SEASONAL CHANGES IN HYDROGRAPHIC AND CHEMICAL PROPERTIES OF INDIAN ARM AND THEIR EFFECT ON THE CALANOID COPEPOD *EUCHAETA JAPONICA*

by

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ABSTRACT

This study examines seasonal changes in the relationship between a test organism and changes in the hydrographic and chemical properties of Indian Arm, a coastal fjord. There is a close relationship between changes in the hydrographic properties of the water and changes in the metal complexing ability of water in the inlet, as determined with the test organism.

The relationship between the organism and the availability of metals changes with time; the complexing ability of natural water increases at the time of the major intrusion of water from the Strait of Georgia into Indian Arm, and then decreases. The addition of a variety of metals under experimental conditions affects the relationship between the organism and the complexing ability of the water.

Additional studies examine the effect of material extracted from sediment samples on the toxic effect of copper enrichment. The ability of the extracted material to reduce the toxic effect changes and is related to the seasonal productivity in the surface waters of the inlet.

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INTRODUCTION

The ability of naturally occuring organic material to reduce the toxic effects of some metals has been suggested by several authors (Barber and Ryther, 1969; Barber et al., 1971; Tabata and Nishikawa, 1969; Lewis et al., 1972; 1973). Further, it has been suggested that organic material is responsible for maintaining a certain level of metal in solution and preventing it from being removed by precipitation (Johnson, 1964). Several authors have suggested that the predominant form of certain metals in natural waters may be an organic complex (Slowey et al., 1967; Williams, 1968; Slowey and Hood, 1971) although there is considerable disagreement (Zirino and Healy, 1970; Zirino and Yamamoto, 1972). It has been suggested that the action of some organic material present in natural waters alters the availability of a metal or metals (Barber and Ryther, 1969; Steeman-Nielsen and Wuim-Anderson, 1970; Lewis et al., 1971; 1972; 1973). Barber and Ryther (1969) suggested that the conditioning of newly upwelled water for phytoplankton growth occurs as the result of increases in the organic content of that water. Lewis et al. (1971) showed that the addition of a synthetic chelating agent increased survival of a test organism during certain periods of the year in nearshore waters and more frequently in near-oceanic waters, which suggested a relationship similar to that described by Barber and Ryther (1969). Lewis et al. (1972; 1973) showed that certain naturally occurring organics could act to reduce the toxic effect of increased copper concentration.

Davey et al. (1973), used a bioassay and measured the ability of natural waters to complex amounts of copper above that which was already present. There are certain drawbacks to this type of work, however, as

copper will displace many other metals from complexes with organic molecules, obscuring the fine detail of the system and possibly giving erroneously high values. High values may result from the exchange of copper for the complexed metal. If the metal which is displaced has little or no biological effect, it will not be adequately measured by the bioassay. This situation also holds for chemical analyses.

Although all of these studies indicate that organic material is important to the organisms because of its ability to alter the availability of metals, they fail to accomplish two things. First, they do not work at natural levels of either the metal or the organic material, which is necessary to prevent alterations of the effect on the organism which may occur as the result of metal interactions and concentration effects. Second, they do not indicate those interactions which may affect the equilibrium system in natural waters. The present study demonstrates some of the features of the relationship between an organism and the metals which are present in natural waters, and shows that organic material can alter this relationship.

(i) The chemistry of the complexing of metals in seawater

In the preceding paragraphs, the term metal availability has been used. Availability, in the context of this thesis, indicates the condition or quality of a metal being present in a form which the organism recognizes as that metal. This infers that metals can exist in forms which are not recognized by the organism, so that the organism is not dependent upon the total metal present but only upon that fraction which is available to it (Lewis and Whitfield, 1974).

In the natural environment, chemical reactions of the general forms

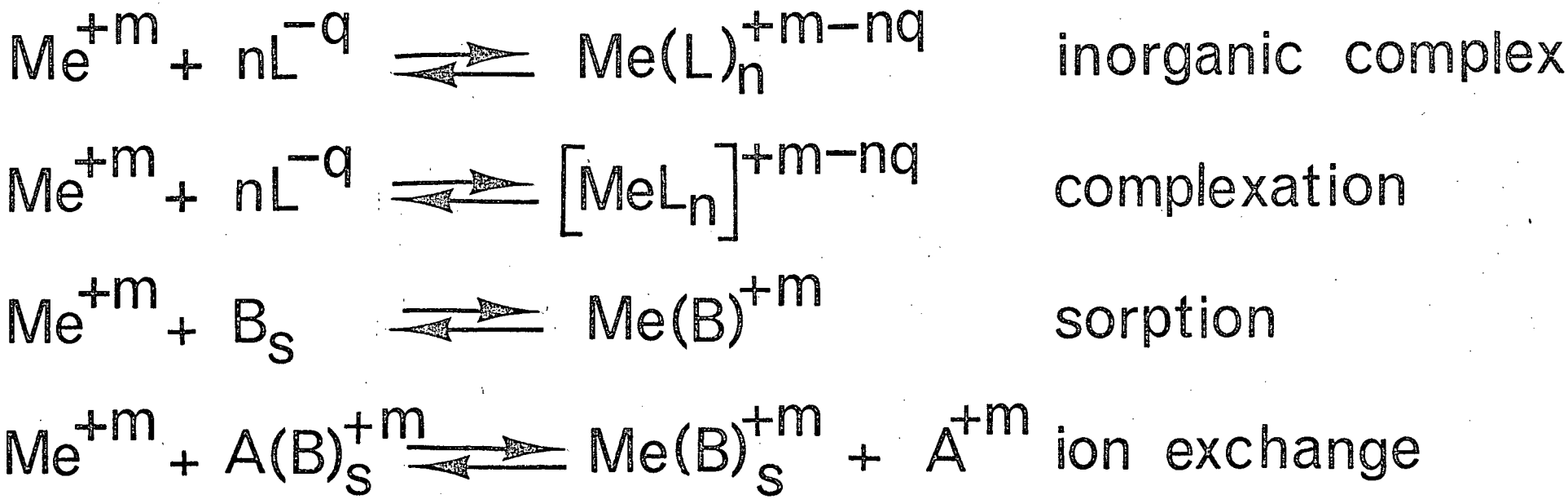
shown in Figure 1 are thought to occur. Each of these has some importance in the chemistry of transition metals in the natural environment (Lewis and Whitfield, 1974). One of the problems which is at present unanswered is the relative biological importance of each of these components. Essentially every biogenous organic molecule has the potential to act as an electron donor molecule, some having stronger affinities for specific metals. Electron donors in organic molecules include the sulphydryl, carboxyl, carbonyl, and amino groups.

The formation constant (k_f) of stable complexes of metals with organic molecules is generally much greater than that for inorganic ligands (Albert, 1950; 1952). Although the concentration of inorganic ligands far exceeds that of the organic ligands, the difference in the stability of the formation constants is sufficiently great to more than counteract the concentration difference (Spencer, 1958; Marchand, 1974).

The term complex describes the end product of a physico-chemical interaction between an electron donor which forms a ligand for an electron acceptor, which is the heavy metal. There are problems associated with the use of the term complex with respect to its biological implications because an organic molecule may affect the availability of a metal to an organism in three ways:

(1) For metals which tend to form precipitates in water (e.g., iron and manganese) the organic molecule may act to increase the metal's solubility by binding the metal in a complex, hence increasing the total amount of metal which can remain in solution. As the level of free metal in solution decreases, the complex will become less stable, releasing the metal from the complex as the result of the chemical

Figure 1. Generalized chemical reactions which are believed to influence the chemistry of transition metals in seawater. Me^{-m} - a metal with an ionic charge of +-m, L^{-q} - an electron donor (ligand) with an ionic charge of -q, B_s - solid particle (e.g., clay particle), and A - an exchangeable metal.


$Me^{+m} + nL^{-q} \rightleftharpoons Me(L)_n^{+m-nq}$
inorganic complex
$Me^{+m} + nL^{-q} \rightleftharpoons [MeL_n]^{+m-nq}$
complexation
$Me^{+m} + B_S \rightleftharpoons Me(B)^{+m}$
sorption
$Me^{+m} + A(B)_S^{+m} \rightleftharpoons Me(B)_S^{+m} + A^{+m}$
ion exchange

equilibrium which exists between the forms of the metal. In this way the organic material is acting as an agent which buffers the level of the metal which is present in a form other than an organic complex.

(2) With some of the more toxic metals (e.g., copper and zinc) the binding of the metal by the organic molecule alters the chemical properties of the metal to such a degree that, in the form of an organic complex, the metal will not have the same biological effect as the ionic form (Avakyan, 1971; Avakyan and Rabotnova, 1971). A well documented example of this type of mechanism in biological systems is the effect of metallothioneins. These proteins are produced in the liver of vertebrates and act to make a group of metals (cadmium, copper, mercury and zinc) non-toxic to organisms even when the metals are present in very high concentrations (Olafson and Thompson, 1974).

(3) The effect of the complexation of a metal may also be to change the chemical properties of the metal in such a manner that an organism may make direct use of the metal, such as allowing the metal to pass through a cell membrane when in the form of an organic complex but not when in an ionic form. Thus, it is possible that the organism requires some metals which it can only utilize when in the form of an organic complex.

Organisms have been shown to be dependent upon geochemical processes to varying degrees (Barber and Ryther, 1969; Phelps et al., 1969; Lewis et al., 1971). The present study examines some geochemical relationships of

biological importance, and relates them to hydrographic events.

(ii) Sediment extracts

Although the complexing ability of the water is the controlling factor, the source of organic complexing agents in the water is important. Water soluble material extracted from sediments has been shown to reduce the toxic effect of a copper enrichment and has been suggested to be naturally diffused into the water column (Lewis et al., 1973). The present study monitored changes in the ability of sediment extract to reduce copper toxicity throughout one year, examining seasonal changes and associating them with events in the rest of the water column. The chemical composition of the material which is extracted is not known but it may be similar to humic acids which have previously been extracted from marine sediments (Rashid, 1972). Trask (1939) and Gross et al. (1972) have suggested that organic material in sediments is from three sources: terrigenous input, phytoplankton production, and material produced by bacterial activity on either or both of the preceeding two sources.

(iii) Measuring complexing ability of natural water

In measuring the ability of a sediment extract to reduce copper toxicity, both the copper stress and the ability of the sediment extracts to reduce that stress are determined as the result of additions to the culture water. This method cannot be used in determining the complexing ability of organic material in natural water. Ultra-violet irradiation of seawater, however, causes photo-oxidation of the organic material (Beattie et al., 1961; Armstrong et al., 1966). This treatment results in the elimination of organic-metal complexes (Strickland, 1972). As the result

of the destruction of organic material, the availability of the metals will change (see page 3). Increases in the concentration of the ionic form of particular metals can be predicted to cause a decrease in the survival of the test organism (Lewis et al., 1972). EDTA (ethylenediaminetetraacetic acid) will, if the organic material acts to complex metals in natural water, be able to replace the active component of the organic material which has been destroyed. The addition of EDTA in sufficient amounts should restore the survival of the organism to that attained in natural seawater. The amount of EDTA required to restore this survival is a measure of the relationship between the test organism and the metals present in the water. Additional evidence about this relationship can be found from experiments which test the effect of increases in the concentration of metals on the relationship between the survival of the organism and the concentration of EDTA. The addition of small amounts of metals which have a high k_f for the formation of a complex with EDTA can be predicted to alter the relationship between the concentration of EDTA and the survival of the organism. Large amounts of metals with lower k_f should also be able to alter this relationship.

Although the nature of the natural complexing agents is not the subject of this study, one attempt was made to obtain an estimate of the molecular size of the material. Large molecular weight organic material has been shown to be able to reduce the toxic effect of copper enrichment (Lewis et al., 1972). Khailov and Finenko (1970) used a chloroform extraction to concentrate large molecular weight organic material for chemical analyses. Chloroform extracts have been used as an indication of organic complexed copper in waters from the Gulf of Mexico (Slowey et al., 1967). If large molecular weight organic material was present in sufficient

amounts, some indication of its ability to bind metals could be obtained from differences in the metal content of water samples as a result of chloroform extraction.

In summary, this study attempts to assess further the complex relationship between an organism and some of the microchemical aspects of its environment. The measuring and monitoring of certain geochemical features of the natural environment are intended to provide an indication of effect on the ecology of an organism.

