and affecting other environmental variables, which collectively determined which species would be present.

A reasonable variety of infauna (83 species exclusive of nematodes) was found in 1965 among the four sampling sites in Shilshole Bay, but adjacent Puget Sound areas exhibited a greater number of species. Some species were present in the Bay in extraordinarily great numbers, this suggesting enrichment had favored these species.

In 1965 the intermediate condition sampling station, closest in enrichment to the clean site, exhibited the greatest number of species and the highest total biomass. On the basis of number of species and total biomass, the sludge bed exhibited the poorest biota. Species number and total biomass in most areas were lower in 1968 than in 1965, presumably due to overall loss of organic enrichment.

The Effects of Sewage on the Distribution and Abundance of Benthic Animals in a Puget Sound Bay

by

William L. Shapeero

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Gene Collias provided assistance to solve several problems, as did Jim Reese, who offered technical assistance in many phases of the study.

To Richard Sternberg and several technicians at the University of Washington Oceanography laboratory, I am indebted for the loan of the current rate meter and advice in construction of the current direction meters used in the study. I am appreciative of the Coast and Geodetic Survey in Seattle, Washington, for helping with a survey of the study area.

It takes a special kind of individual with a special kind of talent to identify to species organisms encountered in studies of this type. Without them such studies would not be possible. Therefore, it is with deep appreciation I now acknowledge the following people and their contributions. Harry Yeatman and Mary Wicksten worked with the harpacticoid copepods; Robert Bowman identified Munnogonium waldronense; Eric Mills worked with the amphipods; Fred Gunther measured and identified the ostracod Euphilomedes carcharodonta; Josephine F.L. Hart identified the cumaceans and other larger crustaceans. Robert Dunnill identified the species of Macoma, Eugene Coan the Tellina, and

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As a time-saving measure, it was deemed desirable to evaluate community diversity by computer. I gratefully acknowledge the expertise of Jim Holgersen, who designed the appropriate computer program and instructed the writer on its use.

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The writer's sons, Mike and Pat Shapeero, spent long hours during several summers diligently and tediously picking animals from sediment and made counts and weights. My deepest thanks to them and their mother, Joan, who put up with all this activity year after year with a smile, advice, and encouragement. She also proofread the entire manuscript.

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TABLE OF CONTENTS

IV. RESULTS

A. Physical factors

V. INTERPRETATION OF RESULTS

A. Physical factors

Metals

Organic material

B. Biological

Crustacea

Mollusca

VIII. APPENDIX

LIST OF TABLES

LIST OF FIGURES

THE EFFECTS OF SEWAGE ON THE DISTRIBUTION AND ABUNDANCE OF BENTHIC ANIMALS IN A PUGET SOUND BAY

I. INTRODUCTION

A. Purposes of the present investigation

The marine environment, once considered by many to contain an inexhaustible supply of food with which to feed the world's starving millions, and to be a "bottomless pit" in which to dispose of unwanted materials, today faces a crisis that threatens its very existence as a medium capable of sustaining life.

Pollution has spread from the rivers to the sea. Rivers that once cleansed themselves to some degree are now overloaded and carry considerable quantities of agricultural, industrial, and domestic wastes unchanged to estuaries. Today many coastal cities dump indiscriminately all effluents into marine waters. The increasing number of oil spills in navigable rivers and at sea are extensive and frequent (Marine Pollution Bulletin; ZoBell, 1964) and often devastating to marine organisms. Nuclear and steam power plants may use marine waters as coolants; in some areas this heated water threatens the very existence of life, or at the least, causes considerable changes in the biota. Unscrupulous land developers, unwilling to recognize the essential nature of estuaries as nurseries for commercial and other fish species, relentlessly fill and sell newly reclaimed "worthless" estuarine lands for real estate developments. All these abuses and declining numbers of fish caught in some areas have led some authorities to believe the ocean has begun to fail. For additional information on the abuse of marine resources see Føyn (1965), Marx (1967), Report of The Environmental Pollution Panel, President's Science Advisory Committee (1965), Council on Environmental Quality (1973), and U.S. Congress (1972).

The desirability of preservation of local marine habitats and the essential nature of the Ocean itself is only now becoming more widely recognized by legislators. Federal and state governments are making an attempt through legislation to halt destruction of marine environments. Through research and tighter controls, pollution has become one of the main targets.

In the past, the majority of pollutional investigations in fresh and marine waters have been carried out by engineers, who understandably, because they are not trained in biology, have looked to measurable physical features of the environment to quantify environmental degradation. Biologists, on the other hand, believe the living organisms reflect, through their distribution and abundance, more accurately the changes that have occurred in the environment as a function of time (Hynes, 1963). They recognize the difficulty of the "biological approach" to pollution investigations because the sensitivity to minute environmental changes that make living organisms the best indicators of environmental conditions also make them exceedingly difficult to study. Add to this the lack of taxonomic experts to identify the animals present in areas under study, and it is readily seen why, until recent years, the task of pollutional investigations has fallen mainly to the engineers. Puget Sound, the location of the study presented here, has not been an exception to the above generalities.

It is well understood by biologists that in each environment a multitude of factors operate on each kind of organism present to determine its distribution and abundance. The range of tolerance to each of these factors within which each species can exist ultimately resides within their genetic makeup. When the environment experiences a subtile or extreme shift in one or more of its variables, the organisms experience stress; their response as individuals and as a species must be either adaptation to the change, or extinction. Biologists often see environmental changes that have occurred as a shift in species composition without understanding the nature of the change involved. They are painfully aware that if the "biological approach" is to be used to help solve estuarine and coastal problems, an attempt must be made to carefully define the relationships that exist between organisms and the physical environment. When this has been accomplished, the understanding of the effect of environmental alterations on organisms may be predictable, and changes in faunal composition might be extrapolated backward in time to the alterations that caused the species shift.

The biological approach requires answers to many questions. The present study was conceived, planned, and modified to gain some insight to a rather broad question: What are the relationships that exist between the physical environment and the distribution and abundance of marine benthic animals in areas of different organic enrichment, all provided by the same sewage source. After the initial data were collected in 1965, the sewage source (North Trunk Sewer) was shut down. Almost as an afterthought, for it is seldom that sewers are shut down, an opportunity came to study reinvasion of the heavily polluted areas by organisms and to see how the areas deprived of their organic enrichment responded biologically.

As sampling proceeded and analysis was undertaken, a third important goal became apparent, that of developing satisfactory techniques and analyses that would yield valid results. Thus the writer feels that in some respects this portion of the report may exceed the value of the others for those about to undertake a similar study.

B. Literature

The literature related to the present study is of three types: 1) The abundance and distribution of animals at different locations. This may take the form of tabulations of extensive sampling of species in a restricted area, such as a bay, or as sporadic or pattern sampling of larger bodies of water. Many such studies have been made worldwide and on the Pacific Coast and specifically for the Pacific Northwest by Lie (1968) in his report of research in Puget Sound. 2) Investigations which deal with the relationships between animals and environmental variables. Here the useful literature is sparse since it consists mainly of laboratory or insitu studies in which only a small number of environmental factors are manipulated or measured to assess their affects on animals. 3) Observations on natural communities of animals in areas that have undergone or are in the process of undergoing discharge of some type of material. The more important of these have been summarized by McNulty (1970) in his studies of Biscayne Bay, and additional studies for California have been reported by Young (1964), Turner et al (1964, 1966), and Grigg and Kiwala (1970).

Investigations for Puget Sound are few and have little bearing on the present study with the exception of Lie (1968) and to a lesser extent Wennekens (1959). In some locations of the Sound, the pollutional affects of pulp and paper mill wastes on animals have been evaluated (F.W.P.C.C. and W.S.P.C.C., 1967). In the actual area of study--Shilshole Bay--Wilson (1957) worked in the vicinity of the outfall of the North Trunk Sewer and reported the gross appearance of the sediment and the kinds of animal present. These were identified only to phyla and in some cases to order. His study was undertaken in preparation for the construction of the West Point treatment plant (Fig. 2) which was to replace the North Trunk submarine outfall. More recently, Domenowske and Matsuda (1969) studied the distribution of seven species of animals near the new, deep-water, West Point sewer outfall. They found no measurable sludge deposition in the vicinity of the outfall and diffusers and no substantial change in the seven benthic populations studied during the first two years of operation and discharge of the effluent to deep water.

C. Topography, sedimentology, and hydrology

Puget Sound

Puget Sound is one of the deepest saltwater basins in the United States. Its pertinent physical features have been summarized by Lie (1968).

Study area--Shilshole Bay (Fig. 1).

The topography and climatic conditions for the Seattle area have been described in the Brown and Caldwell Report (1958).

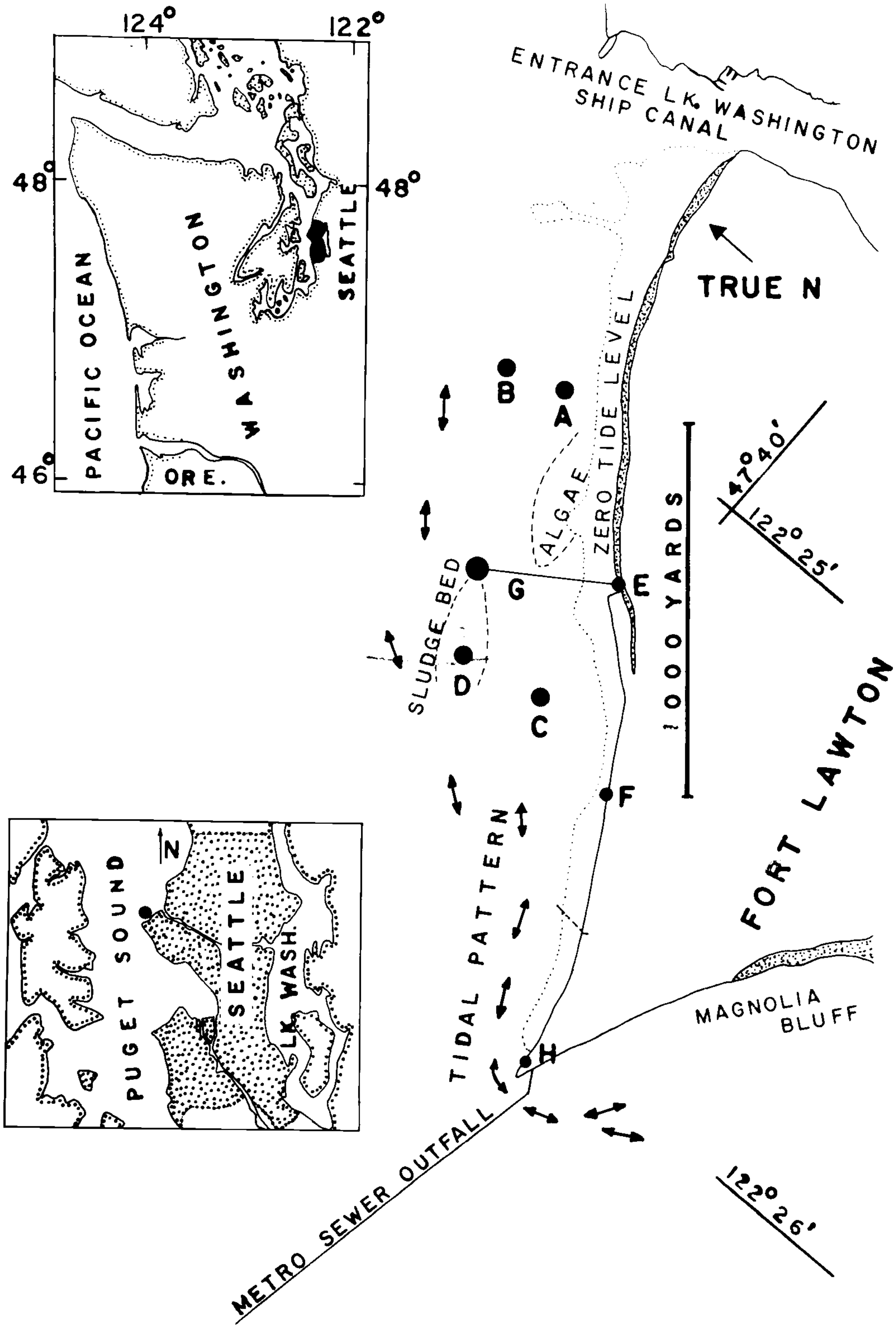


Figure 1. Map of the area of study.

The area involved in the present study lies within Shilshole Bay, off of Fort Lawton, Seattle, Washington. It is bounded on the northeast by the channel leading to the entrance to the Lake Washington Ship Canal and on the southwest by West Point (Pt. H). The distance between the two boundaries is approximately 2530 yards (2313 meters).

The gross tidal pattern is indicated on the map and is shown in detail in the Brown and Caldwell Report.

The more northern portion of the study area is characterized by relatively shallow water--usually less than 50 feet (15.2 meters)--while the southern portion drops off rapidly close to shore to well over 150 feet (45.7 meters) and off shore to more than 800 feet (244 meters). At a distance approximately midway between the two boundaries, a four-foot diameter, submarine outfall pipe, extends some 1100 feet (335 meters) into the Bay, perpendicular to the shore. For more than 50 years it discharged raw, untreated sewage at a depth of about twenty-five feet (7.6 meters) (Pt. G). During the rainy season, storm drainage added to the sewage often exceeded the carrying capacity of the pipe and emptied directly on shore over a weir at the end of a concrete tunnel (Pt. E; Fig. 3).

Study Stations in Shilshole Bay

Four stations of varying degrees of organic encirhment were located by extensive underwater exploration. Their descriptions follow.

1. Sludge bed (Pt. D; 47° 40' 8.4" N, 122° 25' 33.4" W).

This station represents the extreme in organic enrichment. The undisturbed deposit was a snowy-white when viewed by diving; it was very light in weight and easily disturbed, revealing a dark color beneath. Bubbles--presumably methane gas--frequently escaped from the sediment. The margin of the sludge bed was marked with quantities of toilet paper and sanitary napkins in varying stages of decomposition. The extensive bed had the shape of a broad mound so the depth of water varied at different locations. The area selected to sample was 11.8 feet (3.6 meters) at 0.0 water.

The sewage was lighter in weight than seawater because it was warmer and made from freshwater. After discharge it traveled rapidly and directly to the surface as a "boil", where it spread out to form a plume whose configuration reflected the prevailing currents (Fig. 4). Once at the surface, some of the materials settled to the bottom, the heaviest closest to the outfall.

2. Clean station (Pt. A; 47° 40' 14.9" N, 122° 25' 01" W).

This station represented the opposite extreme from the sludge bed. It was close to shore and on the shore-side it was bordered by a fairly extensive kelp bed (Fig. 5). The surface was flat, firm, a fine sand and grey-white in color. Its depth at 0.0 water was 9.7 feet (3.0 meters).

3. Between station (Pt. B; 47° 40' 20.7" N, 122° 25' 04" W).

As we traveled under water from the Clean to Between Station, we noticed the sediment's surface had increasingly greater deposits of a brownish material so that, at the latter station, it was quite brown and the material beneath a light grey-black. Sea pens, found only occasionally at the clean station, became increasingly abundant and came to form a striking orange carpet at the Between Station (see "Epifauna"). The sediment, a fine sand, was not quite as firm as the Clean Station and had a number of openings from animal burrows (Fig. 6). The surface was flat and 10.7 (3.3 meters) at 0.0 tide.

4. Organic station (Pt. C; 47^{o} 40' 01" N, 122^{o} 25' 30.6" W).

This sediment possessed spongy characteristics often found in highly organic, boggy soils. When sediment cores were taken, the ground five feet away and more quaked as the corer was moved back and forth. The surface had a number of openings of animal burrows (Fig. 7). The sediment was dark brown, and occasionally wood fibers and light sewage materials collected in slight depressions in the surface. At 0.0 tide the sediment surface was at a depth of 8.8 feet (2.7 meters) and flat.

Figure 2. West Point Sewage Treatment Plant. Shilshole Bay is to the left, Fort Lawton is in the background (trees).

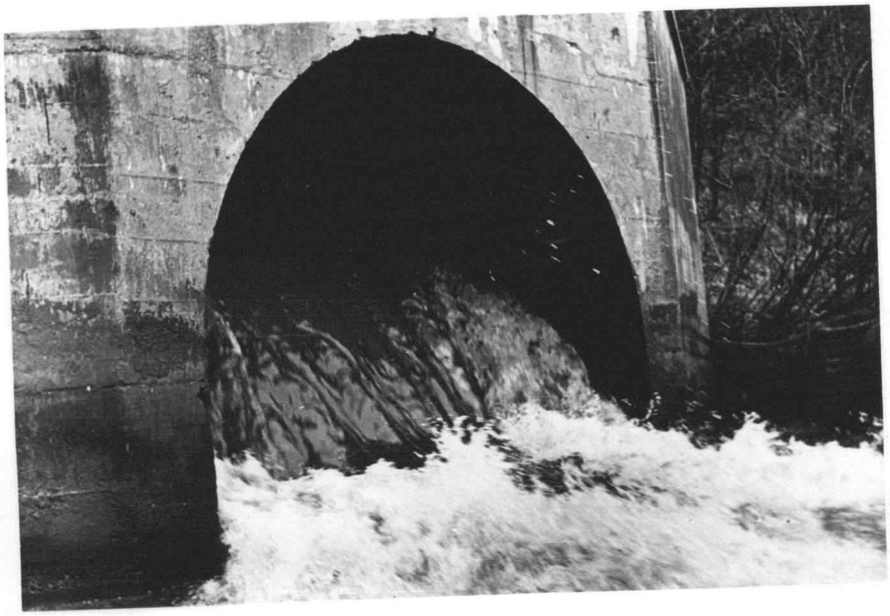


Figure 3. North Trunk Sewer overflow.

Figure 4. Shilshole Bay. Fort Lawton to left rear, West Point to extreme right; North Trunk Sewer boil and plume, center.

Figure 5. Kelp bed of Nereocystis luetkeana, as seen from shore.

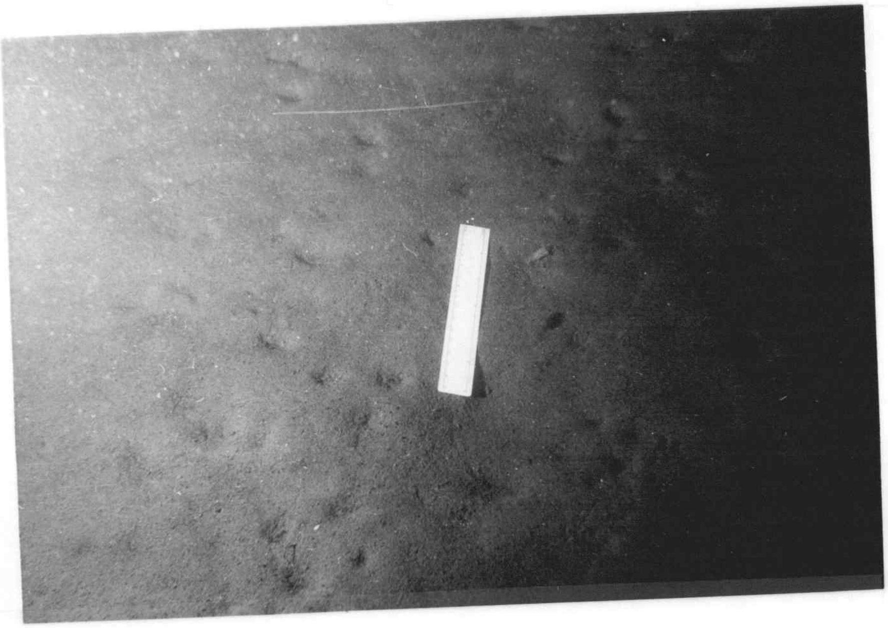


Figure 6. Openings to animal burrows at the Clean Station.

Figure 7. Openings to animal burrows at the Organic Station. Note the detrital skin on the surface of the sediment.

II. CHARACTERISTICS AND SOURCES OF THE ORGANIC ENRICHMENT

The main source of organic enrichment to Shilshole Bay was the effluent of the North Trunk Sewer system. The North Trunk, which was constructed and operated continuously since 1912, serviced in 1957 about 17,000 acres of Seattle, Washington, with a population at that time of about 230,000 people and numerous industries. The sewer consisted of an extensive system of large diameter pipe (to twelve feet), the terminal end of which tunneled at a depth of 200 feet (61 meters) beneath Fort Lawton and then extended as a submarine outfall to discharge some 1100 feet (335 meters) from shore in about 25 feet (7.6 meters) of water.

During dry weather, the effluent consisted of raw domestic sewage, infiltration from ground water through joints and breaks in the pipes, and industrial wastes whose composition and volume has not been determined. During the winter, considerable quantities of storm drainage swelled the total volume.

In October of 1957, the dry weather discharge for seven days was analyzed (Table 1). It was determined to be an average 42 million gallons per day, with a daily biochemical oxygen demand of 32,400 pounds. The total solids delivered daily by the North Trunk was an average of 110,300 pounds. Of this, 87,300 pounds were volatile and supposedly organic in nature; the rest, 23,000 pounds, was inorganic. Of the 87,300 pounds of volatile material, 27,700 pounds would supposedly settle out locally, and the rest--59,600 pounds--would remain in suspension as dissolved or as small particulates to be distributed to this and adjacent areas of Puget Sound according to the currents. The temperature of the effluent was 20° centigrade, somewhat above that of the receiving waters (12-14°C), while the pH of 7.0 was slightly below (7.7). During the winter the dissolved oxygen concentration was 4-8 ppm and in the summer it was 2 ppm or more.

Considerable quantities of inorganic grit entered the study area via the sewer outfall. Its source was street washings including especially materials used to sand streets during icy conditions. During the dry weather an average of two and a maximum of five cubic feet per million gallons was present in the sewage that entered treatment facilities in Seattle. During the winter, the increased flow and velocity of the sewage carried the grit suspended, to be discharged directly via the submarine outfall. The grit varied from small rock to small particles (less than .073 mm).

In addition to the sewage, log rafts, cut lumber, and residues from pulp and paper industries located in Puget Sound contributed wood chips, wood fiber, and bark to the marine sediments of Shilshole Bay.

The North Trunk submarine outfall was inactivated permanently in late 1965, and the new West Point treatment plant began operation in July, 1966. During the period of December 1, 1965 to July, 1966, the sewage was discharged through a new bypass at the shore (Fig. 1, Pt. F). For 41 days following shutdown of the discharge from the submarine outfall, and prior to December 1, the sewage was discharged to the entrance to the Lake Washington Ship Canal, just below the Government Locks. Presently the effluent from the plant is discharged off West Point by a deep water outfall into strong current (Fig. 1, "Metro

Sewer Outfall"). The discharge is approximately 60 million gallons per day with an effluent BOD of 41 ppm. Infrequently, the volume may reach 350 mgd and is then discharged by the bypass (Fig. 1, Pt. F) at the shore (Charles Gibbs, personal communication).

Table 1. Sewage Characteristics* North Trunk Sewer, October 4-11, 1957
(Extracted from the Brown and Caldwell Report)

	Total pounds/day (in ppm)	Volatile pounds/day (in ppm)	Inorganic pounds/day (in ppm)
A. Biochemical oxygen demand (BOD)			
I. Total	32,400 (92)	--	--
II. Settled**	21,500 (61)	-- -	--
B. Solids			
I. Total	110,300 (314)	87,300 (248)	23,000 (65)
II. Settleable material***	32,300 (92)	27,700 (79)	4,600 (13)
III. Suspended material			
a. Total	78,000 (222)	59,600 (170)	18,400 (52)
b. Dissolved (nonfilterable)	47,000 (134)	40,400 (115)	6,600 (19)
c. Particulate (filterable)	31,000 (88)	19,200 (55)	11,700 (33)

* Weighted averages based upon an average daily discharge of 42.1 million gallons (mgd). Pounds/day are rounded off to nearest 100 pounds; ppm to nearest whole number.

** With one hour standing.

***With 15 minutes standing.

III. MATERIALS AND PROCEDURES

A. Introduction

One of the major difficulties encountered in gathering meaningful and valid data for this study has been to find useful and tested techniques and procedures. Indeed, many of the needed, published techniques required modification, and much of the procedural work had to be developed specifically for this study. Even after completion of the study, honesty dictates it be said that there are portions with which there is still dissatisfaction and that would need to be redesigned in any future studies of this type.

Nevertheless, so that others who carry on similar research may benefit from the successes and not repeat the mistakes made, methodology will be explained and evaluated in some detail.

B. The study area: criteria for selection

In order to have the greatest opportunity for success of the expressed goals, it was desirable for the area of study to have certain characteristics which became more apparent during preliminary portions of the study. These were:

1. The depth of water must be shallow and its tidal velocity relatively low so that a diver could work safely for long periods at exploration, placing of equipment, and sampling.

2. To insure comparability of sampling stations, all parts of the area of study must be part of the same water mass so that distribution and abundance of animals would be a reflection of environmental conditions rather than a lack of opportunity for species dispersion.

3. The source of pollution must be from single rather than multiple sewer outlets so that the enrichment effect of the sewage at the different sampling stations would differ only in magnitude and not in composition.

4. To test the effects of organic enrichment on the benthos, the effluent should have a high proportion of domestic sewage.

5. The discharge of sewage should have been of sufficient duration to form well defined, relatively stable environments and their resultant communities.

6. The area of study should have equivalent bottom environments of sand to mud so that infauna could be collected in a uniform manner for comparison between sampling stations.

7. There must be a wide range of amounts of enrichment from "clean" to heavily polluted, to provide sampling stations for comparison of different degrees of pollution.

The area of Puget Sound selected to study provided all the conditions set forth above. The North Trunk Sewer pipe had been discharging continuously for over 50 years an effluent consisting of storm drainage, some unidentified industrial waste, but mostly untreated municipal sewage (Brown and Caldwell, 1958). The prevailing tidal and eddy currents resulted in sufficiently well-defined environmental types to make location of adequate sampling stations possible. These represented the two extremes of organic enrichment--heavy and light--and two intermediates. All four sampling stations were flat and of mud to sand; they were sufficiently shallow and currents sufficiently weak to allow prolonged, safe, underwater work. Furthermore, their depth--mostly under 35 feet (10.7 meters) at high tide--was similar enough to eliminate depth as a probable factor affecting species distribution.

C. The study area: frequency of sampling

Shortly after planning began, it became obvious that all studies have manpower, time, and financial limitations, and that a decision would need to be made regarding the number of environmental factors to be measured and the extent of their sampling. One possibility was to measure periodically throughout the year a few environmental factors thought to be most important and to correlate these with the distribution and abundance of species present at sampling times--a typical seasonal approach. An alternative approach was to examine in detail for a short period of time all measurable environmental factors that might affect the distribution and abundance of the benthic species present at the time of sampling.

The decision to select the second approach was made for the following reasons:

1. One of the prime purposes of the study was to determine, if possible, and with more certainty than ever before, which environmental factors were of importance in determining the abundance and distribution of the animals in an area of organic enrichment. To guess at and measure but a few factors would be self-defeating.

2. Studies of seasonal distribution and abundance of animals are of general scientific interest and of considerable importance. But such information would be of minimal value in evaluating how and which environmental factors are affecting the resident populations. The

study was designed to compare factors at localities, not seasonal fluctuations.

3. Finally, I believe the smaller animals to be extremely important in defining the community and in community dynamics, and perhaps to be more sensitive to environmental changes than the larger animals. But working with smaller animals multiplies many-fold the time required for study; it would not be possible to study the smaller animals with multiple, seasonal sampling.

D. The study area: stages of the study

In retrospect, it is possible to define seven stages in the overall study as follows:

1. Underwater survey: considerable time was spent, through the use of SCUBA gear, to become familiar with the entire area under consideration for study, and to develop a "feeling" for the amount of organic enrichment occurring at different localities. Four sampling stations were selected and the Sludge Bed was mapped with the use of "beer-bottle buoys" (Fig. 8). The 37-foot research vessel Tenas, owned by the University of Washington, was used as a base of operation (Fig. 9).

2. Construction and placement of equipment: Figure 10 shows the overall physical arrangement of the sampling equipment. Heavy concrete anchors were cast, log buoys were constructed, and one unit (buoy and anchor), joined by one-quarter inch steel cable, was placed at each of the four sampling stations, again using RV/Tenas. Current direction meters were constructed (Figs. 11, 12) and placed at three stations; none was placed at the Sludge Bed because of fouling problems. At the

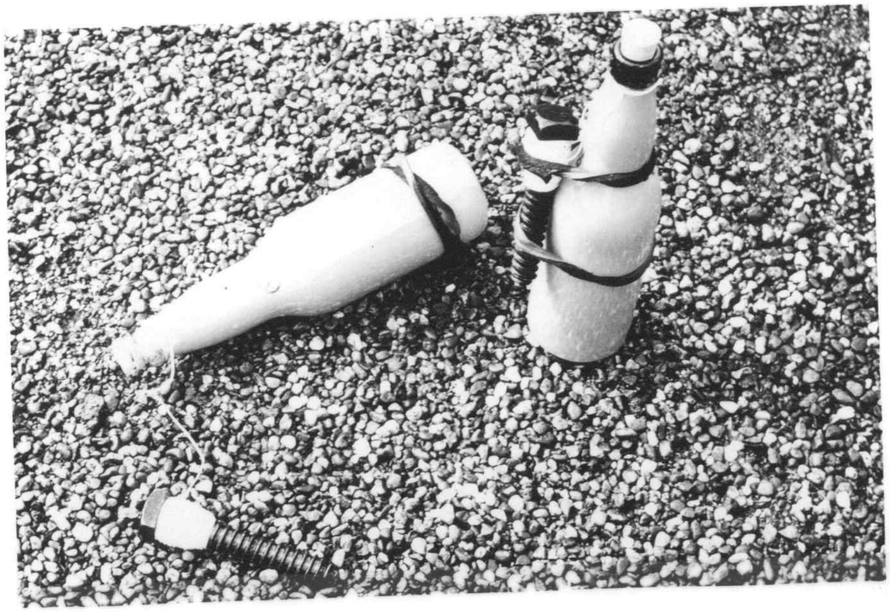


Figure 8. "Beer-bottle buoys" used to mark and map the Sludge Bed Station. Bottles floated and the bolts, which were shoved into the sediment, held the bottles in place.

Figure 9. Research vessel Tenas. Note diving ladder, and winch to lower concrete anchors.

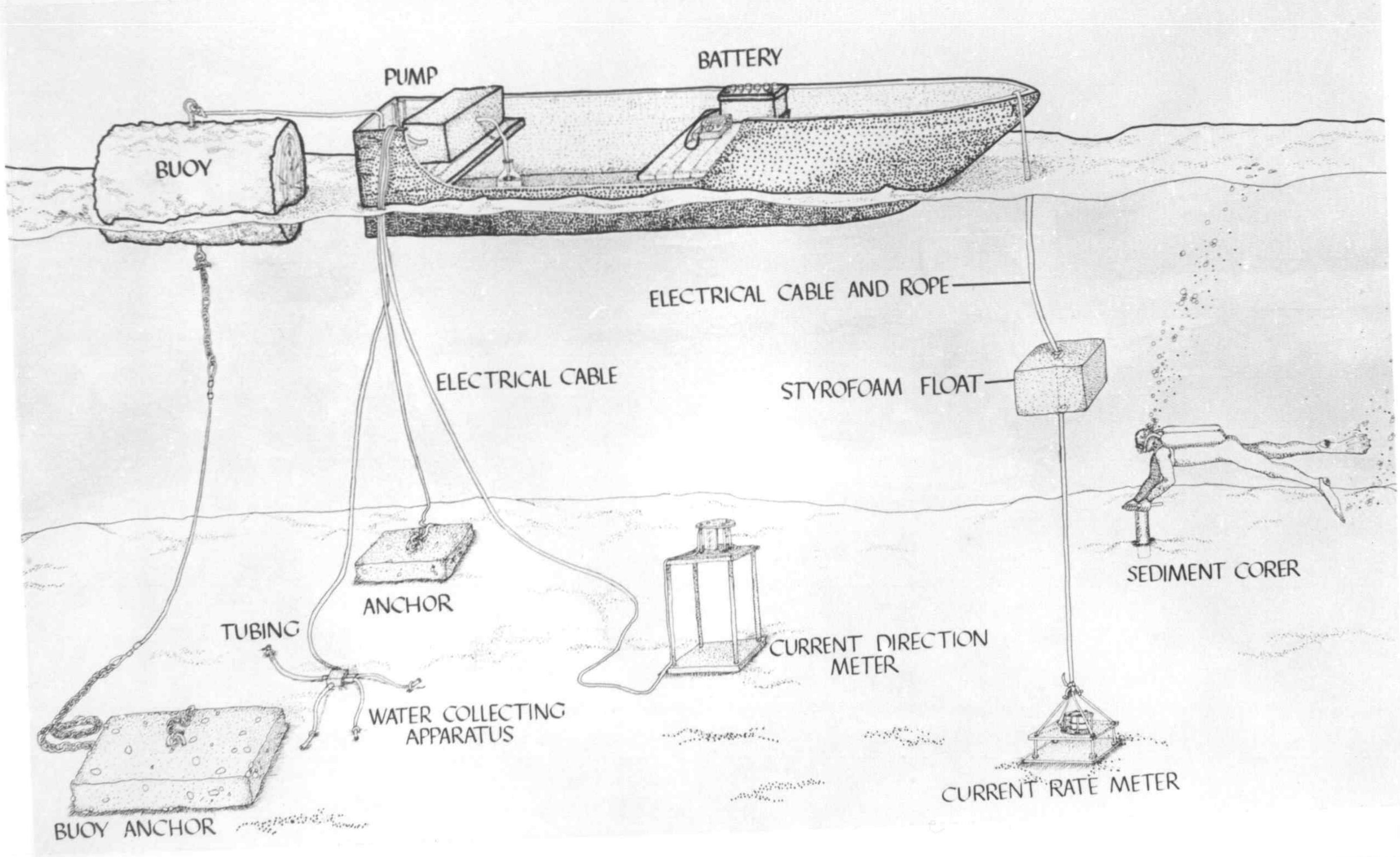


Figure 10. Physical arrangement of sampling equipment.

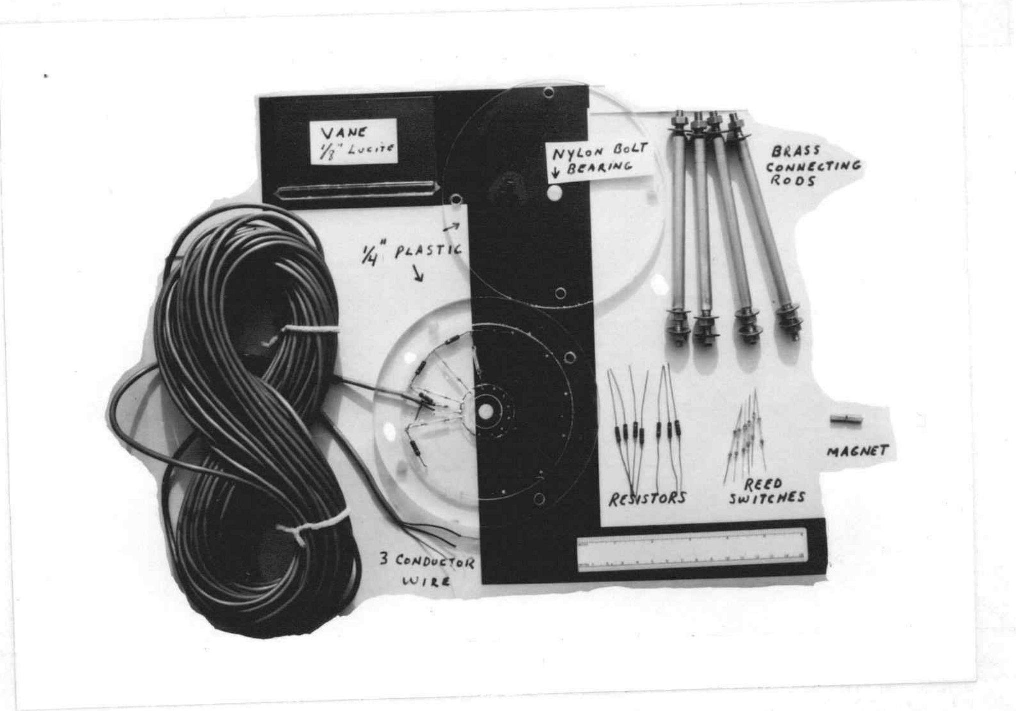


Figure 11. Current direction meter: component parts.

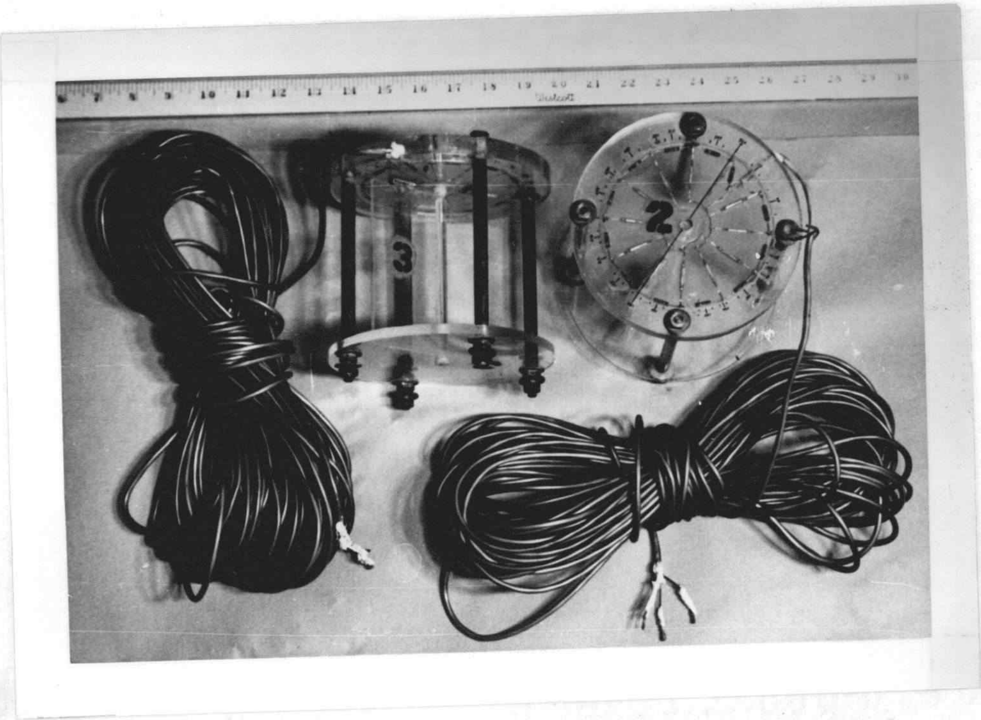


Figure 12. Current direction meters: assembled.

same time, plastic water sampling tubing was installed on the bottom (Figs. 10, 13).

