

AN ABSTRACT OF THE THESIS OF

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(Name of student) (Degree)

in Oceanography presented on May 29 - '73  
(Major department) (Date)

Title: DISTRIBUTION AND ABUNDANCE OF FOUR TRACE METALS IN NET

PHYTOPLANKTON AND SESTON OFF THE OREGON COAST

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Abstract approved: \_\_\_\_\_  
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Phytoplankton were collected in the Oregon coastal waters and the Columbia River Estuary from August, 1969, to October, 1970, and analyzed for their iron, zinc, copper, and manganese content using atomic absorption spectrophotometry. Analysis of the values obtained vs. salinity, temperature, density of seawater, distance from shore, season of the year, phytoplankton biomass, and phytoplankton species composition was performed. Salinity was the parameter most significantly correlated with the zinc, iron, and manganese concentration in the phytoplankton. Temperature modified this relationship for manganese, and residence time in the photic zone did likewise for zinc. Copper concentrations in phytoplankton were poorly correlated with salinity. Distance offshore correlated directly with zinc, copper, and iron. Low winter offshore phytoplankton iron concentrations and the presence of Planktoniella sol were also directly correlated. Copper, in terms of  $\mu\text{g Cu/g}$  phytoplankton ash was found to be specific for

the hydrographic regime where the phytoplankton were collected. Iron results were less reliable than the results with the other three metals due to various iron-specific problems encountered in sampling and processing. Possible explanations for the results are discussed, and several hypotheses are proposed for further investigation.

Distribution and Abundance of Four Trace Metals  
in Net Phytoplankton and Seston off the Oregon Coast

by

Allan Hayes Vogel

A THESIS

submitted to

Oregon State University

in partial fulfillment of  
the requirements for the  
degree of

Master of Science

June 1974

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Date thesis is presented----- 29 May 1973

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## ACKNOWLEDGMENTS

I am deeply indebted to my professor, L. F. Small, for his advice, assistance, and guidance on this work. Much of the field data could not have been collected without the assistance of M. A. Kyte, party chief of the Benthos cruises, and the personnel of the Hydrographic Section of the O.S.U. Department of Oceanography. To them, I give my heartiest thanks. Thanks are also due to H. Vanderploeg, who provided me with salinity data from the inshore areas for August, 1970, and to the ships crews of the R. V. Yaquina and R. V. Cayuse. The flowmeter used in my collection system was supplied courtesy of the Corvallis City Shops. J. Pequegnat and H. Stanford assisted me in the development of the laboratory techniques, and members of the Radioecology Section of the O.S.U. Department of Oceanography were most gracious in permitting me to use their atomic absorption spectrophotometer and in assisting me with the collection of samples and physical data from the Columbia River estuary. Statistical and computational assistance were provided by R. G. Petersen and D. Menzies, respectively. Criticism and valuable suggestions were given to me by many graduate school colleagues, especially P.L. Donaghay.

This work was supported under a U. S. Federal Water Quality Administration Traineeship, Grant Number 5T1-WP-111-04.

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DISTRIBUTION AND ABUNDANCE OF FOUR TRACE METALS IN  
NET PHYTOPLANKTON AND SESTON OFF THE OREGON COAST

INTRODUCTION

Rationale for this study

The hydrography of Oregon coastal waters is highly complex. During the late spring and summer a southward flow of water, induced by prevailing winds, carries the discharge of the Columbia River into the coastal waters off Oregon. There is also a net movement of water offshore at this time. This offshore movement produces upwelling of cooler, more saline waters along the coast. From October to March, net movement is reversed, the Columbia discharge flows north along the Washington coast, upwelling ceases, and there is high precipitation which causes the formation of a narrow band of relatively low-salinity water along the coast. Although the physical changes associated with this annual sequence of water movements have been known for some time, the biological changes accompanying the annual fluctuations are less well known. With the impending industrialization along the Oregon coast, changes might be expected in the concentrations of chemical elements and compounds present in the coastal waters. Such changes have already been noted by Feldman (1970) and Welch (1968) with respect to the concentrations of some of the transition metals in marine receiving waters. The changes noted by Feldman and Welch were caused by the output of secondarily treated domestic sewage and, significantly, all changes to date have been increases in concentration.

Algae have long been known to require more than just the half dozen or so major elemental components of their tissues. Besides the major elements carbon, hydrogen, oxygen, nitrogen, phosphorus, and



sulfur, and the somewhat more minor constituents potassium and magnesium, all algae studied to date require the transition or "trace" metals iron, zinc, copper, and manganese. Some algal species have also demonstrated a need (in the sense that growth and reproduction are halted in the absence of the required element) of silicon, calcium, sodium, boron, chlorine, cobalt, vanadium, and molybdenum (Arnon, 1958a, b; Wiessner, 1962). Probably most of the other elements are also accumulated by algae though no need has been demonstrated for them yet: notable in the group are nickel, titanium, selenium, lead, silver, gold, bromine, iodine, aluminum, and others. None of the so-called minor constituents, with the exceptions of silicon to diatoms and possibly cobalt to certain neritic species (Provasoli, 1963), seem to be more significant to algal growth, reproduction, and metabolism than iron, zinc, copper, and manganese.

The rapid uptake of these four trace metals and of others, in combination with the increases in their concentrations in marine waters which receive domestic sewage, has given Feldman (1970) a deep sense of concern as to the possible effects sewage, both domestic and industrial, is having upon the marine biota. He feels that its effects are unpredictable, and that "weed" species of phytoplankton (species relatively unsuited for zooplankton and benthic grazers, hence a non-usable food base) may grow better in bays and estuaries polluted with domestic sewage. Ryther (1954) documents the takeover in Moriches Bay, Long Island, after a sewage outfall was constructed there, by Stichococcus, a genus of phytoplankton labelled a "weed" genus. Increased concentrations of all required elements for growth (including the trace metals), plus increases in vitamins and other organic growth factors, no doubt acted in concert to create the environment favoring the "weeds."

The orientation of my study is toward providing some information on the present annual succession of trace metals in net phytoplankton and seston in the several different hydrographic regimes off the relatively unpolluted Oregon coast. Such observations might make it possible to predict what the changes due to pollution will be or, at least, after the event, what the gross changes were. Also, no information currently exists on trace metal concentrations in relatively pure phytoplankton samples.

The four trace metals, iron, zinc, copper, and manganese, were selected because they are physiologically important elements accumulated by phytoplankton in low but detectable concentrations. The immediate objectives of the present study were (1) to identify, if possible, causative agents of variation of trace metal concentration in natural phytoplankton populations and the accompanying seston, and (2) to determine whether or not concentrations of any of the four trace metals studied herein can serve to characterize the hydrographic regime in which the sampled phytoplankton population is growing.

#### Definitions

Unfortunately, standardization of methodology in trace metal work does not exist; hence, results by various workers have been reported in various terms, many of which are not interconvertible (given the data reported in the literature). Below are definitions I have used in the present study. Some of the definitions are slightly different from those used by others, mainly because my methods of collection involved net filtration of large samples rather than filtration through small-pore-size membrane filters:

1. ionic: metal in true ionic state in seawater.
2. soluble: metal in either inorganic or organic state, but dissolved and capable of passing through a membrane filter of 0.5u nominal pore size. Soluble metals might be partly or totally ionic, or completely non-ionic.
3. membrane particulate: metal in either inorganic or organic state, but retained by a membrane filter of 0.5u nominal pore size. Membrane particulate metals might be partly net particulate metals.
4. net particulate: metal in or on inorganic or organic particles retained by a mesh of 43u after a prefilter through 351u mesh.
5. membrane total: the sum of all ionic, soluble, and membrane particulate metal in a sample of seawater.
6. net total: the sum of all ionic, soluble, and net particulate metal in a sample of seawater.
7. total: the sum of all ionic, soluble, membrane and net particulate metal in a sample of seawater.

To fractionate further the soluble and two particulate terms the following three terms can be defined:

1. soluble organic: metal in or on dissolved organic matter and capable of passing through a membrane filter of 0.5u nominal pore size. The counterpart is soluble inorganic metal.
2. membrane particulate organic: metal in or on particulate organic matter retained by a membrane filter of 0.5u nominal pore size. The counterpart is membrane particulate

inorganic metal.

3. net particulate organic: metal in or on particulate organic matter retained by a mesh of 43u after a prefilter through 351u mesh. The counterpart is net particulate inorganic metal.

A number of obvious combinations of these definitions also exists.

#### LITERATURE REVIEW

##### Metabolic Roles of Iron, Zinc, Copper, and Manganese

Iron, the most abundant of the micronutrients in algae, was first reported to be essential for algal growth in 1927 (Hopkins and Wann, 1927). It is required for the cytochrome system, nitrogen fixation, ferredoxin, porphyrins, and numerous metalloenzymes (Eyster, 1964, 1968; Nicholas, 1963). Chlorosis occurs in iron-deficient algal cells and can be only slowly reversed (Hayward, 1968; Pirson, 1955, 1958). Davies (1970) noted that the production of chlorophyll may require the presence of organic iron complexes. Pirson (1955) postulated that iron may be essential as a detoxicant of other heavy metals (e.g., copper) within cells, but this has not been proved yet. There also might be a need for iron as a structural material in some algae (Bowen, 1966). The mechanism for iron uptake is not clearly understood. Rate of uptake is very slow in comparison to the division rate in a fast-growing culture (Hayward, 1969), and growth can occur without measurable iron in the culture medium (Davies, 1970), two results which strongly imply the control of growth by intracellular iron reservoirs. The

concentration of iron within algal cells, as well as that of zinc, copper, and manganese, is dependent upon the concentration external to the cells (Bachman, 1963; Davies, 1970; Hayward, 1969; Knauss and Porter, 1954; Riley, 1939). This dependence upon the external media can sometimes lead to algal cells having these metals far in excess of their apparent needs (Pequegnat, et al., 1969). Iron is perhaps not a good example to demonstrate intracellular pooling, however, because of its several oxidation states and many different uses in the cell (Benoit, 1969).

Zinc is a common element in algae, mainly because the ionic form is capable of forming complexes with organic ligands throughout the algal cells. Some zinc is required in the prosthetic groups of several metalloenzymes, but Pequegnat, et al. (1969) calculated that the maximum zinc requirement for enzyme structure, per unit net weight of live marine organisms, is 2.7 ppm. The zinc concentration is usually far in excess of 2.7 ppm in marine organisms. Bachmann and Odum (1960) found, for six species of benthic algae, that zinc uptake is proportional to gross oxygen production, and accumulation is proportional to net oxygen production. Such proportionality could be the result of decreasing pH in the medium with increasing oxygen production. The availability of ionic zinc in solution is strongly pH-dependent (Gutknecht, 1961). Zinc uptake is a continual process (Hayward, 1969), obeying Freundlich's adsorption equation (Bachmann, 1963). Dead cells also take up zinc, again by adsorption. The strong relationship between uptake and adsorption argues against the use of zinc uptake as an index of photosynthetic or other metabolic processes.

Copper is involved in the "light reaction" of photosynthesis (Eyster, 1964; Green, et al., 1939). Its exact role is not clearly

known yet, although Hewitt (1951) and Podder and Saha (1969) suggest that it might be a necessary catalyst for the formation of porphyrin. Enzymatically, it is used in various oxidases; ten have been identified (Bowen, 1966). Copper uptake also obeys Freundlich's adsorption equation (Riley, 1939).

Eyster (1961, 1964, 1968), Pirson (1955, 1958, 1960), and their coworkers (Brown, 1961, et al, 1955, 1958; Eyster, et al, 1956, 1958a, b; Noack, et al., 1939; Pirson, et al., 1952, 1955) have shown that manganese is specifically required for oxygen evolution, Krebs cycle decarboxylations, and the Hill reaction in photosynthesis. Reisner and Thompson (1956) found that it was also required in heterotrophic cells for non-photosynthetic reactions, and Pirson (1958) reported that it was required in nitrate reduction. Manganese uptake has been shown to increase gradually during the lag stage and early log growth of Phaeodactylum tricornutum, then while the culture is still in log growth the uptake declines, and finally it levels off when the population reaches its maximum density (Hayward, 1969). In some marine diatoms (Ditylum brightwellii for one, but not Stephanopyxis palmeriana) a manganese deficiency causes the formation of sexual stages (Steele, 1965).

In general, the lack of any one of these four essential micro-nutrients produces a noticeable lack of cell division plus other metabolic changes.

#### Forms and Concentrations of Iron, Zinc, Copper, and Manganese in the Sea

The average membrane total concentrations of these metals in seawater is 3 ug/l for iron, 10 ug/l for zinc, 3 ug/l for copper, and

2 ug/l for manganese (Chow, 1968; Goldberg, 1957, 1965; Goldberg, et al., 1971; Horne, 1969). However, there are wide variations between water masses, at different times of the year, and in different geographical regions in the ocean with respect to the different physico-chemical forms of each element. It is well known that iron is present in all states in seawater, i.e., as soluble inorganic, particulate inorganic, soluble organic, and particulate organic iron. Membrane particulate inorganic iron is the most abundant form (Cooper, 1935, 1937, 1948; Seiwel, 1935). Membrane total iron varies in concentration from water mass to water mass (Cooper, 1948; Goldberg, 1963, 1965), and shows a decreasing gradient seaward (Gran, 1932; Menzel and Ryther, 1961; Thompson and Bremner, 1935) probably due to land runoff (Schaefer and Bishop, 1958). It might generally increase in concentration with depth (Cooper, 1948; Lewis and Goldberg, 1954), although Menzel and Spaeth (1962) did not find any such increase in the Sargasso Sea. In the offshore northeast Pacific, Thompson and Bremner (1935) reported a membrane particulate iron concentration of 17.1 ug/l between April and September, the annual high period. These authors, as well as Jamieson (1932), noted a membrane total iron concentration in Puget Sound of 30 to 50 ug/l. For the Washington coastal waters in 1932 an average value for membrane total iron over a series of samples collected from 0 to 300 m was 73 ug/l (Lewis and Goldberg, 1954), although the time of year for this collection was not given. Offshore stations in the northeast Pacific have membrane total iron concentrations between 1.7 and 7.3 ug/l (Lewis and Goldberg, 1954; McAllister, et al., 1960). A strong note of caution must be given for all the iron values reported, however, because Betzer and Pilson (1970) have found that Nansen bottles can

increase membrane particulate iron concentration by 15 to 86 times over those concentrations in water retrieved from Niskin bottles. If this is a general source of error, then all particulate iron values to date might be meaningless.

Zinc in seawater, other than its isotope  $Zn^{65}$ , has not been examined in too great detail. Zirino and Healy (1970) report that a substantial portion of added zinc can be partitioned into the inorganic  $ZnCO_3$  and  $Zn(OH)_2$  complexes. Other workers have concluded that most zinc in seawater is found in the form of organic complexes (Macchi, 1965; Piro, et al., in press). The concentration of membrane total zinc in seawater varies between 1.2 and 30 ug/l (Rice, 1963), which is only, at the most, 0.008 the saturation level of ionic zinc in seawater (Park, 1969). From this can be inferred that biogeochemical processes are rapidly utilizing zinc. Morita (1953) found 2.2 to 2.3 ug membrane total zinc/l in Sagimi and Suruga Bays, Japan, decreasing seaward to a concentration of 1.3 to 1.5 ug Zn/l in the Kuroshio Current. Although much work has been performed with  $Zn^{65}$  in Oregon coastal waters, very little has been done with non-particulate, soluble stable zinc. A monthly series of soluble stable zinc values for the Columbia River estuary at Astoria has been taken, and the values ranged from 5.2 to 14.6 ug/l zinc. Such values compared favorably with the values reported by Rice (1963). The soluble stable zinc concentrations represented 37 to 65% of membrane total zinc in the same region (Wagner, personal communication).

The abundances and distributions of copper in natural waters have been studied in more detail than those of zinc. There are two common dissolved states of copper in natural water, ionic and soluble



organic (Foster and Morris, 1971; Hutchinson, 1967). Ionic copper is primarily influenced by land runoff (Chow and Thompson, 1954; Foster and Morris, 1971). The soluble organic copper is a result of primary production, being either a direct excretion product or an in situ interaction product between ionic copper in the water and excretion products (Foster and Morris, 1971). Copper is unlikely to be metabolically limiting in freshwater because of the formation of usable complexes (Hutchinson, 1967); however, in the sea its dissolved concentration is at most only 0.007 of the saturation level (Park, 1969), implying a rapid removal rate which might cause it to be limiting in some areas. Atkins (1932), the first investigator to look for copper in seawater, found only 10 ug soluble Cu/l in the English Channel. He later found it varied seasonally from 25 ug/l in the winter to a minimum of 3 ug/l in October, with the decline starting in February with the first phytoplankton bloom (Atkins, 1953). This seasonality has also been noted in freshwater (Hutchinson, 1957; Riley, 1939). The concentration of soluble copper does not vary with tidal cycle (Chow and Thompson, 1954; Galtsoff, 1943), but does vary between water masses and decreases with distance offshore (Chow and Thompson, 1952; Galtsoff, 1943; Harvey, 1955; Riley, 1937). The concentration of soluble copper has been found to decrease with increasing salinity, sigma-t, phosphate, and silicate (Chow and Thompson, 1954; Riley, 1937). Its concentration increases with increasing depth (Riley, 1937). Riley (1937) reports that the Mississippi River plume has higher concentrations in it than the seawater around the plume.

