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OXIDATION OF ORGANIC MATTER IN SEDIMENTS

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OXIDATION OF ORGANIC MATTER IN SEDIMENTS

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ABSTRACT

A suitable sampler for taking undisturbed sediment samples was developed. Techniques were worked out for measuring (a) oxygen uptake by intact sediment cores, (b) dehydrogenase activity of sediment bacteria, and (c) their actual metabolic heat release. Dehydrogenase activity as a relative measure of anaerobic metabolism was calibrated by direct calorimetry for use in determining natural rates of sediment metabolism. The concentration of reduced end products of anaerobic metabolism was determined by an iodometric and dichromate method. Laboratory experiments were conducted to determine the equivalents between rates of oxygen consumption on the one hand and loss of organic carbon of sediments and liberation of nutrient salts, e.g. nitrates, phosphates, silicates, and ammonia, on the other. Seasonal measurements of oxygen consumption at 33 stations in Puget Sound provided benchmark information for an area that may be subject to worsening conditions due to the impact of increasing human population.

In situ oxygen uptake by the sediment can be estimated by shipboard measurements with sufficient accuracy. The original working hypothesis, however, that total oxygen uptake represents a measure of total metabolism, aerobic plus anaerobic, in the sediment column appears erroneous, at least in organically rich sediment. The rate of total oxygen uptake by intact cores represents aerobic plus part of the anaerobic metabolism in a surface layer of indeterminate thickness. At present the only practical way to estimate total aerobic and total anaerobic metabolism in sediments is to combine the rates of respiratory oxygen uptake by undisturbed sediment cores with estimates of anaerobic metabolism derived from dehydrogenase assay of subsurface sediment layers.

The rate of oxygen uptake by the sediment, however, remains a useful index of equilibrium conditions among the various factors that affect this rate: oxygen tension, temperature, salinity, turbulence, available metabolizable energy, size and composition of the community, compactness and porosity of sediments and perhaps more. As sedimentation rate of oxidizable organic matter increases, e.g. in cases of organic pollution and eutrophication, anaerobic metabolism becomes a relatively more important process in the mineralization of organic matter in sediments. In this situation, the estimation of anaerobic metabolism by the dehydrogenase assay technique is particularly desirable.

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SECTION I

CONCLUSIONS

In situ oxygen uptake by the sediment can be estimated by shipboard measurements with sufficient accuracy.

The rate of total oxygen uptake by the sediment represents the sum of aerobic plus anaerobic metabolism in a surface layer only of indeterminate thickness.

In situ rates of anaerobic metabolism in the sediment column can be determined by means of a TTC method of total dehydrogenase assay.

In Lake Washington, which has had a long and well-documented history of eutrophication and deposition of sewage effluent, anaerobic metabolism by bacteria alone in the sediment column far exceeds total metabolism as estimated by the rate of total oxygen uptake by undisturbed cores.

As the rate of sedimentation of organic matter increases, e.g. as a consequence of eutrophication or organic pollution, anaerobic metabolism becomes a relatively more important process in the mineralization of organic matter in sediments.

Benchmark measurements of oxygen consumption by the sediment are useful indices of equilibrium conditions among the various factors that affect the rate of uptake, such as oxygen tension, temperature, salinity, turbulence, available metabolizable energy, size and composition of the community.

Benthic community metabolism decreases with decreasing supply of oxidizable organic matter.

Accumulated reduced products of anaerobic metabolism may be measured by a dichromate oxidation technique.

Reduced end products of anaerobic metabolism near the sediment surface are in a state of dynamic equilibrium between the rate of formation and the rate of oxidation.

Below a few centimeters reduced end products of anaerobic metabolism are no longer effectively oxidized; they accumulate, as shown by an increase in concentration with depth of sediment layer.

If sediment layers can be dated, such that the time they are removed from

the surface zone of oxidation can be ascertained, then total anaerobic metabolism from that time can be determined from the concentration of reduced substances in that layer.

There is no easy single method for measuring total benthic community metabolism in the sediment column. Direct calorimetry may be the only means of measuring aerobic plus anaerobic metabolism of undisturbed sediment cores, but it does not look promising for field studies.

At present the only practical way to estimate total aerobic and anaerobic metabolism in sediments is to combine the rate of respiratory oxygen uptake by undisturbed sediment cores with estimates of anaerobic metabolism derived from dehydrogenase assay of subsurface sediment layers.

It is extremely difficult to quantify the effect of each variable on benthic community metabolism (e.g. total oxygen uptake) because of factor interactions.

SECTION II

RECOMMENDATIONS

The described research has shown the usefulness and limitations of benchmark information on the rates of oxygen consumption by the sediment. The rate of oxygen uptake is a characteristic parameter of an area and indicative of equilibrium conditions. Thus, where conditions that affect the rate of uptake, e.g. supply of organic matter to the bottom as a result of pollution or eutrophication, is expected to change, the effect of such changes can be assessed in terms of changes in benthic oxygen consumption. The rate of uptake, however, is affected by many other factors, such as oxygen tension, temperature, salinity, composition of the community, and factor interactions. For example, acclimatization to seasonal or long-term temperature changes by some communities and varying degrees of acclimatization make it difficult to quantify the effect of temperature. In spite of this difficulty, it is recommended that areas susceptible to organic pollution be subjected to a study of benthic community metabolism. In addition to usual measurements of benthic oxygen uptake, the following routine field measurements are also recommended:

1) Anaerobic metabolism in the sediment column to be determined by its dehydrogenase activity;

2) the concentration of total reduced substances in the sediment column.

These latter measurements are especially relevant and particularly imperative in areas designated for dumping of sewage sludge such as the New York Bight, because they would indicate the degradation of quickly buried organic matter that the rates of oxygen uptake alone would not reveal. To better understand the degradation of organic matter in bottom deposits, more work is called for on the following problems and I suggest that these problems are in the interest of the Environmental Protection Agency:

1) Development of a chemical method for the determination of anaerobic metabolism of benthic macrofauna, meiofauna, and microfauna.

2) The quantitative relationship between the level of anaerobic metabolism in sediments and the resulting accumulation of total reduced substances.

3) The relationship under a wide range of conditions between measures of dehydrogenase activity and actual rates of metabolic

heat release of undisturbed sediments.

4) The measurement of the rate of sedimentation of particulate organic matter in different areas and its relationship to the level of benthic community metabolism.

5) Long-term experiments on the quantitative relationship between anaerobic metabolism and the loss of organic carbon in sediments.

6) The carbon equivalent of total humic fraction of organic matter in sediments and the relative oxidizability of organic matter containing various fractions of humic substances.

7) Laboratory experiments on temperature acclimation by benthic communities.

SECTION III

INTRODUCTION

The general cycle of elements and their compounds, the biological, physical, and chemical processes involved therein, and the concomitant flow of energy through terrestrial and aquatic ecosystems are generally understood. The biggest gaps in our knowledge concern the rates of the various processes and the quantitative effect of conditions in nature that affect these rates. It is now widely recognized that these gaps are critical in considering many aspects of man's use and management of the earth's natural resources, e.g. understanding of biological productivity, decisions concerning domestic and industrial use of waters in streams, lakes, estuaries, and the open ocean, shoreline management, barging of wastes to the oceans, etc. One of the least understood processes takes place in the basins of bodies of water, after organic and inorganic materials have settled on the bottom or been taken up by the sediment. These include the degradation and mineralization of organic matter and the exchange of substances and by-products of metabolism between the sediment and the overlying water.

The oxidation of organic matter in the sediment may be measured by a number of ways: by the rate of decrease in organic matter content during incubation, by the rate of evolution of carbon dioxide, by different measures of microbial activity such as uptake of C-14 in labeled organic substrates, enzyme activity, such as dehydrogenase or oxidase, etc., by direct calorimetry and by the rate of oxygen consumption. We chose to develop the latter technique by virtue of its directness, its sensitivity, the state of the art in oxygen measuring techniques, its natural involvement in in situ processes, and its applicability to all aerobes. The interpretation of oxygen consumption by the sediment is, however, far from being straightforward.

RATIONALE OF DESCRIBED WORK

In situ Measurements-In view of envisioned difficulties and likely sources of errors in the measurement of oxygen uptake by the sediment, Pamatmat and Fenton (1967) developed an instrument system for making in situ measurements to 180 m depth. A disadvantage, besides that of cost, became evident when results showed no correlation with measured parameters of the sediment but indicated a significant correlation with bottom water temperature and a seasonal fluctuation in oxygen consumption rate (Pamatmat and Banse 1968). The in situ method does not easily allow any manipulation of water temperature which was necessary if the effect of temperature was to be isolated from other possible seasonal effects. The problem demanded shipboard measurements of oxygen uptake which could be done under

controlled temperature, i.e., the same temperatures throughout the year.

Development of a Shipboard Method-A shipboard method requires first of all that representative samples of the benthic community (sediment plus inhabitants) be brought aboard the ship undisturbed. No satisfactory sampling gear existed at the time and it was necessary to invent one. A multiple coring device (Pamatmat 1971a) resulted from this effort and was subsequently used for taking samples in Puget Sound in all kinds of sediment, offshore from the continental shelf with hard-packed sand to the deep sea with soft ooze, the Aleutian Trench, and in Lake Washington with its gyttja type of sediment. The coring device is described in greater detail together with later modifications in this report. Detailed plans for its construction are to be found in the Appendix.

Comparisons of shipboard measurements of oxygen uptake by sediment cores with in situ measurements have shown that at the same temperature the shipboard method yields the same estimate of total uptake and inorganic chemical oxidation as the in situ technique (Pamatmat 1971a, 1971b).

Significance of Reduced Substances in Sediments-The total oxygen uptake by the seabed is partly due to respiratory uptake by aerobic organisms and partly due to abiotic chemical oxidation of reduced substances like ferrous, manganous, sulfides, etc. In order to understand differences in the rate of abiotic chemical uptake, it is necessary to measure the concentration of these reduced substances. A technique for their estimation was therefore developed.

Humic Acid Content of Sediments-It is well known that only a fraction of the organic matter in sediments is metabolizable; the refractory portion of organic matter has been called humus (Waksman 1933). One might expect that the difference between total organic matter and the humus fraction would therefore represent the amount of oxidizable organic matter. This would be a better measure of available food to the benthic community.

Effect of Sediments on Oxygen Tension of Bottom Water -The amount of reduced substances in the sediment is proportional to its oxygen debt (Pamatmat 1971b). Hence, one would expect that the spreading of such substances in the water would affect the oxygen tension. Observations via underwater television revealed that the concentration of suspended particles in bottom water increased with intensity of tidal currents (Pamatmat 1971b). Hence, we attempted to show that resuspension of bottom deposits lowers the oxygen tension of the water.

Dehydrogenase Assay for Measuring Anaerobic Metabolism in Sediments-

The problem of clarifying the quantitative relationship between the rate of abiotic oxygen consumption and anaerobic metabolism in the sediment requires the direct measurement of anaerobic metabolism. An imposing difficulty in measuring anaerobic benthic metabolism is the diversity of metabolic types of heterotrophic bacteria, which could include various fermenters, nitrate reducers, denitrifiers, sulfate reducers, and methane bacteria, in addition to anaerobic macrofauna, meiofauna, and microfauna. Quantitative chemical analysis of metabolic end products would not be practicable.

We turned to a method of dehydrogenase assay (Lenhard 1956) which measures the rate of hydrogen production during intermediary metabolism by means of triphenyltetrazolium chloride (TTC) which, in the absence of oxygen, reacts with hydrogen to form a red-colored compound, triphenylformazan (TPF), whose amount is measured colorimetrically. The concentration of formazan produced is a function of incubation time, pH, temperature, kind of substrate added if any, substrate concentration, plus the population density of microorganisms. The relative measure of metabolic activity was calibrated by direct calorimetry to enable its use in determining the actual rate of metabolism under natural field conditions.

Direct Calorimetry -The measurement of metabolism by direct calorimetry circumvents the complexities of dealing with mixed metabolic types (Forrest et al. 1961). ZoBell et al. (1953) observed that sediment bacteria liberated enough heat to raise the temperature of organically rich sediment. Ordinarily, however, very sensitive and expensive microcalorimeters (Calvet and Prat 1963) would be required to detect the heat output by sediment bacteria. We describe here our experience with a gradient type of microcalorimeter and assess the present outlook for its use in ecological studies. The microcalorimeter would be indispensable for calibrating such a chemical method as the dehydrogenase assay.

Laboratory Experiments on Organic Matter Oxidation -In addition to an overall measure of benthic community metabolism, it is equally desirable to know the concomitant loss of organic carbon and recycling of nutrient salts during the mineralization of organic matter. The possibility of determining these equivalents was examined in a long-term experiment involving the periodic measurement of oxygen consumption, the carbon content of the sediment, and the nutrient concentration of the overlying water.

Sediment and Other Environmental Parameters-Relying on the usual approach of attempting to explain differences in benthic community metabolism between places by searching for correlations with possible, known, and suspected factors we made the following measurements:

total carbon, organic carbon, organic nitrogen, sand-silt-clay fractions of the sediment, biomass of macrofauna, temperature, salinity, oxygen concentration of bottom water, and depth of station. Later on, when techniques had been developed, the concentration of total reduced substances was also routinely determined. Humic acid content of sediments was measured in a few samples. All these measurements and analytical determinations were of value in a negative way: they served to emphasize the fact that the rate of benthic oxygen consumption although affected by some of them was also greatly influenced by other factors.

Unplanned Field Experiment-By the end of the first year of research, it was evident that a major factor influencing the rate of oxygen uptake by the sediment was the rate of supply of oxidizable organic matter to the bottom. Just then, a project on pen-rearing of salmonids was started in Clam Bay. The aquaculture operation called for artificial feeding of the fish with formulated pellets. An undetermined quantity of uneaten food plus fish feces settled to the sediment -- a clear case of increased rate of supply of organic matter to the bottom. Rates of oxygen uptake were measured along a transect of stations running across the floating fish pens. The measurements were repeated after the fish were harvested and the floating pens had been removed.

Benchmark Survey of 33 Stations in Puget Sound-Even before we were certain that the rate of total oxygen uptake did not represent total metabolism in the sediment column, it became apparent that the rate of oxygen uptake was characteristic of each area studied and probably was a useful overall indicator of equilibrium conditions among all the prevailing factors at the time of measurement. It was then imagined that measurements over a wide area of Puget Sound would be of value in later assessing possible eutrophication of the estuary as a result of direct discharge of sewage sludge or indirectly through some physical modification of the body of water.

OBJECTIVES

1) To develop a technique suitable for routine measurements of benthic community metabolism in large-scale surveys.

2) To develop and adapt analytical methods to the study of sediment parameters which may lead to better understanding of decomposition of organic matter in sediments.

3) To evaluate the rate of oxygen uptake by the sediment as a measure of benthic community metabolism.

4) To perform a base-line survey of benthic oxygen consumption in Puget

Sound.

5) To develop a method suitable for routine measurements of anaerobic metabolism in sediments.

6) To perform laboratory experiments that would clarify certain aspects of benthic community metabolism and material exchange between the sediment and the overlying water.

SECTION IV

METHODS

CONSTRUCTION OF CORER

The need for an appropriate sampler is discussed by Pamatmat (1971a) who describes a multiple coring device that seemed to meet necessary requirements of the problem on hand. Detailed plans for the construction of such a gear are presented in the Appendix.

A full description of the corer's principle of operation is as follows:

While suspended from the winch cable during descent, the cylinder (A, Fig. 1a) is full of water. As soon as the frame has settled on the bottom and the cable becomes slack, the weights (B) force the piston (C) to descend at a slow speed as regulated by the size of the opening on the top of the cylinder through which water escapes. The coring tubes (D) at full penetration are shown in Fig. 1b. When the corer is being pulled off the bottom, water is sucked in through the same opening and a half-inch check valve on top to refill the cylinder. When the coring tubes are again all the way up (Fig. 1c), the external core catchers (E) flip underneath to seal them. The principle of operation is essentially similar to that of Craib (1965), which is, however, a single-barreled corer with a more complicated core catcher. A Niskin bottle (F) attached to the frame is automatically tripped when a line attached to a descending weight stand pulls a pin (G) that releases the messenger (H).

The corer is presently used with fiberglass-reinforced epoxy tubes ("green thread" or "red thread" pipe made by A. O. Smith, Little Rock, Arkansas) of 5.7-cm inside diameter. They are impermeable to dissolve oxygen and are of uniform bore. The only objectionable feature of the material is its opacity; but the sediment surface is seen clearly from the top and, when held against a light, the wall is translucent enough to allow a check for the presence of unwanted air bubbles. "Red thread" pipe is thinner, more translucent, and cheaper.

The tube is attached to the corer by means of a polyvinyl-chloride (PVC) plastic coupling (Fig. 1d). A tight seal is provided by an inside O-ring. The coring tube is secured to the coupling by means of three set screws with wing nuts for quick detachment afterwards.

The guides (I) for the tubes serve to seat the core catchers firmly out of the

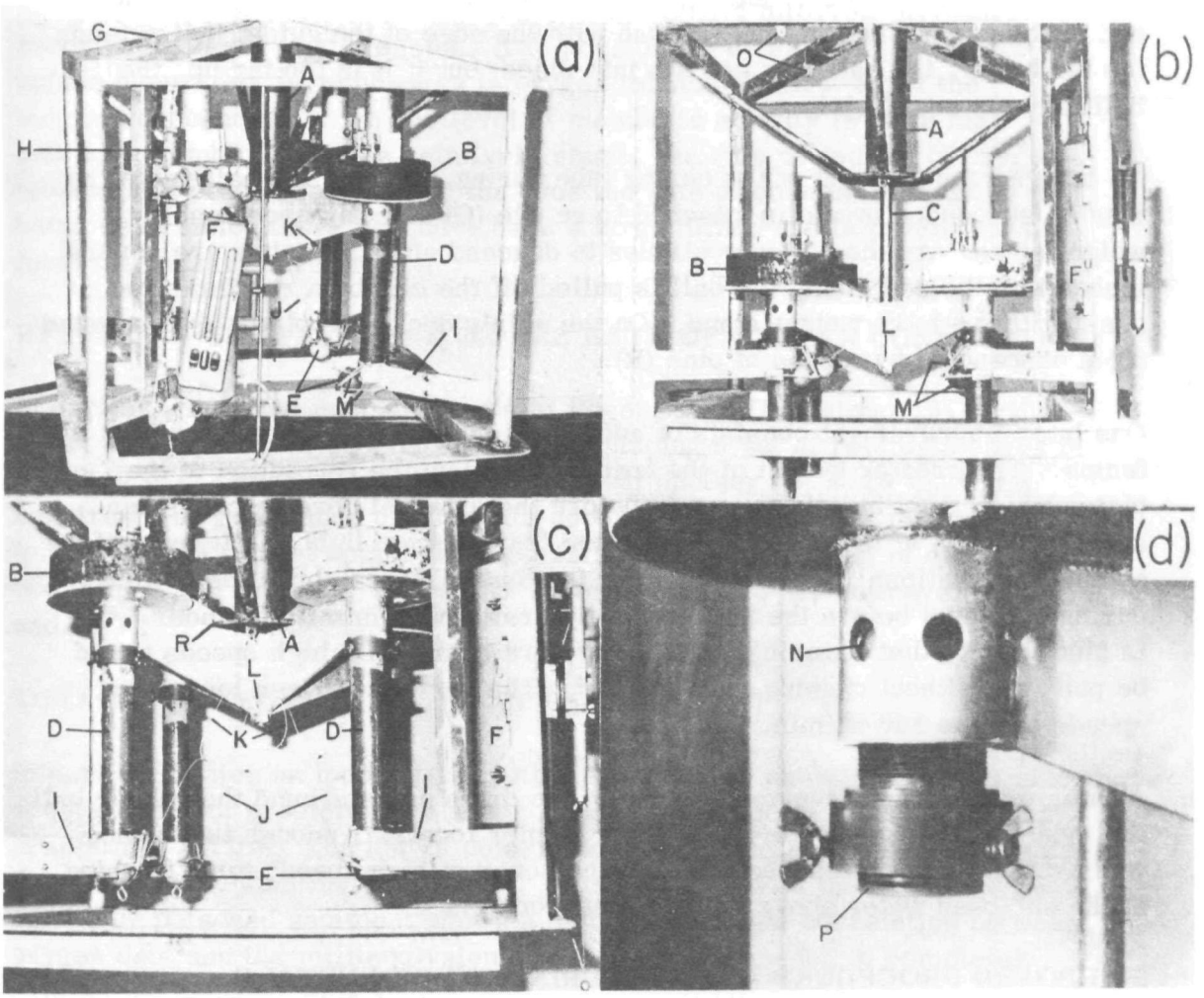

Figure 1. The multiple corer (a) ready to be lowered, (b) when the piston is fully extended and the coring tubes are in full penetration, (c) when the piston and coring tubes are retracted, and with an external core catcher sealing the bottom of each tube, (d) Close-up of PVC coupler to which a coring tube is secured by set screws.

way initially. Each catcher, a solid rubber ball (E), is held out of the way of the tube by a taut stainless steel wire (J) hooked to the lower end of the piston. As the piston descends, the previously slack upper section of the wire (K), which is secured to the bottom plate of the cylinder, gets taut and pulls the now slack lower section (J) off a hook (L). The total length of the wire provides enough slack for the rubber ball to flip into place when the tube is fully retracted into the guide (Fig. 1c). Rubber tubings (M) pull the ball to center and up tight against the lip of the tube. The lower end of the coring tube is ideally flush with the edge of the guide; if it extends too far below, the ball may not flip into place; but if it is too far up, the ball may not seal tightly.

To insure good flushing of the coring tube during descent, the ball valve (N) may be suspended by a clip attached to an eye (O) directly above each weight stand. As the piston continues to descend after the tubes are several inches into the sediment, the ball is pulled off the clip by a retaining rod passing through the weight stand. On the ship's deck, the piston is prevented from descending by means of pins (R).

One later improvement consists of additional weights (100 kg or more) on the frame. The greater weight of the frame speeds up the retraction of the piston which must be all the way in before the external core catchers can seal the bottom of the coring tubes; if the frame is too light, as it was before adding the additional weights, the gear is some distance above the bottom during retrieval before the piston is fully drawn back into its cylinder. It is also thought that lowering the heavier core sampler at high speeds would be possible without causing it to "plane". The device has been lowered at speeds of up to 120 m/min.

A later modification involved relocating the drain holes around the rubber ball that seals the top of the core. In their former location, enough turbulence was evidently generated when raising the corer at high speed; some flushing of the enclosed water above the sediment took place.

SHIPBOARD PROCEDURE FOR MEASURING OXYGEN UPTAKE

The method has already been described in detail (Pamatmat 1971a). Briefly, as soon as the corer is aboard, the cores are transferred to a constant temperature bath. Each sample is sealed with an oxygen electrode and stirrer and the concentration of dissolved oxygen in the water is monitored for a sufficiently long time until a steady decline in oxygen tension is noted. Then some of the cores are poisoned with formaldehyde; the residual uptake indicated by another steady but slower rate of decrease in oxygen tension is considered to be inorganic chemical oxidation. At the end of the procedure, which may take up to several hours depending upon the rate of

uptake, the water is measured to the nearest milliliter.

Since the rate of oxygen consumption is dependent on oxygen concentration (Pamatmat 1971a) it is essential that the rate of inorganic chemical oxidation and rate of total oxygen uptake be determined at the same concentration, which should be that of the bottom water. To ensure that there is no significant decrease in the oxygen tension during the experiment, the volume of enclosed water above the core should be adjusted to the expected rate of uptake. By using a millivolt stripchart recorder with zero suppression, it is possible to determine the rate of uptake accurately from changes in oxygen tension of about 5% of the original concentration. If the level of metabolic activity is quite high and the volume of water is relatively small, the rate of decline of oxygen tension will be so rapid that by the time the rate of chemical oxidation is being determined there will have been a large difference in oxygen tension from the initial concentration.

EFFECT OF TEMPERATURE ON THE RATE OF OXYGEN CONSUMPTION

In earlier shipboard measurements in Puget Sound (Pamatmat 1971b) the effect of temperature was determined by placing a set of replicate cores in a bath at $5^{\circ}C$, another set at $10^{\circ}C$, and still another set at $15^{\circ}C$. This requires a large number of replicate cores. In a later work (Pamatmat, in press), the rate of uptake was determined of the same set of cores at two temperatures, e.g. at 3 and $8^{\circ}C$, or at 8 and $13^{\circ}C$, but never at 3 and $13^{\circ}C$.

TOTAL REDUCED SUBSTANCES IN THE SEDIMENT

In earlier studies an iodometric method was used to determine total reduced substances, but problems are associated with the use of iodine as oxidant (Pamatmat 1971b). The use of potassium dichromate appears to eliminate the problems (Pamatmat, in press). Comparisons with the actual oxygen uptake by poisoned sediment shows a highly significant correlation between oxygen debt and the milliequivalents of dichromate needed to completely oxidize the reduced substances.

The procedure consists of adding 1 ml of 10 N H_2SO_4 and 10 ml of 0.01 N (0.02 N with highly reduced sediments) $K_2Cr_2O_7$ to 50 ml of distilled water. The solution, in a bottle train, is stripped of dissolved oxygen by bubbling with nitrogen gas for about 5 min. Sediment samples are added to the solution with a special sampler (Pamatmat 1971b) to avoid exposure to air; the mixture is stirred continuously with a magnetic stirrer for 5 min and allowed to settle for about 15 min. To a 20-ml aliquot of the supernatant, 1 ml saturated KI is added. After the mixture has been allowed to stand

for 5 min the liberated iodine is titrated with thiosulfate. The total reduced substances determined by this method apparently do not include organic carbon as highly organic sediment samples that had been oven-dried at 90°C and finely ground did not reduce dichromate by this procedure. It is possible, however, that in the drying process volatile organics that would have reduced the dichromate had been lost; in any case, their amount is considered negligible in comparison with the total inorganic reduced substances.

HUMIC ACID CONTENT OF SEDIMENTS

This was determined strictly according to the procedure of Lenhard et al. (1962). For blanks we used ground dried mud that had been combusted in a furnace at 500°C. Less than 1 g of dry sediment is refluxed with 100 ml of 10% v/v HCl for 2 hr to destroy carbonates and hydrolyze non-humic organic matter. The sample is filtered, the insoluble fraction is washed free of chloride and heated with 0.5% w/v NaOH for 2 hr on a boiling water bath. After cooling, the extract is made up to 250 ml and centrifuged for 10 min at 3800 rpm. The optical density of the extract is measured in a 1-cm cell at 436 nm with a Beckman spectrophotometer.

DEHYDROGENASE ACTIVITY OF SEDIMENT BACTERIA

During a cruise in July, following preliminary tests in the laboratory, dehydrogenase activity of sediment bacteria was determined by the following procedure: Sediment samples were incubated with 5 ml Tris buffer and 5 ml of triphenyltetrazolium-chloride-glucose solution at 37°C in a water bath for one hr. The reaction was stopped and the formazan simultaneously extracted with absolute ethanol. The extract was centrifuged before reading its optical density at 483 nm. The results seemed to show significant differences between stations and between layers of a sediment core. Then it was decided that the effect of incubating at 37°C was difficult to assess and the incubation had better be done closer to environmental temperature.

The method finally worked out is an adaptation of the method of Lenhard et al. (1965) which was modified according to results of laboratory experiments with the use of different substrates and incubation at 10°C. On the basis of those findings, the following concentrations of reagents and procedure were established: (1) TTC solution -- 1 g of 2, 3-5 triphenyltetrazolium chloride in 100 ml of distilled water; (2) Tris buffer -- 6.037 g of tris (hydroxymethyl) aminomethane plus 20 ml of 1.0 N HCl in 1 liter of distilled water, pH adjusted to 8.4; (3) sodium citrate solution -- 74 g of sodium citrate in 1.0 liter of distilled water; (4) saturated mercuric chloride ($HgCl_2$) solution; (5) absolute ethyl alcohol.

To each graduated 50-ml Erlenmeyer flask are added 3 ml of Tris bugger, 3 ml of sodium citrate solution, 2 ml of TTC, and 2 ml of sediment. The total volume is made up to 20 ml with distilled water, the flask is swirled a few times, and then allowed to stand undisturbed in the dark for 3 hr at 10^{o}C. Triplicate samples are run. For blanks, duplicate samples are prepared similarly except for the addition of 1 ml saturated $HgCl_2$. After 3 hr the activity is stopped with 20 ml of absolute alcohol, which also extracts the formazan. The mixture is shaken every 15 min for 1 hr to complete the formazan extraction. The mixture is centrifuged and the clear supernatant is read at 483 nm in a spectrophotometer. The sediment is dried overnight at 90^{o}C and the optical density of each sample is normalized to 1 g of dried sediment. The difference between the untreated and the $HgCl_2$-treated samples represents the dehydrogenase activity of the sediment.

DIRECT CALORIMETRY

The instrument used is a gradient or conduction type of calorimeter (Evans 1969; Hammel and Hardy 1963; Benzinger and Kitzinger 1963) with 12,000 copper-constantan junctions. Fig. 2 shows a cross-section of the system arrangement as it was finally used during the experiments. The calorimeter is buried in sand in the stainless steel vacuum Dewar vessel; the sand serves as the heat sink. The temperature control system consists of the closed 0.11-m^3 double-walled tank connected to the 0.19-m^3, floor-model, externally circulating temperature bath. The Forma bath used has a rated pumping rate of 38 liters/min. The temperature in the bath was constant to within 0.02^{o}C with no drift. During the experiments the water bath was set at 10^{o}C. The calorimeter was electrically calibrated as described by Berger (1969).

The procedure for direct calorimetry of sediment samples was as follows: The sediment sample is packed inside a 60-ml, screw-capped culture tube. This tube is wrapped in plastic and immersed in the water bath. After temperature equilibration, the dry culture tube is quickly transferred to the calorimeter. After the calorimeter restabilizes (at least 12 hr) the sample is removed. The empty calorimeter restabilizes much more rapidly (4 hr) and the decrease in thermal emf following removal of the sample represents the rate of metabolic heat release.

In calibrating the dehydrogenase assay by direct calorimetry, the assay was performed immediately or within a few hours after the sample was removed from the calorimeter.