3. Water sampling: during a 48-hour period beginning September 20, 1965, the four stations were visited in sequence every two to three hours, at which time water was collected and analyzed and also stored for later analysis. Simultaneously, the current direction was determined and the current rate was measured through the use of a special meter lowered to the bottom (Figs. 10, 14, 15).

4. Sediment sampling: one week later, through the use of SCUBA gear, interstitial water samples were collected at each station with specifically designed equipment (Fig. 16). A total of 70 large and several small sediment cores were taken, using hand-held corers (Figs. 10, 17). All were preserved with formaldehyde or frozen for analysis.

5. Laboratory study: water, sediment, and fauna were processed and analyzed in the laboratory, involving a significant portion of the research time.

6. Resampling the stations: exactly three years later (1968) the sampling stations were revisited. Observations were made on gross changes, and 15 large and several small sediment cores were secured for sediment analysis.

7. Analysis of data and preparation of report.

Figure 13. Current direction meter unit, battery, volt-amp meter, and water sampling tubing.

Figure 14. Current rate meter, battery, and volt-amp meter, ready for use.

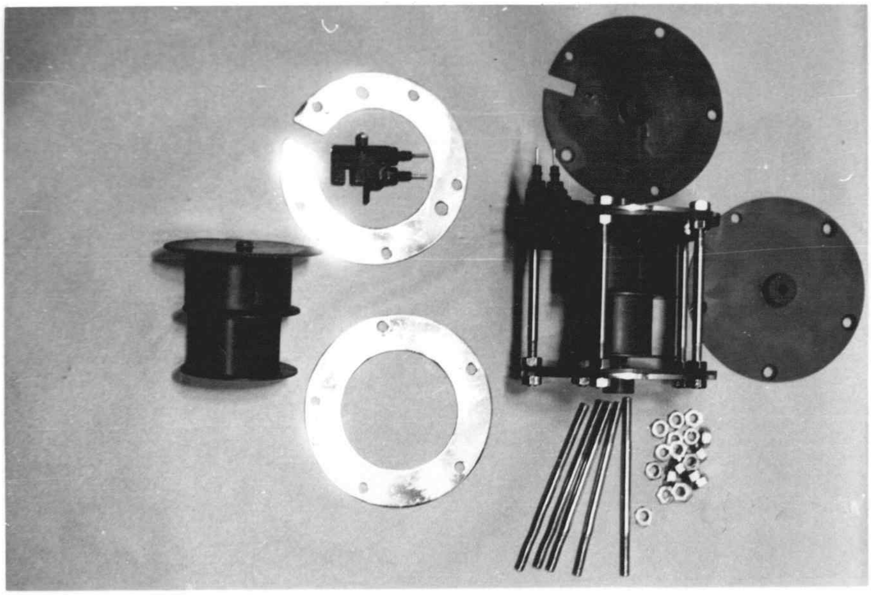


Figure 15. Current rate meter: component parts.

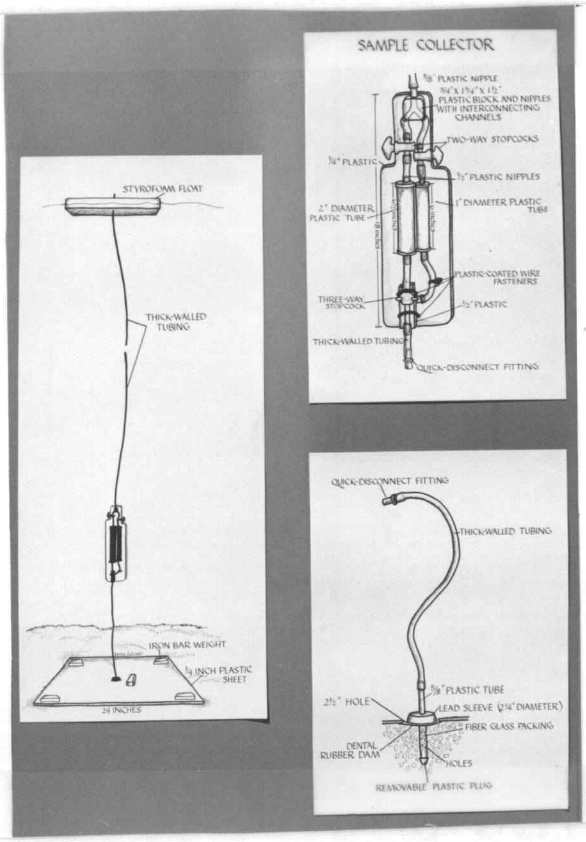


Figure 16. Interstitial water collecting device.

Figure 17. Sediment corer, bag of sediment, and plastic ties for bag.

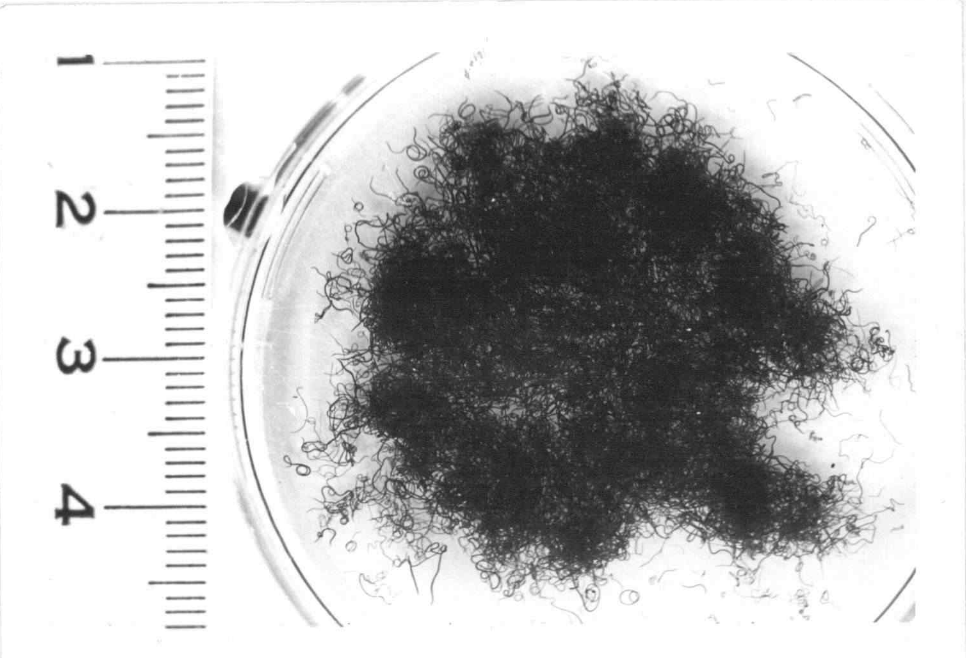


Figure 22. Nematode worms from one core, Between Station, 1965. Grid marks are one millimeter apart.

E. Methods of sampling

Sampling the sediment-water interface

Animals that live in the sediment but draw water from above depend upon the properties of the water that exist at the sediment-water interface. To analyze this water, equipment was designed, installed, and operated to collect water at each sampling station every two to three hours in sequence for a period of 48 hours. A description of construction and operation of the water sampler is provided in the Appendix. Because of fouling problems at the Sludge Bed, it was necessary to use a special tube to draw water. This was attached to the platform holding the current velocity meter and was raised and lowered with the meter.

The amount of water drawn when sampling each station and its uses are summarized in Table 2.

Sampling the interstitial water

Animals that live entirely within the sediment depend upon the properties of the interstitial water. To analyze this water a special apparatus was designed to remove water from between sediment particles. For a description of construction and operation, see the Appendix.

Portions of the collected water were used for oxygen, ammonia, hydrogen sulfide, dissolved organics, salinity, and pH determinations; temperature was taken by pushing a thermometer into the sediment.

Collecting sediment cores

To mark the site of each sediment sample to be taken, and to insure randomness in their collection, a number of small marking devices were thrown in a random manner from the boat while anchored to the buoys. Each marker consisted of a 3 x 2 x 1 inch piece of styrofoam, attached

Table 2. Collection, Analysis, and Measurements of Water from Sediment-Water Interface

A. Every two-three hours pump water for use as follows:

1. 300 ml B.O.D. bottles; add reagents → analyze for dissolved oxygen on shore.
2. 50 ml plastic bottle; add reagents; develop in dark box on boat → analyze for hydrogen sulfide on shore.
3. 120 ml plastic bottle; acidify, freeze with dry ice immediately on boat → analyze for ammonia later in laboratory.
4. 60 ml serum bottles → analyze for salinity in laboratory.
5. 4000 ml acid-washed glass bottles; acidify on boat → to laboratory.
    - a. In laboratory, filter, glass filter.
    - b. Filtrate:
        - 1'. 10 ml saved for dissolved organics analysis.
        - 2'. Rest, add KOH to co-precipitate dissolved metals with magnesium hydroxide floc; stand overnight; draw off supernatent (discard); acidify precipitate and read content with atomic absorption spectrophotometer for chromium, lead, copper.
    - c. Undissolved solids on glass filter:
        - 1'. Burn in muffle furnace.
        - 2'. Residue = particulate inorganics
        - 3'. Material lost = particulate organics
6. Temperature
7. Read pH with color comparator.

B. Measure current direction and current rate.

by two feet of nylon string to a three-inch iron bolt. The bolts settled slowly to the bottom, but to eliminate the possible effect of disturbing the animals when landing, each sample was taken one foot north of the bolt. Using SCUBA equipment, the sediments were taken with a hand-operated, six-inch (15.24 cm) diameter corer (Fig. 17) to eliminate the variability inherent in operating characteristics of samplers used from a boat. Sediment was taken to a depth of 9.25 inches (23.5 cm) for a volume of 261.6 cu inch (4286.7 cu cm). Cores were transferred to plastic bags underwater and preserved with formaldehyde in the boat.

Collected at the same time were smaller sediment cores, taken with 35 mm inside diameter plastic cylinders. Aboard the boat, the standing water was allowed to drain through the cores which were then frozen quickly with dry ice. In the laboratory, the top 5 cm was removed. Part was dried at 103° C for analysis of organic and nitrogen content; the rest was analyzed for the metals copper, chromium, and lead, and for sediment size profile.

Sedimentation jars

To estimate the net deposition of inorganic and organic material as a function of time, one-gallon jars were buried in the sediment almost to their mouths and left for 11 to 14 days. To prevent introduction of sediment while placing, or loss of sediment during recovery, the jars were capped with lids until these activities were over. For preservation, formaldehyde was added in the boat. In the laboratory, water was removed by siphoning and drying over a waterbath. The inorganic and organic portions were determined on the 103° C oven-dried sediment by ashing at 600° C.

Current measurements

To determine current direction, small meters were constructed, mounted on sturdy iron frames, and placed on the bottom. The meters worked on the principle of a weathervane and at all times reflected the direction of water current near the bottom. Because of fouling problems at the Sludge Bed, current direction was determined in a different manner. A small, weighted drogue, attached to a hand-held line, was lowered almost to the bottom. From the surface, the position the line pointed was noted with a compass. A description of construction and function of the meters is provided in the Appendix.

To determine current velocity a meter was lowered from the boat to the bottom for each measurement. A description of construction and function of the meter is provided in the Appendix.

F. Techniques of analysis: physical factors

Dissolved oxygen

Dissolved oxygen was determined by the Alsterberg modification of the Winkler method (American Public Health Association, 1962). Because of the presence of hydrogen sulfide in some samples, which produces low oxygen values, it was necessary to use duplicate water samples and treat these by the Ohle method (Ohle, 1952). The smaller interstitial water samples with their low dissolved oxygen required the use of proportionally smaller amounts of reagents and N/1000 potassium thiosulfate.

Hydrogen sulfide

Cline (1969) reported a sensitive, spectrophotometric procedure for the determination of hydrogen sulfide in water. A mixed reagent (N,N-dimethyl-p-phenylenediamine sulfate and ferric chloride in hydrochloric acid) when added to water in the presence of sulfide-sulphur, produces methylene blue. The color developed follows Beer's Law. Concentrations as great as 1500 ppb could be determined with a one-centimeter cell; with a 10-centimeter cell, values as low as five ppb above spectrophotometer background could be measured. The mixed reagent is added immediately upon collection of the sample and placed in the dark for 30 minutes to allow for full color development. The stable color formed can be measured in the laboratory days later.

Ammonia

Ammonia was determined by a modification of a procedure developed by Richards and Kletsch (1964). Their method does not distinguish between naturally occurring ammonia and ammonia derived during the analytical process from the decomposition of labile amines and urea. As a result, the possibility exists for inflated values in an area receiving sewage. For a discussion of the modification, its reasons, and other procedures developed for the handling of ammonia in water, see the Appendix.

Salinity

Water from the sediment-water interface and interstitial water were stored, respectively, in 60 ml and 12 ml serum bottles with tight-fitting rubber caps. In the laboratory, salinity was determined by the low precision method outlined by Strickland and Parsons (1960).

pH

Malfunctioning, portable Beckman pH meters forced the use of color comparator discs for the determination of pH in the field. The disc colors were checked against a laboratory pH meter and were found to provide accuracy within 0.1 pH units.

Temperature

Temperature was read to the nearest tenth degree with a centigrade thermometer. Interstitial water temperature was taken by pushing the thermometer into the sediment; sediment-water interface water was measured as the water was pumped into containers aboard the boat.

Particulate materials in water

Water from the sediment-water interface was pumped into four-liter, acid-washed, glass bottles; acidified with redistilled nitric acid; and stored for analysis. Acidification was used to minimize adsorption of metals to the bottle wall and to inhibit bacterial decomposition of organic materials present.

In the laboratory, samples were filtered through gooch crucibles containing 0.3 micron mesh glass filters that had been acid-washed and oven dried at 103° C. After reaching a constant weight at 103° C, the filter unit and particulates were ignited at 600° C for two hours. The weight loss was defined as particulate organic, that remaining as particulate inorganic. The filtrate was saved for metals analysis.

Dissolved organic material in water

A small portion of sample previously acidified was passed through a millipore filter to remove particulate matter. The filtrate was sparged with nitrogen gas to remove any residual carbon dioxide, and injected into a Beckman I.R. 315 Carbonaceous Analyzer, where at a temperature of 850° C all organic matter was converted to and measured as carbon dioxide. A standard curve was made from sodium oxalate standards.

Organic content of the sediment

Problems in defining the organic content of sediments are legion. It became necessary to measure the organic content by two methods, each of which has faults. The implication of organic content and problems of analysis are discussed elsewhere. At the beginning of the study, organic content was determined by loss of weight of oven dried (103° C) sediment upon combustion at 600° C. Later, duplicate samples were measured with a LECO automatic, 70-second carbon analyzer. In this procedure, at 1800° C, all organic carbon is converted and measured as carbon dioxide.

Nitrogen content of sediments

The nitrogen content of two-gram samples of sediment was determined by a semimicro-Kjeldahl method (Bremner, 1965) by the Agricultural Experiment Station, Oregon State University.

Copper, chromium, and lead in seawater

The four liters of filtrate containing the metals was treated as outlined by Joyner et al (1967); this allowed the metals in the water to be concentrated from 20 to 60 times. Briefly, the procedure involves the addition of potassium hydroxide to produce a natural magnesium hydroxide floc. The sample was agitated for about an hour and allowed to stand overnight. The supernatent was drawn off and discarded; the magnesium hydroxide with its co-precipitated metals was redissolved with acid and analyzed with an atomic absorption spectrophotometer.

Copper, chromium, and lead in sediment

For reasons to be discussed later, a double extraction procedure was performed on each sediment sample to obtain the metals copper, chromium, and lead. Ten grams dry weight of sediment was treated for 24 hours with 25 ml of 0.1 N redistilled hydrochloric acid. The sediment was filtered, washed several times with the acid, and the filtrate taken to 50 ml volume. The second extraction on the same sample was like the first except the extracting agent was 5% E.D.T.A. neutralized with sodium hydroxide. Metals content for each extraction was determined with an atomic absorption spectrophotometer.

Sediment size profile

The sediment particle profile was determined by a standard technique used in the Departments of Oceanography at Oregon State University and University of Washington. The method involves determination of the weights of the various size fractions by passing the sediment through a geometrical series of U.S. Standard Sieves starting with 1000 microns (1000, 500, 250, 125, 62 microns). Sediment passing the 62 micron sieve is further separated into sizes of 31.5, 16, 8, 4, 2, and less than 2 microns by a procedure that relies on settling velocity of particles in distilled water as a function of size (Stokes Law). Weights recorded were for inorganics only, the organics being destroyed. In part of the samples, the organics were destroyed by incineration at 600° C after fractionation, and in the rest by a wet oxidation pretreatment with 30 percent hydrogen peroxide.

G. Techniques of analysis: organisms

Extraction of animals from sediment

Large sediment cores containing the animals were preserved in the field with formaldehyde. In the laboratory, the sediment was first screened through a two-millimeter sieve to remove large material and then through a 0.42 mm sieve to eliminate the smallest unwanted inorganic and organic fractions. Material remaining on the smaller sieve was placed in formaldehyde strongly colored with rose bengal for 24 hours to stain the animals red, at which time it was placed in a special apparatus designed to separate inorganic and organic fractions (Fig. 40). Finally, the animals were removed from the organic fraction with dissecting scope and forceps and separated into taxonomic groups.

Biomass and number of animals

With some notable exceptions, the number of animals in a sample was determined by direct count; and the wet, dry, and ash-free dry weights by direct weighing. Prior to weighing the preserved animals, they were placed in seawater for 24 hours to, as much as possible, reestablish their previous saltwater composition. They were then gently blotted to remove surface water, and weighed on a Mettler balance. Larger bivalves were removed from their shells to prevent high wet weights due to entrapment of water; tiny bivalves were simply decalcified with a few drops of hydrochloric acid placed in their containers.

Following the suggestions of Lovegrove (1966), animals were dried to constant weight in an oven at 62 - 64^{o} C (usually overnight). Ash-free dry weight was determined as loss of weight following ignition at 600^{o} C for three hours. In some cases, ash-free dry weights were obtained indirectly from wet weight after working out a wet weight to ash weight ratio from a portion of a sample or from a composite of several to many samples. This was done for any of three reasons: 1) Some organisms were so small as to be individually unweighable in the dry or ash condition. 2) Some organisms had not been fully identified so that sacrifice of only a portion of the specimens was advisable; wet weighting does not damage them. 3) Possible new or otherwise rare species were saved for their descriptive and taxonomic value.

Because of the very large number of nematode worms present in some samples, it was considered to be too time-consuming to count the number of individuals. Instead, one representative sample from each of the four different stations was individually counted and weighed in the wet, dry, and ash condition. A value of wet weight to number of individuals was established for each station and applied to the other similar samples to arrive at numbers of individuals. Spot checks of several of the smaller samples showed the method to be remarkably accurate.

To provide more realistic biomass and number values on the basis of a square meter, the single species values obtained for the cores at one sampling station were added together and then converted to the larger unit. This was done to prevent inflated values for rarer species.

Selection of minimal screen size

In most sediment samples, organisms span a wide range of sizes. It is typical in sediment studies to retain only those animals larger than one millimeter. Yet Reish (1959a) has shown that for his mud sediment samples, a mesh much smaller than 1 mm must be employed to obtain at least 95 percent of the number of species present and to retain 95 percent of their numbers. Large screens will account for 95 percent of the total biomass which is due mainly to the larger species. However, in evaluating the effects of organic enrichment (or other forms of pollution), I believe the animals smaller than 1 mm must be considered. Trial screening with various size sieves showed that in our study area a sieve of 0.42 mm would retain all but a small number of tiny nematodes, copepods, and interstitial coelenterates. Therefore, material smaller than 0.42 mm was discarded, animals larger than this were removed, sorted, and identified for analysis.

IV. RESULTS

A. Physical Factors

Oxygen

The lowest and highest values for dissolved oxygen found in the water at the sediment-water interface, during the period of sampling in 1965, was 4.02 and 6.72 milligrams per liter (Table 3). Neither of these, or any other amount found, had any relationship to the station at which they were collected. In fact, the lowest value was collected at the Between Station, and the highest at the Sludge Bed.

It was not possible to detect any dissolved oxygen in the interstitial water of the Between, Organic, and Sludge Bed Stations. The amount found at the Clean Station was small, only 0.1 mg/liter.

Hydrogen sulfide

Hydrogen sulfide was not present in the sediments of the Clean or Between Stations but was present at the Organic Station, and was very abundant at the Sludge Bed (Table 4). The water at the sediment interface of these last two stations only showed small amounts of H_2S (Table 3).

The pH affects the amount of measured sulfide that is in the form of toxic H_2S. On this point see Interpretation of Results.

Ammonia

The actual amount (see Interpretation of Results) of ammonia present in the interstitial water was very small, not exceeding just under 0.5 parts per billion for the Organic Station and to just over 0.05 ppb at the Between Station. The Sludge Bed had about one-half

TABLE 3. CHARACTERISTICS OF WATER TAKEN AT THE SEDIMENT-WATER INTERFACE, SEPTEMBER 20-21, 1965

Parameter	Clean Station		Between Station		Organic Station		Sludge Bed	
	Average	Range	Average	Range	Average	Range	Average	Range
Salinity (ppt)	29.58	29.36-29.66	29.62	29.45-29.70	29.60	29.45- 29.71	29.62	29.45- 29.71
Actual Hydrogen sulfide (ppm)	.000	-	.000	-	.002	0 - .007	.034	.004- .151
Dissolved oxygen (ppm)	5.71	5.15- 6.19	5.56	4.02- 6.12	5.58	4.93- 6.26	6.16	5.23- 6.72
Temperature (degrees centigrade)	12.95	12.3 -14.0	12.84	12.3 -13.4	12.87	12.5 - 13.8	12.83	12.3 - 13.4
pH	The pH of all samples during the sampling period was 7.7							
Current velocity (ft/sec.)	.123	.30*	.130	.32*	.163	.34*	.298	.605*
Particulate organic material (ppm)	2.18	1.27- 4.74	1.63	.95- 2.75	1.60	.92- 3.11	8.68**	1.19- 76.41
Particulate inorganic material (ppm)	4.85	1.12- 8.42	.86	.23- 2.37	1.18	.39- 2.26	14.41***	.26-159.63
Dissolved organic material (ppm)	1.38	.7 - 3.0	1.7	.4 - 4.2	1.7	.7 - 3.3	1.3	.4 - 2.2
Copper (ppb)	21.5	3.4 -74.0	19.1 ⌿	3.4 -90.3	40.9 ⌿	3.6 -360.3	30.3 ⌿	5.0 -209.9
Lead (ppb)	16.2	13.1 -21.1	16.3	13.2 -21.7	16.0	12.4 - 25.2	26.4 ≠	14.2 -127.4
Chromium (ppb)	29.8	11.3 -52.1	28.2	6.8 -47.7	30.5	13.8 - 60.8	31.4	4.6 - 71.2

* Maximum velocity recorded.

** Average of Sludge Bed would be 3.03 without high value of 76.41.

*** Sludge Bed average would be 1.20 without high value of 159.63.

⌿ Between Station average would be 13.6 without high value of 90.3. Organic Station average would be 18.1 without high value of 360.3. Sludge Bed average would be 16.4 without high value of 209.9.

≠ Sludge Bed average would be 18.7 without high value of 127.4.

TABLE 4. CHARACTERISTICS OF SEDIMENT AND INTERSTITIAL WATER

Parameter	Clean Station		Between Station		Organic Station		Sludge Bed	
	1965	1968	1965	1968	1965	1968	1965	1968
Depth of Water (feet below 0.0 ft.)	9.7	9.7	10.7	10.7	8.8	8.8	11.8	11.8
Salinity (ppt)	29.69		29.79		29.80		27.98	
Temperature (degrees centigrade)	11.7		11.6		11.7		11.7	
pH	6.9		6.9		6.8		5.9-6.1	
Dissolved oxygen (ppm)	0.1		0.0		0.0		0.0	
Actual Hydrogen sulfide (ppm)	0.0		0.0		21.2		423.2	
Actual Ammonia (ppb)	0.0		0.054		0.446		0.242	
Dissolved organic material (ppm)	2.3		4.7		6.8		8.5	
Organic material deposited (gm/m^2/day)	3.7		11.7		36.7		*	
Inorganic material deposited (gm/m^2/day)	75.28		81.06		91.18		*	
Total Nitrogen (%)	0.059	0.028	0.048	0.020	0.078	0.025	0.160	0.050
Organic Carbon (%)	0.50	0.23	0.92	0.45	1.11	0.44	3.28	1.36
Weight loss on ignition - volatile material (%)**	1.31	1.35	2.46	1.38	4.35	1.74	8.37	3.25
Copper (ppm; micrograms/gram sediment) 0.1 N HCl extraction EDTA Extraction Total	 1.60 1.60 3.20	 3.25 0.60 3.85	 3.55 2.75 6.30	 3.10 0.70 3.80	 3.00 8.40 11.40	 6.90 0.80 7.70	 2.10 6.55 8.65	 14.95 15.00 29.95
Lead (ppm; micrograms/gram sediment) 0.1 N HCl extraction EDTA Extraction Total	 8.10 3.65 11.75	 0.0 0.55 0.55	 11.80 7.45 19.25	 2.30 2.75 5.05	 13.05 25.85 38.90	 6.80 7.65ƚ 14.45	 46.15 159.50 205.65	 7.95 8.25ƚ 16.20
Chromium≠								

* Very heavy, too much material like toilet paper that clogged mouth of collecting jar.

** Upper 5 cm sediment

ƚ Readings are essentially the same - due to machine drift.

≠ Just barely detectable - all stations about the same.

that of the Organic Station, and none was measured at the Clean Station.

Unfortunately, the analysis results of the interface water for ammonia were lost.

Water temperature

The temperature of the water at the sediment-water interface, flowing past all stations during the period of sampling, averaged about 12.8 degrees centigrade. From Table 3 and Figure 18, it can be seen that the widest fluctuation experienced at a station, 1.7 degrees (12.3 to 14), was more than the differences seen between sampling stations. The highest temperatures occurred during afternoon hours when insolation was at its greatest; lowest temperatures occurred during evening and early morning hours. The temperatures at the four sampling stations closely paralleled each other, although the Organic and Clean Stations experienced temperatures a few tenths of a degree higher, perhaps reflecting that they were closer to shore and a foot or two more shallow.

The temperatures of interstitial water were essentially the same at all stations, but were a few tenths of a degree lower than the circulating water.

pH

The pH at the sediment-water interface at all stations was a constant 7.7, for the period of sampling.

Interstitial water pH was below neutral. At the Sludge Bed it ranged between 5.9 and 6.1, at the Organic Station 6.8, and at the Clean and Between Stations 6.9.

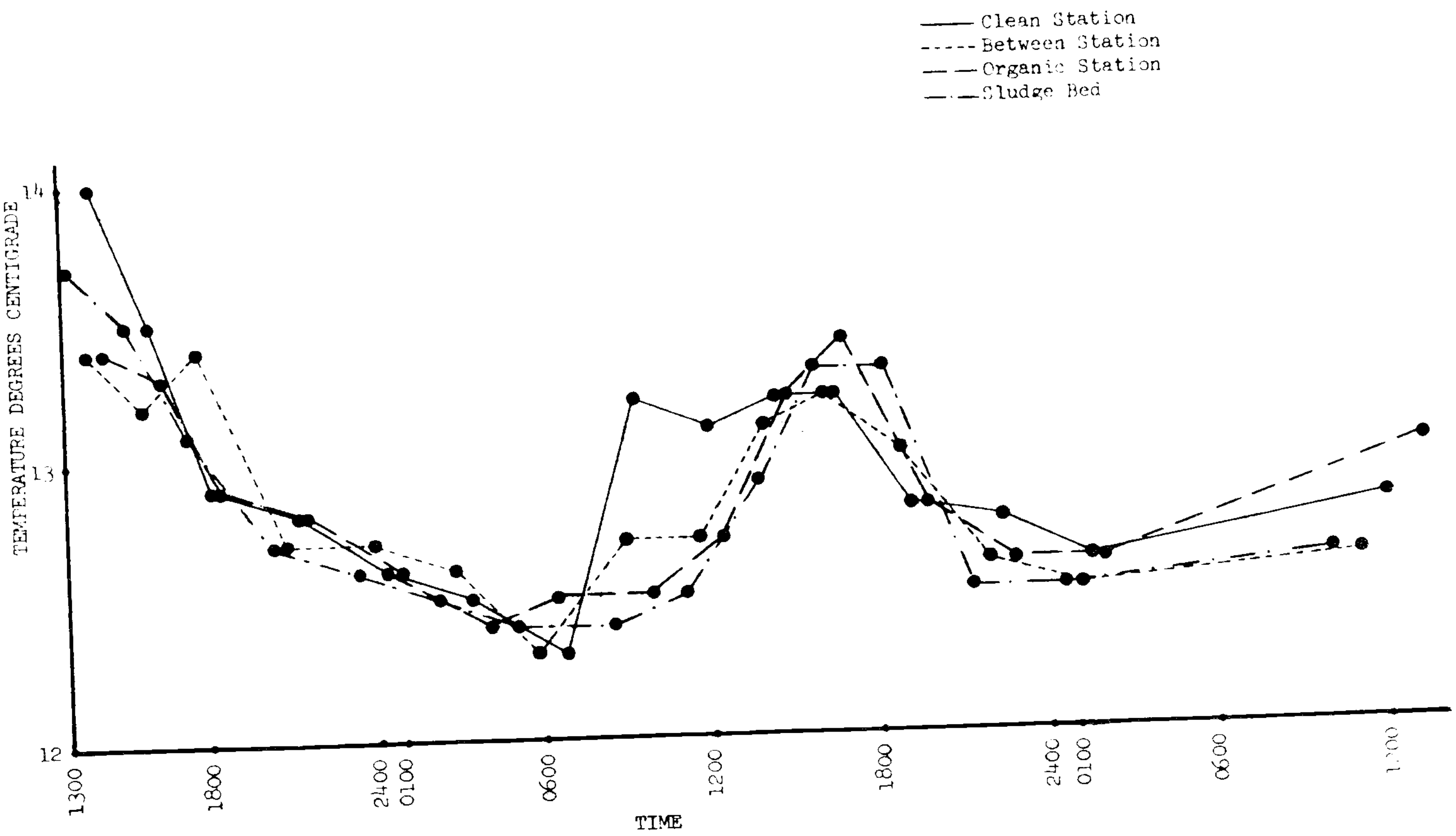


Figure 18. Temperatures at the sediment-water interface, September 20-21, 1965.

Salinity

The measurements of this variable were quite constant at the four sampling stations for the water at the sediment interface. Overall they averaged 29.60 parts per thousand, with the lowest measured at 29.45 and the highest at 29.71.

Salinity in the interstitial water was constant for the Clean, Between, and Organic Stations, and very close in value to that of the water immediately above the sediment surface. However, interstitial salinity at the Sludge Bed was almost two parts per thousand less than the others.

Inorganic materials

The average and range of amounts of particulate inorganic materials collected in the water at the sediment-water interface in 1965 are shown in Table 3. The Sludge Bed had the highest average and the highest value measured. The high value of about 160 ppm was collected only one time during the sampling. Without this value, the average of the Sludge Bed would have been only 1.20 ppm instead of 14.41. The Between and Organic Stations were similar to each other in average and high and low values. But the Clean Station was higher than the last two stations in all values.

The amount of material deposited in 1965, as measured by sediment collectors while the sewer was operational, increased from the Clean to Between to Organic Station. The Sludge Bed, which could not be measured because of fouling problems with the collector, was presumably much higher than the others; visual observations substantiate this assumption. A comparison of the amount of inorganics deposited in the collectors immediately before and after sewer shutdown will be made later.

Sediment particle size

The particle size distribution of the four stations is shown in Table 5 and Figure 19. In 1965 the Organic Station contained the largest amounts of smaller silt-clay fractions, but by 1968 it was similar to the Clean and Between Stations which had changed little between the two years. The amount of sand, silt, and clay fractions at the Sludge Bed was about the same for both years, but in 1968 the sand fraction was of a larger size. Of the four stations, the Sludge Bed contained the largest particle sizes in both years.

Organic materials

Because of the importance and complexity of the results on the organic materials, the information of this section has been presented as one unit under Interpretation of Results.

Metals

1. Copper: The values obtained at the sediment-water interface during the period of sampling were extremely variable and fluctuated greatly at each station. The low values at the four stations were constant, but the high values were not (Table 3; Fig. 24).

In like manner, considerable variability was experienced for copper in the sediment not only between stations for one year, but between years at one station (Table 4). There appears to be no relationship between the results of the hydrochloric acid and EDTA extraction procedures.

TABLE 5. SEDIMENT PARTICLE SIZE ANALYSIS

Parameter	Clean Station		Between Station		Organic Station		Sludge Bed	
	1965	1968	1965	1968	1965	1968	1965	1968
PERCENT WEIGHT OF SEDIMENT								
Very coarse sand (more than 1000 microns)	.11	.20	.16	.00	.20	.29	.76	14.27
Coarse sand (1000-500 microns)	.42	.53	.56	.22	1.12	.63	2.07	29.70
Medium sand (500-250 microns)	13.02	11.00	7.74	12.26	8.70	5.25	43.53	45.70
Fine sand (250-125 microns)	54.81	49.18	60.88	53.19	19.88	65.09	46.14	7.02
Very fine sand (125-62.5 microns)	24.29	28.90	23.41	30.46	26.19	18.55	5.19	.75
Coarse silt (62.5-31.2 microns)	5.42	7.03	2.80	2.20	22.55	5.89	1.06	.58
(31.2-15.6 microns)	.88	1.39	2.14	.47	7.56	1.70	.21	.53
Fine silt (15.6-7.8 microns)	.18	.24	.66	.23	3.99	.77	.21	.40
(7.8-3.9 microns)	.35	.56	.49	.05	3.39	.44	.42	.36
Clay (3.9-1.95 microns)	.35	.06	.49	.11	1.60	.02	.21	.28
(Less 1.95 microns)	.18	.90	.66	.81	4.79	1.35	.21	.42
CUMULATIVE PERCENT WEIGHT OF SEDIMENT								
Percent sand	92.65	89.81	92.75	96.13	56.09	89.81	97.68	97.44
Percent silt	6.83	9.22	6.09	2.95	37.51	8.80	1.90	1.87
Percent clay	.53	.96	1.15	.92	6.39	1.37	.42	.70
OTHER								
Median Particle Size (microns)	148	140	138	142	72	158	245	470
Weighted, mean particle size (microns)	182	171	174	177	119	171	282	623
Mode of particle size (microns)	54.81% wt. between 125-250	49.18% wt. between 125-250	60.88% wt. between 125-250	53.19% wt. between 125-250	26.19% wt. between 62.5-125	65.09% wt. between 125-250	46.14% wt. between 125-250	45.70% wt. between 250-500
95% of particles (by weight) between (in microns)	37-400	29-420	19-370	50-420	1.3-410	13-330	80-500	62.5-more than 1000
68% of particles (by weight) between (in microns)	92-236	78-222	98-195	103-228	20-197	84-179	170-360	308-930

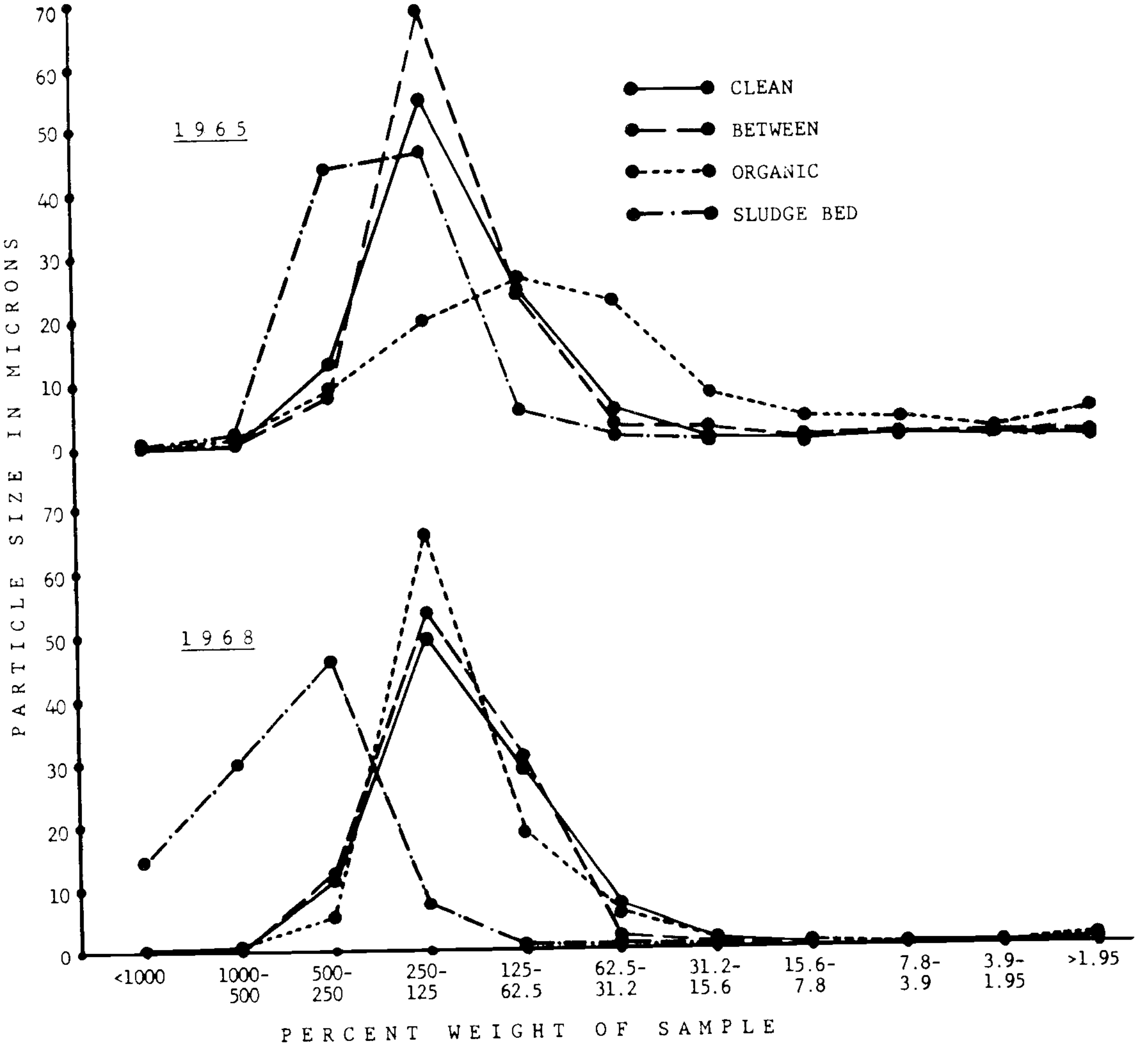


Figure 19. Inorganic sediment profile, 1965 and 1968.

