AN ABSTRACT OF THE THESIS OF

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Title: TRACE ELEMENTS IN OYSTER BIODEPOSITS



Abstract Approved: Norman H. Cutshall

The contents of the trace metals copper, zinc, manganese and iron in adult Pacific Oyster (Crassostrea gigas, Thunberg, 1795) feces, pseudofeces and suspended particles were investigated. The biodeposits were collected on a continuous basis for 28 weeks with a specially designed apparatus. Seston samples were obtained in a sedimentation chamber. The biodeposition rate was calculated from the relative contributions of feces and pseudofeces. During the experiment, samples were periodically withdrawn and analyzed for:

a) trace metal content, by flame atomic absorption spectrophotometry (AAS);

b) organic carbon and total carbon and nitrogen, by combustion gas chromatography; and

c) total organic matter, by the difference between dry and ash weights.

d) Biogenic opal and quartz were analyzed by X-ray diffraction in selected samples.

The biodeposition rate for adult oysters under the experimental conditions was 0.63 g dry weight/oyster/week. The feces production rate was 0.33 g dry weight/oyster/week, and the pseudofeces rate 0.30 g dry weight/oyster/week. The mean feces/pseudofeces ratio was found to be 0.90. Both biodeposits were composed mainly of a loose agglomeration of very fine particles. The average trace metal concentrations on a dry weight basis were 38.5 µg Cu/g, 117 µg Zn/g, 686 µg Mn/g, and 34.4 mg Fe/g for feces. For pseudofeces: 39.6 µg Cu/g, 121 µg Zn /g, 778 µg Mn/g and 37.2 mg Fe/g and for biodeposits: 39.2 µg Cu/g, 119 µg Zn/g, 731 µg Mn/g, and 35.9 mg Fe/g. These concentrations were all higher than a group of mud samples from Yaquina Bay, Oregon. This condition appears to be due to the higher proportion of fine particles in the biodeposits than in the bay mud. In the gravitationally settled seston, with a particle size distribution comparable to that of the biodeposits, the metal content was 35.9 µg Cu/g, 106 µg Zn /g, 688 µg Mn/g, and 36.6 mg Fe/g.

The organic carbon and total nitrogen content for feces and pseudofeces were comparable. The mean values for the biodeposits on a dry weight basis were 58.4 g/kg organic carbon and 7.4 g/kg total nitrogen. The total organic matter for the biodeposits was 15.6%. This relatively high content of organic matter creates an important nutritional substrate for deposit feeders. Biodeposition

is potentially important in the distribution and cycling of trace elements in estuaries, through the translocation of particles bearing trace metals from the water column to bottom communities.

# Trace Elements in Oyster Biodeposits

by

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Typed by Gerri A. Riley for Katsuo A. Nishikawa-Kinomura

"I believe that the secret lies in the role particles play as the sequestering agents for reactive elements during every step of the transport process from continent to ocean floor."

K.K. Turekian 1977

"It should be emphasized that the normal oyster is enormously more efficient in clearing ordinary turbid sea water than is gravity."

E.J. Lund 1950

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# TRACE ELEMENTS IN OYSTER BIODEPOSITS

## PROLOGUE

During the last few decades, major changes have been brought about in the environment by a single species, Homo sapiens, which has gained dominance over much of the biosphere. This species "success" and its interactions with the other living forms have created threats to the ecosphere and brought about a so-called "Worldwide Pollution Problem." Some would hold that man, the producer, will provide the solution to this problem. But, all too often in his attempts to resolve the situation he has found that this is not simple. The problem has roots in space and in time and is related to other complexities of our civilization.

The human attitude toward pollution has evolved away from the plutocratic approach of the last century, via anthropocentrism, to an ecosystem view. Increasingly we consider pollution as a whole ecosystem threat and not just as a local human health problem or an economic problem.

This more recent approach, even though it provides a more realistic view of the situation, requires that we understand the functioning of each particular ecosystem. We have to identify and evaluate its functional basis, as well as the interaction in time and space of a pollutant or mixture of pollutants with the ecosystem.

The research described here is focused on the role of a suspension feeder of estuaries in transferring metal from the seston to the benthic boundary layer.

# INTRODUCTION AND LITERATURE REVIEW

The hydrosphere has been the dumping ground for many refuse materials and of energy expelled by the anthroposphere. The ocean is the ultimate recipient of these aquatic discharges, and coastal zones, especially estuaries, are the main route of entry. For this reason, estuarine ecosystems are among the environments most severely affected by human activities (Duce, 1974).

Pollutants are introduced to coastal zones both directly as a result of human use and indirectly through atmospheric fallout, river input, and tidal exchange with the adjacent neritic zone.

The interaction of an ecosystem with pollutants is often complex. Aquatic biota can influence the chemical state, transport and the ultimate fate of pollutants which enter the sea (Martin, 1977). Thus, the fate and effect of any pollutant in the estuarine environment depends not only on its initial chemical state, but also on chemical changes occurring in the estuary. Biological activities such as feeding, burrowing and fecal material production, may also have geochemical effects (Rhoads, 1974). Quantitative comparisons of these processes with non-biological ones are difficult although an interaction presumably exists between the biotic and abiotic processes to form the homeostatic control mechanism of any ecosystem.

Among the estuarine pollutants that have caused concern are the metals, especially the "heavy metals," some of which may have undesirable ecological effects. In the last two decades, this group of

elements has been the focus of extensive environmental research stimulated both by their importance as toxic agents to many forms of life and by their significance to radioactive pollution problems (Waldichuk, 1974). In this group, four of them, copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn), are the most commonly studied metals involving the biogeochemistry of estuaries and nearshore environments. These heavy metals have been studied because of their significance as trace nutrients, their toxicity to the biota (Waldichuk, 1974), and their role in geochemical studies as indicators of the fate of other heavy metals in such transient environments (Elderfield, 1976; Evans, 1977).

## Ecological Considerations of Estuaries

Estuaries are defined by Clark (1974) as "any confined coastal water body with an open connection to the sea, and measurable quantities of salts in the water." This definition includes all hyper-, oligo-, meso-, and polyhaline environments found in different latitudes regardless of the river contribution.

The hydrology in such coastal physiographic structures, as well as the biology, defines a unique system. Most estuarine ecosystems are characterized by: a) high organic productivity, associated with low species diversity and b) steep gradients of environmental conditions (Odum, 1971). Consequently, variability and complexity of environmental conditions are more pronounced than those found in neritic zones or in freshwater habitats (Perkins, 1974).

The material cycling processes associated with this unique habitat are as complex and variable as its environments. Thus, each estuarine ecosystem has its own way of distributing those materials. The water, biota and sediment systems interact to establish a material flux by means of a series of poorly known transference steps which gives the estuary its singular dynamic properties. One result is that nutrients and many pollutants can be entrapped within its confines. The unique homeostatic processes prevailing in each estuary determine, to some degree, the residence time of materials therein. Therefore, in order to assess the role of estuaries in marine pollutant transfer we must carefully evaluate each of the steps through these transition environments.

## TRACE METALS IN ESTUARIES

Trace metals, upon their introduction to the estuaries by the river, ocean, atmosphere or industrial and municipal outfalls, may be in solution or associated with the suspended particles or the bed load. Our understanding of their fate under these conditions is highly speculative (Martin, 1977) The estuary may act as a sink (Turekian, 1974) or as a transient medium to the ocean. In either case, depending upon the specific element and the suite of physical and chemical conditions in the estuary, metals experience alterations to various degrees during their residence in this environment. Their ultimate fate may depend on those alterations.

Partitioning of trace metals into particulate and soluble phases in an estuarine environment is complex and normally in disequilibrium (Parks, 1975). According to Stumm and Brauner (1975), metals can be present in the dissolved phase, as charged or uncharged complexes formed by organic or inorganic ligands, as ion pairs, as free cations or as oxy-ions. Similarly, in the solid phase these metals are present as insoluble precipitates or coprecipitates on a normally complex and heterogenous surface, as biogenic components, or bound on or within the crystalline lattice of inorganic detritus (Elderfield, 1976; Evans, 1977).

In a very simplistic view, trace metal cycling in estuaries can be conceptualized as a series of mass transferences and complex chemical reactions taking place within five major components; biota,

water, abioseston, bottom sediment, and interstitial water (Figure 1). Exchanges of trace metals among these components are basically reactions of solubilization and particulation and are superimposed upon mass transport processes such as flushing of the estuary, river runoff, tidal currents, and biological activity.

Bottom sediment and suspended particles in this environment contain the overwhelming reservoir of trace metals. However, the trace metals dissolved in the water, as well as those associated with seston (both riverborne and tidally suspended) are the most readily mobile metals, since they are subject to the hydraulic regimen of the estuary.

Some trace metals associated with the fluviatile derived sediment particles are partially displaced by the more abundant cations $Ca^{+2}$, $Mg^{+2}$, and $Na^{+}$ upon mixing with sea water (Elderfield, 1976; Martin, 1977; Evans, 1977). The enrichment of trace elements in the aqueous phase by these mechanisms is considered transient because many of them are subject to precipitation, coprecipitation, and adsorption. Thus the particulate phase in this more saline environment will ultimately be enriched by metals and this will result in a downstream precipitation of metals to the sediment (Martin, 1977). The complete understanding of exchange reactions between the soluble and particulate phase is difficult because it depends on the estuarine condition. Moreover, we are dealing with an environment out of thermodynamic equilibruum where incomplete reactions are common.

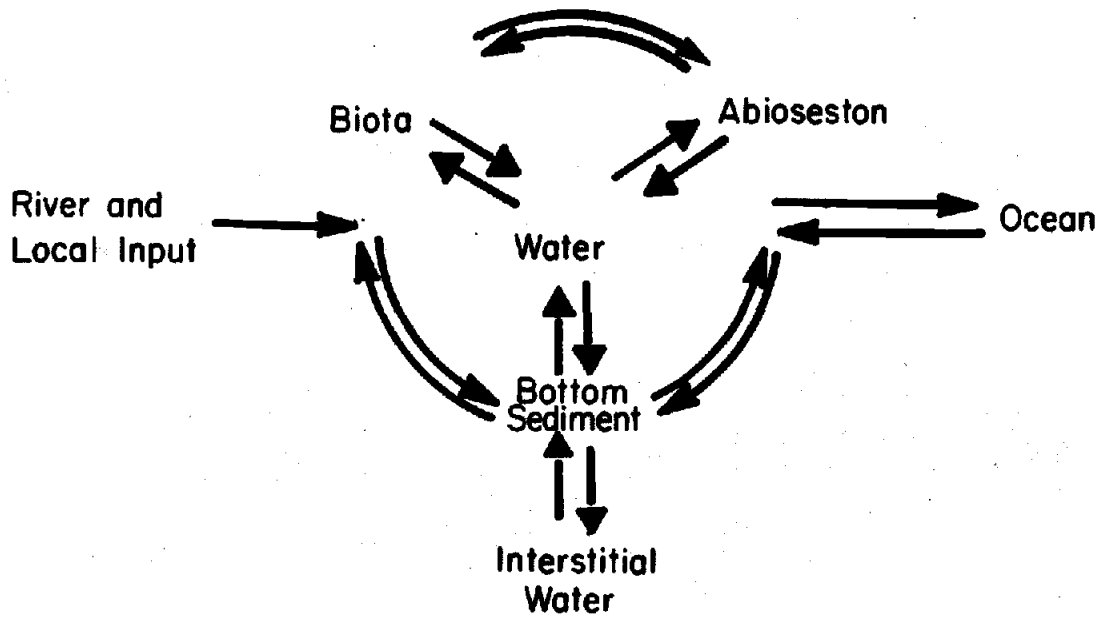



Figure 1. Material cycling in estuarine ecosystem.

## Behaviour of the Trace Metals Cu, Fe, Mn, and Zn in Estuaries

The trace metals considered in this work can be divided into two groups, which tend to behave similarly, Fe, Mn, and Cu, Zn. According to Evans (1977) and Martin (1977), higher pH estuarine environments will favor the oxidation and precipitation of $Mn^{+2}$ as $MnO_2$ and $Fe^{+2}$ as $Fe(OH)_3$, while, inorganic precipitation of Cu and Zn should not occur because both are undersaturated with respect to their supposed solubility limiting compound.

The transport of these trace metals from the water column to the bottom sediments (Figure 1) is mainly due to flocculation and sedimentation of suspended colloids (Dyer, 1972) and to biodeposition by suspended matter feeders (Haven and Morales-Alamo, 1968; Martin, 1977). Similarly, the inverse process takes place as tidally resuspended bottom sediments (from which certain elements such as Mn may desorb), bioturbation and resuspension by benthic organisms (Elderfield, 1976), and dissolution and diffusion from the sediment.

The mobilization of Cu, Fe, Mn, and Zn in bottom sediments and interstitial water (Figure 1) takes place in a more or less closed system under anoxic conditions usually along with low pH. Cu and Zn, as well as Fe and Mn, can desorb from the particulate fraction into solution. This may be due to the influence of pH on the surface properties of hydrous oxides or be due to the low redox potential, which causes the reduction of coprecipitated oxides (e.g. Mn and Fe) with the consequent release of trace elements to the interstitial water (Elderfield, 1976). Thus, Cu and Zn are normally precipitated as sulfides and become trapped by the sediments, while Mn and Fe,

with more soluble sulfides, can maintain high dissolved concentrations in reduced interstitial waters (Evans, 1977). Consequently, Fe and Mn can diffuse to the overlying waters where they reprecipitate as oxides, and enrich the superficial sediments.

## Estuarine Food Web

The pathways of energy flow in estuarine ecosystems are complicated; their characteristics depend on temperature, nutrient supply, water exchange rates and other environmental factors. Consequently, the trophic relationships within an estuary are not fixed, but rather change seasonally and from one year to another, depending on the food source.

From the functional point of view, according to Mann (1972) and Odum and Heald (1975), there are two basic types of food webs: the grazing food web, in which the primary energy source is the macrophytes and microphytes; and the detrital food web, where the dead plant material and other organic aggregates are the primary energy source.

In the former case, plant material generated within the estuary is consumed directly by herbivorous organisms, which in turn are grazed by predators. In the latter case, organic detritus originated either within the estuary or imported is consumed by a group of detritus feeders and omnivorous invertebrates from which the carnivores obtain nourishment (Mann, 1972). These two pathways of energy flow are extremes and most estuaries have both of them, even though one may dominate under a given set of environmental conditions. In any case, it is important to point out that each heterotrophic

organism, besides consuming part of the organic matter available in the estuary, will relocate and transform the material associated with its food.

Figure 2 represents a conceptual model of the food web in an estuary where both grazing and detrital food bases are important. The sources of energy in this case can be either autochthonous or allochthonous. The diagram is similar to the one presented by Odum and Heald (1975), and has been modified to represent a more "general estuary," based on the works of Teal (1962), Newell (1965), Johannes and Santonni (1966, 1967), Riley (1970), Mann (1972), and Parsons and LeBrasseur (1972).