The concentration of soluble copper in Pacific waters varies from 0.8 to 0.9 ug/l in Sagimi and Suruga Bays, to 0.5 to 0.7 ug/l in

the Kuroshio Current (Morita, 1953), to 1.0 to 1.8 ug/1 in San Juan Channel, Washington (Chow and Thompson, 1954). In offshore waters near the Washington coast the range is from 1.0 to 1.3 ug/1 (Chow and Thompson, 1954). Chow and Thompson (1954) report one anomaly in their copper data, the fact that the seasonal pattern is the reverse of that reported by Atkins (1953), Hutchinson (1957), and Riley (1937), but they give no explanation for this difference.

The concentration of membrane total manganese in seawater varies between 0.25 to 10 ug/1 in the Atlantic Ocean and Gulf of Mexico (Harvey, 1949, 1955; Wangersky, 1963), and between 0.27 and 14 ug/1 in the Pacific (McAllister, et al., 1960; Wangersky, 1963). It is much lower offshore, only 0.27 to 0.93 ug/1 in the Pacific (McAllister, et al., 1960). One cause of this inshore-offshore difference is rapid deposition of manganese in the inshore waters (Wangersky, 1963), and another is rapid uptake by phytoplankton (Thompson and Wilson, 1935). Both Harvey and Wangersky feel that manganese might be limiting primary production offshore, but this has not been proved. McAllister, et al. (1960) did not detect any limitation of growth in the northeast Pacific, while Hutchinson (1957) found it clearly limiting to phytoplankton growth in several English lakes, and Harvey (1947, 1949) found additional manganese necessary for marine phytoflagellates grown in unenriched English Channel waters.

It becomes evident from the discussions above that trace metal concentrations in natural phytoplankton populations are limited principally by the concentrations of utilizable forms of the element in the water. As a corollary, the concentrations of utilizable forms might be functions of the amounts of chelators available to put the metals into

utilizable forms (Johnston, 1963; Provasoli, 1963; Schelske, 1962; Schelske, et al., 1962; Wetzel, 1966). Although at times the elemental concentration itself can be the limiting factor (Harvey, 1947, 1949; Hutchinson, 1957), one must be cautious in assigning this interpretation to field enrichment experiments which seem to suggest limiting concentrations. The lack of a usable form of an element might be the limiting factor in many cases (Ryther and Guillard, 1959; Menzel and Ryther, 1961; Menzel and Spaeth, 1962; Menzel, et al., 1963). In addition to general population growth limitation, the availability of certain trace metals in natural waters is known to influence phytoplankton speciation. Iron (Ryther and Kramer, 1961) and manganese (Hutchinson, 1957) have been identified as causative agents in species changes in phytoplankton communities.

#### Concentrations of Iron, Zinc, Copper, and Manganese in Algae

Trace metal concentrations in marine benthic algae, on a dry weight basis, range from 4 to 3380 ppm Fe, from 7 to 2800 ppm Zn, from 1 to 243 ppm Cu, and from 4 to 800 ppm Mn (Black and Mitchell, 1952; Fukai and Meinke, 1959; Wort, 1955; Young and Langille, 1958). Trace metal concentrations on a dry weight basis in marine phytoplankton previously reported in the literature includes 300 ppm Cu for a mixed plankton sample of Calanus finmarchicus and neritic phytoplankton (Nicholls, et al., 1959), and 200 ppm Cu, 3500 ppm Fe, 75 ppm Mn, and 2600 ppm Zn for a natural phytoplankton population consisting mainly of diatoms (Bowen, 1969). Vinogradov (1953), giving the most comprehensive coverage on a dry weight basis to date, reported 500 ppm Fe and 0.3 to 0.4 ppm Mn for a Microcystis bloom in the Sea of Azov in 1939; 9800 to 13,300 ppm Fe

for several Perdinieae (mostly Ceratium tripos) blooms in the Bay of Kiel from 1914 to 1922; 31,400 ppm Fe for diatom blooms in the Bay of Kiel for the same time period; 11,300 ppm for a diatom bloom in the Gulf of Kola in 1933; 1000 to 2300 ppm Fe for a Rhizosolenia bloom and 6300 ppm Fe for a Coscinodiscus bloom, both in the Caspian Sea in 1939. Manganese and copper concentrations on a dry weight basis were 0.4 to 0.7 ppm for Rhizosolenia blooms in 1934 and 1935, and near 240 ppm for a Coscinodiscus bloom in 1938. Nearer to the Oregon coast, Thompson and Wilson (1935) reported a manganese concentration of 700 ppm on an ash weight basis in diatom blooms in San Juan Channel, Washington, in 1932 and 1933.

Cooper (1935) reported 2.3 ug of phytoplankton iron/l during a bloom in the English Channel. He did not report the dry or ash weight of the phytoplankton per liter, unfortunately.

The extreme variability of the above values is somewhat of a problem to explain. Differences in the concentration of the utilizable metal in the water and age of the sampled population are two possibilities. Data from Knauss and Porter (1954) supports the first possibility and from Young and Langille (1958) the second. There are no reports in the literature in which metal concentrations in relatively pure phytoplankton samples have been related to conservative hydrographic properties of the water.

## METHODS AND MATERIALS

### Field Collections

There were three systems used for phytoplankton collection from the sea. The first system employed a 1/2 hp, 115 v, 8-stage Teel deep-well submersible pump (capacity of 775 gal/hr at a 50 ft head) which

pumped seawater through a 351  $\mu$  prefilter and 43  $\mu$  collecting net, then past a flow meter. This system was used solely on the August 1969 cruise. The hosing on the pump was all plastic and rubber, and the pump itself was made of stainless steel with brass fittings. Water contact with the fittings was minimal, and statistical tests on the results later showed no obvious differences between the samples collected this way and with the other two systems. Water could be gathered with this pump from the surface to 55m; however, phytoplankton in sufficient concentration to make harvesting worthwhile was collected only from the top 10 m. Contamination of the phytoplankton collections by zooplankton and large detrital particles was reduced by using the prefiltering net and by pumping only during the daylight hours near the sea surface. The collecting net was cleaned of its phytoplankton harvest by scraping into a glass collecting jar, using a plastic spoon and a squeeze bottle of seawater. Subsamples were taken for species counts and preserved in Lugol's solution ( $KIO_3$  and  $I_2$  in glacial acetic acid and distilled water).

A towed net was the second collecting system. The net was used only for the October 1969 and March 1970 inshore samples. It was also used for a comparison with the third system in the May 1970 Inshore sample. The net had a fixed diameter and was towed at 0.5 knot over a north-south track parallel to a line between two shore markers with a known distance between them. The collected material was processed similarly to that collected in August 1969.

The third system was used to collect all of the remaining samples. It consisted of a 1/4 hp Flotec Rotolator pump, a brass flow-meter, and three sections of clear plastic hose, 2 cm in diameter. The

body and impeller of the pump were made of Epoxy, the seals of plastics, and the shaft, cone and backplate of stainless steel. The flowmeter was accurate to 0.01 cubic foot, and its interior was coated with a teflon compound to prevent corrosion and sample contamination. An examination of the inshore samples collected by the second and the third systems in May 1970 revealed no significant differences at the 90% level, so apparently the teflon coating was sufficient to insure negligible contamination. The October inshore samples were collected in 1968 and 1969 by the second system and in 1970 by the third system. Concentrations of the four metals in phytoplankton in the October 1970 samples were not significantly different from those of preceding years, further evidence that at least does not negate the hypothesis of insignificant difference in trace metal values between the two systems.

For the operation of the third collection system, a 3 m length of the plastic hose was connected to the intake of the pump and the opposite end, joined to a plastic and teflon-coated brass foot valve, was lowered into the water from the deck of the research vessel. The pump was connected to the flowmeter by a 25 cm-long section of the plastic hose. The effluent flow from the flowmeter was directed through the filtering nets on the ship's deck by means of another 3 m length of the plastic hose. All phytoplankton collected by this system came from within 1 m of the surface. It was processed similarly in the field to that collected by the first system.

Because of the great volume of seawater required to be filtered to obtain a single concentrated phytoplankton sample, most of the samples were integrated cruise track samples rather than point samples.

The August 1969 samples are an exception to this, being several point samples combined into a single sample.

Two physical parameters, water temperature and salinity, were monitored during most phytoplankton collections. On a few occasions no salinity data were available. Sigma-t values were calculated from the temperature and salinity values.

#### Identification of Hydrographic Regimes

The total area sampled is bounded by the Oregon coast on the east,  $46^{\circ}30'N$  latitude on the north,  $126^{\circ}30'W$  longitude on the west, and  $43^{\circ}55'N$  latitude on the south, plus the Columbia River estuary as far up as the Astoria bridge (Fig. 1).

Identification of the water being sampled was based on four criteria: time of year, geographic location, salinity, and temperature. Because the Columbia River plume moves north from October to April and upwelling is absent (with one possible two-week exception in February which was not sampled), only the distance offshore is critical in determining hydrographic regime for over half the year. Approximately 15 to 20 nautical miles offshore represented the break between the Inshore and the Offshore hydrographic regimes; most of the Offshore stations were over 50 nautical miles from the shore. The 15 to 20 mile break represented the approximate division between "green" water with its high phytoplankton productivity and "blue" water with a low productivity. This break also approximated the 200-meter depth contour off the Oregon coast, the approximate edge of the continental shelf.

During the summer (June through mid-September) salinity and temperature become extremely important in assessing hydrographic regime. The Columbia River Plume is defined as any surface water contiguous with the river mouth having  $\leq 32.5\%$  salinity, while Upwelling regimes are defined as any water adjacent to the coast that has  $\geq 33.25\%$  salinity and  $\leq 10^{\circ}\text{C}$  temperature. The normal distribution of the marine regimes during the summer is likely to be: (1) a shallow tongue of Plume water, overlying Offshore waters, extending southwest from the Columbia River mouth for several hundred miles; (2) several Upwelling fronts in the Inshore area (the number dependent upon bottom topography and recent local weather); (3) Offshore waters approximately 145 to 185 nautical miles off Newport; that is, beyond the seaward edge of the plume; and (4) a thin compressed wedge of Offshore water occasionally on the surface between the Plume and the Inshore/Upwelled areas.

The Columbia River Estuary was the fifth hydrographic regime sampled. The selection of the Columbia River Estuary as a hydrographic regime was done because it is sharply defined hydrographically from the marine areas. If there were no difference between the Columbia River Estuary metal concentrations in phytoplankton and the marine values, then trace metal concentrations in phytoplankton could not be allied to conservative properties of seawater.

#### Laboratory Processing

On board ship the phytoplankton samples were allowed to separate into water and phytoplankton phases. The water was then decanted off and the phytoplankton was stored in a freezer. In the laboratory the



samples were placed in a drying oven at 28 to 30°C. The dry samples were then transferred to a crucible, weighed, ashed at 475 to 500°C, and reweighed. The transfer from sample jar to crucible was difficult as the dried material showed a tendency to stick on the sides of the sample jar; however, a glass rod and a plastic spoon uniformly removed 97 to 100% of the material. The average weight loss on ashing was about 15%; the range was from 10 to 35%. The ashed samples were stored in a desiccator until treated for silicate removal. They could be stored as long as the desiccant was good.

The bulk of the phytoplankton sampled was diatoms; therefore, it was necessary to desilicify the samples before analyzing for the four metals, because silicon interferes with the determination of these metals by atomic absorption analysis, the method used in this research. Desilicification was begun by placing between 0.3 and 0.4 grams of the ashed sample into a teflon beaker. This subsample was then washed with double-distilled water (DDW). Preliminary experiments demonstrated that, for the four metals studied here, DDW provided less contamination than deionized DDW and considerably less than water distilled only once. In sequence, 1 ml of 30%  $H_2O_2$ , a few drops of concentrated  $H_2SO_4$ , and 0.5 ml of concentrated HF were added to the ash and water. This mixture was then heated to a slurry. Another 0.5 ml of HF and some DDW were added, and heating to a slurry was repeated. This process was repeated twice more, first with the addition of 1 ml of  $H_2O_2$  and then without the  $H_2O_2$ . Finally, 1 ml of  $H_2O_2$  was added by itself and the solution heated to a slurry. There was a total of 3 ml of  $H_2O_2$  and 2 ml of HF added to the ash and DDW. To the final slurry was added 1.25 ml of 12 N HCL. The resultant solution, silicon-free by all measurement criteria, was put

in a 25 ml volumetric flask, and the flask was filled to the mark with DDW. This sample could then be stored, prior to the next stage of processing, for up to nine weeks without any detectable change in trace metal concentration.

If extreme care were not exercised during desilicification to insure low and even heating, splattering of the sample occurred, causing loss from that sample and possibly addition to any other sample being processed at the same time. Examination of the results seemed to indicate that splattering is a greater source of error than all other sources combined. Too much heat also could warp and destroy the teflon beakers.

The next stage of processing was the determination of the concentration of the metals in solution by means of a Perkin-Elmer model 303 Atomic Absorption Spectrophotometer (AAS). The AAS was first calibrated with a number of standards covering the range of expected metal concentrations, and a DDW blank was determined. Multiple measurements of each sample then followed, with DDW blank determinations spaced between them. During long AAS runs, standards were also redetermined. At the end of each run standards and DDW blank determinations were performed to check for instrumental drift. The AAS readings were converted into ppm of metal in phytoplankton ash. For the theory and general techniques behind the AAS, see Allaway (1965), Angino and Billings (1967), Elwell and Gidley (1962), Mizuike (1965), Morrison and Skogerboe (1965), Robinson (1966), and Weberling and Cosgrove (1965).

Because of the presence of other metals and materials in the phytoplankton ash, interference with the iron, zinc, copper, and manganese peaks measured by the AAS was frequently, but not always,

encountered. To strip out these "matrix effects," multiple samples were necessary, with some of the samples having a series of standard metal additions made to them. Multiple samples also made it possible to check for processing and AAS malfunctions. Manganese consistently had very little interference at the absorption wavelength of 2800Å, and copper and zinc were not badly affected at some of their absorption wavelengths; however, iron had serious "matrix effects" at all wavelengths utilizable by the AAS. As a result, single iron values potentially had great error associated with them. As a rule in my samples, however, the "matrix effect" remained small and approximately constant, so that uncorrected values usually were no more than 5% above the matrix-corrected value for iron in all hydrographic regimes sampled. The effect changed only with time of year, being slightly greater in the spring; however, the change was not significant. Also, multiple samples were usually available, so that the "matrix-free" iron concentration could be calculated.

Standards for the AAS were prepared by dissolving 1.000 g of reagent grade metal in 11 ml of 6 N HCL, after which 25 ml of DDW and 5 ml of concentrated  $\text{HNO}_3$  (reagent grade) were added and the resultant solution boiled for 10 minutes. The solution was then cooled and diluted with 50 ml of 12 N HCL and DDW to make 1 liter. The resultant stock solution had a concentration of 1000 ppm of the metal. Laboratory standards were then made from the stock solution, DDW, and enough 12 N HCL to keep the acidity at 0.6 N. The laboratory standards had concentrations of 0.1, 0.5, 1.0, 2.0, 3.0, and 5.0 ppm for manganese, copper, and zinc, and 2, 5, 10, and 20 ppm for iron. Preliminary experiments demonstrated that the laboratory standards remained at these

concentrations for at least nine weeks; however, to be safe, fresh standards were prepared every time a run was made on the AAS.

Trace metal concentrations in each phytoplankton sample were examined as functions of volume of water pumped. This was done in order to determine whether there was rapid sorption or desorption of metal when the phytoplankton cells already trapped in the net were subjected to a steady renewal of water. Hynes (1970), in an excellent review on the effects of flowing water upon attached algae, notes that velocity of flow, hence rate of replacement of nutrient-containing waters, has a marked long-term effect upon algal production. There was no correlation at the 90% significance level between the water volume pumped and the concentrations of any of the metals in my collected phytoplankton.

Taxonomic identification to species was performed in conjunction with the metal analyses. The planktonic diatom identification was based on Cupp (1943), the dinoflagellates on Lebour (1925) and Wood (1954), and the silicoflagellates on Geminhardt (1930). No attempt was made to identify any other forms.

All cells within a microscope field were identified and counted. A number of randomly chosen fields (from 6 to 20 in number, depending on the density of the preserved subsample) were enumerated until there were several hundred cells counted. The number of species for these samples was independent of the number counted, or at least there was no statistical correlation between the two factors. Relative abundance was determined in percentage of the total count, and summed percentage contribution of the four most abundant species was calculated. If this index were less than 80%, then the sample was considered to have

no predominant species. Sometimes only two or three species added to over 80% of the total count. This identification effort was more in the nature of a data sweep than any preplanned hypothesis-testing. Its function was mainly to note any large-scale species differences which might be associated with the different hydrographic regimes, thereby yielding species-specific trace metal concentrations.

## RESULTS

### Relationships between trace metal concentrations and the measured parameters

Zinc in net phytoplankton is negatively related to water salinity in the range 30.0 to 33.8‰, and apparently the relationship holds for salinities as low as 24.9‰ (Fig. 2). The predictive linear equation, based on the salinity (S, in ‰) of seawater from which the phytoplankton was collected, is  $Zn(\text{ug/g ash}) = 3270.25 - 93.80 S$ . Manganese in phytoplankton, like zinc, is negatively related to salinity, but with somewhat lower correlation (Fig. 3). The predictive equation for manganese is:  $Mn(\text{ug/g ash}) = 2703.06 - 80.58 S$ . Phytoplankton iron content also is reasonably related to salinity (Fig. 4) with a correlation coefficient of 0.74. The equation is  $Fe(\text{ug/g ash}) = 4546.2 - 1302.5 S$ . Phytoplankton copper showed no meaningful relationship to salinity.