2. Lead: Results for lead were not quite as erratic as those for copper in the interface water. The low values for all stations were similar, as were the high values measured at the Clean, Between, and Organic Stations (Fig. 26). The Sludge Bed was somewhat higher, but this was due to one high value.

The sediment lead paralleled the degree of pollution in 1965:

Clean Station < Between Station < Organic Station < Sludge Bed

In 1968 the same parallel held, but the lead content was significantly lower than during the earlier year (Table 3).

3. Chromium: Although the average values for the interface water were similar for the four stations, their low and high values varied somewhat (Table 3; Fig. 25). Evidently the extraction procedures used for chromium in the sediment were not effective or chromium was not present (see Interpretation of Results). Quantities measured were so small as to be barely detectable.

Current direction and velocity

The average and maximum current velocities experienced at each of the four sampling stations are shown in Table 3. The Clean, Between, and Organic Stations are very similar for these values, while at the Sludge Bed the average is about one and one-half and the maximum about two times as great as the others.

The Current Vector Diagrams (Fig. 20) show the actual direction and relative velocity of water flow near the sediment surface during the period of sampling. The length of the lines indicates relative velocity; multiple arrows on the same line show reoccurring direction of flow. From the diagrams it is clear that the current past the

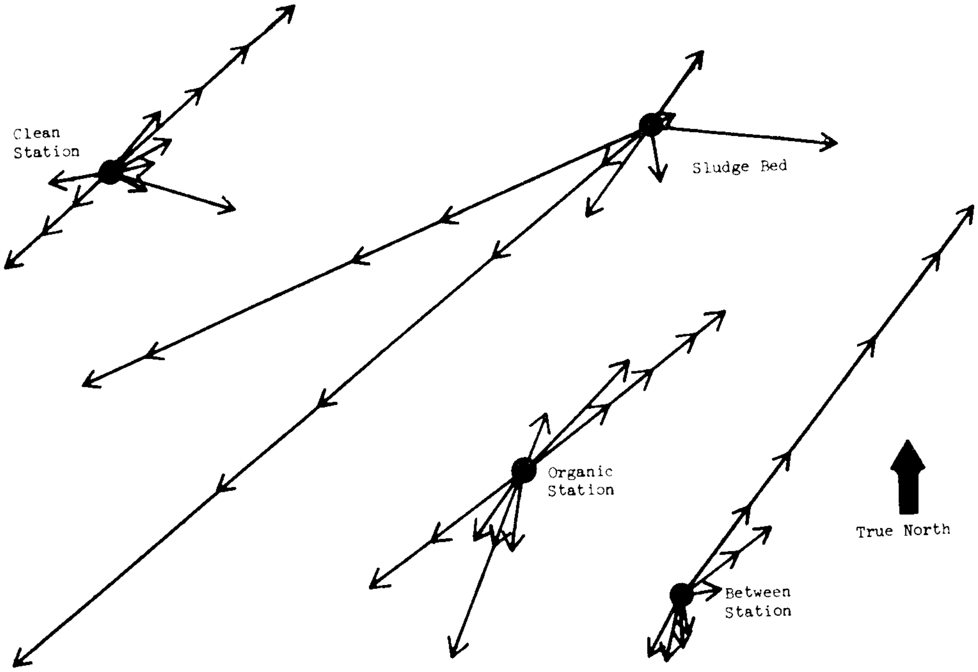


Figure 20. Current direction and relative velocity, Sept. 20-21, 1965.

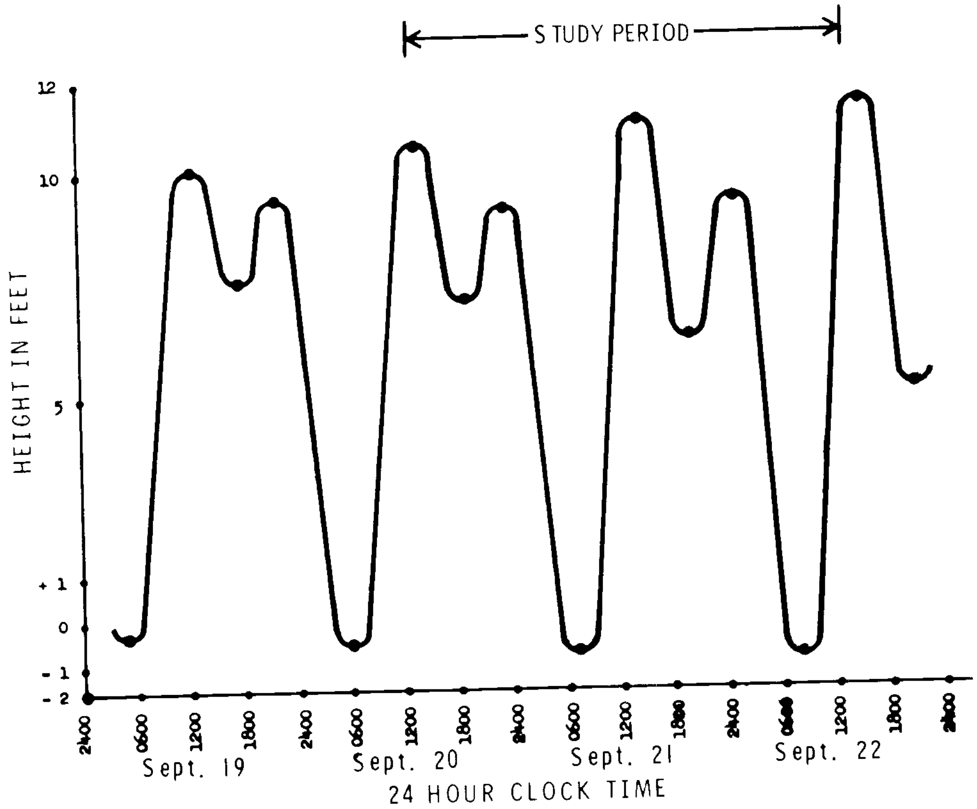


Figure 21. Tidal fluctuations, September 19-21, 1965.

Sludge Bed was strong and mostly in a south-westerly direction. The Between Station experienced an unexpected predominance of north-easterly currents. At the Organic Station the net water movement was somewhat cancelled out by moving back and forth in a NE to SW direction. The water moved similarly at the Clean Station but a greater number of times it moved north-east and on several occasions it moved to the east and west.

Slack tides or currents too slow to be measured are not indicated on the vector diagrams. During sampling these were not detected at the Sludge Bed, but very infrequently at times before and after the sampling, they had been observed while diving. Slack tides or immeasurable currents were experienced 20 percent of the sampling time at the Clean and Between Stations and 13 percent at the Organic Station.

Tides influence current directions and velocity. The overall direction of movement of a mass of water is partly a function of whether the tide is at ebb, flood, or some stage between. The velocity of water movement is to some degree a function of the tidal magnitude, the greater the difference between consecutive high and low tides, the greater the velocity. Tidal fluctuations experienced during the period of water sampling are shown in Figure 21.

B. Biological

1. For the more abundant species, several expressions of population distribution and abundance have been calculated (Table 10). These include population dispersion ratios (variance/mean), standard error of the mean of number of animals per sample, and the range of number of animals per sample at a 90% confidence interval. The values are

reasonably valid only when a larger number of samples are used in the calculations. In some cases the values were calculated where the number of samples was small, but the number of animals per sample was relatively high. This was done for comparative purposes only. For a consideration of the significance of these values, see Interpretation of Results.

It is worth noting that only one species, the isopod *Munnogonium waldronense*, seemed to have a uniform distributional pattern in 1968, but this may be an error due to the small number of samples taken. This species in 1965, with more samples collected, showed a clumped distribution. There were eight species whose ratio values ran from just below one to below two, this suggests random distribution with some clumping. Some species had values so high as to leave no doubt they were strongly clumped in distribution.

2. Biomass values of the animals are presented as ash-free dry weight (Tables 6, 7, 8, 9, 33). The values of the ratio of *dry weight* to *wet weight* ranged between 14.42 and 28.09 percent, with most centering around 22 percent (Table 11). The lowest value belonged to the seapen *Leioptilus guerneyi*, while the higher values belonged to those animals with inorganic tubes or calcium-impregnated skeletons, or which may have been non-selective sediment feeders.

The values of the ratio of *ash-free dry weight* to *dry weight* ran as high as almost 88% for nematode worms and the organic-shelled snail *Alvinia*, while the lowest values were for small clams that were weighed with their shells. For further information see Interpretation of Results.

TABLE 6. DISTRIBUTION AND ABUNDANCE OF HARPACTICOID COPEPODS

		Clean Station			Between Station			Organic Station			Sludge Bed		
Species		% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)
Acrenhydrosoma karlingi	1965 1968	4.5 25	3 115	.01 .45	73.9	234	.93	5 33.3	3 726	.01 2.87			
Amonardia pertubata	1965 1968							66.7	115	.45	25	86	.34
Bulbamphiascus imus	1965 1968	100 25	1827 14	7.22 .06	100 25	2703 29	10.68 .11	100 66.7	29871 631	123.65 2.49	100 100	17219 488	68.02 1.93
Dactylopodia vulgaris	1965 1968	9.1 25	8 29	.03 .11				5	115	.45	25	14	.06
Diagoniceps N. Sp.	1965 1968				4.2	5	.02						
Heterolaophonte discophora	1965 1968	9.1	5	.02				33.3	19	.08			
Huntemannia jadensis	1965 1968							25 100	37 402	.15 1.59	25	14	.06
Intermediate between *Parathalestris bulbiseta* and *P. californica*	1965 1968				4.2	29	.12						
Tisbe furcata	1965 1968	36.4	60	.24	50	60	.24	50 66.7	259 57	1.02 .23	100 25	10194 14	40.27 .06
Tisbe gracilis	1965 1968	13.6	10	.04	4.2	2	.01	30 33.3	32 19	.12 .08			
Typhlamphiascus pectinifer	1965 1968	45.5 75	44 72	.18 .28	100 75	1460 57	5.78 .23	45 33.3	72 57	.28 .23			

TABLE 7. DISTRIBUTION AND ABUNDANCE OF CRUSTACEA

Species		Clean Station			Between Station			Organic Station			Sludge Bed		
		% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)
AMPHIPODA													
Aoroides columbiae	1965				4.2	2.4	.004				50	43	6.31
	1968	100	86	.51	25	28.7	1.92						
Corophium sp.	1965							33.3	19.1	.216	25	14.3	.431
	1968												
Hyale sp.	1965				4.2	2.4	.20						
	1968												
Orchomene sp.	1965										25	14.3	1.35
	1968												
Parapleustes pugettensis	1965				4.2	2.4	1.26						
	1968												
Photis californica (?)	1965				50	573.5	12.48	66.7	898.5	25.91	100	1281.7	59.98
	1968												
Photis conchicola (?)	1965				25	157.7	5.09						
	1968												
Photis sp.	1965				25	16.7	.036	10	5.7	.016			
	1968				25	200.7	2.82	33.3	38.2	.505			
Synchelidium shoemakeri	1965				25	19.1	.99	5	22.9	.13	50	28.6	.59
	1968	75	129	2.84				33.3	95.6	1.84			
Westwoodilla caecula	1965				20.8	11.9	1.13				25	14.3	1.08
	1968												
CHELIFERA (Tanaidacea)													
Leptochelia dubia (?)	1965				25	14.3	.64				50	71.7	3.20
	1968				50	43	1.92						
CUMACEA													
Cumella vulgaris	1965				70.8	69	.81	66.7	57	.76	25	29	.29
	1968												
Diastylopsis tenuis (?)	1965				4.2	2.4	.04						
	1968												
Lamprops quadriplicata	1965				4.2	2.4	.05				25	14.3	.29
	1968	50	28.6	.57									
ISOPODA													
Munnogonium waldronense	1965				95.8	712	8.53	66.7	153	1.72	50	57.4	.72
	1968	25	28.6	.28	100	330	3.87						
OSTRACODA													
Euphilomedes carcharodonta	1965	4.5	2.6	.49	79.2	301	48.79	15	17.2	3.25	25	14.3	2.71
	1968	100	143.3	13.91	100	3713.4	295.34	100	1262	73.58	100	100.4	16.36

TABLE 8. DISTRIBUTION AND ABUNDANCE OF MOLLUSCA

Species		Clean Station			Between Station			Organic Station			Sludge Bed		
		% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)
GASTROPODA													
Alvinia compacta	1965 1968	25	14.3	1.72	70.8	115	13.79	66.7	153	18.35	50	57.4	6.88
PELECYPODA													
Clinocardium nuttallii	1965 1968				8.3	4.8	.65	100	152.9	20.07	75	100.4	16.63
Lucina (*Lucinoma*) *annulata*	1965 1968				16.7	9.6	5823.91						
Luciniaca tenuisculpta	1965 1968				8.3 75	4.8 143.3	12.86 81.15				25	14.3	4.44
Macoma carlottensis	1965 1968	77.3 100	117.3 129	58.52 4.16	100 100	1082.5 315.4	147.15 49.32	65 66.7	134.8 86	44.07 9.37	50	43.0	3.58
Macoma nasuta	1965 1968	68.2 100	83 71.7	9547.93 20259.88	79.2 50	96 43	17115.27 19217.99	10	8.6	1488.81			
Mysella tumida	1965 1968	18.2 100	18.2 301.1	.96 6.88	100 100	645.2 759.9	52.86 25.95	10 66.6	5.7 325	.26 6.12	100	817.2	25.38
Protothaca staminia	1965 1968										50	28.7	7.74
Psephidia lordi	1965 1968	50	71.7	2.01	50 100	76.5 8287.1	2.44 200.01	100	325	25.43	25 100	28.7 1347.7	1.15 105.1
Tellina modesta	1965 1968	4.5 100	2.6 387.1	16.66 495.79	100 100	1756.4 1777.9	437.85 320.59	5 100	2.9 1911.7	.66 190.98	75	100.4	8.60

TABLE 9. DISTRIBUTION AND ABUNDANCE OF POLYCHAETA

Species		Clean Station			Between Station			Organic Station			Sludge Bed		
		% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)
Paleanotis bellis	1965 1968				4.2	2*	.05	5	3*	.06*			
Dorvillea rudolphi	1965 1968	54.5	52	.63	66.7	62	.79	15	34	1.52	25	29	.29
Glycera capitata	1965 1968	18.2 25	10 14*	88.87 66.24*	100 100	662 172	145.45 195.85						
Glycera tenuis	1965 1968										75	86	76.71
Glycinda polygnatha	1965 1968	75	43	1.00	20.8 50	19 72	5.50 17.64	66.6	115	37.09	50	29	3.01
Goniada acicula	1965 1968				8.3	7	7.65				50	29	15.20
Gyptis brevipalpa	1965 1968	27.3	21	.34	100	2753	33.98	20 66.6	17 38	.23 .76	75	358	4.01
Lumbrineris latreilli	1965 1968	25	14*	10.32*				33.3	38	13.57	25	14*	2.58*
Aglaophamus erectans	1965 1968	25	14*	1.43*									
Nephtys caeca	1965 1968	25	14*	153.55*	12.5	10	64.52	33.3	38	82.20			
Nephtys ferruginea	1965 1968	25	14*	.29*	100 50	160 57	51.33 80.58				25	14*	115.13*
Platynereis bicanaliculata	1965 1968	27.3 25	26 14*	3.23 1.15*	87.5	208	68.82	65	292	23.17	75	129	17.21
Nereid species A	1965 1968	4.5	3*	3.68*	33.3 75	24 43	23.99 113.27	10 33.3	9 19*	1.20 2.10*	25	57	24.23
Nereid species B	1965 1968	9.1	8	4.48	25	24	37.18	5	3*	1.00*			

*One specimen only was collected

TABLE 9. DISTRIBUTION AND ABUNDANCE OF POLYCHAETA CONTINUED

Species		Clean Station			Between Station			Organic Station			Sludge Bed		
		% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)
Diopatra ornata	1965				8.3	5	1.96						
	1968												
Nothria elegans	1965				12.5	7	105.74						
	1968	25	14*	55.06*	50	43	1602.07	33.3	19*	68.44*	25	14*	18.93*
Lepidasthenia longicirrata	1965	4.5	3*	.34*									
	1968				25	14*	14.77*						
Anaitides williamsi	1965	59.1	57	.68	100	375	29.97	50	141	1.72	50	72	1.66
	1968				100	115	10.47	33.3	19*	.38*	50	158	2.01
Eteone californica	1965	4.5	3*	.05*	16.7	19	.33	15	17	.17	100	803	76.13
	1968				25	14*	28.53*	33.3	19*	.38*	25	14*	.29*
Eumida bifoliata	1965				16.7	10	.29						
	1968												
Pholoe glabra	1965	4.5	3*	.05*	41.7	26	.62						
	1968				25	14*	.29*						
Langerhansia sp.	1965				4.2	2*	.05*						
	1968												
Capitella capitata	1965	100	8081	650.24	100	1439	86.22	100	10,406	497.37	100	11,814	2663.91
	1968	25	43	4.87							100	387	7.31
Notomastus tenuis	1965	9.1	8	4.43	70.8	122	310.43						
	1968				50	29	49.75						
Mesochaetopterus taylori	1965							5	3*	.06*			
	1968												
Spiochaetopterus costarum	1965				8.3	5	2.17						
	1968				25	14*	82.73*						
? Caulleriella hamata	1965				4.2	2*	.05*						
	1968												
? Praxillella gracilis	1965												
	1968	25	14*	27.24*									
Armandia brevis	1965	36.4	21	.89	100	2002	124.90	35	32	.69			
	1968				50	29	9.32	33.3	19*	.38*	75	115	34.12
Haploscoloplos elongatus	1965				45.8	43	19.07						
	1968	25	14*	12.62*	75	43	446.76	33.3	19*	.38*	25	29	19.36
Owenia fusiformis	1965												
	1968							33.3	19*	.38*			

*One specimen only was collected

TABLE 9. DISTRIBUTION AND ABUNDANCE OF POLYCHAETA CONTINUED

Species		Clean Station			Between Station			Organic Station			Sludge Bed		
		% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)	% Samples with species present	# Animals per meter^2	Ash-free Dry wt. (mgs/m^2)
Cistenides brevicoma	1965	4.5	3*	1.30*	41.7	50	42.06	15	11	10.61			
	1968				25	14*	4.30*	33.3	19*	26.76*	25	14*	83.16*
Pectinaria belgica	1965				4.2	2*	2.63*						
	1968												
Boccardia proboscidea	1965				4.2	2*	22.68*						
	1968												
Polydora brachycephala	1965				29.2	79	11.42						
	1968												
Polydora near socialis	1965												
	1968	25	14*	.86*									
Polydora A	1965				4.2	2*	.05*						
	1968												
Prionospio cirrifera	1965	95.5	336	4.87	100	366	2.60	10	6	.11			
	1968	50	358	7.03	100	215	1.72	100	115	1.53	50	72	.72
Prionospio malmgreni	1965				12.5	10	.14						
	1968	75	72	7.17	75	158	7.31	33.3	19*	1.53*	25	43	8.75
Rhynchospio arenicola	1965												
	1968	25	14*	.29*				33.3	38	.57			
Spio filicornis	1965	9.1	5	.10				5	23	.37	25	14*	.43*
	1968												
Spionid-like	1965												
	1968										25	14*	.29*
Polychaeta unusual	1965				4.2	2*	.05*						
	1968												

*One specimen only was collected

TABLE 10. POPULATION DISPERSION, STANDARD ERROR OF MEANS, AND CONFIDENCE INTERVALS

Species	Station	Date	No. of Samples	Variance	Mean	Dispersion Ratio	Standard Error of the Mean	90% Confidence Interval (# animals/core)
MOLLUSCA								
Alvinia compacta	Between	1965	24	12.67	2.00	6.33	.73	.8 - 3.2
Macoma carlotensis	Clean	1965	22	3.68	2.05	1.80	.41	1.4 - 2.7
	Between	1965	24	26.44	18.88	1.40	1.05	17.1 - 20.6
	Organic	1965	20	6.83	2.35	2.91	.58	1.8 - 2.9
Macoma nasuta	Clean	1965	22	1.61	1.45	1.11	.27	1.0 - 1.9
	Between	1965	24	1.56	1.67	.93	.25	1.3 - 2.1
Mysella tumida	Between	1965	24	22.85	11.25	2.03	.98	9.6 - 12.9
	Between	1968	4	15.19	13.25	1.15	1.95	10.0 - 16.5
	Sludge Bed	1968	4	27.19	14.25	1.91	2.61	10.0 - 18.5
Psephidia lordi	Between	1965	24	3.47	1.33	2.60	.38	.7 - 2.0
	Between	1968	4	728.75	144.50	5.04	13.50	122 - 167
	Sludge Bed	1968	4	119.25	23.50	5.07	5.46	14.5 - 32.5
Tellina modesta	Between	1965	24	137.48	30.63	4.49	2.39	26.7 - 34.6
	Between	1968	4	29.00	31.00	.94	2.69	26.6 - 35.4
NEMATODA								
All species	Clean	1965	22	387,498	1762.86	219.81	132.72	1545 - 1981
	Between	1965	24	372,472	1268.04	293.74	124.58	1063 - 1473
	Organic	1965	20	8,149,480	5822.95	1399.55	638.34	4773 - 6873
	Sludge Bed	1965	4	3,062	88.50	34.60	27.67	43.0 - 134
	Clean	1968	4	65,535	559.25	117.19	128.00	349 - 770
	Between	1968	4	31,856	628.00	50.73	89.24	481 - 775
	Organic	1968	3	12,880	317.00	40.63	65.52	209 - 425
ARTHROPODA								
Acrenhydrosoma karlingi	Between	1965	24	50.95	4.09	12.47	1.46	1.7 - 6.5
Bulbamphiascus imus	Clean	1965	22	893.48	31.86	28.04	6.37	21.4 - 42.3
	Between	1965	23	1461.06	45.26	32.28	7.97	32.1 - 58.4
	Organic	1965	20	49,134	520.85	94.32	49.57	439 - 602
	Sludge Bed	1965	4	13,037	300.25	43.42	57.09	206 - 394
Cumella vulgaris	Between	1965	24	1.33	1.21	1.10	.24	.8 - 1.6
Euphilomedes carcharodonta	Between	1965	24	36.52	5.25	6.96	1.23	3.2 - 7.3
	Between	1968	4	199.69	64.75	3.08	7.07	53.1 - 76.4
Munnogonium waldronense	Between	1965	24	99.00	12.00	8.25	2.03	8.7 - 15.3
	Between	1968	4	.69	5.75	.12	.42	5.1 - 6.4
Photis californica (?)	Sludge Bed	1968	4	15.19	21.25	.71	1.95	18.0 - 24.5
Tisbe furcata	Between	1965	24	1.71	1.04	1.64	.27	.6 - 1.5
	Organic	1965	20	75.75	4.50	16.83	1.95	1.3 - 7.7
	Sludge Bed	1965	4	5667.69	177.75	31.89	37.64	114 - 240
Typhlamphiascus pectinifer	Clean	1965	22	1.18	.77	1.52	.23	.4 - 1.2
	Between	1965	24	367.17	25.46	14.42	3.9	19.0 - 31.9
ANNELIDA								
Anaitides williamsi	Clean	1965	22	1.36	1.00	1.36	.25	.6 - 1.4
	Between	1965	24	36.33	6.54	5.55	1.23	4.5 - 8.6
Armandia brevis	Between	1965	24	130.74	34.92	3.74	2.33	31.1 - 38.8
Capitella capitata	Clean	1965	22	1633.36	140.91	11.59	8.62	127 - 155
	Between	1965	24	206.91	25.08	8.25	2.94	20.2 - 29.9
	Organic	1965	20	5132.15	181.45	28.28	16.02	155 - 208
	Sludge Bed	1965	4	2976.50	206.00	14.45	27.28	161 - 251
Dorvillea rudolphi	Clean	1965	22	.90	.91	.99	.20	.6 - 1.2
	Between	1965	24	1.08	1.08	.99	.21	.7 - 1.4
Glycera capitata	Between	1965	24	17.08	11.54	1.48	.84	10.2 - 12.9
Gyptis brevipalpa	Between	1965	24	442.92	48.00	9.23	4.30	40.9 - 55.1
Haploscoloplos elongatus	Between	1965	24	1.02	.75	1.36	.21	.4 - 1.1
Nephtys ferruginea	Between	1965	24	2.83	2.79	1.01	.34	2.2 - 3.4
Notomastus tenuis	Between	1965	24	5.44	2.13	2.56	.48	1.3 - 2.9
Pholoe glabra	Between	1965	24	.33	.46	.72	.12	.3 - .7
Platynereis bicanaliculata	Between	1965	24	9.73	3.63	2.69	.64	2.6 - 4.7
	Organic	1965	20	55.86	4.80	11.64	1.67	2.1 - 7.5
Prionospio cirrifera	Between	1965	24	31.40	6.38	4.93,	1.14	4.5 - 8.3
	Clean	1965	22	23.07	5.45	4.23	1.02	3.8 - 7.1

TABLE II. WET, DRY, AND ASH-FREE DRY WEIGHT RATIOS

Species	Dry wt./wet wt. (percent)	Ash-free dry wt./dry wt. (percent)	Ash-free dry wt./wet wt. (percent)
MOLLUSCA			
Alvinia compacta	*	87.88	*
Clinocardium nuttalli	**	10.02	**
Lucinoma annulata****	20.36	76.74	15.62
Lucinisca tenuisculpta****	23.15	85.91	19.89
Macoma carlotensis****	23.57	71.03	16.74
Macoma nasuta	***	65.26	***
Mysella tumida****	24.37	81.59	19.88
Protothaca staminea	**	16.77	**
Psephidia lordi****	22.35	86.74	19.38
Tellina modesta****	23.05	72.01	16.60
ARTHROPODA			
Amphipoda	24.06	78.48	18.88
Copepoda (_Bulbamphiascus imus_)	19.60	83.88	16.44
Cumacea (_Cumella vulgaris_)	27.30	56.94	15.53
Euphilomedes carcharodonta †	17.90	59.93	10.73
Munnogonium waldronense	27.24	76.66	20.34
ANNELIDA			
Armandia bioculata	21.47	79.80	17.13
Capitella capitata	18.15	84.62	15.36
Cistenides brevicoma ‡	28.09	34.06	9.57
Glycera capitata	19.44	82.86	16.11
Pectinaria belgica	28.03	32.35	9.17
COELENTERATA			
Leioptilus guerneyi	14.42	63.24	9.12
NEMATODA			
All Species	22.98	87.83	20.18

* Wet weight not taken.

** Wet weight not taken. Weighed with shells.

*** Wet weight not taken. Weighed without shells.

**** Weighed without shells.

† Shells decalcified before weighing.

‡ Case removed before weighing.

3. Table 12 provides a listing and classification of all species collected and identified as infauna. The epifauna are treated in the Interpretation of Results. More than 45% of the infauna species found were polychaetes, the Crustacea 37%, and the remainder were Mollusca. Many nematode species were present in the samples, but at this time they have not yet been identified, which prevents their inclusion in the figures just presented.

4. Nine species of Mollusca were collected. One, the snail *Alvinia compacta*, was the only gastropod collected and was never abundant. The remaining eight species were Pelecypoda, half of which were known only from small numbers (*Clinocardium*, *Lucinoma*, *Lucinisca* and *Protothaca*). The other four were fairly abundant at different times in 1965 and/or 1968, in the cases of *Tellina modesta* and *Macoma carlottensis*, 8,287 and 1,082 per meter square respectively at the Between Station in 1965. Size distribution graphs (Figs. 31, 32) show these high values to be due mainly to large numbers of juvenile clams. Because of the adverse conditions at the Sludge Bed in 1965, no species of clam was present at all stations, although *Tellina* and *Macoma carlottensis* were found at all other times and places.

Eleven species of Harpacticoid copepods were collected, four of which were known only from one to several specimens. Additional species were moderately represented, and two--*Bulbamphiascus imus* and *Tisbe furcata*--were present in sizable numbers in at least one location.

Among the other Crustacea, the isopod *Munnogonium waldronense*, the ostracod *Euphilomedes carcharodonta*, and the amphipod *Photis californica* (?) appeared in large numbers at times. The rest were represented

TABLE 12. SPECIES OF INFAUNA COLLECTED IN STUDY AREA, 1965 AND 1968

Phylum *Arthropoda*, Class *Crustacea*

Amphipoda
Aoroides columbiae Walker
Corophium sp.
Hyale sp.
Orchomene sp.
Parapleustes pugettensis (Dana)
Photis californica (?) Stout
Photis conchicola (?) Alderman
Photis sp.
Synchelidium shoemakeri Mills
Westwoodilla caecula (Bate)

Chelifera
Leptochelia dubia (?) Kröyer

Cumacea
Cumella vulgaris Hart
Diastylopsis tenuis (?) Zimmer
Lamprops quadriplicata Derzhavin

Copepoda
Acrenhydrosoma karlingi Lang
Amonardia pertubata Lang
Bulbamphiascus imus (Brady)
Dactylopodia vulgaris (Sars)
Diagniceps N.Sp.
Heterolaophonte discophora (Willey)
Huntemannia jadensis (Poppe)
Intermediate *Parathalestris*
bulbiseta & *californica*
Tisbe furcata (Baird.)
Tisbe gracilis (T. Scott)
Typhlamphiascus pectinifer Lang

Isopoda
Munnogonium waldronense
George and Stromberg

Ostracoda
Euphilomedes carcharodonta (V.Z. Smith)

Phylum *Mollusca*

Class Gastropoda
Alvinia compacta (Carpenter)

Class Pelecypoda
Clinocardium nuttalli (Conrad)
Lucinoma annulata (Reeves)
Lucinisca tenuisculpta (Carpenter)
Macoma carlottensis Whiteaves
Macoma nasuta (Conrad)
Mysella tumida (Carpenter)
Psephidia lordi (Baird)
Tellina modesta Carpenter
Protothaca staminea (Conrad)

Phylum *Nematoda* (Class of others)

Species not yet identified

Phylum *Protozoa*

Order Foraminifera

Trochammina inflata
Elphidium sp.

Phylum *Coelenterata*

Leioptilus guerneyi (Gray)

Phylum *Annelida*

Polychaeta Errantia

Family Chrysopetalidae
Paleanotus bellis (Johnson)

Family Dorvilleidae
Dorvillea rudolphi (delle Chiaje)

Family Glyceridae
Glycera capitata Oersted
Glycera tenuis Hartman

Family Goniadidae
Glycinde polygnatha Hartman
Goniada acicula Hartman

Family Hesionidae
Gyptis brevipalpa (Hartmann-Schröeder)

Family Lumbrineridae
Lumbrineris latreilli Audouin and Milne Edwards

Family Nephtyidae
Aglaophamus erectans Hartman
Nephtys caeca (Fabricius)
Nephtys ferruginea Hartman

Family Nereidae
Platynereis bicanaliculata (Baird)
Nereid species A
Nereid species B

Family Onuphidae
Diopatra ornata Moore
Nothria elegans (Johnson)

Family Polynoidae
Lepidasthenia longicirrata Berkeley

Family Phyllodocidae
Anaitides williamsi Hartman
Eteone californica Hartman
Eumida bifoliata (Moore)

Family Sigalionidae
Pholoe glabra Hartman

Family Syllidae
Langerhansia sp.

Polychaeta Sedentaria

Family Capitellidae
Capitella capitata (Fabricius)
Notomastus tenuis Moore

Family Chaetopteridae
Mesochaetopterus taylori Potts
Spiochaetopterus costarum (Claparede)

Family Cirratulidae
? *Caulleriella hamata* (Hartman)

Family Maldanidae
? *Praxillella gracilis* (Sars)

Family Opheliidae
Armandia brevis (Moore)

Family Orbiniidae
Haploscoloplos elongatus (Johnson)

Family Oweniidae
Owenia fusiformis (delle Chiaje)

Family Pectinaridae
Cistenides brevicoma (Johnson)
Pectinaria belgica (Pallas)

Family Spionidae
Boccardia proboscidea Hartman
Polydora brachycephala Hartman
Polydora near *socialis*
Polydora A
Prionospio cirrifera Wiren
Prionospio *malmgreni* Claparede
Rhynchospio arenicola Hartman
Spio filicornis (O.F. Müller)
Spionid-like

by one to a few animals.

Nematode worms were the most abundant animals by numbers (Fig. 22) and, next to the mollusks, in biomass for some stations. They were ubiquitous, appearing in reduced numbers even at the Sludge Bed Station in 1965 and 1968.

The polychaete worms have proven to be one of the most important groups of animals because of their diversity and the large number in which they existed. A total of 43 species were found. Table 12 lists these, and Table 9 shows their distribution and abundance. Because of their importance, further consideration of this group has been deferred to the Interpretation of Results section, where they are considered at some length.

V. INTERPRETATION OF RESULTS

A. Physical Factors

Oxygen

1. The amount of oxygen dissolved in water is a function of temperature and salinity. From these can be calculated the solubility of the oxygen (American Public Health Association, 1962). The percent saturation of the water with oxygen is the quotient of the oxygen measured in the water, divided by the oxygen solubility. These were calculated for the high and low oxygen values. Only the Clean Station had measurable amounts of dissolved oxygen in the interstitial water, 0.1 mg/liter.

Table 13. Oxygen Solubility and Saturation in Interface and Interstitial Water

Water location	Amount Oxygen (mg/liter)	Solubility (mg/liter)	Percent Saturation
Sediment-water interface			
a. High value	6.72	8.7	77.2
b. Low value	4.02	8.9	45.2
Clean Station, interstitial	0.1	9.1	1.1

2. As noted in Results, the amount of dissolved oxygen was high in the interface water over the anoxic Sludge Bed, even though the sediment was undoubtedly drawing oxygen from the ambient water. In fact, the highest dissolved oxygen measured was from the Sludge Bed interface water. It must be kept in mind, however, that even though the sediment

probably had a high oxygen demand (Hayes, 1964), the relatively high dissolved oxygen in the interface water, plus the large amount of water moving past the Sludge Bed could supply all the oxygen needed without much apparent change.

3. It would appear (Table 13) that the amount of dissolved oxygen of the circulating water was adequate for animal life and would probably be of little value in explaining differences in species distributions. But the quantity of the oxygen in the circulating and interstitial water raises several interesting questions:

a. Does the overall reduction of oxygen, that is, somewhat less than saturated, in the ambient water of the area of study, Shilshole Bay, explain the overall reduction in variety of organisms observed?

b. Is the difference in interstitial dissolved oxygen of significance at the four stations?

c. How are the animals able to survive at zero or greatly reduced oxygen concentrations in the interstitial environment?

4. With regard to the first question, Richards (1957, page 212) notes that in general, ". . . in most aquatic organisms, respiratory consumption of oxygen is essentially independent of the oxygen tension down to some lower limit of the latter. Below this limit, the respiratory consumption may decrease with decreasing oxygen tension, until the tension becomes so low the organisms can no longer survive." Papers cited by Richards show clearly the lower tension to which he refers is considerably less than that experienced in the circulating water during the present study. Furthermore, while there is an

overall reduction in number of species, the diversity of animals here referred to as epifauna, suggest that oxygen in circulating water was adequate at all stations and was not a factor in species reduction or distribution.

5. The interstitial water presents a special problem in interpretation. Dissolved oxygen was not found at the Organic Station and Sludge Bed, but hydrogen sulfide and ammonia were. During analysis it was not possible to measure the presence of dissolved oxygen at the Between Station. But it was not possible to measure the presence of hydrogen sulfide either; its presence would have been of anaerobic respiration by bacteria. Therefore, whether the Between Station with its variety of species is in fact truly anoxic is questionable. There may be a delicate balance between oxygen consumption by organisms and the replacement process from above. Similar conditions occur widely in the so-called oxygen-minimum zones of the eastern tropical Pacific, where the oxygen content of the water column is so low as to be scarcely detectable and yet hydrogen sulfide is <u>not</u> present.

As mentioned, interstitial water at the Organic Station and Sludge Bed is devoid of oxygen and contains appreciable amounts of hydrogen sulfide and ammonia. Whether the reduction of species diversity at these two stations is due to the anoxic conditions, to the presence of toxic hydrogen sulfide, to a combination of both, or to neither, is unknown. But since the Between Station with its greater number of species of animals, lacks oxygen and hydrogen sulfide, it would appear that the presence of the latter is partially responsible in determining the abundance and distribution of the species (see Interpretation of Results for H_2S).

Interstitial water at the Clean Station resembles that at the Between Station in its lack of hydrogen sulfide, but possesses small amounts of dissolved oxygen. The biota too is similar, but the Between Station has a greater number of species and organisms, suggesting again that interstitial oxygen here is not the limiting factor. It is likely that the amount of deposited organic material acting as an organic food source is the main difference between these two stations.

6. How the animals are able to survive under the greatly reduced oxygen content or the complete absence from the interstitial water is a question of wide biological interest. There are at least four possibilities:

a. The animals in greatly reduced oxygen tension are respiring at a greatly reduced rate.

b. The animals may be able to live anaerobically as do bacteria, as long as oxidizable organic material is available. A paper by Hochachka and Mustafa (1972) substantiates this premise.

c. The animals commute periodically to the sediment water interface, to relieve their oxygen debt.

d. Immobile forms such as bivalves, really live aerobically by drawing oxygen from the water column while their body is in anaerobic surroundings.

There are articles in the literature in support of each of the possibilities above. Further examination of this question is beyond the scope of this paper.

Hydrogen sulfide

1. Hydrogen sulfide, according to McGilvery (1970), is as toxic as hydrogen cyanide. It is a product of the reduction of sulfate, which is normally present in sizable amounts in seawater (Hayes, 1964; Richards, 1965). Certain bacteria utilize sulfate as the terminal electron acceptor in anaerobic respiration and produce large amounts of sulfide. The species believed to be the most responsible is the strictly anaerobic bacterium <u>Desulfovibrio</u> <u>aestuarii</u>, which obtains its energy from anaerobic reduction of sulfate accompanied by the simultaneous oxidation of organic matter.

2. Hydrogen sulfide was not present in the sediments of the Clean or Between Stations, but it was present at the Organic Station and very abundant at the Sludge Bed.