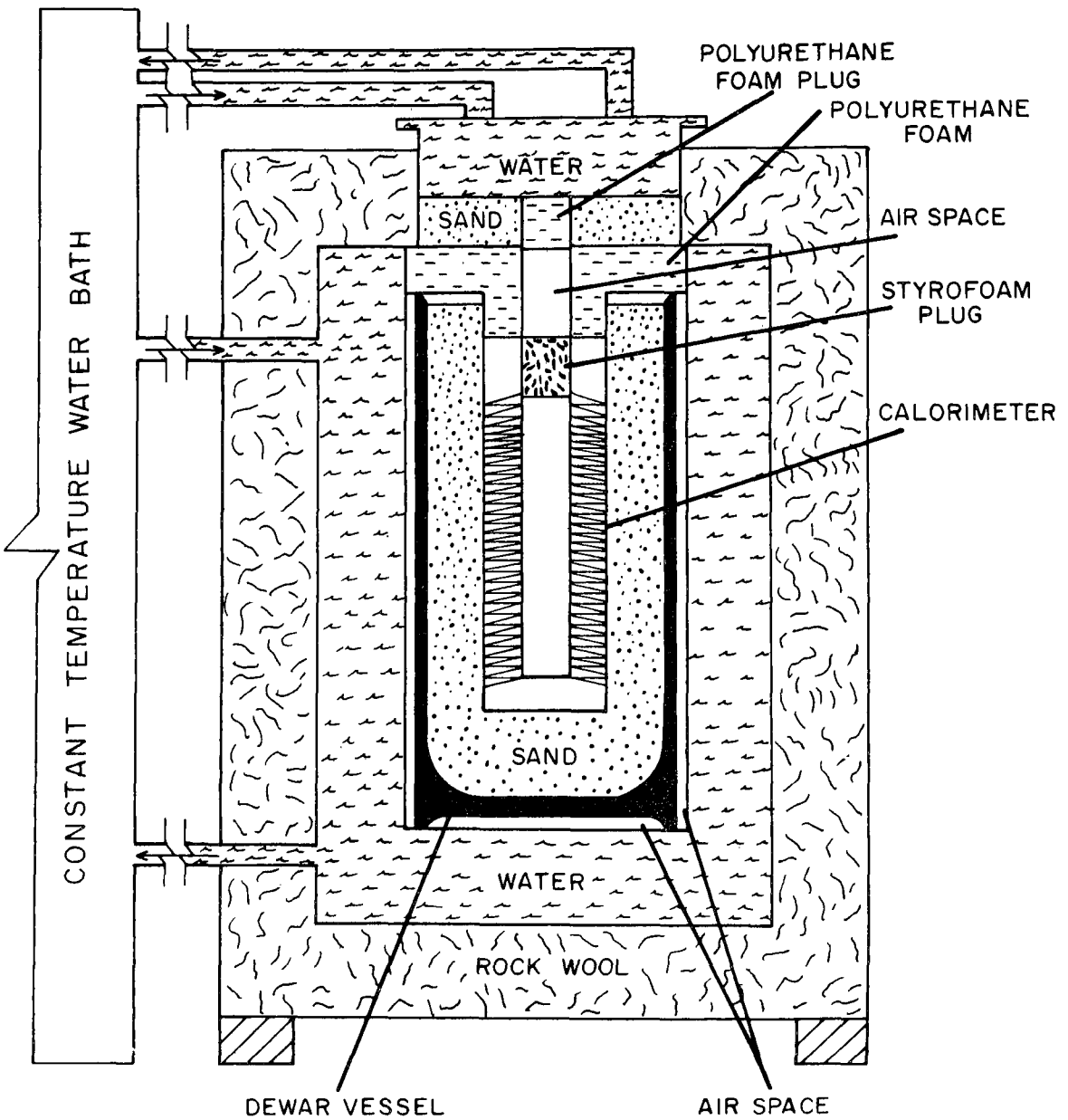

Figure 2. Cross-section of microcalorimeter and environmental-temperature control system.

COMPARISON BETWEEN OXYGEN UPTAKE BY CHEMICAL OXIDATION AND ANAEROBIC METABOLISM IN THE SEDIMENT COLUMN AS DETERMINED BY DEHYDROGENASE ASSAY

Samples were taken from 19 stations (Fig. 3) in Lake Washington with the multiple corer. Oxygen uptake by the intact cores from all stations was measured immediately at 10°C while replicate cores from five stations were sectioned and some layers were assayed for dehydrogenase activity on board ship. The core from a sixth station (station 19) was stored at 10°C in the dark for one week before it was assayed for dehydrogenase activity. Each layer, except the already liquid 0-1 cm layer, was mixed with a small amount of distilled water to facilitate homogenization and measurement of replicate samples. These layers also were analyzed for concentration of reduced substances by the dichromate oxidation procedure.

LABORATORY EXPERIMENTS

A preliminary experiment to work out a method for determining the relationship between oxygen uptake, loss of organic carbon from the sediment, and liberation of nutrients was conducted over a two-month period. In order to eliminate any complication with anaerobic metabolism and anoxic conditions in the sediment, a small amount of sediment was allowed to settle in a thin layer on the bottom of a 250-ml Erlenmeyer flask. A large batch of sediment was mixed well into an homogeneous paste; all discernible large particles, e.g. broken shells, macrofauna, gravel, were removed. Then 2 ml of the slurry was distributed into each of 20 replicate flasks which were slowly filled with Millipore-filtered sea water and allowed to stand until the water had cleared completely, except for two randomly picked flasks whose oxygen uptake was determined within a few hours. The rest were kept in a cold room at 9.5°C and bubbled with air which had been bubbled through a larger flask of water. On designated dates replicate flasks were picked at random for determination of oxygen uptake, both total and residual uptake after poisoning with $HgCl_2$, the nutrient content of the water, and the organic carbon content of the sediment.

GRADIENT OF DISSOLVED OXYGEN ABOVE THE SEDIMENT

During previous cruises to a station at the entrance to Port Madison (Fig. 4), an increase in suspended sediment in the bottom water during ebb tide was observed with underwater television. This resuspension of sediment could increase *in situ* benthic oxygen uptake, which could be reflected by a tidal change in the vertical distribution of oxygen in the bottom water. Electrodes, each equipped with a magnetic stirrer, were mounted on a vertical angle iron at 3, 33, and 93 cm above a broad plastic plate attached to the bottom end of the angle iron. The whole mount was loosely fixed to a tripod (the same one used in the *in situ* experiments on benthic metabolism and described by Pamatmat and Fenton, 1968) so that when the tripod landed on the

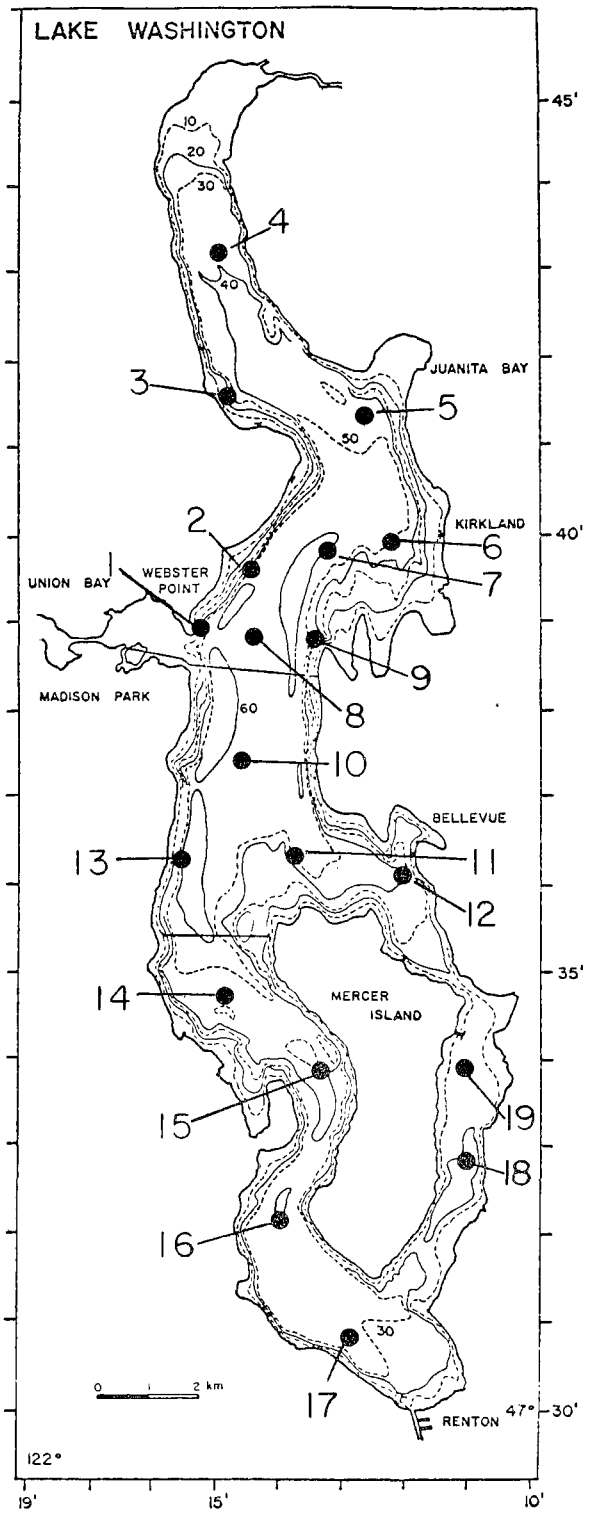

Figure 3. Chart of Lake Washington showing 10-m isobaths and location of stations.

bottom, the plastic plate rested on the sediment surface and as the tripod settled into the soft bottom, the vertical angle iron was free to slide up. Hence the lowermost electrode was positioned as close to the bottom as possible without getting buried. The three electrodes were monitored continuously with a multipoint recorder.

SEDIMENT AND WATER ANALYSES

The sediment size analysis was done according to standard geological methods (Krumbein and Pettijohn 1938). The determinations of total and organic carbon were made with the LECO analyzer (Laboratory Equipment Co., Michigan). Organic nitrogen was determined by the COLEMAN analyzer (Coleman Instrument Co., Illinois).

Five-cm layers of the sediment cores (0 to 5 cm, etc.) were squeezed with Reeburgh's (1967) device, and the interstitial water samples were analyzed for dissolved phosphate, nitrate, silicate, and ammonia with an AUTOANALYZER using an adaptation of the method of Armstrong et al. (1967) for nitrate and silicate, by the method of Murphy and Riley (1962) for phosphate, and by Koroleff's (1970) method for ammonia. When nutrient values were very high, the samples were diluted with filtered sea water of known nutrient concentration and re-analyzed.

Dissolved nutrients in all other samples were determined according to the foregoing methods. All samples were frozen after addition of a few drops of saturated mercuric chloride ($HgCl_2$) and stored in the freezer for up to three months before they could be analyzed.

SECTION V

RESULTS

ENVIRONMENTAL PARAMETERS

The location of 33 stations in Puget Sound are shown in Fig. 4. All the measured parameters at the first 23 stations are shown in Tables 1 and 2. The variations in depths of stations with each cruise, corrected to mean lower low water when greater than 3 m, are perhaps indicative of navigational error, which is estimated to be a radius to 200 m. Some stations are located on a slope (station 12), or at the bottom of a small depression (stations 15 and 19), while others are on relatively broad flat bottom (stations 8 and 9).

The mean organic carbon content of the sediment in the 23 stations during 5 cruises ranged from 0.4 to 3.7% of dry sediment. Carbonate-carbon was present in a significant amount only at station 13; at all other stations organic carbon was not significantly lower than total carbon values. There was no detectable seasonal change in the organic carbon content of the sediment surface, nor was there any difference between the organic carbon content of the 0-1 and 5-6 cm layers. Organic carbon and organic nitrogen are strongly correlated ($r = 0.97$) with the following regression equation:

$$\% \text{ organic nitrogen} = 0.105 \times \% \text{ organic carbon} - 0.002\%,$$

which signifies essentially a C:N ratio of 10.

A significant correlation ($r = 0.89$, d.f. = 21) was found between organic carbon and percent silt plus clay (and therefore significant negative correlation between organic carbon and percent sand), but there is considerable variability indicating heterogeneity of the samples or their sources in terms of other factors. There are deep as well as shallow sandy stations so that the sediment type is not strictly a function of depth.

At nearly all stations, salinity dropped from October 1969 to January 1970 and still further to the lowest values in April; then it increased again until October 1970. The bottom water was significantly more saline in October 1970 than in October 1969. The seasonal change is small and is presently considered of negligible importance as a physiological factor in benthic community metabolism.

With few exceptions, temperature was lowest in January and highest in July.

Table 1. DEPTH AND MEASURED PARAMETERS OF THE SEDIMENT AT DIFFERENT STATIONS

Station	Depth range	Total[a] carbon	Organic[a] carbon	Carbonate[a] carbon	%Sand	%Silt	%Clay
1	7-11	2.85	2.65	0.20	10	68	23
2	10-13	2.95	2.95	0	5	78	17
3	25-29	1.90	1.90	0	87	8	5
4	45-55	1.30	1.30	0	41	47	12
5	28-33	0.80	0.65	0.15	63	28	9
6	25-29	2.60	2.60	0	5	70	25
7	74-76	2.45	2.30	0	3	70	27
8	14-14	2.40	2.40	0	14	68	17
9	204-209	2.50	2.50	0	4	68	28
10	187-189	2.45	2.45	0	13	61	26
11	250-252	2.00	1.85	0.15	53	34	13
12	181-221	0.20	0.20	0	96	2	2
13	10-13	4.45	2.80	1.65			
14	10-12	3.70	3.70	0	7	73	20
15	36-41	2.65	2.50	0.15	17	64	19
16	31-35	0.90	0.90	0	54	39	7
17	206-229	2.15	1.85	0.30	4	70	26
18	187-204	2.10	1.85	0.25	9	66	25
19	259-299	1.55	1.40	0.15	41	48	11
20	221-224	0.40	0.40	0	87	9	4
21	204-241	1.95	1.85	0.10	10	67	23
22	118-121	2.40	2.25	0.15	2	68	29
23	177-182	2.55	2.35	0.20	2	67	31

[a]Average percent of dry sediment.

Table 2. SEASONAL CHANGES IN DISSOLVED OXYGEN (ml/liter), TEMPERATURE (^{o}C) AND SALINITY (^{o}/oo) OF BOTTOM WATER AT THE DIFFERENT STATIONS.

Station	Dissolved oxygen				
	Oct	Jan	Apr	Jul	Oct
1	4.50	6.55	7.15	6.50	5.55
2	4.20	5.95	6.65	4.35	4.85
3	4.25			5.85	4.95
4	4.00	5.90	5.85	5.45	5.10
5	4.40	5.20	5.95	6.15	4.65
6	3.75	6.15		4.70	4.45
7	3.90	5.55	5.40	4.55	3.95
8	3.90		5.50	4.65	4.95
9	3.55	5.25	5.45	4.60	4.50
10	3.95	5.15	5.45	4.65	
11	3.95	5.80	5.45	4.60	4.35
12				5.75	4.80
13	4.20	5.50	6.30	5.80	4.65
14	5.50	6.00	6.60	6.60	5.05
15	3.90	6.10	6.25	5.80	4.95
16		5.20	5.50	4.50	4.40
17	3.95	5.30	5.45	4.65	4.75
18	3.90	6.10	5.60	6.30	4.20
19	3.95	5.20	5.35	4.80	4.65
20	3.95		5.10	4.10	4.75
21	3.70	5.60		4.60	4.25
22	3.00	2.70	1.60	2.65	3.35
23		4.20	2.55	3.60	3.80

Table 2 (continued). SEASONAL CHANGES IN DISSOLVED OXYGEN (ml/liter), TEMPFRATURE (^{O}C) AND SALINITY (^{O}/oo) OF BOTTOM WATER AT THE DIFFERENT STATIONS.

Station	Temperature				
	Oct	Jan	Apr	Jul	Oct
1	14.60	7.74	9.23	15.62	12.56
2	13.28	8.22	8.92	13.49	12.44
3	13.12			13.29	12.29
4	12.40	8.66	8.75	12.04	11.93
5	12.39	8.61	8.67	12.85	11.92
6	13.26	8.46		12.18	12.33
7	12.10	8.82	8.68	11.57	11.82
8	11.94		8.88	12.40	11.58
9	11.04	8.54	8.47	10.00	10.72
10	10.73	8.32	8.47	10.56	
11	10.54	8.46	8.46	10.72	10.20
12	11.50			12.29	11.15
13		8.30	9.24	13.84	11.92
14	12.97	8.05	9.64	15.39	11.45
15	12.37	8.26	8.94	12.46	11.30
16		8.54	8.69	11.14	11.04
17	10.41	8.28	8.45	10.74	10.49
18	10.36	8.20	8.46	10.66	10.62
19	10.36	8.76	8.40	10.77	9.94
20	10.44		8.40	10.88	9.95
21	10.43	8.30		10.67	9.92
22	11.04	8.84	9.23	9.08	10.76
23		9.44	9.60	9.18	10.14

Table 2. (continued) SEASONAL CHANGES IN DISSOLVED OXYGEN (ml/liter), TEMPERATURE (°C) AND SALINITY (°/oo) OF BOTTOM WATER AT THE DIFFERENT STATIONS.

	Salinity				
Station	Oct	Jan	Apr	Jul	Oct
1	28.99	28.40	27.33	29.14	29.80
2		29.06	28.26	29.27	30.09
3	29.65			29.44	30.18
4	29.91	29.60	28.64	29.57	30.36
5	29.83	29.29	28.74	29.61	30.36
6	29.59	29.32		29.45	30.30
7	30.07		28.88	29.60	30.48
8	30.15		29.03	29.63	30.57
9	30.66	30.12	29.94	30.22	30.86
10	30.65	30.16	29.95	30.39	
11	30.69	30.12	30.02	30.46	31.11
12	30.38			29.80	30.63
13	29.76	29.47	28.76	29.76	30.45
14	29.50	28.94	28.58	29.45	30.15
15		29.68	28.97	29.56	30.44
16			29.36	30.00	30.79
17	30.79	30.16	30.10	30.51	30.96
18	30.85	30.24	30.08	30.51	30.86
19	30.83	29.86	30.25	29.80	31.29
20	30.70		30.36	30.49	31.24
21	30.78	30.20		30.48	31.29
22	30.69	30.40	29.97	30.08	30.91
23		30.47	30.37	30.16	31.04

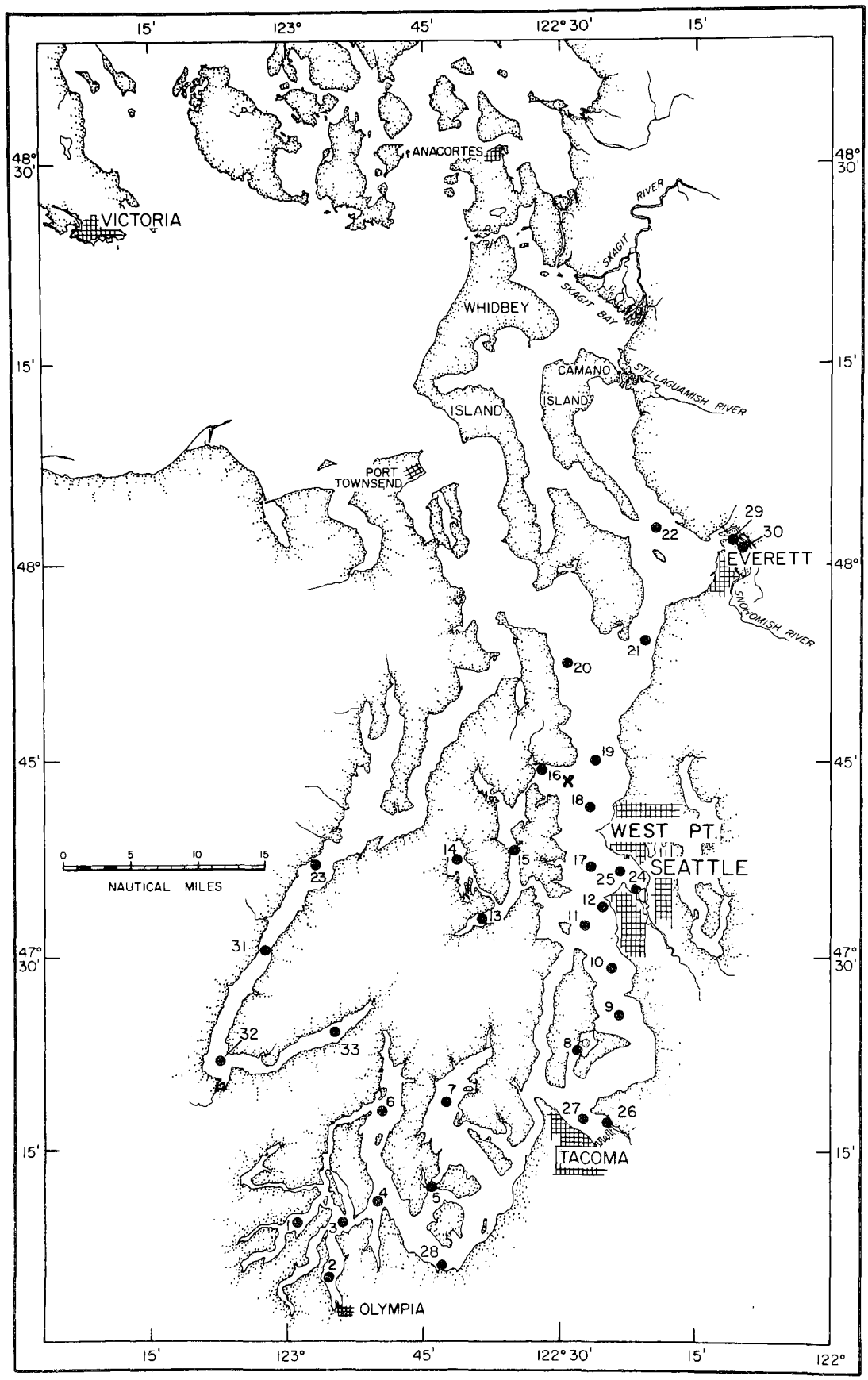

Figure 4. Chart of Puget Sound showing location of 33 stations. X marks the location of station where the effect of scouring on oxygen tension of bottom water was observed.

In October 1969, it was significantly higher than in October 1970. The effect of temperature is discussed more fully later.

There was some variation in the seasonal cycle of oxygen concentration in the different stations, some stations having the highest concentrations in January and others in April, but in general the values dropped in July and October. The oxygen tension of the bottom water in October 1970 was significantly higher than in October 1969; this may be related to the significantly lower temperatures in October 1970. The shallowest stations had the highest oxygen tension, station 1 becoming supersaturated in April. Stations 22 and 23, being located behind threshold sills, had the lowest oxygen tension and exhibited a reverse seasonal cycle. The oxygen data indicate flushing of the bottom water at both stations between April and July and again between July and October. The influence of the seasonal cycle in oxygen tension of the bottom water on benthic oxygen consumption is discussed later.

TOTAL OXYGEN CONSUMPTION

The rates of measured total oxygen uptake in Puget Sound during the year ranged from 4 to 56 ml $0_2/m^2$ per hr. The average rates at each of the stations where measurements were made at three temperatures are shown for January and July (Figs. 5 and 6). There is a significant regression of standard deviations of the rates of uptake versus the means ($P < 0.001$) as the following regression equation shows:

$$\text{standard deviation} = (0.147 \times \text{mean rate of uptake}) + 0.160.$$

The variability between replicate measurements at each temperature increased with increasing rates but remained reasonably small. However, the data show a fairly high variability in the apparent effect of temperature on the rates at each station. In January, for example, stations 4 and 7 show no significant effect between 5 and 10°C but show a greater temperature coefficient between 10 and 15°C than the other stations. Ordinarily this type of data is considered as evidence for temperature adaptation between 5 and 10°C and lack of adaptation between 10 and 15°C, e.g. the findings of Duff and Teal (1965) regarding the rates of gas exchange by the intertidal sediment at different latitudes. While there may be certain interesting differences between stations that would account for apparent differences in their Q_{10}'s of oxygen consumption, these differences may be attributed to chance until they can be verified. The seasonal change is evident from the increases in the elevation of the various curves from January to July.

Because of the significant regression of standard deviations on the means, an analysis of variance to determine any significant differences between stations, seasons, and temperatures was performed after logarithmic transformation of the data. The results of the analysis show significant differences between

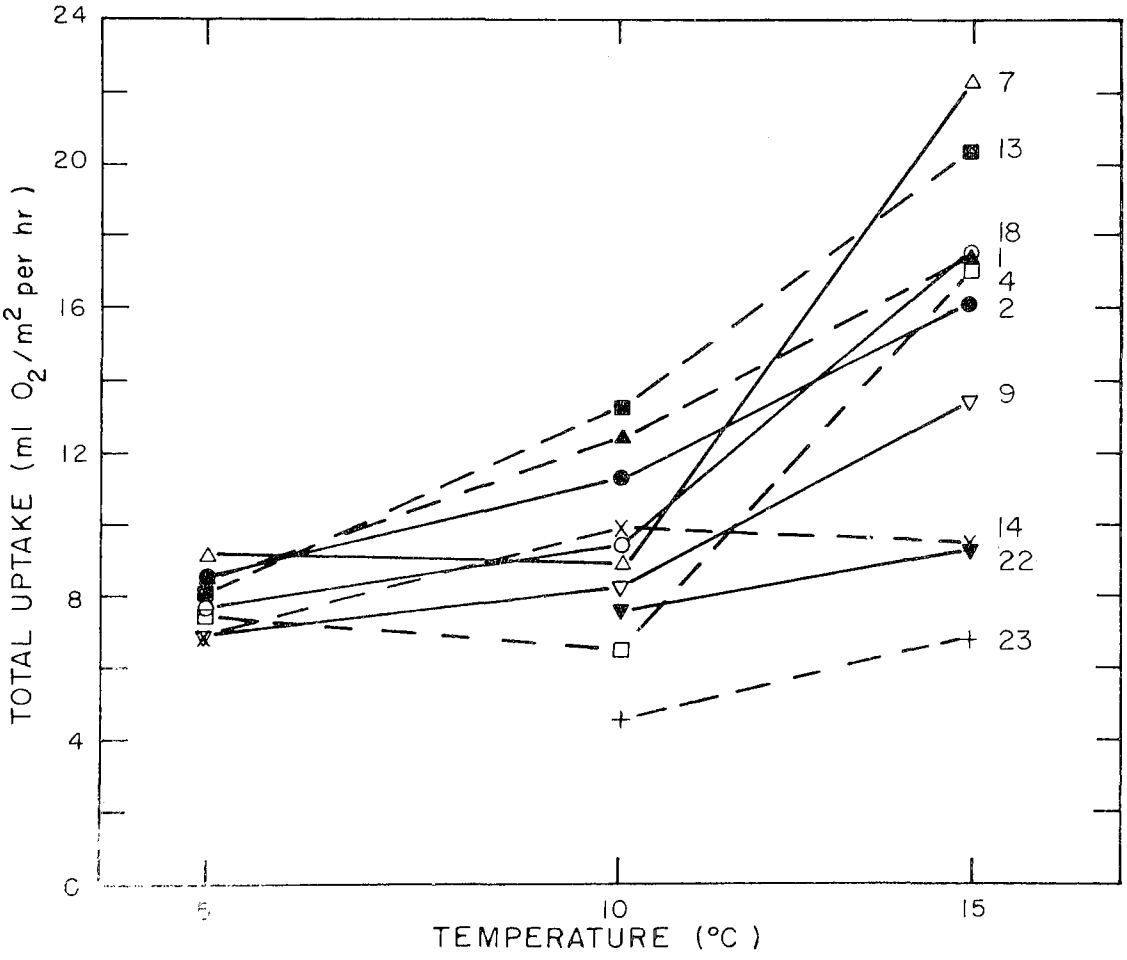

Figure 5. Total oxygen uptake versus temperature at various stations in January.

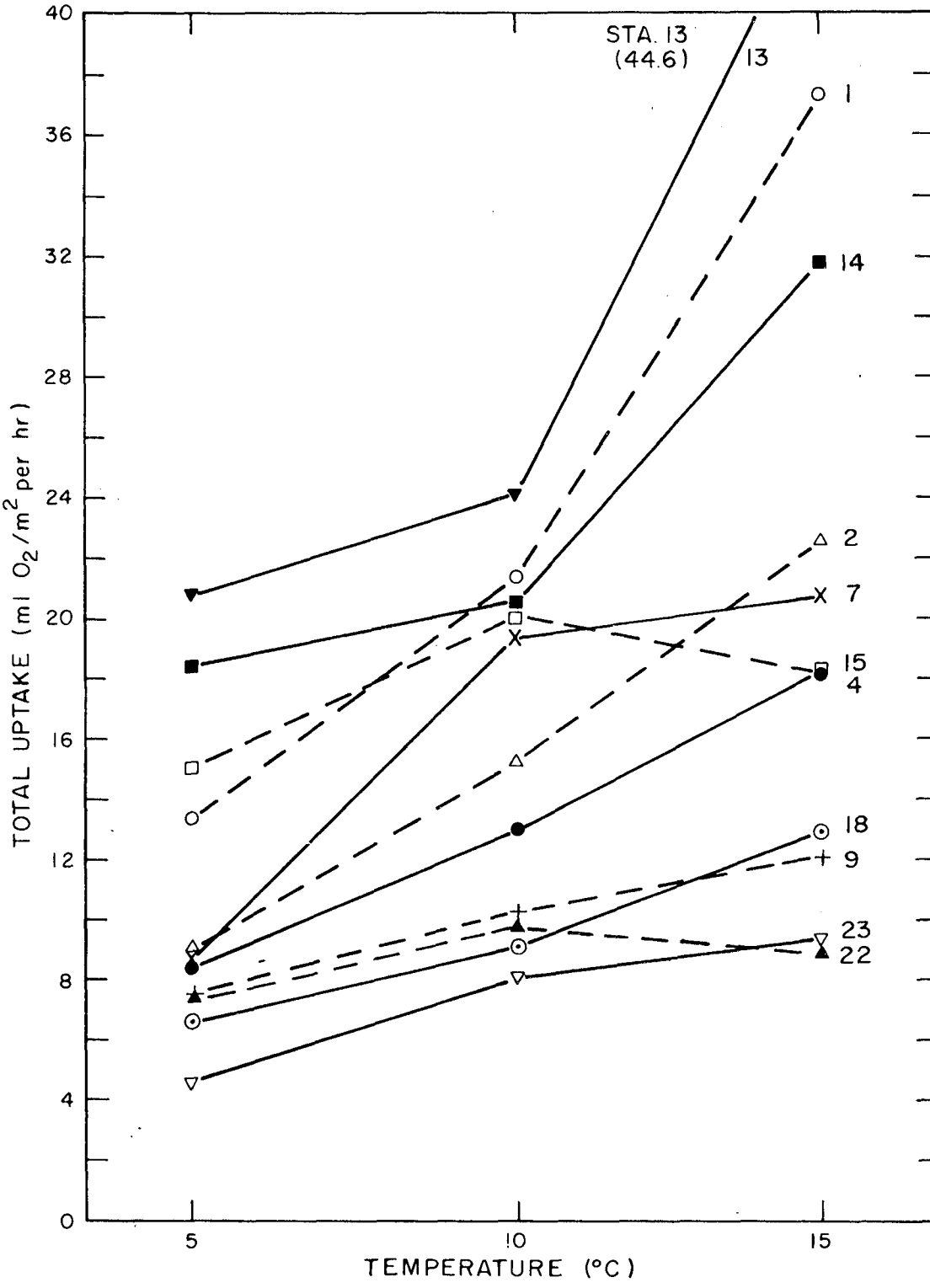

Figure 6. Total oxygen uptake versus temperature at various stations in July.

stations, seasons, and temperatures (Table 3). One output of computer program BMDX 64 (general linear hypothesis, version of July 27, 1965, Health Sciences Computing Facility, University of California at Los Angeles) which was used in the analysis of variance is a matrix of predicted rates of uptake for each station, each season, and each temperature (Appendix). These predicted rates are summarized for station 1 (Fig. 7) and station 23 (Fig. 8), which have the highest and lowest rates, respectively. The 95% confidence limits are predicted rates multiplied or divided by 1.17.