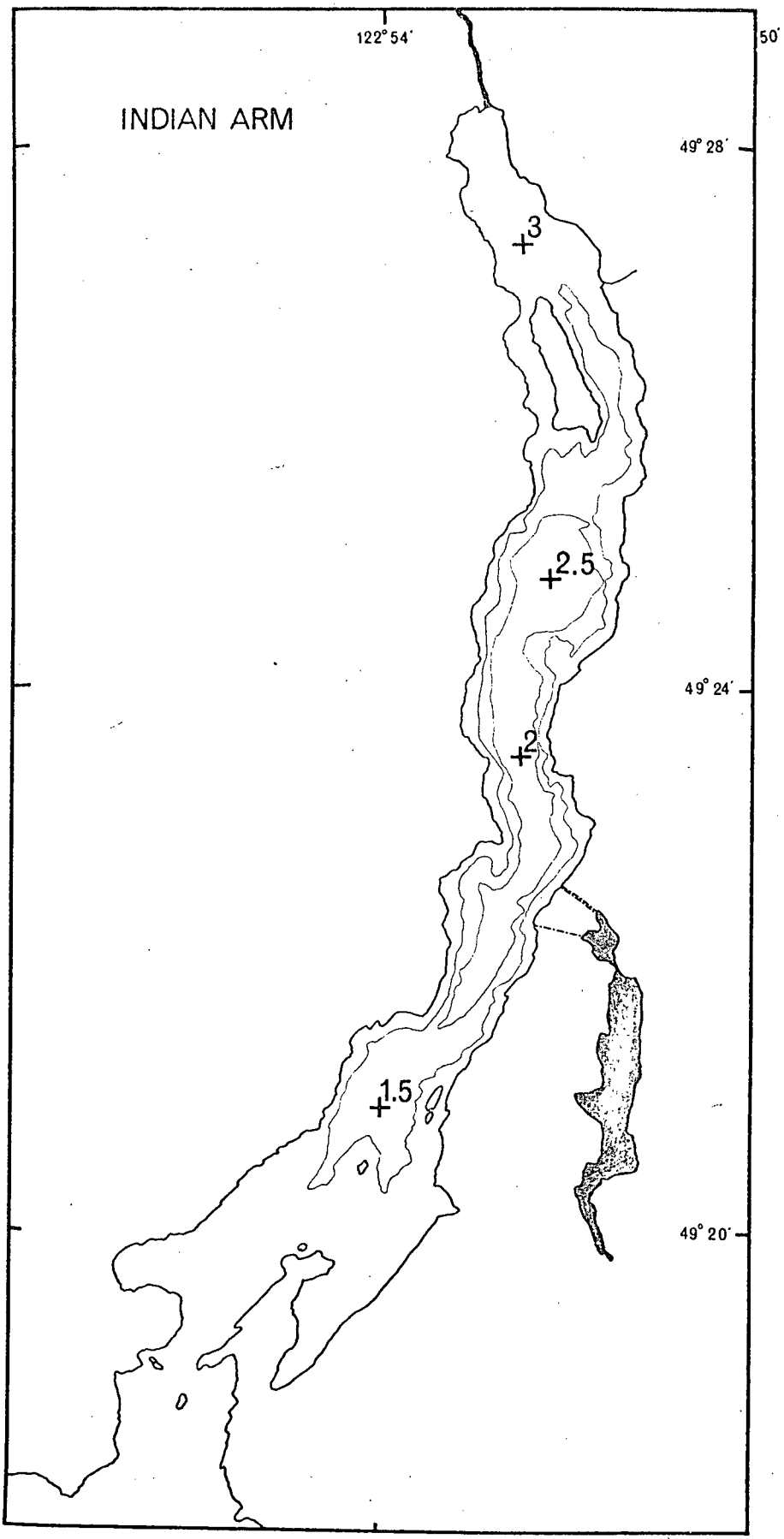
MATERIALS AND METHODS

(i) Field studies

This study was conducted in Indian Arm, a fjord type inlet located 15 km to the northwest of Vancouver (Figure 2). This inlet has been described in relatively great detail by Gilmartin (1962). The total length of Indian Arm is 22 km, with an average width of 1 km (Gilmartin, 1962). In common with most fjords it has a deep basin occupying a large portion of its length. This basin is bounded at the south by a sill 26 meters deep and at the north by the delta of the Indian River. Oceanographic properties of Indian Arm are controlled by the addition of freshwater from stream and river runoff, and by the intrusion of saline water from and the loss of brackish water to the Strait of Georgia, by way of Burrard Inlet (Gilmartin, 1962). A high level of organic production has been shown for the near-surface waters of Indian Arm (Gilmartin, 1964). Indian Arm is less affected by freshwater input and consequently the effects of entrainment, than high runoff inlets (Pickard, 1961), so that the deep water remains relatively stable. When compared with those inlets which have a glacial influence or high river input, Indian Arm is less affected by the massive amounts of sedimentary material which may be important in sorption and ion exchange type reactions. Indian Arm is in an area of high mineralization (personal communication, Anaconda Mines) and has relatively high concentrations of trace metals (Erickson, 1973). This inlet also sustains a relatively large population of the test organism Euchaeta japonica (Evans, 1973).

Biological availability of metals has been studied with chemical techniques (e.g., anodic stripping voltammetry, Erickson, 1973) and bioassay

Figure 2. Indian Arm showing station locations. Also indicated are the 50 and 100 fathom bottom contours.


INDIAN ARM
122° 54'
50'
49° 28'
49° 24'
49° 20'
3
2.5
2
1.5

techniques (Barber and Ryther, 1969; Barber et al., 1971; Lewis et al., 1972; Davey et al., 1973). The present study utilizes the bioassay technique with Euchaeta japonica, a holoplanktonic calanoid copepod. This organism is found throughout the North Pacific Ocean (Davis, 1949). The life cycle consists of an embryo, six naupliar, and six copepodite stages, the last of which is the adult. The morphology of the developmental stages has been described by Campbell (1934). The number of eggs produced ranges from eight to twenty-four per cluster, and the number is thought to be a function of the food supply of the adult (Evans, 1973). Until the organism reaches the third naupliar stage it feeds entirely upon the yolk of the egg. Campbell (1934), Pandyan (1971), and Evans (1973) showed that the naupliar and first copepodite stages of Euchaeta japonica are found in deep water (greater than 100 meters), while the later stages are found higher in the water column. As it is the first three naupliar stages (prefeeding stages) which have been shown to be sensitive to changes in "water quality" (Lewis and Ramnarine, 1969), the field sampling program was biased towards the deeper portion of the water column.

The four stations selected for this study are Ind-1.5 ($49^{o}20.9'N$, $122^{o}54.1'W$), located midway between the sill and the deepest portion of the basin, in 140 meters of water; Ind-2 ($49^{o}23.5'N$, $122^{o}52.5'W$), located at the southern end of the deepest portion of the basin, in 223 meters of water; Ind-2.5 ($49^{o}24.8'N$, $122^{o}52.2'W$), located at the northern end of the deepest portion of the basin, in 225 meters of water; and Ind-3 ($49^{o}27.3'N$, $122^{o}52.5'W$), located just south of the Indian River delta, in 78 meters of water (Figure 2). At each station, horizontal tows were made at specific depths (Table 1) with Clarke/Bumpus opening-closing samplers (Clarke and Bumpus, 1940). The Clarke/Bumpus sampler allows collection of discrete

Table 1. Sampling depths and sampling dates for the collection of information in the field, and water and animals for laboratory experiments.

(a) Sampling Depths

	STATIONS			
Depth (m)	Ind-1.5	Ind-2	Ind-2.5	Ind-3
0	h	h	h	h
10	h b m	h b m	h b m	h b m
20	h	h	h	h
30	h b	h b	h b	h b
50	h b	h b	h b	h b m c
65				h b m c
75	h b m c	h b m c	h b m c	
100	h b m c	h b	h b	
125	h b m c	h b m c*	h b m c	
150		h b	h b	
175		h b m c	h b m c	
200		h b m c*	h b m c	

(b) Sampling Dates

September 14, 1972	July 18, 1973
October 12, 1972	August 15, 1973
November 7, 1972	September 12, 1973
December 5, 1972	October 17, 1973
January 16, 1973	November 7, 1973
February 13, 1973	December 5, 1973
March 13, 1973	January 22, 1974
April 16, 1973	February 19, 1974
May 15, 1973	March 15, 1974
June 16, 1973	April 3, 1974

h - indicated hydrographic samples taken (temperature, salinity, dissolved oxygen).

b - indicates biological samples taken with Clarke/Bumpus (C/B) samplers.

m - indicates samples taken for analysis of dissolved metals and particulate material.

c - indicates samples taken for organic carbon determination during the period from May, 1973 to October, 1973.

* - water collected for laboratory culture studies.

samples and monitoring of the approximate volume of water filtered (Yentsch and Duxbury, 1956; Regan, 1963; Tranter and Heron, 1965). The samplers were towed for 15 minutes at a speed sufficient to maintain a wire angle of 35 ± 5^{o} to the vertical. This speed is approximately two knots depending upon the wind and tide conditions.

Samples taken from the Clarke/Bumpus nets were transferred to 4 oz. screw top jars identified with respect to depth of sample, cruise number, date and sample number. Samples were preserved by the immediate addition of 3-5% (V/V) of borax buffered formalin. Nets were washed between uses to prevent cross-contamination of the samples.

Hydrographic data were collected from a series of depths (Table 1), using NIO sampling bottles (National Institute of Oceanography, Wormley, U.K.). Oxygen determinations were done on board ship using a modified Winkler technique (Carritt and Carpenter, 1966). Temperatures were read from reversing thermometers ($\pm0.01^{o}C$) mounted in frames on the sampling bottles, and water samples were drawn for salinity determination in the laboratory. Surface samples were obtained with a bucket for a salinity sample and for determination of temperature ($\pm0.1^{o}C$). A bathythermograph (BT) cast was also made at each station to the depth of the deepest hydrographic sample. The trace provided from the BT was used to check temperature fluctuations, and to determine the depth of the thermocline.

Samples for chemical analyses were also collected with the NIO bottles (because these are for the most part plastic, contamination problems are reduced). A two liter sample was collected at each of the depths specified in Table 1. This sample was then passed through a preweighed 0.45 μm Millipore filter. This pore size was chosen as it is the accepted limit between dissolved and particulate material (Lewis and Goldberg, 1954).

This was done to determine the relative concentration of copper in particulate phase as compared to that in the dissolved phase. The filters were immediately placed in a freezer and returned to the laboratory for analysis of weight of particulate material and the amount of copper associated with the particulate material. One liter of the filtrate was collected into an acid-cleaned polyethylene bottle, which had been rinsed with approximately 300 ml. of the filtered sample. The sample was then refrigerated at $8^{o}C$ until analysed.

Samples for organic carbon determination were drawn from the NIO bottles into a 200 ml. acid-cleaned, glass stoppered bottle. Samples were drawn from this through a prefired ($600^{o}C$) glass fiber filter, mounted in a Millipore syringe adapter (XX3002500), into a 10 ml. glass syringe. This apparatus was rinsed twice with 7-8 ml. of the sample. A 5 ml. sample was drawn and injected into a 10 ml. pyrex ampoule which had been precombusted at 600^{o} for four hours in a muffle furnace. The ampoules were kept covered with precombusted aluminum foil at all times to prevent airborn contamination. Three replicates of each sample were drawn, and stored frozen until analysis.

(ii) Analysis of field samples

Each C/B sample was sorted, using a Wild M5 Stereomicroscope. The naupliar stages of Euchaeta japonica and adult females bearing eggs were removed. These were then identified to stage of development and a record of the number of each of the stages was kept. The sorted animals were then placed in a 2 dram screwtop vial which was kept in the original sample jar. All counts were recorded initially as total numbers and later converted to numbers/m^3 with the calibrated C/B flowmeter recordings. Naupliar stages were grouped into prefeeding stages (first through third nauplius), total

naupliar stages, and sixth naupliar stage (the numerically predominant stage). These groupings serve to remove some of the variability of numbers due to the length of time spent in each stage.

The salinity of samples was measured with an Autolab Inductively Coupled Salinometer (Model 601, MK3). Temperatures were corrected to give the temperature at the depth of the sample. Oxygen readings were converted to give dissolved oxygen concentrations, in ml./l. Densities were calculated from the temperature and salinity of the sample and expressed as a sigma-t value. Sigma-t = (specific gravity - 1) x 10^3. Initially the conversions of data were done by hand but later the raw data was converted on a PDP12 computer, with a program written by J.R. Buckley (IOUBC).

The filters which were used in the field for trace metal samples were removed from the freezer, dried at 60°C for one hour and weighed. The difference between the weights of the unused and the used filters is a measure of the particulate material which has been removed onto the filter. Subsequent to the weighing process the filter was digested in hot nitric/perchloric acid (1/1, V/V). After the filter was digested, hydroxylamine buffer was added to bring the pH to approximately 7.0. Five ml. of 0.0025 M bathocuproine in ethanol was added and the bathocuproine-copper complex extracted into chloroform. The chloroform layer was then transferred into a 10 cm cell and the absorbance at 474 nm measured with a Perkins-Elmer double beam spectrophotometer. Concentrations were determined from a standard curve, and expressed as µg Cu/mg particulate material. Unused filters were treated in the same manner to determine background levels of copper. The precision of the copper determination is about ±20% of the mean.

Seawater samples collected for trace metal analysis were removed from the refrigerator and placed in a one liter glass erlenmeyer flask, and

the metals extracted into isoamylacetate with diethyldithiocarbamate. After the layers separated subsequent to the water and reagents being stirred, the upper layer (isoamylacetate) was removed. This process was repeated three times. The isoamylacetate was removed to a 100 ml. separatory funnel, the metals being back extracted into an aqueous phase with chlorine in 0.1N HCl. The aqueous phase was collected into a 25 ml. glass stoppered graduated cylinder. This was then analysed for zinc and manganese (when greater than 10 µg/l) on a Techtron Atomic Absorption Spectrophotometer (no. AA-4). Of the remaining aqueous material, 15 ml. was retained for further analysis. To the 15 ml. of concentrated metals was added 2 ml. of a 1.0 M tris buffer (pH approximately 7.0) and 1 ml. of a 2% solution (by weight) of diethyldithiocarbamate. The metal-diethyl-dithiocarbamates were then extracted into 5 ml. of methylisobutyl ketone (MIBK). The MIBK phase was then subjected to atomic absorption analysis for copper, nickel, cadmium, and manganese (where less than 10 µg/l). The amount of absorption obtained for each metal was compared to a standard curve prepared for each metal and a concentration for the original sample determined (Grill, unpublished). This technique has been shown to have a greater than 95% recovery for all metals analysed (Grill, unpublished).