In this conceptual model, the autochthonous materials are transferred in the following manner. The bulk of organic material synthesized by autotrophic organisms (phytoplankton, macroalgae, benthic diatoms, and vascular plants) enters the next trophic level of consumers by direct grazing. But, the detritus produced by vascular plants and microalgae goes through an intermediate step where microorganisms decompose the material and create the bulk of organic detritus. This, in turn is consumed by omnivores and detritivores. In the mixed trophic level, as well as the middle and higher carnivores, not all the ingested food is utilized. Portions pass undigested or unassimilated as feces and are voided to the environment, as shown in the lower portion of the diagram. This introduction of the fecal material supports important recycling mechanisms for the organic material. A large portion of these substances is reutilized as organic detritus by many benthic

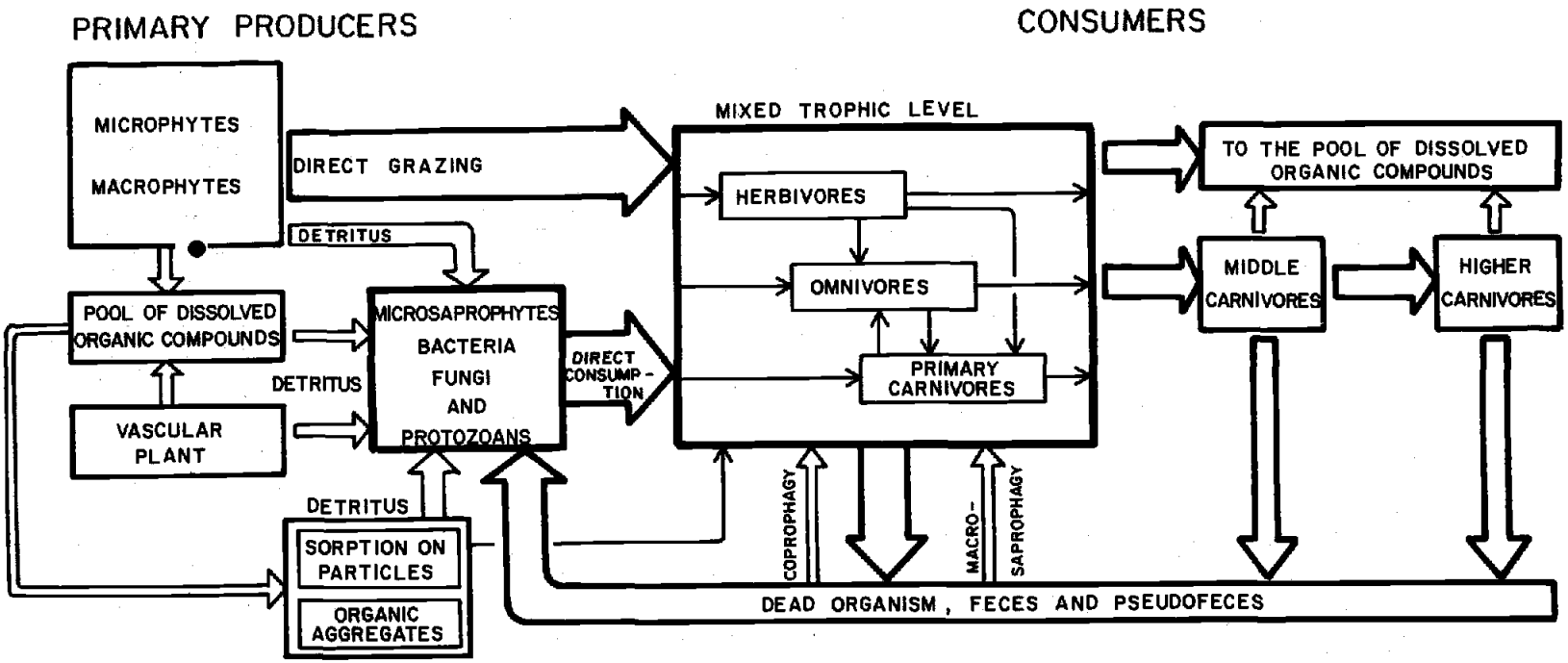



Figure 2. Conceptual model of the estuarine food web.

organisms and consequently introduced back to the food web.

Utilization of allochthonous material generated in the river or the ocean depends on how and where it is introduced, as well as its quality and the presence of opportunistic feeders. It can be incorporated in almost any level of the grazing food web or the detrital food web.

## BIODEPOSITS IN ESTUARIES

In aquatic communities (Figure 3), feeding activity of heterotrophic organisms leads to the formation of byproducts and waste material. These substances are released to the surrounding medium as agglomerated bodies, with features that are characteristic of the organism involved and the specific food resources utilized (Krauter and Haven, 1973).

In this work, the term oyster feces is used for the compact material ejected at the end of the digestive tract. The term pseudofeces refers to the material ejected by oysters from the shell cavity in a loosely compacted mass before ingestion (Haven and Morales-Alamo, 1972; Bernard, 1974b). Both feces and pseudofeces settle to the bottom where they are termed biodeposits, the process involved is known as biodeposition (Haven and Morales-Alamo, 1966; Bernard, 1974b).

Biodeposits are ubiquitous in the aquatic environment, they can be found wherever feeding animals are present, either in the water column as part of the seston, or in the bottom sediments. The presence of biodeposits, however, is not restricted to the location of the organisms. Fecal material can be transported passively in the water column for long distances before being trapped in the sediments or disaggregated. In this regard, the process involved in the formation of fecal material by the organisms, as well as their biodeposition, are important factors with biogeochemical

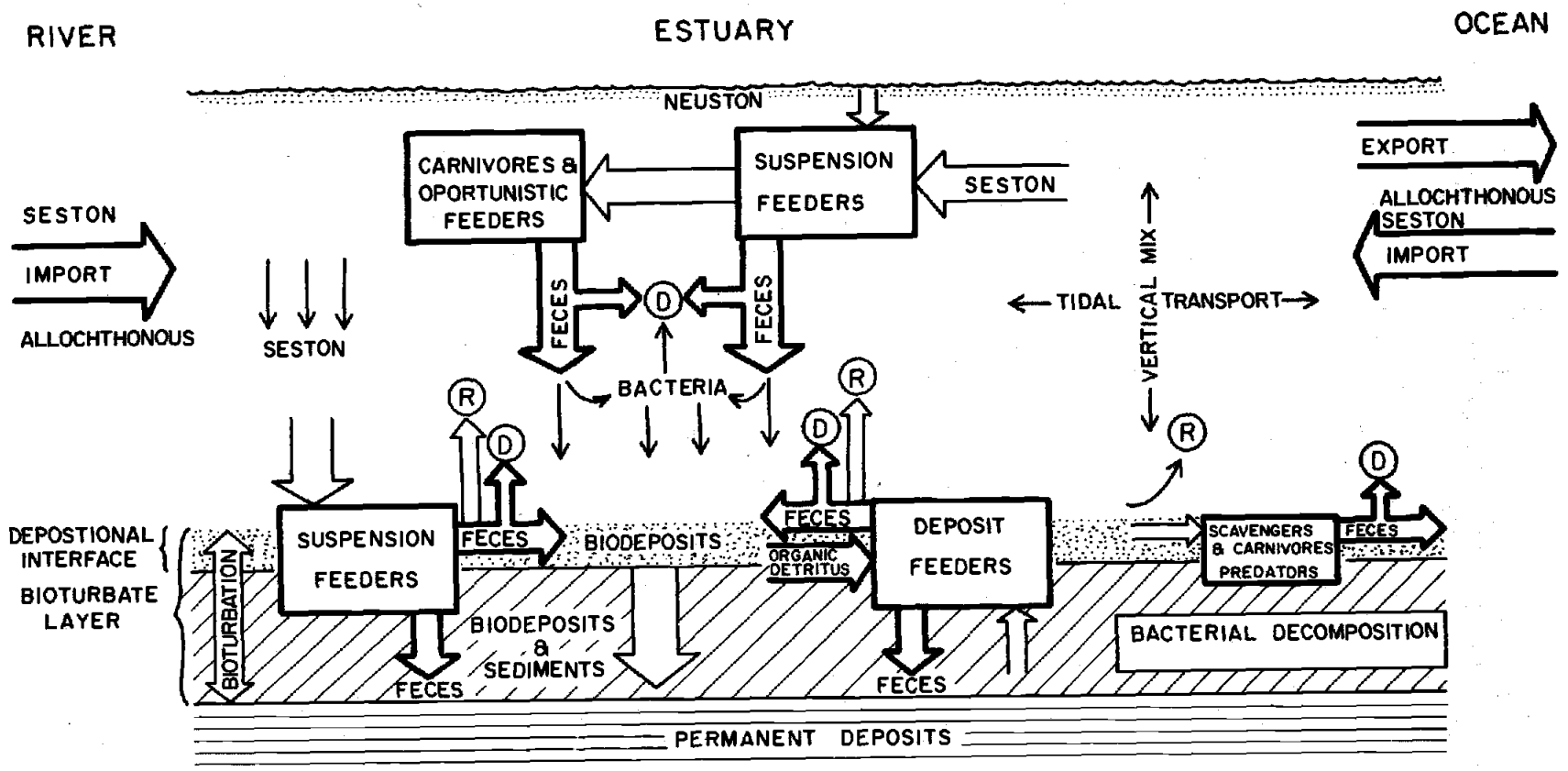



Figure 3. Conceptual model of biodeposit production in estuaries.

significance in the transference of material and energy within the hydrosphere.

Although the importance of these processes in marine environments has not yet been evaluated completely (Rhoads, 1974), it is possible to infer their role in many biogeochemical processes (Kuenzler, 1965). Most of the information that is found elsewhere in the literature concerns a few studies of biodeposits in coastal regions. Thus, the organisms in estuaries and bays are among the most completely studied, particularly certain suspension feeders, such as oysters. For estuarine ecosystems, practically all published results are related to autochthonous source biodeposits, while the allocthonous contribution has virtually been ignored.

In most eutrophic estuaries, the impact of fecal material on the environment takes place through a complex and poorly evaluated process. The sources of autochthonous biodeposits and their composition are as diverse as the different feeding mechanisms present. Consequently, the trophic structure of each community is of major concern in studying the estuarine biodeposit dynamics.

Figure 3 gives a diagrammatical representation of biodeposit formation in a hypothetical estuary. Only known significant mechanisms are shown (Haven and Morales-Alamo, 1966; Young, 1971; and Rhoads, 1974). These diagrams indicate that the source of biodeposits in the estuary can be either allochthonous or autochthonous. The allochthonous contribution is carried into the estuaries from the river and the ocean, probably as coprogenic detritus in the seston. Export processes to the ocean are similar.

The autochthonous contribution occurs through heterotrophic organisms, mainly as a result of five basic feeding types; carnivores, scavengers, browsers, suspension feeders and deposit feeders (Walker and Banbach, 1974). Predators and suspension feeders are commonly found in the water column (Odum, 1971). On the other hand, the benthic community includes all five types (Rhoads, 1974; Walker and Banbach, 1974).

Biodeposits produced by each feeding type are potentially important in the food web and in biogeochemical processes in the estuarine ecosystem. The carnivores, scavengers and browsers, by the nature of their food, produce feces relatively rich in organic compounds but unstable in the aquatic environment. They are important contributors to the detrital food web as well as to the formation of organic rich deposits. Suspension feeders, which feed upon sestonic materials in the water column, produce biodeposits of widely variable composition depending on the degree of food selection. Those organisms are important as sedimentation agents for fine sediments of clastic and biogenic origin (Haven and Morales-Alamo, 1966). On the other hand, deposit feeders in most cases ingest complex food resources composed of organic detritus and mineral grains, with their associated micro and meiofauna. These organisms feed at the depositional interface or within the sediment column. Therefore, they are important in the bioturbation of sediment deposits by pelletizing, resuspending, and selecting the grain size of the sediments (Rhoads, 1974).

From the five feeding mechanisms discussed earlier, the deposit

feeders and suspension feeders have been considered the major bio-deposit sources. According to Rhoads (1974) and Walker and Banbach (1974), these are the primary feeding mechanisms found in the many benthic communities of protected shallow waters. This work is limited to suspension feeders.

## Suspension Feeders

Suspension feeding is a mechanism employed by many invertebrates in feeding upon dispersed food particles which are too small to be sensed and collected individually (Rhoads, 1974). Suspension feeders are common in pelagic as well as benthic communities, while in the latter environment they may be epifauna or infauna. In each case, the particles that they remove from the water column have different characteristics depending on proximity to the bottom (Jorgensen, 1966). The specific mechanisms involved in suspension feeding are diverse, but two basic types are found: 1) Filter feeders which remove seston by passing the surrounding water through structures that retain particles mainly according to size and shape; and 2) "Whirlers" which retain suspended particles touching the particle collecting surfaces (Jorgensen, 1966).

The efficiency of retention is different in each case. For filter feeders the minimum size is restricted by the pore size of the filtering mechanisms, while the upper size limit is determined by screen-like structures that remove large particles from the water before they enter the feeding organ. For the "whirlers," there are no systematic studies, but the retention does not decrease abruptly at a certain minimum size of particles, as in the case of some filter

feeders (Jorgensen, 1966).

## Biodeposits

Feces and pseudofeces of suspension feeders leads to the formation of agglomerate particles larger than the original suspended seston. The settling velocity of fecal material is many times greater than that of the original seston (Lund, 1957; Haven and Morales-Alamo, 1966). In this regard, the biodeposits of this group of organisms are quantitatively important to the formation and quality of bottom deposits in many aquatic systems.

Among the best known effects is the formation of large accumulations of organic rich coprogenic mud. Verwey (1952) calculated that the population of Cardium in the Waddenzee deposits about 10,000 metric tons (dry weight) of fecal material each year, and the mussel (Mytilus edulis) population, between 25,000 and 175,000 metric tons (dry weight). In the Clyde Sea, Moore (1931) found that fecal pellets from Calanus and euphausiids are deposited during spring at a rate of 33.4 $mg/cm^2$ per week. In Cape Cod, Massachusetts, the cirriped Balanus balanoides produces from 0.2 to 0.5 ml/individual/day (Rhoads, 1974).

The production of considerable amounts of biodeposits in estuaries and protected bays by commonly occurring suspension feeders is better documented. In Sendai Bay, Japan, Ito and Imai (1955) calculated that a raft of Pacific oysters (Crassostrea gigas), 60 $m^2$, surface produces a miminum of 0.6 to 1.0 metric tons (dry weight) of fecal material per year. On the other hand, Lund (1957) estimates that a

single layer of the American oyster (C. virginica) deposits about 12 metric tons (dry weight) of solids/hectare/week. However, Haven and Morales-Alamo (1966) calculated that one hectare of a culturing bed of the same oysters, in Lower York River, Virginia, produces 1 to 2 metric tons (dry weight) of biodeposits per week. This latter figure is substantially different from those given by Ito and Imai (1955). However, this discrepancy is probably caused by the culturing techniques used; that is, the values for the Pacific oyster are for suspension cultures, while the American oysters are applied to bottom cultures where the available seston is considerably higher. Haven and Morales-Alamo (1966) also measured the biodeposition rates of other suspension feeders is Chesapeake Bay. For invertebrates they found: soft shell clams (Mya arenaria) produced 25 mg dry weight of solids/individual/day, ribbed mussels (Modiolus demissus) 125 mg dry weight individual/day; cirriped crustacea (Balanus eburneus) 18.4 mg dry weight individual/day, tunicates (Molgula manhattensis) 60 mg dry weight/individual/day. These values are small, but when multiplied by the population sizes they become considerable.

In freshwater ecosystems, biogenically produced organic rich muds are not uncommon. In eutrophic lacustrine environments, these types of sediments are called gytja or copropel (Cole, 1975), and are composed of large proportions of fecal material from invertebrate organisms. As an example, according to Iovino and Braddley (1969), the soft ooze, 1 m thick found in the bottom of Mud Lake, Florida, is composed almost exclusively of minute pellets of the filter feeding larvae of the insect Chironomus spp.