In effect, the computer program has extracted for all the stations together the average trend of the seasonal cycle and of the relationship between uptake rates and increasing temperature; hence, only the magnitude of the changes differs between stations. The calculated Q_{10}'s are 2.3 between 5 and 10°C and 1.5 between 10 and 15°C. The seasonal trend as shown in Figs. 7 and 8 is an increase from January to April to a maximum in July and a decrease to October to a minimum in January. The rates were the same in October 1969 and October 1970 in all stations except 4 and 6, where the rates were significantly lower and higher, respectively, in 1970. Nevertheless, station 6 still showed a decrease from July to October 1970. Note that the seasonal cycle described is of the rates versus temperature curves. From these four seasonal curves for each station, the in situ rates could be read against the actual temperature of the bottom water each season, resulting in the actual seasonal cycle in situ in each station as depicted in Figs. 9 and 10 for stations 1 and 23. The effect of temperature was to increase the amplitude of the cycle in total uptake.

From the results of the long-term experiments on partitioning (Pamatmat 1971), a positive effect of higher oxygen tension of the water on the rates during January and April is to be expected, i.e., if the experiments in January and April had been conducted at the same oxygen tension as in July and October, the rates would have been even lower than they were. However, the effect of oxygen tension on the rate of uptake has not been adequately studied. All cores showed steady rates of uptake for at least 2 hr, during which period the oxygen tension dropped by several tenths of a ml/liter. Over longer periods the rates declined slowly, evidently depending on the relative rates of respiration and chemical oxidation. The effect of the 1-2 ml/liter difference in oxygen tension between summer and winter can be quite large.

The significant differences between stations in total uptake still cannot be explained fully in terms of oxygen tension, temperature, or sediment properties. There are suggestive trends of decreasing rates with depths of stations and increasing sand fraction, and of increasing rates with increasing carbon content of the sediment or increasing silt and clay fraction; however, none of these correlations is significant, indicating the influence of one or more factors not related to these measured parameters.

Table 3. ANALYSIS OF VARIANCE ON MEASUREMENTS OF TOTAL UPTAKE (LOGARITHMICALLY TRANSFORMED DATA)

Source	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio (F)
Mean	1942.83	1	1942.83	26462.57
Temperature	28.79	2	14.40	196.07[a]
Season	8.69	3	2.90	39.47[a]
Station	38.42	22	1.75	23.79[a]
Error	40.97	558	0.073	

[a] $P < 0.01$

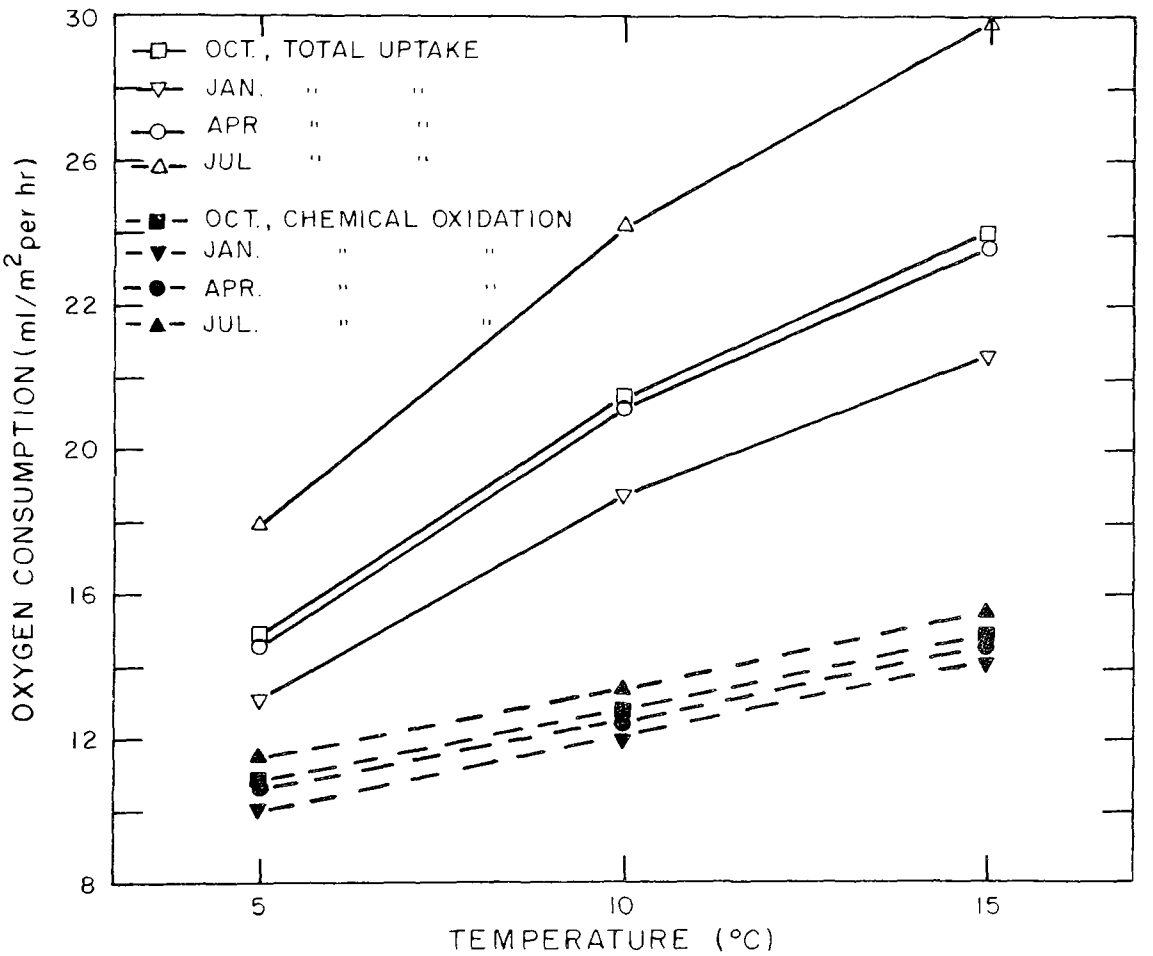

Figure 7. Predicted rates of total oxygen uptake and chemical oxidation at station 1 as functions of temperature and season. The differences in elevation between regression lines represent seasonal changes independent of temperature effect.

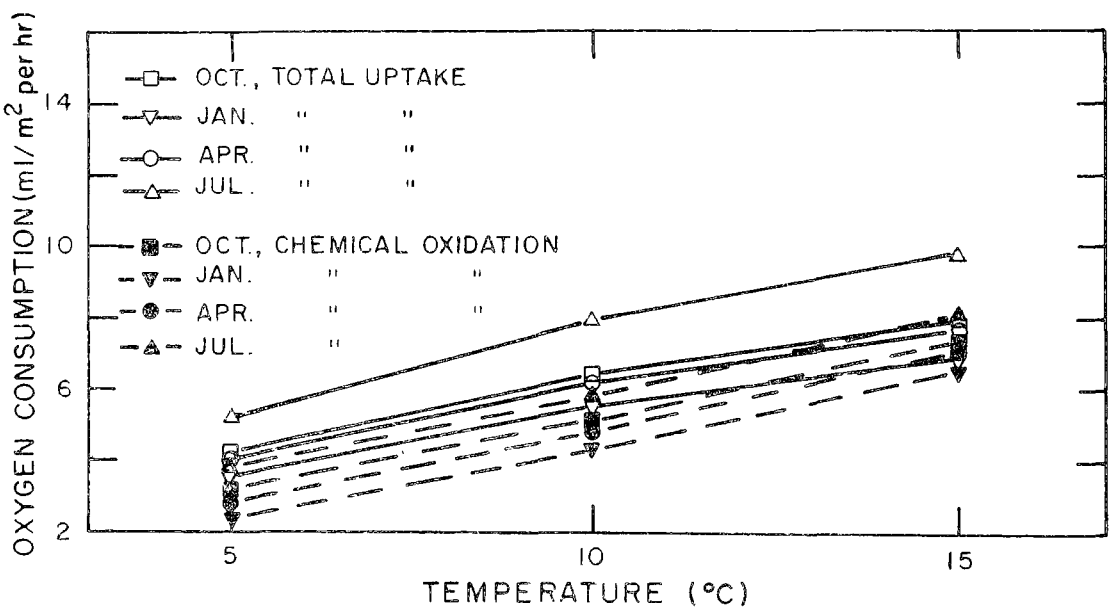

Figure 8. Predicted rates of total oxygen uptake and chemical oxidation at station 23 as functions of temperature and season. The differences in elevation between regression lines represent seasonal changes independent of temperature effect.

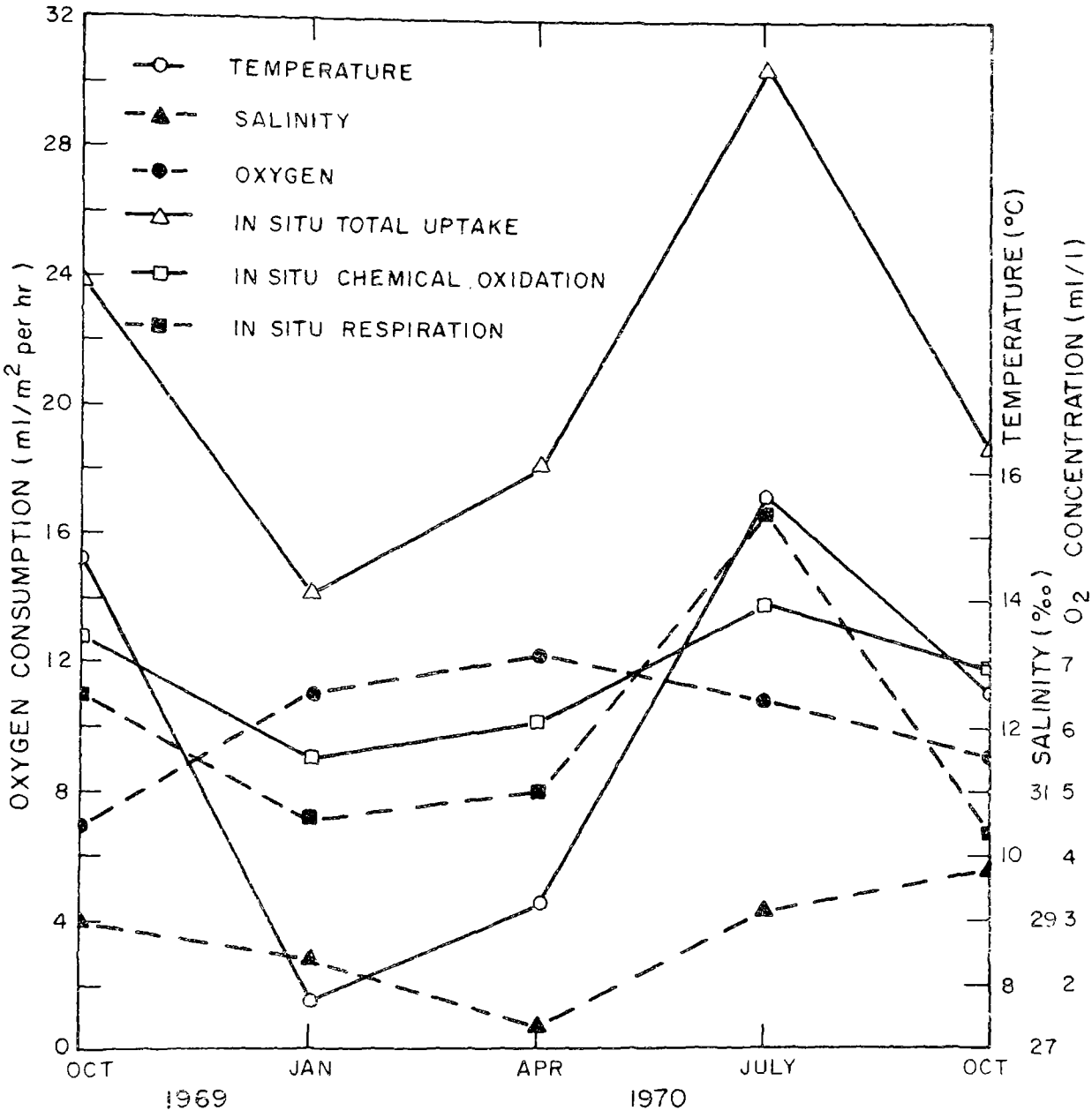

Figure 9. Seasonal cycle of in situ total oxygen consumption, chemical oxidation, respiration, oxygen tension, salinity, and temperature at station 1.

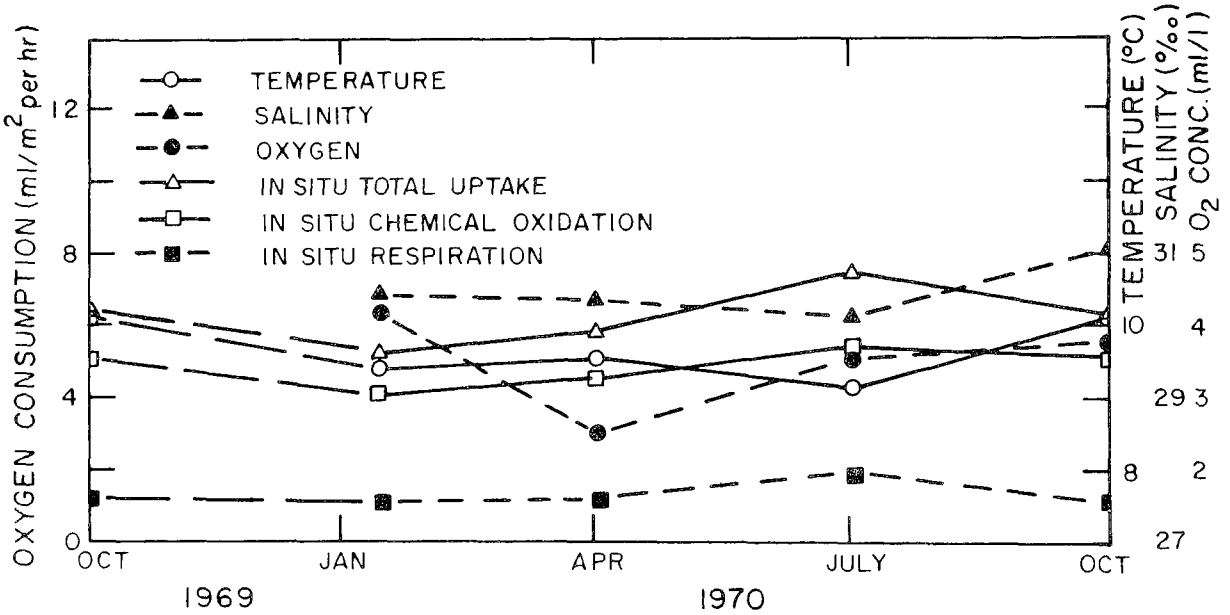

Figure 10. Seasonal cycle of in situ total oxygen consumption, chemical oxidation, respiration, oxygen tension, salinity, and temperature at station 23.

The situation in Puget Sound is depicted in Fig. 11. In January at 10°C, total oxygen uptake was almost significantly correlated with organic carbon concentration ($r = 0.53$ versus $r = 0.57$ at $P_{0.05}$). Part of the variability here is probably due to differences between stations in the oxygen concentration of the bottom water (Table 2). An eye-fitted regression line for the January data would have a high positive Y-intercept and a rather flat slope. Since it is reasonable to assume that in the complete absence of organic matter in the sediment there cannot be metabolic activity, the regression line should pass through the origin. As for the slope, there is no theoretical basis for predicting it although the indicated low increments of oxygen consumption with increasing organic matter content of the sediment conform with the now common notion that much of the organic matter in the sediment is not only nonliving but refractory to biochemical oxidation.

The same scatter plot for July at 10°C shows a much increased variability. At the same temperature, but generally lower oxygen tension, the rates were almost invariably higher in July; if the experiments in July had been run at the same oxygen tension as those in January, the rates would have been even higher. It is interesting that the shallow stations (arbitrarily designated as those less than 100 m deep) increased by an average of 9 ml/m^2 per hr from January to July, while the deep stations on the average increased by only 3 ml/m^2 per hr. This difference between shallow and deep stations is statistically significant ($P < 0.01$). As will be discussed later, the increased scatter in July may be attributed to different rates of supply of organic matter to the various stations.

INORGANIC CHEMICAL OXIDATION

What was said about variability in the measurements of total uptake may also be said about the measurements of chemical oxidation and the effect of temperature. However, unlike the rates of total oxygen uptake, the standard deviations in the rates of chemical oxidation are much smaller and are independent of the means. The average standard deviation is 1.01 ml/m^2 per hr. The analysis of variance was therefore done on the untransformed data, giving the results shown in Table 4. There are highly significant differences between stations, seasons, and temperatures. The predicted rates are shown in Figs. 7 and 8 for stations 1 and 23. The 95% confidence limits are predicted values ± 1.9 ml/m^2 per hr. It is clear that chemical oxidation is very much less variable than total oxygen consumption.

The curves of chemical oxidation versus temperature show that the relationship cannot be described in terms of the usual temperature coefficient of metabolic rates. The curves are parallel and would indicate decreasing Q_{10}'s with increasing elevation. In fact the relationship shows that chemical oxidation is a linear function of temperature. It appears to increase by 0.08

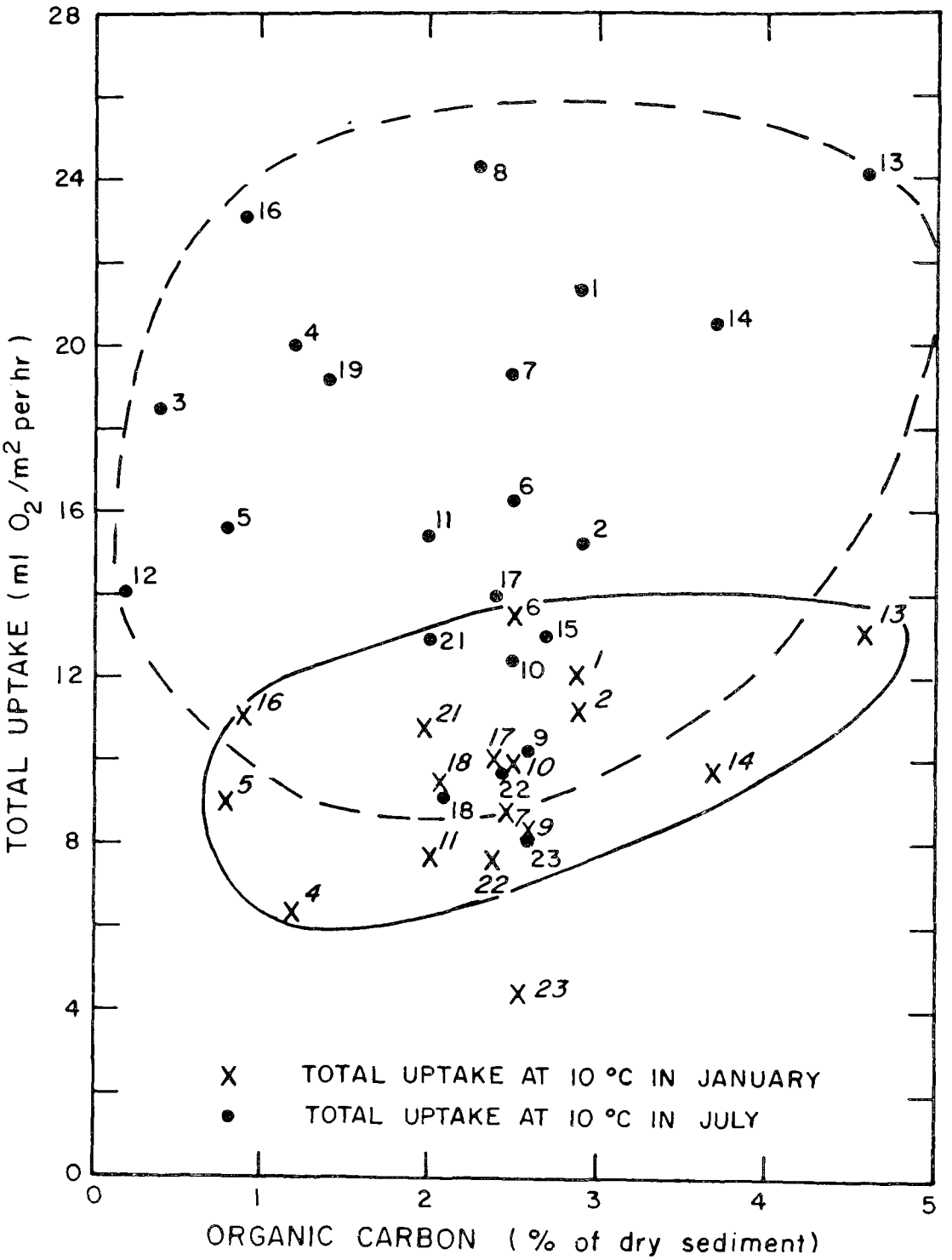

Figure 11. Rates of total oxygen consumption at 10°C in January and July versus organic carbon content of the sediment.

Table 4. ANALYSIS OF VARIANCE ON MEASUREMENTS OF CHEMICAL OXIDATION (UNTRANSFORMED DATA)

Source	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio (F)
Mean	10195.50	1	10195.50	2827.75
Temperature	623.35	2	311.68	86.44[a]
Season	76.47	3	25.49	7.07[a]
Station	1008.64	22	45.85	12.72[a]
Error	1085.26	301	3.6055	

[a] $P < 0.01$

ml O_2/m^2 per hr for each degree centigrade increase in temperature from 5 to 15°C. The unusual temperature effect on chemical oxidation rates may be attributed to the fact that the chemical reactions take place within the sediment and are controlled by diffusion as well.

The seasonal cycle is similar to that of total uptake, increasing from January through April to a maximum in July and decreasing through October to a minimum in January. The seasonal cycle is likewise enhanced by the temperature cycle (Figs. 9 and 10).

The significant differences between stations will be discussed in connection with the concentration of reduced substances in the sediment.

RESPIRATION

The greater portion of variability in total oxygen uptake is obviously variability in respiration. The rate versus temperature curves of the different stations are even more variable than those of total uptake. The analysis of variance for respiration could have been done on the actual differences between measured total uptake and its corresponding chemical oxidation. Instead, since there were many values of total uptake without corresponding values of chemical oxidation, the analysis of variance was performed on the differences between actual total uptake and predicted chemical oxidation corresponding to that station, temperature, and season. This is justified by the accuracy of the predicted values of chemical oxidation. The analysis of variance was done on log-transformed data since their standard deviations have a significant regression on the means, with the following equation:

$$\text{standard deviation} = (0.211 \times \text{mean}) + 0.877$$

The analysis of variance showed significant differences between stations, seasons, and temperatures. The seasonal trend is the same as that shown by both total uptake and chemical oxidation. The seasonal cycle of in situ respiration is shown as the difference between the total uptake and chemical oxidation curves (Figs. 9 and 10). There is a highly significant correlation between respiration and inorganic chemical oxidation at 5°C ($r = 0.34$, d.f. = 34), 10°C ($r = 0.47$; d.f. = 74), and 15°C ($r = 0.56$; d.f. = 43). However, there were certain cruises whose results (grouped separately into the three temperatures) were not significantly correlated.

The much greater variability in respiration suggests that possibly the 5.7-cm corer is less than the optimum size for sampling the aerobic organisms. Since the 27-cm diameter bell jars do not show any less variability in their estimates of oxygen consumption than the cores, it is presumed that the two methods have different sources of variability (Pamatmat 1971).

OXYGEN UPTAKE AT OTHER STATIONS

Ten more stations (stations 24-33, Fig. 4) were studied seasonally in 1971-72, but all measurements were done at 10°C only. The data are presented in Table 5 while dissolved oxygen, temperature, salinity, total carbon, and sand fraction are given in Table 6. They clearly show a seasonal cycle except that stations 29 and 30 in the Snohomish river remained high in oxygen uptake from September to February and showed a sharp drop in metabolic activity from February to July, while station 27 at the mouth of the Puyallup river showed a dramatic increase from February to July 1972 to an average value much greater than that of July 1971. None of the environmental parameters appear to explain these changes (see HUMIC ACID CONTENT OF SEDIMENTS).

Whereas most stations with soft sediment show that aerobic respiration is smaller, sometimes much smaller, than the rate of chemical oxygen uptake, station 29 indicates that sandy sediment in shallow water that is washed by currents have relatively much higher aerobic respiration and correspondingly low concentrations of reduced substances. In inland and estuarine waters, however, this will be the exception. They are to be observed in shallow coastal areas exposed to surf and tidal currents (Smith 1970; Smith et al. 1971).

DIRECT MICROCALORIMETRY

The results of the electrical calibration of the calorimeter at 10 and 20°C is shown in Fig. 12. The thermal emf is proportional to power or rate of heat production. Furthermore, the calibration factor of 0.62×10^{-6} cal/sec per microvolt is independent of temperature.

At low rates of heat production the high sensitivity of the calorimeter was not enough to produce reliable results at all times. It is subject to baseline instability as it is affected by erratic changes in room temperature. The environmental temperature control system was inadequate in insulating the calorimeter from ambient temperature drifts of more than 2 or 3°C. Under this condition, the calorimeter exhibited long-term baseline fluctuations of as much as ± 10 microvolts. Furthermore, although an empty calorimeter under constant temperature should show zero emf, the present calorimeter in its present set-up always showed a baseline above zero, anywhere from a few tens to a few hundreds of microvolts.

The calorimeter was designed to measure low but steady levels of metabolic activity. The original plan was to place a whole sediment core (5.7 cm in diameter and up to 30 cm long) in its original coring tube. It soon became evident that such a large thermal mass unduly prolonged equilibration time to an impractical number of days. In short, the instrument could not be used for the purpose that it was designed.

Table 5. RATES OF TOTAL OXYGEN UPTAKE, INORGANIC CHEMICAL OXIDATION, AND RESPIRATION (ml/m^2 per hr) AT STATIONS 24 TO 33 IN PUGET SOUND.

Station		July	September	February	July
24	Total Uptake	14.5, 12.4, 14.8, 10.3, 15.1		14.2, 7.9, 8.0, 13.9	16.7, 15.5, 12.2, 13.5
	Chemical oxidation	7.0		9.1, 7.0	6.8, 7.7
	Respiration	7.5		5.1, 1.0	9.9, 5.8
25	Total uptake	21.2, 23.4, 20.3, 13.4, 12.5		14.5, 14.6, 12.8 15.0	13.4, 14.3, 15.8 14.4
	Chemical oxidation	10.3		6.1, 9.8	7.7, 7.0
	Respiration	10.9		8.4, 5.2	5.7, 8.8
26	Total uptake	42.2, 44.8, 59.5, 38.1, 41.3		25.9, 33.2, 23.3, 26.4	47.4, 31.7, 22.2
	Chemical oxidation	19.4		15.7, 13.1	22.2
	Respiration	22.8		10.2, 10.2	9.5
27	Total uptake	13.7, 11.8, 13.4, 11.5		11.5, 12.1, 11.7 13.8	56.5, 37.7, 48.6 31.0
	Chemical oxidation	7.4		6.6, 5.8	18.1, 15.3
	Respiration	6.0		5.5, 5.9	38.4, 22.4
28	Total uptake	16.7, 13.9, 21.2, 22.2		10.1, 8.7, 7.5, 5.6	16.8, 16.0, 15.8 17.6
	Chemical oxidation	9.4, 9.1		5.3, 3.4	11.2
	Respiration	7.3, 13.1		3.4, 4.1	5.6

Table 5. (continued) RATES OF TOTAL OXYGEN UPTAKE, INORGANIC CHEMICAL OXIDATION, AND RESPIRATION (ml/m^2 per hr) AT STATIONS 24 TO 33 IN PUGET SOUND.

Station		July	September	February	July
29	Total uptake		61.2,49.4,48.2	58.2,86.5,57.9 61.7,	29.6,37.9,31.1
	Chemical oxidation		12.3,15.2	5.0,15.6	10.3,16.0
	Respiration		48.9,34.2	52.9,46.1	19.3,21.9
30	Total uptake		23.0,23.0	28.2,30.5,39.5 30.6	6.9,5.7,8.9 7.7
	Chemical oxidation		19.4	7.5,6.9	5.5
	Respiration		3.6	23.0,32.6	0.2
31	Total uptake		7.2,10.3,7.7	8.1,16.5,13.7 5.9	8.6,6.5,6.0 6.4
	Chemical oxidation		7.6	7.9.9.8	3.8
	Respiration		0	8.6,3.9	2.2
32	Total uptake		11.6,8.6	7.0,8.5,7.7,4.9	13.4,14.1,10.5 15.0
	Chemical oxidation		5.4	5.9,6.6	4.0,4.1
	Respiration		6.2	2.6,1.1	6.5,10.9
33	Total uptake		13.2,12.9,7.6	10.8,12.2,9.9 14.1,	13.3,12.3,13.6 14.5
	Chemical oxidation		8.6	7.8,10.6	8.1,7.8
	Respiration		4.6	4.4,3.5	4.2,6.7

Table 6. DISSOLVED OXYGEN (ml/liter) TEMPERATURE (°C), SALINITY (0/00) TOTAL CARBON (%) AND SAND FRACTION (%), AT STATIONS 24 TO 33 IN PUGET SOUND.