Table 14. Hydrogen Sulfide in Interstitial and Interface Water

Station and water	pH	% sulfide as H_2S	Average (PPM)		Range (PPM)	
			Sulfide Measured	Actual H_2S	Sulfide Measured	Actual H_2S
Clean						
Interstitial	6.9	-	0	-	0	-
Interface	7.7	-	0	-	0	-
Between						
Interstitial	6.9	-	0	-	0	-
Interface	7.7	-	0	-	0	-
Organic						
Interstitial	6.8	53	40	21.2	0-.060	0-.007
Interface	7.7	12	.017	.002		
Sludge Bed						
Interstitial	5.9-6.1	92	460	423.2	.03-1.26	.004-.151
Interface	7.7	12	.280	.034		

The percentage of the total sulfide present as hydrogen sulfide decreases with increasing pH (Fig. 23). At the Organic Station the interstitial pH was 6.8, so only about 50 percent of the sulfide existed as the toxic hydrogen sulfide; the rest existed as hydrogen sulfide (HS^-) and sulfide ($S^=$) ions. The somewhat lower pH of about 6.0 at the Sludge Bed resulted in about 90 percent of the sulfide being hydrogen sulfide.

At the Sludge Bed and Organic Station, small amounts of sulfide were measured (Table 3) in the water just above the bottom, from which it had escaped. Since the pH of this water was 7.7, only a small part of sulfide was present as hydrogen sulfide. It is doubtful that any hydrogen sulfide would remain higher in the water column for long, since sulfide-sulphur is rapidly oxidized (Cline and Richards, 1969) and the water was anywhere from 45 to 77 percent saturated with oxygen during the period of sampling.

3. The extent to which hydrogen sulfide limits the presence of marine animals is not known. McGilvery (1970) states that it combines with cytochrome oxidase so the mitochondrial electron transport system is blocked at its terminal reaction. As a result, electrons are not transferred to oxygen, the previous electron carriers in the chain accumulate in their reduced state, and the generation of high-energy phosphate ceases. Death quickly follows. In addition to this, it should be remembered that hydrogen sulfide in the sediment means that anoxic conditions exist, which may be severely limiting in and of itself.

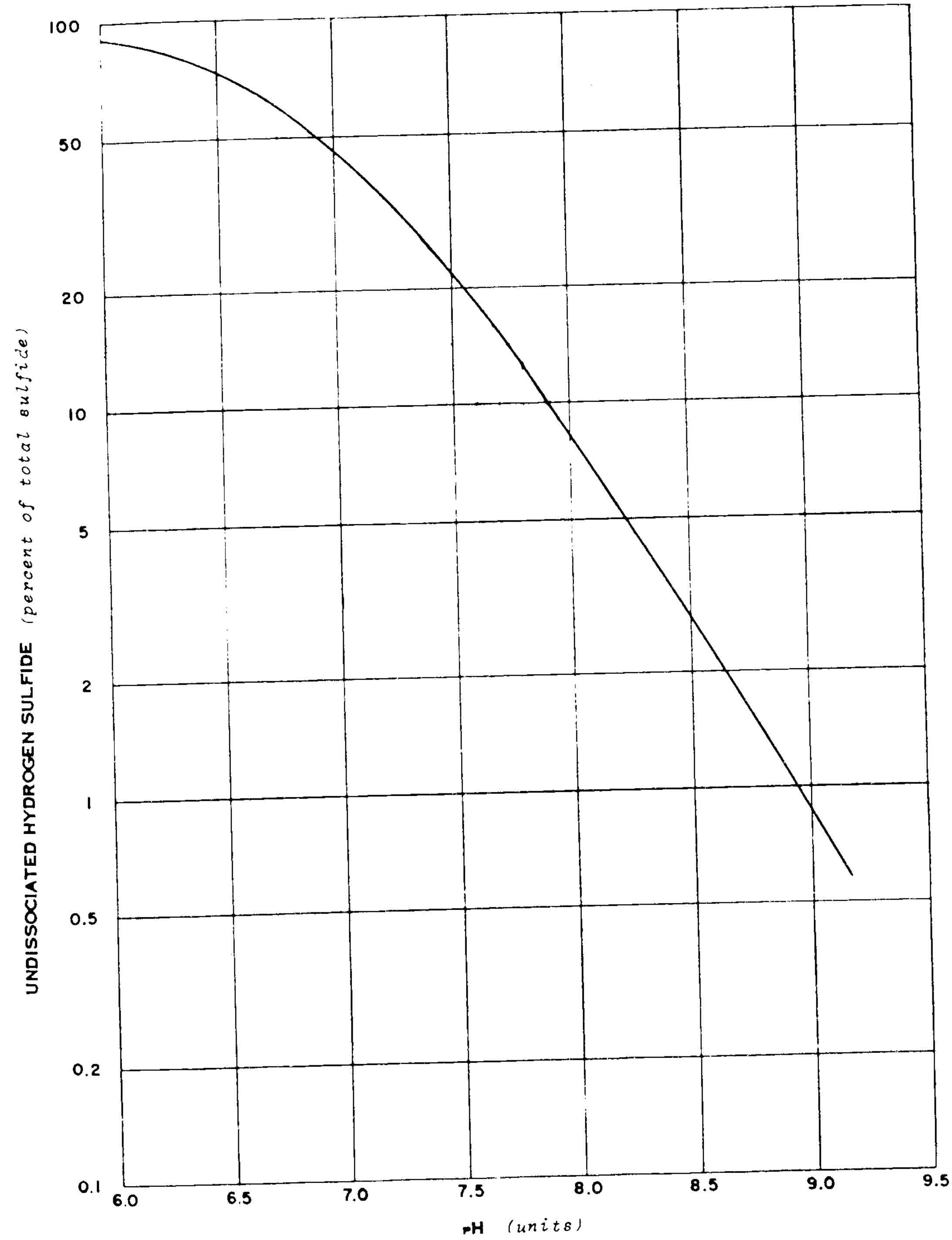


Figure 23. Percentage of total sulfide as undissociated hydrogen sulfide in aqueous solution as a function of pH.

The large quantity of hydrogen sulfide-sulfide in the Sludge Bed is undoubtedly partially responsible for the small number of species able to survive there. The quantity of hydrogen sulfide at the Organic Station, where many more species were present, was some twenty times less than that at the Sludge Bed. Many other studies of sediments have associated reduced numbers of species with this presence of hydrogen sulfide.

Ammonia

1. The procedure used to measure un-ionized ammonia gas was modified because it did not distinguish between naturally occurring ammonia and that produced from labile amines during the analytical process (see Appendix). Neither method distinguished between the un-ionized ammonia and the ammonium ion. Since only the former is toxic to animals, the amounts of each had to be determined to arrive at some conclusion as to its toxicity in the environment.

The prime factor as to the ionic state of ammonia is pH. When the measured ammonia is recalculated on the basis of pH, the actual amount of un-ionized ammonia is found to be extremely small, most of which is in the ionic form.

Thus, although the measured amount of ammonia in the Sludge Bed was over three times that at the Organic Station, because of the higher pH, the actual amount of un-ionized ammonia at the latter was twice the former. From Table 15 we see the amount of measured ammonia was consistent with the amount of organic material deposited (Table 4). The small amount of un-ionized ammonia at the Between Station gives no indication as to the presence of oxygen in the interstitial water since

Table 15. The Amount and Chemical State of Ammonia in the Interstitial Water

Station	Measured ammonia (ppb)	pH of the interstitial	$NH_4^+ : NH_3$ ratio at pH	Actual un-ionized NH_3 (ppb)
Clean	0.0	6.9	590:1	0.0
Between	32	6.9	590:1	.05
Organic	275	6.9	590:1	.47
Sludge Bed	900	6.1	3720:1	.24

ammonia can be produced either aerobically or anaerobically. Gameson and Barratt (1958) for the Thames Estuary, found reduction of nitrate to ammonia occurred even when the concentration of dissolved oxygen amounted to as much as several percent of saturation in water.

2. The literature on the toxic effects of ammonia on fish have been summarized by Doudoroff and Katz (1950). They found the lowest lethal values reported ranged from 2 to 7 ppm. The literature on ammonia toxicity to invertebrates is sparce. From Table 15, the actual amounts of toxic, un-ionized ammonia in the interstitial environment is too small to have had much if any affect on the distribution and abundance of the organisms.

Water temperature

1. Temperatures of water at the sediment-water interface were sufficiently close to each other at the four stations to eliminate temperature as a factor influencing the distribution and abundance of the animals between stations (Fig. 18). This conclusion is reinforced by the fact that diurnal fluctuations at each station was much greater

than between stations. Interstitial water temperatures were essentially the same at all stations, a few tenths of degrees lower than the circulating water.

pH

1. The open ocean is chemically buffered (Moberg, et al, 1934) and resists much change in pH, which is usually between 7.5 and 8.4. Under restricted or stagnant conditions, the pH of non-sediment water may change to less than seven (Sverdrup, Johnson, and Fleming, 1942). In sediment where there is high organic enrichment and restricted circulation, elevated production of carbon dioxide and organic acids results in reduced pH. It is not surprising then to find that the pH of the sediment at all sampling stations was reduced to below neutral, the Sludge Bed being reduced the most, the Clean and Between Stations the least, and the Organic Station intermediately.

2. It is unlikely the reduced pH--even the 6.0 recorded at the Sludge Bed--had any sizable, direct effect on the animals. But the cause of the low pH--restricted circulation or stagnant anoxic conditions--most certainly did affect the animals.

3. The water circulating just over the sediment was constant at pH 7.7 for all stations and for the entire period of sampling. It appears unlikely that pH at the sediment-water interface influenced the distribution and abundance of the animals in the study area.

Salinity

According to Pearse and Gunter (1957), the salinity of the mid Pacific is about 35.5 parts per thousand. The average salinity measured at the four Shilshole Bay sampling stations was 29.605, with little variation between samples and stations. The measured extremes of 29.36 and 29.71 provided a total range of less than 0.4 ppt. This variation is less than one might expect for an estuary which was receiving injections of fresh water continuously from a sewer, and frequently each day from a lake. It would appear that by the time any entering fresh water flowed past the sediment interface at the four sampling stations, it had become well mixed and evenly distributed with the seawater.

Although it is well known that salinity can regulate the distribution and abundance of animals, the constancy of this environmental variable in our study area would seem to preclude its importance here.

Particulate inorganic materials

Large quantities of particulate inorganic materials entered Shilshole Bay in 1965 primarily through the North Trunk Sewer. According to the Brown and Caldwell Report (1958), the source of the inorganics was street washings and materials from sanding icy streets in the Seattle area during the winter months. During the summer months, much of the inorganics was recovered at sewage treatment plants and did not enter the Bay. These amounts varied from two to a maximum of five cubic feet per million gallons of water. During the rainy and winter months, the sediment was carried by increased water velocity all the way out into the Bay. Other particulate inorganic materials entered Shilshole Bay from the Ship Canal and from construction activities on shore.

It is most likely that most of the larger-sized particles from the Sewer were deposited relatively close to their point of discharge, the smaller fractions being distributed more widely in the Bay. Particulate inorganic concentration in the water column (Table 3), particle composition of the sediment (Table 5), and rate of deposition (Tables 4, 17) have been presented.

1. Inorganics in the water column in 1965

It may be recalled the Between and Organic Stations were similar to each other in amount of particulate inorganics in the sediment interface water, but the Clean Station was unexpectedly higher than both of them. This is inconsistent with the rate of sediment deposition in the sediment collectors, which was lowest at the Clean Station. Rate of sediment deposition will be discussed later, but for now we can suggest that perhaps during the two days when water samples were collected, there was an unusual amount of small, particle-laden water passing over the Clean Station, perhaps coming from the nearby Lake Washington Ship Canal.

From Table 3, we notice the Sludge Bed interface water had a high average particulate inorganic content. This is a result of one very high sample collected during the sampling period, and suggests the smaller inorganic fractions are prevented from regularly settling at the Sludge Bed due to the strong currents experienced at this station. This is substantiated by the small amounts of silt and clay present in the sediment.

2. Composition of the sediment 1965-1968

The particle size distribution for 1965 and 1968 has been presented in the results section. An explanation is in order as to why the Sludge Bed had larger sand particles in 1968 than in 1965. While it was possible in 1968 to relocate the earlier sampling sites for the Clean, Between, and Organic Stations, and thus insure comparability between years, this was not possible for the Sludge Bed. That the actual Sludge Bed was resampled is not in question, but it is likely the samples in 1968 were taken a bit closer to the end of the former sewer outfall, where the particles sizes were larger.

Between the years of 1965 when the sewer was shut down, and 1968, all stations but the Sludge Bed experienced a change in amount of the smaller, silt-clay portions of their sediments.

Table 16. Amount of Silt-clay in Sediments in 1965 and 1968

Station	% Silt-clay (1965)	% Silt-clay (1968)
Clean	7.36	10.18
Between	7.24	3.87
Organic	43.90	10.17
Sludge Bed	2.32	2.57

The Organic Station experienced a drastic reduction in three years. This was most likely due to completion of construction activities on shore near the Station, which it is believed had contributed much of the finer fractions. The sediment present in 1965 was of course still present in 1968, but because it was now deeper, it obviously constituted a lesser, unknown part of the sample of which only the top five centimeters was analyzed. Unfortunately, the rate of sediment accretion to Shilshole Bay is unknown.

In 1965 strong currents prevented large amounts of natural and sewage-introduced silt and clay from settling at the Sludge Bed. After the sewer ceased discharging, the Sludge Bed stayed the same in sediment composition. This was due to the continuing, strong currents preventing, as before, the natural source of silt and clay from settling out.

The Between Station experienced a slight decrease in finer fractions by 1968. Current patterns show this station was partially in the tidal pattern of the outfall of the North Trunk Sewer. When it ceased discharging, the Between Station was deprived of these smaller fractions carried by the sewer effluent to the Bay.

With shutdown of the sewer, the Clean Station experienced an increase in its finer inorganic fractions through 1968. The reason is unknown. It may be this station experienced new current patterns created by the freshwater discharge from the nearby Ship Canal that were not experienced when the North Trunk Sewer was operating. However, it is not really known what effect the sewage discharge had on current patterns in Shilshole Bay.

3. Rate of deposition of inorganics

After the North Trunk Sewer was shut down in late 1965, the sewage was diverted to the Ship Canal for 41 days, and the rate of deposition of inorganic materials increased. Sediment collectors installed and used just before and just after the shutdown provided the information below.

Table 17. Deposition of Inorganic Materials Immediately Prior to, and Just After Shutdown of the North Trunk Sewer (1965)

Station	Inorganics deposited before sewer shutdown ($gms/m^2/day$)	Inorganics deposited after sewer shutdown ($gms/m^2/day$)	After X 100 Before
Clean	75.28	132.33	176
Between	81.06	97.86	121
Organic	91.18	160.13	176
Sludge Bed	Collector fouled, sediment not collected		

An increase in the rate of deposition at first seems strange until the amount of rainfall in the Seattle area is considered. During the period before the North Trunk Sewer submarine outfall was inactivated, and at which time the collectors were in place, rainfall was 0.91 inches. But after sewer inactivation, the rainfall for a comparable period of time was 2.13 inches. As noted earlier, the greater the rainfall, the greater was the amount of inorganic materials that was contributed to the North Trunk effluent from the increased storm drainage. The Clean Station increased in inorganic materials probably because it was near the Ship Canal, which was now receiving both the North Trunk's sewage and the storm drainage with its increased inorganic loading caused by the rain. The increase at the Organic Station was probably due to increased water runoff with its inorganic materials from the construction site nearby. The Between Station also exhibited an increase in inorganics, but this was considerably less than at the other stations, probably because it was further from shore and the Ship Canal discharge point.

Sedimentation rates at the Sludge Bed could not be measured because of fouling of the mouth of the collecting container by toilet paper. Instead, visual observations were used. There can be no question but that this station in 1965 received the largest amount of inorganic as as organic material. Many times while diving on this Station, I was aware that the area below the sewage plume was quite turbid from the surface to the sediment. Close to the sewer outfall, the larger, heavier inorganic particles rain to the bottom. Further away, where our sampling station was located, these largest particles were not present, but other sizable sandy particles swept by strong currents settled fairly rapidly to the sediment. Silt and clay were kept suspended in the upper waters by the strong current. This explains why the Sludge Bed sediment had larger particles than the other stations and why the water at the sediment-water interface was not excessive in the finer suspended inorganic particles. It was the larger sand to gravel sized particles plus the large amounts of organics that caused this station to take the form of a large, broad, convex mount. Immediately after sewer shutdown, the water over this station became clear, even with the increased rainfall noted above. It was evidently too far from shore to be influenced as much as were the other stations.

4. Effects of particulate inorganic materials on animals

The rate of deposition of sediment and its constitution are important to animals. Numerous studies (Sherk and Cronin, 1970) indicate that if sufficient suspended inorganic materials are present in the water column, many species of invertebrate eggs, larvae, and adults are affected by smothering. High turbidity due to inorganics can kill fish by fouling their gills, and photosynthesis may also be affected.

Was the amount of inorganics in Shilshole Bay water sufficient to have these adverse effects? The concentrations seen at our sampling stations is considerably below the levels reported to do damage elsewhere (Sherk and Cronin, loc. cit.), and it is concluded the inorganic content of the water column was not an important factor directly in the distribution and abundance of the animals.

What the effect of the settling inorganics is upon the infauna is unknown. At the Sludge Bed, where the rate of deposition of larger inorganic particles was high, there were other environmental variables that were more important. The high deposition of organic materials, with its attendant problems of anoxia, precluded all but a few very tolerant species that presumably were not affected adversely by either the inorganics or organics. In fact, one might ask if these tolerant species might have benefited from these unusual conditions at least indirectly through reduction or elimination of competition.

For the Organic Station, the story is much different. Since there were very large amounts of silt and clay deposited in 1965, the sediment was sealed off from the water above and it had become anoxic. We may assume the amount and kind of particles deposited had a significant effect on the animals.

The distribution of marine animals has been correlated with sediment characteristics such as particle size and degree of sorting.

Some workers look for sediment effects in terms of whether filter or sediment feeders predominate; others compare distribution of specific kinds of organisms with these sediment characteristics. For some species, the relationships to the sediment composition is clear, but for others relationships are not so obvious or are non-existent.

There has been published a sizable literature on the subject of sediment-animal relationships. For further information the reader is directed to Pratt (1953), Bader (1954), Barnard and Reish (1959), Sanders (1958, 1960), Wieser (1959a, 1960), McNulty, Work, and Moore, (1962), Harrison, Lynch, and Aftschaeffl (1964), Newell (1965), Lynts (1966), Tenore, Horton, and Duke (1968), and Lie (1968). Many of the afore mentioned papers have been summarized by Sherk and Cronin (1970).

Metals

1. Physical - Chemical Considerations

a. Goldberg (1957) listed the concentrations of the various elements found in sea water. A comparison of his values and those found at the sediment-water interface in Shilshole Bay, show the content of waters of the Bay averaged many times the amount of copper, chromium, and lead found in more open waters.

Table 18. Metals Content of Open Sea Water and Shilshole Bay

Metals (average)	Open waters	Clean Station	Between Station	Organic Station	Sludge Bed
Copper (ppb)	3	21.5	19.1	40.9	30.3
Chromium (ppb)	0.05	29.8	28.2	30.5	31.4
Lead (ppb)	3	16.2	16.3	16.0	26.4

Such increases are expected in an estuary where the runoff from the land carries quantities of these materials, and the dilution factor is not as great as in open waters.

b. The effluent from the North Trunk Sewer entering the study area was known to contain undetermined quantities of industrial wastes (Brown and Caldwell, 1958). There are in Seattle metal plating and fabricating plants that utilize quantities of copper, chromium, and other metals. Storm drainage, known to be high in lead from auto exhausts, also emptied by the Sewer into Shilshole Bay. Nearby locations in Puget Sound also received these metals via freshwater runoffs. Because these metals are toxic, it was decided to examine their concentrations in water and sediments.

c. The procedure for metals analysis in sea water have been established (see Materials and Procedures), but there is no chemical extraction procedure for metals in the sediment that exactly duplicates their availability to sediment feeders. Cross, Duke, and Willis (1970) attempted to evaluate "available"magnesium, iron, and zinc to sediment-feeding organisms by extraction with 0.1 N HCl, which they concluded to be roughly equivalent to biological extraction. For reasons listed later in this section, it is questionable if 0.1 N HCl is equivalent to biological extraction, but for purposes of comparison, the same procedure was used. In addition to acid treatment, a follow-up extraction of the same sediment with EDTA, a strong chelating agent, was performed to determine the concentration levels of the remaining metals that could be extracted by animals should the conditions in their digestive tracts be more efficient than the 0.1 N HCl treatment.

d. From the results (Tables 3 and 4; Figs. 24, 25, 26), the amounts of copper, chromium, and lead at the sediment-water interface water can be seen to be quite variable. There may be two possible reasons: (a) the process of extraction and analysis was faulty with possible contamination, or (b) the erratic results obtained reflect a series of injections of metals into the Bay, which were then moved about by currents. Let us consider each briefly:

1'. All glassware used for collecting and analysis of metals was washed with aqua regia, followed by several rinses with doubly distilled water. All acids used during collection and analysis were redistilled to eliminate metal contamination. The potassium hydroxide used to create the magnesium hydroxide floc to co-precipitate the metals at the sediment-interface water was first cleansed of metals contamination with dithizone. With these precautions, it seems reasonable the metals present were from the sea water only. The extraction of metals, according to Joyner (1967), whose procedure of co-precipitation was utilized, recovered better than 99 percent of copper and most of the lead and chromium. Equal precautions were taken for extraction of metals from the sediment and their analysis.

2'. The second possibility seems more likely. There is considerable variation in current patterns, so that movement of water inside Shilshole Bay is difficult to predict (see Interpretation of Results). The Brown and Caldwell Report recorded a sizable number of chemical and metal plating plants and other related industries in the Seattle area. In addition to plants that emptied directly into Shilshole Bay via the North Trunk Sewer, other plants located nearby

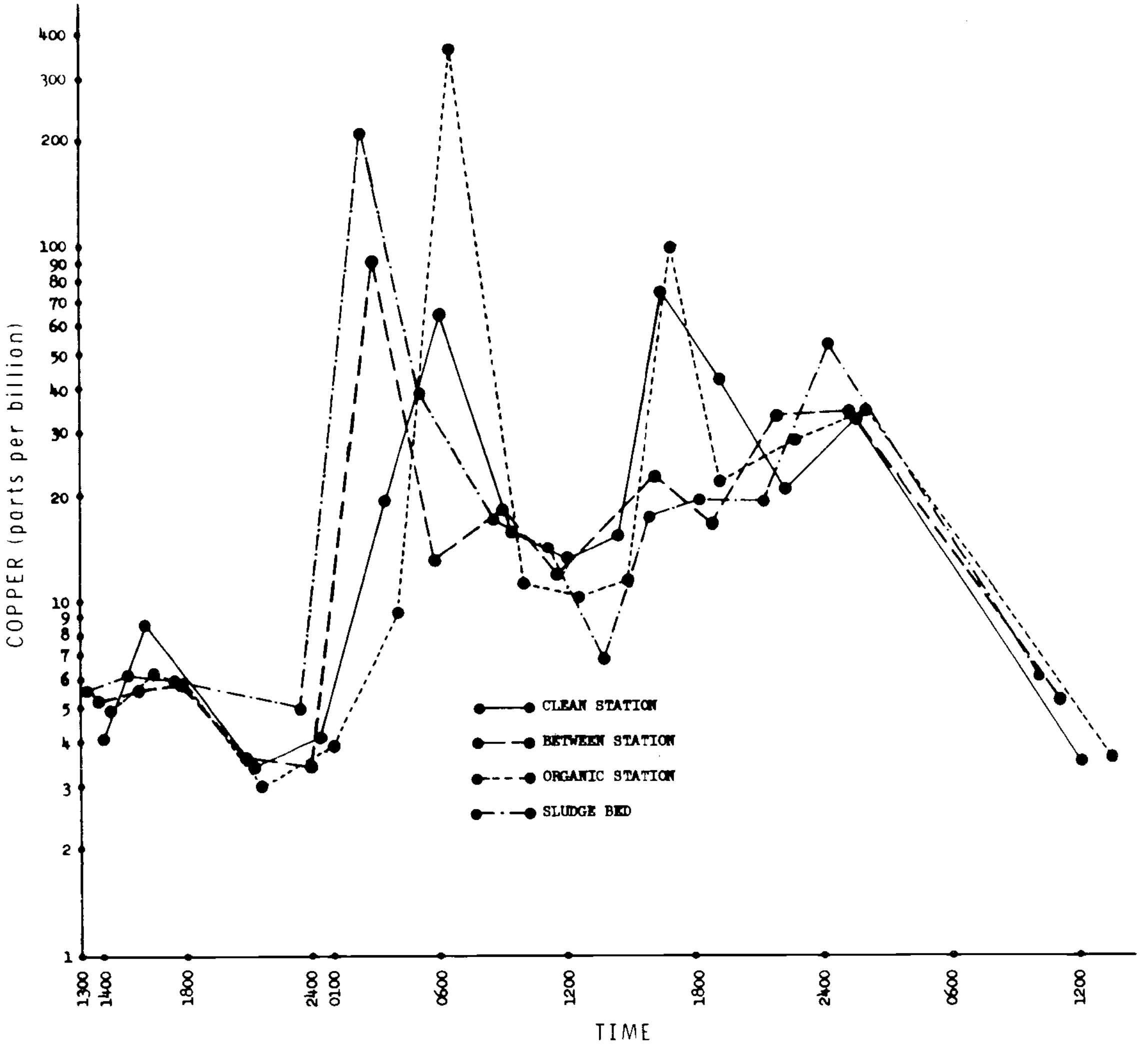


Figure 24. Copper at the sediment-water interface, September 20-21, 1965.

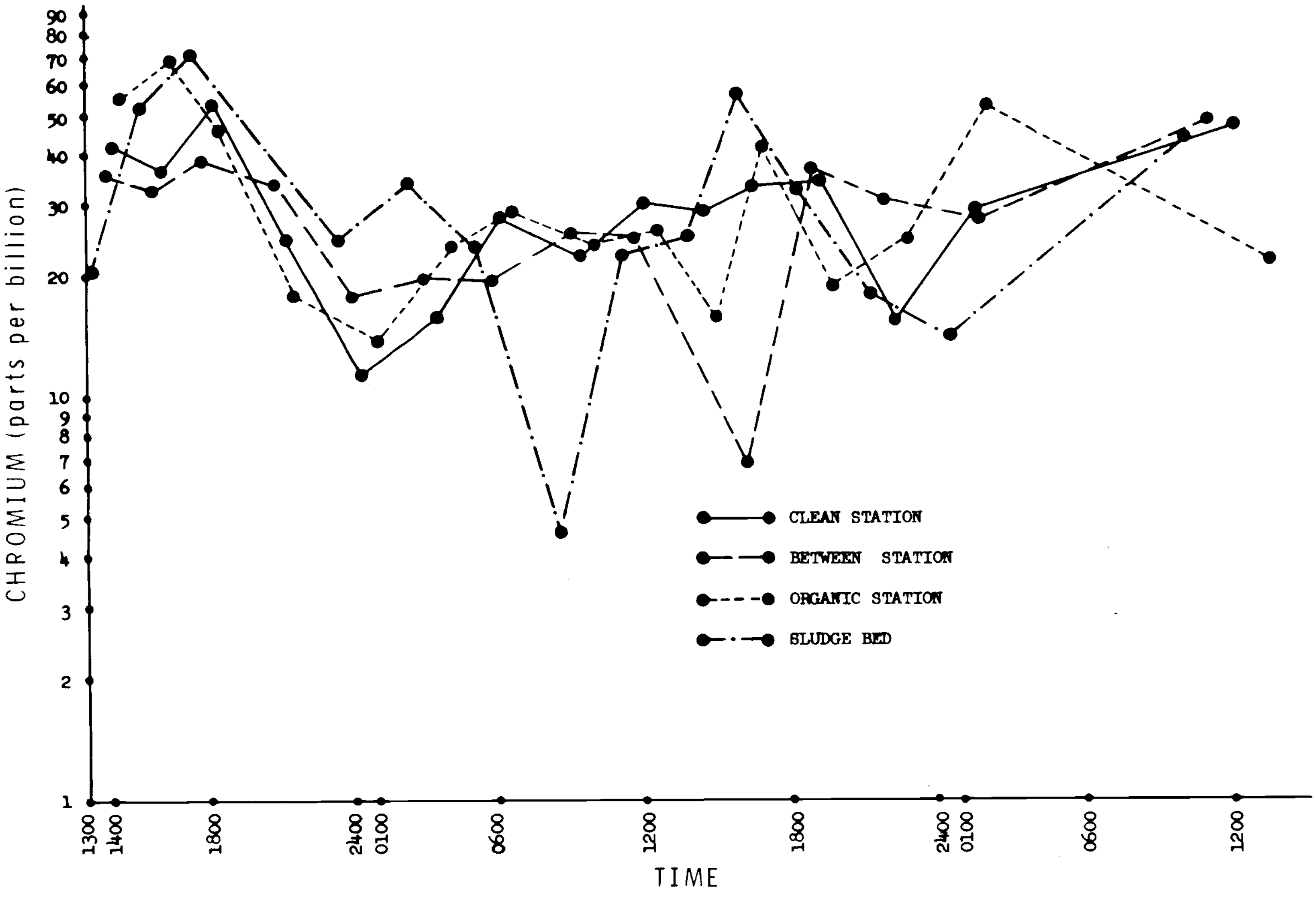


Figure 25. Chromium at the sediment-water interface, September 20-21, 1965.

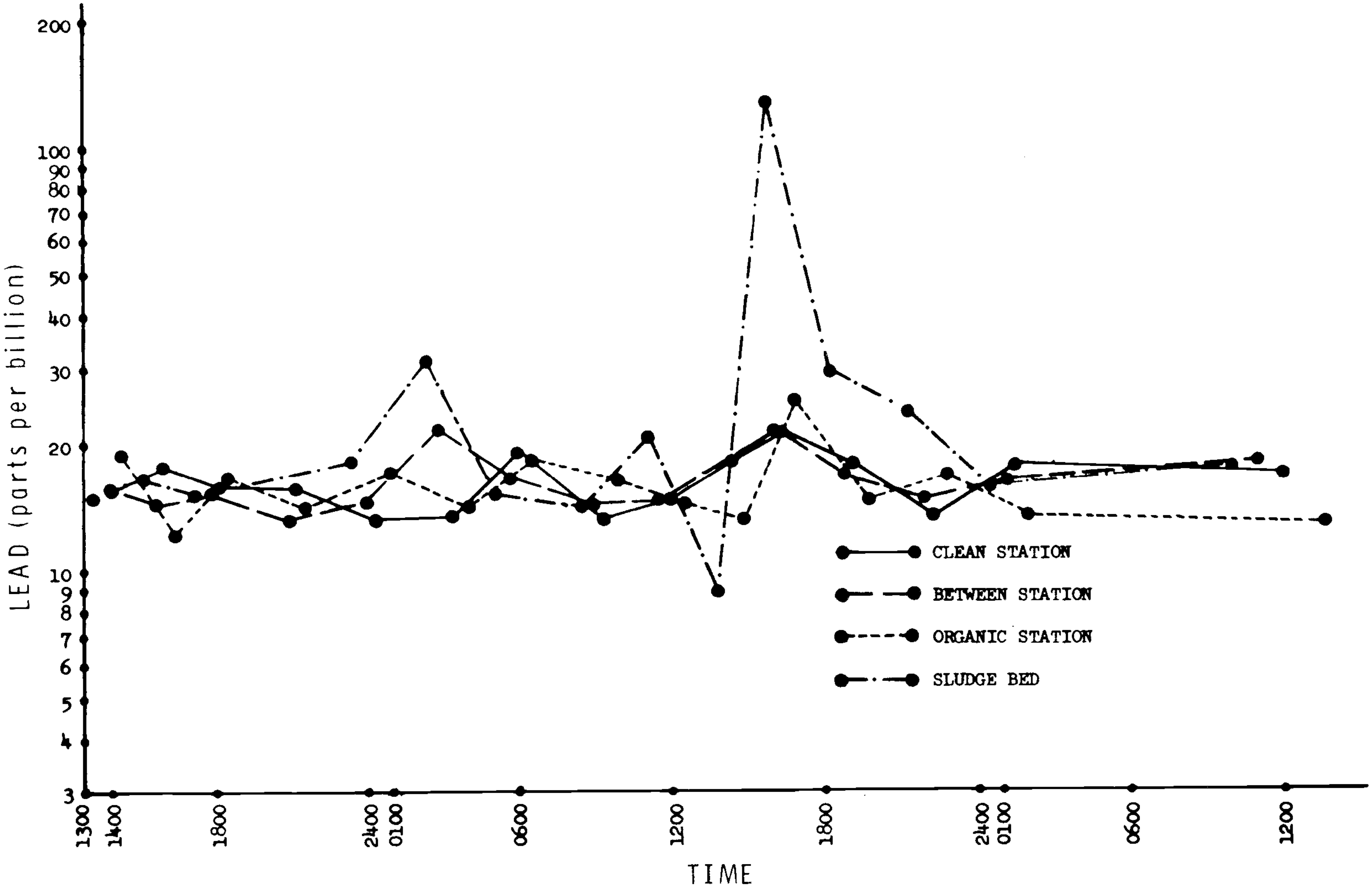


Figure 26. Lead at the sediment-water interface, September 20-21, 1965.

released effluents to adjacent waters of Puget Sound, which could be carried to the Bay by currents.

e. It would appear the effect of the current and effluents was to create a bay-water environment having varying and unpredictable amounts of metals. Nevertheless, some trends are apparent in the graphs and tables.

1'. For <u>copper at the sediment interface</u>, the average values exceeded by many times the value of the open ocean. The minimum values of 3.4 to 5 ppb for the stations, which are close to the natural values of the open ocean, suggest there were periodic discharges of copper rather than continuous.

There were at least three distinct peaks of copper discharge where all stations experienced an increase. From Figure 24 it would appear that the Sludge Bed experienced the discharge first, followed by the appearance of the metal at the other stations.

The average values for copper at the four stations were quite different. The Organic Station experienced the highest amount instead of the Sludge Bed. It is possible that sampling of the latter occurred after peak discharge, resulting in a lower than actual value, producing an average lower than actual.

2'. For <u>lead at the sediment interface</u>, both the average and minimum values consistently exceeded the open ocean values, suggesting the Bay was experiencing chronic lead pollution.

On two sampling occasions, the Sludge Bed exhibited significant increases in lead, and these seemed to be reflected in a very minor way at the other stations. The Clean, Between, and Organic Stations were close to each other in average values, the Sludge Bed was slightly more.

3'. The average and minimum values of chromium at the sediment interface of the Bay greatly exceeded open waters. The minimum value for the Bay was 92 times the latter. There did not seem to be any overall pattern of distribution; at times some stations increased while others declined. The average values of the four stations were quite similar.

f. The concentrations of metals in the sediment greatly exceeded their concentrations in the ambient water.

Table 19. Copper, Chromium, and Lead in the Sediment

Station and metal	Sediment-water interface average (ppb) 1965	Total Sediment 1965 (ppm)	$\frac{\text{Sediment}}{\text{Interface}} \times 100$ 1965	Total Sediment 1968 (ppm)	$\frac{\text{Sediment 1968}}{1965} \times 100$
Clean					
Copper	21.5	3.20	148.8	3.85	120.3
Lead	16.2	11.75	725.3	.55	4.7
Chromium	29.8	*	*	*	*
Between					
Copper	19.1	6.30	329.8	3.80	60.3
Lead	16.3	20.25	1243.3	5.05	24.9
Chromium	28.2	*	*	*	*
Organic					
Copper	40.9	11.40	278.7	7.70	67.5
Lead	16.0	38.90	2431.3	14.45	37.1
Chromium	30.5	*	*	*	*
Sludge Bed					
Copper	30.3	8.65	285.5	29.95	346.2
Lead	26.4	205.65	7789.7	16.20	7.9
Chromium	31.4	*	*	*	*

* Just barely detectable--all stations about same.

1'. In the sediment the copper was concentrated about 150 to 330 times greater than in the sea water. There appears to be no correlation between the amount of copper in the interface water and the amount in the sediment. However, the sediment copper clearly increased from Clean to Between, to Organic Station. For unknown reasons, the Sludge Bed copper was below that of the Organic Station.

By 1968, copper in the sediment had changed considerably. The Clean Station, which was lowest of all stations in 1965, stayed essentially the same. The Between and Organic Stations were reduced over 30 percent, but the Sludge Bed increased almost 350 percent. One might expect a copper reduction due to the discharge of the sewage elsewhere, but not the increase observed at the Sludge Bed.

2'. In 1965, the quantity of lead paralleled the amount and deposition of organic material, though not in exactly the same proportion. The Clean Station, with the lowest organics, had the lowest lead; the Sludge Bed had the highest, the other stations being intermediate. The amount of lead at the Sludge Bed was surprisingly high, supporting the idea that most lead made its way into Shilshole Bay via the North Trunk Sewer from storm drainage. The fact that so much of the lead remained at the Sludge Bed, instead of being carried to the other parts of the Bay, suggests it was adsorbed to larger organic or inorganic particles, which were then deposited locally. By 1968, three years after shutdown of the Sewer, lead at the Sludge Bed had been reduced to but 7.9 percent of its former value.

3'. Chromium was barely detectable in the sediment. From this we may assume it was either present in very small amounts or it did not lend itself to the extraction procedures used. Its presence in the sediment might be suggested by its presence in theinterface water in amounts equal to or exceeding the amounts of lead and copper, which did appear in quantity in the sediment. But had chromium been in the sediment, surely the 48-hour extraction treatment with hydrochloric acid and EDTA would have yielded some amounts of chromium from hydroxide or sulfide precipitates that presumably would have been formed at least at the Organic and Sludge Bed Stations.

The answer seems to lie in what chemical form chromium entered Shilshole Bay and in what form it existed at the sediment-water interface. Chipman (1967) found that if trivalent chromium was added to seawater, there was rapid formation of colloidal chromic hydroxide which then precipitated and adsorbed to surfaces. If hexavalent chromium was added to sea water, it remained unchanged in a true ionic solution.

It appears likely hexavalent chromium entered Shilshole Bay and remained in that state to become more diluted as the water moved about and out of the Bay. According to Zajic (1969), hexavalent is changed to trivalent chromium in the presence of organic matter and reducing agents, both abundant in some Bay sediments. It may be the sediments of the Organic and Sludge Bed Stations were sufficiently stagnant to prevent mixing of interstitial and interface waters and formation first of the trivalent ion and then chromic hydroxide precipitate. At the Between and Clean Stations the sediment may not have been in a reduced state; therefore, chromium would remain in the hexavalent form in the interstitial water.

g. For metals, the use of acid and then EDTA was to provide two levels of extraction: one level to simulate the animal's handling of the sediment, and the other to provide a more complete extraction to give a total amount of metal present the animals might obtain.