Organic carbon determination was performed by the method of Menzel and Vaccaro (1964), as described in Strickland and Parsons (1972). Values determined by this method are considered accurate to ±0.13 mg/l (this being the standard deviation of the mean of 10 replicate samples). This accuracy exceeds that achieved by Slowey and Hood (1971) (±0.44 mg/l) but is not as accurate as that suggested by Strickland and Parsons (1972) (±0.06 mg/l).

(iii) Laboratory studies

Animals and water for the laboratory studies were collected at Ind-2. The water was collected with a 96 liter fiberglass and lucite sampler (lewis et al., 1971). The collection depth was 200 meters, although additional samples have been collected from 125 meters. The water was subjected to pressure filtration through a membrane filter (0.45 μm mean pore diameter) within 2 hours of collection and stored at 8°C in 23 liter polyethylene containers. Animals were collected with a 1 meter diameter conical net (mesh aperture approximately 0.7 mm square) towed vertically to the surface from near the bottom. The plankton was sorted and egg clusters as well as females bearing eggs were removed and transported to the laboratory in a 3 liter thermos flask filled with filtered seawater collected from 200 meters. Egg clusters were sorted in the laboratory, and those containing eggs of the same dark blue colour as eggs present in the oviducts of the females, were retained for use in the culture experiments. This choice ensures the use of embryos during an early stages of development.

The prefeeding stages of the organism were maintained in 1 liter polypropylene erlenmeyer flasks containing 600 ml. of filtered seawater. The water used was in a series of treated and enriched states, as discussed elsewhere. The flasks were kept at 8°C in a controlled environment chamber (Sherer Gilett Co. Model E2). Water was changed every third day and the condition of the embryos and nauplii was recorded. Dead organisms, as well as those which had reached the third naupliar stage (the first feeding stage), were removed at the time of the water change.

The 1 liter erlenmeyer flasks were filled with 600-700 ml. of 0.1 N HCl in distilled water and stored between experiments. At all times

these flasks were kept covered with Parafilm, ensuring that the flasks were not contaminated.

The effects of various treatments and enrichments of the culture water were measured by comparison of the percent of the organisms used per test which survive until the third naupliar stage. Four egg clusters (one in each of four flasks) were used for each test in a monthly series. Under experimental conditions this number of replicates allows examination of the effect of the treatment of the media, while maintaining a standard error of the mean of less than 5%. This estimate of error was obtained by running 12 replicates and calculating a mean survival of all possible combinations of increasing numbers of flasks until the 5% level had been reached. However, because of the small number of replicates, the standard deviation of each mean is relatively large. In evaluating the results a difference between means greater than 10% was considered to be statistically significant.

The test materials used to enrich the seawater were prepared in a manner such that a 1 or 2 percent mixture (i.e., 1 or 2 ml. of enrichment media to 100 ml. of seawater) of the stock solution would yield the desired concentration in the culture media. All metal solutions used were prepared from the chloride salt of the metal, rather than the sulphate, as there is some evidence that the sulphate anion changes the effect of certain metals. All metal concentrations used are expressed as µg/l of culture media.

Metal additions were made on the basis of doubling or tripling the concentration of the metal which was believed to be present, based on previous measurements. For zinc the additions were either 4 or 8 µg/l. For manganese the additions were 50 or 100 µg/l. Additions of copper were made at several different concentrations. A copper addition of 5.4 µg/l was

used in the copper stress series and in determining the complexing ability of material extracted from sediments. Copper concentrations of 1, 2, and 5 µg/l were used in some of the ultra-violet experiments.

EDTA (ethylenediaminetetraacetic acid) enrichment media were prepared by dissolving the disodium salt of EDTA in deionized water to obtain the desired concentration. The final culture concentrations in the copper stress series were 0.125 and 0.250 µM, while 0.125, 0.250, and 0.500 µM were used in the ultra-violet series.

Sediment extracts were prepared from 125 ml. of sediment taken from a sample collected with a Shipek grab sampler. This sediment was mixed with 800 ml. of filtered seawater collected at Ind-2 from 200 meters. This was then shaken periodically during the first two hours of the extraction, and left to stand for a further twenty-two hours. After this time the supernatant was filtered through a 0.45 µm Millipore filter and then stored at $8^{o}C$. This material was used in enriching culture water (previously enriched with 5.4 µg/l of copper) at a 2% dilution.

The high wattage lamp used for irradiating seawater samples is operated under a nitrogen environment within a glass immersion well. The immersion well is double walled with a controlled water flow passing between them to allow transfer of heat away from the lamp. Controls for the components of the system are electrically interlocked with the power supply to the lamp. In addition to the electrical supply to the lamp, the electrical components include switches and solenoids which extinguish the lamp in the event of failure in one of the associated systems, thus reducing the danger involved in the operation of such a lamp. Also included in the electrical system is a timing clock which allows control of the duration of sample treatment. The lamp is suspended with a 50 l chromatographic chamber

covered on the outside with aluminum foil. This apparatus is shown schematically in Figure 3. This system is contained in an environmental chamber which is kept at 8°C. Further description of this apparatus is given as an Appendix.

The necessary duration of ultra-violet treatment of the culture water was determined from the results shown in Figure 4. After four hours, the changes induced by the ultra-violet light are essentially complete, as indicated by the change in the slope of the line. On the basis of these results it was believed that a sufficient duration of treatment would be six hours. This allows for some deterioration of the efficiency of the lamp, and changes in the amount of material which the lamp is acting upon. Measurements of dissolved organic carbon, before and after the treatment of water with ultra-violet light, provides further support for the use of this duration of exposure.

Chloroform extractions were performed by the method of Khailov and Finenko (1970) on one liter samples of seawater used in culture experiments. The metal content of the samples after extraction was analysed with the method described elsewhere in this paper.

(iv) Statistical analysis

Several statistical treatments were used to examine field measurements. These included correlation analysis, cluster analysis and regression analysis. Analysis resulted from the use of statistical packages made available by the UBC Computing Center, for use with the IBM 360/70 and later with the IBM 370/168 computers.

All of the filed data collected was subjected to a correlation analysis (UBC CORN). This program computes correlations between pairs of

Figure 3: Diagrammatic representation of the ultra-violet apparatus

Key to Figure 3

Water Cooling System

1. Water inlet valve
2. Filter (Cuno type, 5 microns)
3. Water pressure reducing valve
4. Solenoid valve
5. Inlet pressure gauge
6. Well inlet
7. Well outlet
8. Outlet pressure gauge
9. Pressure switch
10. Drain

Electrical System

11. Off-On switch
12. Timing clock
13. Circuit breaker
14. Pilot light
15. 220 volt stabilized ballast
16. 12 inch 1200 watt mercury arc lamp

Nitrogen System

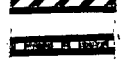
17. Nitrogen tank regulator
18. Outlet needle valve
19. Erlenmeyer flask with water

Additional Parts

20. Vycor immersion tubes
21. 50 liter treatment chamber
22. Aluminum foil reflector
23. Well head assembly
24. Plate glass covers

Water lines 

Electrical lines 

Nitrogen lines 

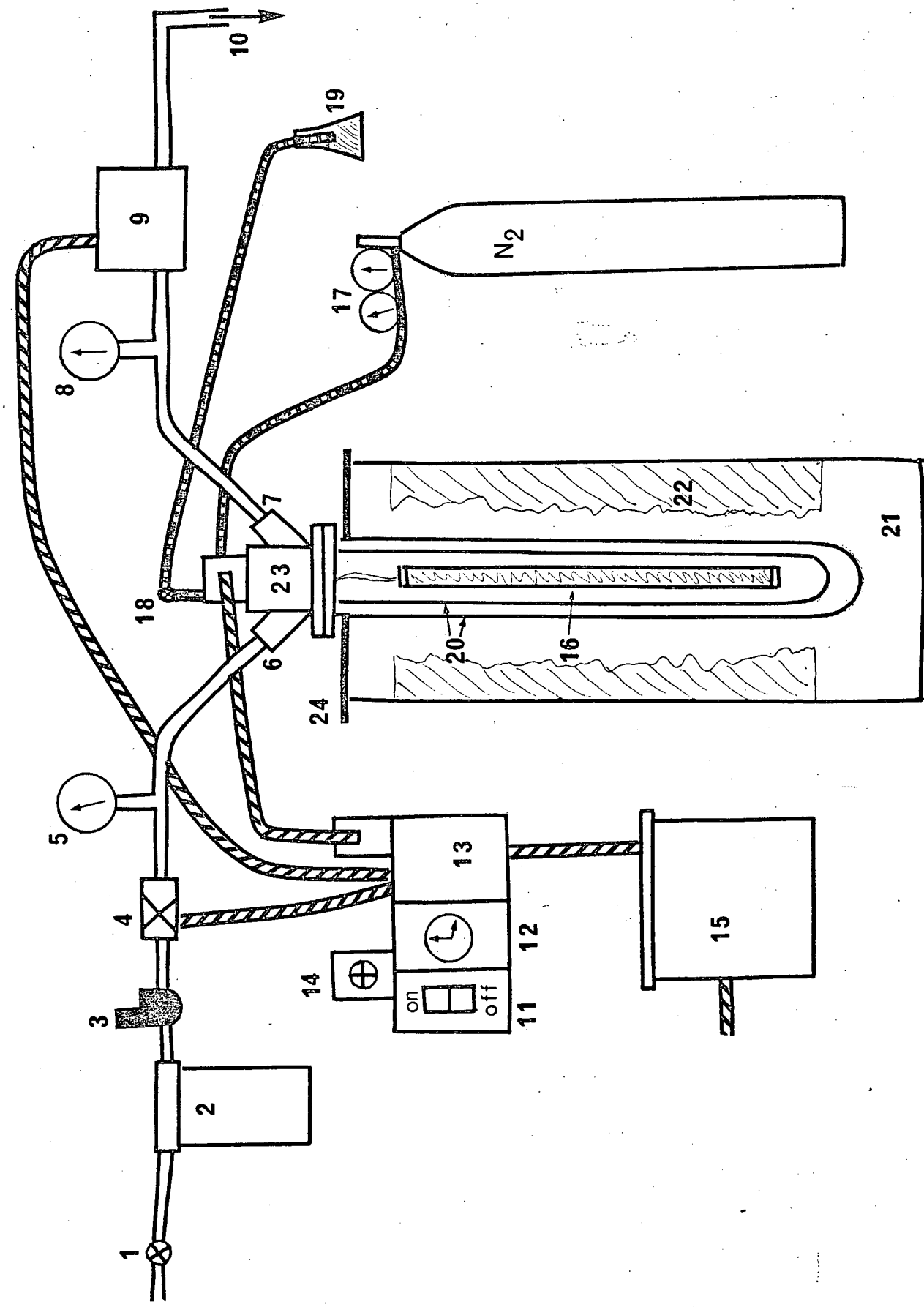
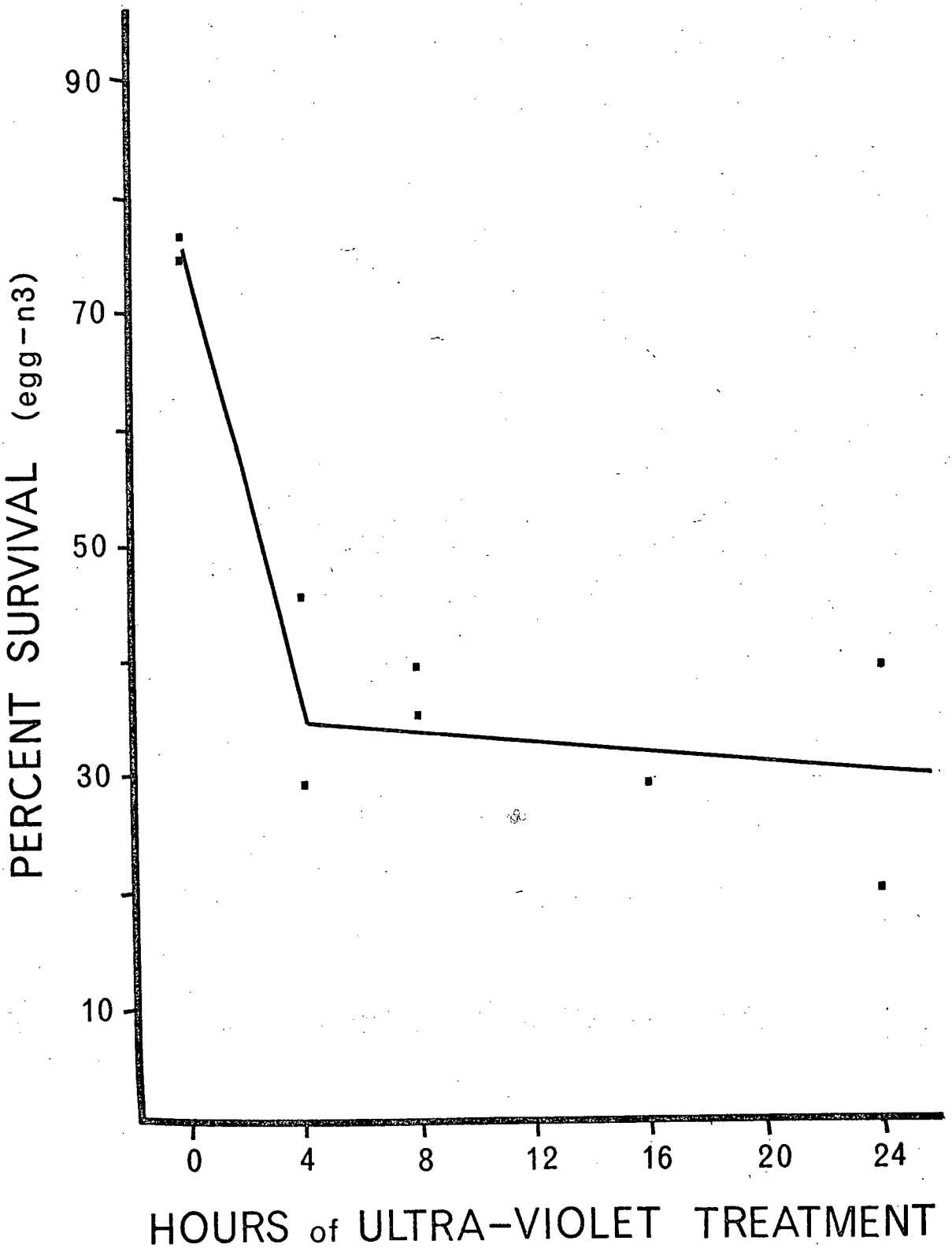

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
N_2
on
off

Figure 4: Percent survival from the egg through to the third nauplius as a function of the duration of treatment of the culture water with ultra-violet light. Each point represents the mean survival determined from approximately 60 animals. The curve is fitted by eye.