Station		July	September	February	July
24	Dissolved oxygen	6.15		5.50	5.50
	Temperature	10.18		6.91	10.53
	Salinity	28.34		29.06	28.38
	Total carbon	1.60		0.89	1.80
	Sand fraction	37		59	35
25	Dissolved oxygen	4.90		6.00	4.45
	Temperature	9.47		6.70	9.33
	Salinity	29.88		29.66	29.66
	Total carbon	2.15		2.54	2.19
	Sand fraction	15		14	21
26	Dissolved oxygen	5.60		7.40	5.20
	Temperature	9.74		7.12	9.99
	Salinity	29.15		29.31	28.91
	Total carbon	0.97		1.59	0.96
	Sand fraction	57		47	66
27	Dissolved oxygen	4.80		6.80	4.55
	Temperature	8.97		7.36	9.16
	Salinity	29.85		29.76	29.58
	Total carbon	1.91		0.99	1.32
	Sand fraction	15		20	17

Table 6. (continued) DISSOLVED OXYGEN (ml/liter) TEMPERATURE (^{o}C), SALINITY (0/00) TOTAL CARBON (%) AND SAND FRACTION (%), AT STATIONS 24 TO 33 IN PUGET SOUND.

Station		July	September	February	July
28	Dissolved oxygen			8.65	5.75
	Temperature	12.18		6.52	11.06
	Salinity	28.58		28.01	26.86
	Total carbon	0.03		0.45	0.41
	Sand fraction	96			53
29	Dissolved oxygen		4.35	8.90	7.00
	Temperature		13.59	3.90	13.16
	Salinity		20.96	2.12	11.54
	Total carbon		0.85	1.16	1.52
	Sand fraction			85	63
30	Dissolved oxygen		5.55	6.15	8.00
	Temperature		14.04	3.74	10.76
	Salinity		6.00	0	0
	Total carbon			0.29	0.14
	Sand fraction			96	100
31	Dissolved oxygen		2.50	3.10	3.80
	Temperature		8.66	9.14	8.28
	Salinity		29.99	30.30	29.82
	Total carbon		2.99	3.95	2.67
	Sand fraction			5	3

Table 6. (continued) DISSOLVED OXYGEN (ml/liter) TEMPERATURE (°C), SALINITY (0/00) TOTAL CARBON (%) AND SAND FRACTION (%), AT STATIONS 24 TO 33 IN PUGET SOUND.

Station		July	September	February	July
32	Dissolved oxygen		0.90	3.15	3.50
	Temperature		8.97	9.49	7.76
	Salinity		29.85	30.34	29.35
	Total carbon			2.19	2.25
	Sand fraction			4	3
33	Dissolved oxygen		0.70	2.85	2.60
	Temperature		8.53	9.14	8.34
	Salinity		28.48	29.80	28.66
	Total carbon			3.94	3.88
	Sand fraction			9	10

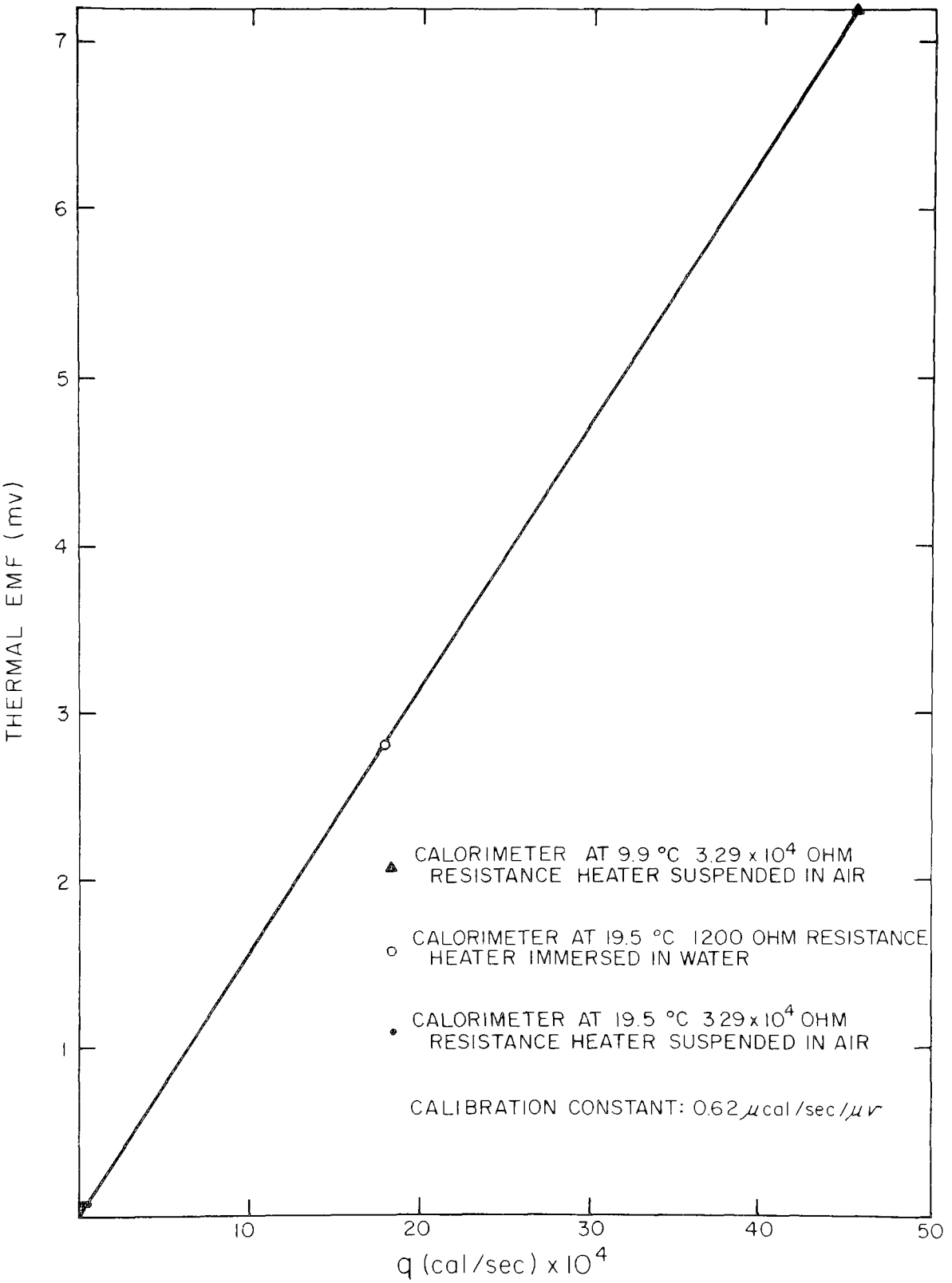

Figure 12. Calibration line for the microcalorimeter at 9.9°C and 19.5°C.

The most reliable measurement of metabolic heat release is obtained with small samples suspended in the middle by a thin monofilament and which may be left inside until the signal emf is steady. This takes about 12 hr. Then the sample is quickly removed. The empty calorimeter re-stabilizes much more rapidly (its signal is within 5% of the total change in 3 hr) and the decrease in thermal emf following removal of the sample represents the rate of metabolic heat release.

DEHYDROGENASE ACTIVITY OF PUGET SOUND SEDIMENTS

Any dehydrogenase assay technique by itself yields only relative measures of metabolic activity. The results from Puget Sound (Table 2, Appendix) merely indicate that there is fairly active anaerobic metabolism in station 33, the level of activity is generally detectable in most places, and very low or not significantly different from zero in some.

Other tests performed with Puget Sound sediment at 37°C indicated that aerating, grinding, autoclaving, and treating the sediment sample with lysozyme resulted in a reduction or complete cessation of apparent metabolic activity. The effect of aeration points to a deleterious effect of oxygen and therefore the apparent activity without aeration must be anaerobic. The effect of grinding, autoclaving, and addition of lysozyme means that the production of formazan must be the result of live metabolic activity and not of a chemical reaction.

The results of the assay at 37°C may or may not be correlated with the natural activity at much lower temperatures, i.e., the enhancement of activity at 37°C may or may not be in direct proportion to the natural activity. The thermal shock of a sudden temperature change of 20 or 30°C is difficult to assess. Hence, incubation of samples at or close to in situ temperatures would be desirable. Puget Sound samples incubated at 10°C with glucose for as long as 6 hr, however, did not produce any detectable formazan.

DEHYDROGENASE ACTIVITY OF LAKE WASHINGTON SEDIMENTS

The following results were obtained with Lake Washington sediments during the course of our investigation to increase the sensitivity of the assay so that positive results may be obtained at 10°C.

Selection of Substrate - Besides glucose, we tried citrate, malate, lactate, succinate, and pyruvate. Sodium citrate gave the highest activity during a 3-hr incubation at 10°C (Table 7); glucose yielded on the average only 39% of the activity due to citrate. This is in contrast to the findings of Lenhard et al. (1965) that glucose was more effective than the sodium salts of lactic, citric, succinic, and glutamic acids. Evidently there is a question

Table 7. RELATIVE LEVELS OF DEHYDROGENASE ACTIVITY RESULTING FROM THE USE OF THE SAME CONCENTRATION OF DIFFERENT SUBSTRATES ADDED TO REPLICATE SEDIMENT SAMPLES FROM LAKE WASHINGTON

Substrate	Dehydrogenase activity (absorbance/gram dry sediment)	
	Replicates	Average
Sodium citrate	1.17, 1.18, 1.23	1.19
Sodium malate	0.89, 0.89, 0.93	0.90
Sodium lactate	0.66, 0.77, 0.70	0.71
Sodium succinate	0.57, 0.57, 0.58	0.57
Sodium pyruvate	0.01, 0.02, 0.01	0.01
Glucose	0.45, 0.46, 0.48	0.46

of dehydrogenase specificity, which in Lake Washington sediments differs from that in activated sludge.

Dehydrogenase activity increases with increasing concentration of sodium citrate, reaching a peak at 0.15 M and declining at a higher concentration (Fig. 13). The effect of additional substrate appears to be to enhance or amplify the activity in direct proportion to the natural dehydrogenase activity. Fig. 14 shows that at a final concentration of 0.04 M the measured activity is on the average 5.3 times that of replicate samples without any substrate, while at 0.20 M it is 1.4 times the activity at 0.04 M.

Sodium citrate has an unexplained effect on the absorbance of the $HgCl_2$-treated blanks (Fig. 15). The blanks with 0.04 M citrate have about 43% higher absorbance than replicate blanks with 0.20 M citrate or without substrate, and there appears to be no difference between the two latter treatment replicates.

Amount of Sediment - The measured activity increases but the increment of activity decreases with increasing amount of sediment (Fig. 16), which may be an indication of nutrient limitation with increasing amount of sediment. Therefore, normalizing the result to unit weight of dry sediment is computationally inadequate. To minimize error, we have tried using as close to 0.3 g dry sediment as possible but the final weights still varied from 0.1 to 0.5 g; this wide range may be a source of variability in the final results. Obviously the amount of sediment should be limited to a quantity within which the activity is proportional to quantity. If the problem is caused by nutrient limitation, it could be solved by periodic stirring of the sediment during incubation, provided that this does not oxygenate the sample.

Exclusion of Oxygen - The presence of oxygen causes an underestimate of dehydrogenase activity. We have tried guarding against the presence of oxygen during incubation by flushing and sealing the incubation flask with nitrogen. We have not detected a significant difference between nitrogen-flushed and unflushed replicates. Evidently the concentration of reduced substances in the sediment is enough to consume oxygen in solution and keep the settled sediment anoxic during the course of the reaction. The formation of formazan itself is prima facie evidence of anaerobic metabolism; no dehydrogenase activity is detected when the sample is aerated.

Blanks - Lenhard et al. (1965) and others have used as their blanks the reaction mixture minus TTC. We were concerned, however, with the possibility of a chemical reduction of TTC since sometimes we are dealing with highly reduced sediments. Although Casida et al. (1964) have shown that chemical reduction of TTC appears to occur only at much higher temperatures (above 65°C), we think that an appropriate blank is one that contains TTC

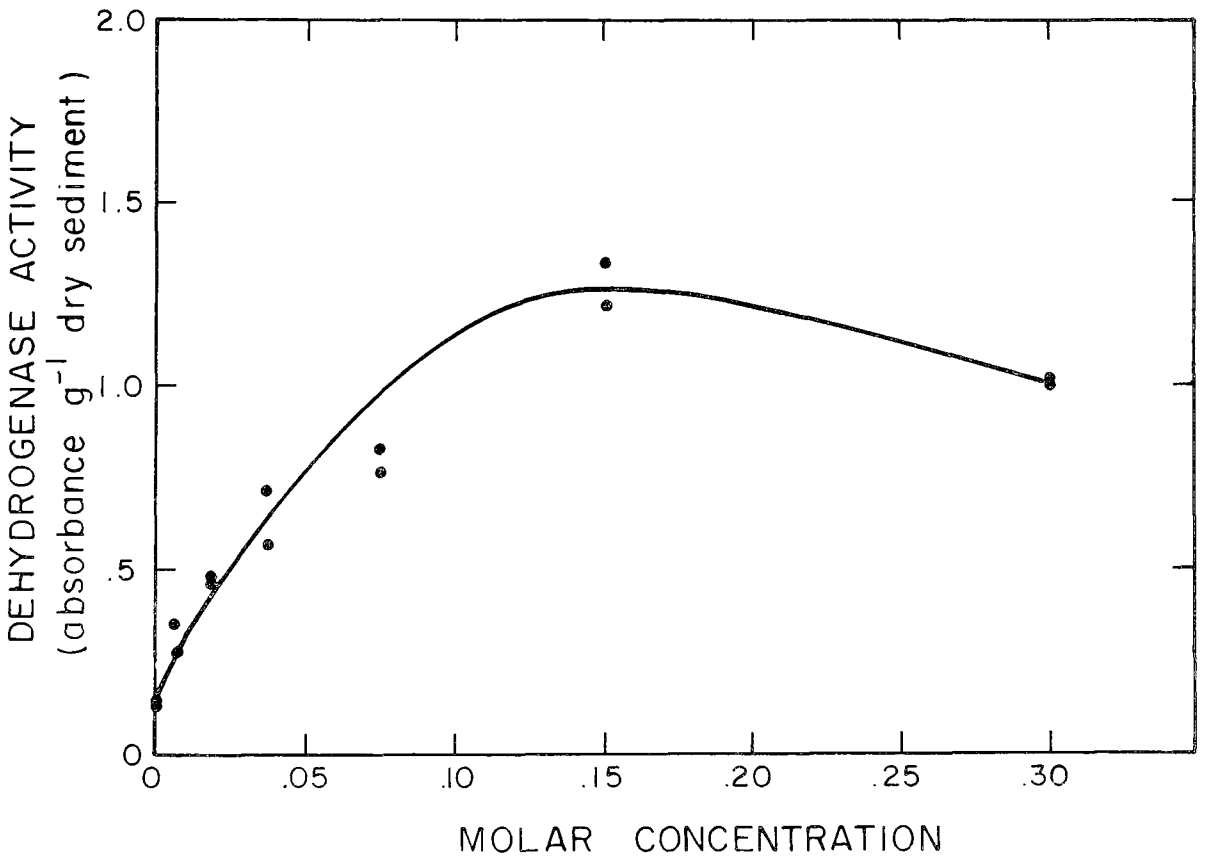

Figure 13. Dehydrogenase activity as a function of sodium citrate concentration.

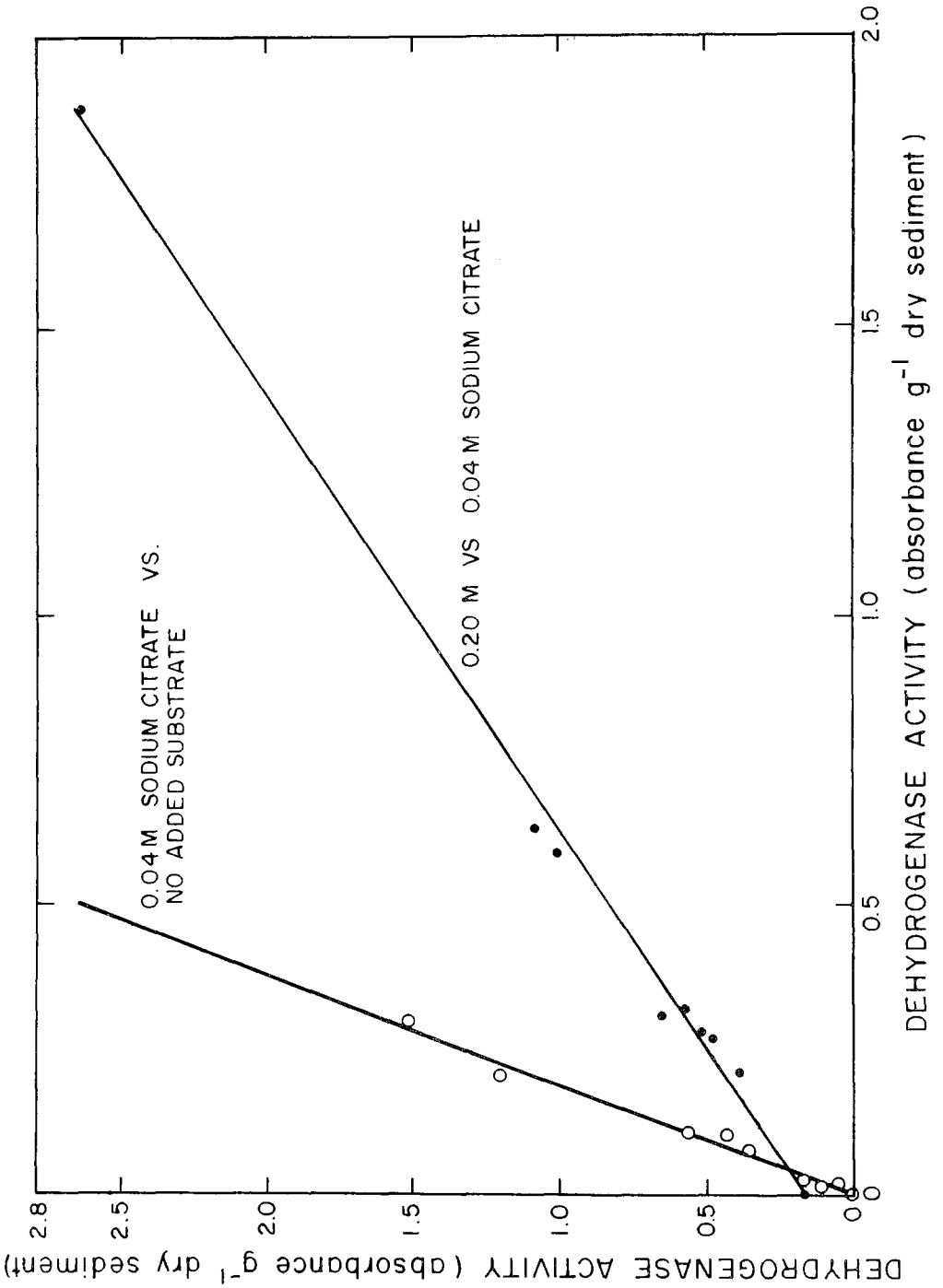

Figure 14. Dehydrogenase activity at 0.04 M sodium citrate as compared to dehydrogenase activities at 0.20 M sodium citrate and without added substrate.

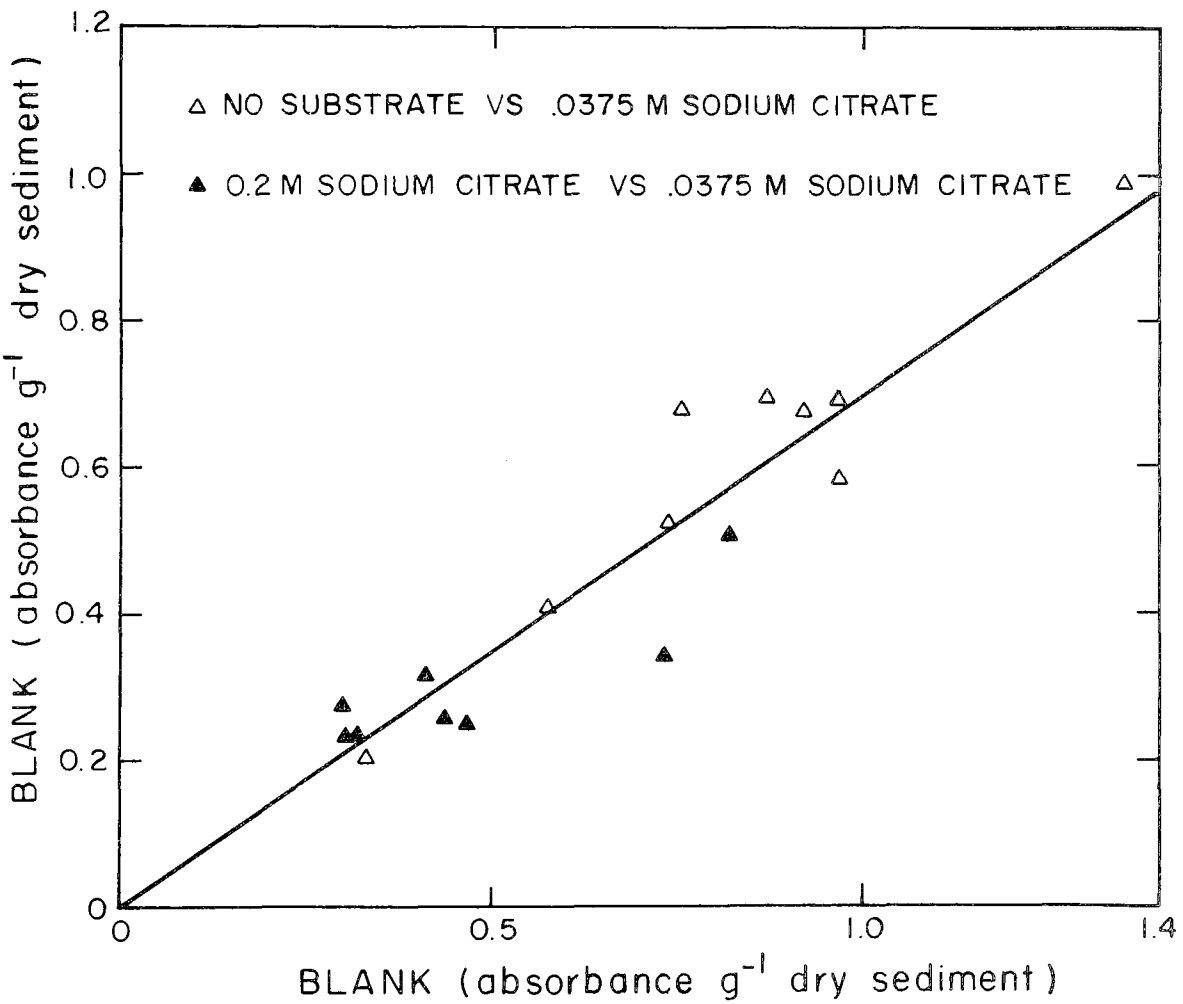

Figure 15. Effect of substrate concentration on the absorbance of mercuric-chloride-treated blanks.

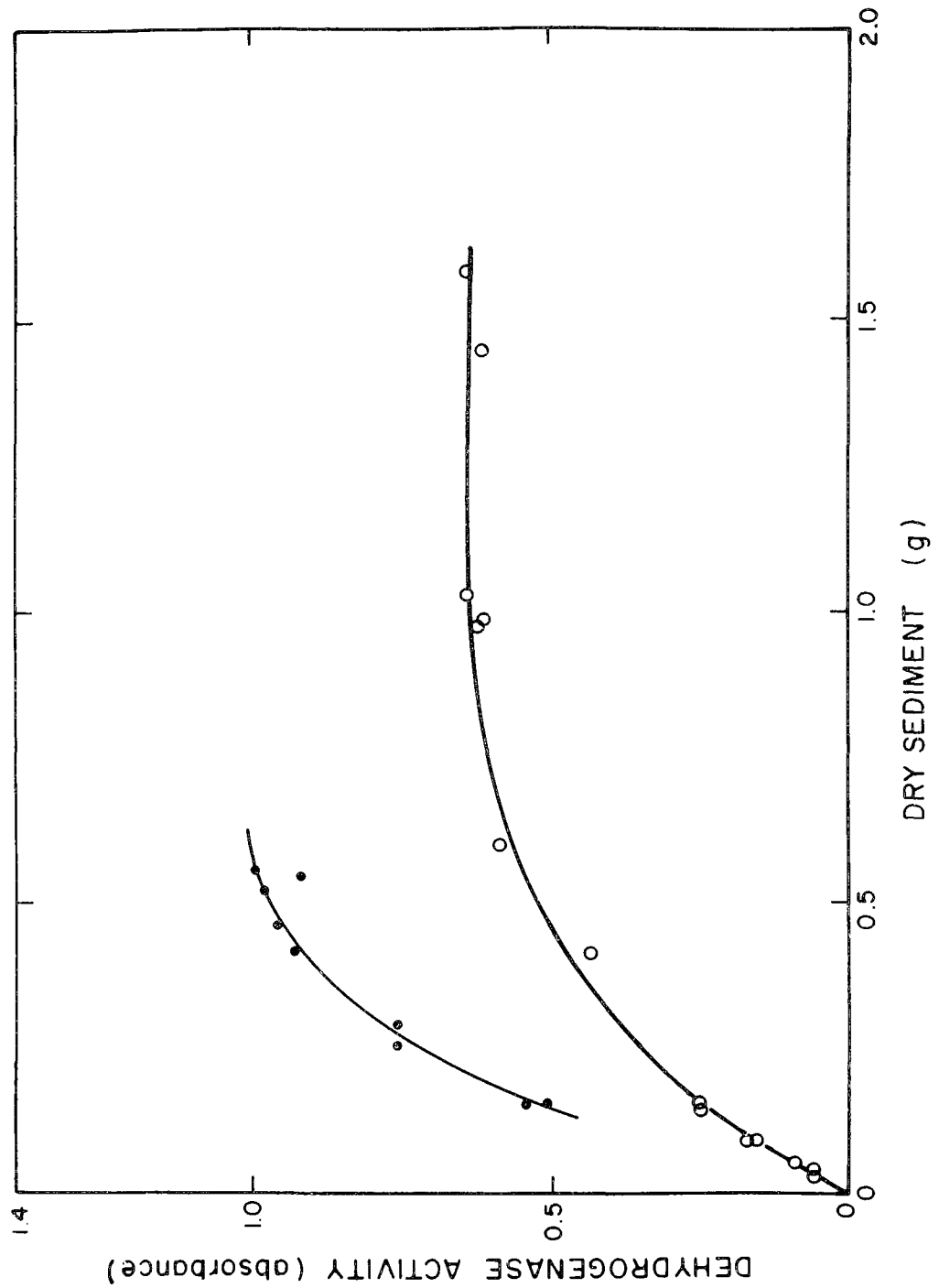

Figure 16. Dehydrogenase activity as a function of amount of sediment of two different levels of metabolic activity.

but has been poisoned to kill any dehydrogenase activity. Mercuric chloride has been shown to be the most effective agent in stopping dehydrogenase activity (Lenhard 1965). We obtained the same blanks with $HgCl_2$-treated and autoclaved samples.

MICROCALORIMETRIC CALIBRATION OF DEHYDROGENASE ACTIVITY

The significant regression of dehydrogenase activity on the actual rate of metabolic heat release (Fig. 17) signifies a fairly high degree of functional relationship between the two measures of community metabolism. The sediment samples from Lake Washington had metabolic rates of 3-17mcal g^{-1} hr^{-1}, corresponding with relative dehydrogenase activities giving absorbances of 0.27-0.86 g^{-1}. In this range, the regression of dehydrogenase activity on metabolic heat release is

$$\underline{Y} = 0.037 + 0.046\,\underline{X}$$

where $\underline{Y}$ is dehydrogenase activity in absorbance units per gram and $\underline{X}$ is metabolic heat release in millicalories per gram per hour. The regression coefficient is significant at $P < 0.001$ with 95% confidence limits of 0.039-0.053. The $\underline{Y}$-intercept is not significantly different from zero; this may signify that purely chemical exothermic side reactions in the sediment, e.g. neutralization of organic acids (Forrest et al. 1961) are negligible.

To obtain samples with even higher rates of metabolic heat release, two sediment samples from Lake Washington were treated with dried ground zooplankton and glucose and allowed to ferment for two days. These samples were then placed in culture tubes for calorimetry measurements. After their removal from the calorimeter, their dehydrogenase activity was immediately assayed. The regression line with these samples included with the untreated samples is given by the equation

$$\underline{Y} = 0.166 + 0.030\,\underline{X}$$

The regression is significant at $P < 0.001$ with 95% confidence limits of 0.025-0.035. The $\underline{Y}$-intercept is significantly greater than zero. Furthermore, there is a significant difference between the slopes of the two regressions. The difference between the two regressions may be attributed to the effect of the added substrate and consequent increase in exogenous metabolism. When removed from the calorimeter the culture tubes containing the treated samples were under internal pressure due to the liberated gaseous fermentation products. The bacterial populations in the treated samples may have been in a logarithmic phase of growth whereas those in the untreated samples may have been closer to steady state. Although both regressions indicate that the enzyme activity measured in 3 hr is proportional to the

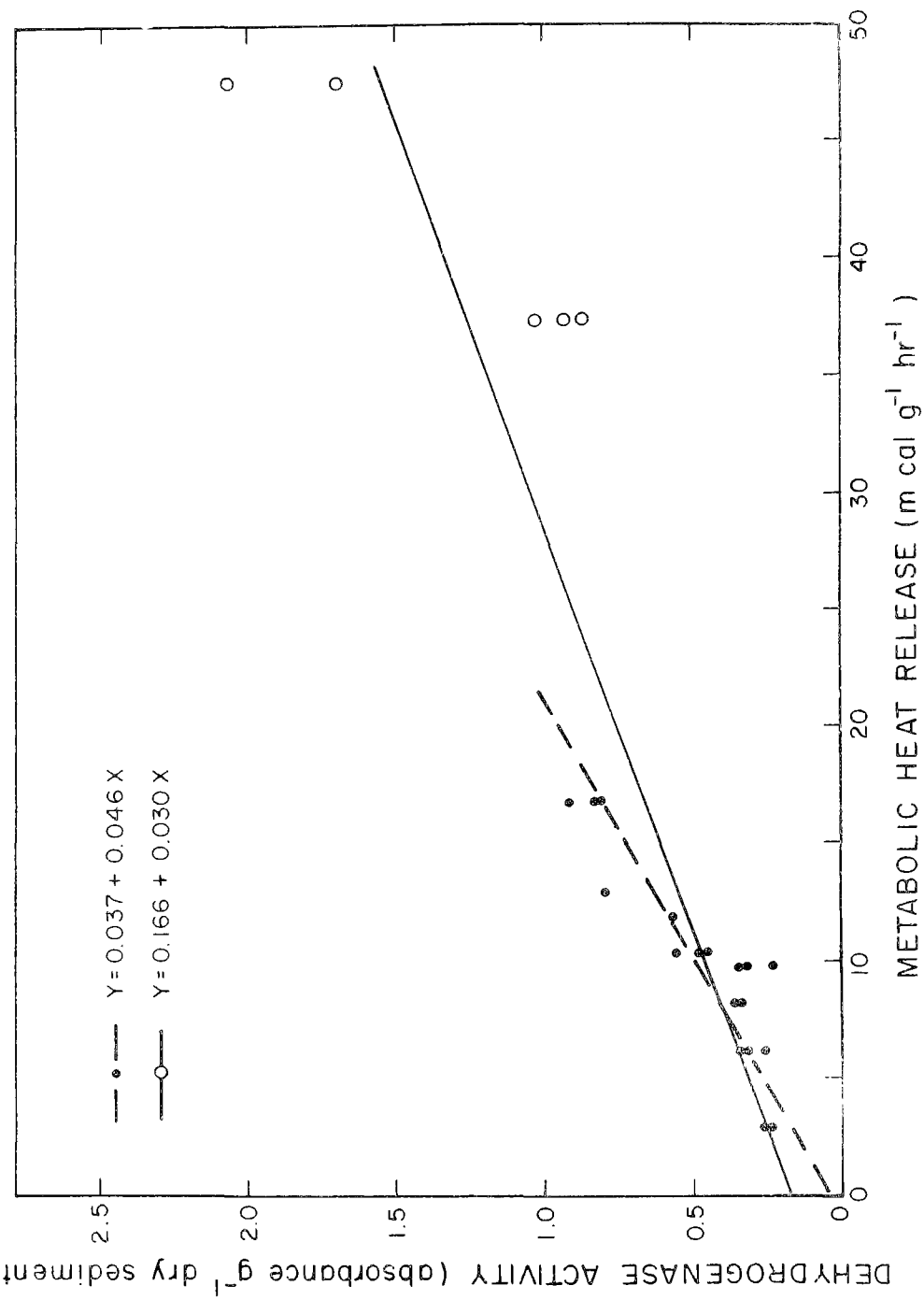

Figure 17. Dehydrogenase activity as a function of the rate of metabolic heat release.

undisturbed rate of anaerobic heat release by the microbial community, it appears that the relationship between dehydrogenase activity and metabolic heat release may vary according to the microbial population's growth phase.