Table 20. Copper and Lead Extracted from Sediment by EDTA and 0.1 N HCl

Metal and Extraction Procedure	Clean Station		Between Station		Organic Station		Sludge Bed	
	1965	1968	1965	1968	1965	1968	1965	1968
Copper (ppm)								
0.1 N HCl	1.60	3.25	3.55	3.10	3.00	6.90	2.10	14.95
EDTA	1.60	0.60	2.75	0.70	8.40	0.80	6.55	15.00
Total	3.20	3.85	6.30	3.80	11.40	7.70	8.65	29.95
Lead (ppm)								
0.1 N HCl	8.10	0.00	11.80	2.30	13.05	6.80	46.15	7.95
EDTA	3.65	0.55	7.45	2.75	25.85	7.65	159.50	8.25
Total	11.75	0.55	19.25	5.05	38.90	14.45	205.65	16.20

In 1965, hydrochloric acid yielded more or equal amounts of both copper and lead than did EDTA at the Clean and Between Stations; at the Organic and Sludge Bed Stations, it yielded less. The meaning of this is not clear, but it should be remembered that the last two stations had higher organic content. In 1968 acid extraction yielded approximately equal or more copper than EDTA, but the reverse was true for lead. Since the organic content had been reduced in 1968, the meaning of these results is not clear.

With the exception of the copper at the Sludge Bed, the total amounts of both metals became reduced from 1965 to 1968. It is likely the metals had leached from the sediment. But the Sludge Bed experienced an unexplained increase.

2. Biological Considerations

a. It is generally accepted by biologists that most heavy metals are essential or they are at least not harmful in trace amounts to organisms; in larger quantities they are toxic. As mentioned earlier, copper, chromium, and lead, known to enter Shilshole Bay via the North Trunk Sewer, were measured as possible toxicants both in the sediment and in the water at the sediment-water interface.

During sediment sampling and analysis no attempt was made to distinguish metals associated with organic and inorganic particles and those dissolved in the interstitial water. Since the interstitial water was allowed to drain through the sediment cores after collection, any metals measured in the sediment were associated with the particles. It was unfortunate that the interstitial water was not collected and analyzed, for it provides an environment in which the organisms are bathed and from which they may absorb metals directly through the epithelial surfaces. It is likely that the amounts of metals in the interstitial water of Shilshole Bay sediments were very small. Bryan and Hummerstone (1971) worked with sediments very high in copper and found that compared with the total concentration of copper in the sediments, the concentration in the interstitial water was extremely low.

b. The question of relevance is whether the quantities of the metals found in Shilshole Bay waters and sediments were sufficient to influence the distribution and abundance of the animals.

Much of the pertinent literature was brought together by Eisler, (1973) who assembled 567 abstracts of papers that deal with the biological effects of metals on both fresh water and marine organisms. Earlier Doudoroff and Katz (1953) provided a critical review of the literature on the effects of metals on fish. At least two other important papers have been published. Bryan and Hummerstone (1971) considered the question of copper toxicity in British estuaries, and Bryan (1971) has an excellent review paper covering such topics as absorption, concentration, excretion, storage, and toxicity of copper, lead, and other metals. Examples will be selected from these papers.

c. Before each metal is considered separately, some general remarks about the toxicity of metals and their measurement is in order.

1'. The literature shows that larval stages and often juveniles of a species are more susceptible to metals than the adults. Thus while the concentration of a certain metal may not be limiting to the adults, it may through its action on younger forms prevent a species from living in an area.

2'. The degree of sensitivity of animals to a particular metal may be extremely variable. The surface of the organism may also be a factor in the sensitivity to the metal. Soft bodied animals like polychaete worms readily absorb some metals through the epidermis, while chitin-containing surfaces, as seen in crustaceans, slows absorption.

3'. Metals may have synergistic or antagonistic effects with other metals or materials that increase or decrease their toxicity. Changes in pH may increase or decrease toxicity. Increased metabolic activity, caused by increased temperature, may significantly increase the effect of certain metals. Bryan (loc. cit.) is of the opinion that most of the factors affecting toxicity owe their influence to changing rates at which the metals are absorbed. These factors may affect the ionic state of the metals. Thus, if an environmental factor enhances the toxicity of a particular metal it is because through its influence the offending metal is absorbed more rapidly or readily by the organism.

d. Chromium

The uptake and toxicity of chromium varies greatly between species. For example, the threshold for long term toxicity in *Nereis virens*, a polychaete worm, was 600 to 700 parts per billion (ppb). For the crab *Carcinus maenas*, 20,000 to 40,000 ppb and for the small shrimp *Leander squilla*, 5,000 ppb. A variety of estuarine mollusks, including *Mercenaria mercenaria*, *Mya arenaria*, and *Crassostrea gigas* and *virginica* experienced normal mortality at about 100 to 200 ppb chromium.

It would appear from the values given by Bryan (loc. cit.), Eisler (1973), and Doudoroff and Katz (1953), that chromium in Shilshole Bay does not constitute a hazard to animals. The highest value measured of 71 ppb in the interface water appears to be considerably below any recorded short or long term toxicity studies, and of course the average and most of the rest of the values were much lower yet. Since chromium was not measurable in the sediment it does not constitute a hazard in terms of uptake via the digestive tract of sediment-feeding infauna.

e. Copper

Copper is essential for the respiratory pigment in the blood of some annelids, crustaceans, and mollusks; in excess it is toxic as are the other metals. In general it is more toxic than lead, which is more toxic than chromium. The amount of copper toxicity varies not only with the species but certain physical and chemical factors, which have been discussed by Bryan (loc. cit.).

The levels of toxicity have been recorded for many species, but a few examples will serve to illustrate these amounts. At more than 100 to 500 ppb it was toxic to oysters; but with long-term exposure to more than 20 ppb the clam *Mya arenaria* was adversely affected. The threshold of toxicity for Nereis virens was about 1000 ppb, for *Carcinus maenas* 1000 to 2000 ppb, and for *Leander squilla*, about 500 ppb. The marine copepod *Nitocra spinipes* experienced 1.3 percent of mortality in 24 hours with 26 ppb while *Daphnia magna's* growth was inhibited with 56 ppb. For other examples see the paper cited earlier.

Larval and young stages are more susceptible to copper than the adults. Adult crayfish (*Orconectes rusticus*) were killed by 1000 ppb in 16 days, while juveniles were killed in six days, and younger stages in .5 days. Embryos of the same species exposed for 13 to 14 days to 60 ppb copper had about 1 percent mortality. The pluteus larvae of the sea urchin *Paracentrotus lividus* was affected by low concentrations: at 10-20 ppb body growth was retarded; 30 ppb affected growth of the larval arms; 50 ppb was lethal.

Was the amount of copper present in Shilshole Bay sufficient to have been involved in the distribution and abundance of the animals? For the water at the sediment-water interface, the answer is possibly; for the sediment, we don't know. From Table 3 and Figure 24, we see in the interface water the average amounts of copper plus their high values exceeded the tolerance values for some adult, juvenile, and larval animals. According to Bryan (loc. cit.) many animals are affected by concentrations as low as only an order of magnitude higher than normal sea water.

It is extremely difficult to assess the effect of copper in the sediment on the animals. All bio-assay tolerance values are based upon metals dissolved in water into which are placed the organisms. We do not know the extent to which metals are obtained from the sediment through the digestive tract. But Bryan, (loc. cit.) and Bryan and Hummerstone (1971) working in English estuaries, found *Nereis diversicolor* lived successfully in sediments containing almost 4,800 parts per million copper, which was over 420 times the maximum found in Shilshole Bay in 1965, and about 160 times in 1968. The lowest values these workers found was 38 ppm. They found the concentration of copper in *Nereis* increased with the increase in the sediment. We do not know if this species is representative of the other animals. The relatively low amount of copper in our sampled sediments suggests its effects are probably minimal.

f. Lead

The literature is sparse on the toxicity of lead to invertebrates, especially those living in marine waters. Several estuarine bivalves (Bryan, loc. cit.) were subjected to various concentrations of lead. It was found they concentrated the metal from sea water and accumulated it in various organs. Depletion from the tissues was a relatively slow process. For the oyster *Crassostrea virginica* there was a 12-week TL_m value of 500 ppb and an 18-week TL_m of 300 ppb. Concentrations of from 100 to 200 ppb induced noticeable changes in mantle and gonadal tissues in 12 weeks of exposure. Fresh water tubificid worms experienced respiratory difficulties due to production of mucus caused by presence of lead.

As mentioned earlier, it appears that Shilshole Bay was suffering from low-level, chronic lead poisoning where the minimum values measured for the interface water exceeded by 5 to 9 times the amount found in open, unpolluted waters (Table 18). Even though the highest value measured of 127 ppb was substantially less than the toxicity values reported (Bryan, 1971; Eisler, 1973), the effects of the lead may have produced subtle effects in younger organisms.

The remarks made concerning the difficulty in evaluating the copper in the sediment apply equally to the lead. However, the amounts of lead at the Sludge Bed, for reasons discussed elsewhere, were sizable and it may be that this material was one more environmental variable that was responsible for the reduced number of species able to survive at this station.

Current velocity and direction

1. Current velocity affects organisms by creating or alleviating stagnant water conditions, and by determining the amount of inorganic and organic materials that are able to be deposited on the sediment. Sediments beneath strong and persistent currents tend to be characterized by low sediment organics, larger and well sorted (even sized) inorganic particles, and an aerobic interstitial environment. When currents are weak there is a settling out of organic and the finer inorganic materials which tend to clog the interstitial spaces and drive

the bottom anoxic. Each of the two environments would host their own kinds of organisms.

2. The currents measured during 1965 over the Organic, Clean, and Between Stations were quite similar and weak. This produced at the Clean and Between Stations an inorganic sediment profile that was practically the same (see Table 5, Fig. 19). The Organic Station experienced about the same maximum velocity, but showed a higher average current velocity during the period of sampling because fewer slack currents entered into the calculations.

Table 21. Current Velocity, September 20-22, 1965

Station	Current velocity (ft/second)		% Slack or Immeasurable Currents
	Average	Maximum	
Clean	.123	.30	20
Between	.130	.32	20
Organic	.163	.34	13
Sludge Bed	.289	.605	0

On the basis of velocity, one would expect the Organic Station sediment profile to be similar to the Clean and Between Stations; instead it had large amounts of silt and clay. It would appear, as mentioned elsewhere, the silt and clay were coming from construction activities on shore nearby. In 1968, long after these activities were over, the newly deposited sediments at the Organic Station had become quite similar to the other two stations, tending to corroborate the source of the silt and clays.

3. The current meters measured the velocity and direction of water past the sampling station within a few inches of the sediment. While the meters provided some useful information at the sampling stations, they did not provide information as to where the water over the sampling stations came from or where it was going. This is to say, it was not possible to determine the net magnitude or direction of water movement.

Drogue studies (Brown and Caldwell, 1958) show the water of Shilshole Bay has an overall well defined pattern of flow, at least at the outer periphery (see Fig. 1), and also eddy currents. Since the water in the Bay was part of the same water system, the lack of a species at a sampling station must be explained on the basis of unfavorable environmental conditions, not upon physical isolation of the sampling stations from each other.

4. Examination of current vector diagrams show the Sludge Bed was in the direct path of tidal flow and the water moved past the station as expected. But the other three stations show peculiarities in their patterns that warrant some explanation. The Clean and Between Stations, being close to shore and sheltered somewhat by the land north of the entrance to the Ship Canal, were out of the main tidal pattern. This produced, as noted in results, water movements not fitting the overall pattern. A similar situation existed at the Organic Station, which was quite close to shore, but it also was close to West Point, and so evidently experienced some influence of the general tidal pattern.

Organic Material

1. Introduction

The purpose of measuring the organic content of sediment was to provide an index of comparison between sampling stations and to assess the amount of potential food available to organisms.

The evidence in this and other studies (Hynes, 1963) suggests that the deposition of organic material on the bottom is one, if not the prime factor, altering all other sediment variables and thus affecting the distribution and abundance of the organisms. The deposition of organic material on ocean bottoms is continuous, as is its utilization for food by organisms. Thus one might consider the upper layers of the sediment to be a dynamic microcosm. It is ironic, that while the organic content may be the prime factor of all the variables measured in this study, it is the one most subject to sampling and analytical error. Nevertheless, the results obtained are valuable, and meaningful trends are evident upon examination of the data.

Our ecosystem--Shilshole Bay--was a dynamic, open system, with organic energy input via a sewer pipe. It had been receiving organic enrichment for some 50 years and presumably had developed well established and stable environmental variables and probably communities.

2. Sources of food for benthic animals

Under natural conditions, benthic animals obtain food 1) by feeding upon other organisms in their community, 2) by filtering plankton and organic debris from the water column, 3) by consuming dead materials produced locally, and 4) by consuming organic debris deposited in the sediment from the water column.

In the last case, as the quantity of the usable organic debris falling upon the bottom is increased, the numbers of some kinds of organisms will increase. But other species may be affected unfavorably. With heavy deposition of organic material, species composition may change considerably.

3. Since the organic enrichment came to the sediment via the water column, the quantity of particulate organics in the water should be considered.

Table 22. Particulate Organics in the Water at the Sediment-Water Interface in 1965

Station	Average Value (PPM)	Range of Values (PPM)
Clean	2.18	1.27 - 4.74
Between	1.63	.95 - 2.75
Organic	1.60	.92 - 3.11
Sludge Bed	8.68*	1.19 - 76.41

*Average would be 3.03 without the high value of 76.41.

At the Sludge Bed the average particulate organic is not much larger than the other stations if the former's larger values are not included (Table 22). But evidently the several high values measured are the ones responsible for the difference between stations. It appears that the sewage, being basically of fresh water, is lighter and travels to the surface as a boil where it layers over the salt water. Evidently the persistent strong currents at the Sludge Bed (Table 3, Fig. 20) prevented most of the lighter sewage from settling to the bottom, whereas the larger, heavier materials--organic and inorganic--settled rapidly to the bottom and did not appear in the water at the sediment-water interface, where the samples were taken. But under a certain set of circumstances, which cannot be explained solely on the basis of current velocity, a quantity of finer sewage particles settled to the bottom periodically (Fig. 27). Whether this was due to a higher organic content of the sewage at the time, or to some peculiar current or lack of current which was undetected, is unknown. The latter seems more likely.

The data show other peculiarities. The Clean Station has an average and range slightly greater than the Between and Organic Stations. It may be the tides and currents at the time of sampling were such as to create conditions to carry sewage-laden water over the Clean Station. Whatever the cause, it is reasonable to assume this was not a frequent event, else the Clean Station would exhibit a buildup of sediment organics, which it did not.

4. Problems of analysis for organics in sediment

We know that each analytical method commonly in use today has serious deficiencies that raise a number of questions that must be considered in evaluating our results. For example, should living organisms, presently unavailable to sediment feeders but which will of course eventually die, be considered an available organic part of the sediment? How is one to consider the refractile organic materials, which according to some authorities (Bader 1954a) constitutes a fairly sizable portion of the organics?

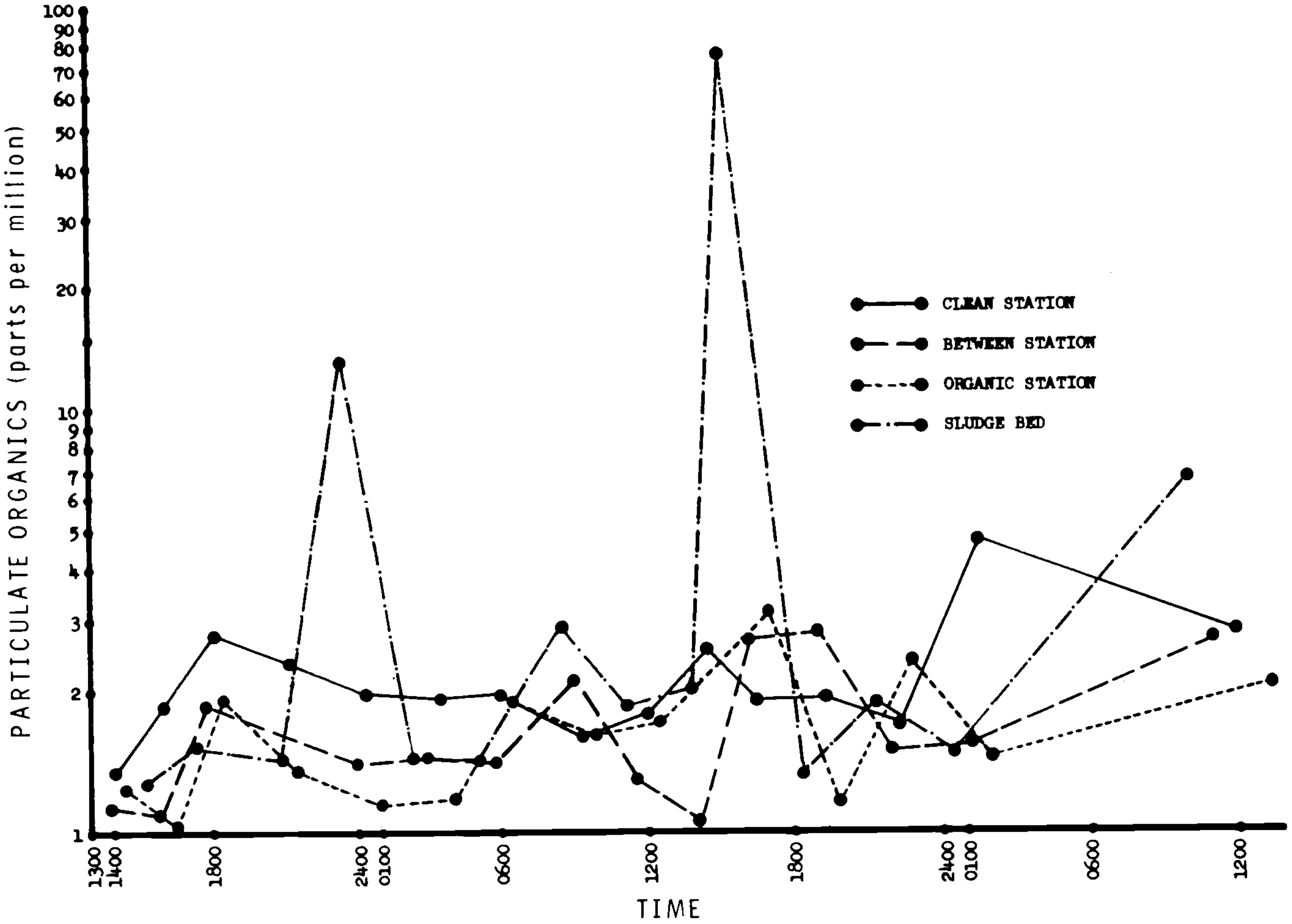


Figure 27. Particulate organic content of water from sediment-water interface, September 20-21, 1965.

It seems to the writer, ideally, a measurement of organic content of the sediment would include that material which is biologically degradable and usable in a relatively short period of time, so that it is used before deep burial makes it unavailable. It should not include, at least, the larger living organisms. It should not include the refractile organics such as lignin, which according to Bader (1956), is extremely resistant to microbial decomposition and as such has negligible value as a food source. It follows that areas with large amounts of wood wastes will give false high values for usable organic material.

5. Methods of analysis

There are at least three methods widely used for quantitative analysis of sediment organics, and a fourth that measures organics indirectly by measuring the nitrogen component and then converting the value. Each of the three has its own shortcomings, and none distinguishes between sources of the organics present or their usability in the food web. The nitrogen method, according to Bader (1954b), is unacceptable to define the amount of organic material present. He found the conversion factor of nitrogen to organic material varied from 5.2 to 50.

As noted in the Materials and Procedures section, one method (volatile materials) was initially used exclusively in this study but later a second (organic carbon analyzer) was added to attempt to remedy the deficiencies of the first. To understand the results in this paper, a few words about each method is warranted.

a. Wet Oxidation, also known as chemical, or dichromate oxygen demand, fails to include some organic compounds that are biologically available, while including some others that are not readily available to organisms. The test does not measure the presence of a specific entity, but rather the ability of the dichromate acid mixture to oxidize the organic matter in the sample.

b. The Organic Carbon Analyzer measures all organic material as carbon dioxide produced when the sediment is combusted at 1800°C. No atoms of hydrogen, oxygen, sulphur, nitrogen, or others present as part of the organic molecule are measured, resulting in values for organic material which could be considerably lower than actual. Bader (1954b) found in sediment samples taken from the Gulf of Maine, that only 40 to 67.2 percent of the organic material was carbon. He further concluded the amount of organic carbon to be quite variable between samples with conversion factors to total organics, ranging from 1.49 to 2.50, making conversion an inaccurate procedure.

c. All Volatile Materials are destroyed when sediment is combusted at 600°C in a muffle furnace. The weight reduction is considered as organics lost. The values can be somewhat elevated because any water held by soil particles, not removed by 103°C pre-treatment, is now lost and recorded as organics. This is especially true of soils with larger amounts of the finer clay and silt fractions.

Even though the organic carbon analyzer has its drawbacks to define organic content of the soil, it appears to be less susceptible to error than the volatile materials methods, if no attempt is made to convert the organic carbon to total organics. If the particle size composition of the sediments were uniform at all sampling stations so equal amounts of bound water were present in the samples, then the volatile materials method would have greater value than it does in this study. Finger and Wastler (1969), working in Charleston Harbor, found a sufficiently linear relationship between organic carbon and volatile materials to conclude the latter as present in each of their samples serves as an estimate of the total quantity of organic material present.

The problems of analysis and interpretation of the results insure that it will be some time before a completely acceptable method of organic analysis is available. There is a need for detailed, quantitative analysis of carbohydrates, lipids, and protein in sediment, and their usability to sediment feeding organisms.

7. Usability of the data collected

In spite of the deficiencies of the two methods used, they have value when the four sampling stations are compared to each other and the methods are also compared. Table 23 presents data related to the organic content of the sediment. A bit of manipulation of the figures shows some interesting trends.

Table 23. Organic content of the sediment.

Station	1965				1968				Org. Car.	Vol. Mat.	Silt-Clay	Total Nitrogen
	Percent Volatile Material	Percent Organic Carbon	$\frac{\text{Vol. Mat.}}{\text{Org. Car.}}$ X 100	% Silt-Clay	Percent Volatile Material	Percent Organic Carbon	$\frac{\text{Vol. Mat.}}{\text{Org. Car.}}$ X 100	% Silt-Clay	$\frac{1968}{1965}$ X 100	$\frac{1968}{1965}$ X 100	$\frac{1968}{1965}$ X 100	$\frac{1968}{1965}$ X 100
Clean	1.31	.50	262	7.36	1.35	.23	587	10.18	46	103	138	47
Between	2.46	.92	267	7.24	1.38	.45	307	3.87	49	56	53	42
Organic	4.35	1.11	392	43.9	1.74	.44	395	10.17	40	40	23	32
Sludge	8.37	3.28	255	2.32	3.25	1.36	239	2.57	41	39	111	31

8. Trends

a. The amount of organics significantly varies between sampling stations.

Table 24. Amount of Volatile Materials and Organic Carbon in the Sediment

Station	1965 Percent Volatile Materials	1965 Percent Organic Carbon	1968 Percent Volatile Materials	1968 Percent Organic Carbon
Clean	1.31	.50	1.35	.23
Between	2.46	.92	1.38	.45
Organic	4.35	1.11	1.74	.44
Sludge Bed	8.37	3.28	3.25	1.36

1'. 1965: the amount of both volatile materials and organic carbon were different from station to station as follows:

Sludge Bed > Organic Station > Between Station > Clean Station

2'. 1968: the differences were not as obvious.

For volatile materials:

Sludge Bed > Organic Station > Between Station = Clean Station

For organic carbon:

Sludge Bed > Organic Station = Between Station > Clean Station

b. The organic content became reduced between 1965 and 1968.

For all stations the total quantities of volatile materials and organic carbon were not as great in 1968 as 1965. This is to be expected, since the areas had been deprived of their organic enrichment, and the measurable sediment organics--both dead and living--had been depleted or reduced. The reductions are more obvious using the ratios of the 1968/1965 values:

Table 25. Yearly Ratios of Organics of the Sediment

Station	% Organic Carbon $\frac{1968}{1965}$ X 100	% Volatile Materials $\frac{1968}{1965}$ X 100
Clean	46	103
Between	49	56
Organic	40	40
Sludge Bed	41	39

Thus we see for organic carbon, at all stations there had been a reduction to not more than 49 percent of 1965 values. For volatile materials, reduction had been to less than 56 percent, except at the Clean Station where the ratio remained the same. This last value is deceptive, because the organic carbon content was reduced almost 50 percent, but it experienced a 38 percent increase in water-holding, silt-clay that probably elevated the volatile materials and affected the ratio.

c. <u>Volatile materials always exceeded organic carbon</u>.

Table 26. Ratio of Volatile Materials and Organic Carbon

Station	1965 $\frac{\%\text{ Volatile Material}}{\%\text{ Organic Carbon}}$ X 100	1968 $\frac{\%\text{ Volatile Material}}{\%\text{ Organic Carbon}}$ X 100
Clean	262	587
Between	267	307
Organic	392	395
Sludge Bed	255	239

In all cases, 1965 and 1968, the amount of volatile materials exceeded organic carbon from about 250 to almost 600 percent. The higher value was most likely due to the increased silt-clay fraction at the Clean Station that holds water during preparation for volatile materials analysis.

d. <u>The sediment nitrogen reflects the three-year reduction in organic carbon</u>.

Table 27. Comparison of Sediment Organic Carbon and Nitrogen

Station	% Organic Carbon $\frac{1968}{1965}$ X 100	% Nitrogen $\frac{1968}{1965}$ X 100
Clean	46	47
Between	49	42
Organic	40	32
Sludge Bed	41	31

The nitrogen content of the sediment has been reduced in all cases to less than 47 percent of its previous 1965 value. The decrease approximates the decreases of organic carbon. This is to be expected since nitrogen is a component atom of protein, a nutrient fully and rapidly utilized by organisms.

e. The quantity of silt-clay fraction affects the values for volatile materials.

The silt-clay fraction of sediment holds water, some of which is not removed by 103°C pretreatment for volatile materials analysis (Van Andel, 1969). Combustion at 600°C liberates the water and produces inflated values for organic content. The more silt-clay, the higher the volatile values and the larger becomes the value for the ratio: $\frac{\text{\% Volatile Materials}}{\text{\% Organic Carbon}}$. The effect can be seen for both 1965 and 1968 although it is not as obvious for the latter.

Table 28. Comparison of Fine Inorganic Fractions with the Organic Materials in the Sediment

Station	1965 $\frac{\text{\%Vol. Mat.}}{\text{\%Org. Car.}}$ X 100	Percent Silt-Clay	1968 $\frac{\text{\%Vol. Mat.}}{\text{\%Org. Car.}}$ X 100	Percent Silt-Clay
Clean	262	7.36	587	10.18
Between	267	7.24	307	3.87
Organic	392	43.9	395	10.17
Sludge Bed	255	2.32	239	2.57

The $\frac{\text{\% Volatile Materials}}{\text{\% Organic Carbon}}$ ratio may also be affected by the atoms unmeasured by the organic carbon analyzer, in essence, the kind of organics present. In 1965 it is probable the kind of organic material in the sediment was not a big factor affecting the ratio, because all areas were receiving organic enrichment to varying degrees from the same source. But perhaps in 1968 we need to look to a change in the quality of organic content of the sediment to help explain the ratio values. Visual observations show clearly by 1968 there had been considerable change in the sediment and presumably in its organic composition, since the organic enrichment had ceased three years previously. For example, in 1965 the Sludge Bed was a dynamic pool of high organic enrichment, open to the water above; in 1968 it had become sealed off and highly stagnant.

f. <u>The organic material dissolved in the interstitial water reflected the quantity of the sediment organic carbon</u>.

In 1965 the organic material dissolved in theinterstitial water paralleled the organic carbon in the sediment so that:

Sludge Bed $>$ Organic Station $>$ Between Station $>$ Clean Station

Table 29. Comparison of Dissolved Interstitial Organics with Sediment Organic Carbon, 1965

Station	Dissolved Organics (PPM)	Organic Carbon (%)
Clean	2.3	.50
Between	4.7	.92
Organic	6.8	1.11
Sludge Bed	8.5	3.28

I am unable to say what, if any, effects the dissolved organics had on the distribution and abundance of the organisms.

g. <u>In 1965 the quantity of sediment organics reflected the rate of deposition of organics</u>.

The rate of deposition of organic material on the bottom was determined by sediment collector as suggested by Reish (1961). The rate paralleled the quantity of sediment organics noted earlier, that is, Organic Station is greater than Between Station, which is greater than the Clean Station. It was not possible to measure rate of deposition at the Sludge Bed because of the fouling of the collector jar by heavy deposition of toilet paper and other organic materials. But it is clear from observation that the rate at this station far exceeded that at the other three.

Table 30. Relationship of Rate of Deposition of Organics and Percent Organics in Sediment, 1965

Station	Organic Carbon (%)	Organic Material Deposited ($Gms/m^2/day$)
Clean	.50	3.7
Between	.92	11.7
Organic	1.11	36.7
Sludge Bed	3.28	container fouled

These results are completely expected since they form one of the basic assumptions of this study, namely, the amount of organic material in the sediment is a function of the rate of fallout enrichment via the North Trunk Sewer.

Support for the importance of the North Trunk Sewer in contributing to the organic enrichment of the area is seen for the rates of deposition just before and after the sewer was shut down. These results are summarized.

Table 31. Deposition of Organic Materials Before and After Sewer Shutdown, 1965

Station	Before (Gms/m^2/day)	After (Gms/m^2/day)	$\frac{\text{After}}{\text{Before}}$ X 100
Clean	3.7	10.7	290
Between	11.7	5.4	46
Organic	36.7	12.2	33
Sludge Bed	Container fouled		

When the sewer was shut off, the Between and Organic Stations behaved as expected--their rates of deposition were greatly reduced. The Clean Station increased however, almost 300 percent. Simultaneous with sewer shutdown, the sewage was discharged to the Ship Canal where the tides and the freshwater discharge from the Government Locks, undoubtedly, caused the sewage to be carried over the Clean Station.

h. <u>The organic carbon to nitrogen ratio increased from 1965 to 1968</u>.

At the Between, Organic, and Sludge Bed Stations, both organic carbon and nitrogen decreased in amount between the two year samplings, but the ratio of the carbon to nitrogen increased suggesting a greater decrease in the nitrogen. This too is to be expected since a sizable part of the nitrogen was tied to biologically usable organics such as protein, which became reduced or exhausted when the sewer shut down.

At the Clean Station, the ratio remained the same for the two years. This is not surprising for the Station received most of its enrichment, before and after the sewer was shut down, from natural organics in the water rather than from sewage.

Table 32. Relationship of Sediment Organic Carbon and Nitrogen

Station	% Organic Carbon / % Sediment Nitrogen 1965	1968
Clean	8.47	8.21
Between	19.17	22.50
Organic	14.23	17.60
Sludge Bed	20.50	27.20

The usability of C/N ratios is subject to question. Bader (1954, 1955), after an extensive review and series of experiments, concluded the particular type of relationship between carbon and nitrogen in marine sediments varies considerably with the result that data from different areas must be treated separately and do not necessarily relate to each other. It should be noted that Bader was dealing with samples in which the organic content was basically natural. However, Finger and Wastler (1969), working in the Charleston Harbor drainage, tried with some success to define the type of pollution by the ratio obtained. They concluded that a ratio of less than 15 would indicate organic sediments of primarily domestic wastewater origin, while above 15 would indicate primarily vegetable material such as wood or other cellulosic material.

For the later kinds they found ratios up to 40 and more. For natural areas they found values of around 12 and the total sediment organics to be low.

The C/N data presented in this paper do not compare closely to the values of Finger and Wastler. Instead, they seem to lie somewhere between the extreme values they found for the two primary sources of enrichment, sewage and vegetable material; they too noted this tendency for their areas of mixed effluents. These data are probably to be expected for Shilshole Bay, since the organic enrichment is both sewage and wood wastes such as bark and shredded wood from log rafts, which were frequently moored there. Even the Sludge Bed contains vegetable matter in the form of seeds and large amounts of decomposing toilet paper and sanitary napkins, whose origin is plant fibers.

B. Biological

Infauna vs. epifauna

In this study the fauna have been divided into two groups: those taken by cores; and those that are larger and motile or are attached and associated with the surface of the sediment. The animals in the cores were identified and their numbers and biomasses were measured. They constituted the major portion of the study and are commonly referred to as infauna (Thorson, 1957). The others were studied in a qualitative manner only, animals being observed and collected only for identification. Members of this last group are often referred to as epifauna, but because of the variety of ways these animals use the sediment surface, some of them may be technically infauna, so the term epifauna is used here in the broadest sense. The prevalent nektonic species were recorded.

Literature

The literature on benthic organisms is copious, a sizable portion of which is taxonomic or of a survey nature. The most useful publication has been the comprehensive study by Ulf Lie (1968) titled: "A Quantitative Study of the Benthic Infauna in Puget Sound, Washington, USA, in 1963-1964." This paper presents an in-depth discussion of various aspects of sampling and also the ecology, distribution, and abundance of dominant species encountered in the Sound. Lie (1968) is the source of most ecological information on species presented here unless otherwise stated.

Species distribution patterns

1. Benthic animals in their distribution may be random, clumped, or uniform. A population dispersion ratio has been calculated for species that were numerically most abundant and when sufficient numbers of samples were collected. As noted by Lie (1968), the degree of patchiness (dispersion) is a function of the number of samples; the results are more reliable when using a larger number of samples.

I have chosen to use the dispersion ratio of variance/mean. When values are less than one, the animals are said to tend toward a more uniform distribution, and values equal to one show random distribution. Values greater than one show clumping, the larger the number, the greater the degree (Smith, 1974).

2. The arithmetic means of number of animals per core sample have been calculated (Table 10). The means, however, do not reflect the

scatter of the data about them. Standard error of the mean was also calculated and this does provide an indication of the scatter, in fact, it is a summation of the scatter. It gives the distance by which the position of the arithmetic mean must be shifted in order to be centered in the data. The smaller the standard error, the more uniform is the scatter around the arithmetic mean.

Since standard error of the mean is a reflection of the number of samples collected $\left(\sqrt{\frac{\text{variance}}{\#\text{ samples}}}\right)$, some of these values provided for species with only a few samples are of limited value.

3. How many individuals of each species would be expected to be found in each core sample at each sampling site? To answer this question double-headed confidence intervals at the 90 percent level were calculated. The two values provide an expected number of animals in 90 out of every 100 cores taken, or it may be considered that 90 percent of the time the collected animals per core will fall within the predicted range. Again, the confidence intervals are of minimal value where the number of samples taken was small.

Adequate sampling

How many samples should be taken to collect sufficient species to define the community? Too few samples may miss some species; too many waste time to process.

From Figure 27a, it appears adequate samples were taken at all stations in 1965 to collect most species present. However, in 1968, the four samples collected at each of three stations (Clean, Between, Sludge Bed) do not appear to have been adequate to define the communities

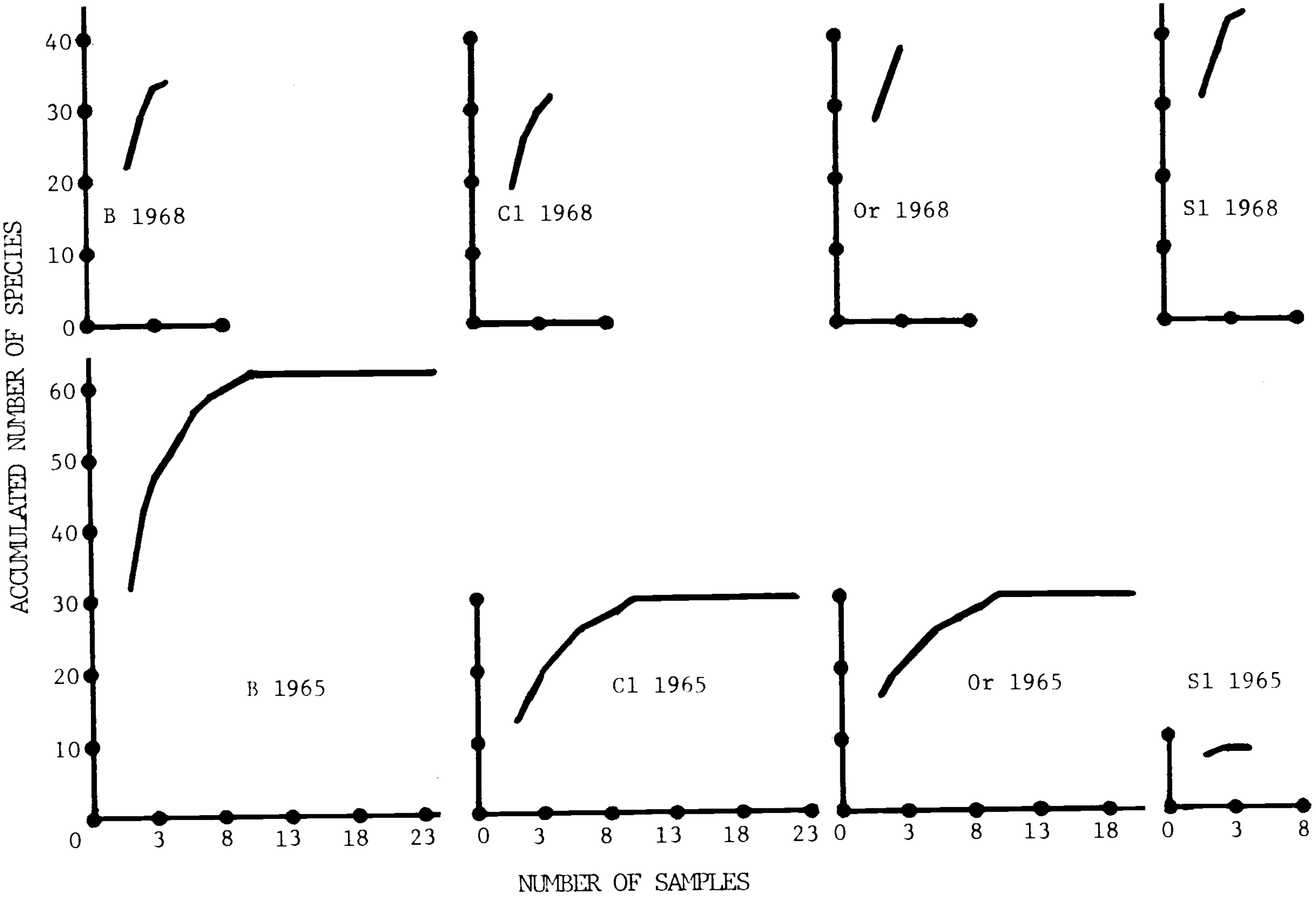


Fig. 27a. Addition of new species with additional sampling

thoroughly. There are indications that the first three samples at each of these stations contained most species and the addition of new species was leveling off with the fourth. Because only three samples were collected at the Organic Station, it is not possible to tell how many species were present in the community.