90
70
50
30
10
PERCENT SURVIVAL (egg-n3)
0
4
8
12
16
20
24
HOURS of ULTRA-VIOLET TREATMENT

variables and performs significance tests on the correlation coefficients. This program was used also to treat several subsets of the data. The data were divided into subsets by several means. First, the entire data series was treated. Next, all samples where the naupliar stages had been found were analysed (i.e., all samples taken from deeper than 75 meters). The data were divided again into subsets by stations and then by periods of the year as determined from Figure 9. The assumption behind correlation analysis, that the pairs of variables are linearly related, was tested using the UBC STRIP-SIMREG routine to check for linearity between variables.

Cluster analysis was done with UBC BMDP1M, on a data subset which consisted of all observations where all of the 15 variables had been measured. This program groups variables into clusters on the basis of similarity, in this case from a correlation matrix. The program progressively groups pairs of variables from high correlations to low correlations.

RESULTS

(i) Hydrographic properties

Figures 5-8 summarize tabulated data published in IOUBC Data Reports (1972; 1973; 1974), and are included here to provide evidence of the occurrence of intrusions. Ind-2 data was selected for presentation because it is the station where animals and water were collected for laboratory experiments. The other stations exhibit patterns which are similar to those shown. Figure 5 shows the distribution of temperature through the water column over the period of this study. Similarly, Figure 6 presents salinity data; Figure 7, density data; and Figure 8, dissolved oxygen data. Figure 9 shows temperature salinity envelopes for three periods of the year. Intrusions of water into the inlet from the Strait of Georgia occurred at two times during the first year. Between the cruises of December, 1972 and January, 1973 there was a large intrusion of water which had a higher temperature, higher salinity, and higher dissolved oxygen than that of the deep water of Indian Arm. Prior to the July, 1973 cruise there was a small intrusion of very dense water which caused the deeper portion of the water column to become more homogenous with respect to temperature, salinity, and dissolved oxygen. Another intrusion may have occurred at mid-depth in January, 1974, but adequate hydrographic data are not available for verification of this. Such intrusions have previously been noted in this inlet (Gilmartin, 1962).

(ii) Chemical properties

The samples taken for chemical analyses were biased towards the deep water as this is where the organism is most abundant (Evans, 1973). Figure

Figure 5. Distribution of temperature (^{o}C) through the water column at Ind-2 during the study period.

Note: In figures 5-8 the sampling depths are given in Table 1, and the sampling was done at monthly intervals. Arrows indicate time of intrusion.

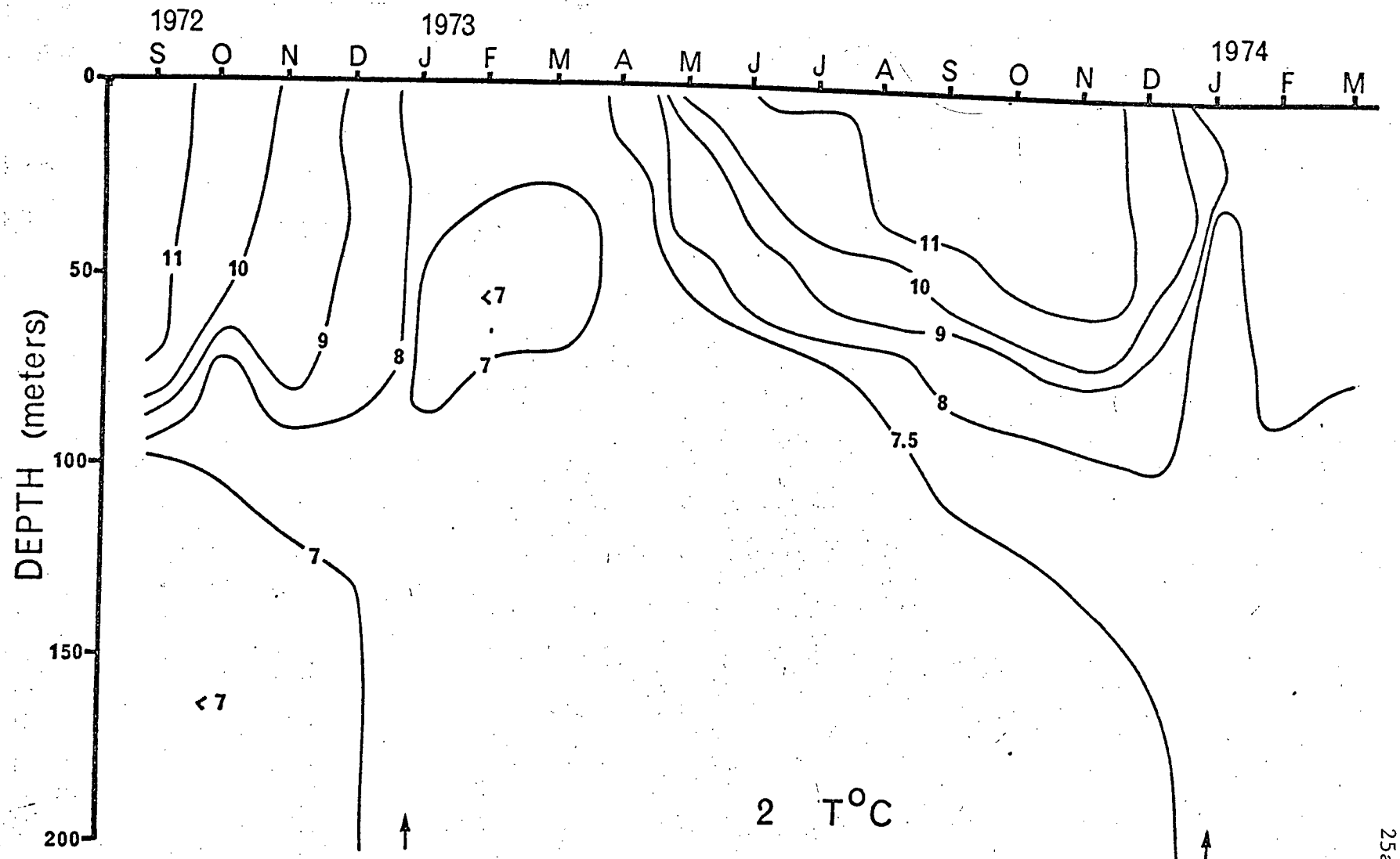

1972
1973
1974
S O N D J F M A M J J A S O N D J F M
0
50
100
150
200
DEPTH (meters)
11
10
9
8
7
<7
11
10
9
8
7.5
7
<7
2 T°C

Figure 6. Distribution of salinity ($^{o}/_{oo}$) through the water column at Ind-2 during the study period.

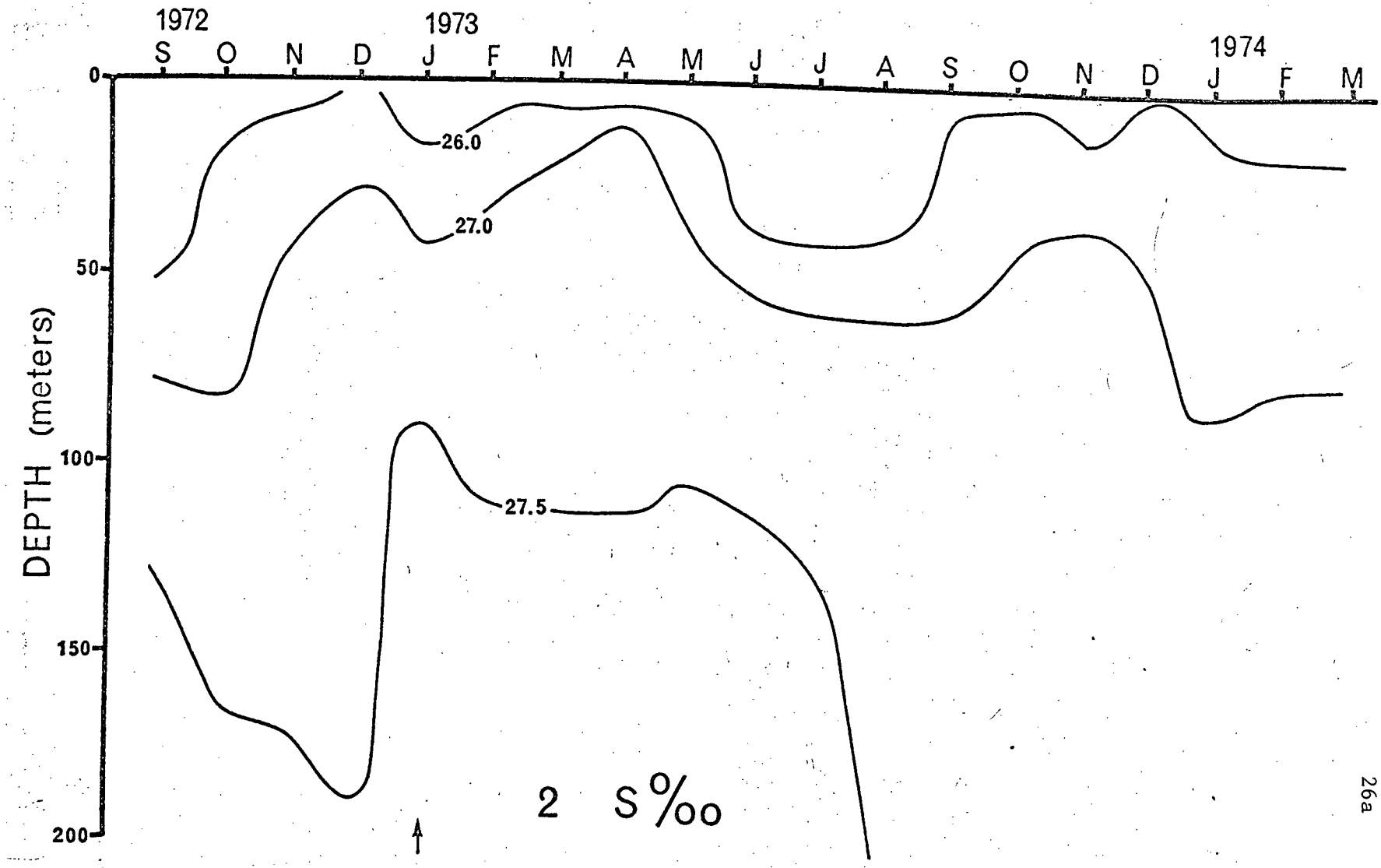

1972
S O N D
1973
J F M A M J J A S O N D J F M
1974
0
50
100
150
200
DEPTH (meters)
26.0
27.0
27.5
2 s‰

Figure 7. Distribution of density (sigma-t) through the water column at Ind-2 during the study period.