Phytoplankton trace metal concentrations were very poorly related to water temperature. Particulate manganese showed the best simple correlation ( $r = 0.36$ ), but the relationship was not good enough to predict adequately manganese concentration by itself. Using multiple linear regression techniques, the equation  $Mn(\text{ug/g ash}) = 3482.05 - 95.74 S - 26.48 t$ , where t is the temperature in degrees C, was found to increase the coefficient of determination (or percent of the

variation explainable by the variables tested) from 0.75 (considering salinity only) to 0.81, or by 6%. None of the other three metals had any significant relationship to temperature, and multiple regression analyses did not improve the equations relating phytoplankton metal concentrations to salinity alone.

Trace metal concentrations in phytoplankton were negatively related to water density ( $\sigma_t$ ), although as expected, the relationships usually were not as good as with salinity. Multiple linear regression showed a very high correlation between salinity and  $\sigma_t$  (0.9998), while temperature, by comparison, was more poorly correlated with  $\sigma_t$  (0.9745). Because the  $\sigma_t$  relationship to trace metal concentration is dependent upon the primary physical parameter, salinity, equations using  $\sigma_t$  for predicting trace metal concentrations of net phytoplankton in Oregon coastal waters may be used; however, where salinity data is available, those data should be used preferentially.

The concentration of phytoplankton ash dropped sharply during the summer months between 12 and 20 nautical miles from shore, separating the Inshore from Offshore regimes (Fig. 5). The ash concentration at 12 miles was 7.7 mg/l and it dropped to 0.6 mg/l at 20 miles. There was only a slight decrease from 20 miles seaward, although at 100 miles phytoplankton ash was less than 0.1 mg/l regardless of season. Inshore of 20 miles, summer concentrations were almost always higher than those in winter, although on three occasions upwelled water and Columbia plume water had concentrations equally as low as winter and offshore concentrations. The far-inshore (<5 miles) ash concentrations are undoubtedly low because of freshly-upwelled water devoid of

phytoplankton cells. The rapid proliferation of phytoplankton to maximum concentration at 10 to 12 miles is easily observed in Figure 5, and coincides with the physical upwelling model proposed by Mooers and Smith (1967) and with the distributions of chlorophyll, oxygen, and nutrients given for the same region by Small and Ramberg (1971). Phytoplankton ash in the Columbia River Estuary was low in winter, at essentially the same concentrations as in the Winter Inshore and Winter Offshore regimes. Summer concentrations in the estuary were roughly four times higher than winter values, and tended to increase with proximity to the mouth. The increased fertility of waters immediately seaward of the estuary mouth undoubtedly contributed to the enhancement of phytoplankton growth at the mouth.

Zinc (and possibly copper) concentration in phytoplankton ash in the estuary apparently is positively, linearly related to salinity in the estuary, although only five data points are available (Fig. 6). Some data from Droop (1961) are plotted for comparison to my copper data. Zinc concentration in ash is also positively related to zinc concentration in the estuarine water, but statistical variation is large, particularly at the higher zinc concentrations (Fig. 7). The relationship suggests that the phytoplankton ash in the estuary concentrated zinc about 94,000 times. Neither iron nor manganese in phytoplankton ash bore any relationship to estuarine salinity, although only five data points were available.

Phytoplankton zinc showed the best direct correlation with increasing distance offshore (Fig. 8), but this relationship was rather poor ( $r = 0.45$ ) and specifically emphasizes the great variability in offshore samples in seasons other than summer. When only the 16

samples collected during high-radiation periods (May plus the three summer cruises) were considered, the correlation coefficient increased to 0.66, which was significant at the 99% level. Phytoplankton iron and manganese showed poor correlation with distance from shore, with the non-summer offshore concentrations varying widely. Using all 22 samples, the correlation coefficient for iron was 0.34; however, without the four winter offshore samples in the computations, the coefficient increased to 0.68 (Fig. 9). The winter offshore samples were all from Davidson Current water, with characteristic phytoplankton species such as Planktoniella sol (see Appendix B) and characteristically low metal concentrations in the phytoplankton. The other two offshore samples in Figure 9 were taken from typically offshore water in May and October, when the Davidson Current is not off the Oregon coast. Phytoplankton copper also showed poor correlation with distance from shore; however, in a multiple linear regression equation with salinity and miles from shore (d), the coefficient of determination ( $R^2$ ) doubled to 0.28. The equation was  $Cu \text{ (ug/g ash)} = 2309.57 - 64.40 S + 3.58 d$  with 20 degrees of freedom.

Trace metal concentrations in phytoplankton ash tended to decrease with increasing phytoplankton ash concentration in the water, as might be expected from a standpoint of competition for metal ions on a surface area-to-volume basis. The relationship appeared to fit a log-log model, as exemplified by zinc (Fig. 10). Zinc/g phytoplankton ash was always lowest in upwelled waters, regardless of the concentration of phytoplankton ash in the water, hence, the four upwelling data points were not included in the computation of the regression line. The equation of the line was  $\log Zn \text{ (ug/g ash)} = 2.338 - 0.202 \log A$ , where A is the



concentration of the phytoplankton ash in mg/l of seawater. The correlation coefficient was 0.80 with 17 degrees of freedom.

#### Relationships between trace metal concentration and hydrographic regimes

The Columbia River Estuary is clearly different from the marine regimes in terms of zinc, manganese, and copper concentrations in the phytoplankton ash collected during the summer (Tables 1 and 2). On a year-round basis, all four metal concentrations in estuarine phytoplankton differ from marine values, on a ug/g ash basis (Tables 3 and 4). On the basis of average phytoplankton trace metal concentration per liter of water, the Columbia River Estuary is again separable from the marine regimes with respect to zinc, manganese, and copper (but not iron) during the entire year. Iron, thus, is clearly different between the estuarine samples and the marine ones only in terms of ug Fe/g phytoplankton ash on a year-round basis.

On a ug/g ash basis, zinc concentrations in phytoplankton in the marine regimes exhibit an inshore-offshore division both during the summer and the year-round (Tables 2 and 4). However, on a ug Zn/l basis, there is no clear difference. There is a significant manganese difference between the Inshore and other ocean regimes on a per liter basis during the summer.

In only two situations are the concentrations of copper the same; however, it would be difficult to confuse the concentrations spatially in one of these situations (the CRE vs. the Offshore during the entire year). The other situation is the Plume vs. the Inshore during the summer. Copper is reasonably similar to manganese on a per liter basis, although the levels of significance vary somewhat in summer.

### Seasonality of the trace metal concentrations

Seasonality appears to be a factor influencing ug of phytoplankton trace metal/l for the combined Inshore-Offshore regimes sampled year-round, although sampling variability does not allow statistical separation (Table 5). The highest values are always in the summer and the lowest in the winter. On the basis of ug/g ash, zinc is highest in winter (March) and tapers off through the fall, manganese peaks in the summer and is lowest in winter, and copper and iron are highest in spring (May) and lowest in fall. The details of the pattern vary from metal to metal in terms of intensity, but only manganese appears to be totally out of phase with the other metals in regard to seasons of high and low concentration.

### Species predominant in the hydrographic regimes

All Columbia River Estuary and Plume samples were dominated (80% of the total cells or more) by four or less species. In the CRE, Fragilaria crotonensis and Melosira spp. were predominant at all times, while Tabellaria sp. and Asterionella formosa were subdominants in the spring-summer and fall-winter, respectively. The species composition of the Plume flora was not as clear. At least 7 species predominated at one time or another and at one position in the Plume or another. F. crotonensis and Melosira sp. were the only estuarine dominants to maintain their dominancy in the Plume at any time. Nitzschia spp., Rhizosolenia alata, Skeletonema costatum, Synedra ulna, and Thalassiosira decipiens were the other Plume dominants.

Only the May Offshore sample was dominated by four or fewer species. Chaetoceros convolutus, R. alata, and Thalassiothrix longissima

made up 96% of the cells counted. In the winter Offshore samples from the Davidson Current water, Planktoniella sol made up to 44% of the cells counted, and in the January sample over half of the material collected in terms of mass was P. sol.

The August, 1969, and June, 1970, Inshore samples were dominated by Chaetoceros constrictus, S. costatum, and T. decipiens. No other Inshore samples were dominated by four or fewer species. All Upwelling samples were dominated by four or fewer species, the main ones being Chaetoceros spp., S. costatum, and T. decipiens. Nitzschia spp. also was heavily represented in two samples.

#### DISCUSSION

Zinc and manganese in phytoplankton ash are strongly (but negatively) related to salinity above 25‰, iron is less related, and copper is poorly related (Figs. 2, 3, 4). Data from Riley (1937) also indicated increasing concentrations of metals with decreasing salinity, but this general relationship is exactly opposite the one predicted by Droop (1961). However, it must be noted that Droop's work was with lower salinity (0 to 11 ‰) than my non-estuarine data, and there could easily have been a peaking of his curve, then a decline which he never resolved at high salinities. My data for phytoplankton zinc and copper in the Columbia River Estuary (up to 5‰ salinity) also showed increases with increasing salinities (Fig. 6). If the curves of Figures 2 and 6a are extrapolated linearly, the maximum occurs at about 10‰ salinity. It seems likely there is some salinity range, perhaps 10 to 15‰, over which ug Zn/g ash reaches a maximum value. Data from Evans and Cutshall (in press) tend to support a maximum

for soluble zinc at about 10‰ salinity. Their work further strengthens the idea of decreasing soluble zinc concentrations as the salinity increases, over a range of higher salinities.

The concentration of zinc in marine phytoplankton probably is a function not only of salinity, but of residence time of the population in the waters being sampled. It follows that if salinity is the predominant external factor influencing zinc concentration in cells (in a negative fashion), and salinity generally decreases with distance offshore in the summer, then zinc concentration in phytoplankton should increase with distance offshore, which it does (Fig. 8). However, it increases at a greater rate than it should were salinity the only parameter influencing zinc concentration. Because the surface waters adjacent to the Oregon coast can at times move offshore in the summer, the phytoplankton populations associated with these waters will be carried seaward. The population that have been in the photic zone and growing longer (those farther offshore) are likely to have greater zinc concentrations than those populations in newly upwelled waters. Alternatively, cells offshore might be allied more to Plume waters (with the Columbia River as a high zinc source), while inshore cells are allied to upwelled water which does not readily mix with Plume waters. This latter alternative might not apply at distances beyond about 40 miles from the river mouth, however (see later). From Tables 1 and 2 some support for the residence-time differences exists. For example, there is a significant difference between the combination of Inshore-Upwelling (IU) and the combination Offshore-Plume (OP), and the differences are not significant between the components within either combination. Also, the March and October, 1970, Inshore samples have higher zinc concentrations than

the June and August ones, which supports the idea of shorter residence time at the surface for the summer samples. It should be noted that the March and October Inshore samples are not greater than the March and October Offshore samples, however. This similarity is expected because salinities are very similar in all four samples (range: 31.44 to 32.69%), and the salinities are similar because March and October are transition months off Oregon, when winds are variable and waters reasonably well mixed. Residence time is not as important an effect under these conditions. Another indication of support for the residence-time concept is found in Figure 10. There, three of the four samples from upwelled waters were well below the general slope describing the relationship between  $\mu\text{g Zn/g ash}$  and  $\text{mg ash/l}$  of non-upwelled seawater. Apparently those populations in upwelled water had not had enough time at the surface to reach the zinc concentrations of comparably dense populations in non-upwelled water.

Another possible mechanism or additional factor influencing zinc concentration might be the absorption mechanism itself. Williams (1971) demonstrated that if the concentration of an element in phytoplankton is related to the two-thirds ( $2/3$ ) power of the biomass of the phytoplankton, then it is an example of surface-limited absorption. Using all of the data where paired values of salinity and biomass existed for a given zinc concentration in phytoplankton, a multiple regression of salinity and per-liter ash biomass to the two-thirds power against zinc concentration (in  $\mu\text{g/g ash}$ ) was performed. The increase in the multiple correlation coefficient was only 0.014 over that for the simple regression using only salinity and zinc concentration. This

increase was not significant; therefore, the two-thirds power relationship had no measurable effect.

The strong correlation between salinity and certain trace metal concentrations in marine phytoplankton has never been satisfactorily explained. Several possible mechanisms suggest themselves with respect to zinc. The first possibility is that the salts in seawater interfere with the uptake of zinc, either by presenting ions of a competitive nature or by disrupting absorption or adsorption sites on the cell. Another possibility is that there is less available zinc in waters of greater salinity off the Oregon coast. A third possibility is that the increased salt content beyond a certain salinity might be symptomatic of conditions in which chelation plays a major role in making the metal unavailable to the cells. The fact that the relationship with salinity is positive in the Columbia River Estuary but negative off the coast suggests that neither salt interference nor zinc availability can satisfactorily explain the zinc-salinity relationship over all salinity ranges. At the higher salinities (about 25‰), however, a further possible explanation for the salinity-trace metals relationship might be a simple dilution of fresh water (containing high trace metal concentrations) with seawater (containing small concentrations of trace metals). It would then follow that with continued dilution of this semi-fresh water by seawater (i.e., as salinity increases), the available metal would be diluted further, and the cellular concentrations would reflect this dilution with lower amounts of trace metals/g ash. Evans and Cutshall (in press) provide strong support for this idea, in that zinc-65 concentration in estuarine water discharging into the sea decreases linearly with increasing salinity, as the fresher water mixes with

seawater. Apparently at very low salinities the zinc does not desorb at once from particles, however, so that the zinc-65 concentration in the estuary itself increases with increasing salinity. These features of zinc-65 sorption suggest that some physico-chemical property of salt solutions separates zinc-65 from particles, and increasing salt content signals increased separation.

Iron concentrations, although not as strongly influenced, are still reasonably related to the effects of salinity. There seem to be three possible explanations for the differences between the iron and zinc relationships. First, Hayward (1969) postulates a different mechanism for phytoplanktonic assimilation of iron as opposed to zinc. Second, the commonest states of iron in seawater are different from the commonest states of zinc in seawater. These different available metal concentrations might induce different uptake rates in phytoplankton, because of different concentrations or because of different external chemical equilibria between utilizable states and non-utilizable states of the metals. Finally, Glooschenko and Curl (1971) have found that iron can be limiting in the inshore environment off the Oregon coast, which could modify the concentration of iron in phytoplankton from time to time and place to place. The non-significant differences found in my study, in terms of the amount of phytoplankton iron/l of seawater (Tables 2 and 4) would seem to support the third explanation, for if there is only a limited supply of available iron for use by phytoplankton, the phytoplankton growth would be limited by the rate of regeneration of the utilizable iron. However, the lack of precision in determining iron in phytoplankton makes all interpretation very speculative. Note, for example, the order of magnitude differences in ug/l values in Tables

1, 3, and 5, which were not great enough to produce significant differences.

Iron determination has a number of problems that are not serious with the other metals; e.g., sampling is done from steel ships, contamination at sea or in the laboratory can occur quite easily and is hard to prevent, the most abundant form of iron in natural waters is particulate and differentiating between it and phytoplankton iron is next to impossible, and finally, the AAS does not handle iron determination as well as other metals (the peaks are cluttered, the testable linear range of concentrations are small in comparison to the concentration in the samples, and baseline drift occurs with iron more than with any of the other metals). In all, it is not surprising there is so little information to be had from the iron data, both here and in the literature; therefore, all analyses of the data on iron must be considered to be of a low reliability. The problem of differentiation of particulate inorganic iron from phytoplankton iron probably was the greatest problem in my iron determinations. The total iron concentration in seawater probably varies more with randomly imposed and seasonal weather changes and by chemical processes than it does through biological uptake.

While salinity seems to be the primary external factor influencing the concentration of manganese in marine phytoplankton, it is not the only one. If salinity were the sole important factor there would likely be higher correlation of phytoplankton manganese with distance offshore because of the sequence and frequency of sampling in relation to upwelling patterns and the offshore transport of water during the summer. From multiple linear regression analysis, I have found that an additional 6% of the variation in ug Mn/g ash can be



explained by including temperature as a factor. A hypothetical explanation for the effect of temperature is that manganese concentration is not overwhelmingly controlled by simple, passive adsorption, but is partly physiologically regulated. Because manganese in phytoplankton is significantly correlated with both salinity and temperature, it could be expected that there would be characteristic phytoplanktonic manganese concentrations for the different hydrographic regimes. Analysis of variance does not reveal, with one exception, any significant differences between the four marine hydrographic regimes, however (Tables 2,4). The one exception, besides in the CRE, is for phytoplankton manganese/l of seawater between the Inshore and the other three summer regimes (at the 90% level). The difference might merely reflect the higher phytoplankton population densities in Inshore waters. Sampling variation masks any other differences.