Abundance error

To be able to compare the abundance of animals between sampling stations and between years, it was necessary to multiply the values for biomass and density at each station by a factor to convert them to square meter area.

Station		Multiplication factor
Clean	1965	2.6068
Clean	1968	14.3375
Between	1965	2.3896
Between	1968	14.3375
Organic	1965	2.8675
Organic	1968	19.1168
Sludge Bed	1965	14.3375
Sludge Bed	1968	14.3375

The use of the multiplication factors assumes, perhaps erroneously, a greater number of cores taken from a station would have given a proportionally greater number of animals. In the case of the bivalves, where graphs are provided of the size distribution by species, use of multiplication factors assume the increases would be in the sizes already collected. But not all size classes were collected at stations even when the animals were abundant. Thus by multiplying our values we are denying the existence of the missing size classes which presumably would be collected with more samples.

In multiplication of the size classes already collected we are also assuming the animals have uniform distribution and thus additional cores would have provided the same number and size as those already collected. Seldom is it true that marine benthos are uniformly distributed, rather they are randomly or probably more frequently clumped. Such is the case for most of our animals (Table 10). In the case of clumped distribution, multiplication of the size data may produce much inflated or reduced values.

Nevertheless, for purposes of comparison, all graphs of bivalve abundance as well as biomass and numbers of other species are reported as per square meter.

Percentage of samples with species error

Another possible source of misunderstanding is the reporting of percentage of samples that have the species present. When many samples are collected at a station there is a number of percentage values that could be used to give their occurrence. With but a few samples taken, as seen at all stations in 1968 and the Sludge Bed in 1965, only a limited number of values can be used to tell the frequency of a species in the samples. Thus if there were only four samples taken, the animals could be reported with only 0, 25, 60, 75, or 100 percent frequency; there are no middle values possible.

Biomass error

Biomass of animals is presented as ash-free dry weight. This provides a means of comparing the weight of animals that may be fleshy and watery with those having heavy endo-or exoskeletons of inorganic composition.

Ash-free dry weight is a measure of the amount of dry, organic material present in an animal, but small amounts of water always remain bound to the organics. The weight does not include inorganic materials that remain after incineration, even though some may have been part of the organic molecule.

Because of the difficulty of blotting animals uniformly before weighing and because animals may contain greatly different percentages of water, wet weight measurements have their greatest value in obtaining a weight where it is not feasible or desirable to dry the animals. Dry weight values are more useful than wet weights because they do not contain the water, but since they include all inorganic materials present, they are less useful than ash-free dry weights.

Wet, dry, and ash-free dry weight may be manipulated to provide several ratios (Table 11). The exact meaning of these ratios is subject to interpretation. For example, if the ratio of dry to wet weight is low, it may be assumed we are weighing amounts of water trapped within the animal's tissues. This is certainly the case for the seapen Leioptilus, which, like all coelenterates, contains large amounts of unbound water. On the other hand, if the ratio value is higher than average, one might assume the elevated dry weight is due to presence of inorganic tubes or calcium-impregnated skeletons. But it may also be due to quantities of inorganic materials found in the digestive tract of animals that are non-selective, sediment feeders.

Animals that do not possess inorganic tubes, skeletons, and/or sediments in the digestive tract have high values for the ratio of ash-free dry weight to dry weight. For example, we found ash-free weight almost 88 percent of the total dry weight in the nematodes and in the organic-shelled snail Alvinia. As one might suspect, the lowest values were found for the small clams, which were weighed with their shells.

The figures obtained for the ratio of the ash-free dry weight to wet weight are the least reliable, but were very useful at times. As noted elsewhere, there were many instances where it was not desirable to dry the animals to obtain a weight. In these cases, a portion of the animals was sacrificed to obtain wet and ash-free dry weights, and then the value of the ratio was used to convert wet weight to ash-free dry weight for the remaining animals.

Foraminifera

Foraminifera are large protozoans that may be present in the ocean in tremendous numbers.

In two cores from the Organic Station in 1965, small numbers ($69/m^2$) of the species Trochammina inflata were recovered. No other station that year had the species. In 1968 they were very abundant, reaching a total of 43,490 per square meter for 100 percent presence in the samples at the Organic Station; they were not observed at the other stations.

A second foram, Elphidium sp., was present in small numbers ($1223/m^2$) in 1968 at the Organic Station and in lower numbers in a few of the Clean and Sludge Bed samples. Unfortunately, the delicate shell of this species is of calcium carbonate and subject to being dissolved by the acids formed in formaldehyde. Therefore, the specimens probably represent only a portion of those present, and the counts are unreliable.

The shells of *Trochammina* are of an organic material with properties similar to chitin (Vinogradov, 1953) and therefore are resistant to formaldehyde damage.

I am unable to explain the distribution of these organisms. If conditions at the Organic Station were too drastic in 1965, why were they not found at the Clean and Between Stations? If the answer involved the need for the fine sediment type found at the Organic Station, then why did they become so abundant in 1968, after the sediment at this station had become so very much like that of the Clean and Between Stations?

The only statement one can make with certainty about the distribution and abundance of *Trochammina* is that in 1965 it was present in small numbers in a restricted area, whereas in 1968 it became very abundant but still in the same restricted area.

Amphipoda

1. During the entire period of study, 1965 and 1968, and at all sampling stations, only ten species of Amphipoda were collected. Lie (1968) at his nearby Golden Gardens collecting site found 29 species, five of which were the same as ours. Wieser (1959b) at Golden Gardens and at nearby Alki Point found nine species, of which only three were the same.

Of the ten Shilshole Bay species, only one specimen each of four were collected: *Parapleustes pugettensis* (1965 Between), *Hyale* sp. (1965 Between), *Photis conchicolor* (?) (1968 Between), and *Orchomene* sp. (1968 Sludge Bed). One specimen of *Corophium* sp. was collected in 1968 at each of the Organic and Sludge Bed Stations. Two of the remaining five species, Photis californica (?) and Aoroides columbiae, appeared for the first time in 1968 in sufficient numbers to consider them to be a regular part of the community. Photis was present in 67 percent of Organic Station cores, 50 percent of the Between, and 100 percent of the Sludge Beds, for a maximum of $1282/m^2$ at the latter Station to be called the dominant amphipod species. For reasons unknown, it was not present at the Clean Station. Aoroides was present in small numbers in 100 percent of the Clean, 50 percent Between, 50 percent of Sludge Bed samples, but was not present at the Organic Station. This species is listed as being associated with various kelp holdfasts and coralline algae (Ricketts and Calvin, 1962), but its distribution in the Bay, suggests other life styles.

Of the three remaining species, Westwoodilla caecula was present only in 1965 (except one specimen, Sludge Bed, 1968) in 21 percent of the Between Station cores. The other two, Synchelidium shoemakeri and another Photis sp. were present in relatively small numbers in 1965 and 1968.

2. Little is known about the habitat needs and food preferences for our west coast species. In an excellent paper by Enequist (1949), feeding habits and other ecology were presented for amphipods of soft-bottom sediments in Scandinavian marine waters. Although only one of our species was present in his area (Westwoodilla caecula), other representative genera or families provide some insight to amphipod requirements.

He found *Westwoodilla caecula* and *Synchelidium* move freely in the water or on the bottom where they burrow in the superficial layer and utilize the deposited detritus of the mudwater interface for food. According to Wieser (1959b) Puget Sound *Synchelidium* are almost always in mud, on muddy sand, or in fine sand. He found *Westwoodilla* restricted to mud and muddy sand.

Enequist notes that members of the family to which *Aorides* belongs (Aoridae) have glands that produce a secretion with which the animals build tubes or line burrows. Other genera are listed as detritus feeders. *Photis* species he observed build short tubes of clay or detritus and eat detritus. He found *Corophium* to be a highly specialized detritus feeder. In species *C. volutator*, there appear to be two modes of obtaining food: sifting suspended detritus and scraping decomposed detritus with the second pair of antennae. This species builds tubes or lines burrows.

Barnard (1954) lists *Parapleustes pugettensis* in Oregon as found on hydroids, which might explain its one appearance only at the Between Station.

3. Species not associated with larger vegetation evidently derive their nourishment from eating microflora or some type of detritus, the scum which is composed largely of decaying plant material, and the bacteria that are breaking it down (MacGinitie and MacGinitie, 1968). In an area of organic enrichment like Shilshole Bay, there should have been an abundance of this material in certain areas. Evidently in 1965 the Clean Station had inadequate amounts of detritus, and the Sludge Bed and Organic Stations with abundant food were too toxic for the amphipods.

The Between Station more closely approximated their needs and thus more animals were found.

In 1968 the four stations were utilized to varying degrees, the Clean Station the least, again perhaps due to lack of sufficient, proper food. In 1968 the degree of occupancy at the Sludge Bed and Organic Stations depended upon the ability of the species to utilize the thin crust that had sealed off the undesirable sediment below.

4. In summary, in 1965 essentially only the Between Station was used by the amphipods; in 1968 all four stations were occupied to varying degrees. We have seen this pattern of distribution for other animals in Shilshole Bay.

Cumacea

Three species were found at different times and locations: *Cumella vulgaris* Hart, *Diastylopsis tenuis* (?) Zimmer, and *Lamprops quadriplica* Smith. Judging from the distribution of the dominant cumacean, *Cumella vulgaris*, it is a sensitive indicator of environmental conditions and change.

Diastylopsis was represented by one juvenile taken in 1965 at the Between Station. We may assume it was an infrequent visitor.

Only one juvenile *Lamprops* was taken in 1965 at the Between Station. In 1968, one juvenile was taken at the Sludge Bed and two--one female, and one subadult male--at the Clean Station. Again the small number suggests this species to be an infrequent visitor.

Cumella, while not very abundant, was in sufficient numbers in 1965 to show some preference in stations. The animals were found in 71 percent (17 of 24) of the core samples taken at the Between Station;

ten cores had only one animal each, four had two animals, and one core each had three and four animals. The Clean, Organic, and Sludge Bed cores did not contain any. Of the 29 animals collected, 6 were males, 17 females, and 6 were juveniles; they came from 17 of 24 stations. This would suggest a resident rather than transient population. A dispersion ratio value just above one, suggests this species had a random distribution.

In 1968, three years after the sewer was shut down, there were no _Cumella_ at the Between or Clean Stations, but now three animals total were found (67 percent) in the Organic Station cores, and one (25 percent) in the cores for the Sludge Bed.

Admittedly the number of animals is small, but the distribution of _Cumella_ does suggest its preference for 1965 Between Station conditions. It may be food is the limiting factor (Wieser, 1956). The Sludge Bed and Organic Stations with their abundance of food may not have been habitable because of adverse sediment conditions. The Clean Station in both years may have lacked adequate food. Evidently in 1968, when the sediment of the Sludge Bed and Organic Stations was sealed off, a few animals found these stations more suitable.

Lie (1969) recorded eight species from Golden Gardens, just north of Shilshole Bay, but none were ones I collected. Wieser (1959a and b) ten years earlier from Golden Gardens just north, and Alki Point just south of Shilshole Bay, found only three species of cumaceans. These were the same species that I collected. He found that sediments in which mediam diameter exceeded 200 microns tended not to attract the animals.

In Shilshole Bay, all stations had such a smaller diameter except the Sludge Bed, which was also unacceptable because of its highly anoxic condition and toxicity.

When Wieser (1956) observed Cumella vulgaris for its feeding habits and sediment particle size relationships, he found: "In sand predominantly coarser than 149u C. vulgaris feeds as an 'epistrate-feeder' by scraping food off the surface of individual grains" which they can handle with mouth parts, but when in "sand predominantly finer than 149u and in mud it feeds as a "deposit-feeder".

Chelifera (Tanaidacea)

Leptochelia dubia (Fig. 28) is a tube builder and occurs in mud, particularly in the vicinity of algae when the substratum is rich in debris (Wieser, 1959b). Hatch (1947) recorded it from algae and hydroids. It was found at Golden Gardens by Wieser (1959) and by Lie (1969), who found it in Puget Sound to be abundant on fine sandy bottoms in shallow water.

In Shilshole Bay, Leptochelia appeared in 1965 at the Between Station in 25 percent of the samples (6 of 24 cores), with only one specimen per core. In 1968 three animals total appeared in two of the four Between Station cores, and also that year at the Sludge Bed, where five animals were collected from two of the four samples.

In these small numbers we must consider Leptochelia dubia in our area of the Bay to be a visitor from the kelp bed, or a very minor member of the bottom community. Evidently, where the environment is suitable, such as Golden Gardens, the species may reach 1103 per square meter (Lie, 1968).

Figure 28. Leptochelia dubia. Ruler markings are one millimeter apart.

Harpacticoid copepods

In 1965 and 1968, eleven species of harpacticoid copepods were collected. Two were exceedingly abundant, one abundant, and the other eight, including a new species, were in small numbers or rare.

The most abundant copepod was Bulbamphiascus imus. It reached just under 30,000 individuals per square meter at the Organic Station to rank as the most numerous of all animal species collected in 1965, aside from the Nematoda. At the Sludge Bed it reached $17{,}219/m^2$; at the Between Station, $2{,}703/m^2$; and at the Clean Station, $1{,}827/m^2$. All cores taken had Bulbamphiascus present. The presence of this species is valuable to us because it shows adverse conditions, such as anoxia or high hydrogen sulfide at the Sludge Bed, are not limiting to its distribution, and allows us to look for other factors that may be. The way Bulbamphiascus imus is distributed in numbers suggests food is a main factor, the more food, the more organisms. However, even though there was more food at the Sludge Bed, its occurrence in greatest numbers at the Organic Station suggests it prefers the smaller sediment types. It was extremely patchy in its distribution everywhere. In 1968 it was drastically reduced to but 631/m^2 at the Organic Station and fewer elsewhere. This would tend to reinforce the idea that organic enrichment in the form of sewage was an important factor in the animal's abundance in Shilshole Bay. Chappuis (1957) found *Bulbamphiascus imus* off Vashon Island, near Seattle. Lang (1948) found it to be a polyhaline species living in mud mixed with sand, but reports others as finding it associated with algae.

The second most abundant copepod collected in Shilshole Bay was *Tisbe furcata*. Its distribution was very patchy and also more restricted, reaching 10,194 animals per square meter at the Sludge Bed in 1965 and very few at the Organic and other stations. Such a restricted distribution would qualify the species to be considered an "indicator" in Shilshole Bay. How it behaves elsewhere is unknown. Its restriction to the Sludge Bed suggests some other factor was limiting because food was sufficiently abundant at other stations for the animals to be present. In 1968 when the sewage food was no longer available and the Sludge Bed had been sealed off by fine sediment, the species all but disappeared from the Sludge Bed and Organic Stations and was no longer present at the Clean or Between. It may be the species prefers the larger type of sediment found at the Sludge Bed although its cosmopolitan distribution has been reported by Lang (1948) from tide pools, common in the algal zone, and fine sand bottoms as well as mud and clay. It appears to be able to live successfully in a wide variety of habitats. Yeatman (1971) and Johnson and Olson (1948) found *Tisbe furcata* to be abundant in fish aquaria which contain large amounts of both plant and animal organic debris.

A second species of *Tisbe*, *T. gracilis*, was not found at the Sludge Bed and never became abundant in the Bay. It is evidently cosmopolitan and common, being reported from algae (Barnard and Reish, 1960) and elsewhere. Their food has been reported as wood (Barnard and Reish, loc. cit.), possible flesh (Lang, 1948), organic plant and animal debris in an aquarium, and mucus in the bronchial chambers of spiny lobsters and mussels (Yeatman 1962, 1963, 1971). With this wide variety of food and habitat preferences, it is difficult to understand why *Tisbe gracilis* did not become more abundant in Shilshole Bay by utilizing the organic debris. Perhaps it is a poor competitor, or as pointed out by Johnson and Olson (1948), its numbers may have been controlled by predators.

Another species that shunned the Sludge Bed both years is *Typhlamphiascus pectinifer*. Unlike *Tisbe gracilis*, it reached a population peak in 1965 of 1,376 animals per square meter at the Between Station, with 100 percent occurrence in sediment cores. At the Clean and Organic Stations it was present in just under 50 percent of the cores and considerably lower in number. This distribution suggests one station was too clean and one was too organic. By 1968 it was drastically reduced at the Between Station ($57/m^2$) to approximate the Clean Station in numbers and percent occurrence in cores, which had increased to 75 percent. Its presence had remained about the same at the Organic Station. Lang (1965) found it in Monterey Bay, off Hopkins Marine Station, associated with tidal pools, shell-sand, stones, and algae.

Huntemannia jadensis was present in small numbers ($37/m^2$) in 25 percent of the cores at the Organic Station in 1965. It was not present elsewhere, this suggesting rather narrow environmental requirements. By 1968, it reached $402/m^2$ at the same location and was found in 100 percent of the cores. That year, one animal was also taken from the Sludge Bed. Chappuis (1957) found Huntemannia at Golden Gardens and Alki Point, while Wieser (1959b) found it to occur at the first location and to be the most abundant copepod in his Puget Sound study. He noted the animals extended their range into fairly clean and medium fine sand to a diameter of 250u, which causes me to wonder why it was not present at the Clean Station in 1965. Lang (1948) notes that Huntemannia is definitely a brackish water form coming only from sand bottoms.

Land (1965) described the distribution of Acrenhydrosoma karlingi in Monterey Bay off Hopkins Marine Station, from tidal pools and coarse sand. I found the species at the Organic Station, with its organic silty-clay, and in the clean, fine sand sediment at the Clean Station. The species was never very abundant, reaching $225/m^2$ at the Between Station in 1965; it was absent there in 1968. Its numbers increased from one animal in 1965 to $726/m^2$ in 1968 at the Organic Station. A similar, though not as large, increase occurred at the Clean Station.

Amonardia pertubata made its first appearance in 1968 in small numbers at the Organic and Sludge Bed Stations. Lang (1965) records its presence at Dillon Beach, Tomales Bay, from rinsings of algae.

Dactylopodia vulgaris was found by Lang (1965) in Monterey Bay, off Hopkins Marine Station, in tidal pools, shell-sand, stones, and algae. In Shilshole Bay it had a spotty distribution both years, appearing in small numbers in 1965 at the Clean and Organic Stations, and in 1968 at the Clean and Sludge Bed Stations.

Two specimens of Heterolaophonte discophora were taken at the Clean Station in 1965; one was taken at the Organic Station in 1968. Lang (1965) records its presence in Monterey Bay, off Hopkins Marine Station, in tidal pools, shell-sand, stones, and algae. Wieser (1959b) found it in the high intertidal at Golden Gardens. By their locations in Shilshole Bay, Heterolaophonte, Dactylopodia, and Amonardia extend their recorded habitats to fine sands.

Two specimens of a new species of Diagoniceps were taken in 1965 at the Between Station. Two specimens of Parathalestris, intermediate between species bulbiseta and californica, were collected in 1968 at the Between Station. The new species and intermediate are currently being described by Dr. Harry Yeatman, College of the South, Sewanee, Tennessee.

From the literature it would seem the Harpacticoid copepods are excellent animals to show habitat preference. They can be very motile and as such could select the habitat type most "desirable" to them.

The greatest number of the animals in 1965 and 1968 was as follows:

Acrenhydrosoma karlingi - 1965, Between Station; 1968, Organic Station.

Amonardia pertubata - 1968 Organic Station.

Bulbamphiascus imus - 1965 and 1968, Organic Station.

Dactylopodia vulgaris - 1965, Organic Station; 1968, Clean Station.

Heterolaophonte discophora - 1965, Clean Station; 1968, Organic Station.

Huntemannia jadensis - 1965 and 1968, Organic Station.

Tisbe furcata - 1965, Sludge Bed; 1968, Organic Station.

Tisbe gracilis - 1965, and 1968, Organic Station.

Typhlamphiascus pectinifer - 1965, Between Station; 1968, Clean Station.

From this information, it is seen that no copepod was most mumerous in 1965 at the Clean Station, except for the two specimens of *Heterolaophonte* that were collected. *Dactylopodia* found this habitat most desirable in 1968. Thus in 1965 essentially every species found another station (except the Clean) best for it, though the Clean Station had its representative species in reduced numbers. Only *Acrenhydrosoma* and *Typhlamphiascus* were found in greatest abundance at the Between Station in 1965; none were most abundant there in 1968. The Organic Station exhibited the greatest abundance of four species in 1965 and of seven in 1968. Only at the Sludge Bed in 1965 was *Tisbe furcata* most abundant; no species were most abundant in 1968.

If we add the total number of copepods of all species at a station we get results as follows:

Year	Clean Station	Between Station	Organic Station	Sludge Bed
1965	1,957	4,240	30,388	27,413
1968	230	115	1,969	616

All the data presented above suggest the Organic Station supports the highest total numbers and most kinds of species in both 1965 and 1968. The Sludge Bed also had high total numbers but mainly of two species in 1965. Although the Sludge Bed had more food available than the Organic Station, other factors would obviously be involved in determining the number and kinds of copepods present. The Between Station supported more than the Clean in 1965, but by 1968 they were similar in animal numbers, the two stations being more nearly the same in physico-chemical properties by 1968.

Isopoda

Munnogonium waldronense George and Stromberg, is a small isopod (Fig. 29) that is still the subject of some taxonomic confusion and investigation (George and Stromberg, 1968). It evidently does not occur widely distributed, for the entire previously known collection and the description made was from four specimens. Neither Lie (1968) nor Wieser (1958) found the animals in areas adjacent to Shilshole Bay.

Evidently conditions for a local population were adequate only at the Between Station in 1965. Here 95.8 percent of the samples (23 of 24 cores) contained an average of about 13 (range 1-41) animals per core, or 712 per square meter. The animals exhibited a high degree of patchiness. Three years later in 1968, the animals still inhabited the Between Station, with 100 percent occurrence in the samples, though the average number was now about six per core or $330/m^2$. It is particularly interesting to see that by 1968, the species had come to occupy the entire area under study, though not to the same degree at each station.

Although it is difficult to determine what is the limiting factor for this species, it may be food. MacGinitie and MacGinitie (1968) believe that, for the most part, isopods are scavengers, picking up whatever they can find. If this is true for Munnogonium waldronense, then certain portions of Shilshole Bay, with its sewage discharge, would provide optimum conditions for the species.

Figure 29. Munnogonium waldronense. Between Station, 1965. Grid marks are one millimeter apart.

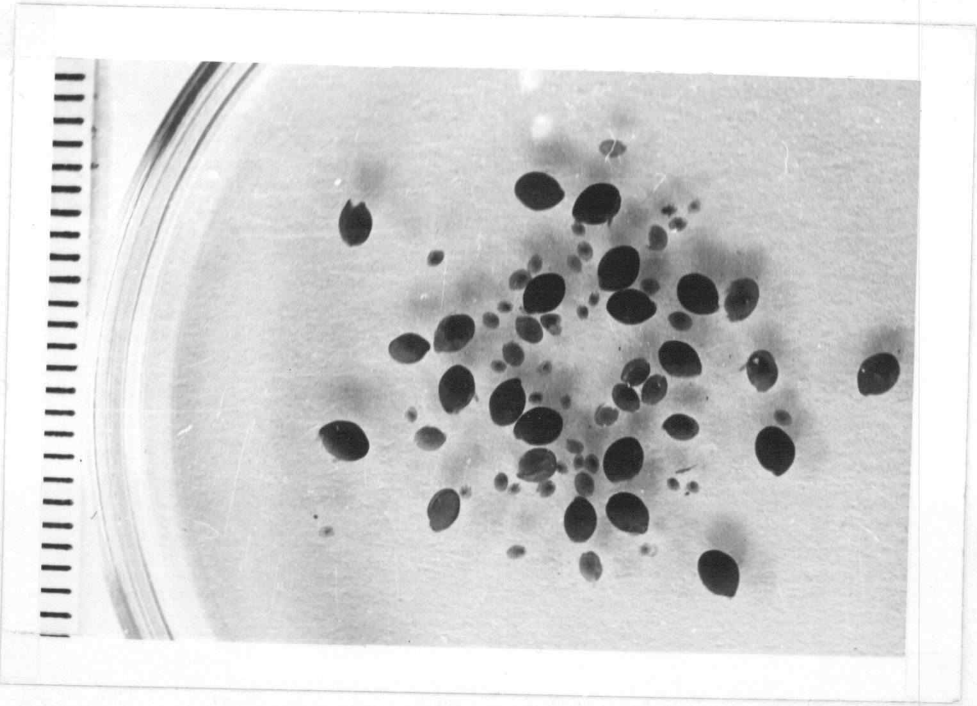


Figure 30. Euphilomedes carcharodonta. Between Station, 1968. Grid marks are one millimeter apart.

Its absence in 1965 at the Clean, Organic, and Sludge Bed Stations may be for the same reasons mentioned for the Cumacea, lack of food and/or unsuitable sediment conditions. On the other hand unlike the Cumacea, in 1968 it seems to have populated all sampling stations to some degree, the greatest number being at the Between, then Organic, Sludge Bed, and Clean Station.

Since Wilson (1957) in a previous study of the Shilshole Bay area records only Isopoda as being collected, we have no way of knowing if *Munnogonium* was present then. It may be this species is a recent addition to the Bay fauna and that the food and sediment conditions at the Between Station were optimum in 1965. Later, in 1968, it had populated larger parts of the Bay, although their reduced numbers indicate less than optimum conditions.

Ostracoda

One of the more abundant species collected in Shilshole Bay in 1968 was the ostracod, *Euphilomedes carcharodonta* (Fig. 30). That year at the Between Station it reached an abundance of over 3,700 per square meter and over 1,260 at the Organic Station. Both Clean and Sludge Bed Stations had relatively small numbers, but cores at all stations exhibited 100 percent occurrence of the species.

In 1965 during sewer operation, *Euphilomedes* was less abundant but did occur at all stations, though only one animal was taken at the Sludge Bed. The Between Station that year had the greatest number ($201/m^2$), with animals in 79 percent (19 of 24) of the cores.

Wieser (1959) did not find Euphilomedes carcharodonta at either Alki Point or Golden Gardens. On the other hand, Lie (1968) found this species in Puget Sound to be the most abundant infauna species, reaching at Golden Gardens over 1,900 per square meter. He found it to be associated with fine sand; I found it to be also present on silts and clays. As did Lie, I found the species to be quite patchy in distribution (Table 10).

Green (1968) notes that many of the ". . . bottom dwelling ostracods appear to be general scavengers, capable of feeding on living algae or on detritus." If Euphilomedes was feeding on detritus, Shilshole Bay in 1965 would certainly provide an abundance of food. Its small numbers in 1965 suggest that if it is a general detritus feeder and conditions were favorable, then other factors such as competition or predation limited its numbers. If it is not a detritus feeder, its increase in 1968 may have been concomitant with an increase of whatever food organism it was utilizing. The answer to its feeding habits is unknown.

Mollusca

Gastropoda

Only one tiny snail, Alvinia compacta, was present in small numbers at the sampling stations in 1965 and 1968. A second snail, Polinices, was seen to occur in shallow water outside the study area near shore (see Interpretation of Results).

Alvinia's appearance and numbers do not show any trend. In 1965 it was present only at the Between Station in about 71 percent (17 of 24) of the cores, from one to four per a core with a high value of 18 in one. In 1968 it was present at all stations but the Between, in numbers per core comparable to 1965, but in fewer percent of the cores.

It is not possible to tie the distribution and abundance of Alvinia to any one environmental variable. It is the most common species of the genus, ranging from the Aleutian Islands to San Martin Island, Baja, California (James H. McLean, 1970). It is reported by McLean to be ". . . found in gravels from eel grass beds along the partially protected open coast and in gravels from sublittoral areas along the open coast. It is not found in bays or on sand bottoms away from rock." The presence of Alvinia in Shilshole Bay on fine sediments without rock suggests the animal has a wider habitat utilization than suspected. It was not found at Golden Gardens or Alki Point (Lie, 1968; Wieser, 1959b).

Pelecypoda

During the period of study, nine species of bivalves were collected. Four of these occurred in such small numbers as to be considered accidental residents, or at best very minor species in the community. Only two juvenile Protothaca staminea were collected at the Sludge Bed in 1968. The distribution of Clinocardium nuttalli was also limited, only two tiny individuals being collected in 1965 at the Between Station. In 1968, small individuals appeared in small numbers in all cores of the Organic Station and 75 percent (3 of 4) of the Sludge Bed cores. Lucinoma annulata had a 16 percent occurrence in the samples, one-animal per core, at the Between Station only in 1965; it was not found anywhere in 1968. Two specimens of Lucinisca tenuisculpta occurred in 1965 at the Between Station, but in 1968 three of four cores had small numbers of small individuals. One individual was found at the Sludge Bed.

The other five species were more abundant and widely distributed. No clams were found at the Sludge Bed in 1965 because of burial problems and general overall toxic conditions of the sediment and interstitial water. But three species were found at every other station and time. These were *Macoma carlottensis*, *Mysella tumida*, and *Tellina modesta*. Of the two remaining, *Macoma nasuta* was mostly restricted to the Clean and Between Stations both years, while *Psephidia lordi* appeared at all stations in 1968, and in 1965 at the Between Station. Two small *Psephidia* were also taken in a core from the 1965 Sludge Bed.

In 1965 the most abundant clam in number of individuals was *Tellina modesta* with over 1,750 individuals per meter square and 100 percent occurrence in samples from the Between Station. The size distribution curve (Fig. 31) shows a variety of sizes present in the population with the majority of the animals as juveniles between less than one millimeter to about three; larger members reached about 13 mm but were not abundant. Three years later the number of individuals was about the same but the larger members were gone and medium-sized animals were more prevalent. Only one animal was collected at the Organic Station in 1965; by 1968 they had experienced a sizable increase, exceeding slightly the number at the Between Station for the same year. The size distribution curves of the two stations were somewhat similar. An increase over the one animal collected in 1965 had occurred at the Clean Station by 1968. The 1965 samples at the Clean Station and all 1968 samples showed the same general overall size profile, larger number of small animals with a few larger members. At most stations, occurrence of this species in samples

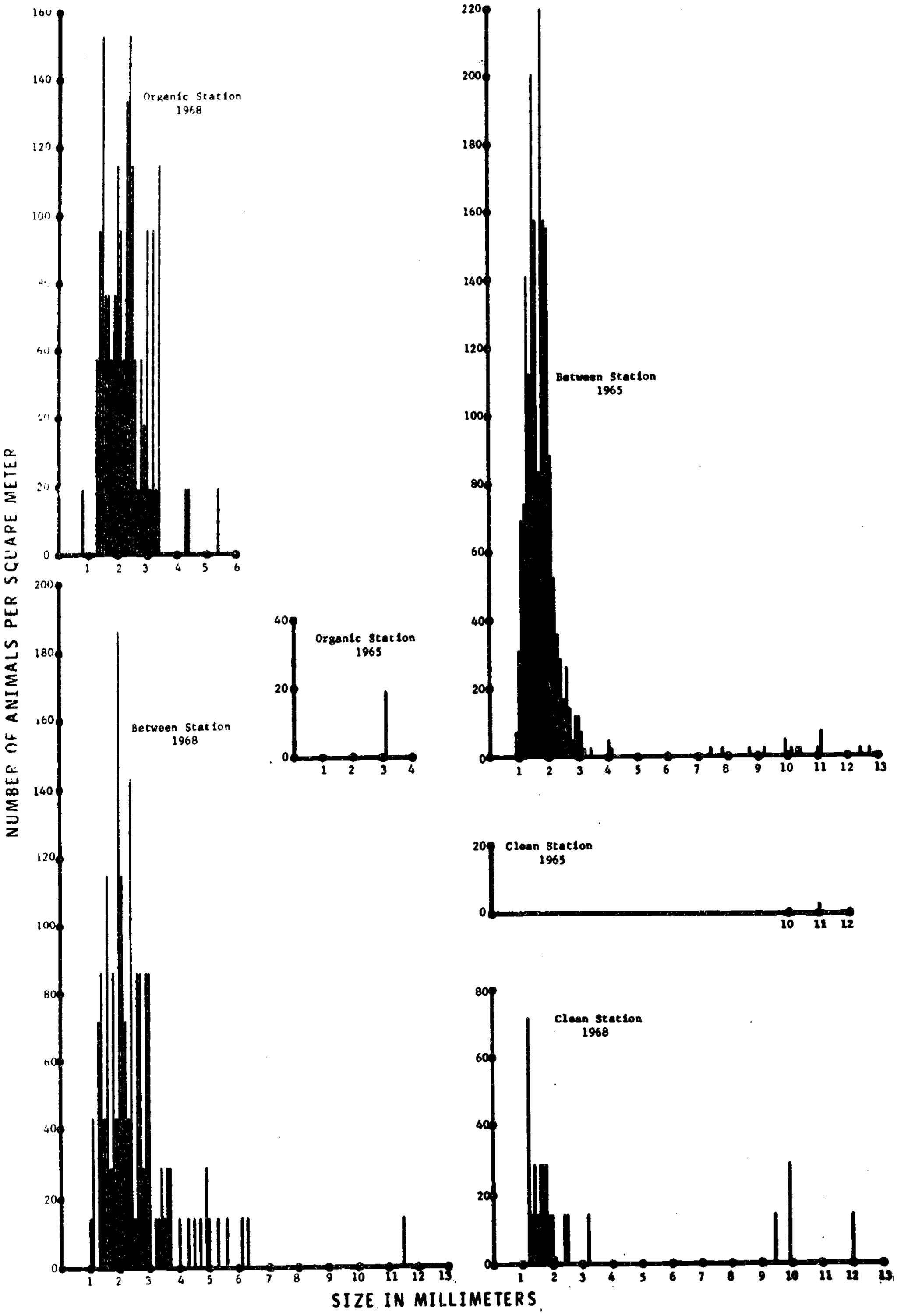


Figure 31. Distribution and abundance of *Tellina modesta*.

was 100 percent. Lie found Tellina present at Golden Gardens but in numbers considerably lower than those in Shilshole Bay.

Macoma carlottensis at theBetween Station was the second most abundant species in 1965, almost 1,100/m^2. It was found in 100 percent of the samples. Aside from the number of animals involved, its size distribution (Fig. 32) was remarkably like that of Tellina for the same year at the same location. By 1968, the number of animals was greatly reduced. The population at the Clean Station in both years was small and similar. The number of animals at Organic Station in 1965 also was small and was reduced further by 1968. By 1968, a small population of this species had become established in the Sludge Bed.

The only station with a sizable number of Mysella tumida in 1965 was the Between; a few were found at the Clean and Organic Stations. The size profile at the Between (Fig. 33) shows a variety of sizes present ranging from .6 to 3.8 mm. In 1968 the populations at the different stations consisted solely of the smaller animals, but the number of animals had increased over 1965 and the Sludge Bed had been populated. Mysella was recorded from Golden Gardens in a size range similar to those I found but in much lower numbers.

In the three years after 1965, Psephidia lordi changed from a very minor bivalve species to the most abundant, reaching at the Between Station 8,387 per square meter. The Sludge Bed also became well populated. The 1968 Sludge Bed and Between Station populations were similar, differing in number of younger animals. Larger sizes were present in small numbers at both (Fig. 34), but these were relatively small animals when compared to those collected by Lie in Puget Sound.