Suspension feeder biodeposits are also often found in riverine environments. Prokopovich (1969) estimates that 6.2 metric tons (dry weight) of the Asiatic clam (Corbicula sp.) can produce 3350 metric tons per year of sediment from water containing only 330 mg/l of suspended solids. In deep sea environments, the biogenic deposits from remains of pelagic planktonic organisms are also probably coprogenic in origin, since fecal pellets of pelagic organisms are the main routes of sedimentation (Jorgensen, 1966; Smayda, 1970). In this regard, Schrader (1971), among others, suggests that the rapid transport of silica from surface waters to deeper waters is through the fecal pellets of planktonic crustaceans. In order to substantiate this statement, the author compared the sinking rate of a single diatom Thalassiosira baltica frustule 50μ in diameter to a fecal pellet 100μ in diameter. The sinking rate of the frustule was 10 m/day and the fecal pellet 100 m/day. In addition to this increase in the sinking rate, the author points out that the frustule is protected by the membrane of the fecal pellets against dissolution during their descent to the ocean floor. Under laboratory conditions Curl, as cited by Osterberg, et al. (1963), also found comparable sinking rates. For fecal pellets of a euphausiid (Euphausia pacifica) fed with diatoms (Skeletonema costatum), he found values of 43 m/day for fecal pellets 100μ x 1000μ to 500μ x 3000μ in size.

## COMPOSITION OF THE BIODEPOSITS

The fact that suspension feeders remove a size range of particles from the water column, and convert them to discrete fecal pellets or fecal strings, allows them to produce biodeposits with a wide range or composition and physical appearance. The literature concerning estuarine and coastal organisms, in this respect, is limited; oyster biodeposits are the best known. Biodeposits collected immediately below an oyster culture rack 2 years old by Ito and Imai (1955) were black colored, with 99.5% grains less than 0.1 mm diameter, 4.95% "humus" and 1.87% total sulfide. On the other hand, the control sediments were brown, with only 56.5% fine grains, 3.97% "humus" and 0.51% total sulfide.

Haven and Morales-Alamo (1966, 1972) found that oyster biodeposits consisted of sand grains, particles of silt and clay, fragments of diatoms, bacteria, sponge spicules and many unrecognizable fragments. The size range of particles in feces and pseudofeces was from less than 0.8μ to about 13μ. About 95% of the particles observed were less than 3μ. The clay minerals made up 70 to 90% of the sample by weight. Total organic carbon ranged from 4-12% and the phosphate content was on the order of 1.0 g/kg of sample. The composition of organic substances in oyster biodeposits is not well known, but, according to Bernard (1974a) for C. gigas in British Columbia, the protein content ranges from about 4% to 11.5%, the carbohydrates from less than 1% to 12%, and the lipids 1.5% to 7%.

Kraeuter and Haven (1970) also studied the morphology and composition of fecal material of various invertebrates in Lower York River and Lower Chesapeake Bay, Virginia. They gave little information concerning composition, but their taxonomy is very useful.

## OBJECTIVES

This research was designed to: a) quantitatively evaluate the role of biodeposits (feces and pseudofeces) of a suspension feeder, Crassostrea gigas, in removing fine seston and associated metals (Cu, Zn, Mn and Fe) from natural estuarine waters; and b) compare the composition of those biodeposits to that of the fine sediments from the same water body settled by the effects of gravity under very low energy conditions.

# MATERIALS AND METHODS

## Experimental Organisms

A suspension feeder, the Pacific oyster Crassostrea gigas (Thunberg, 1795) was chosen for the study of metal content in biodeposits, for the following reasons:

a) The feeding apparatus, which is relatively well known, has the ability to remove fine particles (<1μ of diameter) from the water column, and produce large masses of consistent biodeposits (Bernard, 1974b).

b) The separate ejection of feces and pseudofeces indicates some particle sorting capacity (Bernard, 1974b).

c) The feces and pseudofeces can be collected and studied individually (Quayle, 1969; Bernard, 1974a,b).

d) This species is relatively easy to culture in a laboratory.

e) Finally, this oyster has been artifically propagated beyond its natural distribution by the oyster culture industry. At the present time, they can be found in many estuaries and protected bays of subtropical and temperate coasts of the world (Quayle, 1969; Tanaka, 1975).

These characteristics make C. gigas a useful experimental organism that can be used as a source of biodeposits either in laboratory conditions or in their natural environments.

## Sample Collection

### Oyster Feces and Pseudofeces

This study involved feces and pseudofeces accumulated during different sampling periods, ranging from four days to two weeks. The source of the biodeposits was nineteen mature (83.7 ± 8.6 mm in length) oysters, which were held for 28 weeks (April 24 to November 24 of 1975) at the aquarium facilities of the OSU Marine Science Center in Newport, Oregon. The whole group of organisms was kept in a biodeposit collecting apparatus especially designed for this study.

The instrument, which is described in detail in Appendix I, is composed of four biodeposit collector units. Each holds five oysters in individual chambers and has provisions for collecting the feces and pseudofeces separately on a continuous basis. During the sampling period, one of the oyster holding chambers was always kept with an empty oyster shell, as a control for gravitationally settled sediment. The biodeposit collection apparatus has no metal parts; the instrument itself was acid cleaned and conditioned for the three weeks before the first sample was collected.

Sea water from Yaquina Bay, Oregon was pumped from the near bottom intake and passed through a sedimentation chamber, where larger particles settled out, assuring that only the fine portion of the seston was available to the oysters (see Appendix II). The water flow rate through each individual unit was 200 ml/min. This value was experimentally obtained and was such that the volume of feces and pseudofeces production were not limited. Feces and pseudofeces from the oysters were collected from the biodeposit chambers

(see Appendix I, Figure 2).

Biodeposits were removed from the collection chambers by suction using an acid washed (G.F. Smith 12N redistilled HCl) polyethylene syringe. Excess water was decanted and the biodeposits concentrated by centrifugation at low speeds (1500 rpm) for ten minutes. The centrifuged material was homogenized with a glass rod, weighed, and stored frozen in polyethylene plastic bags until analysis.

### Control Sediment

Concurrent with feces and pseudofeces collection, a control sediment sample was obtained in the settling ponds, using two glass culturing dishes (20.3 cm diameter). Treatment of this sample was similar to that given to the biodeposits. The objective was to compare the gravitationally settled material to that produced by the oysters.

### Yaquina Bay Sediments

Sediments were also collected from a commercial oyster culturing raft in Yaquina Bay and from the adjacent area, using a minidredge sampler (Farrow and Larsen, 1975) covered with plastic. The normal sampling holding net was replaced by a transparent polyethylene plastic bag. This group of samples was analyzed like the others.

## Salinity and Temperature

Salinity and temperature values were obtained from the continuous recording instrument graphs at the OSU Marine Science Center.

# ANALYTICAL TECHNIQUES

## Sample Preparations

Biodeposits and sediments were analyzed for four metals (Cu, Zn, Mn and Fe), total carbon, organic carbon, total nitrogen, quartz, and biogenic opal. Biogenic opal and quartz were determined in selected samples only, while the other parameters were measured in all the collected materials.

As a standard procedure, all the frozen samples were thawed at room temperature, homogenized inside their respective plastic bags and divided into two subsamples. About two thirds of the material was used for metal determinations and the rest for quartz, biogenic opal, total and organic carbon, and total nitrogen determinations.

## Preparation for Metal Analysis

The metal subsample was homogenized and an aliquot containing about 50 g was transferred to an acid cleaned 250 ml Pyrex beaker and the weight was recorded. The sample was then dried in an oven at 105°C $\pm$ 5°C to constant weight ($\pm$ 0.001 gm), usually obtained within 48 to 72 hours. The sample dry weight was calculated by subtracting the empty beaker weight from the one obtained at the end of a drying period.

All glass and plastic material was washed, and then rinsed three times with 6 N HCl acid, and three times with .36N HCl. Finally, the material was rinsed in deionized, glass-distilled water and

allowed to dry in the low temperature oven in dust free conditions.

Destruction of organic material was achieved by ashing the dry sample in the beaker at 450°C ± 25°C in a muffle furnace for 48 hours or until constant weight was obtained. This ashing process was conducted in two periods. During the first three hours of ashing the temperature was gradually increased to about 250°C and the rest of the time was kept as indicated at 450°C. The ash weight was calculated by subtracting the empty beaker weight from the constant weight obtained after the ashing. Weight loss by ignition, a rough estimate of organic matter, was calculated as the difference of ash weight from dry weight. The ashed samples were homogenized to a fine dust, with a porcelain pestle and mortar, and stored in plastic counting tubes.

Preparation for Total and Organic Carbon and Total Nitrogen Analysis

Subsamples for total carbon and total nitrogen were homogenized and transferred to a covered 5 ml porcelain crucible which had been previously cleaned with acetone. To avoid loss of volatile organics, the material was dried in a low temperature (63°C ± 3°C) oven to constant weight, homogenized in an acetone washed mortar, and stored in tightly closed two dram vials. To minimize decomposition of the organic fractions, the vials were transferred to a desiccator and stored in a freezer until analysis.

Preparation for Biogenic Opal and Quartz Analysis

Samples for biogenic opal and quartz analysis were transferred to 40 ml crucibles and treated with buffered acetic acid in order to

eliminate the $CaCO_3$ portion. The X-ray amorphous opaline silica was then converted to a cristobalite by heating the sample at 1000°C for 24 hours. After this, the samples were homogenized and stored for X-ray diffraction analysis.

# METHODS OF ANALYSIS

## Metal Analysis

### Sample Digestion and Dissolution

A portion (≈1 g) of the dry oxidized material was weighed in a 25 ml volumetric flask, and digested on a hot plate at 120°C with 5 ml of high purity redistilled nitric acid (G.F. Smith, 16N Redistilled) for 48 hours. After 40 hours of digestion, 5 to 10 drops of concentrated hydrochloric acid (G.F. Smith, 12N Redistilled) were added in order to dissolve the light colored flocculated material that was formed. At the end of 48 hours, only a dense white residue composed of about 20% quartz and 30% of biogenic opal was left. Four reagent blanks were carried with each run of samples to establish the background. At the end of the digestion, each volumetric flask was cooled to room temperature and brought to volume with deionized glass-distilled water. The contents of each volumetric flask were then transferred to a 30 ml test tube, where the suspended matter was allowed to settle for three to four hours under a positive flow hood.

### Determination

At the end of the settling period, an automatic sampler carousel was loaded with thirty samples along with sixty standards and four procedural blanks randomly distributed among the samples.

The standards were prepared from certified atomic absorption reference standards (Fisher Scientific Company) in the same matrix (20% $HNO_3$) as the samples.

Trace metal concentrations were determined by atomic absorption spectrophotometry (AAS) using a Varian Techtron Model AA-5R. The manufacturer recommended (Varian,1971) operating parameters for each metal were used. The readings of the instrument, which were always within the linear range of absorbance, were automatically recorded on punched paper tape and teletype. Data from the tape were reduced using a computer (Digital Equipment Corporation Model PDP/11/05) by a least squares regression program.

To check the accuracy of the trace metal analysis, samples of mussel (Mytilus californianus) ash, previously analyzed independently by other workers in our laboratory, were also analyzed for Cu, Zn, Mn and Fe. The analyses agreed to within $\pm$ 1.2%. The instrument coefficient of variation Cu, Fe, Mn or Zn was less than 1%.

## Organic and Total Carbon and Total Nitrogen

A small aliquot ($\approx$15 mg) was used in the carbon and nitrogen analysis. The size of the aliquot was chosen to keep the sample values within the linear portion of the calibration line.

The analysis were made with a Carlo Ebra Elemental Analyzer, Model 1100, using acetanilide as the reference standard. A detailed explanation of this technique is given by Pella and Colombo (1973). The analytical error depends on the type of sample and the element in question. The results of replicate analyses of a group of samples

are shown in Table I. The precision for each type of sample differs mainly because of sediment content and the inhomogenity of the sample, considering their size. The weighing error was about 4%.

TABLE I

Overall Analytical Error in Total Carbon and Total Nitrogen Analysis

| Sample | Element | |
|---|---|---|
| | N | C |
| Feces | 10% | 5.4% |
| Pseudofeces | 10% | 5.0% |
| Control Sediment | 16% | 5.0% |

Organic carbon was estimated from the difference between the total carbon in dry samples minus total carbon of ashed sample. Total carbon in ashed samples was determined with a Leco Carbon Analyzer (Van Andel, et al., 1976).

## Biogenic Silica (Opal) and Quartz

The biogenic silica and quartz concentrations were determined by X-ray diffraction with a Norelco X-ray diffraction unit following the technique described by Ellis (1972). Detailed description of this technique can be found in Goldberg (1958) and Calveret (1966). The overall analytical error for the method was less than 2%.

## Sinking Rate

The sinking rate of feces and pseudofeces was estimated directly by measuring settling velocities through 45 cm of estuarine water. All measurements were made on freshly produced materials at 12.5°C and 26 ‰ of salinity. The size and the diameter of the biodeposits were measured under a dissecting microscope with a calibrated eyepiece.

## Statistical Analysis

In order to compare the concentration of each component among the three sample sources and within each sampling period, the two-way analysis of variance (ANOVA) with unequal but proportional subclass number (Sokal and Rohlf, 1969) was used to compare the sample sources.

The paired t-test (Bruning and Kintz, 1968) was used to evaluate the differences among sample sources in each sampling period. Similarly the t-test for differences among several means (Bruning and Kintz, 1968) was used within each sample source to compare the values of each sampling period. The Pearson product moment correlation coefficient (r) (Sokal and Rohlf, 1969) was used to make comparisons among parameters.

# RESULTS

## Oyster Biodeposits

### General Characteristics

Feces of adult oysters, according to Arakawa's (1962, 1963, and 1965) terminology and classification, are of the "cricoid type, simple faeces" which correspond to the sculptured with longitudinal midridge ribbon as described by Kraeuter and Haven (1970). In most cases during the experiment, the freshly collected material was formed by short fecal ribbons with stiff consistency, easily broken into short segments (1-5 mm) and pale green to brown in color. In cross section the feces shows a ventral side unsculptured and smooth surface, but the dorsal side is normally marked by two deep longitudinal grooves, with a low narrow longitudinal ridge in between. The average diameter of feces measured at the beginning of the experiment was 0.75 mm with a range of less than 0.5 mm to slightly more than 1.0 mm. The size depends on the size of the organisms and food source. Pseudo-feces, though similar in color, had no defined form and were normally voided as mucous covered loosely aggregated material.

The sinking rate of each type of biodeposit was different depending on the size and source of the material. In the case of feces, the sinking rate of the two groups of agglomerated particle sizes, that form the bulk of the material, were selected from naturally broken fecal ribbons. For long strings (4-8 mm) the sinking rate was 1.81 $\pm$ 0.28 cm/sec and for the smaller fractions less than

1.0 mm to about 0.4 mm; the mean sinking rate was 0.45 cm/sec. For pseudofeces, the measurements were difficult; however, for the bulk of aggregates, which are from 1.0 to 2.0 mm long, the mean value was 1.54 $\pm$ 0.41 cm/sec and for smaller loose clumps it was 0.25 $\pm$ 0.07 cm/sec. For the mucous associated with the pseudofeces, the sinking rate is slower; for mucous strings of 10-15 mm long, it was 0.88 cm/sec.

Gross Particle Composition

No systematic analysis of particle size and composition of the biodeposits was made. However, random samples of feces and pseudofeces always passed through a 64μ sieve. This estimate of particle size was substantiated by microscopic examination of dispersed samples under normal and polarized light. The matrix composition did not appear to be different except that coarse clastic sediments of about 40μ were seen in the pseudofeces. The remainder was mainly amorphous organic aggregates with fine clastic sediment particles of less than 20μ, partial or undigested diatoms and their frustules, sponge spicules and many other finely divided unrecognizable fragments of less than 4μ in diameter. The gravitationally settled seston was also of similar composition.