The first regression equation was used to estimate the natural anaerobic metabolism of sediments in Lake Washington.

DISTRIBUTION OF DEHYDROGENASE ACTIVITY IN LAKE WASHINGTON

The relative measures of dehydrogenase activity at the six stations are shown in Fig. 18. The core from station 2 was as long as those from the other stations but was assayed to 5 cm only. There is a trend of decreasing activity per unit weight of sediment with increasing depth of sediment layer. There are significant differences between stations and between layers at each station. There is only a poor correlation between dehydrogenase activity and percentage carbon of the sediment ($r = 0.42$, d.f. = 16). It is interesting that when the sediment samples to which zooplankton and glucose had been added are included in the correlation test, r worsened to -0.21 with d.f. = 18. This suggests that when an amount of readily oxidizable organic matter (although insignificant relative to the large amount of old organic matter) becomes available there is a rapid increase in dehydrogenase activity.

There is a highly significant correlation between dehydrogenase activity and ultimate oxygen demand of the sediments (Lenhard et al. 1962; Edwards and Rolley 1965). Ford et al. (1966) and Stevenson (1959) observed good correlations between dehydrogenase activity and the oxygen consumption of replicate samples, which indicate that the organisms were able to use either oxygen or TTC as hydrogen acceptors, though not necessarily with equal efficiency. On the other hand, Edwards and Rolley (1965) found no correlation between the rate of total oxygen consumption by intact sediment cores and dehydrogenase activity; it is not clear how the sediment samples were taken for dehydrogenase assay, but presumably they were from the top 2-cm layer of the sediment cores. The discrepancy between the findings of Edwards and Rolley (1965) and those of Ford et al. (1966) and Stevenson (1959) may be the result of differences in metabolic types of the samples or in the techniques by which oxygen uptake was measured.

In experiments with decomposition of flax residues in soils, Stevenson (1959) found that during the first three days there was an increase in enzyme activity that was not correlated with an increase in bacterial numbers; however, over the long run the change in enzyme activity paralleled the change in bacterial numbers. Casida et al. (1964) also noted that dehydrogenase activity increases with increase in total bacterial numbers in organically treated soils. When dehydrogenase activities of different untreated soils were analyzed, however, Stevenson found no relationship with total number

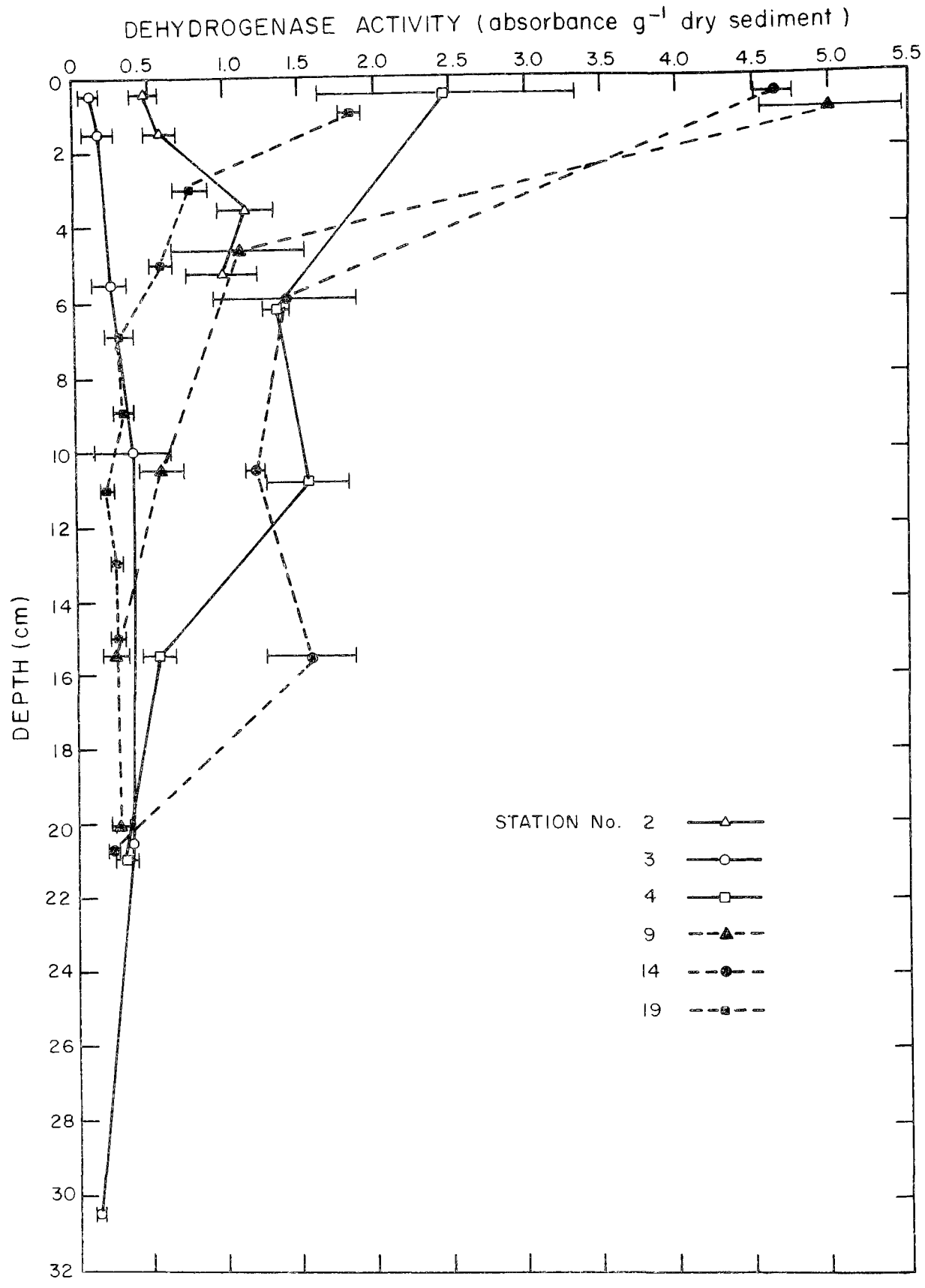

Figure 18. Vertical distribution of dehydrogenase activity at six stations in Lake Washington in October 1972. The horizontal bars denoting ± one standard deviation have been displaced where they would have overlapped.

of bacteria, and Casida et al. found no relationship with Gram-negative bacteria, fungi, or actinomycetes. Evidently different soils harbor microbial species of different metabolic types in sufficient variety to explain Stevenson's findings. The lack of correlation may have resulted more from differential success with different species in plate cultures than from inherent differences in dehydrogenase activities in different species.

METABOLIC HEAT RELEASE OF LAKE WASHINGTON SEDIMENTS

The relative measures of dehydrogenase activity at the six stations were converted to rates of metabolic heat release (in millicalories per gram per hour) according to the regression equation presented earlier. From the measured dry weight of each one-cm layer, units of millicalories per gram per hour were converted to millicalories per layer per hour (Fig. 19). The rate of total heat production by each core was obtained by integrating each curve.

As a result of compaction, sediment weight per layer increases with depth; hence, the actual contribution of deeper layers to total metabolism is greater than is indicated by the decreasing trend of activity per unit weight of sediment. Possibly activity per unit weight of sediment decreases with depth because of decreasing available metabolizable energy, but activity per layer is maintained somewhat because of increasing surface area with depth.

METABOLIC HEAT RELEASE VERSUS OXYGEN UPTAKE

The rates of total oxygen consumption by the intact cores and the residual oxygen uptake or abiotic chemical oxidation after poisoning of the overlying water were converted to rates of heat release by using the factor of 4.8 cal liberated per milliliter 0_2 uptake.

The bottom water temperatures at stations 2, 3, 4, 9, 14, and 19 were 8.2, 8.4, 8.3, 7.9, 8.0, and 13.3°C, respectively. Hence, the in situ metabolic activity may be expected to be slightly less at the first five stations and more at station 19 than the estimated activity at 10°C.

Table 8 shows the results of the two methods of estimating benthic community metabolism. Note that the estimated dehydrogenase activity of the core from station 2 would have been higher if the entire core had been assayed. The estimate by the dehydrogenase method may of may not include the metabolic activity of some aerobes. In the top 1-cm layer, for example, where aerobic microorganisms would be found in nature, their metabolism would be included if they were able to use TTC as hydrogen acceptor in the absence of oxygen. Rarely were Chironomus larvae seen in the sediment, but when they were present we do not know whether their metabolism was included in the enzyme assay.

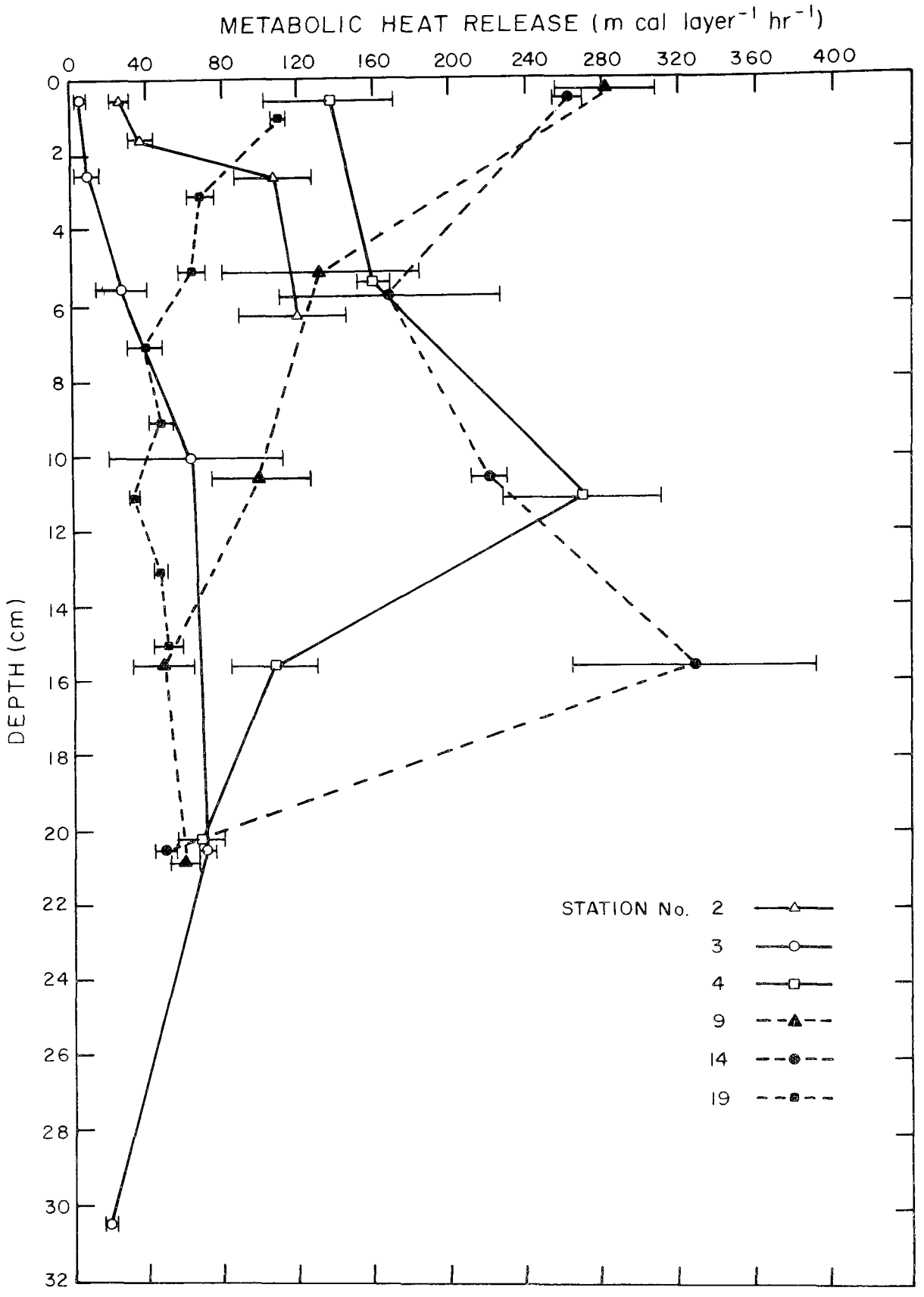

Figure 19. Vertical distribution of metabolic heat release at six stations in Lake Washington in October 1972. The horizontal bars denoting $\pm$ one standard deviation have been displaced where they would have overlapped.

Table 8. COMPARISONS OF DEHYDROGENASE ACTIVITY CONVERTED TO RATE OF METABOLIC HEAT RELEASE (IN CALORIES PER CORE PER HOUR) WITH TOTAL OXYGEN UPTAKE AND CHEMICAL OXIDATION CONVERTED TO METABOLIC HEAT RELEASE (IN CALORIES PER CORE PER HOUR) BY USING THE FACTOR 1 ml 0_2 = 4.8 cal

Station	Core length (cm)	Total 0_2 uptake	Chemical oxidation	Dehydrogenase activity	
				Range	Average
2	6	0.27, 0.34, 0.26, 0.16, 0.16	0.10, 0.11, 0.12	0.37-0.54	0.39
3	32	0.38, 0.42, 0.38, 0.31	0.24, 0.26	0.88-1.6	1.3
4	21	0.41, 0.45, 0.28, 0.33, 0.38	0.18, 0.18, 0.20	2.8-3.6	3.0
9	21	0.35, 0.28, 0.22, 0.19, 0.17	0.12, 0.13	1.7-2.8	2.1
14	21	0.45, 0.39, 0.36, 0.36, 0.32, 0.31, 0.27, 0.24, 0.19, 0.19	0.11, 0.08, 0.11, 0.10, 0.11	3.4-4.7	4.0
19	16	0.36, 0.36, 0.28, 0.25	0.21, 0.23, 0.24	0.39-0.46	0.41

With the exception of station 19, the estimate of benthic anaerobic metabolism by the dehydrogenase method greatly exceeds the estimate of benthic aerobic plus anaerobic metabolism by the total oxygen uptake and even more greatly exceeds the estimate of anaerobic metabolism by chemical oxygen consumption. Even if the estimate of anaerobic metabolism by the former method is reduced by the metabolism of the top 1-cm layer, which may have included some aerobic respiration, a large discrepancy would remain. The discrepancy would be larger still if possible anaerobic metabolism by macrofauna is included. It is clear that the rate of total oxygen uptake does not give an accurate estimate of the total benthic metabolism in the sediment column. It is equally clear that the rate of total oxygen uptake by the sediment surface is equivalent to the rate of dehydrogenase activity in a thinner upper layer.

We do not know if the week-long storage of the core from station 19 has anything to do with the closer agreement between the two methods for that core. This core was also shorter than the others except the one from station 2. The results from station 19, however, indicate that where there is a rapid decline in anaerobic metabolism with depth the rate of total oxygen uptake will be a close estimate of total metabolism in the sediment.

DISTRIBUTION OF TOTAL REDUCED SUBSTANCES

The concentration of reduced substances in the sediment at the first 23 stations in Puget Sound (determined iodometrically) increases linearly with the logarithm of depth of the sediment layer (Fig. 20). Off the coast of Oregon and Washington the concentration of reduced substances (determined by dichromate oxidation), expressed as oxygen debt (Fig. 21), also increases with depth. In Lake Washington, the concentration of reduced substances, also determined by dichromate oxidation, likewise increases with depth (Fig. 22) but only to about 11 to 16 cm and decreases below these layers.

The most reasonable explanation for increasing concentrations of reduced substances with depth is that these products of anaerobic metabolism are no longer effectively oxidized and begin to accumulate when buried under a thickening layer of surface deposit. There is no correlation between the concentration of reduced substances in sediment layers below 4 cm and the rate of oxygen uptake by chemical oxidation.

The concentration of reduced substances is not correlated with the organic carbon content of the sediment. There are, on the average, smaller concentrations in deepwater stations than in shallower stations (Fig. 20). Offshore, the abyssal plains sediments contain less reduced substances than the sandy shelf sediments although the latter contain smaller organic carbon. There is increasing concentration of reduced substances with increasing mud content which increases with increasing depth to the

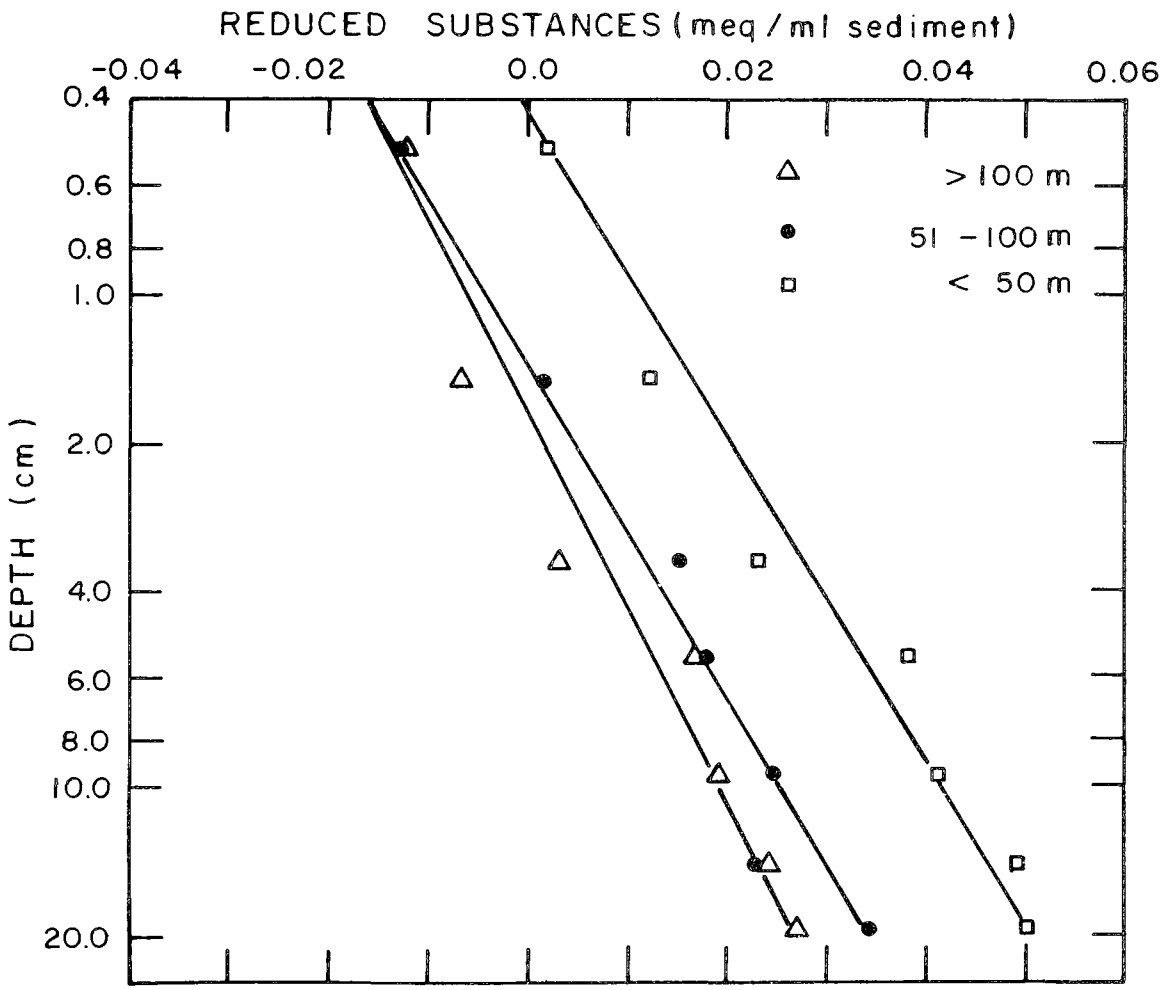

Figure 20. Concentration of reduced substances in Puget Sound versus depth of sediment layer.

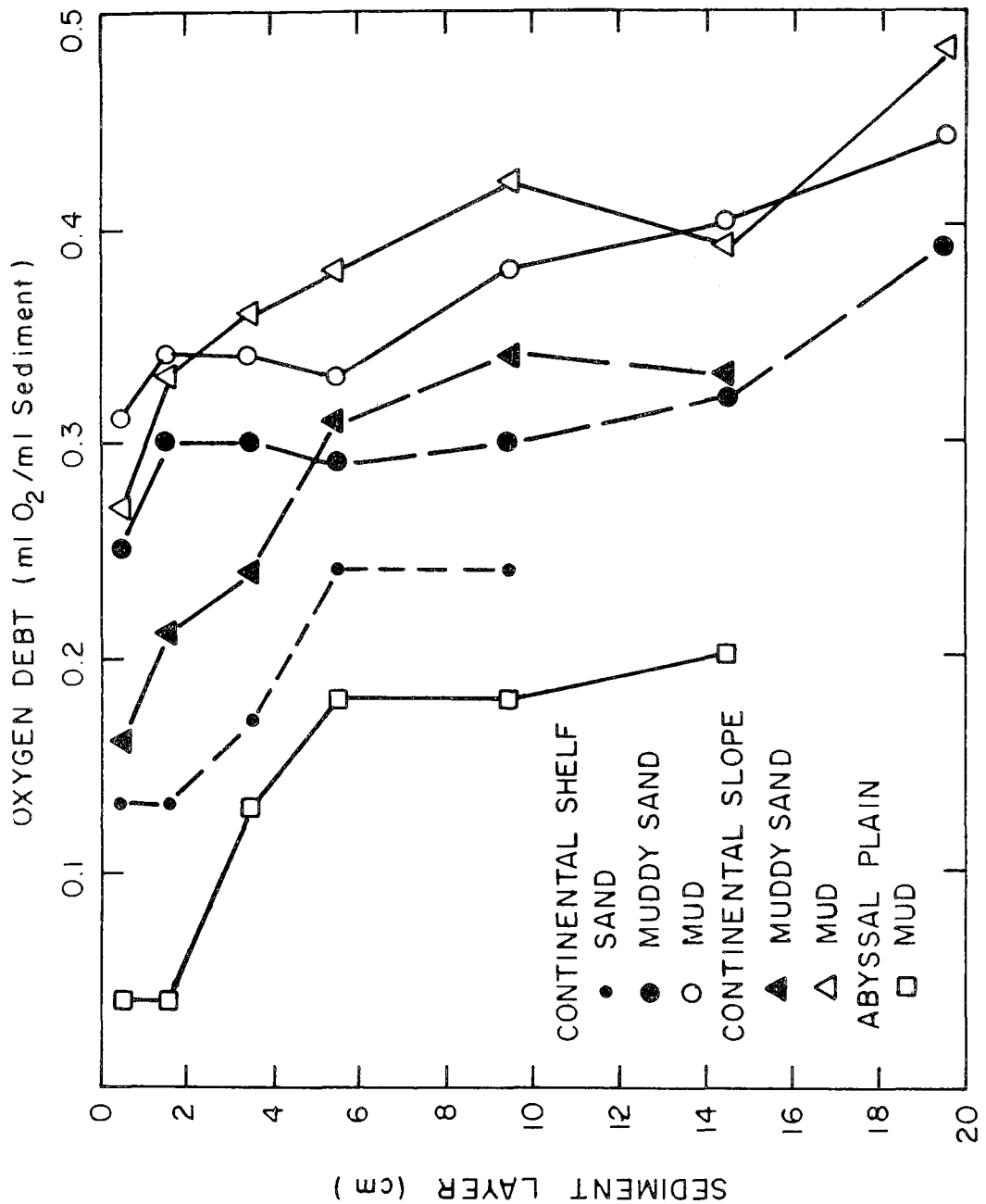

Figure 21. Concentration of reduced substances versus depth of sediment layer off the Oregon-Washington coast.

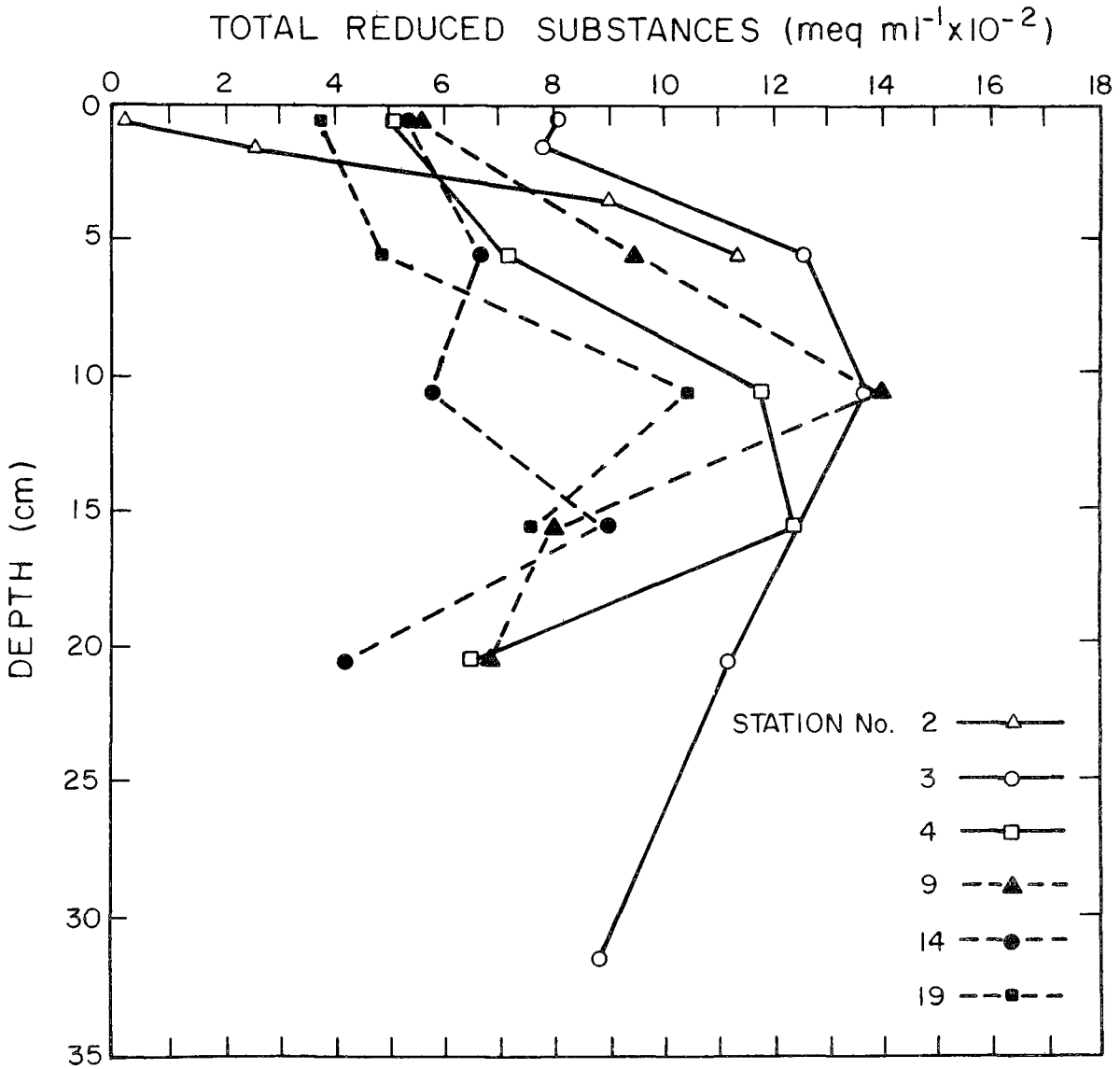

Figure 22. Vertical distribution of the concentration of reduced substances in Lake Washington sediments.

continental slope.

Lake Washington sediments contain higher concentrations of reduced substances than Puget Sound or offshore sediments; they also contain much more organic matter (Shapiro et al. 1971), presumably as a result of highly eutrophic conditions and the discharge of sewage, both raw and treated, over a period of four decades. The history of eutrophication of Lake Washington has been studied and well documented by Dr. W. T. Edmondson and his students (Edmondson 1969a, 1969b, 1970). Sediment below 11 cm which contains lower concentrations may have been deposited before the onset of the period of eutrophication of the lake. It is interesting that the concentration of organic matter, although quite variable, has a decreasing trend with sediment depth (Shapiro et al. 1971). The decrease may be attributed to long-term mineralization through anaerobic metabolism, which in turn produced the accumulated reduced end products.