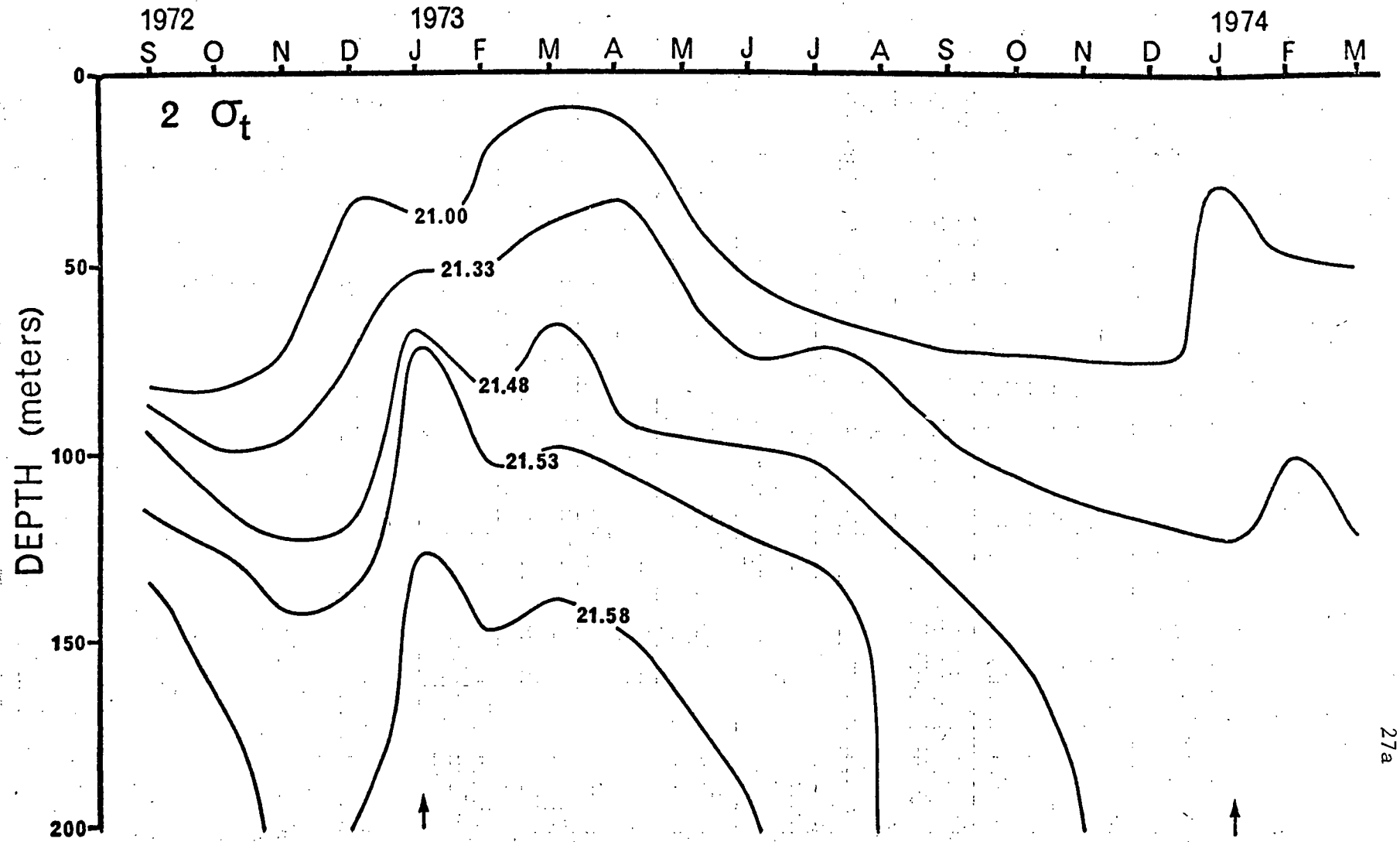

1972
1973
1974
S O N D J F M A M J J A S O N D J F M
0
50
100
150
200
DEPTH (meters)
2 σt
21.00
21.33
21.48
21.53
21.58

Figure 8. Distribution of dissolved oxygen (ml./l) through the water column at Ind-2 during the field sampling period.

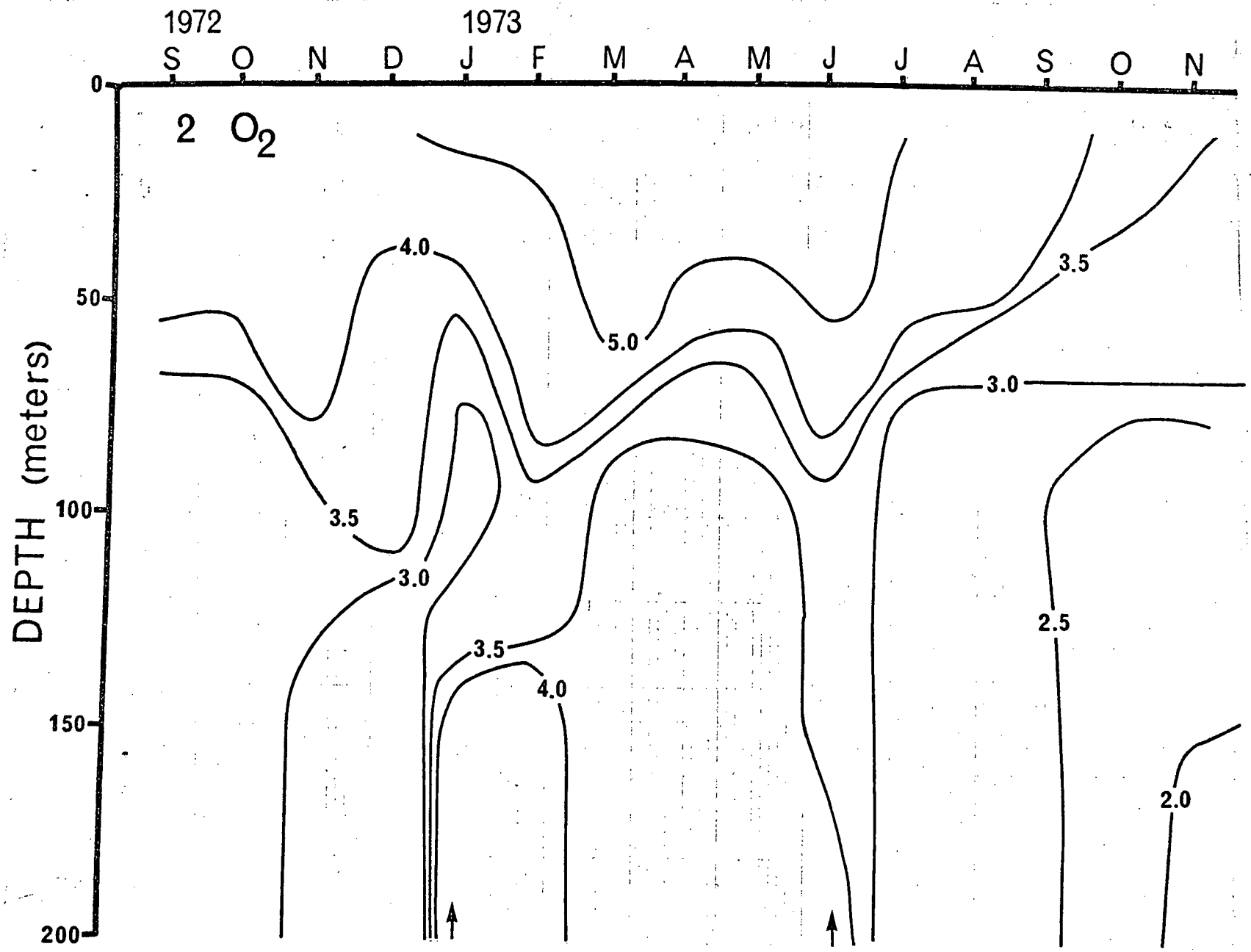
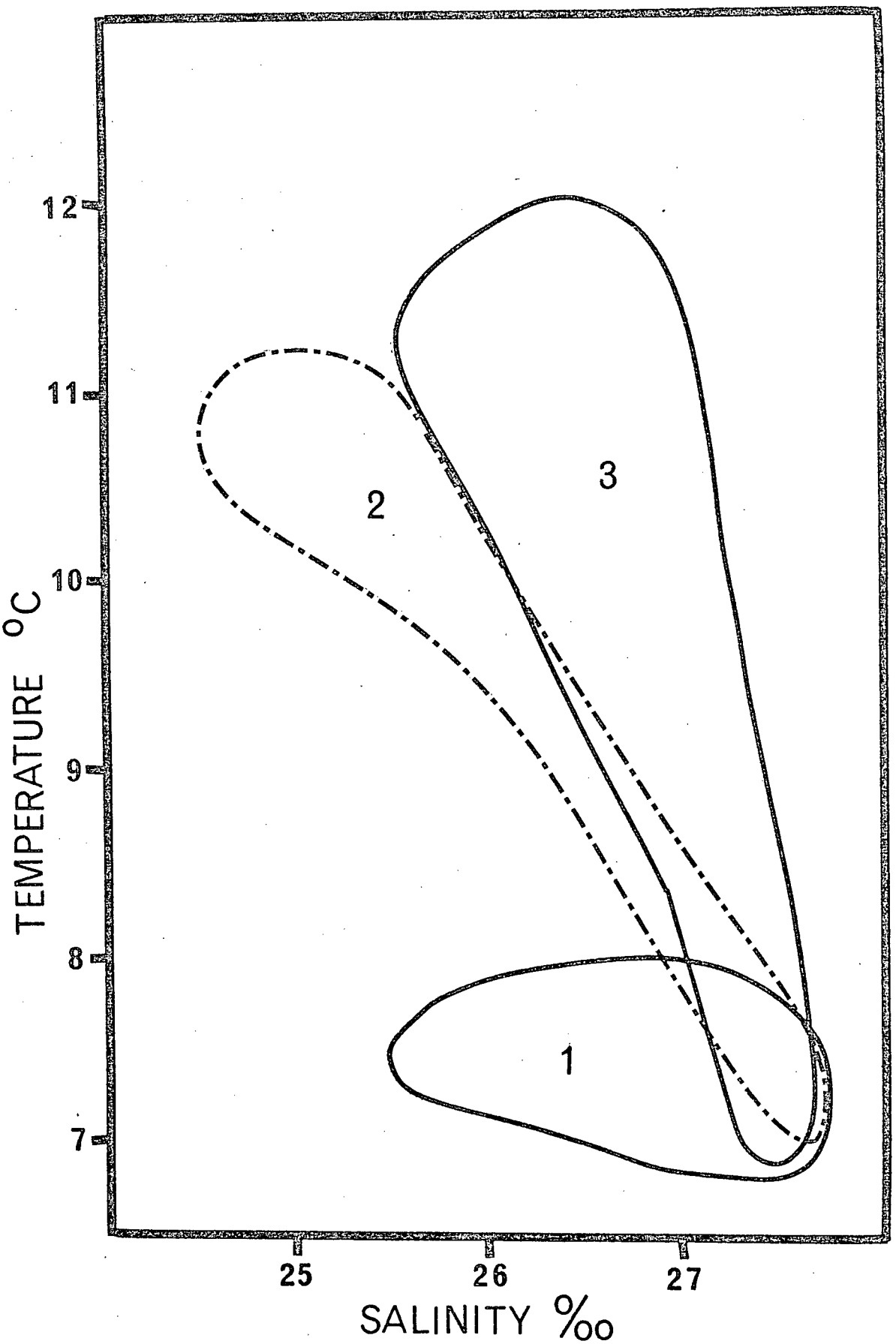

1972
1973
S O N D J F M A M J J A S O N
0
50
100
150
200
DEPTH (meters)
2 O_2
4.0
5.0
3.5
3.0
3.5
3.0
3.5
4.0
2.5
2.0

Figure 9. Temperature salinity envelopes of water below 10 meters for the months sampled during the period of the study.

1.	2.	3.
January 1973	May 1973	September 1972
February 1973	June 1973	October 1972
March 1973	July 1973	November 1972
April 1973	August 1973	December 1972
January 1974		September 1973
February 1974		October 1973
March 1974		November 1973
		December 1973


12
11
10
9
8
7
TEMPERATURE °C
2
3
1
25
26
27
SALINITY ‰

10 shows the distribution of dissolved manganese through the water column at Ind-2 with time. Large changes in the manganese distribution occurred at two times during the year. During most of the year the manganese concentration in the deepest water was 6 to 10 times as great as the concentration at 10 meters. At the time of the intrusions, manganese levels decreased in the deep water (from 10 to 12 times) from the preceding months (e.g., January, 1973 and July, 1973), and the vertical gradient of manganese was reduced (deep water concentrations are approximately 2 to 3 times as great as 10 meter concentration).

Copper, zinc, cadmium and nickel had similar distributions. The concentration of all of these metals remained relatively constant with the exception of the changes at the time of the large intrusion in January. Zinc levels prior to the intrusion ranged from 10 to 15 μg/1. After the intrusion the level of zinc ranged from 2 to 7 μg/1. The distribution of dissolved copper through the water column is shown as a function of time in Figure 11. The levels of copper present in the deep water were higher after the intrusion than they were before it. After the intrusion, the level of dissolved copper declined slightly. Nickel ranged in concentration from 0.8 to 1.4 μg/1, and cadmium from 0.07 to 0.18 μg/1. The intrusion had little effect on the concentration of these metals. The levels of nickel and cadmium determined are near the minimum level detectable by the method used. Some of the variation which the data demonstrate may be attributable to this.

The particulate content of the deep water nearly doubled at the time of the January intrusion. A large increase was also noted in July. The average weight of particulate material prior to January was 4.69 mg/1 while in January it increased to 8.61 mg/1. The level then declined until

Figure 10. Distribution of dissolved manganese (μg/l) through the water column at Ind-2 during the field sampling period.

Note: In Figures 10 and 11 the sampling depths are as given in Table 1, and sampling was done at monthly intervals. Arrows indicate time of intrusion.