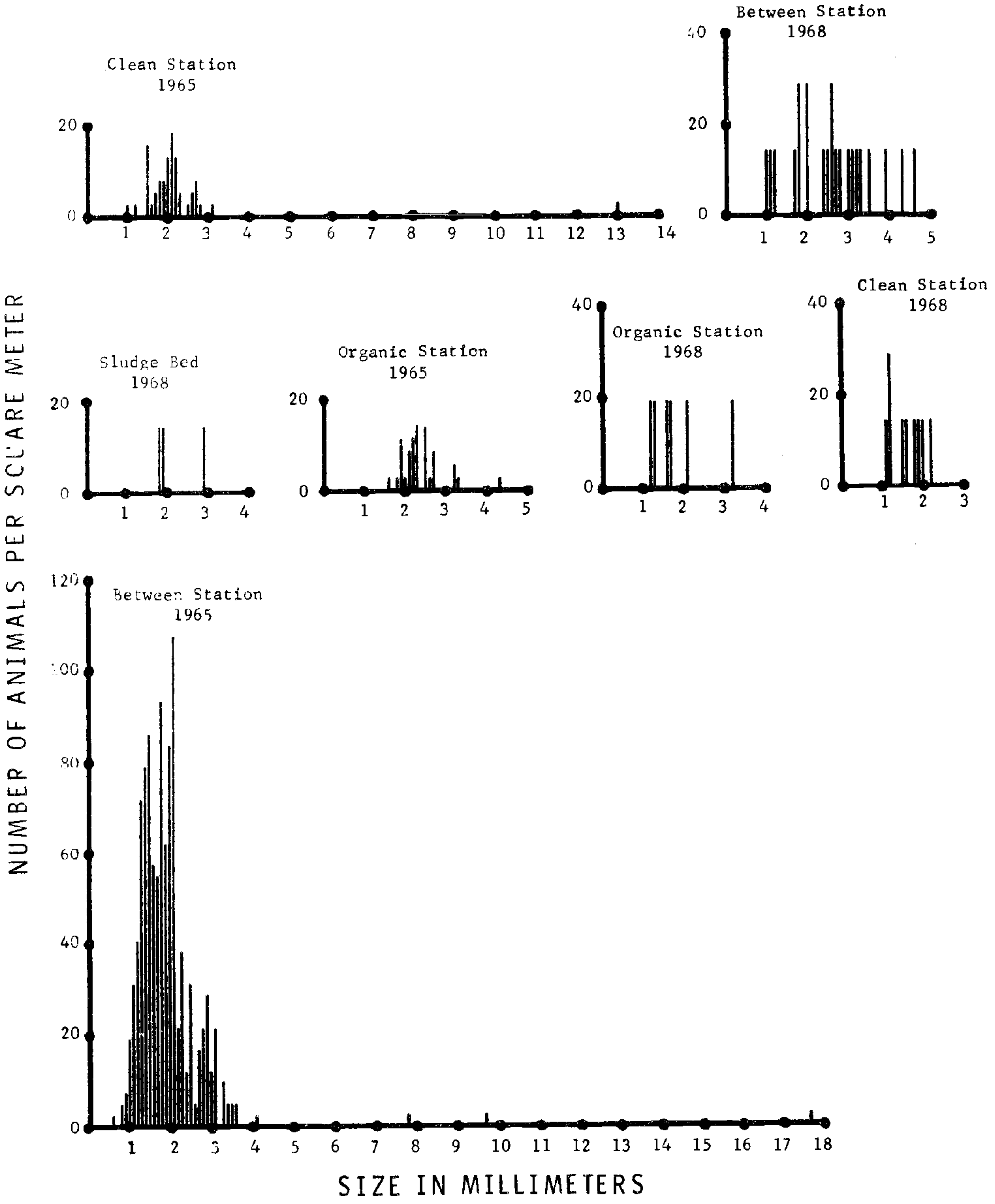
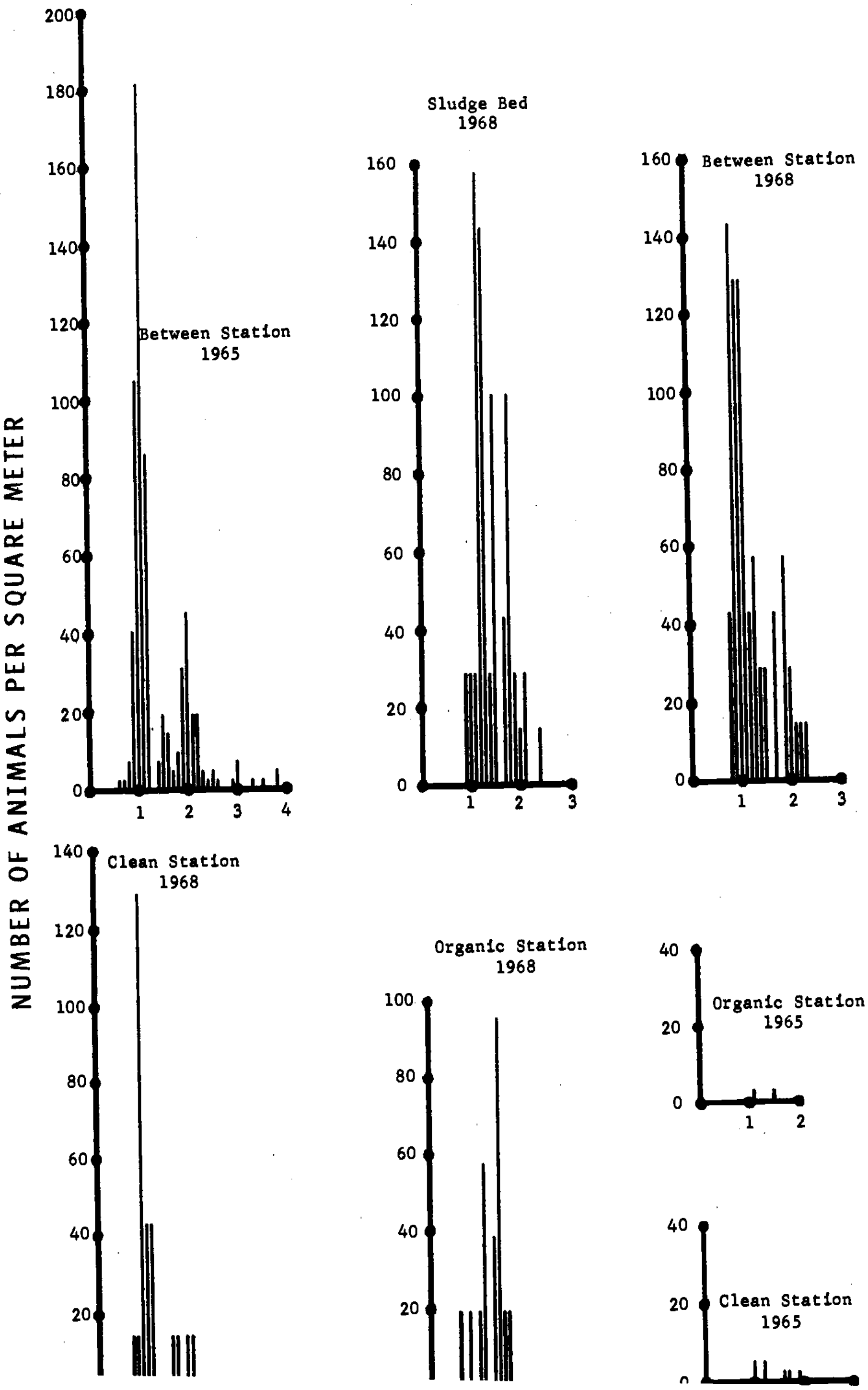


Figure 32. Distribution and abundance of *Macoma carlottensis.*


NUMBER OF ANIMALS PER SQUARE METER
Between Station
1965
200
180
160
140
120
100
80
60
40
20
0
1
2
3
4
Sludge Bed
1968
160
140
120
100
80
60
40
20
0
1
2
3
Between Station
1968
160
140
120
100
80
60
40
20
0
1
2
3
Clean Station
1968
140
120
100
80
60
40
20
Organic Station
1968
100
80
60
40
20
Organic Station
1965
40
20
0
1
2
Clean Station
1965
40
20
0

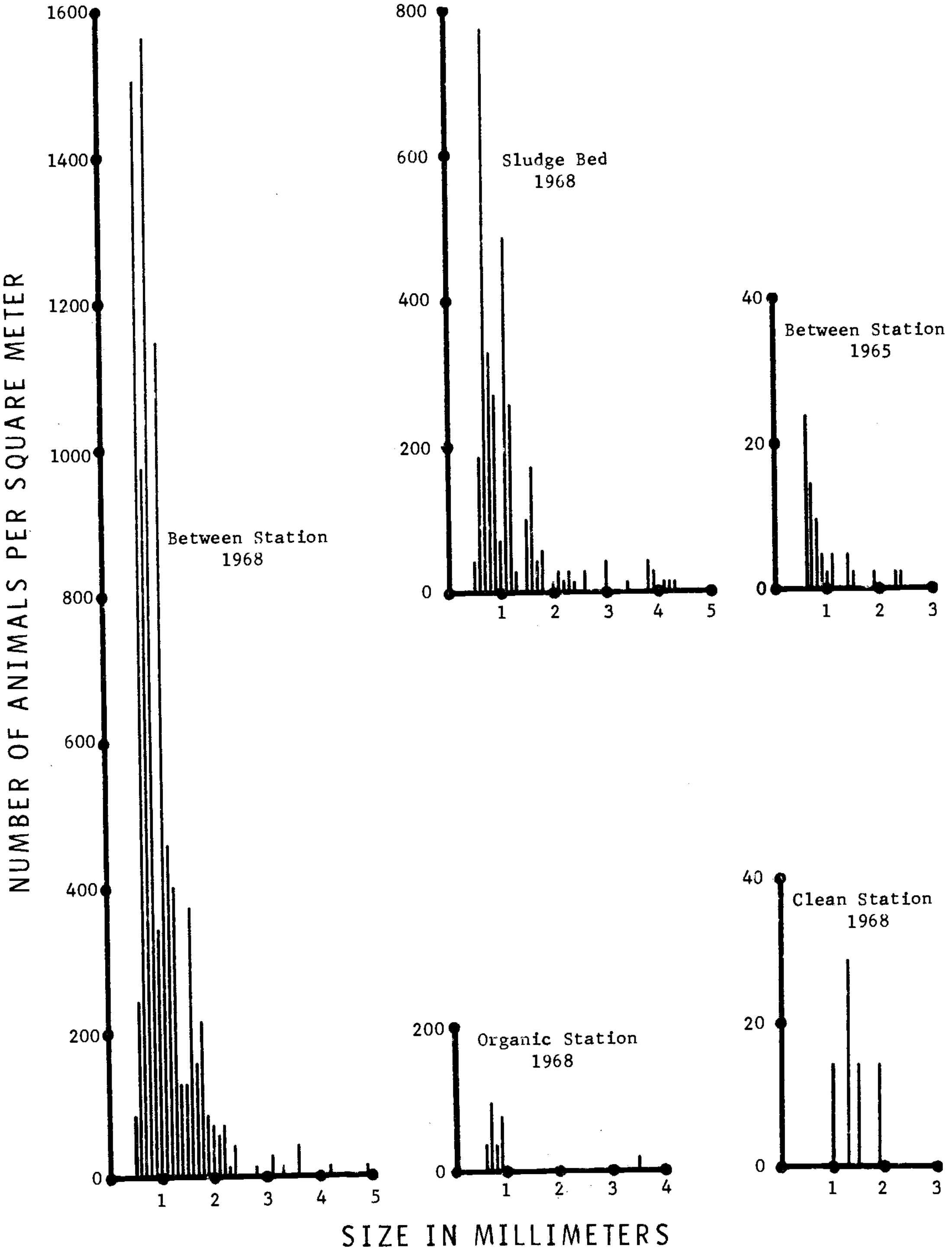


Figure 34. Distribution and abundance of *Psephidia lordi*.

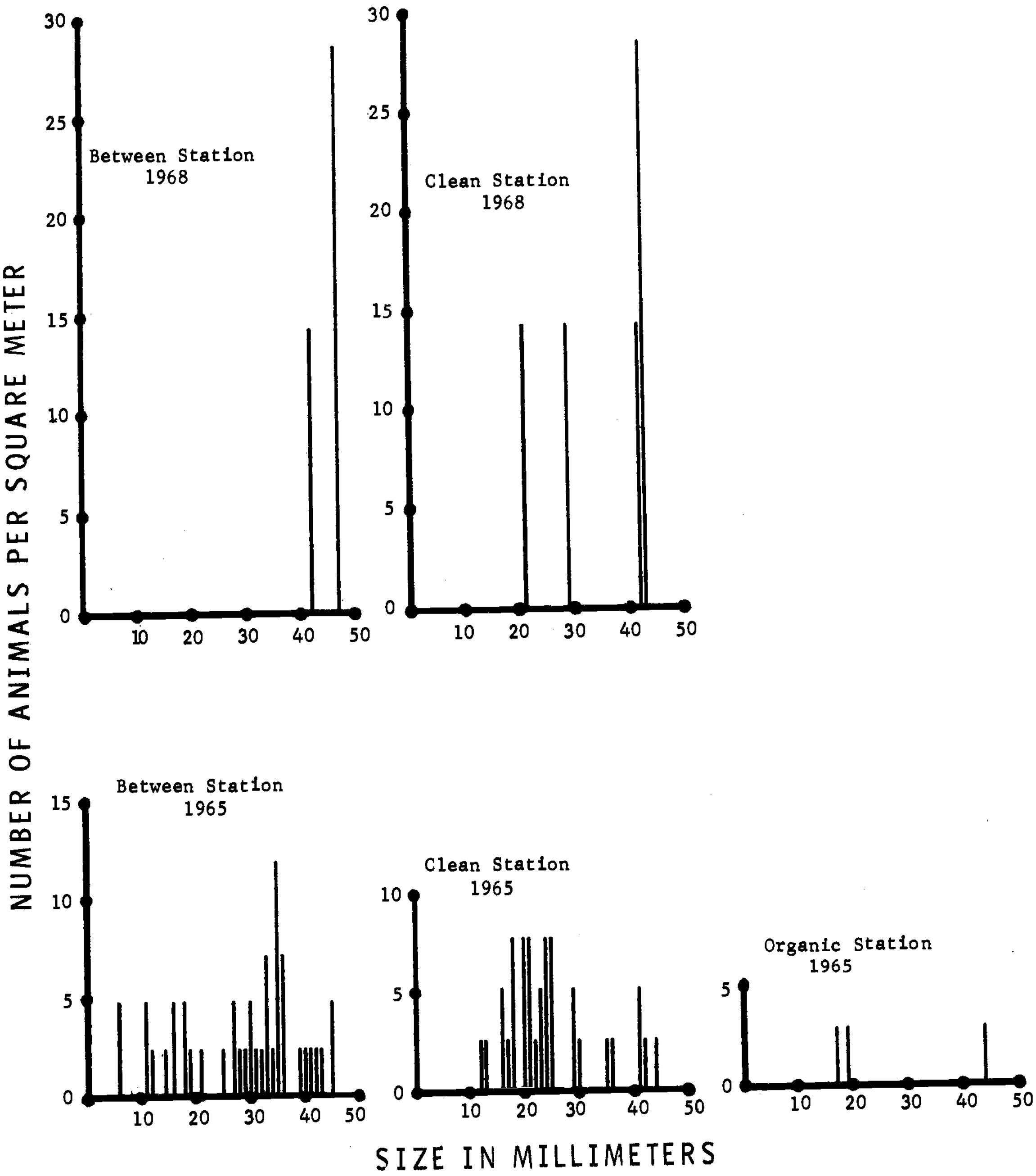


Figure 35. Distribution and abundance of Macoma nasuta.

As in some of the other bivalves, the number in Shilshole Bay considerably exceeded those at Golden Gardens.

Macoma nasuta is one of the few species that showed some indication of a uniform to random distribution (Table 10). The rest showed weak to strong patchiness. It was not present in very great numbers, reaching but 91 animals per square meter in 1965 at the Between Station. But in terms of biomass, it contributed the greatest weight to the standing stock, of all species. The animals ranged from six to 47 mm in size (Fig. 35). Of interest to me was the lack of Macoma at the Organic Station. It has been supposed to be relatively pollution tolerant, but this was not borne out by the animals in Shilshole Bay. It is of interest to note that Lie does not list Macoma nasuta as present at any of his sampling stations; it may be it is found in the higher subtidal sediments.

I was surprised not to find at any station either year any of the much larger clams I had found so abundant in other Puget Sound sediments (also see Wennekens, 1959).

Nematoda

In the introduction of this paper, I stated that finding taxonomists available to work on different groups of animals could be a limiting factor to benthic studies. This statement is particularly true when applied to the nematode worms. They are poorly known as a group. Wieser (1959b) found 106 species in Puget Sound beaches, 76 of which were new including two new genera. The ocean has a large number of free-living and parasitic species, the majority of which are microscopic or near-microscopic in size (MacGinitie and MacGinitie, 1968). Shilshole

Bay is no exception. As a result, up to this time only a cursory taxonomic survey has been made with my collection, so the Nematoda will be treated here not as species, but as a group. Even so, the gathered data are of interest. A more extensive study will be reported later.

Table 33. Distribution and Abundance of Nematoda, 1965 and 1968

Station and year	# of samples	% samples with nematodes	# of animals (m^2)	Ash-free dry weight (mgs/m^2)
Clean				
1965	22	100	101,100	271.37
1968	4	100	32,073	84.59
Between				
1965	24	100	72,723	132.38
1968	4	100	36,016	65.95
Organic				
1965	20	100	333,946	1430.88
1968	3	100	18,180	78.38
Sludge Bed				
1965	4	100	5,075	30.11
1968	4	100	846	3.37

From the data some trends are evident:

1. Nematodes occurred in 100 percent of all samples collected for both years.

2. In all cases, numbers at each station in 1968 were considerably lower than in 1965.

3. The nematodes were most abundant at the Organic Station in 1965, with almost 334,000 animals per meter square (Fig. 22). But by 1968, the number was reduced to but 5.4 percent of the former value. It appears a reduction of food in the form of sewage or more likely as bacteria feeding on sewage was partially responsible for this reduction.

4. The Clean Station in 1965 had the next greatest number, more than 100,000/m^2, followed by the Between Station with about 73,000 and the Sludge Bed with just over 5,000. It would be wrong to assume there were more worms at theClean than Between Station, though the data suggests this is the case. However, the dispersion ratio (Table 10) shows these animals to be extremely clumped in their distribution, so a few cores from either station with high or low number of animals could reverse these second and third ranked stations.

Is it possible the Clean and Between Stations contained species not as dependent upon sewage-related food as the Organic Station? Though this point must wait completion of the taxonomic study, if this were the case, when the sewer was shutdown they would not be as affected. In support of this idea, we see nematodes at the Clean Station in 1968 were reduced to 31 percent, and those at the Between Station to 49 percent of their former values, while those at the Organic Station were reduced to 5.4 percent.

5. Like the Organic Station, the Sludge Bed populations of nematodes experienced a great reduction by 1968, to but 6 percent of its previous value. At this time, it is likely the worms were living only in the newly deposited upper crust since the lower sediment was septic, which made the station marginal at best for nematode occupation.

Wieser (1959b) reported 83 species from Golden Gardens and 78 from Alki Point. Our cursory survey of those found in Shilshole Bay indicates as many species occur there. Wieser had some success in correlating his species with mean sediment size. Such a comparison for Shilshole Bay will first require identification of our species.

Polychaeta

1. Polychaete worms occurred in the greatest number of species in Shilshole Bay, except for perhaps the Nematoda. Because of their considerable variety and numbers, they demand a more extensive analysis than do the other groups.

2. Number of species in Shilshole Bay. Forty-three species have been identified from the Bay, most of which were present in small numbers. Table 12 lists these and Table 9 presents their distribution and abundance. No species showed a distributional pattern that was clearly uniform (Table 10), but several had patterns that were random to weakly patchy, and the rest were clearly patchy.

Of the total 43 species, 35 were found in 1965; 22 of these were also found in 1968. Thus 13 of the 1965 species did not appear in 1968. Of the 30 species found in 1968, eight were not present in 1965. It is not known if the disappearance and appearance of these species was a result of insufficient sampling, of natural population fluctuation, or of habitat changes associated with sewage discharge. There is a strong possibility the drastic habitat alteration that resulted from the shutdown of the North Trunk Sewer in 1965 eliminated some species and encouraged others.

3. The dominant species. In 1965, on the basis of number of individuals, only the species *Capitella capitata* could be called dominant at the Clean, Organic, and Sludge Bed Stations. It was present in 100 percent of the samples taken. In addition, *Eteone californica* was found in abundance in 100 percent of the Sludge Bed samples and

Prionospio cirrifera in moderate numbers in over 95 percent of the Clean Station samples.

At the Between Station, the position of numerical dominance was shared by eight species. In order of abundance they were: Gyptis brevipalpa, Armandia brevis, Capitella capitata, Glycera capitata, Anaitides williamsi, Prionospio cirrifera, Platynereis bicanaliculata, and Nephtys ferruginea. All were present in 100 percent of the samples except Platynereis (87.5 percent).

In 1968, no species existed in sufficient numbers to be classified as truly dominant. In fact, only four species even occurred in 100 percent of samples at any station. These were Capitella capitata (Sludge and Organic), Prionospio cirrifera (Organic and Between), Glycera capitata (Between), and Anaitides williamsi (Between).

4. Species abundance at sampling stations. The Between Station was clearly the most favorable location for polychaete diversity in 1965. It evidently possessed the environmental qualities most needed because 32 species were collected, while the Clean Station was second with only 16. Of the 32 species collected here, 16 were not found at any of the other three stations and only three, which were collected at one or more of the other stations, were not found at the Between. It is possible further collecting at the Between Station would have produced these three species, since they occurred elsewhere in only small numbers.

By 1968 the Between Station had lost 17 of its 32 species and had added a new one for a total of 16. The altered environment had evidently eliminated from the community those species present because of the former organic enrichment.

The alteration of the polychaete fauna at the other stations did not parallel that of the Between. While the number of species at the Clean Station remained about the same, 12 of the 16 1965 species disappeared, so that by 1968 they were replaced by others. The Organic Station experienced a similar trend. Here seven of 14 disappeared and eight appeared.

The Sludge Bed experienced the largest change. Only four species were present in 1965, and three of these were present in 1968. But an additional 16 species were added, all but three of which were present also at other stations in the Bay. Evidently the highly toxic sludge bed of 1965 became acceptable to new polychaetes in small numbers when it acquired a habitable, thin, inorganic outer crust by 1968. Similar observations were made for other taxa at the Sludge Bed.

5. Trends in polychaete distribution and abundance (Tables 34 and 35). When species are dominant or at least abundant at a location, trends are sometimes evident that may be used to shed light on species distribution. Species represented by only one to a few individuals at a sampling site are not useful for this purpose. In fact, apparent absence of a species at a location may be only a result of insufficient sampling. Of the 43 species collected, only nine were in sufficient abundance to be of much value in determining species distribution.

Table 34. Changes in Polychaete Abundance Between 1965 and 1968

Station	Numbers per m^2	Biomass mgs/m^2	Number of Species	Numbers $\frac{1968}{1965}$ x 100	Biomass $\frac{1968}{1965}$ x 100	Species $\frac{1968}{1965}$ x 100	# Species same $\frac{1965-1968}{1965}$ x 100
Clean							
1965	8640	764.18	16	7.8	45.7	93.8	25
1968	670	349.12	15				
Between							
1965	8501	1202.69	32	12.3	221.6**	50	46.9
1968	1046	2665.39**	16		(62.1)		
		(747.29)					
Organic							
1965	10997	538.28	14	5	43.9	107	50
1968	553	236.45	15				
Sludge							
1965	12703	2742.33	4	12.6	15.8	475	75
1968	1605	433.31	19				

**Values high due to samples containing two worms, much larger than usual.
Values in ( ) are without these two animals.

Table 35. Numbers of polychaete worms per square meter, 1965 and 1968

	1965				1968			
Species	Cl	B	Or	Sl	Cl	B	Or	Sl
Paleanotis bellis		2*	3*					
Dorvillea rudolphi	52	62	34					29
Glycera capitata	10	662			14*	172		
Glycera tenuis								86
Glycinda polygnatha		19			43	72	115	29
Goniada acicula		7						29
Gyptis brevipalpa	21	2753	17				38	358
Lumbrinereis latreilli					14*		38	14*
Aglaophamus erectans					14*			
Nephtys caeca		10			14*		38	
Nephtys ferruginea		160			14*	57		14*
Platynereis bicanaliculata	26	208	292		14*			129
Nereid species A	3*	24	9			43	19*	57
Nereid species B	8	24	3*					
Diopatra ornata		5						
Nothria elegans		7			14*	43	19*	14*
Lepidasthenia longicirrata	3*					14*		
Anaitides williamsi	57	375	141	72		115	19*	158
Eteone californica	3*	19	17	803		14*	19*	14*
Eumida bifoliata		10						
Pholoe glabra	3*	26				14*		
Langerhansia sp.		2*						
Capitella capitata	8081	1439	10406	11814	43			387
Notomastus tenuis	8	122				29		
Mesochaetopterus taylori			3*					
Spiochaetopterus costarum		5				14*		
? Caulleriella hamata		2*						
? Praxillella gracilis					14*			
Armandia brevis	21	2002	32			29	19*	115
Haploscoloplos elongatus		43			14*	43	19*	29
Owenia fusiformis							19*	
Cistenides brevicoma	3	50	11			14*	19*	14*
Pectinaria belgica		2*						
Boccardia proboscidea		2*						
Polydora brachycephala		79						
Polydora near socialis					14*			
Polydora A		2*						
Prionospio cirrifera	336	366	6		358	215	115	72
Prionospio malmgreni		10			72	158	19*	43
Rhynchospio arenicola					14*		38	
Spio filicornis	5		23	14*				
Spionid-like								14*
Polychaete unusual		2*						

*One specimen only collected at the station

a. *Abundance of individuals in 1965*:

Sludge Bed $>$ Organic $>$ Clean $\approx$ Between

The Sludge Bed clearly had the greatest number of individuals. It had a very large, exploitable food source in the form of raw sewage. Because of the highly toxic nature of the habitat, only two species (*Capitella capitata* and *Eteone californica*) became abundant, and only the former made up the bulk of the polychaetes present.

The Organic Station too was toxic, but evidently to a lesser degree (see sections on H_2S, D.O., and NH_3), for not only were the Sludge Bed species present, but an additional 12 species found the habitat usable, at least to a marginal extent.

The polychaetes reached their zenith at the Between Station where 32 species were found. Not only were all but two of the Organic Station species present--and they might have been with further sampling--but an additional 20 were found, many occurring as more than one individual per square meter.

The Clean Station had 16 species, all but two of which were found at the Between Station, and these two consisted of only one or two specimens each. All species but *Capitella capitata* and *Prionospio cirrifera* were greatly reduced in number and were less than those found at the Between Station.

b. *Number of species in 1965*:

Between $>$ Clean $\approx$ Organic $>$ Sludge Bed

This was discussed under "a."

c. *Biomass of polychaetes in 1965*:

Sludge Bed $>$ Between $>$ Clean $>$ Organic

Clearly the Sludge Bed polychaetes were able to utilize a source of organic enrichment to produce greater living biomass than were those at the other stations. This type of success is expected where pollution-tolerant species face little competition in an area of essentially unlimited food supply.

As was also expected, the Between Station, with its reduced organic encirhment and greater species diversity, had a greater total biomass than the Clean Station with its little enrichment.

Like the Sludge Bed, the biomass of polychaetes at the Organic Station depended basically upon one species, _Capitella capitata_, which appeared in large numbers, less than the Sludge Bed, but greater than either the Between or Clean Stations. Examination of the specimens of this worm showed a large number of juvenile worms, thus explaining the high numbers but lower than expected biomass.

d. Numbers and Biomass for 1968:

Numbers: Sludge Bed > Between > Clean > Organic

Biomass: Between > Sludge Bed > Clean > Organic

The Sludge Bed experienced the greatest decrease (84.2 percent) in polychaete biomass by 1968, but still had the greatest number of individual worms. Examination of species composition shows a drastic decrease of _Capitella capitata_ the dominant species, with the concomitant appearance of a new species _Gyptis brevipalpa_. Since the individuals of _Capitella_ weighed more than _Gyptis_, percentage decrease in biomass was greater than that in individuals. Because of the many new species, plus the retention of a reduced population of _Capitella_, the Sludge Bed still ranked second in biomass in 1968.

The Between Station was first-ranked in biomass in 1968 because the species it retained were still present in numbers larger than the other stations.

The faunal composition of Clean and Organic Stations consisted mostly of species each with only one to several animals/m^2. Each had one or two species that were "dominant" and constituted the bulk of the numbers and biomass. Thus, these two stations had low values for 1968.

e. Comparison of results for the years 1965 and 1968.

The number of polychaete worms at the four stations had been reduced in 1968 to a low average of 9.5 percent of their 1965 values; the range was 5 to 12.5 percent. Such an overall reduction must certainly have been a reflection of cessation of discharge of organic enrichment from the North Trunk Sewer into Shilshole Bay.

In 1968 the biomass also experienced an overall reduction to an average of about 33.7 percent of what it had been in 1965. The decrease for the Sludge Bed was the greatest, to but 15.8 percent of its former value; the Between Station maintained 62.1 percent of its former value.

The greater decline in percent of number of organisms, over that exhibited by biomass, showed those animals living in 1968 to be larger on the average than those living in 1965.

No trends can be established for loss and recruitment of species between 1965 and 1968. All stations lost some species and all gained others. In most cases, the number of individuals of each kind of added species was but from one to a few. Further discussion on this point appeared earlier in this section.

6. Consideration of the dominant species.

a. The most ubiquitous and numerous species was *Capitella capitata*, a species frequently considered to be an indicator organism because of its tolerance to polluted conditions (Reish, 1959b; Reish and Barnard, 1960). But as noted by Warren (1971, pg. 337), "For an individual species to be useful as an indicator organism, it must have a rather narrow range of suitable environmental conditions that are known and of interest to man." Such would not appear to be the case for *Capitella capitata* in Shilshole Bay. It was found in abundance in 100 percent of all samples collected at all stations in 1965. The greatest numbers were found at the Sludge Bed, followed closely by the Organic and then the Clean Station. The Between Station had only about 11 percent as many as the Sludge Bed. Of particular interest is the fact that by 1968, *Capitella* was greatly reduced or missing at the sampling stations.

The presence of *Capitella* at all stations in 1965, but especially in numbers at the Clean Station, poses some interesting problems. It is obviously extremely pollution-tolerant and may do well for lack of competition at the Organic and Sludge Bed Stations, which have no other dominant species. At the Clean Station there were relatively few species in competition with *Capitella*. Even the species abundant elsewhere were greatly reduced in number or were not present at all. However, at the Between Station where the other polychaetes were most abundant in numbers and species, *Capitella* was reduced considerably.

It appears the species is restricted not by pollution but by competition. The general reduction of Capitella in 1968 was probably due to reduction of suitable food.

b. Perhaps better fitting the concept of indicator species were Glycera capitata, Gyptis brevipalpa, Armandia brevis, and Nephtys ferruginea. In 1965, all four were present in moderate to sizable numbers at the BetweenStation, with just a few or no individuals found elsewhere. All species were found in 100 percent of the cores collected at this station. It may be they did not find sufficient food at the Clean Station, yet found the sediment with its hydrogen sulfide, anoxia, and other properties unacceptable at the Organic and Sludge Bed Stations.

By 1968, Glycera and Nephtys were still present in reduced numbers only at the Between Station. Gyptis was no longer at the Between Station but had taken up residence in the crust of the sludge bed along with Armandia, both at low densities.

c. Prionospio cirrifera was present in moderate numbers in 1965, at both the Clean and Between Stations in approximately equal quantity. They were essentially in all the cores. Its distribution suggests a preference for cleaner environments, similar to the four species discussed, but its presence at the Clean Station also suggests an ability to persist in an environment having relatively little organic matter. By 1968 it still remained in comparable numbers at the two stations, but had expanded its distribution to the Organic and Sludge Bed Stations.

A similar species, *Prionospio malmgreni*, was not present in 1965 except for a few individuals ($10/m^2$) found at the Between Station. By 1968 it had invaded all stations to varying degrees.

d. The second species able to inhabit the Sludge Bed in sizable numbers ($803/m^2$) was *Eteone californica*. Presence of a few individuals at each of the other three stations suggests it to be an extremely pollution-tolerant polychaete, unable to compete well. By 1968 it was all but gone from the four sampling stations.

e. The distribution of *Anaitides williamsi* is of interest, because it was one of the few species able to exist at all stations in 1965. It was most abundant at the Between Station ($375/m^2$), followed by the Organic, Sludge Bed, and Clean. Its existence at the Sludge Bed and Organic Stations suggests it to be a pollution-tolerant species. Its preference for the Between Station shows it is able to compete for a share of the resources. I am unable to explain why the species was not more abundant at the two polluted Stations; perhaps it does not compete well with *Capitella capitata*.

f. *Platynereis bicanaliculata* is a semi pollution-tolerant species, reaching its greatest concentration at the Organic Station ($292/m^2$). Its absence from the Sludge Bed shows either that it cannot compete at all with the Sludge Bed species, or the conditions at this station are too extreme. The latter is suspected, since the food reserve at the Sludge Bed was very adequate to support it at least as a minor species. Reinforcement of this conclusion is its presence in fair numbers ($208/m^2$) at the Between and a few at the Clean Station.

Epifauna, nekton, and birds in the sampling area

The Between Station contained the greatest variety of animals. But a general overall reduction of epifauna and nekton in the total area of study appeared to be due to conditions of the sediment and the lack of proper rocky or other hard materials for attachment. Species and their distributions are summarized by taxonomic groupings. Unless otherwise stated the observations are for 1965, before the sewer was shut down.

The Crustacea

A large hermit crab, *Pagurus ochotensis* Brandt, was observed in small numbers at the Clean Station, and slightly greater numbers at the Between Station. The moonsnail *Polinices*, whose shell the crab frequents, was observed just subtidally near the shore, but not at any of the sampling stations. *Cancer oregonensis* (Dana) was present in small numbers at the Between Station under pieces of bark or trash such as beer bottles or cans; it is normally associated with rocks. Shortly after placement of the concrete anchor blocks and other equipment on the bottom, a very small number of shrimp (Fig. 36) of at least three species took up residence at the Between Station, where they used these materials for cover. They included *Pandalus danae* Stimpson, *Hepacarpus brevirostris* (Dana), and juveniles of a *Cragon* species. Two males of *Pinnixa eburna* Wells and one megalopa and one juvenile of *Pinnixa* sp. were found when a jar containing materials from the Between Station was sorted. A barnacle-encrusted beer bottle in the jar may have provided a home for the pea crabs.

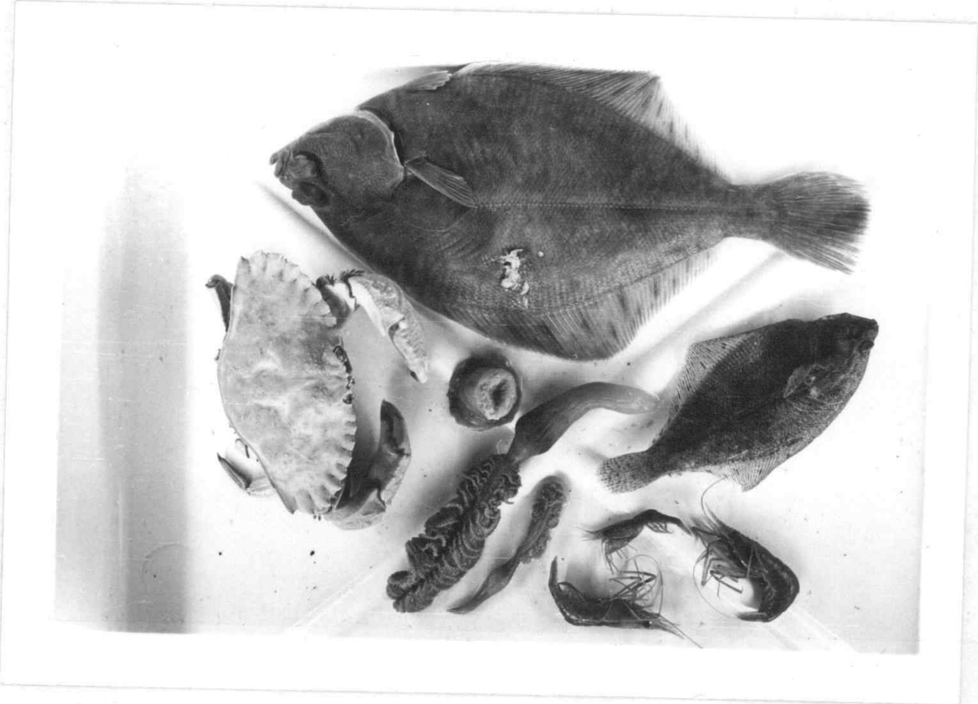


Figure 36. Assortment of epifauna collected at various stations. Red crab, left; sea pen, bottom; shrimp, lower right; flatfish, top and right.

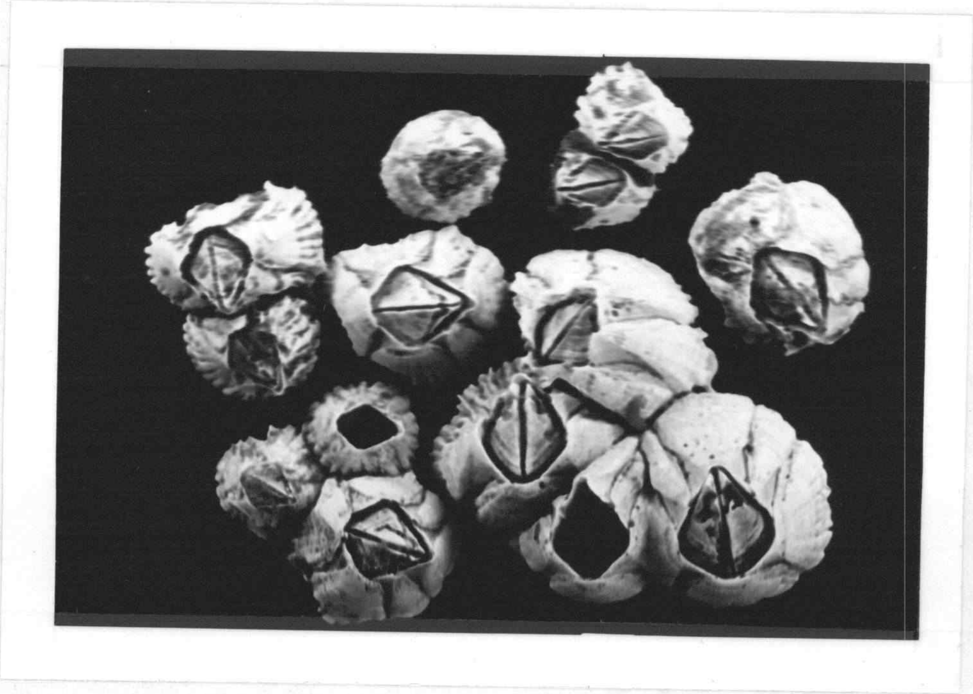


Figure 37. The barnacle, Balanus glandula. Taken from a beer bottle.

Both the Red Cancer Crab (Cancer productus Randall) (Fig. 36) and the Dungeoness Crab (Cancer magister Dana) were found in small numbers at the Clean and Between Stations and the area between them. At times mated pairs were seen. The Red Cancer Crab was the most common.

Any firm object such as beer containers or chunks of bark that had been present for a time at the Clean or Between Stations had the barnacle Balanus glandula Darwin attached to the surface exposed to moving water. A beer bottle taken from the Organic Station was likewise covered (Fig. 37), suggesting survival was possible if the animals had an adequate surface upon which to attach and be elevated from direct contact with the possible toxic sediment surface.

The Coelenterata

At the Between and Clean Stations were found small individuals of both the orange and white color phases of the sea anemone Metridium senile (Linnaeus). Their occurrence was limited to suitable, firm surface such as rock, bark, or bottles for attachment.

One of the most striking and conspicuous members of the benthos was the orange seapen, Leioptilus guerneyi (Gray) (Fig. 36). Each "animal" is in reality a colony of many individuals of three types. The primary individual, from which the other two kinds are budded-off with time, forms the stalk and peduncle by which the colony is anchored into the substratum. Embedded colonies ranged in height above the sediment surface from an inch or less, to more than two feet. At the Between Station prior to shutdown of the sewer, they reached their greatest density, forming when viewed from above a striking orange carpet. At the Clean Station only an occasional small individual colony was seen, their density gradually increasing toward the Between Station. At this time, never were pens seen at the Organic Station or Sludge Bed. Sea pen biomass and numbers were determined for a typical area of the Between Station. In an area of approximately 400 square feet, a total of 125 pens of many sizes were collected. On a square meter basis, the abundance was as follows:

Number of individual pen colonies: 3.36
Wet weight of colonies: 25.65 grams
Oven (103°C) dry weight: 3.70 grams
Ash-free dry weight: 2.34 grams

In 1968, three years after shutdown of the sewer, it was obvious the number of pens at the Between Station had been significantly reduced, though they were not actually counted. At this time it was noticed that there were some tiny, healthy colonies living in the thin crust of inorganic sediment that had settled over the sludge bed. Slightly larger colonies also living in the crust were sick and dying. It would appear as long as the colonies were very small and did not break through to the anoxic, hydrogen sulfide-laden sediment below, they survived.