Each of the water types sampled has phytoplankton with a characteristic copper concentration (Tables 2,4), with the two exceptions noted in the Results section. The two exceptions are, on a ug Cu/g ash basis, the CRE vs. the Offshore during the entire year and the Plume vs. the Inshore during the summer. Because it is very difficult to confuse first pair of regimes spatially, this exception is not important. The Inshore and Plume samples in summer are thus the only ones not distinguishable on the basis of phytoplanktonic copper. The phenomenon of characteristic concentrations of phytoplanktonic copper with hydrographic regime possibly has significance to oceanographers as a water identification device, because determination of copper in phytoplankton is one of the more easily done techniques by the AAS. The phytoplankton Cu levels might also be important in that the plankton, via adsorption,

might reflect the content of copper in the surrounding water, although this needs to be verified.

Seasonal trends in concentration ( $\mu\text{g/g}$  ash) occurred in all four of the trace metals, but it is difficult to reconcile the fact that peak concentrations occurred at different times of the year. Perhaps the March peak for zinc and the May peaks for copper and iron reflect the same general phenomenon of enhanced concentration through a broad period of phytoplanktonic spring blooming in Oregon waters. Interestingly, the major discharge of the Columbia River is in June. If the CRE acts as a trace metal trap, stripping much of the available metal from the water before it can reach the ocean, then the Plume waters would be relatively sterile and would succeed only in diluting the available metals in seawater and ultimately in the plankton. Such an argument runs counter to one proposed to explain Inshore-Offshore differences in phytoplankton zinc in summer. The fact that nutrient elements become exhausted in the Plume within the first 20 to 40 miles offshore along the Plume axis has been documented, however (Hage, 1969; Small and Curl, 1972). This rapid exhaustion also seems to occur in the June, 1970, Plume transect for all four trace metals. Such dilution by Plume waters in summer, plus the onset of water stability and cessation of nutrient resupply to surface waters through deep mixing (except in the upwelling areas), could account for the relatively low concentrations of zinc, copper, and iron in summer phytoplankton and the subsequent decline to lowest values in the fall.

Concentrations of manganese in phytoplankton might not be explained by the above, because highest concentrations occurred in summer, lowest in winter. However, spring and summer concentrations

could not be statistically separated even at the 90% confidence interval. Several other suggestions can be offered for the differences, but without substantial support. The difference in speed of uptake of zinc and manganese has been noted in the literature (Hayward, 1969), with manganese the slowest; however, differences of 3 or 4 months to resolve peak concentrations of manganese after the zinc peaks have been reached appears to be too slow to be explained by differences in uptake kinetics. Competition for sorption sites, particularly with iron, might explain some of the delay; yet copper and iron do not succeed in competitively excluding zinc, even though both ionic copper and iron are higher in the EMF series than zinc. In fact, zinc concentrations in Oregon phytoplankton apparently peak before copper or iron. The apparently tighter relationship between manganese and temperature, as opposed to the weak relationships between the other metals and temperature, suggests that increased manganese concentration might be more strongly favored by increased metabolism by cells in the summer. The low winter concentrations could result partly from reduced metabolism during that season.

In general, the species enumeration reported here is in agreement with that of Hobson (1964) and Haertel, *et al.* (1969). The usefulness of such work with respect to phytoplankton trace metal incorporation must be questioned, however, because it is becoming more apparent that the critical controls on nutrient uptake might not be species differences, but rather clonal differences within species. Carpenter and Guillard (1971) have shown, for example, that while there are species differences in rates of nitrogen uptake and concentration under

given environmental parameters, there can be greater differences between clones of the same species taken from different environments.

Species enumeration can, however, provide an index of the distribution of water types. In this respect, it has some value. In my work, the abundance of viable Melosira and Fragilaria species typical of fresh or brackish waters served to indicate the Plume (although this was not clear from the Melosira data alone, because the various species were not separated in the initial counting of species). The presence of Planktoniella sol, a species characteristic of subtropical waters, indicated the presence of waters from the Davidson Current during the winter off Oregon. The presence of P. sol in samples is correlated 100% to low iron concentrations in the winter offshore samples. Whether such low iron concentrations are typical of subtropical plankton, whether the concentrations merely reflect a change due to transport of the cells to higher latitudes during the winter, or whether the iron values are even valid for those winter samples, are interesting questions worthy of further work.

Although it is likely that, of the four metals, only iron is metabolically limiting, any substantial increase in trace metal concentration in the coastal waters would probably cause some changes in the phytoplankton in those waters. Copper is a well-known algicide (Mackenthun, et al., 1964). Erickson (1970) has shown that the toxic effects of copper start as low as 5 ug/l in the water, which is only four to five times the reported concentrations off the Washington coast (Chow and Thompson, 1954). These effects primarily manifest themselves in a depression of C-14 uptake and growth.

Manganese has been reported by Hutchinson (1957) to reduce species diversity in lakes. There is little other evidence one way or the other on other possible effects of trace metal overabundance in marine waters although Bowen (1966) suggests that "excessive" zinc in the water might reduce primary productivity and that the potential of it happening is very high. It seems safe to assume, at least for the present time, that average levels of the four metals in phytoplankton do not exceed levels at which pronounced deleterious effects occur.

#### SUMMARY

1. The immediate objectives of this study were (a) to determine whether phytoplanktonic concentrations of iron, zinc, copper, and manganese can serve to characterize the hydrographic regime in which the sampled phytoplankton population was growing, and (b) if possible, to identify causative agents of variation of trace metal concentrations in natural phytoplankton populations.
2. Phytoplankton from Oregon coastal waters and the Columbia River Estuary were collected by means of pumps and nets from August, 1969, to October, 1970, and, after certain chemical pre-treatment, the samples were analyzed for their trace metal content using an atomic absorption spectrophotometer.
3. The ranges of values for each metal, in terms of parts per million of ash weight of sample, were 3.2 to 820.0 for manganese, 23.0 to 960.2 for copper, 63.7 to 1762.5 for zinc, and 336 to 12030 for iron.
4. Particularly zinc and manganese in phytoplankton, but also iron, were negatively correlated with salinity above 25‰. Phytoplanktonic copper was not. Distance offshore correlated directly with zinc, copper,

and iron. Descriptive equations for trace metal concentrations vs. salinity were developed with correlations of 0.90 for zinc and 0.87 for manganese.

5. The copper concentration per unit ash weight was found to be diagnostic for the hydrographic regime from which the phytoplankton were collected.

6. Trace metal concentrations in phytoplankton were greatest in the month of March for zinc, in the month of May for copper and iron, and in the month of June for manganese.

7. Although salinity is the dominant physical factor affecting most trace metal concentrations in marine phytoplankton, residence time in the water column seems to modify at least the zinc concentration in the plankton. Temperature does likewise for the manganese concentration.

8. The Columbia River Estuary was always significantly different from the marine hydrographic regimes with respect to phytoplanktonic zinc, manganese, and copper, but not for iron. Iron results must be regarded as questionable because of the various problems associated with sampling the element, its state in natural waters, ease of contamination, and difficulty of processing by AAS techniques. However, low iron concentrations, during the winter offshore, coupled with the presence of a subtropical species of diatom (Planktoniella sol) might be an identifying characteristic of a sixth water type off the Oregon coast, the Davidson Current.

9. Except to identify water types foreign to the local area, species determinations had little relationship to phytoplanktonic trace metal concentrations.

10. Possible explanations for the results were discussed, and several hypotheses to explain the data were proposed.

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## APPENDIX A

Values of independent physical and biological parameters

| Hydrographic regime--<br>Date sampled | Temperature<br>(°C) | Salinity<br>(%) | σ <sub>t</sub> | Distance offshore<br>(naut.mi.) | Volume pumped<br>(l) | Total dry wt<br>(mg/l) | Total ash wt<br>(mg/l) |
|---------------------------------------|---------------------|-----------------|----------------|---------------------------------|----------------------|------------------------|------------------------|
| Columbia River Estuary                |                     |                 |                |                                 |                      |                        |                        |
| 1969 August                           | 13.05               | 24.87           | 19.3           | 0                               | 62.474               | 3.76                   | 2.53                   |
| 1970 January                          | 7.05                | 0.91            |                | -6                              | 3867.123             | 0.07                   | 0.06                   |
| March                                 | 10.3                | 3.97            |                | -3                              | 1305.936             | 0.29                   | 0.25                   |
| June                                  | 16.8                | 5.01            |                | -3                              | 713.564              | 1.89                   | 1.50                   |
| August                                | 20.0                | 2.70            |                | -6                              | 738.483              | 1.84                   | 1.62                   |
| October                               | 15.7                | 2.88            |                | -6                              | 856.561              | 0.57                   | 0.51                   |
| Columbia River Plume                  |                     |                 |                |                                 |                      |                        |                        |
| 1969 August                           | 9.20                | 32.11           | 24.7           | 10                              | 147.084              | 3.10                   | 2.42                   |
| 1970 June (1)                         | 13.7                | 28.4            | 21.2           | 4                               | 1424.297             | 0.62                   | 0.51                   |
| (2)                                   | 12.45               | 30.65           | 23.2           | 10                              | 1496.786             | 0.38                   | 0.25                   |
| (3)                                   | 15.33               | 31.9            | 23.5           | 70                              | 3177.061             | 0.97                   | 0.78                   |
| Offshore                              |                     |                 |                |                                 |                      |                        |                        |
| 1969 August                           | 9.60                | 33.05           | 24.8           | 20                              | 247.701              | 0.48                   | 0.30                   |
| 1970 January                          | 10.75               | 32.50           | 24.9           | 105                             | 9046.128             | 0.01                   | 0.01                   |
| March (1)                             | 10.6                | 32.36           | 24.9           | 65                              | 6467.386             | 0.42                   | 0.36                   |
| (2)                                   | 10.6                |                 |                | 65                              | 13414.729            | 0.06                   | 0.05                   |
| (3)                                   | 11.3                |                 |                | 65                              | 9852.264             | 0.07                   | 0.06                   |
| May                                   | 11.75               | 32.01           | 24.3           | 75                              | 2211.200             | 0.03                   | 0.02                   |
| June                                  | 15.1                | 30.0            | 22.1           | 105                             | 2328.429             | 0.08                   | 0.09                   |
| August                                | 11.8                | 32.4            | 24.7           | 24                              | 2342.587             | 0.56                   | 0.49                   |
| October                               | 14.3                | 31.62           | 23.6           | 57                              | 13792.748            | 0.11                   | 0.09                   |
| Inshore                               |                     |                 |                |                                 |                      |                        |                        |
| 1969 August                           | 9.11                | 33.00           | 25.6           | 6                               | 509.705              | 4.84                   | 3.98                   |
| October                               | 10.0                | 32.12           | 25.4           | 5                               | 1510.                | 0.16                   | 0.13                   |
| 1970 March                            | 9.5                 | 31.44           | 24.2           | 3                               | 3636.                | 0.09                   | 0.08                   |
| May (1)                               | 11.0                | 30.61           |                | 3                               | 2679.548             | 0.14                   | 0.10                   |
| (2)                                   | 10.4                | 32.20           | 24.9           | 1                               | 746.694              | 0.91                   | 0.70                   |
| June                                  | 9.1                 | 32.9            | 25.6           | 5                               | 699.973              | 3.36                   | 2.65                   |
| August                                | 12.1                | 32.7            | 24.8           | 12                              | 674.771              | 7.75                   | 6.78                   |
| October                               | 12.2                | 32.69           | 24.8           | 5                               | 1042.597             | 1.04                   | 0.90                   |
| Upwelling                             |                     |                 |                |                                 |                      |                        |                        |
| 1969 August                           | 9.44                | 33.50           | 25.8           | 4                               | 786.731              | 0.13                   | 0.09                   |
| 1970 June                             | 9.1                 | 33.77           | 26.2           | 3                               | 2951.948             | 2.66                   | 1.65                   |
| August (1)                            | 10.0                | 33.3            | 25.6           | 10                              | 1272.806             | 5.32                   | 4.72                   |
| (2)                                   | 8.7                 | 33.5            | 26.0           | 7                               | 1262.046             | 4.18                   | 3.66                   |

APPENDIX B

Concentration of trace metals in phytoplankton ash (ug/g),  
with 95% confidence limits of all multiple samples

|                           | Mn         | 95% C.L. | Zn            | 95% C.L. | Fe             | 95% C.L. | Cu              | 95% C.L.      |                |
|---------------------------|------------|----------|---------------|----------|----------------|----------|-----------------|---------------|----------------|
| Columbia River<br>Estuary | 69 Aug.    | 820.0    |               | 970.0    |                | 12030    | 610.0           |               |                |
|                           | 70 Jan.    | 405.0    |               | 345.0    |                | 11740    | 312.5           |               |                |
|                           | Mar.       | 237.5    |               | 1762.5   |                | 10490    | 505.0           |               |                |
|                           | June       | 717.4    | (672.5-760.3) | 1057.9   | (531.2-1584.6) | 8094.7   | (7401.8-8787.7) | 721.7         | (661.7-781.7)  |
|                           | Aug.       | 209.0    | (168.0-250.0) | 503.3    | (447.2-559.5)  | 3475.9   | (3027.5-3924.3) | 600.7         | (467.5-733.9)  |
|                           | Oct.       | 575.7    | (290.0-863.4) | 704.9    | (422.8-987.0)  | 1401.1   | (1395.7-1406.5) | 909.2         | (594.6-1223.8) |
| Columbia River<br>Plume   | 69 Aug.    | 110.0    |               | 285.0    |                | 4070     | 185.0           |               |                |
|                           | 70 June(1) | 427.2    | (307.9-546.4) | 326.8    | (135.9-507.7)  |          | 507.75          | (393.9-621.6) |                |
|                           | (2)        | 104.7    |               | 275.8    |                | 6324     | 270.0           |               |                |
|                           | (3)        | 72.1     | (58.2-85.9)   | 224.4    | (192.0-253.3)  | 4997.7   | (3447.9-6547.5) | 79.8          | (30.3-129.4)   |

APPENDIX B Continued

|           | Mn               | 95% C.L.            | Zn                  | 95% C.L.               | Fe                  | 95% C.L. | Cu | 95% C.L. |
|-----------|------------------|---------------------|---------------------|------------------------|---------------------|----------|----|----------|
| Upwelling | 69 Aug.          | 40.1 (0.0-93.2)     | 94.1 (49.1-139.1)   | 5008 (3608-7808)       | 47.2 (38.1-56.3)    |          |    |          |
|           | 70 June          | 20.4 (12.4-28.5)    | 109.6 (81.4-137.8)  | 590.9 (415.4-766.4)    | 58.5 (47.4-69.7)    |          |    |          |
|           | Aug. (1)         | 16.2 (4.6-27.7)     | 63.7 (57.2-70.2)    | 335.5 (202.4-468.6)    | 38.2 (31.2-45.1)    |          |    |          |
|           | (2)              | 34.4 (28.0-40.6)    | 140.5 (122.4-159.6) |                        |                     |          |    |          |
| Inshore   | 68 Oct.          | 16.4 (11.9-20.9)    | 72.5 (58.0-87.0)    | 745.7 (11.2-1380.2)    | 23.0 (0.0-46.4)     |          |    |          |
|           | same*            | 17.8 (10.7-24.8)    | 72.0 (55.6-88.4)    | 682.5 (397.6-967.4)    | 23.0 (9.3-36.7)     |          |    |          |
|           | 69 Aug.          | 207.6 (165.3-251.9) | 165.3 (141.9-192.4) | 1425.8 (1117.6-1734.0) | 87.7 (63.9-111.6)   |          |    |          |
|           | Oct.             | 64.8 (44.3-85.4)    | 272.8 (198.4-347.2) | 783.8 (562.7-1004.9)   | 31.7 (25.1-38.3)    |          |    |          |
|           | 70 Mar.          | 128.8               | 356.5               | 4080                   | 47.2                |          |    |          |
|           | May (1)          | 214.5               |                     |                        | 928.2               |          |    |          |
|           | (2)              | 85.9 (53.6-118.2)   | 224.1 (47.3-398.7)  | 4624.8 (2349.2-6900.4) | 960.2 (732.4-1180)  |          |    |          |
|           | (net 2)          | 20.3 (6.9-33.8)     | 77.0 (46.5-107.6)   | 1182.8 (927.0-1438.6)  | 34.5 (25.9-43.1)    |          |    |          |
|           | June             | 110.1 (100.2-120.1) | 151.8 (145.3-158.3) | 2525.6 (1498.1-3553.1) | 393.5 (361.8-425.2) |          |    |          |
|           | Aug.             | 38.2 (30.2-46.2)    | 149.7 (135.9-163.5) | 1988.9 (1791.0-2186.8) | 148.1 (132.4-163.9) |          |    |          |
| Oct.      | 34.8 (31.2-38.3) | 224.8 (223.2-226.4) | 726.3 (709.2-743.4) | 124.4                  |                     |          |    |          |

\*These values were obtained by John Pequegnat and Hal Stanford, independent of my analyses.