Production Rate

The biodeposit production rate fluctuated daily in an apparently random fashion. The total biodeposit produced per day, as well as the relative amounts of feces and pseudofeces were not predictable. Even on the same day, the biodeposition was not consistent among

oysters. While some were actively producing biodeposits, the ejections of other organisms under similar conditions were almost negligible.

This variability is not uncommon (Lund, 1957; Haven and Morales-Alamo, 1966; Quayle, 1969). According to Haven and Morales-Alamo (1966) and Quayle (1969), the production of biodeposits of Crassostrea-type oysters is highly dependent on the size of the organism, its soft tissue weight, as well as temperature, salinity, and amount of seston in the feeding water. For the Pacific oyster, the optimum temperatures for biodeposit production seem to be about 20°C, and the optimum salinity in the range of 25 ‰ to 35 ‰ (Quayle, 1969). At lower temperature and/or low salinities (≈10 ‰), the feeding of C. gigas is considerably reduced (Quayle, 1969; Breese, 1976). A similar effect on the pumping rate has been observed, in oysters exposed to sea water with high silt content or to an overabundance of microorganisms (Quayle, 1969). Thus, the rate of biodeposition will fluctuate considerably on a short term basis (e.g. daily tidal influence in estuaries), as well as seasonally.

In order to show the more consistent long term fluctuations, rather than short random variations, the experimental period in 1975 was divided into five periods of sampling: late spring (April 20 - June 1), early summer (June 2 - August 6), late summer (August 7 - September 19), early fall (September 20 - November 1) and late fall (November 2 - November 24). Biodeposition rates for the five periods are shown in Table II. The deposition of sestonic material by gravity was tested in the biodeposit collecting

apparatus. The amount found on a weekly basis was less than 5% of the total feces and pseudofeces combined. Table II shows the amount of material, in terms of grams of dry weight deposited by an oyster (83.74 $\pm$ 8.67 mm in length) per week (see Appendix III), from the estuarine water which has about 7-20 mg/l of seston. This latter value was estimated from the organic carbon content of the seston given by Malouff (1976, personal communication), and the gravity settled control sediment.

The rate of biodeposition at the beginning of the experiment in late spring was the highest value recorded. Quantities of biodeposits varied over a wide range, from 1.53 g dry weight/oyster/week at the beginning of the sampling, to 0.27 g dry weight/oyster/week at the end of early fall. For the whole period the mean was 0.63 g dry weight/oyster/week. The feces production had a mean of 0.33 $\pm$ 0.17 g dry weight/oyster/week and pseudofeces 0.30 $\pm$ 0.18 g dry weight/oyster/week.

During early spring the biodeposition rate ranged from 1.53 g to 0.58 g dry weight/oyster/week. The high values correspond to the first group of samples collected at the beginning of the experiments; the mean value was 1.05 g dry weight/oyster/week.

In the next period, early summer, the biodeposition was slight and did not fluctuate. The mean biodeposition was only 0.48 g dry weight/oyster/week, that is 45.71% of the preceeding. However, in the following sampling period, late summer, the amount of biodeposits increased to 0.66 g dry weight/oyster/week which corresponds to 63% of the late spring mean value. The biodeposits were composed of

TABLE II

Mean Biodeposition Rate by the Pacific Oyster (C. gigas)

| Period | Sample Size | g dry weight/oyster/week ± standard deviation | | | $\frac{P}{F}$ Ratio |
|---|---|---|---|---|---|
| | | Feces | Pseudofeces | Total Biodeposit | |
| Late Spring | 4 | 0.53 ± 0.22 | 0.52 ± 0.26 | 1.05 ± 0.26 | 0.97 |
| Early Summer | 3 | 0.27 ± 0.03 | 0.21 ± 0.02 | 0.48 ± 0.03 | 0.78 |
| Late Summer | 4 | 0.37 ± 0.04 | 0.29 ± 0.08 | 0.66 ± 0.22 | 0.80 |
| Early Fall | 5 | 0,20 ± 0.06 | 0.20 ± 0.06 | 0.40 ± 0.25 | 0.98 |
| Late Fall | 3 | 0.25 ± 0.06 | 0.23 ± 0.01 | 0.48 ± 0.25 | 0.97 |
| Average | 19 | 0.33 ± 0.17 | 0.30 ± 0.18 | 0.63 ± 0.26 | 0.90 |

0.37 g of feces and 0.29 g of pseudofeces. From this second high peak of biodeposit production in late summer, the rate of biodeposition decreases to 0.40 g and 0.48 g dry weight/oyster/week for feces and pseudofeces in early and late fall respectively, corresponding to 38% and 46% of early spring values. In each case, the range of values was relatively small, and roughly half of the deposited material were feces and the other half pseudofeces.

From the individual values of feces and pseudofeces, the ratio of the rejected seston (P = Pseudofeces) to the digested (F = Feces) was evaluated. This ratio, $(\frac{P}{F})$, was calculated by using the values of Table II in order to estimate the response of the organisms to the sestonic material present in the sea water. According to Lund (1957), values near one or smaller correspond to oysters feeding on a low load of suspended particles and gives a relative estimation of the amount of material that passes through their digestive system, from the total biodeposited material.

During the experiment, the $\frac{P}{F}$ ratio was constantly less than one (Table II), which indicates that larger proportions of the seston collected in the biodeposits were exposed to the digestive track, and thus composed of small size particles. This finding was also substantiated and measured microscopically.

The overall mean ratios was $0.90 \pm 0.10$ with a mode around 0.97, and mean low values of 0.78 in early summer. Large fluctuations of this ratio were not observed, the simple linear correlation coefficient between feces and pseudofeces production rate was high ($r = 0.966$).

## Major Components

### Total Organic Matter

The content of organic matter was evaluated for feces, pseudofeces and control sediment by calculating the percent loss in weight during ashing of the sample. This "% loss by ignition" is a rough estimate of the organic content and is considered as such in this work.

The amount of organic matter in the three compartments did not show any marked fluctuations during the experiment (Table III). However, a general trend of increasing concentration after the summer period was observed. The percentage of organics in the feces and pseudofeces were very similar. A paired t-test of the two sample means shows no significant differences ($P < 0.05$) but when the biodeposits were compared to the control sediment, a significant difference ($P > 0.05$) was found. The control sediment always had less organic matter. The mean concentration and standard deviation in feces were 15.4% $\pm$ 3.4% in pseudofeces 15.8% $\pm$ 2.7% and the control sediment 13.5% $\pm$ 1.4%. This concentration of organic matter is very similar to those given by Haven and Morales-Alamo (1966) for the American oysters.

### Total Carbon

Although the content of organic matter estimated from the % loss by ignition did not show a seasonal fluctuation, the concentration of total carbon follows a distinctive seasonal trend (Table IV). The general tendency in feces and pseudofeces was to gradually increase

TABLE III

Percent Loss by Ignition (≈ % Organic Matter)

| Sample | % Loss by Ignition ± 1 Standard Deviation | | | | | |
|---|---|---|---|---|---|---|
| | Late Spring | Early Summer | Late Summer | Early Fall | Late Fall | Average ** |
| Feces | 15.3 ± 1.3 | 14.4 ± 2.6 | 14.8 ± 1.4 | 16.0 ± 0.7 | 16.2 ± 0.8 | 15.3 ± 0.8 |
| Pseudofeces | 15.2 ± 1.6 | 15.2 ± 1.7 | 15.1 ± 1.1 | 16.5 ± 0.8 | 16.7 ± 0.2 | 15.7 ± 0.8 |
| Contr. Sed. | N.D.* | 11.5 ± 0.9 | 12.4 ± 0.6 | 14.8 ± 0.9 | 14.7 ± 0.2 | 13.4 ± 1.7 |
| Sample Size | 4 | 3 | 4 | 5 | 3 | 5 |

*N.D.=no data
**mean of the seasonal average

TABLE IV

Carbon Content in Oyster Biodeposits (g carbon/Kg Dry Weight ± 1 Standard Deviation)

| Sampling Period | Carbon | Feces | % From Total | Pseudofeces | % From Total | Control Sediment | % From Total |
|---|---|---|---|---|---|---|---|
| Late Spring | Organic | 54.0 ± 2.3 | 95.7 | 53.3 ± 4.9 | 95.5 | N.D. | |
| n=3 | Inorganic | 2.3 ± 0.4 | 4.3 | 2.4 ± 0.4 | 4.5 | N.D. | |
| | Total | 56.3 ± 1.7 | | 55.8 ± 4.2 | | N.D. | |
| Early Summer | Organic | 54.9 ± 0.8 | 96.2 | 52.6 ± 2.1 | 96.4 | 46.2 ± 5.1 | 95.0 |
| n=3 | Inorganic | 2.1 ± 0.6 | 3.8 | 1.9 ± 0.1 | 3.6 | 2.3 ± 0.1 | 5.0 |
| | Total | 57.1 ± 1.1 | | 54.4 ± 1.9 | | 48.7 ± 5.0 | |
| Late Summer | Organic | 59.4 ± 7.2 | 97.3 | 55.5 ± 1.2 | 94.2 | 50.5 ± 4.8 | 96.4 |
| n=4 | Inorganic | 1.6 ± 0.3 | 2.7 | 3.2 ± 0.1 | 5.8 | 1.8 ± 0.2 | 3.6 |
| | Total | 60.7 ± 7.6 | | 60.9 ± 4.8 | | 52.2 ± 4.7 | |
| Early Fall | Organic | 61.6 ± 3.2 | 96.8 | 64.0 ± 2.2 | 95.0 | 54.3 ± 3.0 | 96.3 |
| n=5 | Inorganic | 2.0 ± 0.3 | 3.2 | 3.2 ± 0.4 | 5.0 | 2.0 ± 0.6 | 3.7 |
| | Total | 63.7 ± 2.8 | | 67.3 ± 2.4 | | 56.2 ± 2.7 | |
| Late Fall | Organic | 58.7 ± 2.7 | 96.8 | 57.5 ± 6.6 | 95.1 | 52.3 ± 6.5 | 94.8 |
| n=3 | Inorganic | 1.9 ± 0.4 | 3.2 | 2.8 ± 0.1 | 4.9 | 2.7 ± 0.1 | 5.2 |
| | Total | 60.1 ± 2.7 | | 60.2 ± 7.0 | | 54.7 ± 6.9 | |
| Whole | Organic | 58.4 ± 4.9 | 96.7 | 57.6 ± 5.6 | 95.1 | 51.2 ± 4.6 | 96.1 |
| Experiment | Inorganic | 1.9 ± 0.4 | 3.3 | 2.8 ± 0.6 | 4.9 | 2.0 ± 0.5 | 3.9 |
| | Total | 60.3 ± 4.7 | | 61.0 ± 6.1 | | 53.6 ± 4.7 | |

in concentration of total carbon, from a mean value of around 56 g/kg dry weight in late spring to values above 63 g/kg dry weight for feces and 67 g/kg dry weight for pseudofeces in early fall. From this maximum, in the following period, late fall, the concentration drops to about 60 g/kg dry weight.

In the control sediment a similar trend was observed although the mean concentration of total carbon in the biodeposits follows a similar trend. This latter amount was calculated based upon the relative contribution in mass of dry weight material by feces and pseudofeces and their mean concentration of total carbon. In each case, the contribution of the associated error term was considered.

The carbon values observed among the feces and pseudofeces in each sampling period was found to be not significantly different ($P > 0.05$); also the two way analysis of variance (ANOVA) did not indicate a significant ($P > 0.05$) interaction among those two sample sources. This condition is probably a result of the little differences among their concentrations in each sampling period, the large variances due to the variability of their individual samples, and the similar trend of fluctuation with respect to the time. The general tendency, however, was to have higher values of total carbon in the feces than the pseudofeces.

The total carbon concentration in the feces and pseudofeces vs. the control sediment, shows some important differences but no significant interaction, according to the ANOVA results ($P > 0.05$). In this case, the paired t-test at $P > 0.10$, indicates for feces vs. control sediment, that with exception of the samples collected during

the late summer, the rest were significantly different. However, the pseudofeces vs. control sediment were only different during the late summer and early fall, due to the large range of concentration in sediments. Therefore, the sediment was found to have less total carbon than the feces, pseudofeces, and the integrated biodeposits.

Within feces and pseudofeces, the total carbon content was positively correlated. The Pearson product moment correlation coefficient (r), was highly significant ($P < 0.001$). In this case $r = 0.976$. The same relation was found with respect to the control sediments. The correlation coefficient for feces vs. control sediment was $r = 0.902$ and for pseudofeces vs. control sediment $r = 0.909$.

Organic Carbon

The proportion of organic carbon with respect to the total carbon, for three sources of samples considered, was found to be 97.3% Therefore, the seasonal change in their content was found to follow the same pattern as the total carbon.

The mean organic carbon concentration in feces and pseudofeces gradually increases from late spring values 54.0 and 53.3 g/kg dry weight respectively, to 61.6 g/kg dry weight for feces and 64.0 g/kg dry weight for pseudofeces in early fall. However, during late fall, the mean concentration for both sources drops to values close to 58 g/kg dry weight (Table IV). Similarly, the general tendency of the control sediment was to follow closely the fluctuations of the feces and pseudofeces, but with consistently less organic carbon.

The two way ANOVA shows no significant difference ($P < 0.05$) among the sample of feces and pseudofeces.

The overall mean concentration of organic carbon for feces was 58.4 $\pm$ 4.9 g/kg dry weight and 57.6 $\pm$ 5.6 g/kg dry weight for the pseudofeces. Both sources of samples were positively correlated ($P > 0.05$) with the control sediment. The mean for the experimental period was lower, 51.2 $\pm$ 4.6 g/kg dry weight. The paired "t"-test between feces and pseudofeces with respect to control sediment over the whole period of sampling, shows that control sediment was always different ($P < 0.01$). Thus the control sediment averaged about 12% less organic carbon than the others. These results are in agreement with the total organic matter findings.

Total Nitrogen

The concentration of the total nitrogen fluctuated during the experimental period similarly to the total carbon (Table V). Thus the general tendency to increase steadily from relatively low late spring values of 6.6 g/kg dry weight for feces and 6.0 g/kg dry weight for pseudofeees, to a peak value of 8.3 g/kg dry weight for both in early fall. A decline to 8.0 g/kg dry weight for feces and 7.20 g/kg dry weight for the pseudofeces in late fall was also observed (Table IV). Nitrogen content in the biodeposits fluctuate in same fashion. The total nitrogen present in all three sample sources was different during each sampling period. But there was an exception in early fall values for feces and pseudofeces when their respective concentrations were similar. The feces always have the

TABLE V

Total Nitrogen Content in Pacific Oyster Biodeposits and Control Sediment (g/Kg Dry Weight ± 1 Standard Deviation)

| Sampling Period | Feces | Pseudofeces | Control Sediment |
|---|---|---|---|
| Late Spring n=3 | 6.6 ± 0.4 | 6.0 ± 0.2 | N.D. |
| Early Summer n=3 | 7.0 ± 0.0 | 6.6 ± 0.2 | 5.6 ± 0.9 |
| Late Summer n=4 | 7.3 ± 1.5 | 7.5 ± 0.9 | 6.4 ± 0.7 |
| Early Fall n=5 | 8.2 ± 0.2 | 8.4 ± 0.3 | 7.1 ± 0.4 |
| Late Fall n=3 | 8.0 ± 0.6 | 7.2 ± 1.4 | 6.7 ± 0.9 |
| Average | 7.5 ± 0.9 | 7.3 ± 1.1 | 6.6 ± 0.8 |

highest values followed by the pseudofeces and then by the control sediment. The test for interaction between compartments during the experimental period failed to show any significant ($P > 0.05$) interaction. This indicates that the fluctuation with respect to the experimental time were similar for each compartment. The Pearson product moment correlation coefficient (r) supports those findings being significant ($P < 0.05$) in all cases. The correlation coefficients were: feces:pseudofeces $r = 0.933$, feces:control sediment $r = 0.956$ pseudofeces:control sediment $r = 0.858$ and biodeposit: control sediment $r = 0.924$.