The Echinodermata

An occasional *Solaster dawsoni* Verill was found at the Clean Station prior to shutdown of the sewer. Three years later, a large *Pycnopodia helanthoides* (Brandt) was found moving across the sludge bed.

The Fish

The variety of fish at all stations was small. One sizable wolfeel (*Anarrhichthys ocellatus* Ayres) inhabited an old tire on the bottom at the Organic Station. Occasionally ratfish (Hydrolagus colliei (Lay and Bennett)) visited all stations, moving as if in transit to other locations. Small numbers of small cottids were seen at the Clean and Between Stations; they were infrequently seen at the Organic Station and never at the Sludge Bed.

By far the most common were flatfish (Fig. 36); these were seen at all sampling stations. Only the Between Station had a great diversity of sizes, and had the largest numbers of individuals. I had constant companionship while working on the bottom from juvenile fish of less than an inch to adults of better than a foot long. The smaller fish were extremely inquisitive, frequently "attacking" bright colored equipment and gloved hands; larger fish were more wary, preferring to watch from a few feet away.

The largest flatfish seen was a 44 centimeter-long adult Sand Sole (Psettichthys melanostictus Girard) at the Organic Station). The most common species identified were the Rock Sole (Lepidosetta bilineata (Ayres)), the English Sole (Parophrys vetulus Girard), and the Speckled Sand dab (Citharichthys stigmaeus Jordan and Gilbert). No attempt was made to quantify these species.

The Birds

At least two species of adult and juvenile seagulls and one species of tern were observed year-round, generally in the area of study and more specifically floating in or at the edge of the sewer boil (Fig. 38). The birds seemed to represent a resident population. A sizable portion of their food, judging from frequent observations, was derived from solid fecal material present in the sewer boil. During periods of

Figure 38. Seagulls and terns feeding at the sewage boil.

stormy weather the same birds were known to fly into Seattle and use the City's water storage basins as resting sites, constituting a potential public health problem. The birds' droppings were always found on the sampling station bottoms and served as an added source of organic enrichment, especially for the Clean Station.

Epifauna and flora at areas adjacent to the sampling area

From the distribution of animals found at the sampling stations, it would appear that the entire Bay was relatively poor in species, but this was not the case. The small number of species seen at the stations undoubtedly reflected the condition of the sediment and the lack of hard materials for attachment. In the area located between the Clean Station and the shore and south to the sewer outfall pipe, quantities of large rocks had produced a sizable kelp bed of *Nereocystis luetkeana* (Mertens) Postels and Ruprecht (see Fig. 5). Associated with the rocks, kelp, and the sewer pipe were a number of rockfish of the genus *Sebastodes*, numerous small cottids, and the large cottid *Scorpaenichthys marmoratus* Girard. Also found were considerable numbers of the very large anemone *Metridium senile* (Linnaeus) in both orange and white color phases, as well as several other smaller species. The seastars *Solaster dawsoni* and *Pycnopodia helianthoides* were present.

The Community

1. Up to this point, I have compared the distribution and abundance of each species of animal as they existed at the four sampling stations in 1965 and 1968. But we know animals do not exist as isolated populations of species but rather as parts of dynamic,

integrated, and very complex systems called communities. These are easily changed by subtle as well as extreme environmental alterations. The nature and importance of the community concept has been discussed by Warren (1971). I wish now to examine my results from Shilshole Bay within the community context. But first I will consider some community characteristics.

2. Community Structure

In most natural habitats, large numbers of species, representing considerable diversity of taxonomic groups, interact to create complicated food webs. The organisms may range in size and complexity from tiny bacteria to the largest Metaphyta and Metazoa. In each habitat there are a multitude of environmental factors that operate on these organisms. As a result of these factors, each kind of organism has come to utilize a particular portion of the habitat and resources present in a manner at least slightly different from all other species. It is this difference in utilization that has allowed for the great diversity of organisms usually present. Because of the sharing of some of the resources, competition results in the presence of dominant and minor species. Most unpolluted environments are characterized by having a few species, dominant by virtue of their greater number of individuals, and many minor species, each with but a few individuals. When the environment is altered, the species composition becomes altered. When the alteration is in any way detrimental, the less tolerant species usually become greatly reduced or disappear, and the more tolerant species increase in number by utilizing the resources for which they no longer have to compete.

3. Ways to evaluate the community

When an organism is selected at random from a community, considerable uncertainty exists as to which species it will belong, unless one species is extremely abundant and the others are present in very small numbers. However, the more species that exist in the community, and the more evenly the organisms are distributed among the species, the more uncertain will be the species selected. One of the parameters used to define the Shilshole Bay communities--diversity--is a measure of this uncertainty.

An index of diversity, based on the Shannon-Weaver (Pielou, 1975) equation for information theory, is considered to be one of the simplest and most promising methods for representing community structure (Wilhm and Dorris, 1968). Pielou (1969) finds the associated parameter, evenness, to be an essential companion without which the Index has little value. Let us consider each briefly.

a. The diversity index based on information theory is relatively independent of sample size and is dimensionless. It can be applied to either biomasses or numbers of individuals of different species in a community.

A diversity index should reflect not only the number of species but also the relative importance of each species in the community. This index is a function of both the number of species and the manner (evenness) in which the number or biomass of individuals is distributed among the species. Maximum diversity exists if all individuals belong to different species, and minimum diversity exists if all individuals belong to the same species. Thus a collection is said to have a high diversity if it has many species and their abundances are fairly even. Conversely, diversity is low when the species are few and abundances are uneven.

b. Evenness is a measure of how the counted organisms are divided among the species present. It too is a dimensionless value and is a ratio of the observed diversity index to the maximum possible diversity for the species observed. Its values range between zero and one, the latter attained when the organisms are evenly distributed between the species, and the former when all organisms present are of one species.

One must remain alert as Pielou has warned (1969, pg. 222): "It will be seen that since diversity depends upon two independent properties of a collection, ambiguity is inevitable; thus a collection with a few species and high evenness could have the same diversity as another collection with many species and low evenness."

4. Community size

In an academic sense, the community consists of the sum total of all populations of all species living in a particular kind of habitat. The kinds of organisms and their numbers may be tremendous, so it is not possible for any one person or team of individuals to count and identify all organisms in natural communities. Thus, in the practical sense, the community becomes whatever the researcher is able to effectively handle. By necessity, the four communities defined for Shilshole Bay were restricted to animals larger than 0.42 millimeters and to animals defined as infauna in this report. While there were undoubtedly many microorganisms present in the bay sediments, the writer found the 0.42 mm sieve retained most individuals of most metazoan groups. These were collected and evaluated and entered into the computations used for diversity values, except the Nematoda, which have not been identified as to species. Upon completion of the nematode taxonomic studies, diversity indices will be recalculated.

In the academic sense, the community occupies a piece of real estate whose environmental qualities are essentially the same for the area containing the community. In the practical sense, the communities were less well-defined in Shilshole Bay because the seabed was continuous and without barriers, and the populations of the four "communities" were interconnected for species distribution by the same water mass. The composition of the four communities really differed because of the effects of the organic enrichment that fell upon the sediment.

5. Biomass versus density for diversity index

Both numbers density and biomass diversity indices have been calculated for this report. Which is best? Wilhm and Dorris (1968, pg. 478) are of the opinion that since "considerable variation in the biomass exists within the among species, the relative importance of the various species is more adequately expressed in biomass units than in numbers." The logic of their reasoning appears sound for if we should use numbers density instead of biomass, one tiny nematode worm would be equated with one large clam, and that certainly does not recognize the impact or contribution the larger organism makes in the community. On the other hand, one might ask whether the demands and contribution of one clam in the community is the same as 10,000 nematodes of the same total weight. While it is not my intent to enter into a discussion of community energetics, it should be at least pointed out that the total metabolic activities of 10,000 tiny nematodes is much greater because of their larger surface area and other considerations than one individual, which they collectively equal in weight.

6. Diversities of communities at the sampling stations

Diversity indices for both numbers density and biomass have been calculated.

Table 36. Community Diversity Indices (D.I.) and Evenness (J) for 1965 and 1968

Station		1965		1968	
		D.I.	J	D.I.	J
Clean					
	Numbers	1.32	.27	4.02	.82
	Biomass	.52	.11	.34	.07
Between					
	Numbers	3.93	.67	2.64	.53
	Biomass	1.46	.25	1.11	.22
Organic					
	Numbers	1.11	.23	3.78	.73
	Biomass	1.39	.29	3.34	.65
Sludge Bed					
	Numbers	1.69	.56	3.90	.72
	Biomass	.47	.16	3.99	.74

In our evaluation of diversity we must keep in mind the warning voiced by Pielou (1975, pg. 14): ". . . a community with a few, evenly represented species can have the same diversity index as one with many, unevenly represented species. It is obviously desirable to keep distinct these two ingredients of diversity, the number of species. . . and the evenness."

a. In 1965, based upon the number of species, the density of individuals, and the evenness of distribution, the community at the Between Station exhibited the greatest diversity index (3.93). Communities at the other three stations had much lower diversity indices, that at the Sludge Bed being the highest. This is an example of the problem about which Pielou warned. The Sludge Bed did not have high taxonomic diversity; it was a community with but eight species, three of which were dominant and similar in density. The relatively high evenness value alerts us to this fact. On the other hand, both Clean and Organic Stations have many, unevenly represented species, their evenness values showing this to be true. These will be discussed below.

b. If we examine diversity indices based upon biomass for 1965, we find an entirely different picture. While the community at the Between Station still has the highest index, it is much reduced (1.46) and is essentially the same as the Organic Station. Both the Clean Station and the Sludge Bed communities have indices considerably smaller for biomass than for numbers density. To find why the indices for biomass and density are so different, we must examine the species composition.

1'. <u>The Between Station</u>

For 1965, we find only 44 clams (<u>Macoma nasuta</u> and <u>Lucinoma annulata</u>), which constituted less than 0.6 percent of the total animals, contributed more than 92 percent of the weight resulting in the low diversity and evenness values. Only the large number of species prevented the diversity index from falling even lower. Obviously the effect of the clam on the index computed with biomass is profoundly different than on that computed with numbers density.

2'. The Organic Station

For a similar reason, the diversity index is reduced at the Organic Station. Three individuals of *Macoma nasuta* (0.02 percent of total specimens) constituted better than 67 percent of the biomass and another 22.6 percent came from the worm *Capitella capitata*, which made up about 25 percent of the total number of animals. An additional factor reducing the diversity index was the presence of a relatively small number of species.

3'. The Sludge Bed

The low diversity index for the Sludge Bed can be explained by the fact that one species, *Capitella capitata*, which constituted only about 29 percent of the numbers density, contributed more than 93 percent of the biomass. This resulted in the greatly reduced diversity index and evenness values, reflective of an almost mono-specific community.

4'. The Clean Station

Wilhm and Dorris (1968, pg. 480) are of the opinion that "Subjective evaluation of numbers of species can lead to erroneous conclusions." To support this statement, they subject the data of other workers to evaluation by diversity index, and conclude the areas considered unpolluted were in fact polluted as indicated by their low indices. Criteria they developed for such evaluation are as follows:

values less than one, from heavily polluted areas; from one to three, moderate pollution; and values exceeding three, clean water.

From the values just presented, one must conclude the station selected as Clean--which represented the unpolluted standard for Shilshole Bay--was in fact moderately (for density index) to heavily (for biomass) polluted. However, considerable underwater exploration with subjective evaluation, followed by sediment analysis, led to selection of the Clean Station as the standard. Was this a mistake in view of the low diversity indices obtained here? If so, one must then accept a sediment characterized by grey-white sand, low organic content, presence of some dissolved oxygen, no hydrogen sulfide or ammonia, and low daily deposition of organic material as _not_ less polluted than sediments characterized by darkened sediments, high organic content, lack of dissolved oxygen, presence of ammonia and hydrogen sulfide, and heavy daily deposition of organic material. Obviously, this must be rejected, for a diversity index value has too many variables upon which to answer this important question with such certainty.

When we look for the cause of the low density diversity index at the Clean Station, we see it is a result of the presence in great numbers of one organism, _Capitella capitata_, which made up almost 75 percent of the organisms present. We also see that the low biomass diversity index was a result of the high weight of one organism, the _Macoma nasuta_; it made up almost 92 percent of the weight although it was only less than 0.8 percent of the numbers. The low evenness values of .27 and .11 respectively, clearly reflect the role of the two species in the diversity calculations.

c. One can guess why Capitella capitata dominated in numbers, and this has been done elsewhere, but it is probably more instructive to examine the indices for 1968 at the Clean Station. Here the diversity value for numbers density reached a high of 4.02. No one species was present in large numbers--including Capitella--and the evenness value too was high at .82. However, the comparable diversity index and evenness for biomass were still low (.34 and .07) which reflects the fact that Macoma still contributed over 95 percent of the total biomass collected.

d. The Between Station in 1968 had a relatively high diversity index for density. But as in the case of 1965, the low diversity index for biomass was due to the biomass of Macoma nasuta which made up about 84 percent of the biomass. Again, this is an example of how the weight of one species can drastically affect the diversity index for a sampling station.

By 1968, both the Organic and Sludge Bed Stations had attained high diversity indices for both biomass and density. Their evenness values show the lack of any one or several species, like clams, that might have contributed heavily to the biomass as seen before at the Organic Station. As noted earlier, both stations experienced an increase in species by 1968, especially the Sludge Bed, as organisms invaded and began to utilize the non-polluted outer crust that had settled over (and sealed off) the polluted sediments.

7. Reflections on total biomass and numbers density

Every piece of real estate has a certain amount of organisms it can accommodate. The amount is a function of how much food and space are available. The kinds of organisms present are determined to a large extent by the physical properties of the environment.

The physical environment can change, as we saw between 1965 and 1968. With change comes alteration in the amount and kinds of organisms. Thus we may say, the community structure and its organism amounts are dynamic. Nevertheless, it is possible to gather biomass and density data that do provide a picture of what is taking place at a location, and these data may be a reflection of the amount of organic enrichment or the conditions it causes. At this point we shall examine these data to see what trends may be present.

Table 37. Total Density and Biomass of Animals per Square Meter, 1965 and 1968

	Density (number/m^2)		Biomass (mgs/m^2)	
Station	with nematodes	without nematodes	with nematodes	without nematodes
Clean				
1965	111,897	10,797	10,667.86	10,396.49
1968	34,367	2,294	21,222.22	21,137.63
Between				
1965	90,642	17,917	25,021.50	24,889.12
1968	53,536	17,520	22,948.89	22,882.94
Organic				
1965	375,490	41,544	3,632.04	2,201.16
1968	26,266	8,086	697.72	619.34
Sludge Bed				
1965	45,234	40,159	2,884.56	2,854.45
1968	7,226	6,380	711.14	707.41

a. The small number of sediment samples taken in 1968 make the biological data much less reliable than those taken in 1965. The unreliability is compounded by the larger factor with which the 1968 samples must be multiplied in order to convert sample data to units per square meter. For example, the conversion factor for the Clean Station in 1968 is 5.5 times that of 1965. If the animals were distributed evenly in the sediment, the error with small sampling would be minimal. But we found most of our species were distributed in a patchy pattern which, when converted to a square meter, produces values that may greatly over or under estimate the size of the populations. Conversely, the larger the number of samples, the smaller is the conversion factor, and the more reliable are the final values. Let us use the Clean Station as an example in support of this conclusion. We note the biomass value for 1968 is more than twice that for 1965. This may be true, but intuition tells us it is not. All other stations experienced a decrease in biomass and an even larger decrease in numbers density--including the Clean Station--with the cessation of discharge of organic enrichment from the North Trunk Sewer. Examination of the species composition for the Clean Station in 1968 shows several heavy clams made up the bulk of the biomass; and these, when translated to weight per square meter, produced high values. The patchy distribution of the clams may have resulted in an overestimation of biomass. Because of the situation just discussed, total biomass data for 1968 will not be used except in looking for broad trends.

b. Biomass in 1965

Between > Clean > Organic > Sludge Bed

It appears a degree of organic enrichment or fertilization, as seen for the Between Station, stimulated not only the number of species as discussed elsewhere but the total amount of biomass. The Clean Station had less biomass, but this was expected since it was less enriched. On the other hand, heavy pollution (fertilization), as seen at the Organic Station, had a depressing effect upon the total biomass, and the extreme pollution at the Sludge Bed suppressed in addition the number of species able to survive.

One would have to conclude that fertilization, short of serious alteration of the physical features of the habitat, stimulates the number of kinds of organisms as well as their biomass.

c. Numbers density of organisms in 1965

Organic > Clean > Between > Sludge Bed

Here is an excellent example how erroneous impressions might be obtained by complete reliance on density of organisms as indication of habitat diversity. The tiny nematodes are in such quantity in certain locations (the Organic and Clean Stations), that while they do not much alter biomass rankings, they do greatly affect the order of density. Without nematodes figured into density (Table 37) the order is considerably different from above:

Organic ≈ Sludge Bed > Between > Clean

d. Comparison of numbers density of organisms 1965 and 1968

With or without the inclusion of nematode values (Table 37), all stations, but the Between, experienced a drastic reduction in numbers density between the two years. The Between Station, with nematodes included in density, experienced less than a 50 percent decrease, and without them, no decrease. The lack of density change without nematodes is corroborated by little change in total biomass between the two years.

Some light has been shed upon the distribution and abundance of animals in Shilshole Bay during this study, but many other questions still remain unanswered. One of the more obvious that needs answering is: Why did all stations <u>but</u> the Between experience a drastic change in biomass and density between 1965 and 1968? The data do not permit an answer.

VI. SUMMARY AND CONCLUSIONS

1. Shilshole Bay, Puget Sound, Washington, in 1965 had been receiving organic enrichment from a domestic-industrial sewer outfall for over 50 years. Later that year the sewer was shut down. The physical features of Shilshole Bay are described, as are four areas selected to be studied. These areas ranged from the extreme pollution of the "Sludge Bed Station" to the relatively unpolluted "Clean Station". Next to the Sludge Bed in organic enrichment, but without a large buildup of sewage materials, was the "Organic Station". A fourth area designated the "Between Station" received mild organic enrichment in amount somewhat between those of the Clean and Organic Stations. The four stations were 8.8 to 11.8 feet below 0.0 water, eliminating depth as a factor contributing to any differences in the distribution and abundance of the animals at the sampling stations.

2. The total solids delivered to the Bay by the North Trunk Sewer was 110,300 pounds daily. Of this, 87,300 pounds were organic, 23,000 pounds inorganic, the latter consisting largely of various sized particles of clay to large sand, depending upon the amount of rainfall in the Seattle area. The sewage traveled to the surface as a boil and spread out as a plume, the pattern dictated by the prevailing currents. Puget Sound pulp and paper industries and log rafts added some woody materials to the Bay.

3. Equipment was designed, built, and installed to permit measurement of current direction and velocity and the collecting of water samples every two to three hours during a 48-hour period. This water plus interstitial water was analyzed for a variety of environmental

variables. Sediment cores were examined for distribution and abundance of animal life and analyzed for particle sizes and other factors.

4. The water at the sediment-water interface from the four stations was analyzed and compared. The results suggest that the environmental variables salinity, temperature, pH, oxygen, nonionized hydrogen sulfide, and dissolved organic matter were not responsible for observed differences of animal abundance and distribution.

5. In the same water, current velocity and particulate inorganic material were interrelated in that the former prevented the latter from settling at the Sludge Bed, but not at the other stations. This was especially true of the Organic Station which was sealed off from the water column by silt and clay to become anoxic.

6. The quantity of chromium in the interface water in 1965 was insignificant; copper and lead were low but may have had some subtle effects, especially on larvae or sensitive species.

7. In 1965 the amount of particulate organic material in the water column varied somewhat between stations. It was probably the most important variable in that after the material settled to the bottom it affected the amount of organic material and a series of environmental factors in the sediment.

8. For the interstitial water, salinity, temperature, and dissolved organic material were similar at the four stations. Hydrogen ion concentration was low at all stations and the small amount of nonionized ammonia was insignificant. While lack of dissolved oxygen in the interstitial water appears not to be of extreme importance, its companion variable, the buildup of hydrogen

sulfide at the Organic and Sludge Bed (especially the latter), should be considered a serious limiting factor for many organisms.

9. In the sediment, the effect of copper and lead in the amounts present was unknown, but the large amounts of the lead at the Sludge Bed may have had some effects on sediment feeding animals. The amount of chromium was very small.

10. Clearly the important factor in the sediment, especially in 1965, was the difference between stations in the organic content. The organic bottom in turn affected oxygen content, hydrogen sulfide generation, and perhaps other factors. Considerable and varied changes occurred between 1965 and 1968 in the organic content of the sediment.

11. The Between and Clean Stations were similar in sediment particle sizes. The Organic Station sediment was heavy with silt and clay, which caused it to become anoxic. The Sludge Bed was characterized by larger particles. Smaller sediment fractions were evidently shunned by some organisms and sought by others.

12. A reasonable variety of animals was present in Shilshole Bay, but a comparison with adjacent Puget Sound areas showed that overall the sampling stations were reduced in number of species of infauna. It also showed that some of those species were present in the Bay in much greater numbers, the organic enrichment evidently having had a fertilizing effect.

13. During 1965 and/or 1968 there were eleven species of Harpacticoid copepods collected, three of which became dominant (_Bulbamphiascus imus_, _Tisbe furcata_, and _Typhlamphiascus pectinifer_). There were ten species of amphipods, one of which became dominant

(*Photis californica*); three cumaceans, none of which became dominant; one isopod, which became moderately dominant (*Munnogonium waldronense*); one ostracod which became moderately abundant (*Euphilomedes carcharodonta*); one Chelifera, not dominant; one gastropod, not dominant; nine pelecypods, four of which became dominant (*Macoma carlottensis*, *Mysella tumida*, *Psephidia lordi*, and *Tellina modesta*); and 43 species of polychaete worms, three of which became most dominant (*Capitella capitata*, *Gyptis brevipalpa*, and *Armandia bioculata*) and six moderately abundant (*Glycera capitata*, *Anaitides williamsi*, *Prionospio cirrifera*, *Platynereis bicanaliculata*, *Eteone californica*, and *Nephtys ferruginea*). Sizable numbers of nematode worms were present.

14. In 1965 the Clean Station had three dominant species (*Capitella*, Nematoda, and *Bulbamphiascus*); the Between Station had 14 (*Capitella*, *Gyptis*, *Armandia*, *Glycera*, *Anaitides*, *Prionospio*, *Platynereis*, *Nephtys*, *Bulbamphiascus*, *Munnogonium*, *Tellina*, *Macoma*, *Typhlamphiascus*, and Nematoda); the Organic Station had three (*Capitella*, *Bulbamphiascus*, and Nematoda); and the Sludge Bed had five (*Capitella*, *Eteone*, *Bulbamphiascus*, *Tisbe*, and Nematoda).

15. In 1968, three years after the sewer was shut down, the Clean Station had no dominant species except nematodes; the Between Station had five (*Euphilomedes*, *Mysella*, *Tellina*, Nematoda, and *Psephidia*); the Sludge Bed had three (*Photis*, *Mysella*, and *Psephidia*); and the Organic Station had five (*Euphilomedes*, *Trochammina*, *Photis*, *Tellina*, and Nematoda).

16. In addition to the dominant species, there was in 1968 a general overall invasion of the thin upper crust of the Sludge Bed by

small numbers of crustaceans, mollusks, polychaetes, and a few copepods.

17. As to number of species in 1965, the Between Station had 59. This does not include the Nematoda. The Clean and Organic Stations each had 28 species, and the Sludge Bed had eight. In 1968 the Sludge Bed had 43 species followed by the Organic Station with 36, the Between Station with 31, and the Clean Station with 30.

18. The four sampling stations were evaluated by use of diversity and evenness indices. In 1965, based upon number of species, numbers density of individuals, and evenness of distribution, the Between Station showed a relatively high diversity index and was highest of the four stations. The Between Station in 1965 also had the highest diversity index based upon biomass, but this value was not greatly different from the Organic Station.

19. The Between Station supported the greatest total biomass, showing little change between 1965 and 1968. The greatest total number of animals was found in 1965 at the Organic Station due to the tremendous density of tiny nematode worms residing there. By 1968 all stations were greatly reduced in density of organisms, with the Between Station having the most.

20. A qualitative examination of the epifauna and nekton at the sampling stations showed the diversity of species to be small. At the periphery of the study area, however, rocks and a kelp bed harbored many species, this indicating lack of suitable substratum at the sampling stations was one of the major limiting factors.

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APPENDIX

VIII. APPENDIX

Ammonia

Ammonia was determined by modification of a procedure developed by Richards and Kletsch (1964). Their method does not distinguish between naturally occurring ammonia and ammonia derived during the analytical process from the decomposition of labile amines and urea. These writers found a 6.4 percent return of apparent ammonia from urea, and the return from different amino acids tested varied between 15.9 and 91.3 percent. Since Painter and Viney (1959) found domestic sewage to contain relatively large amounts of amines that would be labile, it is likely that waters receiving untreated sewage would be found to have inflated values of ammonia by this method.

In an attempt to obtain values closer to the true amount of ammonia present, each sample was divided so that one-half was treated by the method cited, the other half boiled to drive off any natural ammonia present, brought back to volume, and treated in the same manner. The difference in values was assumed to be from the natural ammonia present and not from decomposition of labile amines or urea.

Justification for this modification is based on the assumption that these compounds when boiled at 100°C are not decomposed to any degree and thus are present in the same quantity as before boiling. Data from The Handbook of Chemistry and Physics substantiates this assumption. Twenty minutes of boiling was found to be adequate to drive off all natural ammonia present.

At time of collection, samples were immediately acidified to about pH 2.5 with redistilled hydrochloric acid and frozen with dry ice for analysis later. It is of special interest that frozen, acidified samples held for over a year did not show any changes in ammonia from their counterparts analyzed soon after collection. It seems likely their stability was due to two facts: 1) acidification at pH 2.5 converts and holds all ammonia in the ionic form (NH_4+), and 2) freezing of samples suspends and reinforces the acid's inhibitory effects on bacteria to prevent further decomposition of any dissolved or particulate organics present. The degrading effects of acid on the frozen organics is not known, but is presumably small.

Whatever the possible weaknesses of the double procedure just described, it seems reasonable to assume it gives a more accurate portrayal of the ammonia present as the gas or ion than a method that admittedly partially degrades labile amines and produces high results. From my studies it seems worthwhile to explore further the modification as a standard procedure for determination of ammonia and labile amines in seawater analysis. The method is very sensitive: With a 5 cm cell values lower than 10 ppb above instrument variation can be measured; with a one-cm cell, ammonia concentrations of 900 ppb were measured.

Apparatus to sample the interstitial water

Special apparatus was designed to remove water from between the sediment particles (Fig. 39). It consisted of four parts.

1. A two-foot square of one-quarter inch thick, clear plastic sheet was placed and held tightly to the bottom with five bricks of iron. In the center of the sheet was a two and one-half inch diameter hole; the sediment was sealed from the water above by cementing a thin

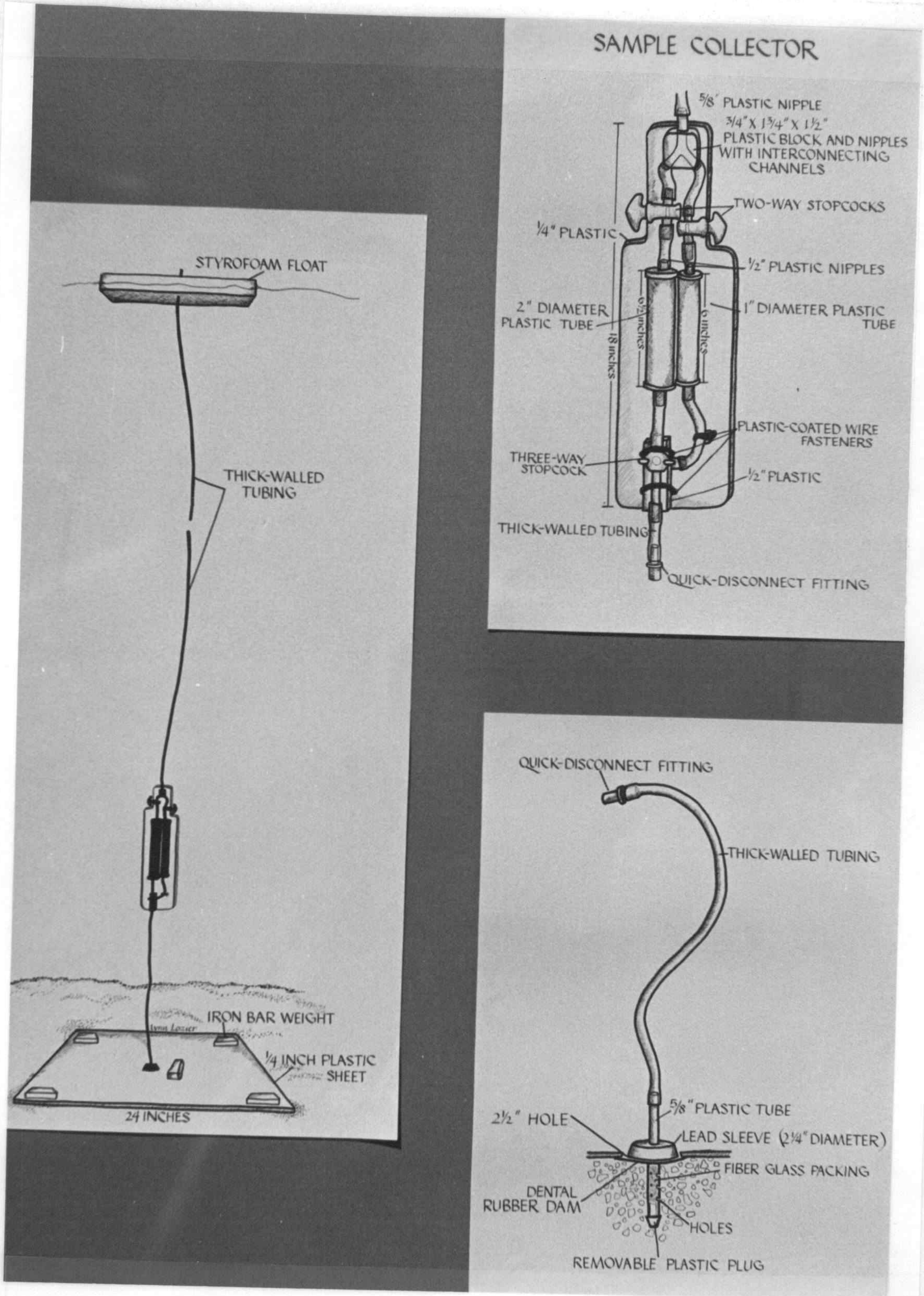

Figure 39. Interstitial water collecting device.

rubber membrane (dental "rubber dam") across the opening.

2. The water collecting unit consisted of a hollow, plastic probe, weighted with a two and one-quarter inch diameter lead collar, located five centimeters above the tip. The probe was simply pushed through a tiny hole in the rubber dam into the sediment.

3. The probe was connected by thick-walled tubing to a unit containing a three-way stopcock attached to two plastic collecting cylinders. The stopcock, located between the cylinders and the tubing from the probe, allowed contaminated residual water to be removed first into the smaller cylinder so that strictly interstitial water could then be collected in the larger.

4. From the top of the collecting cylinders, thick-walled tubing went to the surface where the end was floated with a piece of styrofoam.

The difference in pressure at the surface and at the sediment forced the water rapidly into the cylinders. Red dye injected into the sediment at the edge of the plastic sheet was not drawn into the cylinders, indicating the water collected was truly interstitial and from beneath the plastic sheet.

Current direction meter

Small current direction meters were constructed, mounted on sturdy iron frames, and placed on the bottom (Figs. 11, 12, 13). The meters worked on the principle of a weather vane and at all times reflected the direction of water current near the bottom.

The meters consisted of twelve micro-reed switches arranged like spokes in a wheel and mounted in an upper plate of one-half inch clear plastic. This arrangement provided readings at 30° intervals about a compass. At the periphery of the plate, attached to adjacent reed switches, were small resistors. The vane, with a small magnet on its upper surface to close the switches, swiveled freely on nylon bearings between upper and lower plates.

From the auto battery in the boat, ten volts of current was applied across the current meter on the bottom. The amount of voltage received back on the boat and read on the volt meter depended upon the number of resistors being used. The number of resistors in turn depended upon which reed switch had been closed by the magnet on the vane.

The meters were initially aligned on the bottom by use of an underwater compass so that a predetermined point on the meter pointed magnetic west.

Current velocity meter

A current velocity meter was lowered from the boat to the bottom to obtain velocity measurements. The meter was mounted on the same kind of metal platform that housed the direction meters.

Each meter consisted of a lightweight, savonius rotor swiveled freely between two plastic plates (Figs. 14, 15). A small notch was cut at the margin of the upper edge of the rotor, which was wider, and this edge rotated through a sensing unit in the upper plate. The sensor consisted of a photoelectric cell and a tiny light bulb. Each time the rotor turned once, the passing of the notch resulted in the light reaching the photocell. The resultant change in resistance was recorded as a jump in the needle of the volt meter aboard the boat.

Current velocity in feet per second was calibrated for the meter in a water flume, as the time it took for the rotor to make one revolution.

Sediment-water interface collecting system

In order to be able to collect water periodically, strictly from the sediment-water interface, without having to enter the water by diving, special collecting apparatus was installed on the bottom (Figs. 10, 13).

The apparatus consisted of a small block of plastic fitted with five hollow nipples with interconnecting channels. Four of the nipples were fitted with four-foot pieces of tygon tubing that were installed on the bottom in the pattern of a cross. The distal ends of the tubing were pegged to the sediment. The fifth nipple was fitted with a long piece of the same kind of tubing and this was attached to the electrical cable which ran from the current meter up to the surface.

A battery-operated pump was used to draw the water to the boat. To eliminate residual water in the tubing, the water drawn first was discarded before filling the collecting bottles.

An aid to the separation of small benthic animals from sediment

One of the most time consuming aspects of faunistic studies associated with sediments is separation of the inorganic and dead organic debris from the preserved animals so they can be identified, counted, and weighed. Ultimately final separation involves the use of microscope and forceps, but a device was designed (Fig. 40) that allowed all organic material present to be separated from the inorganic material, so the volume from which the animals needed to be recovered

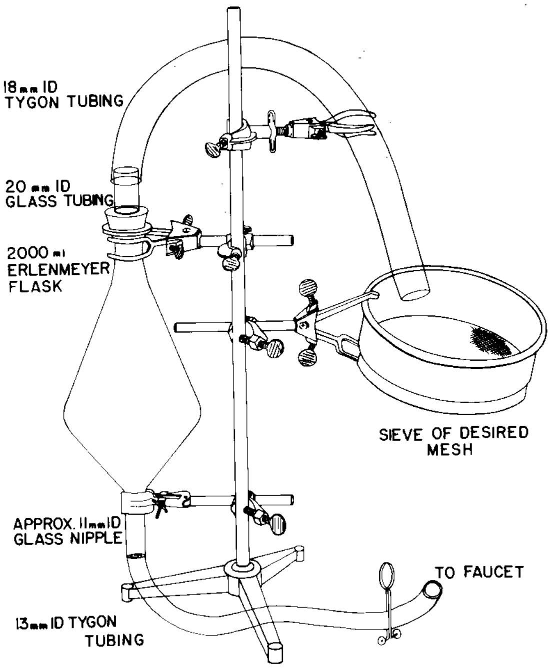

Figure 40. Apparatus to separate organic from inorganic fraction.

was greatly reduced. A staining procedure was used (Mason and Yevich, 1967) that reduced the time factor many fold for recovery of the animals from organic debris.

The apparatus operates on the principle of difference in density between organic and inorganic matter and uses water velocity to separate them. Its advantages are that the vehicle for separation--water--is inexpensive and readily available, recovery of animals is essentially complete, and the apparatus does not require constant attention of the operator while it is being used. The basic part is made from 1000 or

2000 cc Erlenmeyer flask that is thick-walled to minimize breakage. The bottom is drawn out to an angle of less than sixty degrees and provided with a glass nipple to which rubber tubing is attached to admit the water. Any competent glassblower is able to alter a flask at a reasonable cost.

As a jet of water is brought continuously into the bottom of the flask under pressure from a faucet, the contained sediment is thrown up into the water column. The higher the organic material is thrown toward the mouth of the flask, the more likely it is to be caught by the outflowing water at the narrowing mouth. Material carried from the flask is conveyed by tubing to a screen of minimum mesh size desired. The flow of water should be adjusted to throw the inorganics up into the water column, but not high enough to be carried over with the organics; proper flow is easily determined with a few minutes experimentation. Any inorganic particles small enough to be carried over with the organics will usually pass through the receiving screen. The acute angle of the bottom of the flask (less than 60°) is necessary to insure that all the sediment slides equally and continuously into the jet of water.

The procedure used to maximize recovery of animals and minimize time expenditure was as follows:

1. Preserve the sample in buffered formaldehyde; add sufficient one percent solution of rose bengal dye to color the fluid bright red. This will stain all animals and some plant material red, but not wood fibers, bark or inorganics; this makes it easy to distinguish the animals from the plant material or any residual inorganic sediment.

2. Wet sieve the sample first through a one or two millimeter mesh screen to remove larger animals, plant debris, and inorganics, and then through the smallest mesh screen to be used. This removes clay, silt, and small sand fractions.

3. Part of the sample left on the small screen is then placed into the apparatus described herein, and the water flow is adjusted to carry over the organic fraction. The amount of sediment to be placed in the flask is easily determined; too much will result in poor separation because inorganics will be washed over as well.

4. The sample is allowed to wash until little or no stained material is left in the flask; this takes 10 minutes or longer. The residual inorganic fraction in the flask is then drained out through the bottom tube into a white tray where it can be quickly examined with the naked eye for any of the larger, heavier animals, such as bivalves and snails, which have not been separated with the other organics. Usually these will be few in number and easily spotted because the sediment is so clean and the animals have exposed soft parts stained red.

5. Material carried over to the screen is placed in 70 percent alcohol to be stored and picked. The dye will slowly leach from the specimens in the alcohol.

It should be emphasized that this method does not separate animals from other organic material; such a method has yet to be devised. It does separate the smaller organic material from the bulk of the smaller inorganic sediment. The rose bengal dye allows easier separation of animals from plant and other organic debris. Larger samples can be processed by using several flasks simultaneously.