The foregoing indicate that although the concentration of reduced substances might be expected to increase with greatly increasing organic matter content, it is not the concentration of total organic matter but the metabolizable fraction that determines the extent of anaerobic metabolism. Much of the organic matter in sediments is resistant to biochemical oxidation (Waksman, 1933) for perhaps various reasons which are not fully understood. Volkmann and Oppenheimer (1962), who studied the decomposability of organic carbon in different sediments of a shallow marine lagoon, found that organic matter in coarse sediments decomposed more readily than that in fine sediments. The difference in organic matter composition between the sediment types, and compaction and adsorptive capacity of clay minerals, were given as possible reasons. From our results there appears to be a greater fraction of refractory organic matter in deep-water sediment than in shallow-water sediment.

CHEMICAL OXIDATION VERSUS REDUCED SUBSTANCES

Whereas the concentration of reduced substances in deeper layers is not correlated with the rate of chemical oxidation, the concentration in the upper four centimeters is (Fig. 23). This relationship, together with the seasonal cycle of chemical oxidation (Figs. 9 and 10), indicates a dynamic equilibrium between anaerobic metabolism in the surface 4-cm layer, the resulting formation of reduced by-products, and their consequent chemical oxidation.

It appears then that reduced substances below about 4 cm are accumulating while in the upper 4 cm they may be approximating steady-state condition, except when the flux of fresh, more readily oxidizable organic matter increases during the summer; then, anaerobic metabolism in the immediate subsurface layers increases, resulting in higher concentrations of reduced

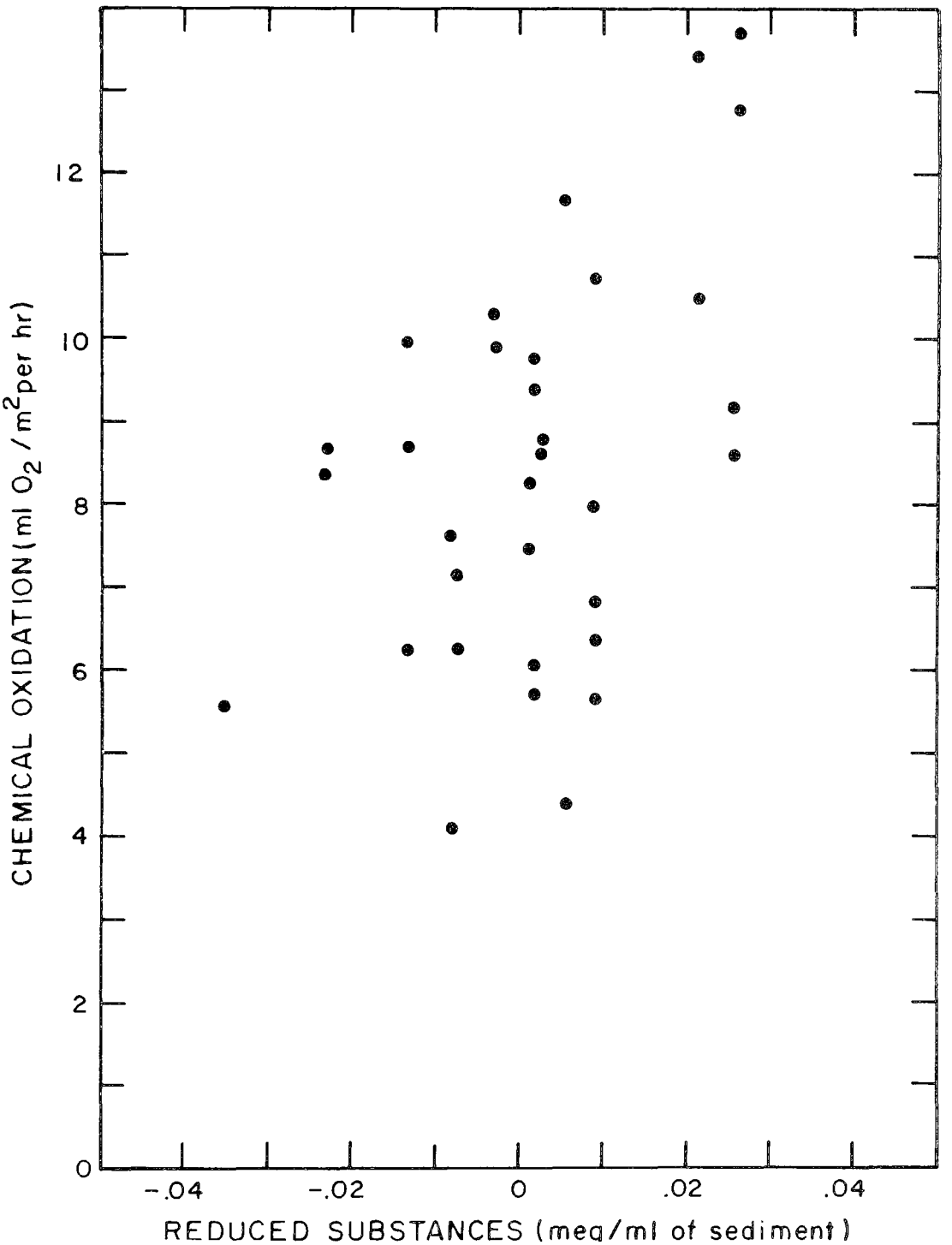

Figure 23. Rates of inorganic chemical oxidation of undisturbed sediment cores versus average concentration of reduced substances in the 0-1, 1-2, and 3-4 cm layers. The correlation was significant between rate of uptake and concentration of reduced substances in any of the three layers, but rate of chemical oxidation did not correlate with concentration of reduced substances in the deeper layers.

substances, and therefore in higher rates of chemical oxygen consumption.

DEHYDROGENASE ACTIVITY VERSUS REDUCED SUBSTANCES

The absorbance of duplicate $HgCl_2$-treated blanks shows little variability. The coefficient of variation is usually less than 5% but occasional large coefficients of up to 20% raised the average coefficient to 5.2%. There is no correlation between the absorbance of the blanks and the concentration of reduced substances ($r = 0.043$, d.f. = 23); hence reduced substances apparently did not reduce TTC to formazan. The absorbance of the blank is probably largely due to phytoplankton pigments and their degradation products as evidenced by the greenish-yellow color of the ethanol extract.

The absorbance of triplicate reaction mixtures shows greater variability than that of the blanks, with an average coefficient of variation of 19%. As explained previously, part of the variability may have been due to differences in sediment weights in replicate samples, although occasional large variations within replicates could not be explained by this cause. There is a significant increase in the variance with increasing level of dehydrogenase activity ($r = 0.52$, d.f. = 31).

There is no correlation between dehydrogenase activity and concentration of reduced substances ($r = 0.024$, d.f. = 23). Effenberger (1966), however, noted a negative correlation between dehydrogenase activity and the redox potential of activated sludge. As the E_h decreased from about +190 to +50 mv the dehydrogenase activity (expressed as relative units) increased from about 120 to 280. Since the effect of anaerobic metabolism is known to be a decrease in E_h of the medium, the lower the E_h (in this experiment approaching some steady-state condition) the greater the metabolic activity and hence the higher the measured dehydrogenase activity. This may seem to contradict our result, indicating no significant correlation between dehydrogenase activity and concentration of reduced substances in Lake Washington. The discrepancy is explained by the fact that the concentration of reduced substances in the sediment is a function of time as well. Deeper sediments have been anaerobic for a longer time than surface sediments and are no longer being oxidized by dissolved oxygen diffusing into the sediment; while surface sediments, especially when being turned over by macrofauna, are still periodically aerobic. Under steady-state conditions, and where no other factors such as differences in sediment compaction and oxygen tension of the overlying water prevail, one might expect a correlation between the concentration of reduced substances in the surface layer and its dehydrogenase activity.

HUMIC ACID CONTENT OF SEDIMENTS

The humic acid content of samples from various stations has been measured in

relative units only, namely, absorbance per gram of dry sediment (Table 9). Assuming that humic acid solution conforms to Beer's law, the ratio of this absorbance unit to per cent organic carbon in the different samples represents a relative measure of the total organic carbon proportion of humic acid in the various samples. The ratios vary by a factor of about 6 at most, e.g. the organic matter in station 29 in Snohomish River consists of proportionately little humic acid as compared to station 1 in Lake Washington. The latter station contains much more organic matter than station 29 and therefore also contains greater absolute quantities of both humic substances and oxidizable carbon.

It may be significant that the three samples that showed the smallest ratios were from stations 29 and 30 in Snohomish River and from Clam Bay, which all showed unusually high rates of oxygen consumption that winter (Table 9). Their rates of oxygen consumption declined the following July (when Puget Sound stations normally exhibit a rise in benthic metabolism) when the sediment smaples showed proportionately greater content of humic acid. With the exception of these three stations, the others showed only slight differences between February and July and between stations. Furthermore, there appears to be only slight and inconsistent differences between the 0-1 and 5-6 cm layers of sediment in Puget Sound.

Recent sediments from various geographical areas contain widely different proportions (4-68%) of organic carbon as humic substances (Nissenbaum and Kaplan, 1972). How these varying proportions of humic acids affect benthic community metabolism is further complicated by the evidence that humic substances originate in situ as well as from terrestrial sources (op. cit.) In any case, our results point to a need for more thorough investigation of humic acids in connection with research on benthic community metabolism. It would also be desirable to establish the quantitative relationship between absorbance and the concentration of humic acid in solution.

EFFECT OF TIDAL CURRENTS ON BENTHIC OXYGEN UPTAKE

The drop in oxygen concentration of bottom water during ebb tide (Fig. 24) probably represents at least partly the effect of oxygen consumption by resuspended sediment, although part of it could have been due to advection. The horizontal mass movement of surface sediment by tidal current at the same location has been observed on other occasions.

Just how important resuspension of sediment is in the total benthic oxygen uptake in Puget Sound is difficult to say until we know how widespread this type of periodic distrubance is. The oxygen tension does not drop by much, but obviously the annual oxygen uptake by the bottom would be underestimated if those periods of disturbance are not taken into consideration.

Table 9. RELATIONSHIP BETWEEN HUMIC SUBSTANCES (absorbance/g dry sediment) AND ORGANIC CARBON (per cent of dry sediment) IN DIFFERENT SEDIMENT LAYERS AND DURING DIFFERENT SEASONS

Location	Station	Absorbance per g of dry sediment		Organic Carbon per cent of dry sediment		A:B Ratio	
		0-1 cm layer	5-6 cm layer	0-1 cm layer	5-6 cm layer	0-1 cm layer	5-6 cm layer
Puget Sound	1	0.40	0.40	2.94	2.58	0.14	0.16
	5	0.19	0.21	1.09	1.20	0.17	0.18
	6		0.19	0.89	1.06		0.18
	8	0.44	0.46	2.54	2.45	0.17	0.19
	10	0.47	0.47	2.54	2.49	0.18	0.19
	21	0.33	0.35	1.64	1.64	0.20	0.21
	23	0.50	0.52	2.68	2.68	0.19	0.19

Table 9. RELATIONSHIP BETWEEN HUMIC SUBSTANCES (absorbance/g dry sediment) AND ORGANIC CARBON (per cent of dry sediment) IN DIFFERENT SEDIMENT LAYERS AND DURING DIFFERENT SEASONS

(continuation)

Location	Station	Absorbance per g of dry sediment		Organic Carbon per cent of dry sediment		A:B Ratio	
		February '72	July '72	February '72	July '72	February '72	July '72
Puget Sound	24	0.10	0.25	0.89	1.80	0.11	0.14
	25	0.42	0.35	2.54	2.19	0.17	0.16
	26	0.17		1.59	0.96	0.11	
	27	0.14	0.20	0.99	1.32	0.14	0.15
	28	0.054	0.047	0.45	0.41	0.12	0.11
	31	0.74	0.47	3.95	2.67	0.19	0.18
	32	0.42	0.44	2.20	2.25	0.19	0.20
	33	0.76	0.72	3.94	3.88	0.19	0.19
	Clam Bay	0.067	0.033	1.02	0.24	0.066	0.14
Snohomish	29	0.039	0.18	1.17	1.53	0.033	0.12
River	30	0.015	0.018	0.29	0.15	0.052	0.12
Lake	1	1.54	2.62	7.55	14.06	0.20	0.19
Washington	8	0.79	0.73	6.29	6.01	0.13	0.12
	9	0.78	0.78	6.79	6.22	0.11	0.13
Blanks[a)]		0.020		0.0			
		0.028		0.0			

[a)] Sediment combusted for 2-3 hr at 500°C

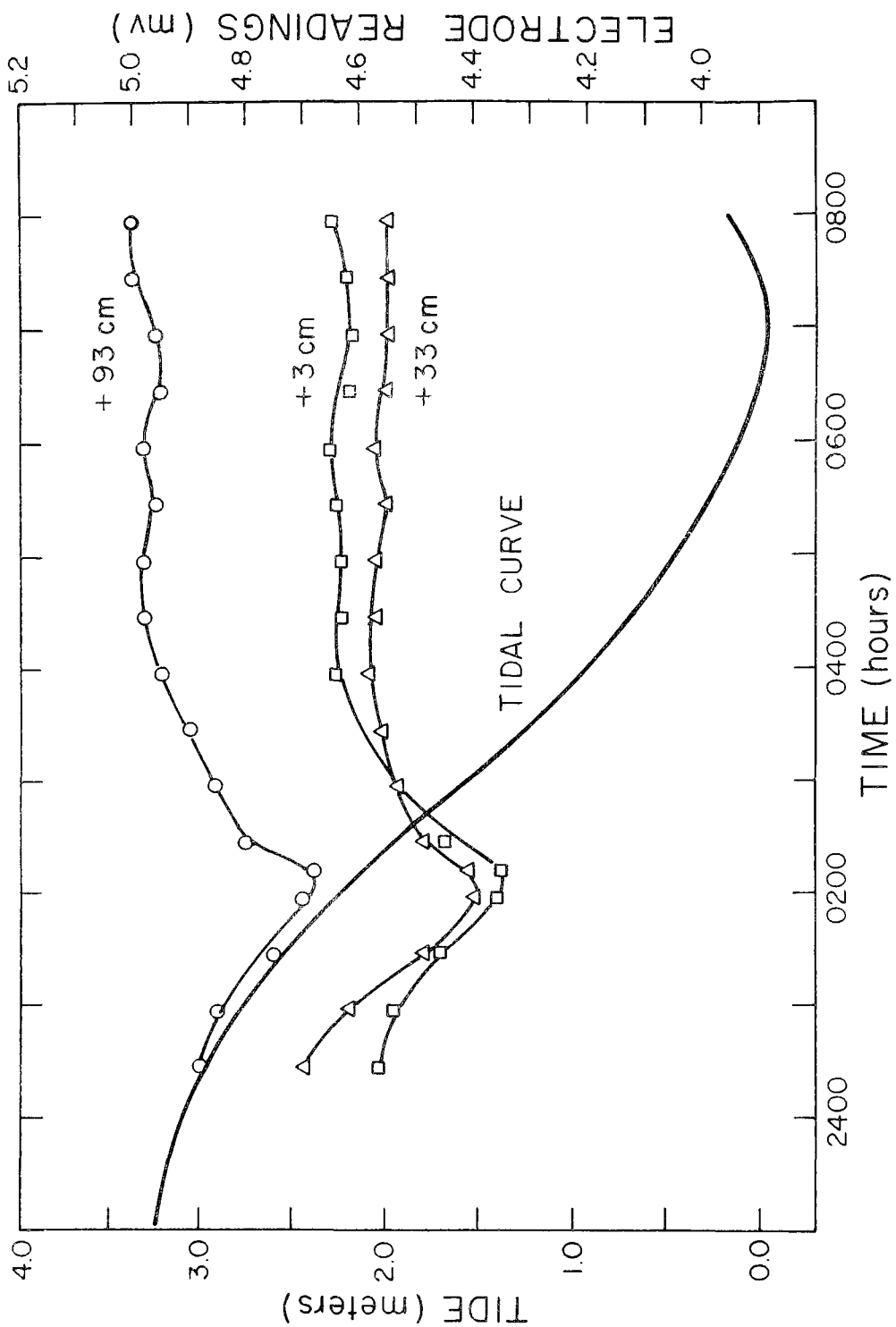

Figure 24. Effect of tidal current during ebb tide on the gradient of oxygen concentration above the bottom at the entrance to Port Madison (122°30.2'W, 47°43.5'N, 104 m depth). The calibration constants for the electrodes at the 3-cm, 33-cm, and 93-cm levels were 1.02, 1.02, and 0.93 ml 0_2/liter per mv, respectively.

EFFECT OF INCREASED ORGANIC SUPPLY TO THE BOTTOM

Directly underneath one of the floating fish pens in Clam Bay the rate of total oxygen uptake averaged 125 ml/m^2 per hr, with respiratory uptake being 86 and chemical oxidation 44 ml/m^2 per hr. The rate of total uptake decreased rapidly to the north and south away from the pens (Table 10). The increased rate of oxygen uptake appears to be confined to the immediate vicinity of the floating pens, although there may have been a slight influence as far as 15 m to the south.

The oxygen debt of the sediment underneath one of the pens is also about twice as high as those to the north and south (Table 11). The increase in reduced substances extended to the 3-4 cm layer. The sediment smelled of H_2S.

The fish were harvested in May and the pens were removed. During a subsequent cruise in July, the rate of oxygen uptake had dropped to 24 ml/m^2 per hr while oxygen debt had decreased to 0.05 meq per ml of sediment. There was no evidence of the highly polluted condition just a few months earlier, except for the persistent absence of macrofauna. Until late summer there were no infauna found; the only benthos to be seen were amphipods on the sediment surface (Mahnken, personal communication).

At least part of the reason for the accumulation of organic matter underneath the pens is believed to be the hindrance of water circulation by the nets. During extreme low tides the bottom of the net would scrape the sediment surface. It appears that once the nets were removed, tidal currents swept away lighter surface deposits and the increased turbulence of the overlying water enhanced the diffusion of dissolved oxygen into and of reduced substances out of the sediment.

RELATIONSHIP BETWEEN OXYGEN UPTAKE, CARBON OXIDATION, AND NUTRIENT RELEASE BY THE SEDIMENT

The rates of oxygen uptake, organic carbon content of the sediment, and nutrient concentrations of the overlying water of individual samples after increasing periods of incubation are shown in Table 12. The initial rates of oxygen uptake, measured when the water was still turbid although most of the sediment had settled to the bottom, show the high rates of oxygen consumption by disturbed sediment. Two days later the rates of uptake appear to have stabilized roughly to a constant rate until the end of the experiment. During this time the organic carbon showed a statistically significant decrease ($P < 0.05$) which averaged about 1.4 mgC/g dry sediment per month. Assuming that respiratory oxygen consumption averaged 2.7 ul/g dry sediment per hr, the respiratory quotient would be about 1.3. This value could mean that not

Table 10. RATES OF TOTAL OXYGEN UPTAKE, INORGANIC CHEMICAL OXIDATION, AND RESPIRATION (ml/m^2 per hr) AT 10^{o}C IN CLAM BAY AT THE SITE OF THE FISH REARING PENS IN FEBRUARY

Station	Distance from fish pens (meters)	Total Uptake	Chemical Oxidation	Respiration
1	15 south	36,42,31	22	14
2	30 south	22,21	13	8
3	45 south	14,10,11	10	4
4	75 south	30,12,14	13	17
5	15 north	11,14	9	5
6	30 north	24,14	14	5
7	45 north	17,17,20	11	9
8	75 north	18,19,10,19	12,7	6,3
9	underneath	130,120	44	86

Table 11. OXYGEN DEBT (ml 0_2/ml sediment) OF THE SEDIMENT IN CLAM BAY AT THE SITE OF THE FISH REARING PENS IN FEBRUARY

Station	Distance from fish pens meters	Sediment Layer		
		0-1 cm	1-2 cm	3-4 cm
1	15 south	0.20, 0.23	0.28, 0.21	0.25, 0.27
2	30 south	0.24, 0.27, 0.24	0.20, 0.17	0.15, 0.17, 0.17
3	45 south	0.15, 0.12, 0.07	0.12, 0.12	0.13, 0.14
4	75 south	0.09, 0.09	0.08, 0.10	0.08, 0.08
5	15 north	0.24, 0.28 0.18	0.27, 0.26	0.23, 0.21
6	30 north	0.10, 0.08 0.11	0.08, 0.09	0.20, 0.14, 0.18
7	45 north	0.05, 0.04	0.08, 0.09	0.25, 0.12, 0.09
8	75 north	0.04, 0.03	0.24, 0.13	0.11, 0.14, 0.16
9	underneath	0.51, 0.63	0.63, 0.50	0.51, 0.58

Table 12. RATES OF TOTAL OXYGEN UPTAKE, INORGANIC CHEMICAL OXIDATION AND RESPIRATION (ml 0_2 g $^{-1}hr^{-1}$)PER CENT ORGANIC CARBON, AND NUTRIENT CONCENTRATION OF OVERLYING WATER (μg-at l^{-1}) DURING A 43-DAY EXPERIMENT

Time Hours	Total Uptake	Chemical Oxidation	Respir-ation	Per Cent Carbon	$Si0_4$	$N0_3$	NH_3	$P0_4$
0	30	18	12	2.18	50	29	0.2	1.9
21	11	7.9	3.1	2.09	110	31	8.1	2.8
44	7.5	5.5	2.0	2.01	250	49	3.0	3.0
68	7.2	4.8	2.4	2.03	145	31	2.6	3.4
140	5.9	5.9	0	2.06	235	46	0.0	5.3
380	7.2	4.6	2.6	1.89	355	70	0.1	10.2
572	3.3	0.9	2.4	1.96	370	60	---	11.9
741	6.4	2.9	3.5	1.94	400	88	0.0	13.3
1030	5.1	2.0	3.1	1.85	---	--	---	18.6

all of the organic carbon loss from the sediment was the result of complete oxidation.

The nutrient values show that ammonia disappeared completely while silicate, nitrate, and phosphate increased with time. On the average, the sediment released silicate, nitrate, and phosphate at the rates of 0.05, 0.008, and 0.002 microgram-atoms/g of dry sediment per hr, respectively. The released ammonia may have been nitrified to nitrate. These amounts were released during respiratory oxygen uptake of 2.7 microliters/g dry sediment per hr.

This preliminary experiment shows a promising approach of establishing relationships between metabolism, decomposition of organic carbon, and nutrient release by the sediment under various conditions.

SECTION VI

DISCUSSION

TOTAL OXYGEN UPTAKE AS A MEASURE OF COMMUNITY METABOLISM IN THE SEDIMENT COLUMN

Total benthic metabolism is a complex of aerobic and anaerobic activity. In order for the rate of total oxygen uptake to be a measure of total metabolism the rate of chemical oxidation of reduced substances must be in equilibrium with the rate of formation of these substances by anaerobic metabolism. Although there is evidence that such an equilibrium may exist in the surface few-centimeters layer, the increase with sediment depth of concentration of reduced substances indicates an accumulation in the deeper layers, i.e., these are not being oxidized and therefore the rate of oxygen uptake by chemical oxidation is underestimating anaerobic metabolism in deeper layers. This underestimation is also indicated by the fact that the rate of oxygen consumption by intact sediment cores is independent of the length of the cores beyond a few centimeters (Edwards and Rolley, 1965).

A direct comparison between the rate of oxygen uptake by chemical oxidation and integrated anaerobic metabolism from dehydrogenase assay of sediment cores from Lake Washington indicates by how much anaerobic metabolism in the sediment column may be underestimated. The large discrepancy between the two measures in Lake Washington may be attributed to the increasing relative importance of anaerobic metabolism with increasingly organic soft sediments as a result of organic pollution or eutrophication. Where anaerobic metabolism is relatively less important then the rate of inorganic chemical oxidation should be a better estimate of total anaerobic metabolism and the rate of total oxygen uptake should accordingly be a better estimate of total metabolism in the sediment column than the results from Lake Washington indicate.

In ecological investigations, we are interested in the quantitative role of the benthos in the annual cycle of materials and energy flow through the ecosystem; that is, we want to know how much of the annual net primary production settles to the bottom and how much of this is mineralized during that year by the benthos. Obviously, if the benthic community in the deeper sediment layers is metabolizing long-buried organic matter, its metabolic activity is not related to the annual sedimentation of organic matter and the energy flow through the rest of the ecosystem. It may be that the true measure of the benthic community's impact on the annually sedimented organic matter is reasonably estimated by the rate of total oxygen consumption by the sediment surface. It would be desirable to obtain direct measurements of sedimentation rates; these plus information on the average annual leftover organic matter in the bottom should allow us to place an upper limit to the annual extent of

degradation of the current year's sedimented organic matter. The limiting values hopefully will agree with the estimated annual oxygen consumption by the sediment. The role of burrowing macrofauna that feed on the sediment surface in mixing and transporting newly sedimented organic material below the surface should be investigated also.

Even if the rate of total oxygen uptake by intact sediment surface represents total metabolism by the aerobic and anaerobic organisms in a surface layer only of indeterminate thickness (a few centimeters in any case), the measure is still probably a useful characteristic parameter of an area; it would seem to be an index of equilibrium conditions among the various factors that affect the rate of uptake, e.g. oxygen tension, temperature, salinity, turbulence, available metabolizable energy, size and composition of the community, compaction and porosity of the sediment, and maybe more. It would be desirable to unravel the quantitative effects of these various factors, but in the absence of such knowledge it is still useful to have a total measure of their combined effects.

There seems to be no easy single method to measure accurately total benthic community metabolism in the sediment column. Direct calorimetry may be the only means of measuring aerobic plus anaerobic metabolism of undisturbed sediment cores, but the outlook for its use in field studies does not look promising. At present the practical way to estimate total aerobic and anaerobic metabolism in sediments will be to combine the rate of respiratory oxygen uptake by undisturbed sediment cores with estimates of anaerobic metabolism derived from dehydrogenase assay of subsurface sediment layers.

GENERAL APPLICABILITY OF THE TTC METHOD FOR MEASURING NATURAL RATES OF ANAEROBIC METABOLISM

A number of questions may be raised about the method of using TTC plus substrate to measure anaerobic metabolism. If TTC is a more efficient hydrogen acceptor than the natural hydrogen acceptors available to the organisms, it may actually stimulate dehydrogenase activity. The added substrate undoubtedly raises the natural level of metabolic activity. Hence, the apparent dehydrogenase activity measured is certainly not the natural level of metabolic activity in situ, even if all other physical and chemical conditions in nature are maintained. If TTC alone without additional substrate is used, the measured activity may still be higher than in situ rates.

By comparing any dehydrogenase method with direct calorimetry, the above questions become immaterial; the effect of TTC and added substrates are in essence systematic errors that are taken care of by the comparison. If the method is to be of wide applicability, however, it is essential to verify that the magnitude of the systematic error remains the same for different microbial

communities. The discrepancy between our results and those of Lenhard et al. (1965) regarding the relative effects of added glucose and sodium citrate presupposes nonuniformity of the systematic error due to added substrate. We have shown, however, a direct proportionality between the results with two concentrations of sodium citrate and with no added substrate. There should be a significant regression of measured dehydrogenase activity without added substrate and metabolic heat release. If the systematic error due to the effect of TTC alone is the same on all microbial organisms, the only problem would be one of low sensitivity in some if not most places. We have not investigated the effect of much longer incubation time without additional substrate on the rate of formazan production. As mentioned earlier, Farkas (1966) has placed a limit of 3 hr on incubation time, but it is not clear why he should find this necessary. Obviously, incubation time may be extended for as long as the rate of formazan production remains constant.

In any case, the regression showing a high degree of functional relationship between dehydrogenase activity and metabolic heat release under one set of conditions (10°C, 3-hr incubation, 0.1% TTC, 0.04 M sodium citrate) warrants further investigation of the relationship under a wider range of conditions. It seems particularly imperative to test the relationship at different temperatures and with the dehydrogenase method carried out at the same or higher temperatures than the calorimetry. With this information it may be possible to obtain positive results with any dehydrogenase method and by comparison with a calibrated method determine the equivalent metabolic heat release in nature.

The dehydrogenase activity of some Metazoa has been shown to be corrleated with their aerobic metabolism (Curl and Sandberg 1961; Packard and Taylor 1968), but the assay is much more difficult than the measurement of oxygen consumption and therefore the latter remains the preferred method of measuring aerobic metabolism. A dehydrogenase assay, however, could be a very useful measure of anaerobic metabolism by metazoans and deserves to be developed.

The metabolism of heterotrophic sediment bacteria has also been assessed in terms of their uptake of radioactive glucose or acetate (Wood 1970; Harrison et al. 1971; Sorokin and Kadota 1972). Hobbie (1969) discusses the significance and limitations of the method. Such a method also might be compared with direct calorimetry. Since the measurement of dehydrogenase activity requires less expensive equipment and materials, it would seem preferable to the measurement of the rate of uptake of a specific substrate.

EFFECT OF TEMPERATURE ON BENTHIC COMMUNITY METABOLISM

There is a problem about comparing benthic community metabolism in various areas that differ in temperature. For example, when comparing deep-sea benthic metabolism at 2°C with shallow sub-tidal or intertidal metabolism at 15°C or greater in summer, we do not know how much of the difference in metabolic rate is due to the temperature difference and how much is due to other factors. The difference in benthic community metabolism between intertidal at 2°C in winter and the deep-sea at 2°C would be due exclusively to factors other than temperature, but it will be most unusual for natural benthic metabolism to be measured at exactly the same temperature in different places.

Any comparison without consideration of temperature differences tacitly assumes that each benthic community is completely acclimatized to temperature at the time of measurement. This assumption may be correct for areas of more or less constant temperature throughout the year but it may not be reasonable for those areas subject to a pronounced seasonal temperature cycle in view of the different degrees of temperature compensation exhibited by different species (Precht, 1958, Prosser, 1958). In the latter areas, it would be desirable to partition the seasonal difference in metabolic rate between the temperature effect and the other factors.

The technique employed in the present study of measuring the rates of oxygen uptake at 5, 10, and 15°C throughout the year resulted in acute metabolism-versus-temperature curves, oxygen consumption rates at the same temperatures throughout Puget Sound, and also rates at prevailing bottom-water temperatures at the time of measurement in the different stations. These stations show differences in metabolism-versus-temperature curves indicating differences in temperature adaptation. Where seasonal temperature fluctuations and temperature differences between study sites are no more than 5°C or so, such as in subtidal Puget Sound, this technique may be all right, but where these are much greater there may be the additional problem of dealing with cold-adapted communities at one time or place and warm-adapted communities at another time or place. The cold-adapted community would be subjected to a large acute temperature rise while the warm-adapted community would be subjected to a large acute temperature lowering. It may be doubtful that their respective metabolism at the same intermediate temperature is devoid of any temperature effect. In such a case it would seem better to measure oxygen consumption rates after acclimation to the same temperature but this would take days and the storage time would then introduce another unknown factor.