Organic Carbon-Nitrogen Ratio (C/N)

During the whole sampling period and among the three sample sources considered in the experiment, the C/N ratio varied by 2.6 C/N units. The overall range was from about 6.9 up to 9.6. In the case of feces, the range of the C/N ratio was from 7.5 to 8.6 with a mean of $7.8 \pm 0.6$, for pseudofeces from 7.3 to 9.5 and a mean of $8.0 \pm 0.6$ and for control sediment from 7.5 to 8.7 with a mean similar to the others of $8.0 \pm 0.4$. Within these restricted ranges, the trend of the C/N ratio was to decrease moderately from late spring to late fall. The ratios of the pseudofeces and control sediment experienced a slight increase in late fall, while that of the feces decreased further.

Biogenic Opal and Quartz

Biogenic opal and quartz were analyzed in only eighteen selected samples from different collection times. Each sample was chosen to

cover the experimental period and give the general behavior of these two parameters in the three sample sources considered.

The results of these measurements (Table VI) show an inverse relation between the biogenic opal and quartz content. The feces are relatively rich in biogenic opal and poor in quartz compared to the sediment.

The general tendency during late spring was for the feces to show low values of opal, about 12%; and moderate values of quartz, around 12.3%. However, the condition reversed in early fall, when the highest values of biogenic opal were recorded, about 29.2%, associated with relatively low quartz 9.8%. The mean concentration in feces of biogenic was opal 20.67 $\pm$ 8.42% and quartz 11.87 $\pm$ 1.89%. In the pseudofeces, no major fluctuations of these two parameters were detected. The biogenic opal content was 9.42 $\pm$ 0.22% and the quartz 13.52 $\pm$ 1.20%. A similar condition was found in the control sediment. Here, the amount of quartz was consistently higher than in previous compartments. The mean value was 19.11 $\pm$ 0.59% for quartz and 8.23 $\pm$ 0.04% for the biogenic opal.

The biogenic opal/quartz ratio in feces of 1.74 compared to pseudofeces 0.70, suggests marked separation by the oysters in favor of the biogenic opal. The feces contain twice as much of this component as the pseudofeces. The control sediment, on the other hand, shows a very low biogenic opal to quartz ratio mainly because of the high quartz content. This condition is not surprising, since control sediments are formed by gravity-settling of particles.

TABLE VI

Biogenic Opal and Quartz, Mean Values ± 1 Standard Deviation

| Sample | n | % Opal | % Quartz | Opal/Quartz |
|---|---|---|---|---|
| Feces | 8 | 20.67 ± 8.42 | 11.87 ± 1.89 | 1.74 |
| Pseudofeces | 6 | 9.42 ± 0.22 | 13.52 ± 1.20 | 0.70 |
| Sediments | 4 | 8.23 ± 0.04 | 19.11 ± 0.59 | 0.43 |

## Salinity and Temperature

Salinity and temperature values during the experimental period were comparable to those described for the Yaquina Bay by Burt and McAllister (1959), and Kulm (1965). According to Burt and McAllister (1959), the estuarine waters undergo marked seasonal variations in response to the tidal mixing of riverine and marine waters. The observed ranges in salinity and temperature during the experiment were primarily produced by the tidal intrusion of coastal water and probably are typical of the bottom water of the bay close to its mouth.

Larger fluctuations in salinity and temperature were observed in the beginning of the experiment in late spring (Table VII). During this period, the high runoff of the Yaquina River superimposed on the spring tide gave a mean salinity of 28.5 ‰ with a range of 16.5 ‰, and mean temperature of 11.0°C with a range of 5.5°C. For the following periods, early and late summer as well as early fall, the salinity was close to 32 ‰ and remained within a small range. However, the temperature fluctuations were always large, near 5°C. This condition might be the result of the alternative intake of cool coastal upwelled water introduced by the high tide and the relatively warm water of the estuary during low tide.

During the late fall the mean salinity reached its lowest values for the experiment period 27.5 ‰, and the temperature was relatively warm, 12.8°C.

TABLE VII

Range and Mean Salinity and Temperature from April 20 to November 24 of 1975

| Sampling Period | Salinity ‰ ± 1 S.D. | Range ‰ Min. Max. | Temperature °C ± 1 S.D. | Range °C Min. Max. |
|---|---|---|---|---|
| Late Spring | 28.5 ± 2.4 | 17.5 ± 34.0 | 11.04 ± 0.9 | 8.5 ± 14.0 |
| Early Summer | 32.3 ± 0.8 | 29.2 ± 34.5 | 11.6 ± 0.9 | 9.0 ± 14.5 |
| Late Summer | 32.2 ± 0.4 | 31.2 ± 33.0 | 12.3 ± 1.8 | 11.0 ± 16.0 |
| Early Fall | 31.6 ± 1.1 | 25.5 ± 33.0 | 12.7 ± 0.6 | 11.0 ± 14.0 |
| Late Fall | 27.5 ± 1.1 | 17.0 ± 30.5 | 12.8 ± 0.8 | 10.4 ± 14.9 |

Note--The daily salinity and temperature were calculated from the mean of six observations every four hours. The mean value for each period was obtained from the daily values.

## Trace Metal Content

Copper, manganese, and zinc concentrations followed a distinctive seasonal pattern; iron did not. Figure 4 summarizes the content of individual metals in each sample source during the experimental period. Trace metal content was analyzed in all samples of feces and pseudofeces that were collected. The control sediment in the first group of samples collected at the beginning of the experiment, late spring, was not analyzed.

In order to present the results of the trace metal analysis in a coherent form, the concentrations are presented in two pairs according to their related behavior: a) copper and zinc, and b) iron and manganese.

## Copper and Zinc

The contents of Cu and Zn within individual samples were positively correlated (Figure 5, Table X and XI). The Pearson product moment correlation ($r > 0.599$) was highly significant ($P < 0.01$). Similar situations were found when the individual metal content was compared among the sample sources. In this case the zinc was the most highly correlated with $r = 0.8935$ while copper was less correlated ($r = 0.872$), Table XI.

The general trend of the metal content in feces and pseudofeces during the experiment period, was a steady increase from late spring low values of 94 and 27.1 µg/g dry weight of Zn and Cu, respectively, in feces and 112 and 31.8 µg/g dry weight Zn and Cu in pseudofeces, to a maximum in early fall of 143 and 49.0 µg/g dry weight of Zn and Cu in feces and 139 and 49.7 µg/g dry weight of

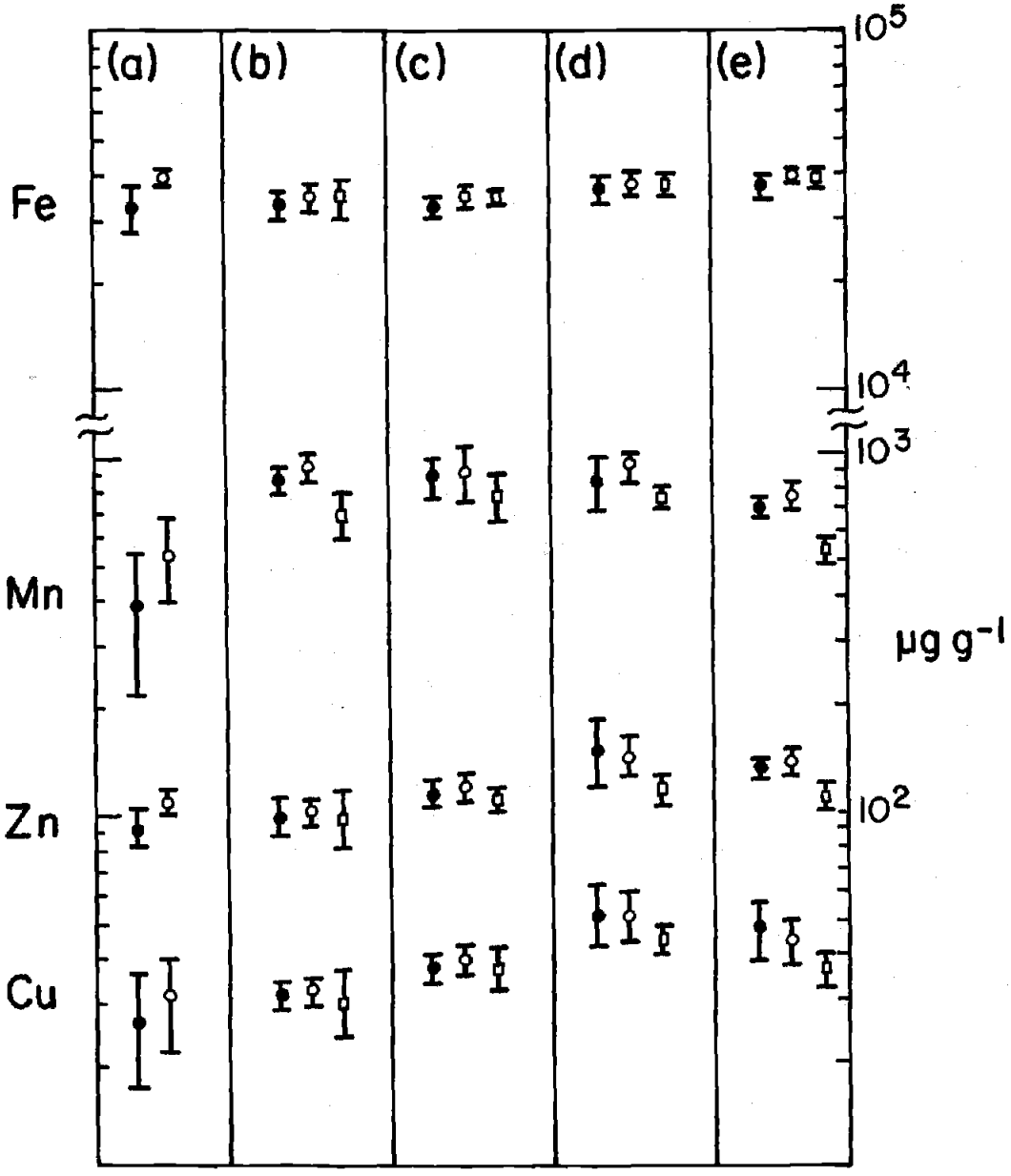
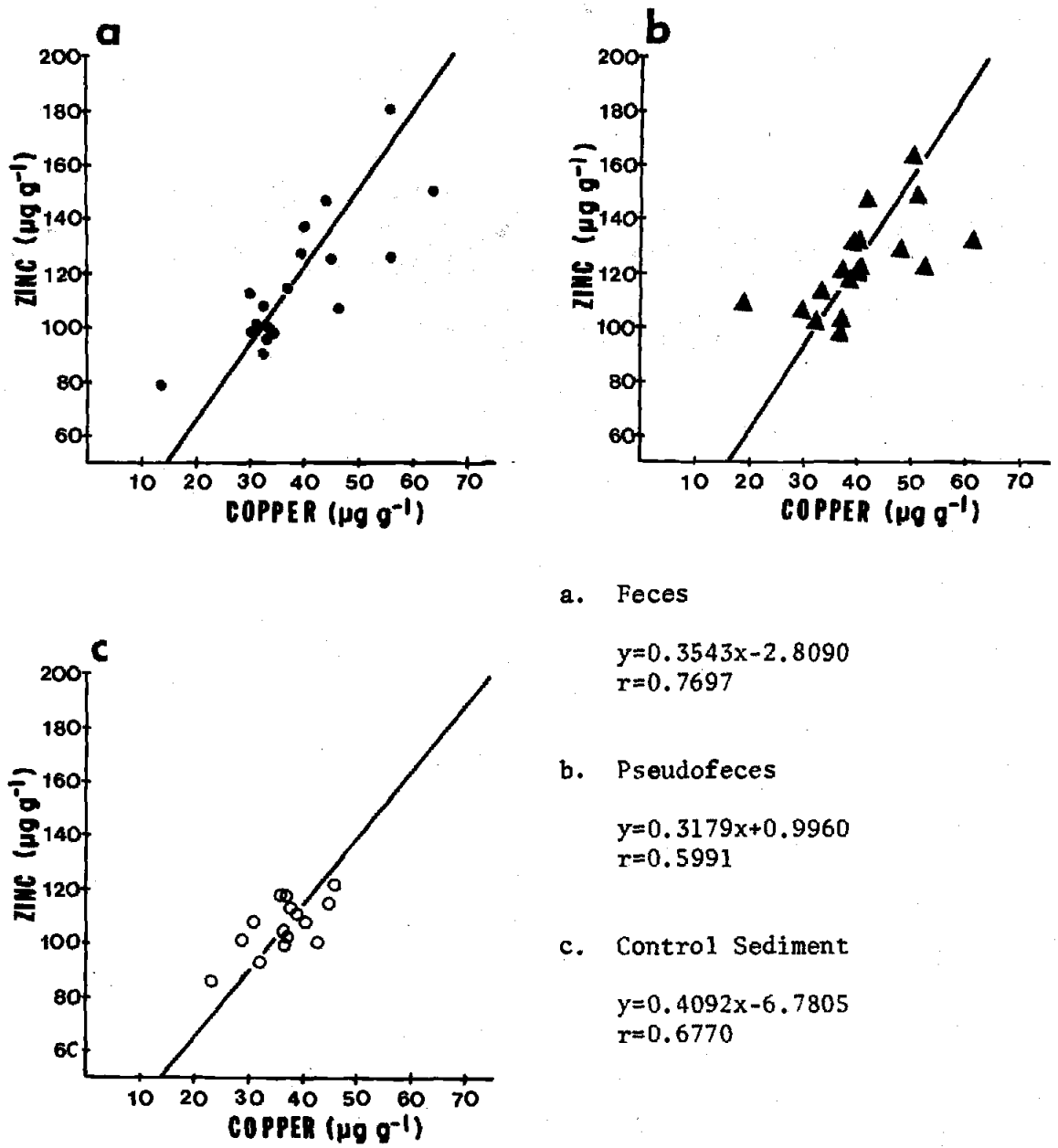



Figure 4. Seasonal fluctuation of trace metal content in feces ●, Pseudofeces o and control sediment ■ . The bar represent one standard deviation. a=late spring; b=early summer; c=late summer; d=early fall; e=late fall.




a. Feces

y=0.3543x-2.8090
r=0.7697

b. Pseudofeces

y=0.3179x+0.9960
r=0.5991

c. Control Sediment

y=0.4092x-6.7805
r=0.6770

Figure 5. Correlation of Cu and Zn content in (a) feces, (b) pseudofeces, and (c) control sediment.