APPENDIX B Continued

|                  | Mn       | 95% C.L.    | Zn     | 95% C.L.        | Fe     | 95% C.L.        | Cu    | 95% C.L.       |
|------------------|----------|-------------|--------|-----------------|--------|-----------------|-------|----------------|
| 69 Aug.          | 210.0    |             | 338.0  |                 | 4980   |                 | 370.0 |                |
| 70 Jan.          | 27.5     |             | 475.0  |                 | 2160   |                 | 927.5 |                |
| Offshore<br>Mar. | (1) 3.2  | (0.0-14.3)  | 154.9  | (136.7-173.1)   | 497.6  | (134.8-860.4)   | 190.8 | (167.5-214.1)  |
|                  | (2) 17.9 | (4.7-31.1)  | 779.0  | (730.4-842.0)   | 863.1  | (334.6-1391.6)  | 706.5 | (663.2-749.7)  |
|                  | (3) 17.9 | (4.7-31.1)  | 1692.5 | (1510.2-1874.8) | 2596.0 | (1127.5-4064.5) | 944.4 | (798.0-1090.8) |
| May              | 325.8    |             | 528.5  |                 | 10120  |                 | 900.6 |                |
| June             | 178.2    |             | 417.2  |                 | 8840   |                 | 721.5 |                |
| Aug.             | 24.7     | (20.4-29.0) | 194.1  | (118.8-269.4)   | 1512.7 | (966.9-2058.5)  | 357.9 | (176.2-539.5)  |
| Oct.             | 42.0     | (5.1-79.0)  | 259.9  | (171.7-348.2)   | 3696.8 | (2696.5-4697.1) | 271.2 | (212.4-330.0)  |



## APPENDIX C

Species count (in percentage of total cell numbers)  
All species with 1% or more in any sample are reported.

|             |           | <u>Asterionella formosa</u> | <u>Bacteriastrum delicatulum</u> | <u>Biddulphia aurita</u> | <u>Biddulphia longicuris</u> | <u>Chaetoceros constrictus</u> | <u>Chaetoceros convolutus</u> | <u>Chaetoceros debilis</u> | <u>Chaetoceros decipiens</u> | <u>Chaetoceros perpusillus</u> |
|-------------|-----------|-----------------------------|----------------------------------|--------------------------|------------------------------|--------------------------------|-------------------------------|----------------------------|------------------------------|--------------------------------|
| C R Estuary | 69 Aug.   |                             |                                  |                          |                              |                                |                               |                            |                              |                                |
|             | 70 Jan.   | 7.0                         |                                  |                          |                              |                                |                               |                            |                              |                                |
|             | Mar.      | 1.8                         |                                  |                          |                              |                                |                               |                            |                              |                                |
|             | June      |                             |                                  |                          |                              |                                |                               |                            |                              |                                |
| C R Estuary | Aug.      | 1.9                         |                                  |                          |                              |                                |                               |                            |                              |                                |
|             | Oct.      | 10.8                        |                                  |                          |                              |                                |                               |                            |                              |                                |
| Plume       | 69 Aug.   |                             | 1.9                              |                          |                              | 4.4                            |                               |                            | 1.3                          |                                |
|             | 70 June 1 |                             |                                  |                          |                              |                                |                               | 1.5                        |                              |                                |
|             | June 2    |                             |                                  |                          |                              |                                |                               |                            |                              |                                |
|             | June 3    |                             |                                  |                          |                              |                                |                               |                            |                              |                                |
| Osshore     | 69 Aug.   |                             | 4.3                              |                          |                              | 11.2                           |                               | 7.4                        |                              |                                |
|             | 70 Jan.   |                             | 2.1                              |                          |                              |                                | 7.6                           |                            |                              |                                |
|             | Mar. 1    |                             | 22.3                             |                          |                              | 25.8                           | 7.2                           |                            | 2.1                          |                                |
|             | Mar. 2    |                             | 15.3                             |                          |                              | 8.0                            | 6.1                           |                            |                              |                                |
|             | Mar. 3    |                             | 30.5                             |                          |                              | 4.4                            | 10.5                          |                            | 2.5                          |                                |
|             | May       |                             | 1.9                              |                          |                              | 25.7                           |                               |                            | 1.5                          |                                |
|             | June      |                             | 6.0                              |                          |                              |                                |                               |                            | 3.2                          |                                |
|             | Aug.      |                             |                                  |                          |                              |                                | 48.5                          |                            | 3.2                          |                                |
|             | Oct.      |                             | 6.7                              |                          |                              |                                |                               |                            | 9.3                          |                                |
| Inshore     | 69 Aug.   |                             | 1.2                              |                          |                              | 11.9                           |                               |                            |                              |                                |
|             | Oct.      |                             |                                  |                          | 1.2                          |                                | 6.4                           | 1.2                        | 9.8                          |                                |
|             | 70 Mar.   |                             | 10.9                             | 1.3                      | 1.9                          | 9.6                            |                               | 16.0                       | 11.5                         |                                |
|             | May 1     |                             | 6.8                              | 1.0                      |                              |                                |                               | 19.5                       | 33.2                         | 5.4                            |
|             | May 2     |                             | 20.4                             |                          | 2.1                          |                                |                               | 18.3                       | 14.0                         | 5.5                            |
|             | June      |                             |                                  |                          |                              | 9.8                            |                               |                            |                              |                                |
|             | Aug.      |                             | 1.1                              |                          |                              |                                |                               | 9.8                        | 2.6                          | 4.7                            |
| Oct.        |           | 1.5                         |                                  | 5.9                      | 17.1                         |                                |                               |                            |                              |                                |
| Upwelling   | 69 Aug.   |                             | 2.6                              |                          |                              | 15.4                           |                               |                            | 7.0                          |                                |
|             | 70 June   |                             |                                  |                          |                              |                                |                               |                            |                              |                                |
|             | Aug. 1    |                             | 2.8                              |                          |                              |                                |                               | 48.6                       |                              | 15.2                           |
|             | Aug. 2    |                             | 2.4                              | 3.2                      |                              |                                |                               | 38.2                       |                              | 4.4                            |

## APPENDIX C Continued

|             | <u>Chaetoceros similis</u> | <u>Coscinodiscus spp.</u> | <u>Ditylum brightwellii</u> | <u>Eucampia zoodiacus</u> | <u>Fragilaria crontonensis</u> | <u>Grammatophora sp.</u> | <u>Gyrosigma sp.</u> | <u>Melosira spp.</u> | <u>Nitzschia spp.</u> |
|-------------|----------------------------|---------------------------|-----------------------------|---------------------------|--------------------------------|--------------------------|----------------------|----------------------|-----------------------|
| C R Estuary | 69 Aug.                    |                           |                             |                           | 72.8                           |                          |                      | 17.4                 |                       |
|             | 70 Jan.                    |                           |                             |                           | 51.5                           |                          | 1.0                  | 30.0                 |                       |
|             | Mar.                       |                           |                             |                           | 72.5                           |                          |                      | 9.9                  |                       |
|             | June                       |                           |                             |                           | 34.6                           |                          |                      | 47.5                 |                       |
|             | Aug.                       |                           |                             |                           | 40.8                           |                          |                      | 28.1                 |                       |
| Oct.        |                            |                           |                             | 40.5                      |                                |                          | 37.8                 |                      |                       |
| Plume       | 69 Aug.                    |                           |                             |                           |                                |                          |                      | 5.0                  | 6.9                   |
|             | 70 June 1                  |                           |                             |                           | 44.4                           |                          |                      | 29.1                 | 1.5                   |
|             | June 2                     | 6.5                       | 1.1                         |                           |                                |                          |                      | 13.0                 | 9.1                   |
|             | June 3                     |                           |                             |                           |                                |                          |                      |                      | 8.3                   |
| Offshore    | 69 Aug.                    |                           |                             |                           | 29.4                           |                          |                      | 1.7                  |                       |
|             | 70 Jan.                    |                           |                             | 5.1                       |                                |                          |                      |                      |                       |
|             | Mar. 1                     | 7.9                       |                             |                           |                                |                          |                      |                      |                       |
|             | Mar. 2                     | 1.6                       |                             |                           |                                |                          |                      |                      |                       |
|             | Mar. 3                     | 1.9                       |                             |                           |                                |                          |                      |                      |                       |
|             | May                        |                           |                             |                           |                                |                          |                      |                      |                       |
|             | June                       | 1.3                       |                             |                           |                                |                          |                      |                      |                       |
|             | Aug.                       |                           | 6.7                         |                           |                                |                          |                      |                      |                       |
| Oct.        |                            | 8.8                       | 6.7                         |                           |                                |                          |                      |                      |                       |
| Inshore     | 69 Aug.                    |                           |                             |                           |                                |                          |                      |                      | 1.9                   |
|             | 70 Oct.                    |                           | 2.9                         | 1.7                       |                                |                          |                      |                      |                       |
|             | Mar.                       |                           | 1.9                         | 1.3                       | 1.9                            |                          |                      | 7.7                  |                       |
|             | May 1                      |                           |                             |                           |                                |                          |                      | 1.0                  | 1.0                   |
|             | May 2                      |                           | 2.1                         | 1.7                       | 2.1                            |                          |                      | 3.0                  |                       |
|             | June                       |                           |                             |                           |                                |                          |                      | 2.8                  | 2.6                   |
|             | Aug.                       |                           | 2.9                         |                           |                                |                          | 4.0                  | 3.6                  | 2.9                   |
| Oct.        |                            | 7.3                       | 5.4                         | 12.2                      | 10.7                           |                          |                      |                      |                       |
| Upwelling   | 69 Aug.                    |                           | 2.6                         |                           |                                |                          |                      |                      |                       |
|             | 70 June                    |                           |                             |                           | 1.6                            |                          |                      |                      |                       |
|             | Aug. 1                     | 1.2                       |                             |                           |                                |                          |                      |                      |                       |
| Aug. 2      |                            | 3.2                       |                             |                           |                                |                          |                      |                      |                       |

## APPENDIX C Continued

|             |           | <u>Planktoniella sol</u> | <u>Rhizosolenia alata</u> | <u>Rhizosolenia spp.</u> | <u>Skeletonema costatum</u> | <u>Stephanodiscus astrea</u> | <u>Stephanopyxis turris</u> | <u>Synedra ulna</u> | <u>Synedra spp.</u> | <u>Tabellaria sp.</u> |
|-------------|-----------|--------------------------|---------------------------|--------------------------|-----------------------------|------------------------------|-----------------------------|---------------------|---------------------|-----------------------|
| C R Estuary | 69 Aug.   |                          |                           |                          | 2.5                         |                              |                             |                     |                     | 2.5                   |
|             | 70 Jan.   |                          |                           |                          |                             | 1.5                          |                             | 1.0                 |                     | 8.0                   |
|             | Mar.      |                          |                           |                          |                             |                              |                             |                     |                     | 14.4                  |
|             | June      |                          |                           |                          |                             |                              |                             | 1.4                 |                     | 15.7                  |
|             | Aug.      |                          |                           |                          |                             | 2.3                          |                             |                     |                     | 23.4                  |
|             | Oct.      |                          |                           |                          |                             |                              |                             |                     |                     | 3.0                   |
| Plume       | 69 Aug.   |                          |                           |                          | 29.0                        |                              |                             | 22.1                |                     |                       |
|             | 70 June 1 |                          |                           |                          | 2.2                         |                              |                             | 3.3                 |                     |                       |
|             | June 2    |                          |                           |                          | 13.0                        |                              |                             |                     |                     |                       |
|             | June 3    |                          |                           |                          | 22.3                        |                              |                             |                     |                     |                       |
| Offshore    | 69 Aug.   |                          | 3.0                       |                          | 24.7                        |                              |                             |                     | 4.8                 |                       |
|             | 70 Jan.   | 44.1                     | 6.4                       |                          |                             |                              |                             |                     |                     |                       |
|             | Mar. 1    | 2.7                      | 3.4                       |                          | 6.5                         |                              |                             |                     | 6.9                 |                       |
|             | Mar. 2    | 3.0                      | 3.0                       |                          |                             |                              |                             |                     | 35.6                |                       |
|             | Mar. 3    | 1.3                      | 2.5                       |                          | 1.0                         |                              |                             |                     | 10.5                |                       |
|             | May       |                          | 57.4                      |                          |                             |                              |                             |                     |                     |                       |
|             | June      |                          | 87.0                      |                          |                             |                              |                             |                     |                     |                       |
|             | Oct.      |                          | 39.5                      |                          |                             |                              |                             |                     |                     |                       |
| Inshore     | 69 Aug.   |                          |                           |                          | 59.4                        |                              |                             |                     | 2.5                 |                       |
|             | 70 Oct.   |                          |                           | 5.2                      | 1.2                         |                              |                             |                     | 7.5                 |                       |
|             | Mar.      |                          |                           | 1.9                      | 17.9                        |                              |                             |                     | 5.1                 |                       |
|             | May 1     |                          |                           | 3.4                      | 17.1                        |                              | 5.8                         |                     |                     |                       |
|             | May 2     |                          |                           | 8.1                      | 13.2                        |                              | 2.1                         |                     |                     |                       |
|             | June      |                          |                           |                          | 41.0                        |                              |                             |                     |                     |                       |
|             | Aug.      |                          |                           |                          | 29.9                        |                              |                             |                     |                     |                       |
|             | Oct.      |                          |                           |                          | 5.9                         |                              |                             |                     |                     |                       |
| Upwelling   | 69 Aug.   |                          |                           | 2.6                      | 30.1                        |                              |                             |                     | 1.3                 |                       |
|             | 70 June   |                          |                           |                          | 26.4                        |                              |                             |                     |                     |                       |
|             | Aug. 1    |                          |                           |                          | 11.8                        |                              |                             |                     |                     |                       |
|             | Aug. 2    |                          |                           |                          | 25.9                        |                              |                             |                     |                     |                       |

## APPENDIX C Continued

|             |         | <u>Thalassionema nitzschoides</u> | <u>Thalassiosira aestivalis</u> | <u>Thalassiosira decipiens</u> | <u>Thalassiothrix longissima</u> | <u>Pediastrum sp.</u> | <u>Scenedesmus sp.</u> | <u>Coelosphaerium sp.</u> | <u>Ceratium furca</u> | <u>Ceratium tripos</u> |
|-------------|---------|-----------------------------------|---------------------------------|--------------------------------|----------------------------------|-----------------------|------------------------|---------------------------|-----------------------|------------------------|
| C R Estuary | 69 Aug. |                                   |                                 | 2.2                            |                                  |                       |                        |                           |                       |                        |
|             | 70 Jan. |                                   |                                 |                                |                                  |                       |                        |                           |                       |                        |
|             | Mar.    |                                   |                                 |                                |                                  |                       |                        |                           |                       |                        |
|             | June    |                                   |                                 |                                |                                  |                       |                        |                           |                       |                        |
| C R Estuary | Aug.    |                                   |                                 |                                |                                  |                       | 2.8                    |                           |                       |                        |
|             | Oct.    |                                   |                                 |                                |                                  | 1.1                   | 5.0                    |                           |                       |                        |
| Plume       | Aug.    |                                   |                                 | 27.1                           |                                  |                       |                        |                           |                       |                        |
|             | June 1  |                                   |                                 | 5.1                            |                                  |                       |                        | 8.7                       |                       |                        |
|             | June 2  |                                   |                                 | 1.3                            |                                  |                       |                        |                           |                       |                        |
|             | June 3  |                                   |                                 | 9.5                            |                                  |                       |                        |                           |                       |                        |
| Offshore    | 69 Aug  |                                   |                                 | 10.8                           |                                  |                       |                        |                           |                       |                        |
|             | 70 Jan. | 10.2                              |                                 |                                | 3.0                              |                       |                        |                           |                       | 10.6                   |
|             | Mar. 1  | 5.5                               |                                 | 2.4                            | 1.7                              |                       |                        |                           | 1.0                   |                        |
|             | Mar. 2  | 11.3                              |                                 | 4.7                            | 2.5                              |                       |                        |                           |                       |                        |
|             | Mar. 3  | 16.2                              |                                 | 4.4                            | 5.1                              |                       |                        |                           |                       | 1.1                    |
|             | May     |                                   |                                 |                                | 12.8                             |                       |                        |                           |                       |                        |
|             | June    |                                   |                                 |                                |                                  |                       |                        |                           |                       |                        |
|             | Aug.    | 20.1                              |                                 |                                |                                  |                       |                        |                           |                       |                        |
| Oct.        | 6.7     |                                   |                                 | 6.3                            |                                  |                       |                        |                           |                       |                        |
| Inshore     | 69 Aug. | 3.8                               |                                 | 16.9                           |                                  |                       |                        |                           |                       |                        |
|             | Oct.    | 59.6                              |                                 | 1.2                            | 6.9                              |                       |                        |                           |                       |                        |
|             | 70 Mar. | 10.2                              |                                 |                                |                                  |                       |                        |                           |                       |                        |
|             | May 1   |                                   |                                 | 3.9                            |                                  |                       |                        |                           |                       |                        |
|             | May 2   | 2.6                               |                                 | 3.0                            |                                  |                       |                        |                           |                       |                        |
|             | June    |                                   | 1.9                             | 40.5                           |                                  |                       |                        |                           |                       |                        |
|             | Aug.    |                                   |                                 | 30.6                           |                                  |                       |                        |                           |                       |                        |
| Oct.        | 5.4     |                                   | 15.6                            |                                |                                  |                       |                        |                           |                       |                        |
| Upwelling   | 69 Aug. | 2.6                               |                                 | 28.8                           | 1.3                              |                       |                        |                           |                       |                        |
|             | 70 June |                                   | 5.1                             | 34.6                           |                                  |                       |                        |                           |                       |                        |
|             | Aug. 1  |                                   |                                 | 12.1                           |                                  |                       |                        |                           |                       |                        |
|             | Aug. 2  |                                   |                                 | 13.9                           |                                  |                       |                        |                           |                       |                        |