More experiments are clearly needed to understand the differences in benthic metabolism between areas of different temperatures. The effect of temperature on metabolism may be further complicated by an interaction with pressure at great depths (Jannasch et al., 1971).

SECTION VII

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SECTION VIII

APPENDICES

Table 1. COMPUTER OUTPUT OF PREDICTED RATES FROM ANALYSIS OF VARIANCE OF TOTAL OXYGEN UPTAKE, INORGANIC CHEMICAL OXIDATION, AND RESPIRATION (ml oxygen $m^{-2}hr^{-1}$) DURING FOUR SEASONS AT 5, 10, AND 15°C

Stations	TOTAL OXYGEN UPTAKE*											
	5°C				10°C				15°C			
	Oct.	Jan.	Apr.	Jul.	Oct.	Jan.	Apr.	Jul.	Oct.	Jan.	Apr.	Jul.
1	12.9	11.1	12.6	15.9	19.5	16.8	19.2	24.2	24.1	20.7	23.7	29.9
2	9.4	8.1	9.2	11.6	14.2	12.3	14.0	17.6	17.6	15.1	17.3	21.8
3	10.0	8.6	9.8	12.3	15.1	13.0	14.9	18.8	18.7	16.1	18.4	23.2
4	10.1	8.7	9.9	12.5	15.3	13.2	15.1	19.0	19.0	16.3	18.6	23.5
5	7.3	6.2	7.1	9.0	11.0	9.5	10.8	13.7	13.6	11.7	13.4	16.9
6	7.4	6.4	7.3	9.2	11.2	9.7	11.0	13.9	13.9	11.9	13.6	17.2
7	8.3	7.1	8.2	10.3	12.6	10.9	12.4	15.7	15.6	13.4	15.4	19.4
8	11.4	9.8	11.2	14.1	17.3	14.9	17.0	21.5	21.4	18.4	21.0	26.5
9	6.5	5.5	6.3	8.0	9.8	8.4	9.6	12.2	12.1	10.4	11.9	15.0
10	6.4	5.5	6.3	7.9	9.7	8.4	9.6	12.1	12.0	10.4	11.8	14.9
11	7.4	6.3	7.2	9.1	11.2	9.6	11.0	13.9	13.9	11.9	13.6	17.2
12	7.3	6.3	7.2	9.0	11.1	9.5	10.9	13.8	13.7	11.8	13.5	17.0
13	10.0	8.6	9.8	12.4	15.3	13.1	15.0	18.9	18.9	16.2	18.5	23.4
14	10.7	9.2	10.5	13.2	16.2	13.9	15.9	20.1	20.0	17.2	19.7	24.8
15	8.3	7.2	8.2	10.3	12.7	10.9	12.4	15.7	15.7	13.5	15.4	19.4
16	8.9	7.7	8.8	11.1	13.6	11.6	13.3	16.8	16.8	14.4	16.5	20.8
17	7.5	6.4	7.3	9.2	11.3	9.7	11.1	14.0	14.0	12.1	13.8	17.4
18	6.7	5.7	6.6	8.3	10.2	8.7	10.0	12.6	12.5	10.8	12.3	15.5
19	8.6	7.4	8.4	10.6	13.0	11.2	12.8	16.1	16.1	13.8	15.8	20.0
20	7.1	6.1	7.0	8.8	10.8	9.3	10.6	13.3	13.3	11.4	13.1	16.5
21	7.7	6.6	7.6	9.6	11.8	10.1	11.6	14.6	14.5	12.5	14.3	18.0
22	5.4	4.7	5.3	6.7	8.3	7.1	8.1	10.3	10.2	8.8	10.0	12.7
23	4.2	3.6	4.1	5.2	6.4	5.5	6.2	7.9	7.9	6.8	7.7	9.7

*The 95% confidence limits of the rate at each station are the predicted rate multiplied and divided by 1.17.

Table 1. (continued) COMPUTER OUTPUT OF PREDICTED RATES FROM ANALYSIS OF VARIANCE OF TOTAL OXYGEN UPTAKE, INORGANIC CHEMICAL OXIDATION, AND RESPIRATION (ml oxygen $m^{-2}hr^{-1}$) DURING FOUR SEASONS AT 5, 10, AND 15°C

	INORGANIC CHEMICAL OXIDATION*											
	5°C				10°C				15°C			
Stations	Oct.	Jan.	Apr.	Jul.	Oct.	Jan.	Apr.	Jul.	Oct.	Jan.	Apr.	Jul.
1	8.8	8.0	8.6	9.5	10.8	10.0	10.5	11.4	12.9	12.2	12.7	13.6
2	6.8	6.0	6.6	7.5	8.7	8.0	8.5	9.4	10.9	10.1	10.7	11.6
3	9.9	9.1	9.7	10.6	11.9	11.1	11.6	12.5	14.0	13.2	13.8	14.7
4	6.2	5.5	6.0	6.9	8.2	7.4	8.0	8.9	10.4	9.6	10.1	11.0
5	5.4	4.7	5.2	6.1	7.4	6.6	7.1	8.0	9.5	8.8	9.3	10.2
6	6.9	6.1	6.7	7.6	8.9	8.1	8.6	9.5	11.0	10.3	10.8	11.7
7	5.5	4.7	5.3	6.2	7.4	6.7	7.2	8.1	9.6	8.8	9.4	10.3
8	9.0	8.2	8.8	9.7	11.0	10.2	10.7	11.6	13.1	12.4	12.9	13.8
9	4.5	3.7	4.2	5.1	6.4	5.6	6.2	7.1	8.6	7.8	8.3	9.3
10	5.7	5.0	5.5	6.4	7.7	6.9	7.5	8.4	9.9	9.1	9.6	10.5
11	5.5	4.7	5.3	6.2	7.5	6.7	7.2	8.1	9.6	8.9	9.4	10.3
12	3.8	3.0	3.5	4.4	5.7	4.9	5.5	6.4	7.9	7.1	7.7	8.6
13	8.4	7.6	8.2	9.1	10.4	9.6	10.1	11.0	12.5	11.8	12.3	13.2
14	5.6	4.9	5.4	6.3	7.6	6.8	7.3	8.3	9.8	9.0	9.5	10.4
15	6.5	5.7	6.2	7.1	8.4	7.6	8.2	9.1	10.6	9.8	10.3	11.3
16	6.5	5.7	6.2	7.2	8.4	7.6	8.2	9.1	10.6	9.8	10.4	11.3
17	4.1	3.3	3.9	4.8	6.1	5.3	5.8	6.7	8.2	7.5	8.0	8.9
18	4.6	3.9	4.4	5.3	6.6	5.8	6.3	7.3	8.8	8.0	8.5	9.4
19	5.8	5.0	5.6	6.5	7.8	7.0	7.5	8.4	9.9	9.2	9.7	10.6
20	4.3	3.5	4.0	4.9	6.2	5.4	6.0	6.9	8.4	7.6	8.1	9.0
21	5.2	4.5	5.0	5.9	7.2	6.4	7.0	7.9	9.4	8.6	9.1	10.0
22	2.0	1.2	1.8	2.7	4.0	3.2	3.7	4.6	6.1	5.4	5.9	6.8
23	3.2	2.4	2.9	3.8	5.1	4.3	4.9	5.8	7.3	6.5	7.0	8.0

*The 95% conficence limits of the rate at each station are the predicted value $\pm$ 1.9 ml 0_2 m^{-2} hr^{-1}

Table 1. (continued) COMPUTER OUTPUT OF PREDICTED RATES FROM ANALYSIS OF VARIANCE OF TOTAL OXYGEN UPTAKE, INORGANIC CHEMICAL OXIDATION, AND RESPIRATION (ml oxygen $m^{-2}hr^{-1}$) DURING FOUR SEASONS AT 5, 10, AND 15°C

	RESPIRATION											
	5°C				10°C				15°C			
Stations	Oct.	Jan.	Apr.	Jul.	Oct.	Jan.	Apr.	Jul.	Oct.	Jan.	Apr.	Jul.
1	4.4	3.6	4.7	6.7	8.1	6.8	8.6	12.0	8.3	7.0	8.8	12.3
2	2.6	2.1	2.8	4.2	5.1	4.2	5.5	7.7	5.3	4.4	5.6	7.9
3	1.4	1.0	1.5	2.4	3.1	2.5	3.3	4.8	3.2	2.5	3.4	4.9
4	3.5	2.8	3.7	5.4	6.6	5.5	7.0	9.9	6.8	5.6	7.2	10.1
5	1.6	1.3	1.8	2.8	3.5	2.8	3.7	5.4	3.6	2.9	3.8	5.5
6	0.9	0.6	1.0	1.7	2.2	1.7	2.4	3.6	2.3	1.8	2.5	3.7
7	2.5	2.0	2.7	4.0	4.9	4.1	5.2	7.4	5.1	4.2	5.4	7.6
8	2.9	2.3	3.1	4.5	5.6	4.6	5.9	8.3	5.7	4.7	6.0	8.5
9	1.4	1.1	1.5	2.4	3.1	2.5	3.3	4.8	3.2	2.6	3.4	5.0
10	1.0	0.7	1.1	1.9	2.4	1.9	2.6	3.8	2.5	2.0	2.6	3.9
11	1.6	1.2	1.7	2.7	3.4	2.8	3.7	5.3	3.5	2.9	3.8	5.4
12	2.6	2.1	2.8	4.1	5.1	4.2	5.4	7.6	5.2	4.3	5.5	7.8
13	1.8	1.4	1.9	3.0	3.8	3.1	4.0	5.8	3.9	3.1	4.1	5.9
14	4.3	3.5	4.5	6.5	7.9	6.6	8.4	11.7	8.1	6.8	8.6	12.0
15	1.9	1.5	2.1	3.2	4.0	3.3	4.2	6.1	4.1	3.3	4.3	6.2
16	2.3	1.8	2.4	3.7	4.6	3.8	4.8	6.9	4.7	3.9	5.0	7.1
17	2.6	2.1	2.8	4.2	5.2	4.3	5.5	7.8	5.3	4.4	5.6	8.0
18	1.6	1.3	1.8	2.8	3.5	2.8	3.7	5.4	3.6	2.9	3.8	5.5
19	2.8	2.3	3.0	4.5	5.5	4.6	5.9	8.3	5.7	4.7	6.0	8.5
20	2.0	1.5	2.1	3.2	4.0	3.3	4.3	6.1	4.1	3.4	4.4	6.3
21	2.2	1.7	2.3	3.5	4.4	3.6	4.6	6.6	4.5	3.7	4.8	6.8
22	1.9	1.5	2.1	3.2	4.0	3.2	4.2	6.1	4.1	3.3	4.3	6.2
23	0.3	0.1	0.3	0.8	1.2	0.8	1.3	2.1	1.2	0.9	1.3	2.2

Table 2. DEHYDROGENASE ACTIVITY OF SEDIMENTS FOLLOWING INCUBATION OF SAMPLES WITH GLUCOSE AT 37°C FOR ONE HOUR

Location	Station	Sediment layer	Absorbance/ml centrifuged sediment
Puget Sound	24	0-1	0
		1-2	0
		5-6	0
	25	0-1	0.01
		1-2	0
		5-6	0.01
	26	0-1	0.01
		1-2	0.02
		5-6	0.03
	27	0-1	0
		1-2	0.01
		5-6	0.01
	28	0-1	0.02
		1-2	0.02
		5-6	0.02
	29	0-1	0.06
		1-2	0.03
		5-6	0.01
	30	0-1	0
		1-2	0
		5-6	0
	31	0-1	0
		1-2	0
		5-6	0
	32	0-1	0
		1-2	0
		5-6	0.05
	33	0-1	0.06
		1-2	0.13
		5-6	0.20
Lake Washington	1	0-1	0.02
		1-2	0.03
		5-6	0.06
	8	0-1	0.12
		102	0.09
		5-6	0.06
	9	0-1	0.12
		1-2	0.20
		5-6	0.08

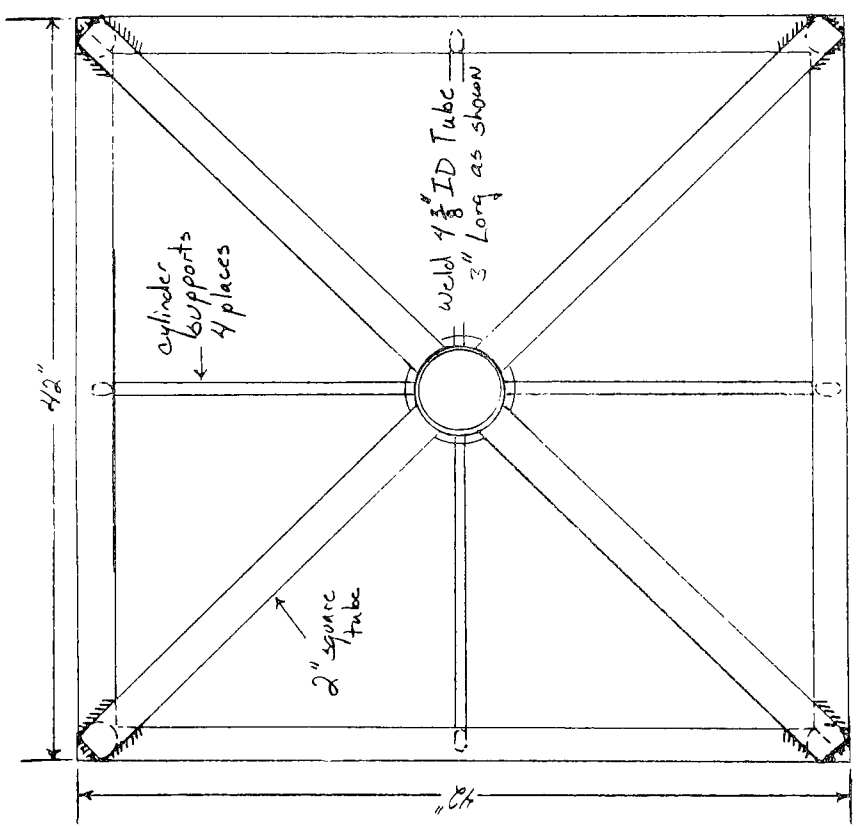

Figure 1. Top view of multiple corer showing four cross braces on top welded to a center piece of 3-inch long cylinder which holds the upper end of the dashpot in place. The four cylinder supports are welded to a center piece of 1/4-inch steel plate onto which the bottom of the dashpot is bolted.

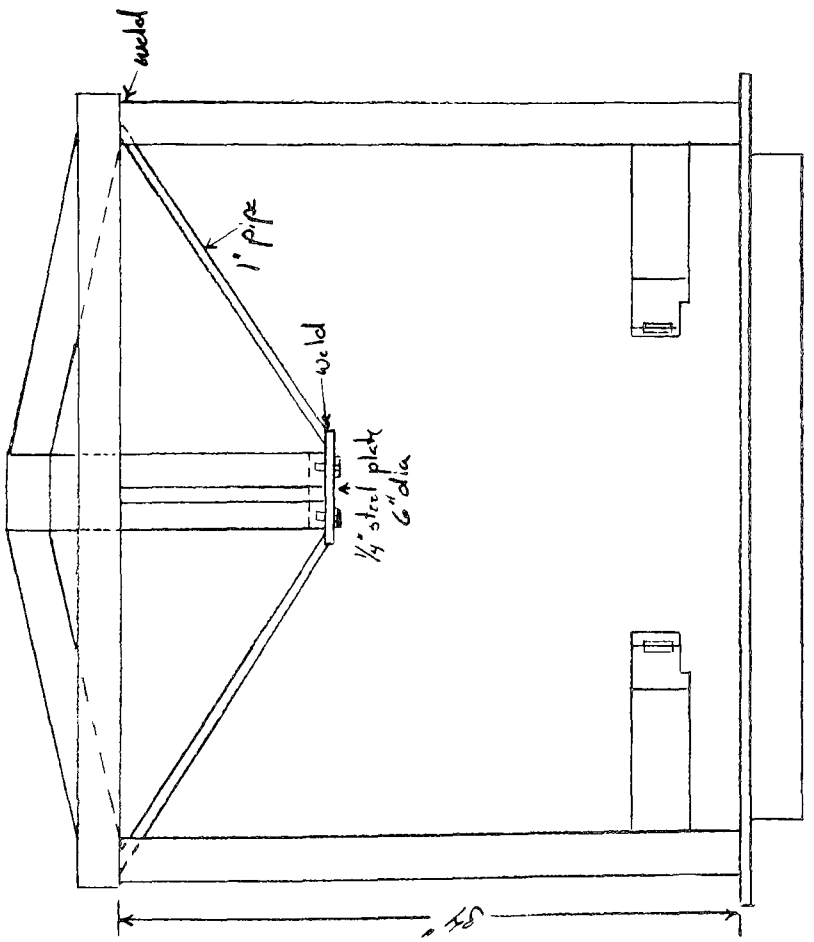

Figure 2. Side view of multiple corer showing cylinder support.

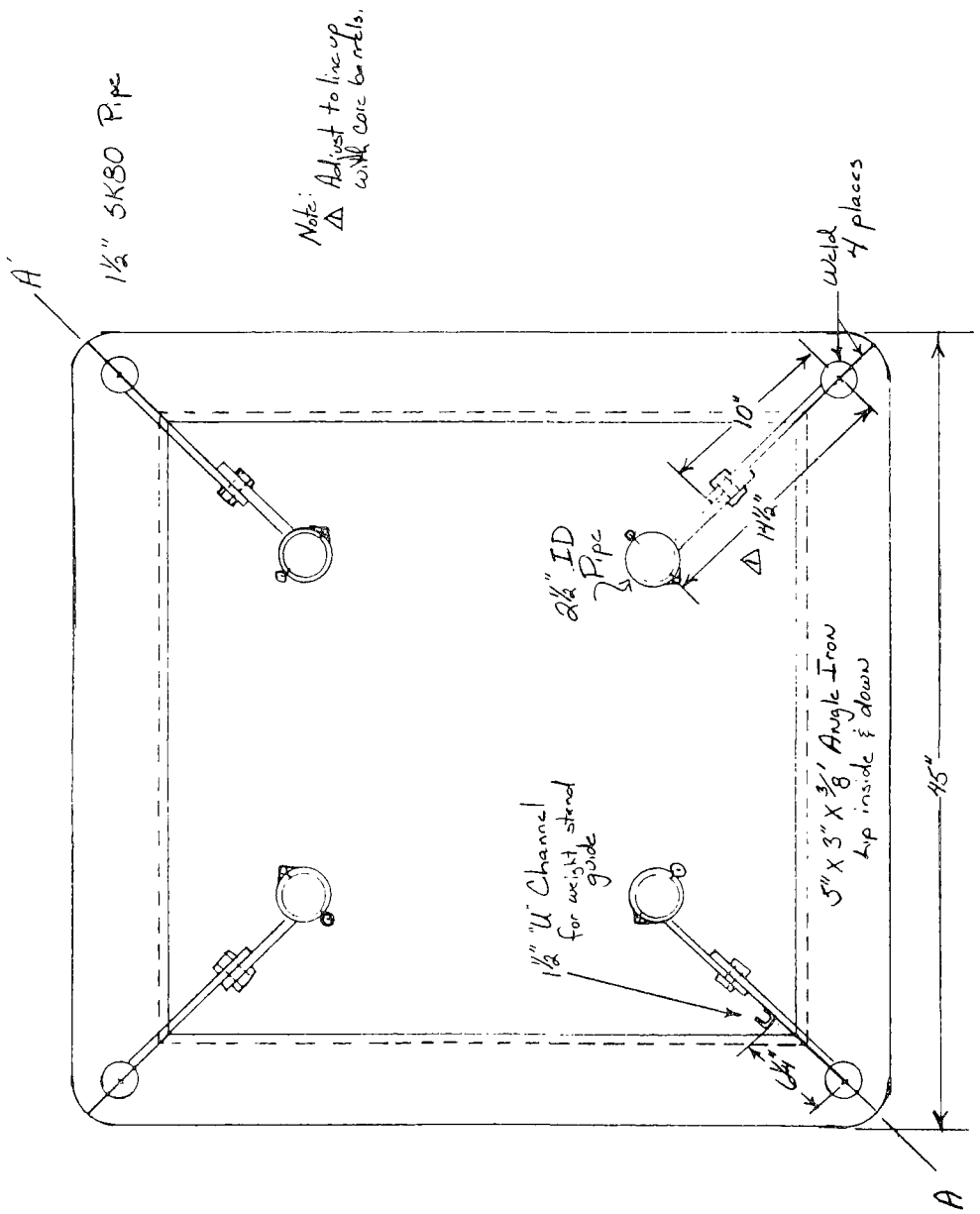

Figure 3. Sectional view across the lower end showing the guides for each of the coring tube s.

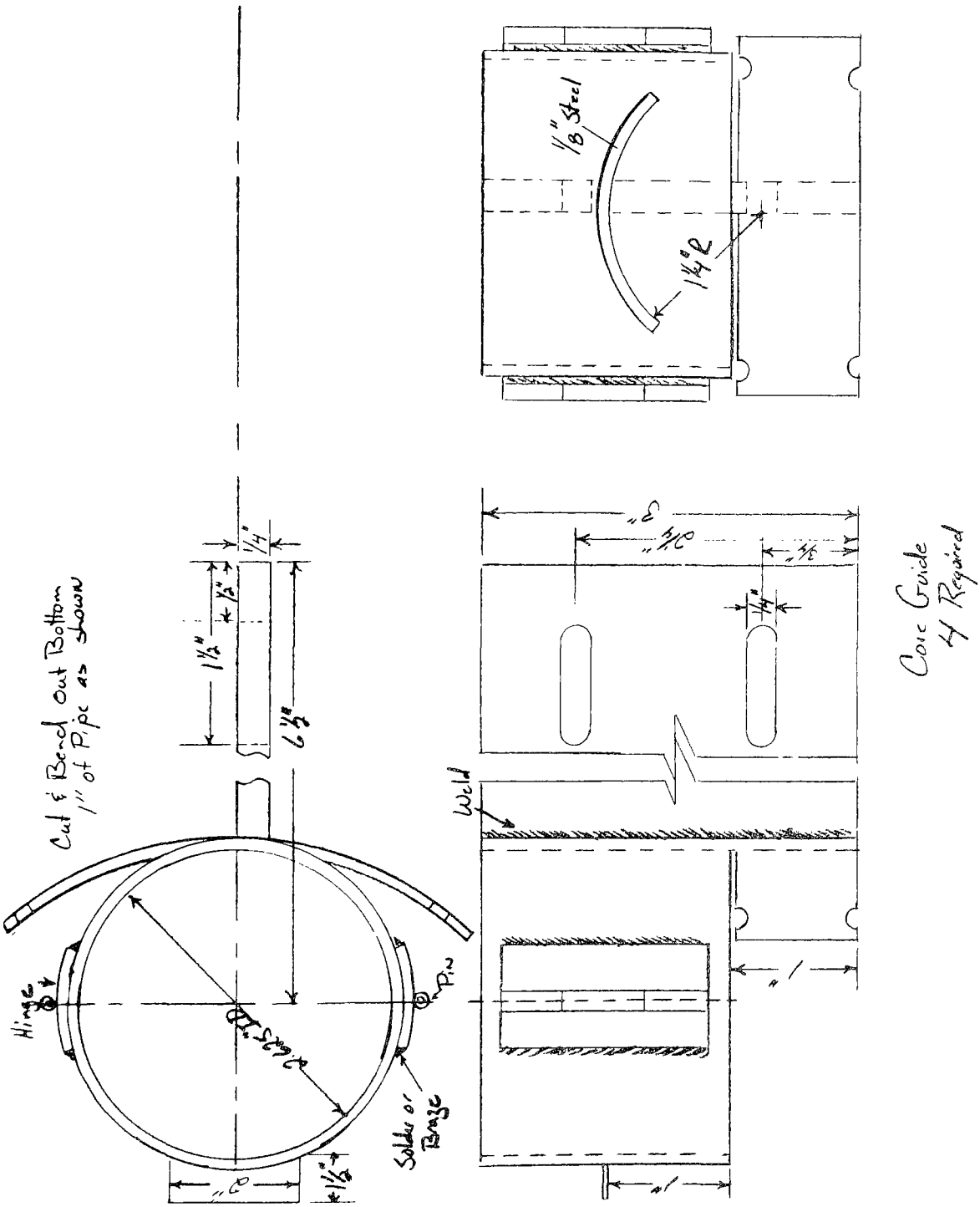

Figure 4. Detailed drawing of a coring tube guide showing the split lower section that is spread out to stretch the rubber tubing away from the path of the coring tube and the curved overhang to keep the core catcher in proper position away from the path of the coring tube.

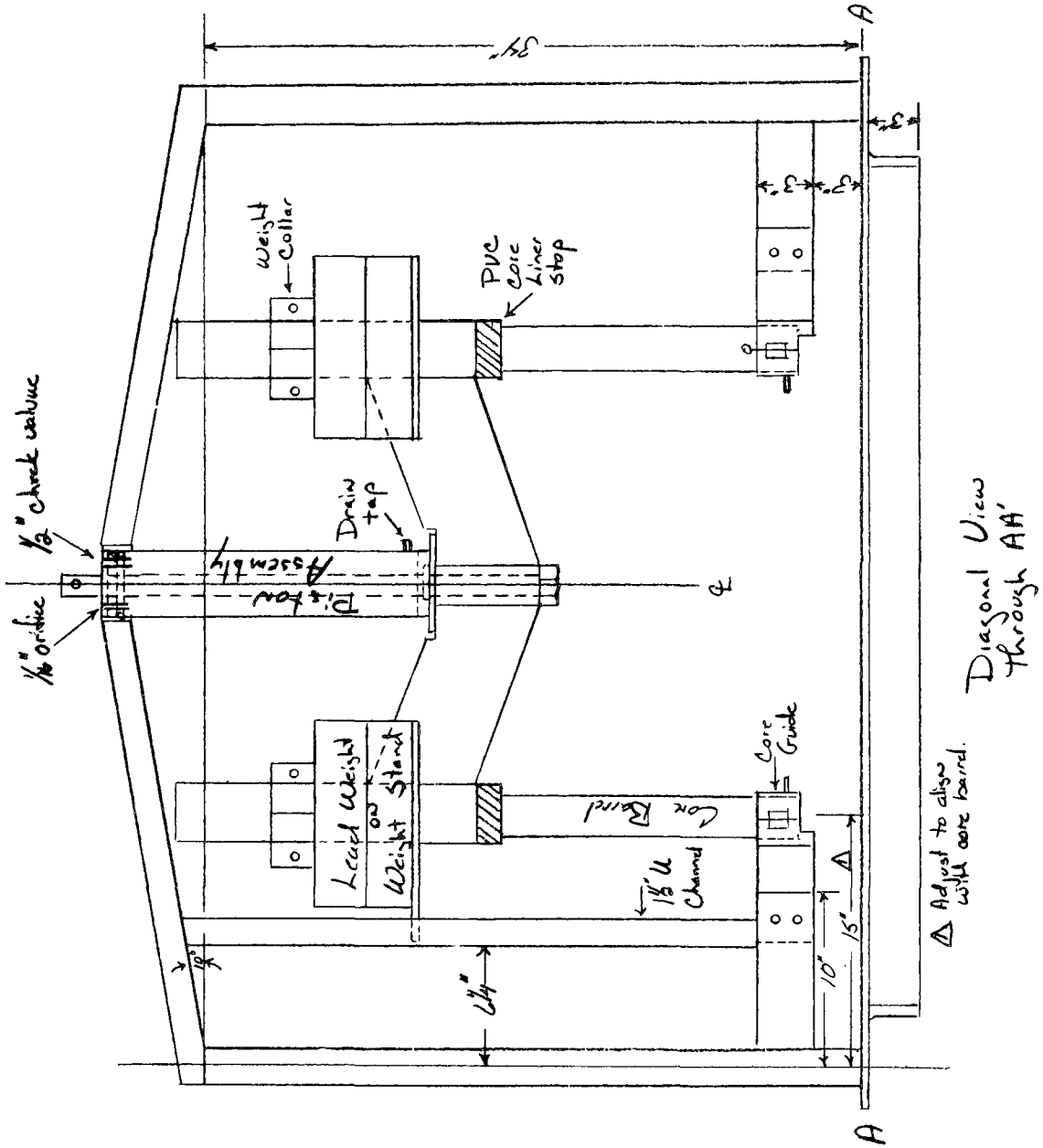

Figure 5. Diagonal section showing two opposite coring tubes and their weight stands.

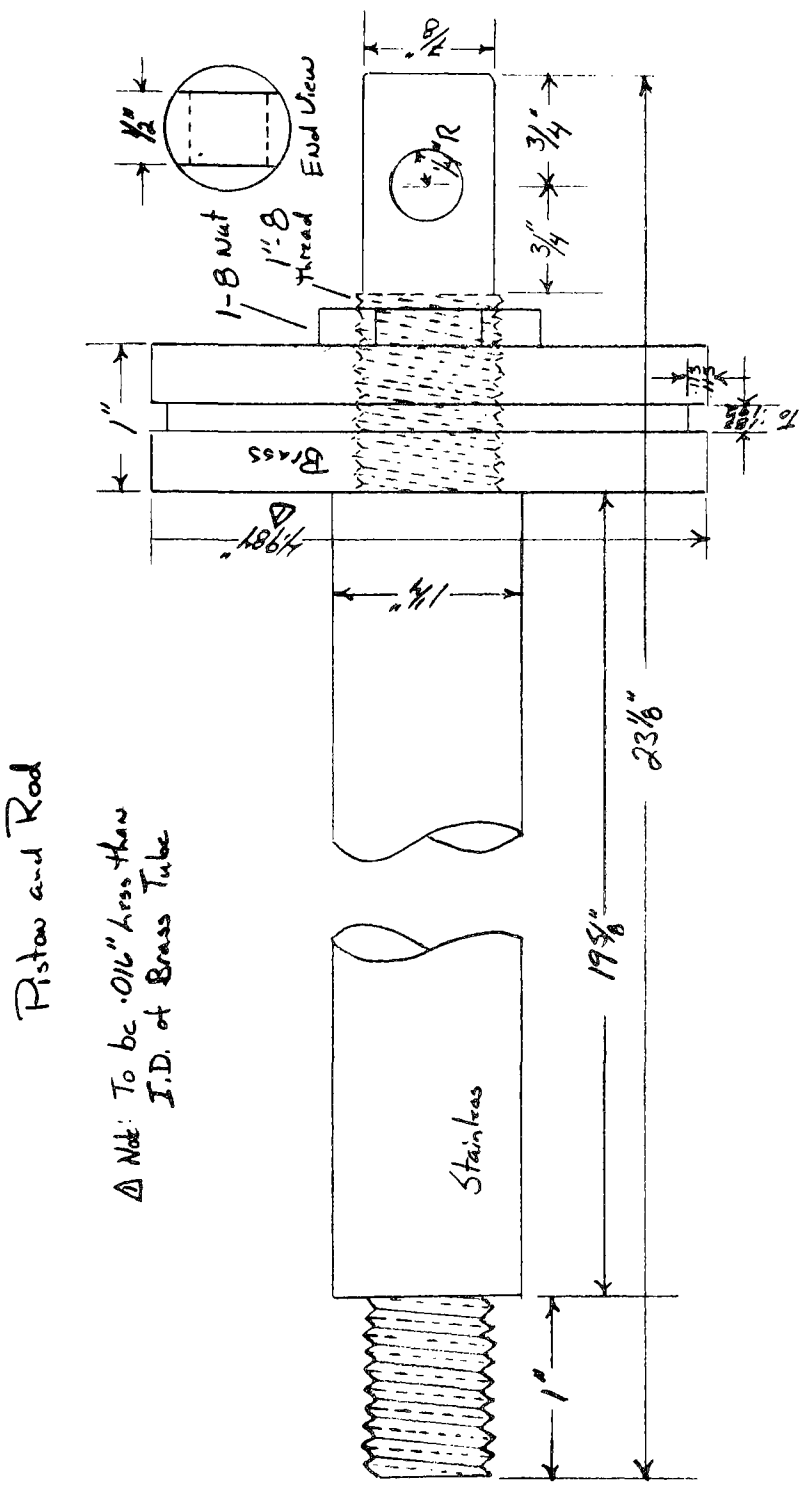

Figure 6. Detailed drawing of piston and rod assembly.