TABLE VIII

Metal Content in Feces, Pseudofeces and Control Sediment (µg/g Dry Weight ± 1 Standard Deviation)

| Season | Sample Size | Source* | Zn | Cu | Mn | Fe** |
|---|---|---|---|---|---|---|
| Late Spring | 4 | F | 94 ± 10 | 27.1 ± 9.6 | 390 ± 168 | 32.8 ± 4.8 |
| | 4 | P | 112 ± 8 | 31.8 ± 9.4 | 554 ± 146 | 39.7 ± 1.7 |
| | - | S*** | N.D. | N.D. | N.D. | N.D. |
| Early Summer | 3 | F | 101 ± 11 | 31.9 ± 2.0 | 878 ± 74 | 32.9 ± 2.2 |
| | 3 | P | 103 ± 6 | 32.8 ± 2.6 | 955 ± 78 | 34.7 ± 2.2 |
| | 3 | S | 99 ± 17 | 30.9 ± 6.4 | 708 ± 106 | 35.2 ± 4.7 |
| Late Summer | 4 | F | 109 ± 6 | 35.5 ± 3.0 | 832 ± 99 | 32.8 ± 1.6 |
| | 4 | P | 113 ± 8 | 37.5 ± 3.3 | 860 ± 155 | 34.7 ± 1.8 |
| | 4 | S | 104 ± 5 | 35.4 ± 4.3 | 741 ± 110 | 35.0 ± 1.0 |
| Early Fall | 5 | F | 143 ± 28 | 49.0 ± 9.7 | 806 ± 123 | 35.8 ± 3.3 |
| | 5 | P | 139 ± 16 | 49.7 ± 7.8 | 898 ± 73 | 37.2 ± 2.2 |
| | 5 | S | 112 ± 8 | 42.6 ± 3.5 | 732 ± 45 | 36.8 ± 2.0 |
| Late Fall | 3 | F | 129 ± 7 | 46.9 ± 8.0 | 693 ± 31 | 37.5 ± 1.8 |
| | 3 | P | 135 ± 10 | 42.7 ± 4.9 | 755 ± 60 | 39.9 ± 1.5 |
| | 3 | S | 109 ± 7 | 35.6 ± 3.3 | 525 ± 36 | 39.7 ± 1.7 |
| Mean Values | 19 | F | 117 ± 24 | 38.5 ± 11.2 | 686 ± 227 | 34.4 ± 3.4 |
| | 19 | P | 121 ± 17 | 39.6 ± 9.2 | 778 ± 208 | 37.2 ± 2.8 |
| | 15 | S | 106 ± 10 | 36.9 ± 6.0 | 688 ± 111 | 36.6 ± 2.6 |

*F=Feces
P=Pseudofeces
S=Control Sediment

**the concentration of Iron are in mg/g dry weight

***ND=No Data

TABLE IX

Trace elements concentration range.

| Metal | Sample Size | Source* | Maximum | Minimum | Range |
|---|---|---|---|---|---|
| Zn | 19 | F | 181 | 79 | 102 |
| µg/g dry weight | 19 | P | 162 | 96 | 66 |
| | 15 | S | 122 | 86 | 36 |
| Cu | 19 | F | 64.3 | 12.9 | 51.4 |
| µg/g dry weight | 19 | P | 60.1 | 18.6 | 42.3 |
| | 15 | S | 46.3 | 23.9 | 22.4 |
| Mn | 19 | F | 963 | 195 | 768 |
| µg/g dry weight | 19 | P | 1042 | 316 | 726 |
| | 15 | S | 857 | 483 | 375 |
| Fe | 19 | F | 39.9 | 26.4 | 13.5 |
| µg/g dry weight | 19 | P | 41.6 | 32.2 | 9.3 |
| | 15 | S | 41.4 | 31.1 | 10.2 |

* F=Feces
P=Pseudofeces
S=Control Sediment

TABLE X

Pearson Product Moment Correlation Coefficient (r) Within Trace Metals for Each Sample Source.

| Trace Metal | Feces n=19 | Pseudofeces n=19 | Control Sediment n=15 |
|---|---|---|---|
| Zn-Cu | 0.7697*** | 0.5991** | 0.6770** |
| Zn-Mn | 0.4809* | $0.1050^{n.s.}$ | $0.0815^{n.s.}$ |
| Zn-Fe | 0.6747** | $0.3193^{n.s.}$ | 0.7150** |
| Cu-Mn | 0.5306* | $0.4423^{n.s.}$ | $0.3372^{n.s.}$ |
| Cu-Fe | 0.6993*** | $0.1799^{n.s.}$ | $0.3395^{n.s.}$ |
| Mn-Fe | $0.3108^{n.s.}$ | $-0.3984^{n.s.}$ | $-0.3438^{n.s.}$ |

Significance Level

P<0.001=*** , P<0.01=** , P<0.05=*

TABLE XI

Pearson Product Moment Correlation Coefficient (r) Within Sample Sources For Each Trace Metal Analyzed

| Sample Source | n | Zn | Cu | Mn | Fe |
|---|---|---|---|---|---|
| Feces vs. Pseudofeces | 19 | 0.8935*** | 0.8721*** | 0.8719*** | 0.5574* |
| Feces vs. Control Sediment | 15 | 0.7020** | 0.5850* | $0.1737^{n.s.}$ | 0.7320** |
| Pseudofeces vs. Control Sediment | 15 | 0.5977* | 0.8528*** | $0.3545^{n.s.}$ | 0.8193*** |

Significant Level:

P<0.001=*** , P<0.01=** , P<0.05=*

Zn and Cu, respectively, in pseudofeces (Table VIII).

Zn and Cu in feces and pseudofeces during this period were respectively 52% and 81%, 24% and 56% higher than late spring values. The concentrations of both trace metals decreased slightly in late fall. Similarly, the variation in concentrations of Zn and Cu in control sediments was also found to follow a related pattern; but the variations were not as large as in the biodeposits. Thus, the Zn and Cu content were only 13% and 38% higher than the values of early summer.

For the entire experimental period, the range of metal content was markedly larger in feces, up to three times as great as the other two sources (Table IX). This indicates a higher variability in these two trace metals in feces compared to pseudofeces and the control sediment. The decreasing order of the mean concentrations were similar for Cu and Zn. The normal sequence of concentrations of Cu and Zn was: Pseudofeces>feces>control sediment. The metal content in the last compartment was always smaller than the other two.

The two way ANOVA, described previously, was used to determine whether the metal distribution among the three sample sources varied with sampling period and also to test for differences within each sample source over the experimental period.

The Cu content did not significantly vary ($P > 0.05$) among sample types except for pseudofeces vs. control sediment in early fall (Table XII). However, some differences existed when the individual mean concentration for each sampling period were compared. The "t"-test for differences among several means indicates that the

TABLE XII

Paired "t" test for differences in trace metal content between sample sources.

A

| Sampling Period | COPPER F-P | F-S | P-S |
|---|---|---|---|
| I | 2.10 | n.d. | n.d. |
| II | 0.41 | 0.22 | 0.62 |
| III | 2.51 | 0.04 | 2.32 |
| IV | 0.37 | 2.11 | 3.20* |
| V | 0.66 | 1.75 | 3.79 |

B

| | ZINC F-P | F-S | P-S |
|---|---|---|---|
| I | 3.96* | n.d. | n.d. |
| II | 0.38 | 0.41 | 0.40 |
| III | 3.48* | 2.42 | 3.46 |
| IV | 0.61 | 3.01* | 3.76 |
| V | 2.97 | 16.78** | 11.68** |

C

| Sampling Period | MANGANESE F-P | F-S | P-S |
|---|---|---|---|
| I | 10.82** | n.d. | n.d. |
| II | 3.26 | 1.64 | 2.48 |
| III | 0.26 | 1.14 | 3.74* |
| IV | 1.76 | 1.05 | 4.32* |
| V | 2.56 | 7.35* | 12.07** |

D

| | IRON F-P | F-S | P-S |
|---|---|---|---|
| I | 3.68* | n.d. | n.d. |
| II | 1.00 | 1.13 | 0.24 |
| III | 2.19 | 2.68 | 1.00 |
| IV | 1.58 | 1.31 | 0.92 |
| V | 2.19 | 1.43 | 0.25 |

NOTE: F=Feces
P=Pseudofeces
S=Control Sediment

| Sampling Period | n | α Level of Significance 0.05 * | 0.01 ** | Code |
|---|---|---|---|---|
| Late Spring | 8 | 3.18 | 5.84 | I |
| Early Summer | 6 | 4.30 | 9.93 | II |
| Late Summer | 8 | 3.18 | 5.84 | III |
| Early Fall | 10 | 2.78 | 4.60 | IV |
| Late Fall | 6 | 4.30 | 9.93 | V |

Cu content during early fall in feces, pseudofeces and control sediment were significantly ($P < 0.05$) different from those collected in spring and summer. Similarly, some of the samples of feces and pseudofeces from late fall were significantly ($P < 0.05$) different than those collected at the beginning of the experiment (Table XII).

The distribution of Zn among the sample sources was different than that of Cu. The two-way ANOVA shows that differences exist in Zn content when the sample sources in each sampling period are compared. The paired t-test for differences among sample sources (Table XII) indicates that the Zn content in feces were different ($P < 0.05$) in two sampling periods; late spring and late summer. Similarly, when both biodeposits are compared against the control sediment, Zn content was different toward the end of the experimental period.

The Zn content in feces vs. control sediment was significantly ($P < 0.05$) different during the fall samples while, the pseudofeces vs. control sediment shows differences in late summer and during the fall sampling. The variation in the Zn content within individual sample sources was similar to those found for Cu, that is, the samples collected toward the end of the experimental period were significantly ($P < 0.05$) different from the early samples. (Table XIII).

## Manganese and Iron

The distribution of iron and manganese among the sample sources in each sampling period and its variation with time were tested in a similar way. During the experiment, the concentrations of Mn and

TABLE XIII

Student's "t" test for difference among the means of each sampling period and by trace elements.

| COPPER IN FECES n=19 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | 4.80 | 8.40 | 21.90** | 19.80** |
| II | | 3.60 | 17.10** | 15.00* |
| III | | | 13.50* | 11.40 |
| IV | | | | 2.10 |
| Critical difference | | p=0.05* | p=0.01** | p=0.001*** |
| | | 12.08 | 16.77 | 23.31 |

| ZINC IN FECES n=19 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | 7 | 15 | 49** | 35** |
| II | | 8 | 42** | 28* |
| III | | | 34* | 20 |
| IV | | | | 14 |
| Critical difference | | p=0.05* | p=0.01** | p=0.001*** |
| | | 26 | 36 | 50 |

| COPPER IN PSEUDOFECES n=15 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | 1.00 | 5.70 | 17.90** | 10.90* |
| II | | 4.70 | 16.90** | 9.90 |
| III | | | 12.20* | 5.20 |
| IV | | | | 7.00 |
| Critical difference | | p=0.05* | p=0.01** | p=0.001*** |
| | | 10.39 | 14.42 | 20.03 |

| ZINC IN PSEUDOFECES n=15 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | 9 | 1 | 27** | 23* |
| II | | 10 | 36*** | 32** |
| III | | | 26** | 22* |
| IV | | | | 4 |
| Critical difference | | p=0.05* | p=0.01** | p=0.001*** |
| | | 17 | 24 | 34 |

| COPPER IN CONTROL SEDIMENT n=15 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | n.d. | n.d. | n.d. | n.d. |
| II | | 4.50 | 11.70** | 4.70 |
| III | | | 7.20* | 0.20 |
| IV | | | | 7.00 |
| Critical difference | | p=0.05* | p=0.01** | p=0.001*** |
| | | 7.17 | 10.11 | 14.45 |

| ZINC IN CONTROL SEDIMENT n=15 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | n.d. | n.d. | n.d. | n.d. |
| II | | 5 | 13 | 10 |
| III | | | 8 | 5 |
| IV | | | | 3 |
| Critical difference | | p=0.05* | p=0.01** | p=0.001*** |
| | | 16 | 22 | 32 |

TABLE XIII (Continued)

| MANGANESE IN FECES n=19 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | 488*** | 442*** | 416*** | 303*** |
| II | | 46 | 72 | 185* |
| III | | | 26 | 139 |
| IV | | | | 113 |
| Critical difference | | p=0.05* | p=0.01** | p=0.001*** |
| | | 183 | 254 | 354 |

| IRON IN FECES n=19 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | 0.10 | 0.00 | 3.00 | 4.70 |
| II | | 0.10 | 2.90 | 4.60 |
| III | | | 3.00 | 4.70 |
| IV | | | | 1,70 |
| Critical difference | | p=0.05* | p=0.01** | p=0.001*** |
| | | 4.95 | 6.87 | 9.55 |

| MANGANESE IN PSEUDOFECES n=15 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | 401*** | 306** | 344*** | 201* |
| II | | 95 | 57 | 200* |
| III | | | 38 | 105 |
| IV | | | | 143 |
| Critical difference | | p=0.05* | p=0.01 ** | p=0.001*** |
| | | 178 | 247 | 343 |

| IRON IN PSEUDOFECES n=15 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | 5.00** | 5.00** | 2.50 | 0.20 |
| II | | 0.00 | 2.50 | 5.20** |
| III | | | 2.50 | 5.20** |
| IV | | | | 2.70 |
| Critical difference | | p=0.05* | p=0.01** | p=0.001*** |
| | | 3.01 | 4.18 | 5.81 |

| MANGANESE IN CONTROL SEDIMENT n=15 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | n.d. | n.d. | n.d. | n.d. |
| II | | 33 | 24 | 183* |
| III | | | 9 | 216** |
| IV | | | | 207** |
| Critical difference | | p=0.05* | p=0.01** | p=0.001*** |
| | | 131 | 185 | 264 |

| IRON IN CONTROL SEDIMENT n=15 | | | | |
|---|---|---|---|---|
| Sampling Period | II | III | IV | V |
| I | n.d. | n.d. | n.d. | n.d. |
| II | | 0.20 | 1.60 | 4.50* |
| III | | | 1.80 | 4.70* |
| IV | | | | 2.90 |
| Critical difference | | p=0.05* | p=0.01** | p=0.001*** |
| | | 4.10 | 5.78 | 8.26 |

Note: I=late spring; II=early summer; III=late summer; IV=early fall; V=late fall.

Fe among the sample sources were not correlated (Table X). However, when each metal was compared within each sample source (Table XI), Fe was always positively correlated while the Mn was positively correlated only between feces and pseudofeces. The mean concentration of Mn during the experiment was always higher in pseudofeces than in the others, with a decreasing order of concentration of pseudofeces> feces>control sediment. The variability in Fe content within each sampling period, considering individually each sample source, was large enough that no defined differences were observed (Table IX).

Concentrations were Mn 686 $\pm$ 227 µg/g dry weight and Fe 34.4 $\pm$ 3.4 mg/g dry weight for feces; Mn, 778 $\pm$ 208 µg/g dry weight and Fe 37.2 $\pm$ 2.8 mg/g dry weight, for pseudofeces; Mn, 688 $\pm$ 111 µg/g dry weight and Fe 36.6 $\pm$ 2.6 mg/g dry weight, for control sediment. The concentration ranges for Mn and Fe in feces during the experimental period were found to be larger than the other two sources. The smallest range for Mn was found in control sediments and for Fe in pseudofeces (Table IX).

According to the two-way ANOVA results, the Mn distribution among the sampling sources was only significantly different ($P < 0.05$) in a small number of cases. The Mn content in feces and pseudofeces was only different during the late spring period, when the lowest values associated with the larger range in concentration were found. For the rest of the period no differences can be shown. A similar situation was also found when Mn content in feces were compared to the control sediment concentration. However, in this case, significant differences were found in late fall. In contrast, due to differences

in Mn content between pseudofeces and control sediment mentioned before, these two sample sources were significantly different in late summer and early and late fall. The iron distribution, on the other hand, was practically the same between the sample sources. Only in late spring can differences between the feces and pseudofeces be observed.

The Mn content in feces among different sampling periods, was essentially similar. No significant difference ($P > 0.05$) was detected in summer and fall sampling. The only exception was in the sample collected in late spring, when significant difference exists in Mn content. In pseudofeces the behavior of Mn was comparable, while, in the control sediment the late fall sample was significantly different than those collected in other sampling periods.