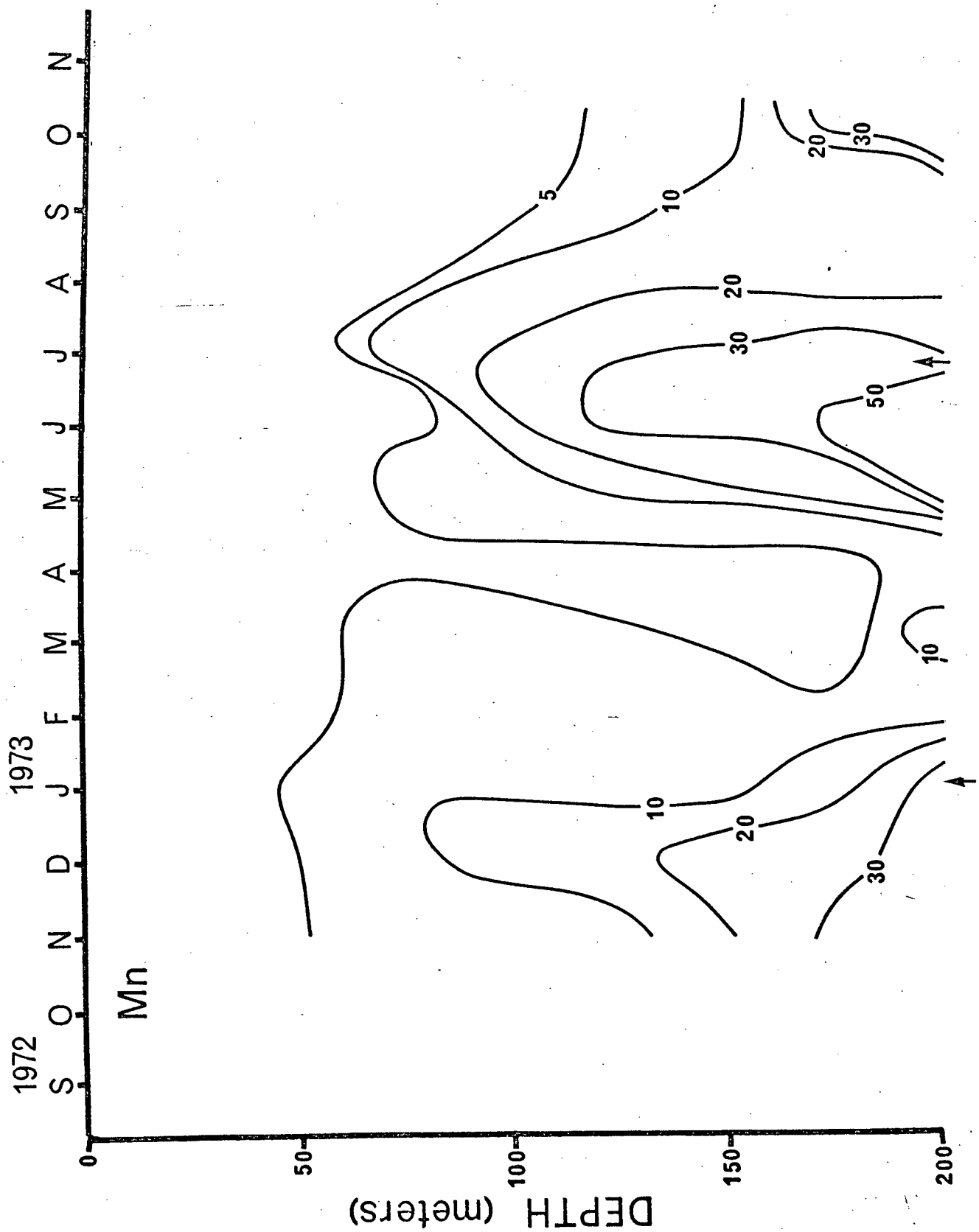
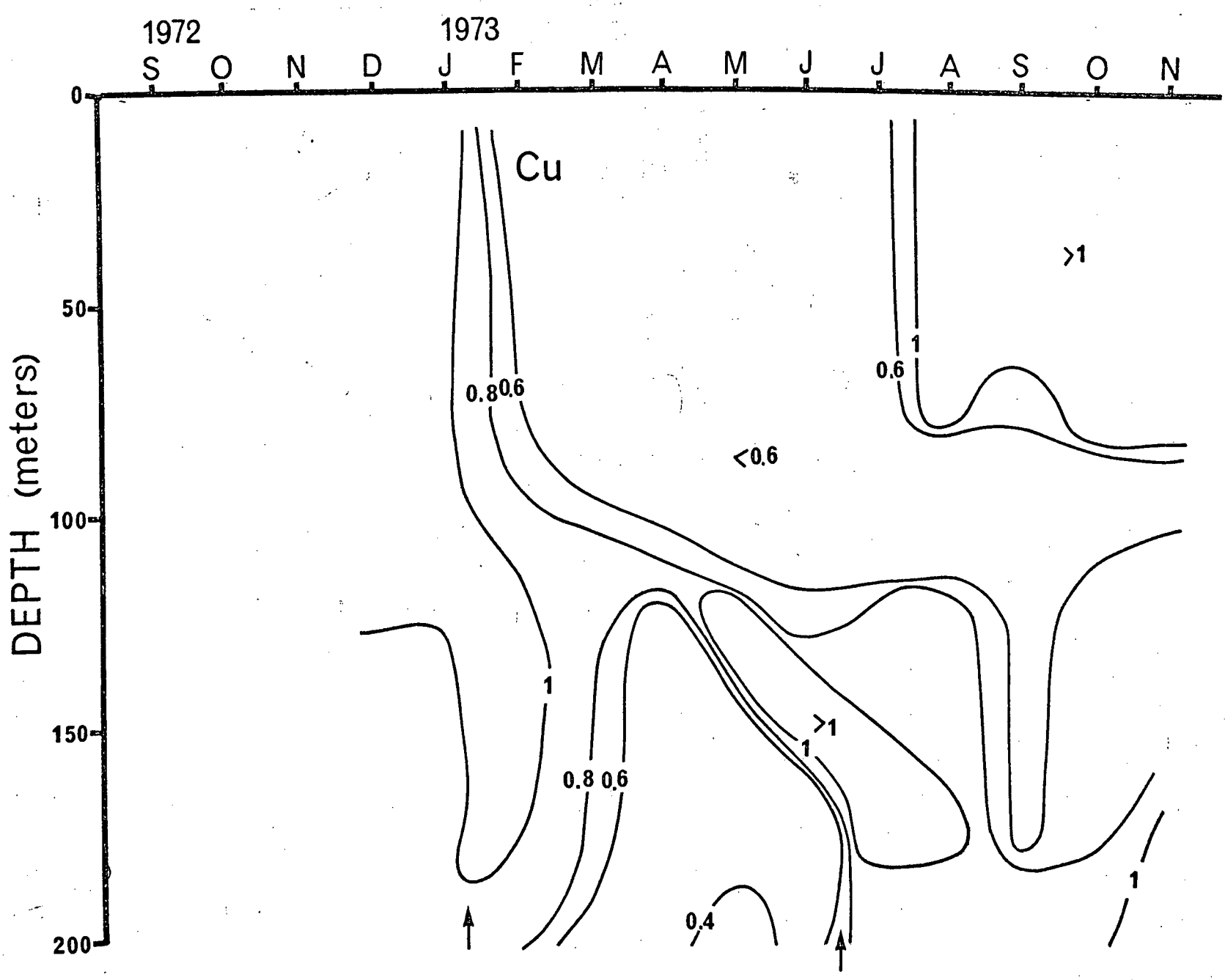

Mn
1972
S O N D
1973
J F M A M J J A S O N
DEPTH (meters)
0
50
100
150
200
5
10
20
30
50
10
20
30
10
20
30

Figure 11. Distribution of dissolved copper (µg/l) through the water column at Ind-2 during the field sampling period.


1972
1973
S O N D J F M A M J J A S O N
0
50
100
150
200
DEPTH (meters)
Cu
0.8 0.6
<0.6
>1
1
0.6
1
0.8 0.6
1
>1
1
0.4
1

June (mean weight of particulate material is 5.50 mg/l) then, in July, again increased (6.50 mg/l) and then decreased through the months following.

Since copper levels associated with the particulate material remained relatively constant during the year, no figure is provided. Copper associated with particulate material represented less than 5% of the total copper measured. The levels (µg/mg particulate material) increased slightly with the increase in particulate material in January.

Organic carbon values are also not shown graphically. Only deep water values were obtained for the period from May, 1973 until October, 1973. There was a decrease in organic carbon levels with time, from 2.69 mg/l (mean of all May samples) to 1.66 mg/l (mean of October samples), except for a small increase in July.

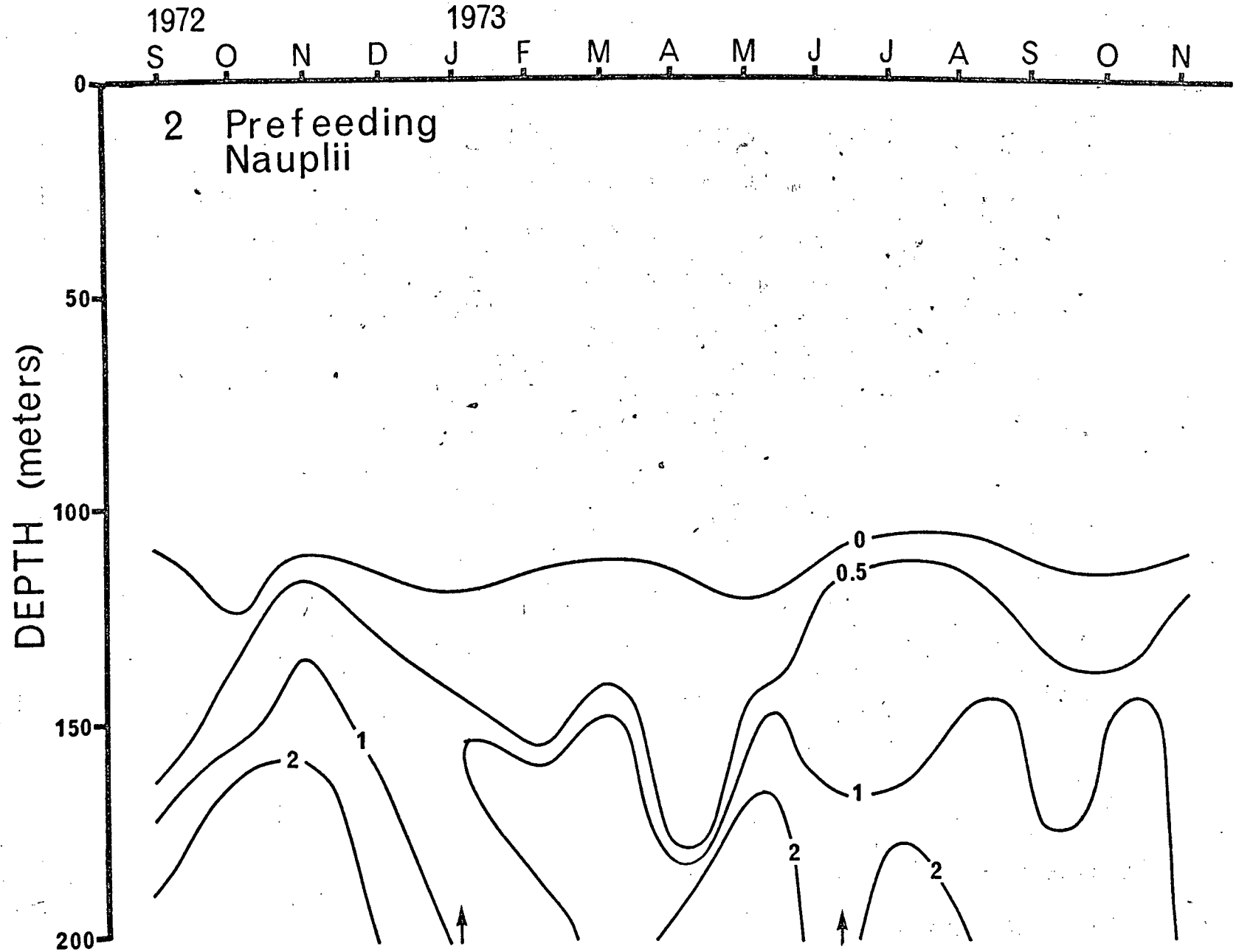
(iii) Abundance of the life history stages

The change in numbers of the prefeeding naupliar stages (first through third nauplius) throughout the sampling period at Ind-2 is shown in Figure 12. Prior to January, 1973 the prefeeding naupliar stages were present in relatively high numbers (greater than two per cubic meter) in the deep water. During and after January the number dropped to less than one per cubic meter. The number of animals then increased. Numbers greater than two per cubic meter were again reached by May and June. During July the animals became more evenly distributed through the deeper portion of the water column. After July the number of organisms in these stages increased in the deepest water which was sampled, as the naupliar stages tend to become concentrated at this depth.

The total number of naupliar stages present in the samples also was examined. Changes which were noted for the prefeeding naupliar stages were

Figure 12. Distribution and abundance of prefeeding naupliar stages (organisms per cubic meter) through the water column at Ind-2 during the field sampling period.

Note: In Figures 12-14 the sampling depths used are as given in Table 1. Sampling was done at monthly intervals. Arrows indicate time of intrusion.