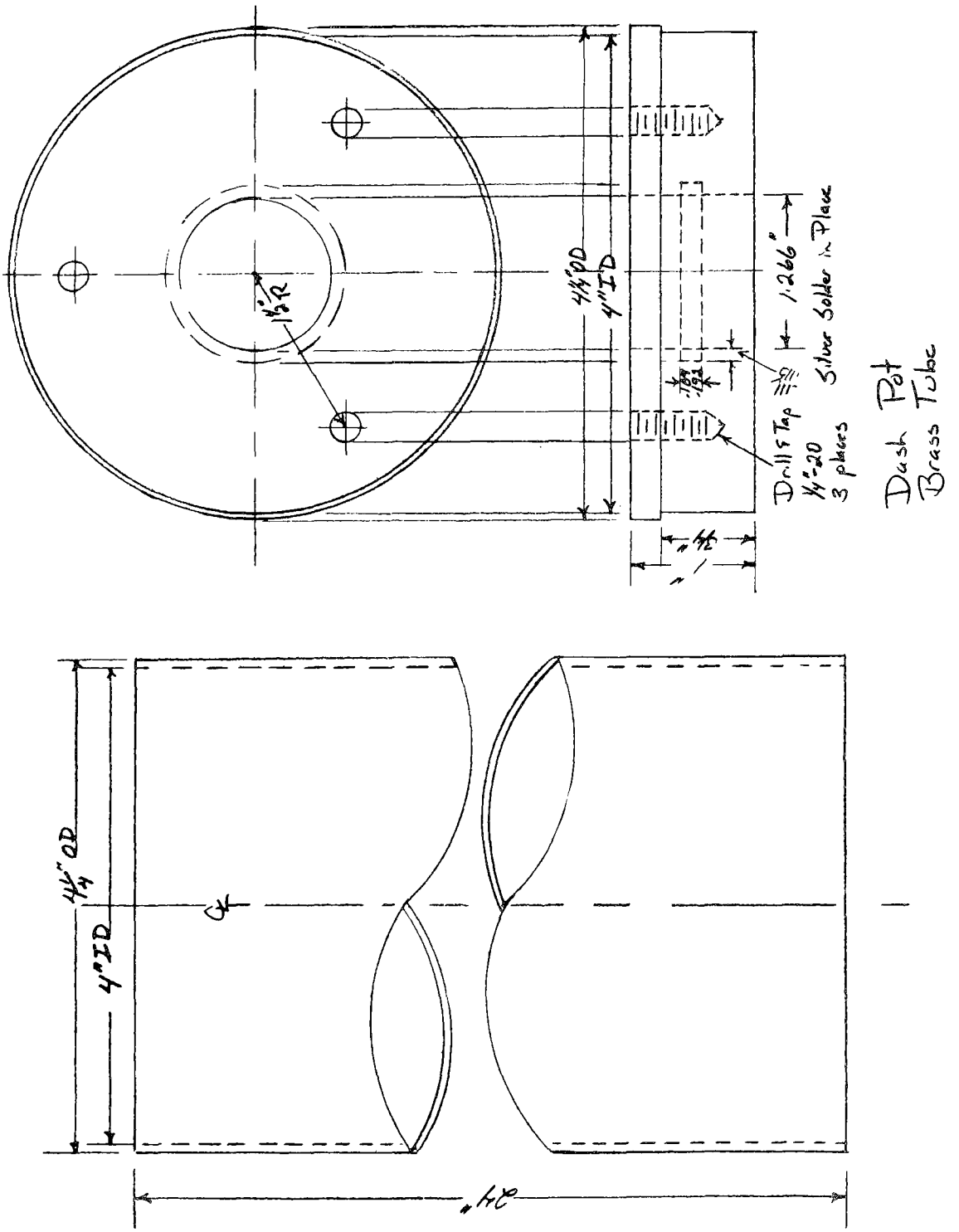

Figure 7. Detailed drawings of the dashpot showing the bottom end which is bolted to steel plate center piece.

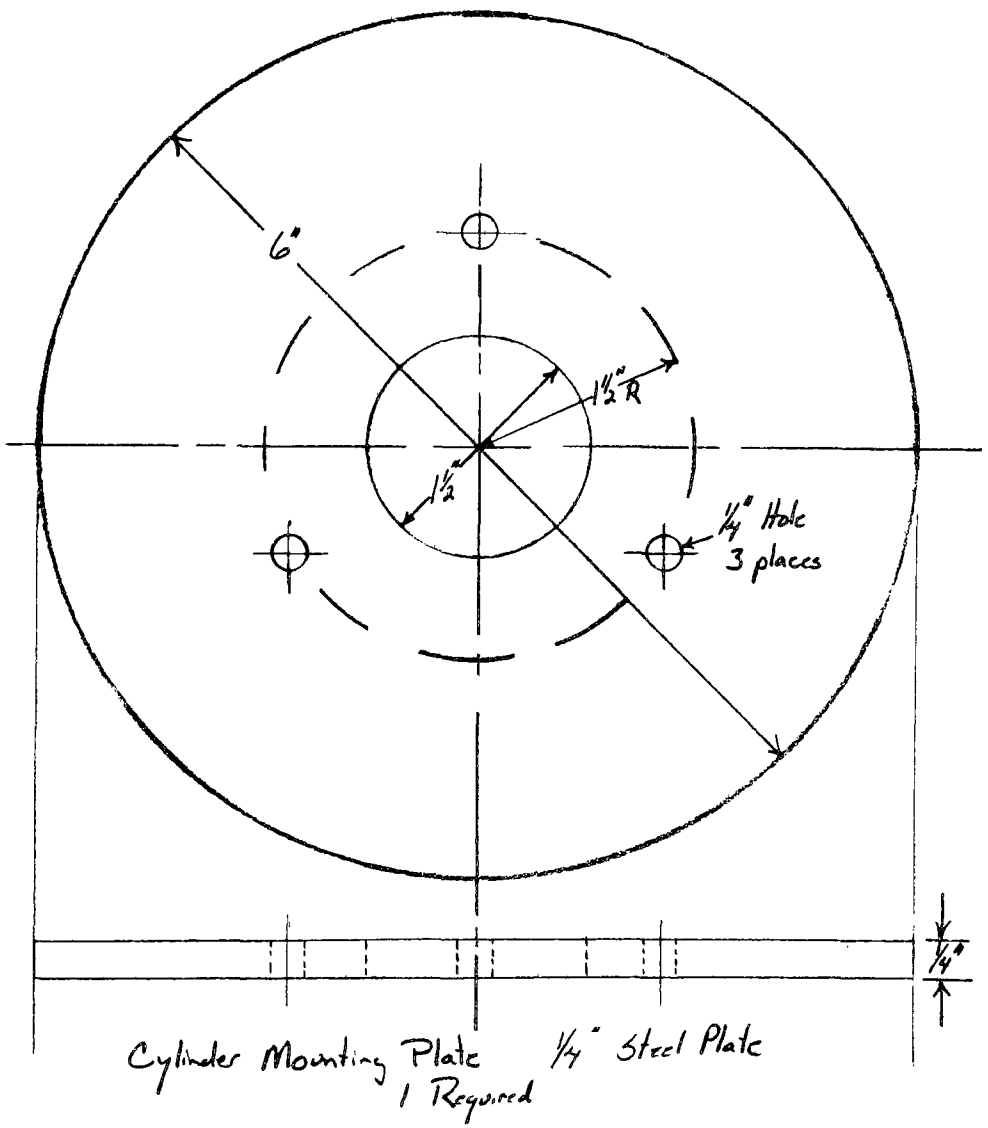

Figure 8. Detailed drawing of the bottom cylinder mounting plate.

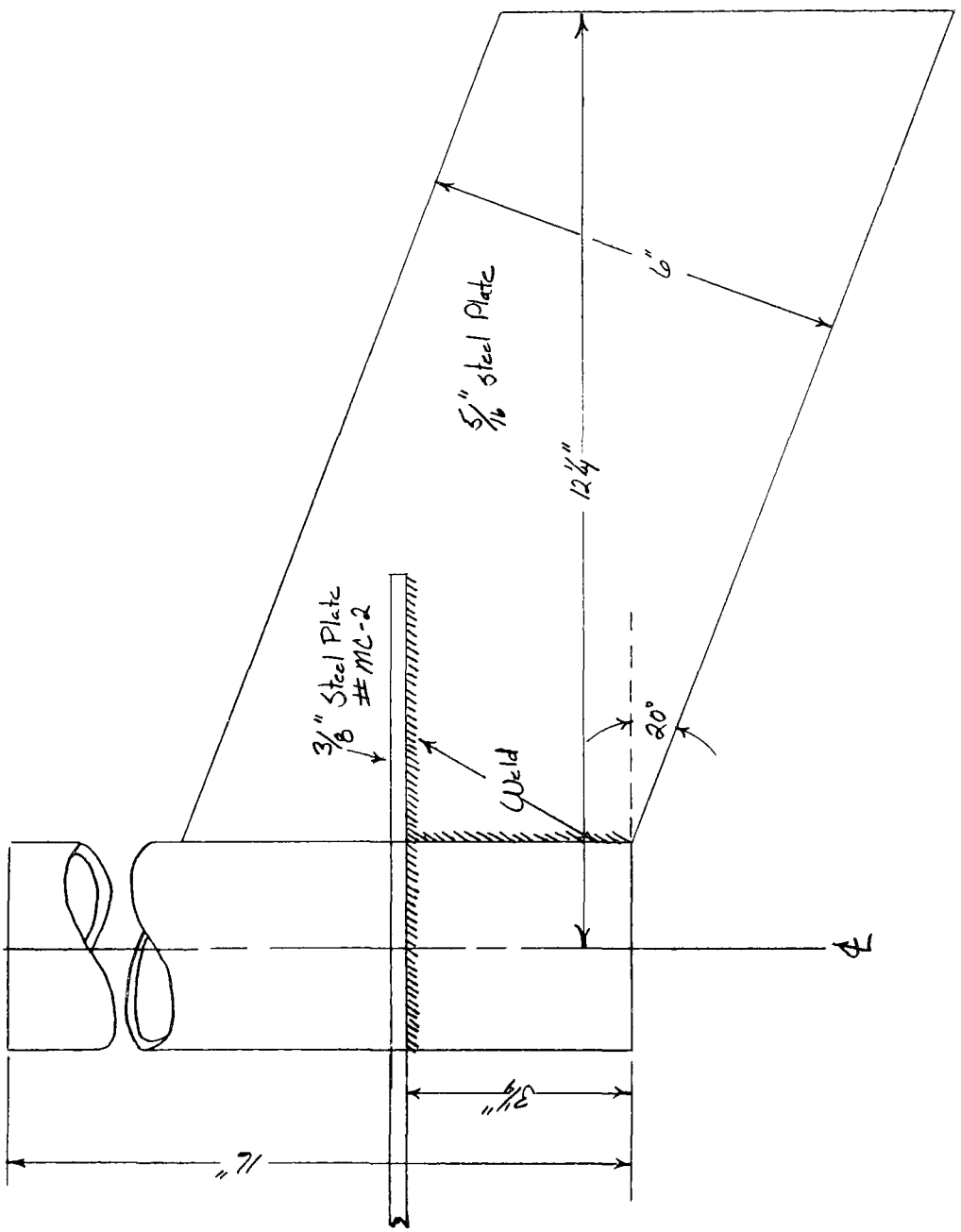

Figure 9. Detailed drawing of the weight stand assembly.

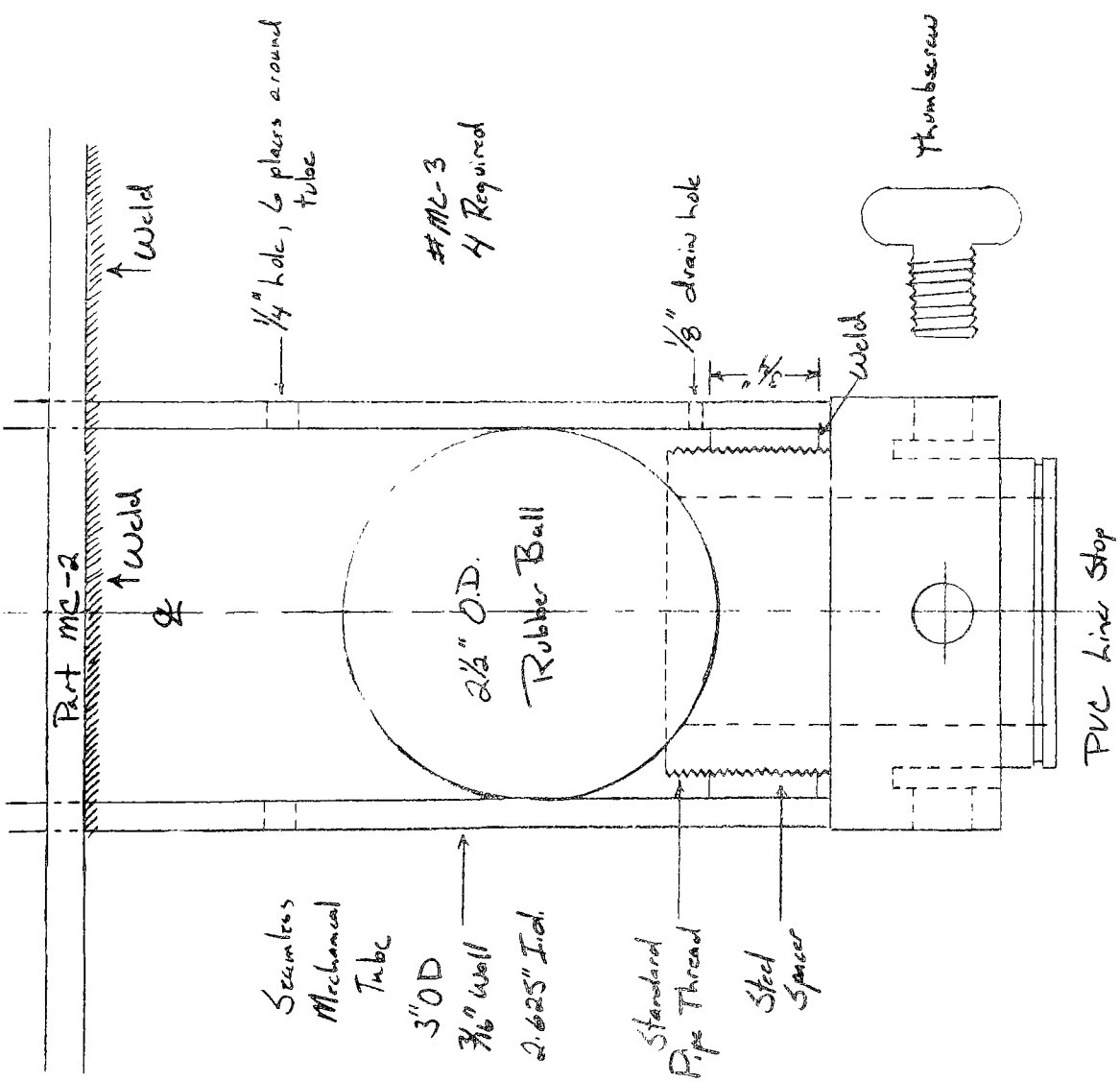

Figure 10. Detailed drawing of the lower end of the wieght stand into which is threaded a PVC coupling piece.

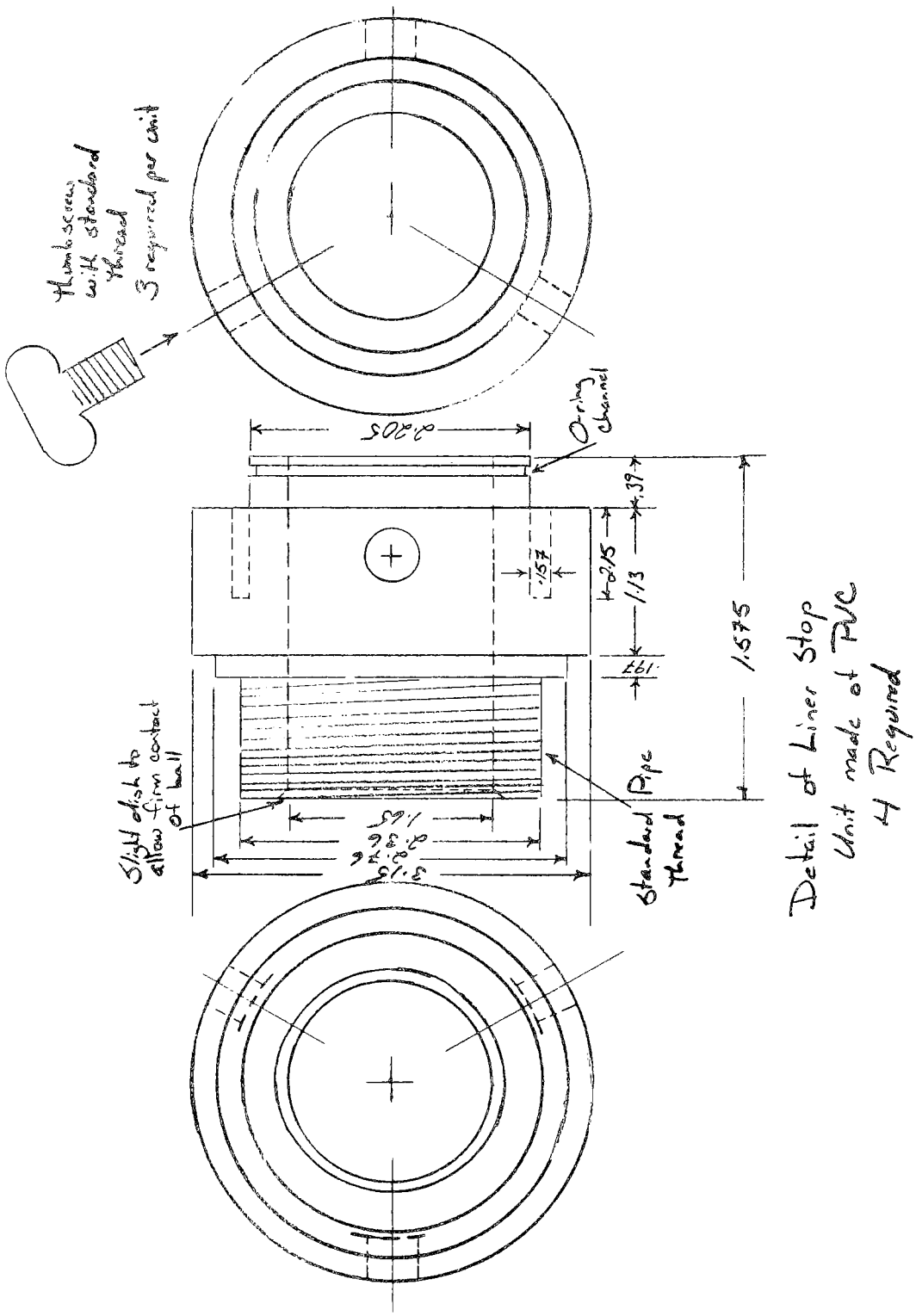

Figure 11. Detailed drawing of a PVC coupling piece that holds a 15-inch long coring tube in place.

ANNOTATED BIBLIOGRAPHY OF UW CONTRIBUTIONS
RESULTING FROM THIS AND RELATED PROJECTS

Pamatmat, M. M. A continuous-flow apparatus for measuring the metabolism of benthic communities. Limnol. Oceanogr. 10:486-489, 1965.

Continuous water flow per se over the sediment surface is not absolutely essential for estimating the rate of benthic oxygen uptake. Practically similar results are obtained by short-term measurements with bell jars. The important point is to minimize underestimation which would result from the development of a steep oxygen gradient next to the sediment surface if the experiment runs too long and the enclosed water is not stirred periodically.

Pamatmat, M. M. Ecology and metabolism of a benthic community on an intertidal sandflat. Int. Revue ges. Hydrobiol. 53:211-298, 1968.

The highest rates of benthic oxygen uptake have been measured in communities with high primary productivity and rapid sedimentation of organic matter, indicating that community metabolism is normally food limited and governed by its rate of supply. There is a pronounced seasonal cycle of community metabolism in False Bay which may be caused partly by temperature effect on metabolism and partly by a seasonal fluctuation in the size of the community. A study of metabolic temperature adaptation on the community level would appear to be useful.

Pamatmat, M. M. and D. Fenton. An instrument for measuring subtidal benthic metabolism in situ. Limnol. Oceanogr. 13:537-540, 1968.

In view of many possible sources of error in shipboard measurements, in situ measurements of benthic oxygen uptake are called for, if only to check initial results of a shipboard method. The in situ instrument was used to depths of 180 m and gave higher estimates of oxygen uptake than measurements with sediment cores aboard ship. The shipboard method was presumed to be in error and work was continued to improve it. This is supposed to be the first paper of a series on oxygen consumption by the seabed.

Pamatmat, M. M. and K. Banse. Oxygen consumption by the seabed. II. In situ measurements to 180 m depth. Limnol. Oceanogr. 14:250-259, 1969.

A summer increase in the rate of oxygen consumption by the sediment in Puget Sound was partly explained by the rise in temperature. Unexplained variability was attributed to the increased activity of small organisms following an increase in the supply of oxidizable organic matter from the plankton. There appears to be a need for understanding the seasonal response of the benthic community to temperature, i.e. whether the community adapts, and to what degree it adapts,

to seasonal temperature cycle. This study would require shipboard experiments.

Pamatmat, M. M. Oxygen consumption by the seabed. IV. Shipboard and laboratory experiments. Limnol. Oceanogr. 16:536-550, 1971.

High initial and operational cost of the in situ instrument, difficulties with and shortcomings of the in situ method made a shipboard method look attractive. Further work on the development of a shipboard method uncovered sources of error. Finally a shipboard method for measuring the oxygen uptake of undisturbed cores of sediment with their overlying water and intact mud-water interface gave the same results as in situ measurements at 22 m depth. Hydrostatic pressures up to 18 atm showed no effect on total oxygen uptake of cores from 180 m depth. Partitioning experiments with intact cores show ed that oxygen uptake by inorganic chemical oxidation is often greater than respiratory consumption. Rates of oxygen uptake off Peru and the coast of Washington and Oregon are given. An attempt to estimate anaerobic metabolism in the sediment by measuring rates of denitrification in different sediment layers was successful in detecting denitrification activity but it was not possible to determine the true level of undisturbed denitrification rates.

Pamatmat, M. M. Oxygen consumption by the seabed. VI. Seasonal cycle of chemical oxidation and respiration in Puget Sound. Int. Revue ges. Hydrobiol. 56:769-793, 1971.

Baseline study of benthic oxygen uptake in Puget Sound. Presents more evidence that benthic community metabolism is forced by the flux of oxidizable organic matter to the bottom. There is a seasonal cycle of oxygen uptake independent of the temperature cycle but the temperature cycle enhances the seasonal cycle of oxygen uptake which is highest in July, decreases through October to a low in January and increases from January through April to a high again the following July.

A correlation was shown between the concentration of reduced substances in the upper 4 cm of sediment and the rate of oxygen uptake by inorganic chemical oxidation. Since some of these reduced substances are metabolic by-products of anaerobic metabolism, it appears that the seasonal cycle of inorganic chemical oxidation may be indicative of a seasonal cycle of anaerobic metabolism. The concentration of reduced substances in the surface layer of sediments appears to be in dynamic equilibrium; deeper in the sediment the concentration increases with depth, indicating an accumulation. This, together with the observation by others that benthic oxygen uptake is independent of the length of the sediment core beyond a few centimeters deep means that the rate of chemical oxidation underestimates the anaerobic metabolism in the sediment column.

Pamatmat, M. M. Benthic community metabolism on the continental terrace

and in the deep sea in the North Pacific. Int. Revue ges. Hydrobiol., In press.

The rates of total oxygen uptake, residual oxygen consumption after poisoning with formaldehyde, and respiration of undisturbed sediment cores from the continental terrace, the abyssal plains, and the Aleutian Trench of the North Pacific were measured aboard ship. The rates of total oxygen uptake decreased with increasing depth of water. Respiratory uptake decreased with increasing depth to undetectable levels in the deep sea except at stations on the Aleutian Trench and relatively close to the Washington coast where there was additional evidence of anaerobic metabolism, especially of denitrification or nitrate reduction. In most of the North Pacific deep-sea stations, interstitial water appears to contain dissolved oxygen, there was no accumulation of reduced by-products of anaerobic metabolism and there was no evidence of denitrification or nitrate reduction; hence metabolic activity appears to be only aerobic. Even though the shipboard technique was not sensitive enough to detect aerobic respiration in most deep-sea core samples, significant metabolic activity in the sediment has taken place as indicated by the nutrient enrichment of interstitial water relative to the bottom water overlying the sediment. These observations emphasize the controlling importance of the rate of supply of oxidizable organic matter to the bottom in the metabolic activity of the benthos.

Pamatmat, M. M. Anaerobic metabolism in Lake Washington sediments. Limnol. Oceanogr. In press.

A dehydrogenase assay technique which gives relative estimates of benthic anaerobic metabolism, primarily of sediment microorganisms, was calibrated by direct microcalorimetry in order to be able to estimate the natural anaerobic metabolic activity in the sediment column. Dehydrogeanse activity, which generally decreased with depth of sediment layer, was detectable to 31 cm. The integrated metabolic heat release based on the measured dehydrogenase activity was invariably greater than the metabolic heat release calculated from the rates of oxygen uptake. Thus, it appears that the rate of total oxygen uptake by the sediment surface underestimates benthic community metabolism in the sediment column. The magnitude of the underestimation should be expected to be large in organic-rich sediments and may be negligible in oligotrophic waters.

Banse, K., F. H. Nichols, and D. R. May. Oxygen consumption by the seabed. III. On the role of the macrofauna at three stations. Vie et Milieu. Suppl. 22:31-52, 1971.

Exemplifies a computational method of determining the fraction of benthic community metabolism that is due to the macrofauna. Weaknesses in the approach involve the usual problem of sampling variability, sampling insufficiency, and the questionable applicability of respiration data from unrealistic experiments on the effects of temperature and oxygen tension on the

rates of oxygen consumption of infauna that are removed from the sediment. With these possible sources of error in mind, the total macrofauna are estimated to utilize 20 to 40 per cent of the oxygen consumed by the benthic community.

May, D. R. The effects of oxygen concentration and anoxia on respiration of Abarenicola pacifica and Lumbrineris zonata (Polychaeta). Biol. Bull. 142:71-83, 1972.

This paper is the fifth in the series on oxygen consumption by the seabed. It considers further the problem of realistically estimating the true rates of oxygen consumption by infauna in nature as distinct from their respiration under laboratory conditions. L. zonata appears able to regulate its respiratory rate down to an oxygen concentration of about 2 ml 0_2/1 while A. pacifica's respiration is a linear function of oxygen concentration at all concentrations up to 7 ml 0_2/1. Both species exhibit an ability to survive anoxia for several days. It is important to know the actual availability of oxygenated water to burrowing forms.

Nichols, F. H. Carbon and energy flow through populations of a numerically dominant macroinvertebrate, Pectinaria californiensis Hartman, in Puget Sound, Washington, with reference to larger, rarer coexisting species. Ph. D. Thesis, Univ. Wash., Seattle. 164 pp. 1972.

A thorough study of a single species' role and importance in the community at several stations in Puget Sound. The author concludes that the small but abundant P. californiensis contributes more greatly to the metabolic processes and to the food-chain dynamics of the seabed of Puget Sound than do the larger but rarer echinoderms, Brisaster latifrons and Molpadia intermedia. More data are presented and discussed relative to the problem of estimating the fraction of benthic community metabolism that is due to the macrofauna.

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27 Abstract

Techniques were developed for sampling undisturbed sediment interface, and measuring oxygen uptake by intact sediment cores, dehydrogenase activity of sediment bacteria, and metabolic heat release by benthic organisms. Dehydrogenase activity, a relative measure of anaerobic metabolism, was calibrated by direct microcalorimetry to provide estimates of actual metabolism under field conditions. The oxygen debt of sediments was determined by a dichromate method. Laboratory experiments were conducted to determine the relationship between oxygen uptake, loss of carbon, and release of silicate, nitrate, ammonia, and phosphate by sediments. The oxygen consumption at 33 stations in Puget Sound was measured each season to provide baseline data for this estuary. The original working hypothesis, that total oxygen uptake represents a measure of total metabolism in the sediment column appears erroneous, at least in organically rich sediment where anaerobic metabolism may greatly exceed aerobic metabolism. As sedimentation rate of oxidizable organic matter increases, as in cases of organic pollution and eutrophication, anaerobic metabolism becomes an important process that is measurable by dehydrogenase assay. In less organic sediments, the rate of oxygen uptake may be a fair estimate of total metabolism. Furthermore, it is a useful index of equilibrium conditions among the various factors that affect the rate of oxygen uptake, e.g. oxygen tension, temperature, turbulence, available metabolizable energy, composition of community, etc.

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