## APPENDIX C Continued

|              |                  | <u>Dinophysis</u> sp. | <u>Gymnodinium</u> sp. | <u>Perdinium</u> spp. | Unidentified dinoflagellate | <u>Dichtyochoa</u> sp. | <u>Distephanus</u> sp. | Unidentified flagellate |
|--------------|------------------|-----------------------|------------------------|-----------------------|-----------------------------|------------------------|------------------------|-------------------------|
| C R Estuary  | 69 Aug.          |                       |                        |                       |                             |                        |                        |                         |
|              | 70 Jan.          |                       |                        |                       |                             |                        |                        |                         |
|              | Mar.             |                       |                        |                       |                             |                        |                        |                         |
|              | June             |                       |                        |                       |                             |                        |                        |                         |
|              | Aug.<br>Oct.     |                       |                        |                       |                             |                        |                        |                         |
| Plume        | 69 Aug.          |                       |                        | 1.3                   |                             |                        |                        |                         |
|              | 70 June 1        |                       | 1.5                    |                       |                             |                        |                        |                         |
|              | June 2           |                       |                        |                       |                             |                        |                        |                         |
|              | June 3           |                       |                        |                       |                             |                        |                        |                         |
| Offshore     | 69 Aug.          |                       |                        |                       |                             |                        |                        |                         |
|              | 70 Jan.          |                       |                        |                       |                             |                        | 5.1                    |                         |
|              | Mar. 1           |                       |                        |                       |                             |                        | 3.1                    |                         |
|              | Mar. 2           |                       |                        | 1.4                   | 1.1                         |                        | 2.8                    |                         |
|              | Mar. 3           |                       |                        | 1.9                   |                             |                        | 5.1                    |                         |
|              | May              |                       |                        |                       |                             |                        |                        |                         |
|              | June             |                       |                        |                       |                             |                        |                        |                         |
| Aug.<br>Oct. |                  | 2.1                   |                        |                       |                             | 8.5<br>1.9             | 3.1                    |                         |
| Inshore      | 69 Aug.          |                       |                        |                       |                             |                        | 1.2                    |                         |
|              | Oct.             |                       |                        |                       |                             |                        |                        |                         |
|              | 70 Mar.          |                       |                        |                       |                             |                        |                        |                         |
|              | May 1            |                       |                        | 1.0                   |                             |                        |                        |                         |
|              | May 2            |                       |                        |                       |                             |                        |                        |                         |
| Upwelling    | June             |                       |                        |                       |                             |                        |                        |                         |
|              | Aug.             |                       |                        |                       |                             | 2.6                    |                        |                         |
|              | Oct.             |                       |                        | 6.8                   | 1.5                         |                        |                        |                         |
| Upwelling    | 69 Aug.          |                       |                        | 1.9                   |                             |                        | 1.3                    |                         |
|              | 70 June          |                       |                        |                       |                             |                        |                        |                         |
|              | Aug. 1<br>Aug. 2 |                       |                        | 1.1                   |                             |                        |                        |                         |

APPENDIX D  
Other species found (less than 1%)

## Columbia River Estuary

Cymbella sp.Frustulia sp.Nitzschia spp.\*Pleurosigma sp. (?)Staurastrum sp.Dinophysis sp.\*Peridinium spp.\*Distephanus sp.\*

## Columbia River Plume

Ditylum brightwelli\*Grammatophora sp.\*Pseudoeunotia dobliolusThalassiothrix longissima\*Dinophysis sp.\*

Unidentified gymnodiniidan

Distephanus sp.\*

## Offshore

Actinopterychus undulatusAsteromphalus heptactisBiddulphia longicruris\*Eucampia zoodiacus\*Ceratium fusus

## Inshore

Chaetoceros teresPleurosigma sp. (?)Dinophysis sp.\*

## Upwelling

Biddulphia longicruris\*Dinophysis sp.\*

Unidentified dinoflagellate\*

Dichtyocha sp.\*

\*These species were more than 1% of a sample in other regimes. See Appendix C.

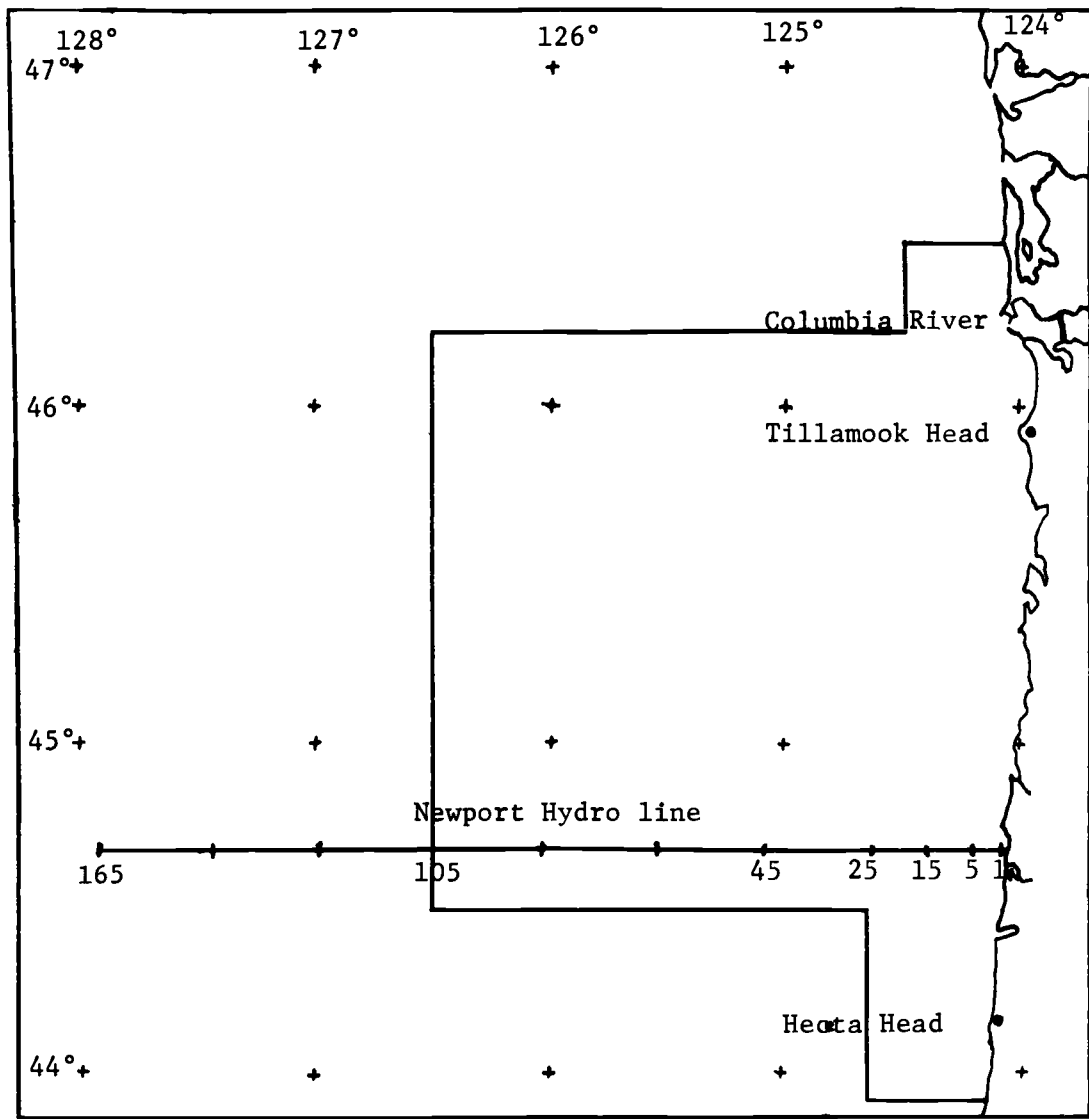


Figure 1: Oregon coastal waters showing the limits of the area sampled.

Figure 2: Zinc vs. Salinity. The descriptive equation of the line (DE) is  $Zn = 3270.25 - 93.80 S$ . Zn = the concentration of zinc in ug/g ash, and S = the salinity in parts per thousand. The correlation coefficient (r) is 0.90 with 18 degrees of freedom (df). The 95% confidence limits around the regression line are given as dashed lines. Symbols used, and their meaning:  $\nabla \blacktriangledown$  = Columbia River Estuary,  $\Delta$  = Columbia River Plume,  $\circ \bullet$  = Offshore,  $\square \blacksquare$  = Inshore,  $\times$  = Upwelling. Solid symbols = winter, spring, and fall; open symbols = summer.



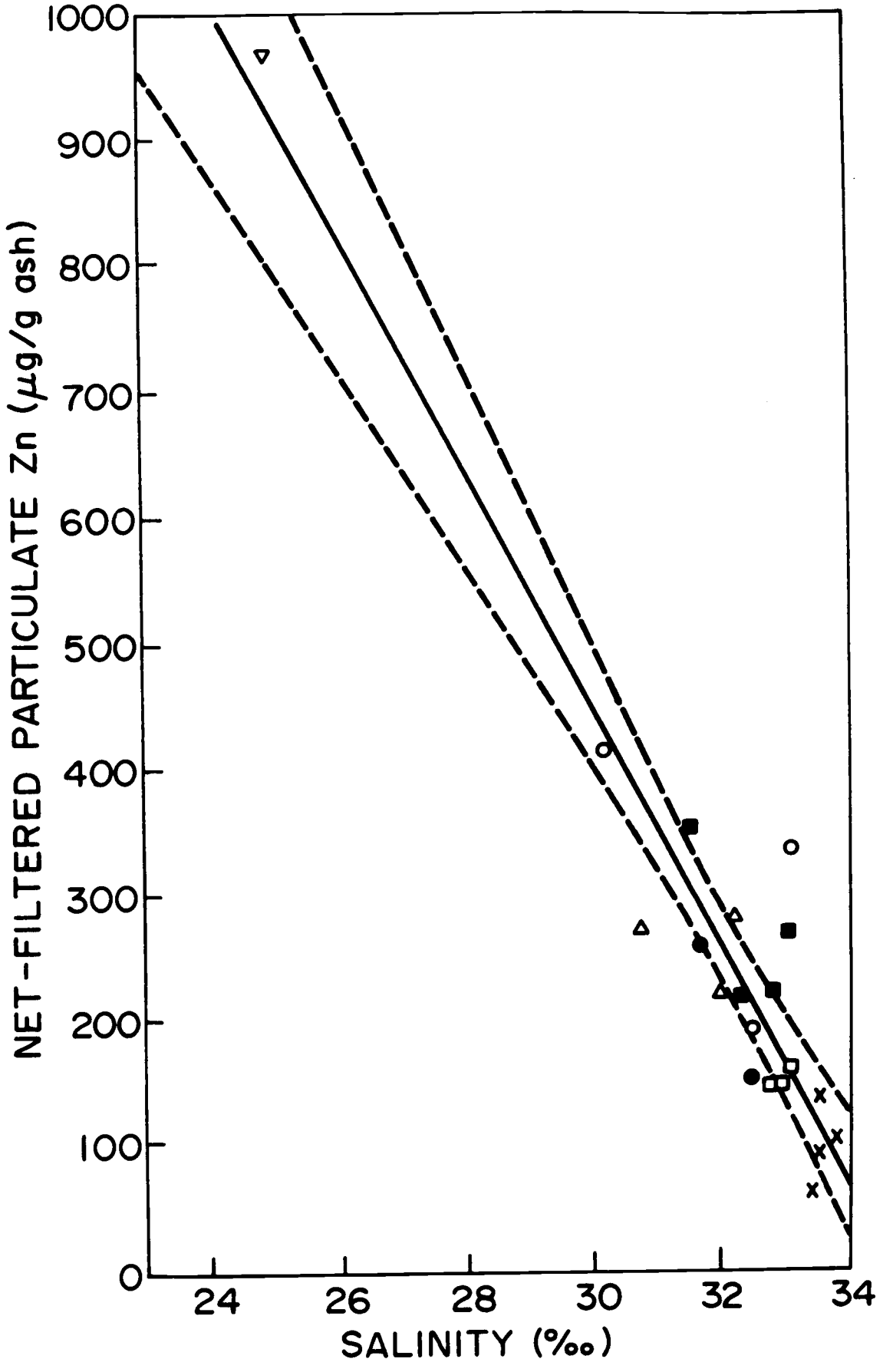


Figure 3: Manganese vs. Salinity. The DE is  $MN = 2703.06 - 80.58 S$ . The  $r$  is 0.87 with 22 df. Mn = the concentration of manganese in ug/g ash. All other abbreviations and symbols have the same meaning as in Figure 2.

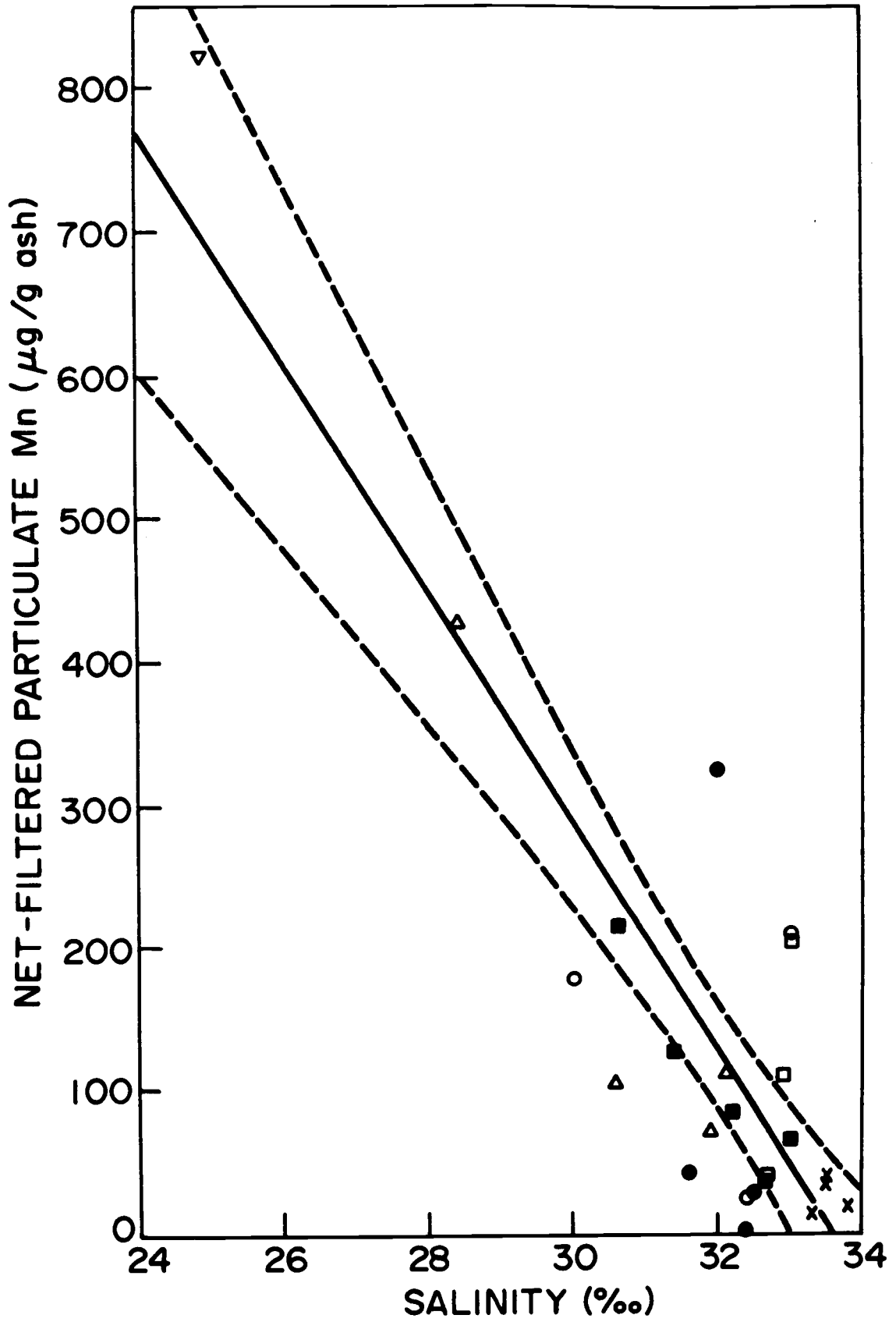


Figure 4: Iron vs. Salinity. The DE is  $Fe = 45465.2 - 1302.5 S$ .  
The  $r$  is 0.74 with 19 df.  $Fe$  = the concentration of iron in ug/g ash.  
All other abbreviations and symbols have the same meaning as those