1972
1973
S O N D J F M A M J J A S O N
0
50
100
150
200
DEPTH (meters)
2 Prefeeding
Nauplii
0
0.5
1
2
1
2
2
2

again evident (Figure 13). Figure 14 shows the distribution of the naupliar stages of Euchaeta japonica through the length of Indian Arm during February, 1973. In this figure it is evident that both the prefeeding (Figure 14a) and the total naupliar stages (Figure 14b) tend to be concentrated towards the mouth of the inlet, being higher in the water column near the mouth than they are further up the inlet. This pattern is typical of that which exists throughout the year, although the absolute numbers fluctuate.

The significance and sign of the correlation coefficients between the number of prefeeding naupliar stages and the other variables measured are given in Table 2. The correlation coefficients are not shown because of unequal sample sizes. Although there are several significant correlations shown in Table 2, prefeeding naupliar stages are only linearly related to total naupliar stages and the number of organisms in the sixth naupliar stage. High correlations between prefeeding naupliar stages and the physical and chemical properties of the water do not result from linear relatiohships. The results of cluster analysis are shown in Figure 15. At the 50% similarity level, three clusters are formed, indicating that there are three groups of similarly related variables.

(iv) Laboratory studies

(a) Copper stressed series

The addition of 5.4 µg/l of Cu^{+2} to filtered seawater, collected monthly from 200 meters, caused a decrease in survival of Euchaeta japonica through the prefeeding stages. This toxic effect could be reduced by the addition of EDTA (a synthetic chelating agent). The toxic effect which resulted from the addition of this amount of copper was not constant, but was generally a 30-40% decrease in survival. The "EDTA equivalence" (i.e.,

Figure 13. Distribution and abundance of total (all six) naupliar stages (organisms per cubic meter) through the water column at Ind-2 during the field sampling period.

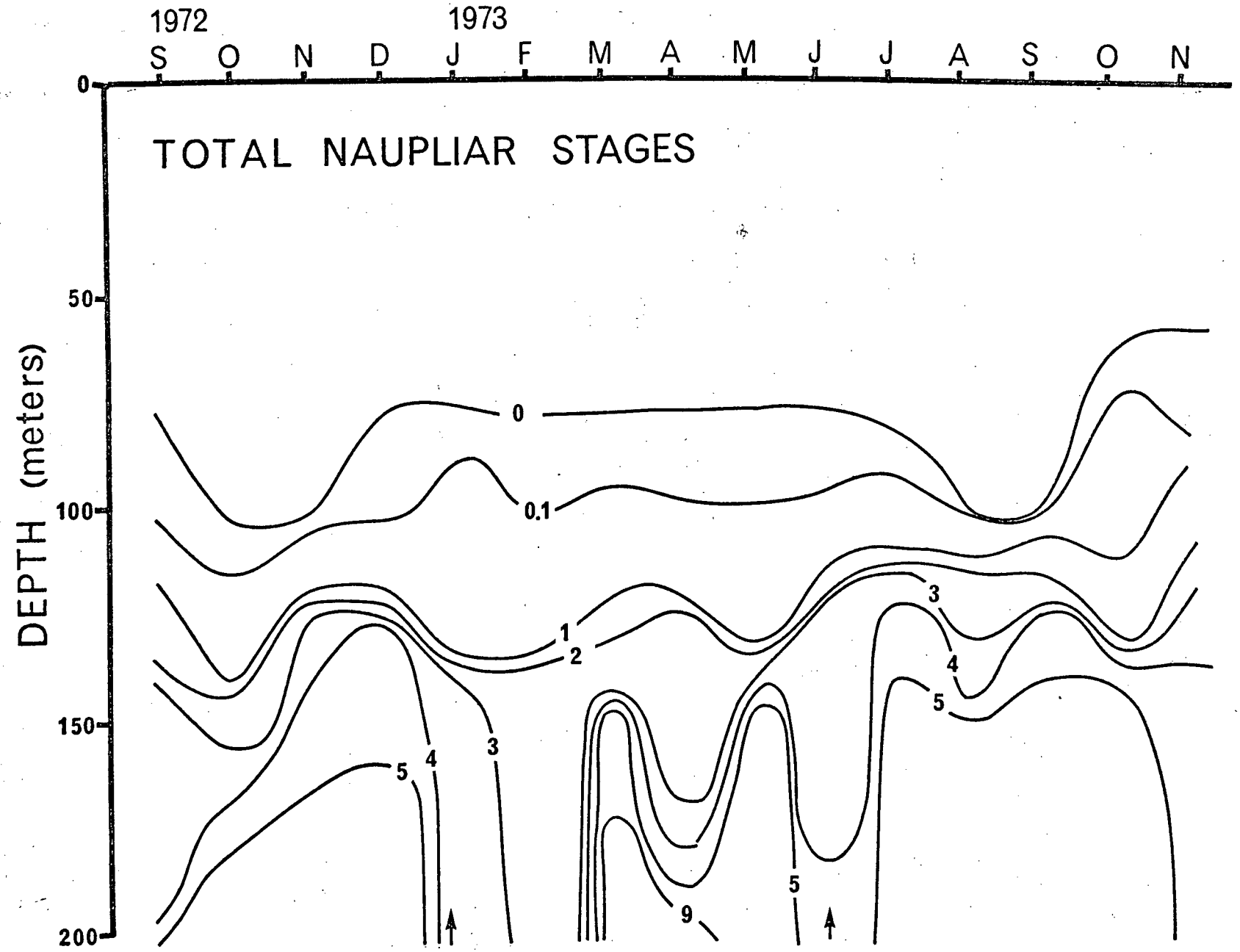
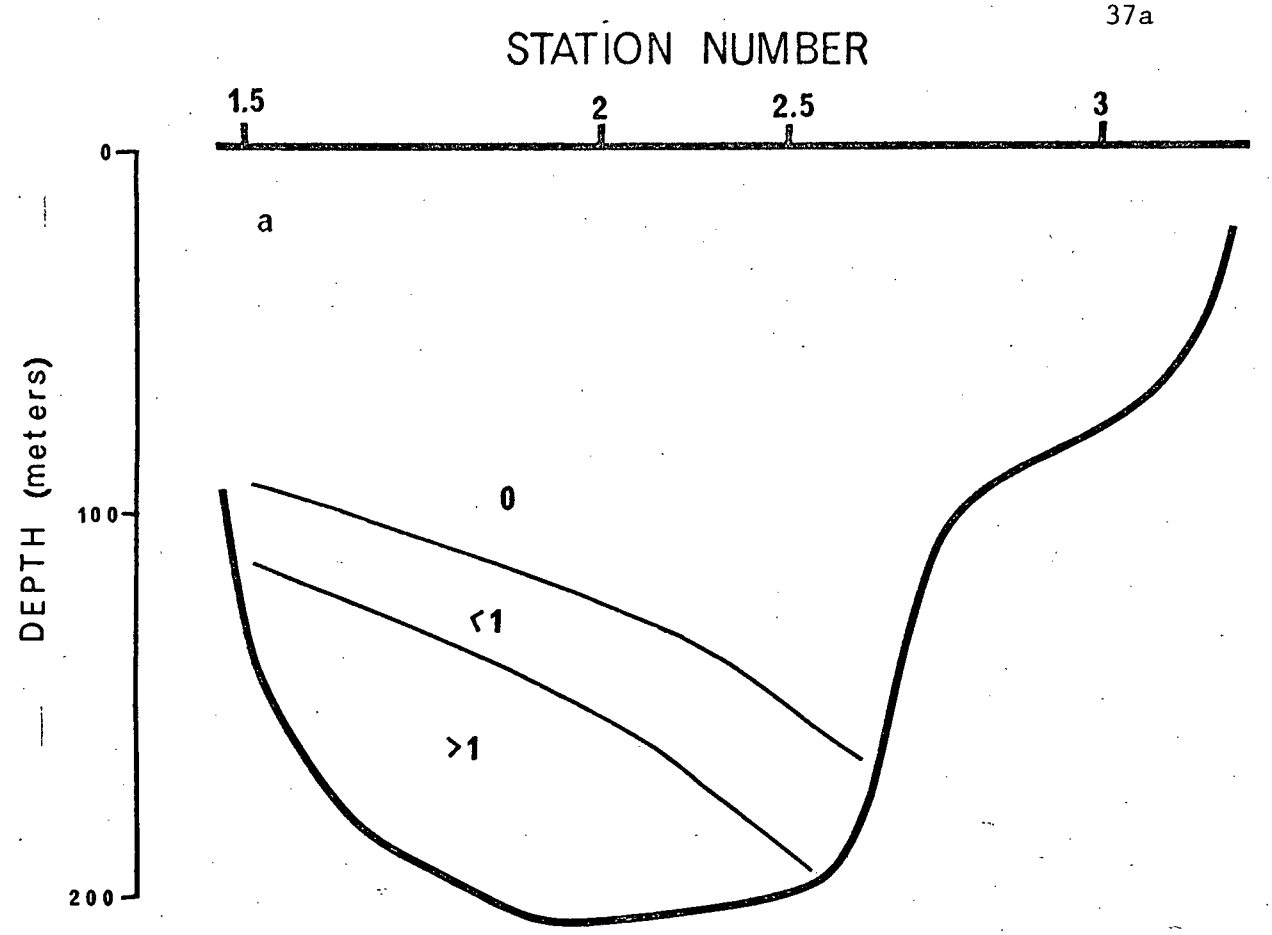

1972
1973
S O N D J F M A M J J A S O N
0
50
100
150
200
DEPTH (meters)
TOTAL NAUPLIAR STAGES
0
0.1
1
2
3
4
5
3
4
5
5
9
5

Figure 14. Distribution of (a) prefeeding, and (b) total naupliar stages through the length of Indian Arm in February, 1973.


STATION NUMBER
1.5
2
2.5
3
0
100
200
DEPTH (meters)
a
0
<1
>1

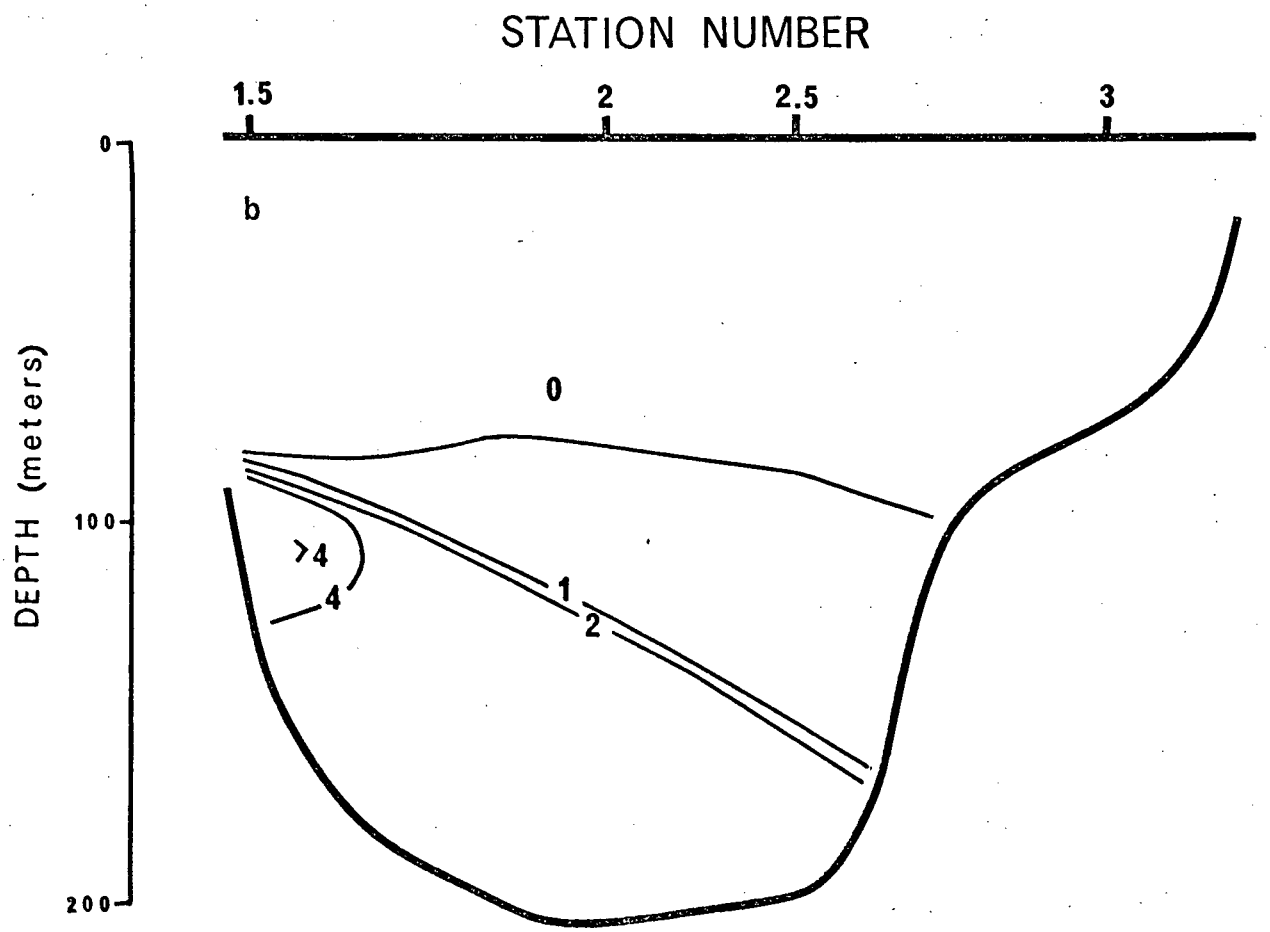

STATION NUMBER
1.5
2
2.5
3
0
100
200
DEPTH (meters)
b
0
>4
4
1
2

Table 2. Significance and sign of the correlation coefficient between prefeeding naupliar stages and the other properties measured in the field.