The Fe content in this regard was similar through the whole experimental period in feces, but in pseudofeces the late spring and late fall samples were significantly different than those found in summer. In the control sediment the samples of late fall were also different from those collected in summer (Table XIII).

# DISCUSSION

## Feces and Pseudofeces Formation

Suspension feeders, as has been pointed out by Bernard (1974a), Jørgensen (1966), and Haven and Morales-Alamo (1966), serve as an important and direct link between the seston in the water column and a wide assemblage of benthic organisms. This coupling mechanism functions basically as downward flux of fecal material into the depositional interface, furnishing an organic rich substratum to the benthic community. The efficiency of this downward flux of particulate matter by suspension feeders is a function of several factors. Among the more important are: the absolute rate of biodeposit production by the suspension feeders, and the resistance of breakdown by physical stress and microbial decomposition during its residence in the water column, and before they accumulate at the depositional interface. On the other hand, the quality of the fecal material is a function of the seston composition as well as the capacity of the organism to select particular fractions of the seston, and its influence on their chemical composition.

In this regard the Pacific oyster, C. gigas, is an exceptional organism. It has a well developed filtration mechanism (Bernard, 1974b) along with the ability to produce distinctive feces and pseudofeces with different resistance to disaggregating forces in the water column. Thus, the role of Pacific oysters in the biodeposition of suspended seston and its associated trace elements depends on the

relative contribution of each biodeposit as well as the oyster's efficiency in removing a large size range of particles from the water and its later distribution among feces and pseudofeces.

According to Bernard (1974b) the particle sorting capacity of C. gigas and thus the formation of the two distinctive biodeposits take place through a highly developed and complex system of filtration and separation processes. At least four particle sorting and rejection steps can be found. Figure 6 shows this process diagramatically along with those involved in the formation of feces and pseudofeces. The first sorting is by the inhalant aperture of the mantle which restricts the passage of large particles. The second, which leads to the formation of pseudofeces, is more complex. The special pallial cavity arrangement has a relatively small inhalant aperture associated with larger ostial aperture. This arrangement produces low water velocity in the inhalant cavity and creates an efficient settling chamber, thus preventing the impingement of high specific gravity particles upon the ctenidium. Bernard (1974b) suggested that a large proportion of the inorganic material rejected in the pseudofeces is the result of this separation. According to his values all particles larger than 14μ and density >2.6 are separated in this chamber. The material ejected by this mechanism has not been in direct contact with the digestive enzymes. The third sorting and rejecting process takes place directly on the ctenidium, before the mucous enters the terminal groove to reach the mouth, and the last rejection takes place in the labial palpi where the volume of the mucous is reduced, and the concentrate-mucous is funneled to the mouth (Jorgensen, 1966;

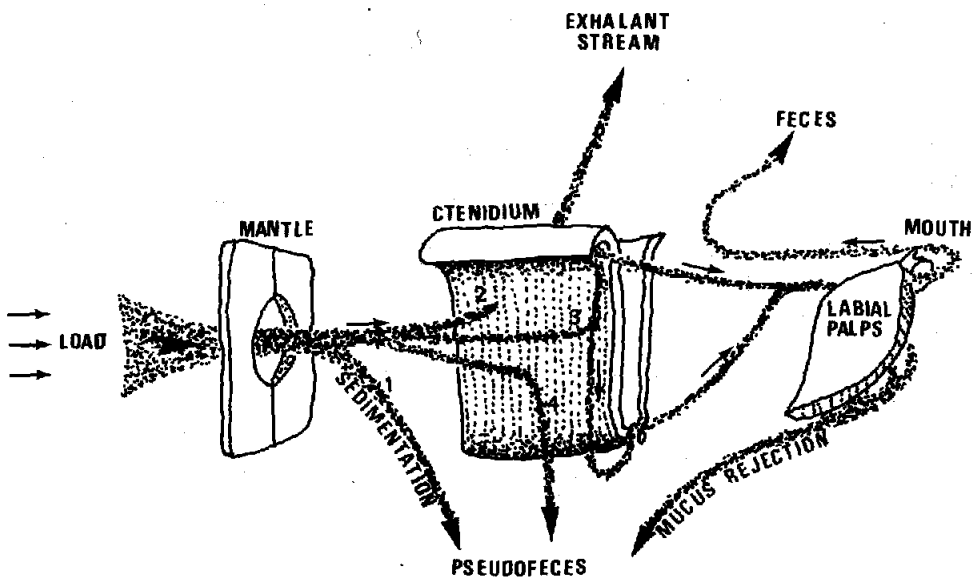



Figure 6. Schematic representation of paths and fate of particulate material drawn into the inhalant pallial cavity; (1) sedimentation of particles of high specific gravity; (2) passage through the ostium; (3) impingement upon ctenidium and transportation on frontal mucus bands to food grooves; (4) rejection of large mucus masses. (Bernard, 1974b).

Bernard, 1974b).

All the rejected mucous material, along with the gravity settled particles in the pallium, form the amorphous mass of pseudofeces. In terms of mass transport from the seston to the bottom, this reject might behave differently than real feces. The pseudofeces are easy to disaggregate and have a specific gravity smaller than feces. Therefore, when both biodeposits are released to the environment the feces are more likely to settle out of the water column than the pseudofeces. Consequently, the feces can accumulate preferentially in the biodepositional interface, while the pseudofeces might return to the detrital food web in the seston directly by disgregation or by resuspension as loosely bound sediment aggregates.

## Significance of Oyster Biodeposits as Food Source

Based upon the percentage of organic matter in biodeposits of various suspension feeders, it has been suggested that this material furnishes an important nutritive substrate to the benthic deposit feeding organisms (Haven and Morales-Alamo, 1966; Bernard, 1974a; Rhoads, 1973). It also may have a significant role in the cycling of organic matter in estuaries (Haven and Morales-Alamo, 1966; Odum, 1975). Biodeposition by oysters (Genus Crassostrea) has been considered as a major mechanism in transferring the food material dispersed in the water column to the bottom community (Haven and Morales-Alamo, 1966; Bernard, 1974a). This downward flux takes place mainly as discrete organic rich agglomerated particles, which according to Haven and Morales-Alamo (1966) sink the sestonic particles seven times faster than if they were to settle by gravity effect.

The efficiency in removing suspended material by C. gigas has been documented by Bernard (1974a). He calculated the percentage of efficiency, taking into consideration the mass of biodeposit ejected in relation to what he calls "available load." This efficiency ranges from 7.5 to 37.1% with an annual mean of 23.6%. The average deposition rate, calculated from the values in Table II is on the order of 32.8 g dry weight/oyster/year. This value is applicable to oysters living under estuarine or enclosed bay environments. For clear water environments, such as the case of oyster farms in Japan, Takana (1975) gives values on the order of 11 g dry weight/oyster/year. The biodeposition rate in an oyster reef, assuming the efficiency rate of 23.6% and a mean biomass of $1.56 \times 10^3$ g wet/$m^2$ is on the order of $1.77 \times 10^3$ g dry weight/year/$m^2$. (Bernard, 1974a). However, for oyster farms where the biomass is higher, Tanaka (1975) gives values of $1.0\text{-}1.7 \times 10^4$ g dry weight/year/$m^2$ for suspended culture rafts. Those biodeposits are equivalent in terms of organic matter for oyster reef to $2.65 \times 10^2$ g dry organic matter/year/$m^2$, when 15% organic matter is assumed, but can also be as high as $2.55 \times 10^3$ g dry weight organic matter/year/$m^2$ if the raft culture values are considered. Thus, in oyster farms this biodeposition of organic matter is more evident, because annual accumulation of organic matter under the culturing rafts, can produce contamination problems that retard the physiological function of oysters (Tanaka, 1975).

From the values of organic matter calculated above, it is evident that the oyster biodeposits are an important potential food source for a large group of estuarine organisms that have the ability to

exploit such a nutritional source. It is also relevant to point out that this biodeposit might have potential implications in the benthic community structure, as well as to the chemistry and particle distribution of the depositional interface. The accumulation of this material, besides creating an organic rich sediment important to many benthic fauna, changes the nature of the substrate creating certain selectivity in its colonization and thereby might modify the species distribution.

## Potential Role of Oyster Biodeposits in the Trace Metal Distribution in Estuaries

The content of Cu, Zn, Mn and Fe in feces and pseudofeces of the Pacific oyster, along with their nutritional significance, suggests that these biodeposits, which are part of the detrital food web, might play an important role in the distribution of trace metals in estuaries.

The most probable implication, is the origin of the biodeposit itself. The oyster, feeding upon the water column with retention efficiency in the order of 23% (Bernard, 1974a) can remove effectively a substantial amount of the very fine fraction (under 3μ; Haven and Morales-Alamo, 1966) of the suspended particulate load. This process results in the direct translocation of trace metals associated with this fraction to the depositional interface. DeGroot (1973) has found an inverse correlation between particle size and the metal content in Ems estuary sediments. Therefore, the feeding process of the Crassostrea-type oysters produces biodeposits rich in fine particles, and consequently high in trace metals.

This biodeposit presents further implications to the trace metal distribution. As shown before, the high organic matter content in these deposits provides an important substrate for many deposit feeders. This group of organisms usually reworks these materials, resuspending certain fractions and producing a sedimentary phase different from those found solely by the gravitational settling of seston. The resulting bioturbation create a soft pelletized deposit, characterized by a high percent of interstitial water associated with a vertical gradient in pH, Eh and $P_{O_2}$ which lead to diffusion of trace elements and nutrients across the depositional interface (Rhoads, 1974). The decomposition of the rich organic materials associated with these biodeposits leads to the formation of considerable amounts of sulfides (Tanaka, 1975) which affect the trace metal distribution within the deposit. Those trace elements with relatively soluble sulfides might tend to diffuse more rapidly out of the deposit. This latter condition will tend to segregate the trace metals, with some being mobilized from these biodeposits.

Another implication is related to the possible changes that might be produced in the adsorptive capacity of particles to trace metals resulting from their exposure to the digestive juices of the organism. These changes in turn might affect the distribution of the biologically available metal content. All these changes deserve further investigation if we want to evaluate more closely the role of organisms in the trace metal cycling processes.

TABLE XIV

Composition of Laboratory Collected Biodeposits and Yaquina Bay Samples

| SOURCE | µg/g dry weight | | | mg/g dry wt. | g/Kg dry weight | | | |
|---|---|---|---|---|---|---|---|---|
| | Zn | Cu | Mn | Fe | Ctot | Corg | Ntot | %organic matter |
| Feces | 117 | 38.5 | 686 | 34.4 | 60.3 | 58.4 | 7.5 | 15.3 |
| Pseudofeces | 121 | 39.6 | 778 | 37.2 | 61.0 | 57.6 | 7.3 | 15.7 |
| Biodeposits | 119 | 39.2 | 731 | 35.9 | 60.9 | 58.4 | 7.4 | 15.6 |
| Sediment from bottom culture | 131 | 29.9 | 386 | 44.6 | 85.9 | 85.6 | 7.3 | 14.9 |
| Sediment from suspended culture | 294 | 30.9 | 389 | 41.7 | 48.1 | 47.9 | 5.6 | 12.9 |
| Suspended sediment | 106 | 36.9 | 688 | 36.6 | 53.6 | 51.2 | 6.6 | 13.4 |
| Yaquina Bay sediment (mud) | 79 | 13.8 | 326 | 28.4 | 22.0 | 21.0 | 1.6 | 6.2 |

TABLE XV

Biodeposition rate of Oyster (Genus Crassostrea) and its associated % organic matter.

| | | American Oyster | Pacific Oyster | Pacific Oyster |
|---|---|---|---|---|
| Suspended Particles in Feeding Seawater | | | | |
| Range: | minimum mg/l | 3.9 | 19.8 | 7.0 |
| | maximum mg/l | 29.0 | 47.5 | 20.0 |
| | average mg/l | 10.0 | 39.2 | 15.0 |
| Biodeposition Rate | | | | |
| | g/oyster/year | 84.24 | 64.4* | 33.0 |
| | g/g wet tissue/year | 4.2* | 8.9 | 2.5 |
| | Feces g/oyster/year | -- | -- | 17.1 |
| | Pseudofeces g/oyster/year | -- | -- | 15.6 |
| % Organic Matter | | | | |
| | Biodeposits | -- | 28.54 | 15.7 |
| | Feces | 15.0 | -- | 15.4 |
| | Pseudofeces | 15.7 | -- | 15.8 |
| | Control Sediment | 14.8 | -- | 13.5 |
| % Organic Carbon | | | | |
| | Feces | 4.6 | -- | 5.84 |
| | Pseudofeces | 5.4 | -- | 5.76 |
| | Control Sediment | 6.8 | -- | 5.12 |
| *Estimated values from the authors data. | | Havens and Morales-Alamo, 1966 | Barnard 1974 | This work |

## Carbon, Nitrogen and Trace Metal Content in Yaquina Bay Samples Compared to Those Obtained in Laboratory

To compare the average composition of the samples obtained in the laboratory to those collected in Yaquina Bay, Oregon (Table XIV), three groups of samples of bottom sediments were analyzed; one was from a bottom oyster culture farm, presumably made up of feces and pseudofeces, the second from a suspension culture oyster farm, composed primarily of feces, and the third was from an area of Yaquina Bay which is representative of sediment unaffected by oyster biodeposits.

Organic carbon, total nitrogen composition, and the percent organic matter in all laboratory samples were similar. However, the sediments from the suspension culture farm were about 17% lower in organic matter. The organic carbon and total nitrogen concentration were also lower (Table XIV). Yaquina Bay mud was significantly different from the other samples. The organic matter content was 60% lower than the laboratory biodeposits, also the content of total organic carbon and nitrogen were 64% and 78% lower, respectively.

The Fe content in both oyster farm sediments were comparable to the laboratory biodeposits. However, the Mn content in oyster farm samples was about half that of the laboratory biodeposit, and Cu in suspended culture farm sediments about twice of the laboratory biodeposit. In Yaquina Bay mud, the content of Cu, Mn, Zn and Fe was roughly half of the laboratory biodeposit value. This lower trace metal content may result from proportionally less fine particles in the mud (Table XIV).

The Mn content in both oyster farm sediments was comparable to those found elsewhere in Yaquina Bay. This value which is relatively low compared to laboratory biodeposit can be caused by the diffusion of relatively soluble $Mn^{+2}$ out of the biodeposit in the presence of sulfides formed by the decomposition of the organic matter. The laboratory biodeposits were not allowed to become anoxic.

The higher Cu content in the samples from the suspension farm are more difficult to explain. However, the Cu and Fe were highly correlated in feces (Table XIV), while in pseudofeces and control sediment they were not. This condition might indicate that Cu and Fe are associated with particles that are successfully retained by the oysters and excreted preferentially in feces.

# CONCLUSIONS

1. The contents of Cu, Zn, Mn and Fe in feces and pseudofeces were comparable to those found in the fine fractions of suspended sediment.
2. No important differences were found when the trace metal concentrations of feces was compared to those in pseudofeces.
3. Concentrations of Cu and Zn in the biodeposits and control sediment were positively correlated, which suggests that the distribution of both metals in the seston might be controlled by similar processes.
4. The content of Cu, Zn and Mn in biodeposits and in control sediments show a seasonal fluctuation. Low values of metal content correspond to period of low salinity in late spring and late fall; higher values of Cu and Zn were found toward the end of summer and early fall when salinities were similar to the marine environment.
5. The Pacific oyster's ability to separate the fine fractions of the seston leads to the formation of biodeposits rich in trace metals.
6. The biodeposition process is potentially important in translocating metallic pollutants associated with the seston from the water column to the benthic community.