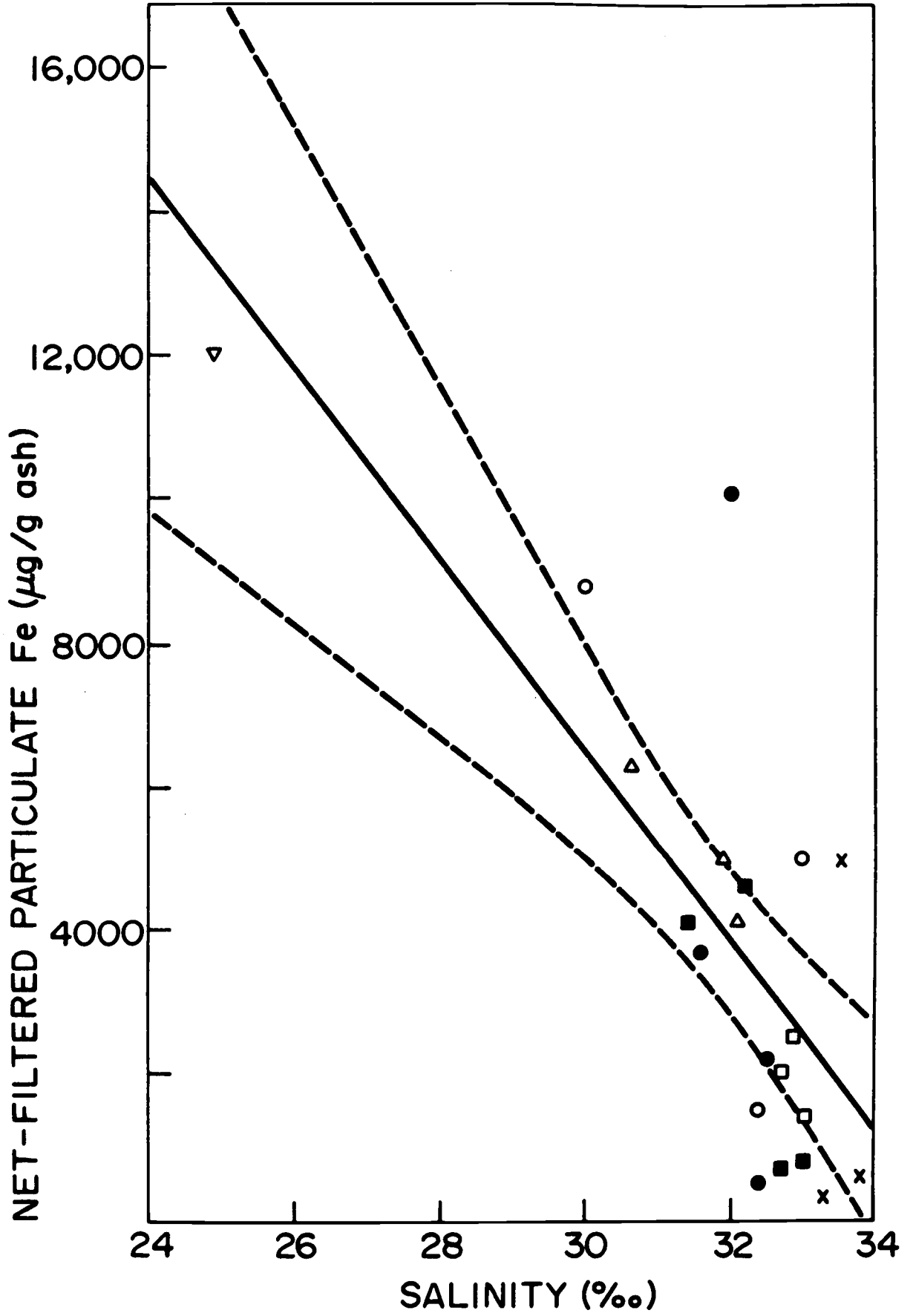



Figure 5: Phytoplankton concentration vs. Distance offshore.

| represents the shoreline and  represents the maximum value at any time. All other symbols have the same meaning as those in Figure 2.

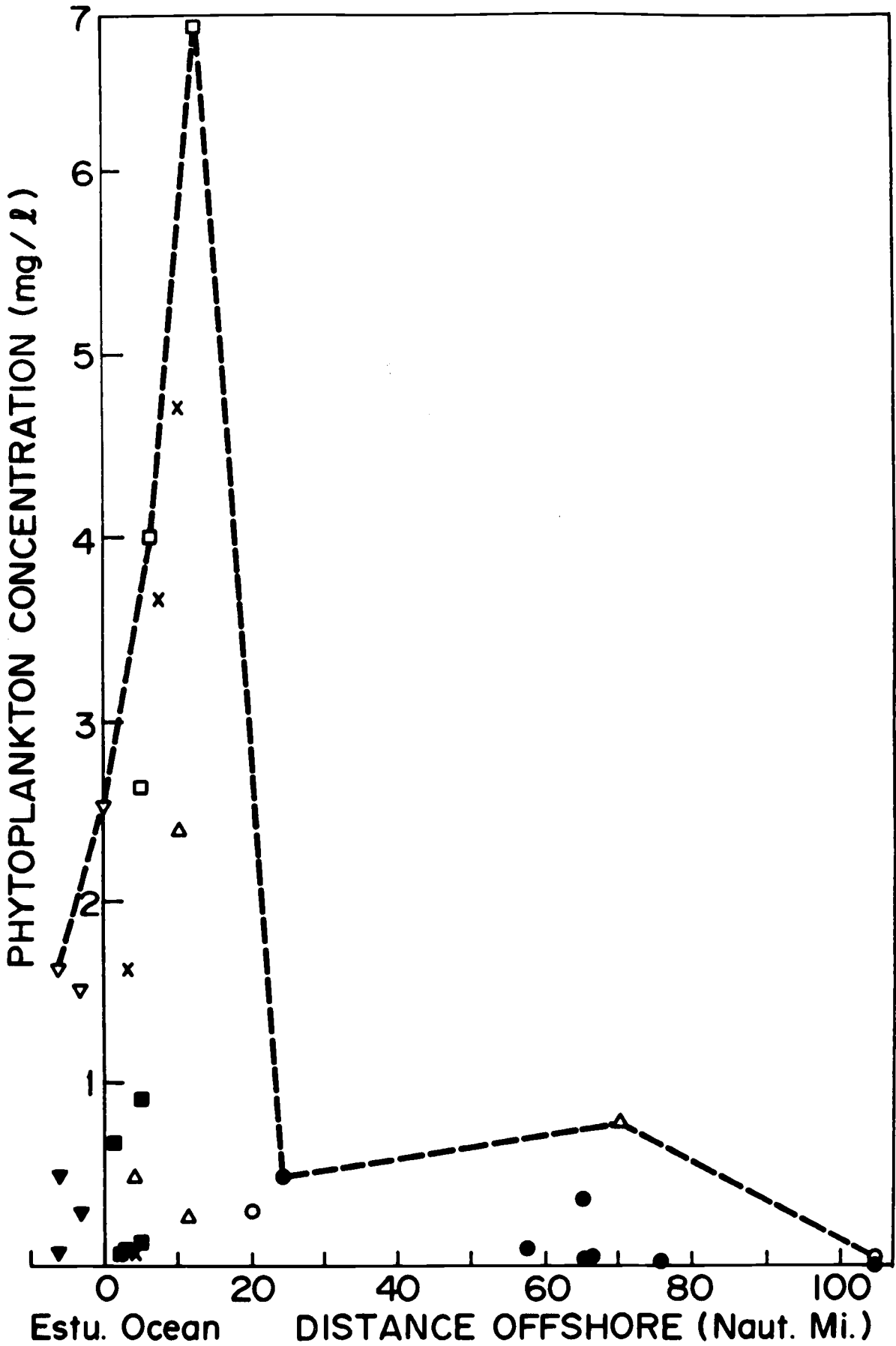


Figure 6: Zinc and Copper vs. Salinity in the Columbia River Estuary. Figure 6a is the zinc concentration vs. salinity in the estuary. Figure 6b is the copper concentration in phytoplankton vs. salinity in the estuary and the copper concentration in water vs. its salinity over the same range as in the Columbia River Estuary according to Droop (1961). Symbols used in Figure 6b and their meaning: ● = sample values from the phytoplankton collected in the estuary at those salinities, / = the slope of the DE for the phytoplankton values, ▼ = values determined by Droop in his artificial seawater, and / = the slope of Droop's values.



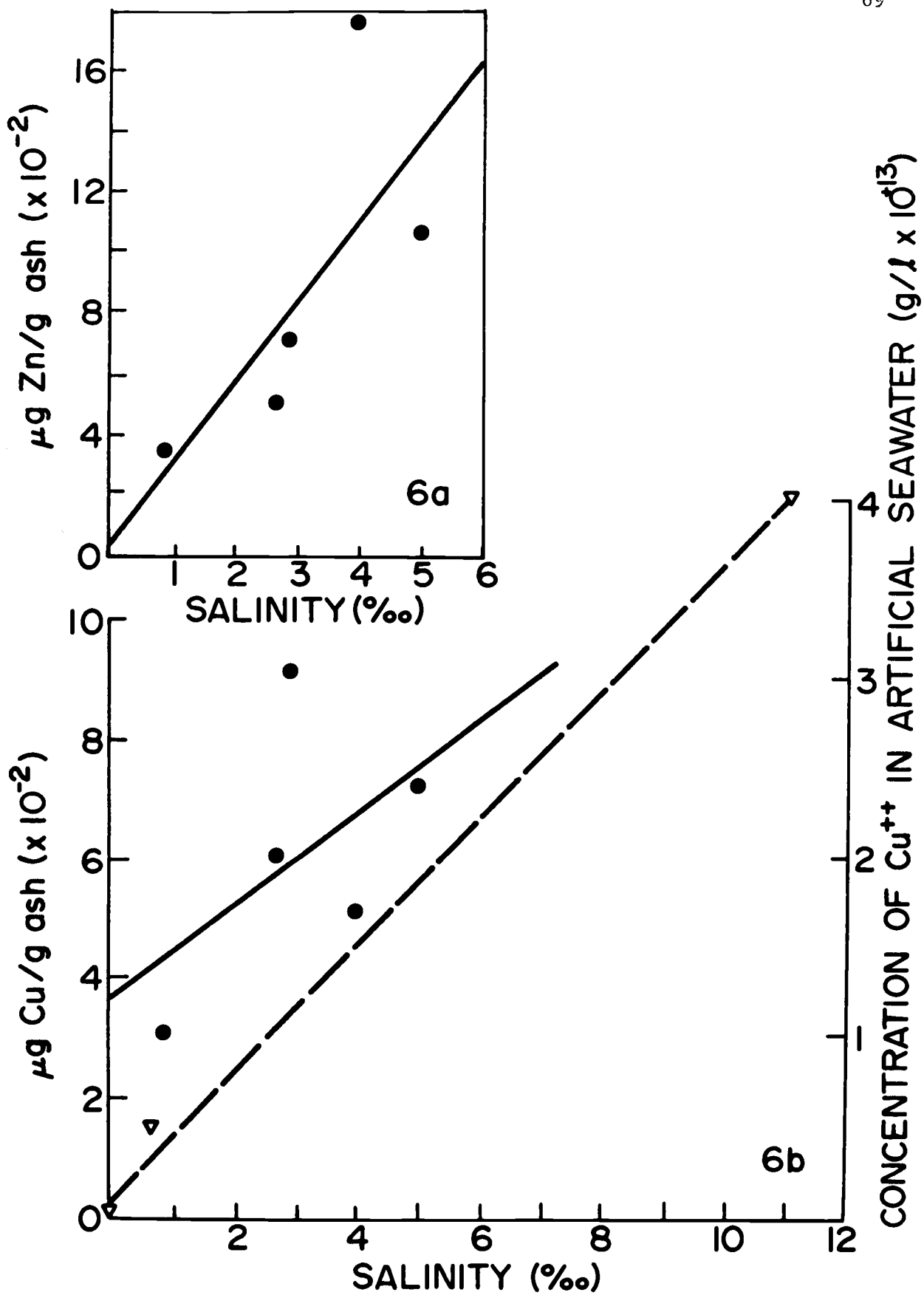


Figure 7: The relationship of zinc in the water of the Columbia River Estuary to its concentration in the net-filtered particulate matter in the water. Symbols used, and their meaning: ● = data points,  $\updownarrow$  = the 95% confidence limits of the matrix-free value,  $\diagup$  = the slope of the relationship, based upon the available data.

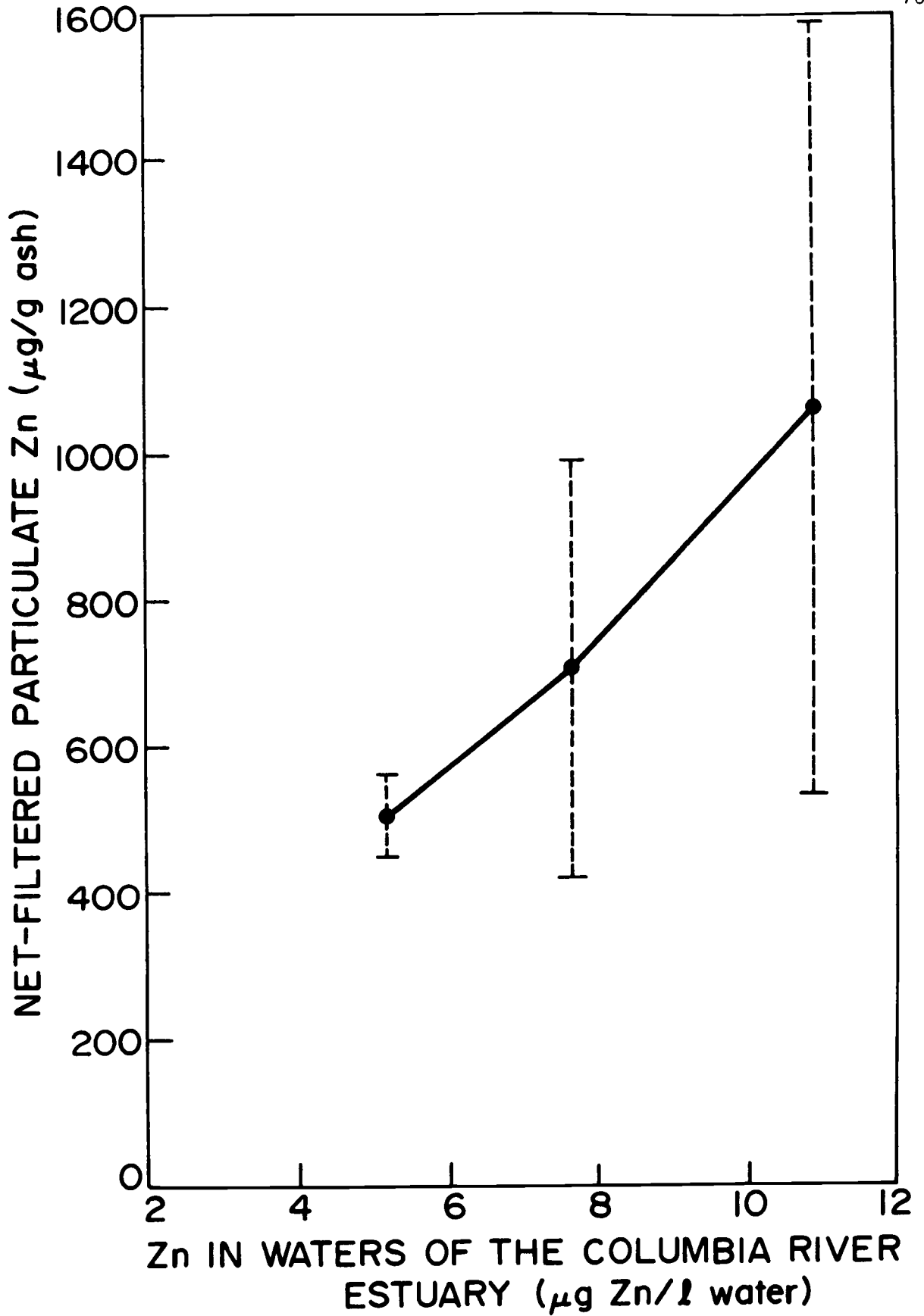


Figure 8: Zinc. vs. Distance offshore. The DE is  $Zn = 195.24 + 4.36 d$ . The  $r$  is 0.45 with 22 df. Abbreviations and symbols used, and their meanings:  $d$  represents the distance offshore in nautical miles,  $M$  = the May sample values, and  $\phi$  = the October Offshore sample values. All other abbreviations and symbols have the same meaning as those used in Figure 2.