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# APPENDIX I

## CONTINUOUS OYSTER BIODEPOSIT COLLECTOR


### ABSTRACT

An apparatus for collecting oyster biodeposits is described. It is made of transparent acrylic plastic and collects the feces and pseudofeces separately on a continuous basis. Different numbers of oysters of variable size can be accommodated.


In order to study the chemical and physical nature of oyster feces and pseudofeces, a simple collecting apparatus has been developed. The apparatus described here is composed of four units. Each holds five oysters in individual chambers and has the ability to collect separately the biodeposits on a continuous basis. Each organism can be kept under different flow rates. The biodeposits are collected according to their source, in two settling receptacles, where they can be removed without disturbing the oysters. Constant flow rates are maintained by constant pressure head of the source water using continuous overflow.

The apparatus has been designed to hold oysters from about 5 cm to 10 cm in length, and has the capacity to work with different oyster shapes. In particular, the instrument described here was tested with the Pacific oyster, Crassostrea gigas (Thunberg, 1795) and its different shell types, ranging from the so called round fluted to the

long smooth type as described by Quayle (1969). The size and the conformation of the holding chamber are such that they do not interfere with the oyster's shell growth, tissue development or other normal life activities.

The apparatus is composed of the following parts (Fig. 1): I) Adjustable double head tank, II) Water distributing manifold, III) Oyster chambers, IV) Oyster biodeposit collectors. Each of these units was constructed of transparent acrylic plastic interconnected with Tygon® and glass tubes.

In Figure 1, the top drawing corresponds to a constant level holding tank, which was used to correct for the uneven flow of sea water from the feed line. From this holding tank, the water flowed to the adjustable water head tanks, which consist of cylindrical containers, 9.5 cm I.D. by 15 cm high. The sea water enters at the top and overflows to maintain a preset water head by opening or closing a series of valves. From here the water flows to the water distributing manifold. This unit consists of a horizontal tube (26 mm I.D.) with ten holds, five drilled in each side. The sea water flows from the manifold to the oyster chambers through individual glass tubes (5 mm I.D.) that are inserted with a rubber stopper on the manifold holes. The glass tubes have a special shape (Figure 2) which by turning can regulate the rate of water flow by changing the water head height.

The oyster chambers, as seen in Figure 2 are made of 3.18 mm thick acrylic plastic sheets on each side, and 1.6 mm thick sheets on the middle and bottom parts. Each chamber is divided in two by a

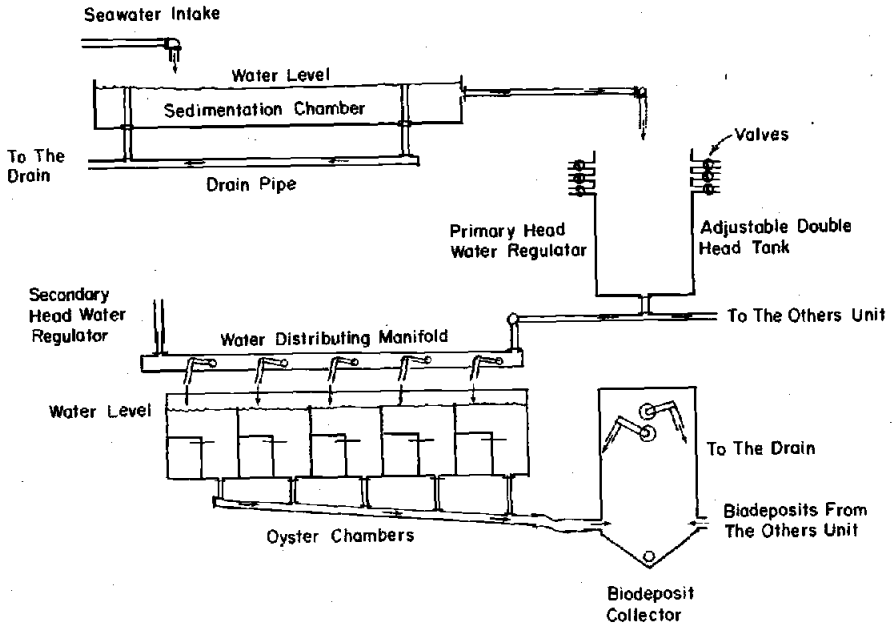


Figure 1. Functional design of the oyster biodeposit collecting apparatus.

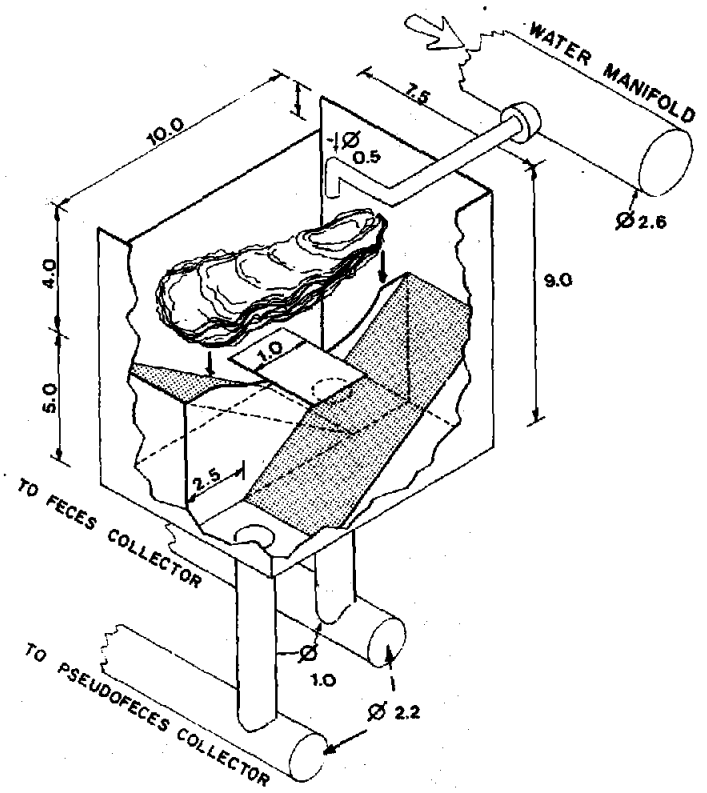


Figure 2. Detail diagram of one oyster chamber. The measurements are in centimeters.

vertical separator as described by Lund (1975), but in this case the separator also functions as an oyster support with the help of a transverse piece of plastic (Figure 2). The bottom of each compartment is not horizontal; instead each compartment slopes in opposite directions to drains that lead to a single collector tube on the side, which conducts the flow to the biodeposit collectors. These latter units are also made of 3.18 mm acrylic plastic sheets and measure 20.3 cm long by 10.12 cm wide and 22.9 cm high. The bottom of this collector is "V" shaped and has four outlets, one per oyster chamber (Figures 1 and 3). The inside is separated by a baffle which disperses the turbulence created by the inflowing water. Finally, the water is discharged through a flow regulating tube localized in the upper portion of the collector, opposite to the inflowing tubes. These discharging tubes control the rate of water flow to the oyster chambers.

Separate collection of feces and pseudofeces takes place in the oyster chamber. The principle of collection is the same as used by Lund (1957) and Haven and Morales-Alamo (1965). It takes advantage of the different sites of excretion of feces and pseudofeces within oysters. Unlike the apparatus of Lund (1957) and Haven and Morales-Alamo (1965) in which the biodeposits settle and are stored within the oyster chambers, the biodeposits are removed by the flowing seawater and settled out in the biodeposit collector, where they can be removed by siphoning or by suction without disturbing the oysters. The samples obtained in this way are pooled samples.

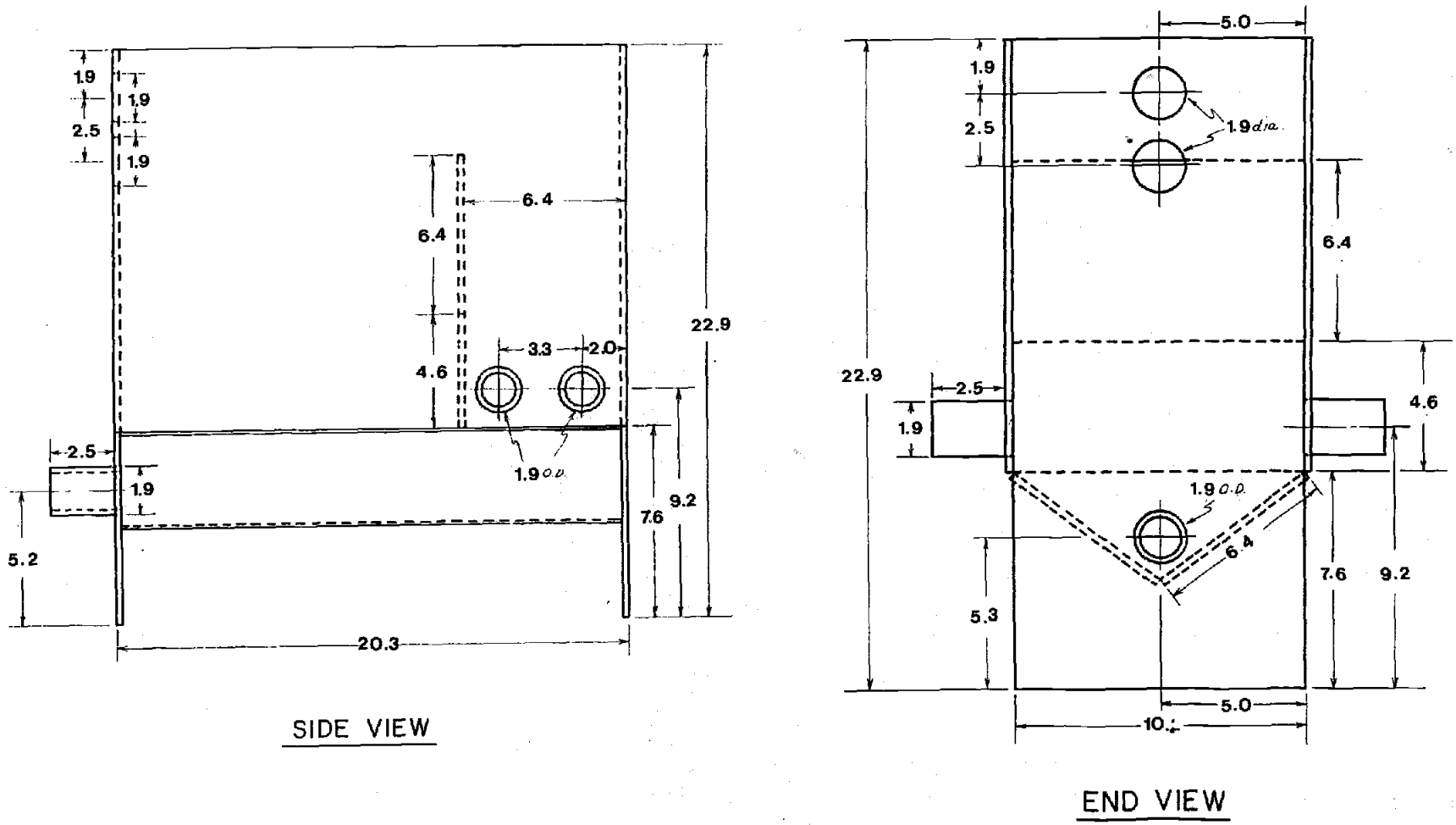


Figure 3. Detail diagram of a biodeposit collecting unit. The measurements are in centimeters.

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# APPENDIX II

## MAXIMUM PARTICLE SIZE AVAILABLE FOR THE OYSTERS: A THEORETICAL COLLECTION

The maximum particle size available for the oysters was calculated theoretically from the characteristics of the settling pond, and the water velocity under representative conditions of salinity and temperature recorded during the experiment.

The water flux in the gravity settling pond was considered to be laminar, and the particles with the density of quartz were assumed.

The terminal velocity of the settling particles were calculated using a modified Stokes equation given by Hutchinson (1967) for particles smaller than 500 μ.

The formula was:

$$Va = 2/9\ g\ r^2(\rho'-\rho)\eta^{-1}\phi_r^{-1}$$

Where:

Va= Terminal velocity of the sinking particle in cm/sec.

g= Acceleration of gravity.

r= Particle radius in mm.

$\rho'$= Particle density.

$\rho$= Sea Water density.

$\eta$= Viscocity of sea water in centipiose (cP).

$\phi_r$= Coefficient of form resistance.

The coefficients of form resistance ($\phi_r$) were obtained graphically from Hutchinson (1967) who discusses various ways of calculating $\phi_r$. In this work, three values of this parameter were considered, one for the sphere ($\phi_r$=1) and two for spheroids ($\phi_r$=1.5 and 1.8). As the value of $\phi_r$ becomes larger than unity, the shape becomes more prolate or oblate.

The salinity-temperature conditions close to the extreme, and mean values found during the experiment were used. Numerical values are shown in Table II-1.

TABLE II-1

Mean and extreme values representative of salinity and temperature during the experiment.

| Temperature (°C) | Salinity (S ‰) | Viscosity (cP) | Density (ρ) |
|---|---|---|---|
| 10 | 20 | 1.5525 | 1.016 |
| 12 | 25 | 1.2903 | 1.019 |
| 12 | 32 | 1.3057 | 1.024 |
| 16 | 34 | 1.1800 | 1.026 |

Hydraulic Condition of the Sediment Pond

The mean water velocity through the gravity settling pond was ca. 0.28 cm $sec^{-1}$. Under these flow conditions, any particle having a terminal settling velocity larger than 0.014 cm $sec^{-1}$ should be removed from the water column before reaching the oyster holding chamber.

The critical particle size (sinking rate of 0.014 cm $sec^{-1}$) assuming the experimental conditions of Table II-1, and the hydraulic conditions of the settling pond, were calculated for three different shapes of particles (Table II-2).

TABLE II-2

Critical Particle Size, Assuming a Density of Quartz (≈2.6)

| Environmental Conditions | | $\phi_r$ Values | | |
|---|---|---|---|---|
| Temperature °C | Salinity ‰ | 1 | 1.5 | 1.8 |
| 10 | 20 | 14.8μm | 18.1μm | 19.9μm |
| 12 | 25 | 14.5μm | 17.7μm | 19.4μm |
| 12 | 32 | 14.6μm | 17.9μm | 19.6μm |
| 16 | 34 | 13.9μm | 17.0μm | 18.6μm |

Any particle, for a given value of $\phi_r$, below those shown in Table II-2 were available for the oysters. That is, they were not settled in the sediment pond.

Hutchinson, G.E. 1967. A treatise on limnology: Vol. II. Introduction to lake biology and the Limnoplankton. John Wiley and Sons, Inc., New York. 1115 pp.

# APPENDIX III

## GENERAL CHARACTERISTICS OF THE PACIFIC OYSTER USED IN THIS STUDY

Scientific Name: Crassostrea gigas (Thunberg, 1795)

Common Names: Pacific Oyster, Japanese Oyster

Race Type: Miyagi and Kumamoto

| | | | |
|---|---|---|---|
| Width: | 58.7 | ± | 9.6 mm |
| Length: | 83.7 | ± | 8.7 mm |
| Condition Index: | 54.92 | ± | 10.03 |
| Internal Cavity Wt.: | 30.82 | ± | 7.97 g |
| Wet Tissue Wt.: | 12.88 | ± | 4.75 g |
| Dry Tissue Wt.: | 1.66 | ± | 0.60 g |
| Ash Wt.: | 0.3729 | ± | 0.1414 g |
| % Solids in Tissue: | 13.04 | ± | 2.04 % |
| % Ash in Tissue: | 2.90 | ± | 0.23 % |

These values are the average of 19 oysters used in the experiment.