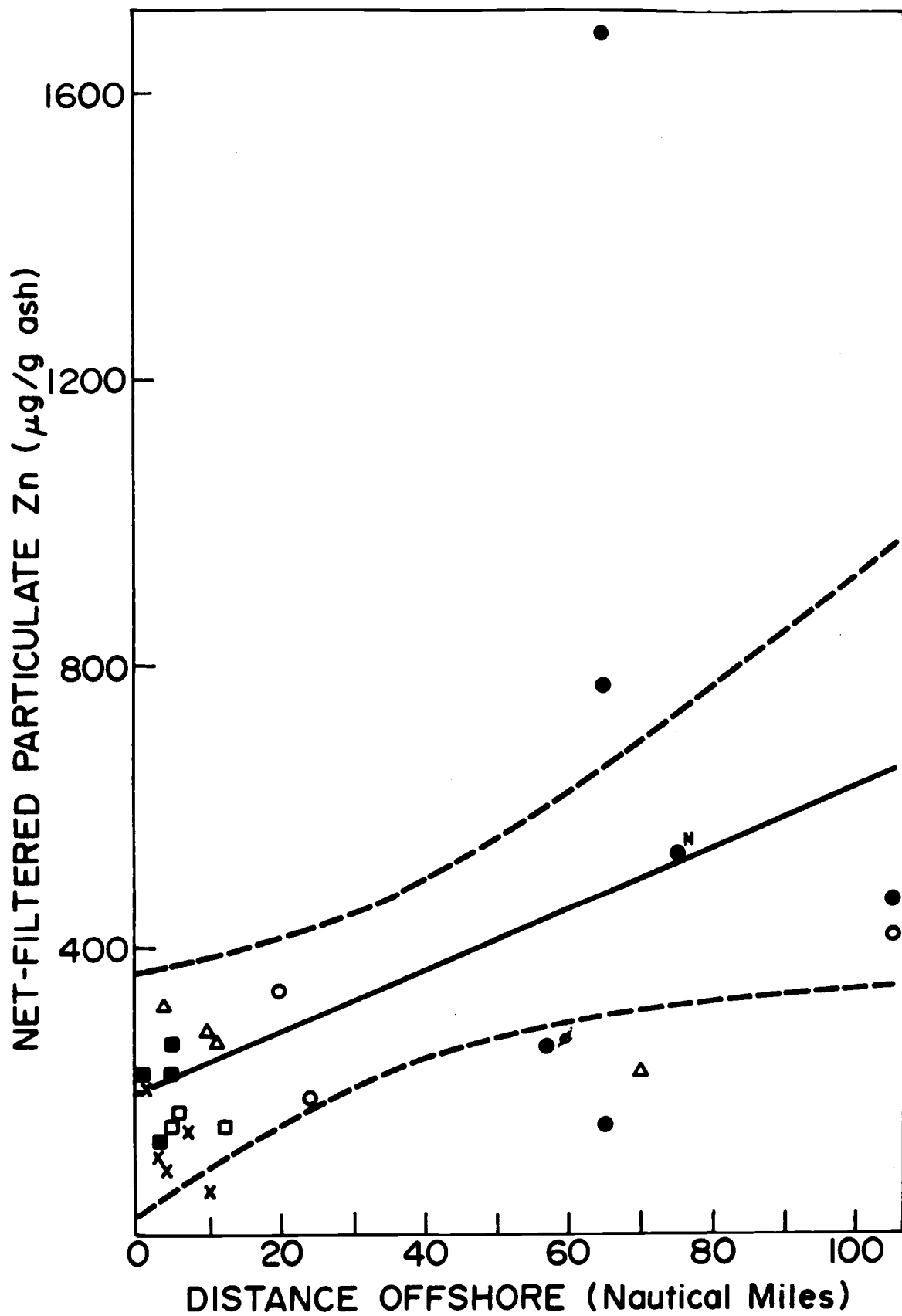



Figure 9: Iron vs. Distance offshore. The DE is  $Fe = 2249.6 + 61 d$ . The  $r$  is 0.68 with 16 df. Abbreviations and symbols used, and their meanings: Fe is the concentration of iron in ug/g ash, d is the distance offshore in nautical miles, M represents the May Offshore sample value,  $\emptyset$  represents the October Offshore sample value, and  Winter encloses the Winter (January and March) Offshore sample values. All other abbreviations and symbols have the same meaning as those used in Figure 2.

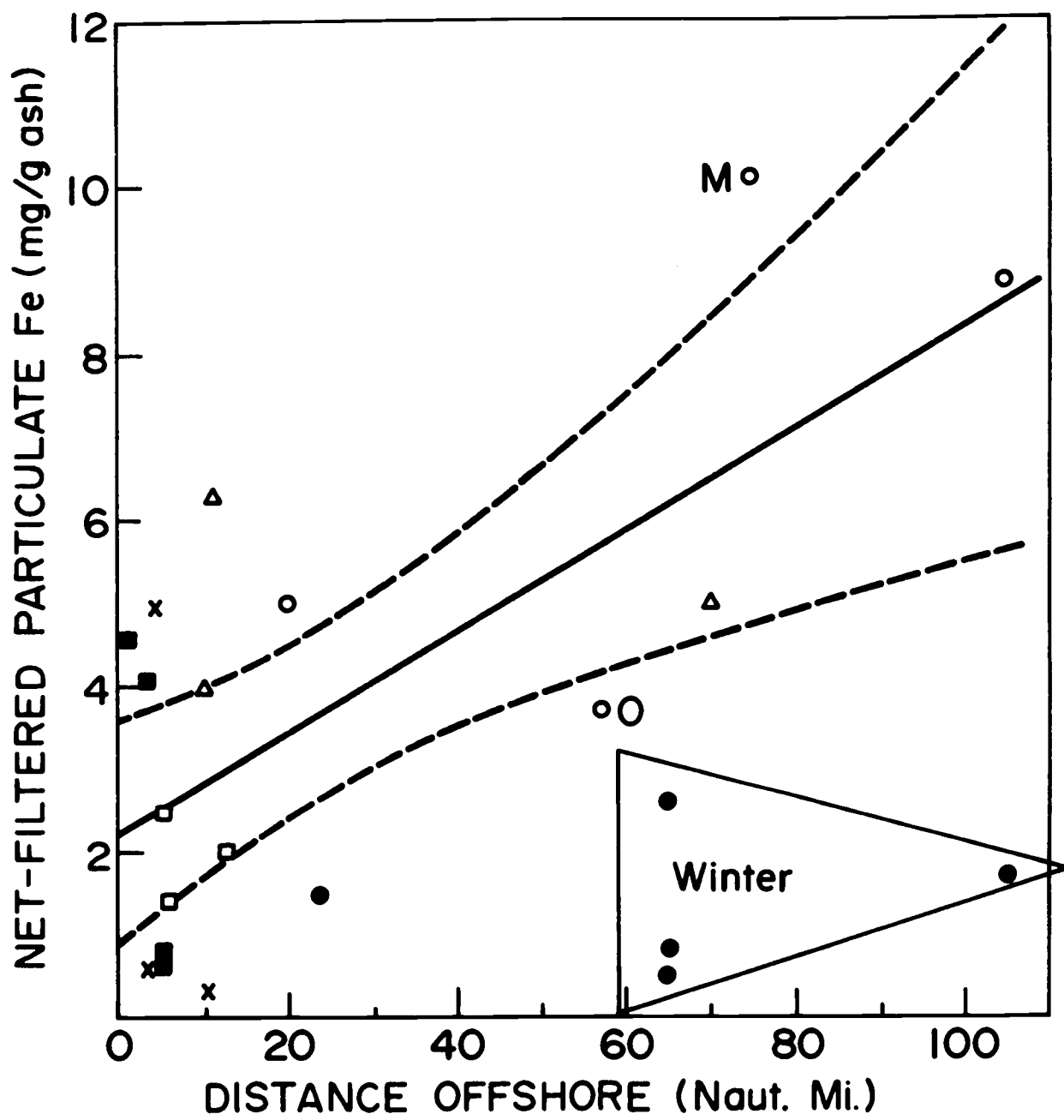


Figure 10: Zinc vs. Phytoplankton Ash Concentration in seawater.

The DE is  $\log \text{Zn} = 2.338 - 0.202 \log A$ . The  $r$  is 0.80 with 17 df.

Abbreviations and symbols used, and their meaning:  $\log$  is the base 10 or common logarithm,  $A$  is the phytoplankton ash concentration in seawater in mg/l, and all other abbreviations and symbols have the same meaning as in Figure 2. The DE was calculated using Plume, Offshore, and Inshore values only.



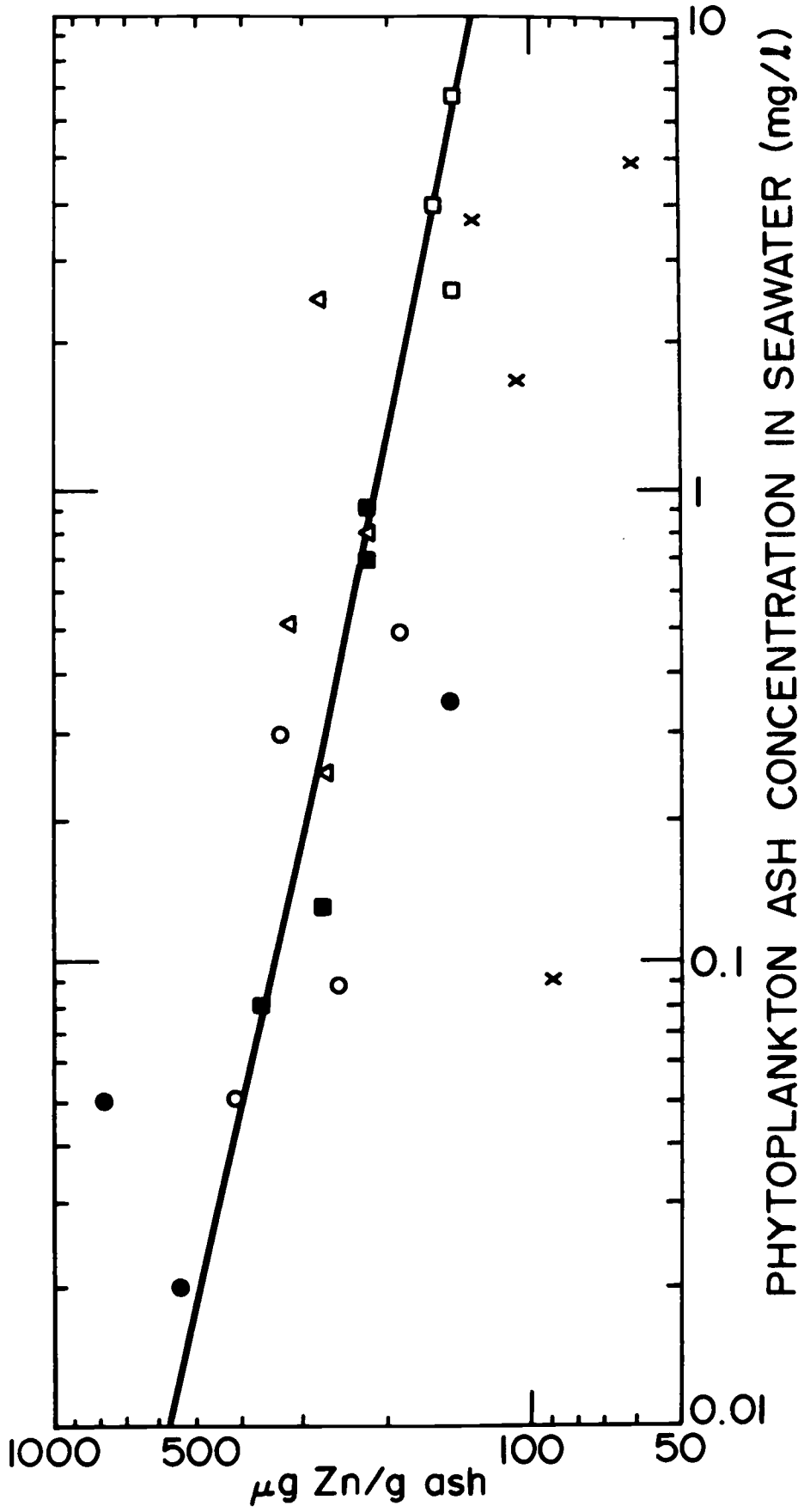


Table 1. --Mean concentration of each metal during the summer in all of the hydrographic regimes (The standard error column in this table and Tables 3 and 5 gives the values which, when multiplied by the t-value for a given significance level at the appropriate number of degrees of freedom, will produce a second value which can be compared to the difference between any two hydrographic regimes. If the second value is higher than the difference, then the significance level between the two regimes is not as high as the significance level set by the t-value. The converse indicates that the difference is at least as high as the set significance level.)

| Metal in       | CRE    |      | Plume  |      | Offshore |      | Inshore |      | Upwelling |      | Standard error |      |
|----------------|--------|------|--------|------|----------|------|---------|------|-----------|------|----------------|------|
|                | ug/g   | ug/l | ug/g   | ug/l | ug/g     | ug/l | ug/g    | ug/l | ug/g      | ug/l | ug/g           | ug/l |
| phyto-plankton | ash    | S.W. | ash    | S.W. | ash      | S.W. | ash     | S.W. | ash       | S.W. | ash            | S.W. |
| Zinc           | 843.73 | 1.62 | 280.90 | 0.39 | 316.43   | 0.07 | 155.60  | 0.69 | 89.13     | 0.16 | 72.4           | 0.26 |
| Manganese      | 582.13 | 1.16 | 107.35 | 0.58 | 137.63   | 0.03 | 118.63  | 0.46 | 28.60     | 0.04 | 82.86          | 0.10 |
| Copper         | 644.13 | 1.20 | 227.50 | 0.30 | 483.13   | 0.11 | 209.60  | 0.80 | 47.97     | 0.09 | 106.93         | 0.16 |
| Iron           | 7867.0 | 16.1 | 5197.0 | 5.7  | 5111.0   | 2.5  | 1980.3  | 8.6  | 1978.3    | 1.0  | 1474.0         | 3.9  |

Table 2. --Significant differences between hydrographic regimes during the summer, by metal and level of significance. Abbreviations used, and their meaning: CRE = Columbia River Estuary, I = Inshore, P = Plume, O = Offshore, U = Upwelling waters, and SW = all seawater hydrographic regimes combined (as opposed to CRE water). Symbols in parentheses indicate combinations of regimes, as (IU), (OP), etc. All differences which are significant at the 99% level are also significant at the 95% and 90% levels; likewise, all differences which are significant at the 95% level are also significant at the 90% level.

| Metal in<br>phyto-<br>plankton | Levels of Significance |           |           |
|--------------------------------|------------------------|-----------|-----------|
|                                | 99%                    | 95%       | 90%       |
| <u>(ug/g ash)</u>              |                        |           |           |
| Zinc                           | CRE-SW                 |           | (IU)-(OP) |
| Manganese                      | none                   | CRE-SW    |           |
| Copper                         | CRE-SW                 |           | P-(IO)    |
|                                | U-(P,I,O)              |           |           |
|                                | I-O                    |           |           |
| Iron                           | none                   | none      | none      |
| <u>(ug/l S.W.)</u>             |                        |           |           |
| Zinc                           | none                   | CRE-SW    | (IP)-(OU) |
| Manganese                      | none                   | none      | CRE-SW    |
|                                |                        |           | I-(U,P,O) |
| Copper                         | CRE-SW                 | I-(U,P,O) |           |
| Iron                           | none                   | none      | none      |

Table 3. --Mean concentration of each metal in the three hydrographic regimes sampled the year round.

Abbreviations and comments are the same as in Table 1.

| Metal in           | CRE    |      | Offshore |      | Inshore |      | Standard error |      |
|--------------------|--------|------|----------|------|---------|------|----------------|------|
|                    | ug/g   | ug/l | ug/g     | ug/l | ug/g    | ug/l | ug/g           | ug/l |
| phyto-<br>plankton | ash    | S.W. | ash      | S.W. | ash     | S.W. | ash            | S.W. |
| Zinc               | 890.6  | 0.77 | 427.4    | 0.04 | 220.7   | 0.32 | 87.9           | 0.19 |
| Manganese          | 494.1  | 0.52 | 118.0    | 0.01 | 95.7    | 0.20 | 57.2           | 0.15 |
| Copper             | 609.8  | 0.57 | 607.9    | 0.05 | 256.1   | 0.42 | 86.0           | 0.14 |
| Iron               | 7872.0 | 7.0  | 4596.0   | 0.4  | 2038.0  | 4.2  | 1120.0         | 2.5  |

Table 4. --Significant differences between hydrographic regimes that are found the year round, by metal and level of significance. Abbreviations and comments are the same as in Table 2.

| Metal in<br>phyto-<br>plankton | Levels of Significance |               |               |
|--------------------------------|------------------------|---------------|---------------|
|                                | 99%                    | 95%           | 90%           |
| <u>(ug/g ash)</u>              |                        |               |               |
| Zinc                           | CRE-SW                 |               | I-O           |
| Manganese                      | CRE-SW                 |               |               |
| Copper                         | none                   | CRE-SW<br>I-O |               |
| Iron                           | none                   | CRE-SW        |               |
| <u>(ug/l S.W.)</u>             |                        |               |               |
| Zinc                           | none                   | none          | CRE-SW        |
| Manganese                      | none                   | none          | CRE-SW        |
| Copper                         | none                   | none          | CRE-SW<br>I-O |
| Iron                           | none                   | none          | none          |

Table 5. --Differences in metal concentrations by season (for the Inshore and Offshore regimes only, combined.) For the seasonal differences reported herein, winter is considered to be December through March, spring is April and May, summer is June through mid-September, and fall is mid-September through November. Abbreviations and comments are the same as in Table 1.

| Metal in       | Winter |       | Spring |       | Summer |       | Fall   |       |
|----------------|--------|-------|--------|-------|--------|-------|--------|-------|
|                | ug/g   | ug/l  | ug/g   | ug/l  | ug/g   | ug/l  | ug/g   | ug/l  |
| phyto-plankton | ash    | S.W.  | ash    | S.W.  | ash    | S.W.  | ash    | S.W.  |
| Zinc           | 691.58 | 0.044 | 376.30 | 0.085 | 236.02 | 0.382 | 207.38 | 0.100 |
| Manganese      | 39.06  | 0.003 | 208.73 | 0.095 | 279.47 | 0.244 | 147.04 | 0.041 |
| Copper         | 563.28 | 0.034 | 929.67 | 0.262 | 346.45 | 0.453 | 112.58 | 0.059 |
| Iron           | 2038.8 | 0.12  | 7372.5 | 1.70  | 3545.7 | 4.72  | 1488.3 | 0.67  |