Characteristic	All Data	All Data below 75m.	DATA SUBSET Ind-1.5[1]	Ind-2[1]	Ind-2.5[1]	Ind-3[1]	PreInt[2]	PostInt[2]	Inter[2]
Temperature	-/0.000	-/0.020	-/0.011	-/0.000	-/0.011	----	-/0.000	+/0.645	-/0.000
Salinity	+/0.000	+/0.000	+/0.003	+/0.000	+/0.002	----	+/0.000	+/0.00	+/0.000
Sigma-t	+/0.887	+/0.000	+/0.002	+/0.000	-/0.765	----	-/0.857	+/0.000	+/0.000
Total nauplii	+/0.000	+/0.000	+/0.000	+/0.000	+/0.000	----	+/0.000	+/0.000	+/0.000
Sixth nauplius	+/0.000	+/0.000	+/0.000	+/0.000	+/0.000	----	+/0.000	+/0.000	+/0.000
Mn	+/0.000	+/0.000	+/0.008	+/0.000	+/0.000	----	+/0.000	+/0.043	+/0.000
Zn	+/0.188	+/0.192	-/0.887	+/0.370	+/0.785	----	+/0.229	+/0.156	+/0.122
Cu	-/0.249	-/0.268	-/0.628	-/0.459	-/0.435	----	-/0.463	-/0.352	-/0.363
Ni	+/0.865	-/0.473	-/0.691	-/0.201	-/0.659	----	-/0.202	-/0.349	-/0.395
Cd	-/0.887	-/0.510	+/0.429	-/0.635	-/0.416	----	-/0.209	-/0.831	+/0.589
Particulates	-/0.122	-/0.071	-/0.268	-/0.248	-/0.604	----	-/0.774	-/0.059	-/0.486
Particulate Cu	+/0.629	+/0.873	+/0.134	+/0.521	+/0.887	----	-/0.845	+/0.002	-/0.471
Organic carbon	-/0.874	-/0.780	-/0.297	+/0.139	-/0.549	----	+/0.924	*******	-/0.801
Oxygen	-/0.000	-/0.303	-/0.000	-/0.001	-/0.046	----	-/0.000	-/0.034	-/0.002

+,- sign of the correlation coefficient.

---- no naupliar stages were found at this station.

******* no organic carbon samples were taken in this period.

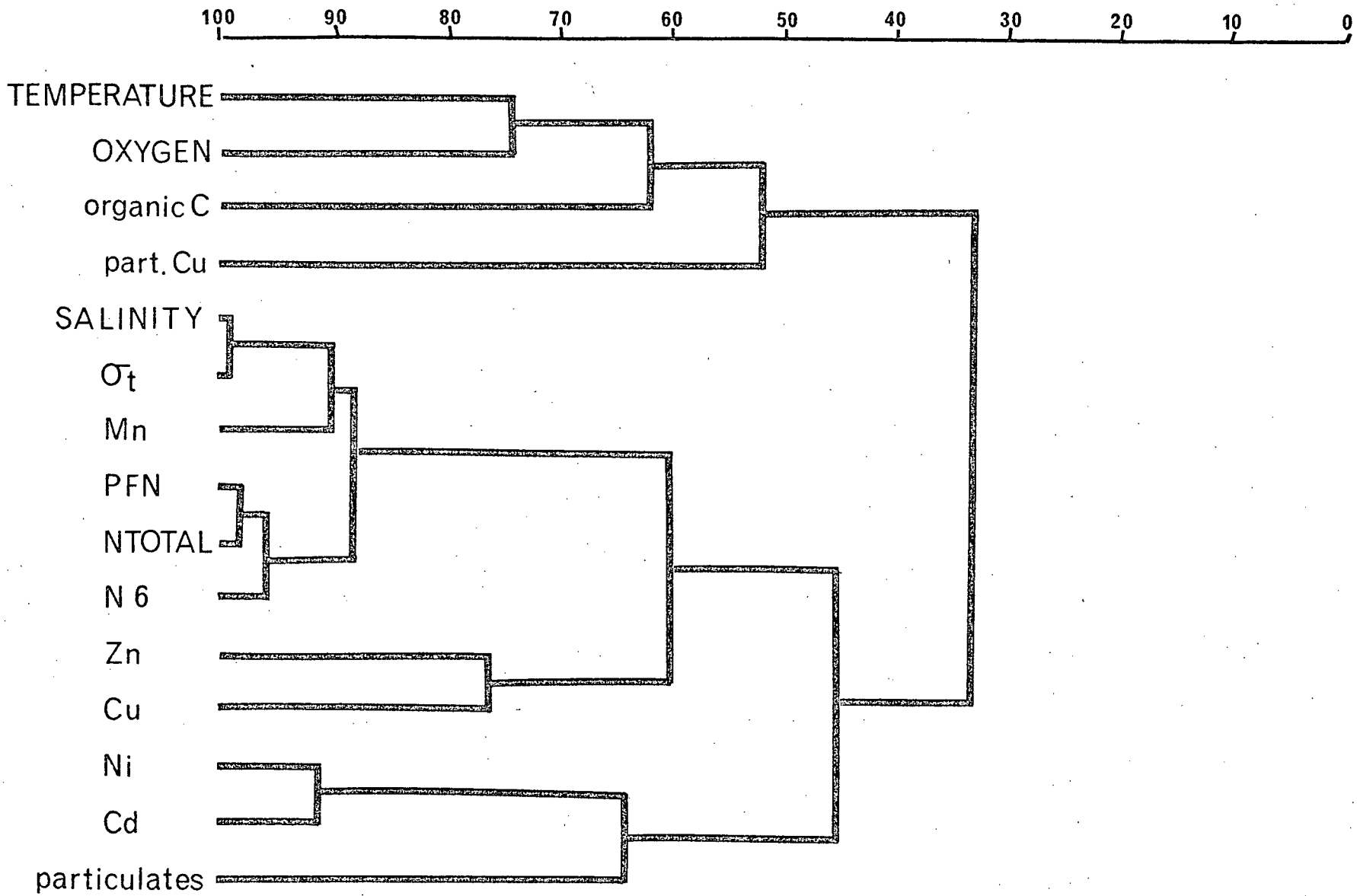
[1]Data Subsets on the basis of stations.

[2]Data Subsets on the basis of period of the year (see Text for details).

Significance is noted at the 10% levle at 0.100, and at 5% at 0.050 or less.

Figure 15. Tree printed from correlation matrix (scaled 0-100). Clustering by average distance method. Data are all observations which have values for the 15 characteristics. The abbreviations used are:

organic C	-	organic carbon
part. Cu	-	particulate associated copper
σ_t	-	sigma-t
Mn	-	manganese
PFN	-	prefeeding naupliar stages
NTOTAL	-	total naupliar stages
N6	-	sixth naupliar stage
Zn	-	zinc
Cu	-	copper
Ni	-	nickel
Cd	-	cadmium
particulates	-	particulate material


100
90
80
70
60
50
40
30
20
10
0
TEMPERATURE
OXYGEN
organic C
part. Cu
SALINITY
σ_t
Mn
PFN
NTOTAL
N 6
Zn
Cu
Ni
Cd
particulates

the amount of EDTA required to negate the toxic effect of the copper enrichment) is obtained from the intercept of the survival value obtained in unenriched water with the line drawn drawn through the values obtained for survival in the copper and EDTA enriched water, as shown in Figure 16. The values of the intercept are shown as a function of time in Figure 17e.

(b) Sediment extract series

Sediment extracts prepared from samples taken at the four stations over the period of one year have been shown to be able to reduce the toxic effect of copper enrichment. The ability of the extracted material to reduce the toxic effect changed with time, and was often different at each of the stations. The ability tended to be greatest in late summer and fall, and least in the late winter (Figure 17a-d). The ability of the sediment extract to reduce the toxic effect is expressed as the amount of EDTA which causes the same reduction in toxicity (Lewis et al., 1973). At times negative values for the EDTA equivalence were obtained. These resulted from a detrimental effect resulting from the addition of the material extracted from sediments, and values were obtained by extrapolation of the EDTA concentration line.

(c) Ultra-violet series

As the concentration of EDTA added to ultra-violet treated water increased, the survival of Euchaeta japonica through the prefeeding stages also increased as shown in Figure 18. The values plotted in Figure 18 are the means of the results shown in Table 3. During the year the slope and intercept of this line changes. The standard deviations of survival at low

Figure 16. The percent survival of the organism from the egg through the third nauplius plotted against the concentration of EDTA which was added to water with 5.4 µg/l of added copper (•). Also shown is the percent survival of the organism in unenriched water (o), and the method of obtaining an EDTA equivalence is shown by the arrows. Values shown are the means and standard deviations for the period of study. The means are based on 12 monthly experiments, each of which involved approximately 60 animals.

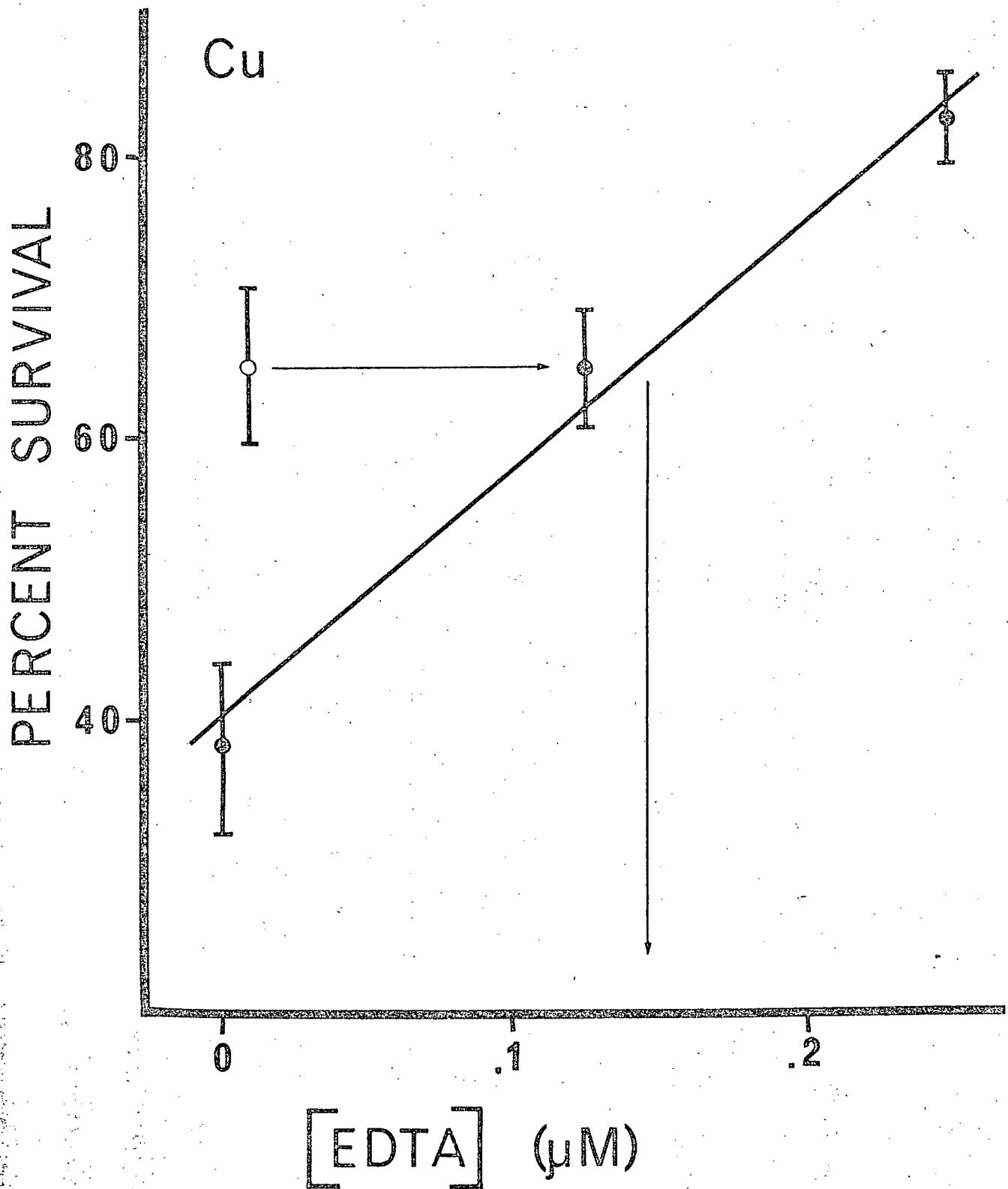

Cu
PERCENT SURVIVAL
80
60
40
0
.1
.2
[EDTA] (μM)

Figure 17. (a-d) EDTA equivalence of the sediment extracts as a function of time, and (e) toxic effect of a copper addition expressed as the amount of EDTA required to negate the effect of the copper addition as a function of time.

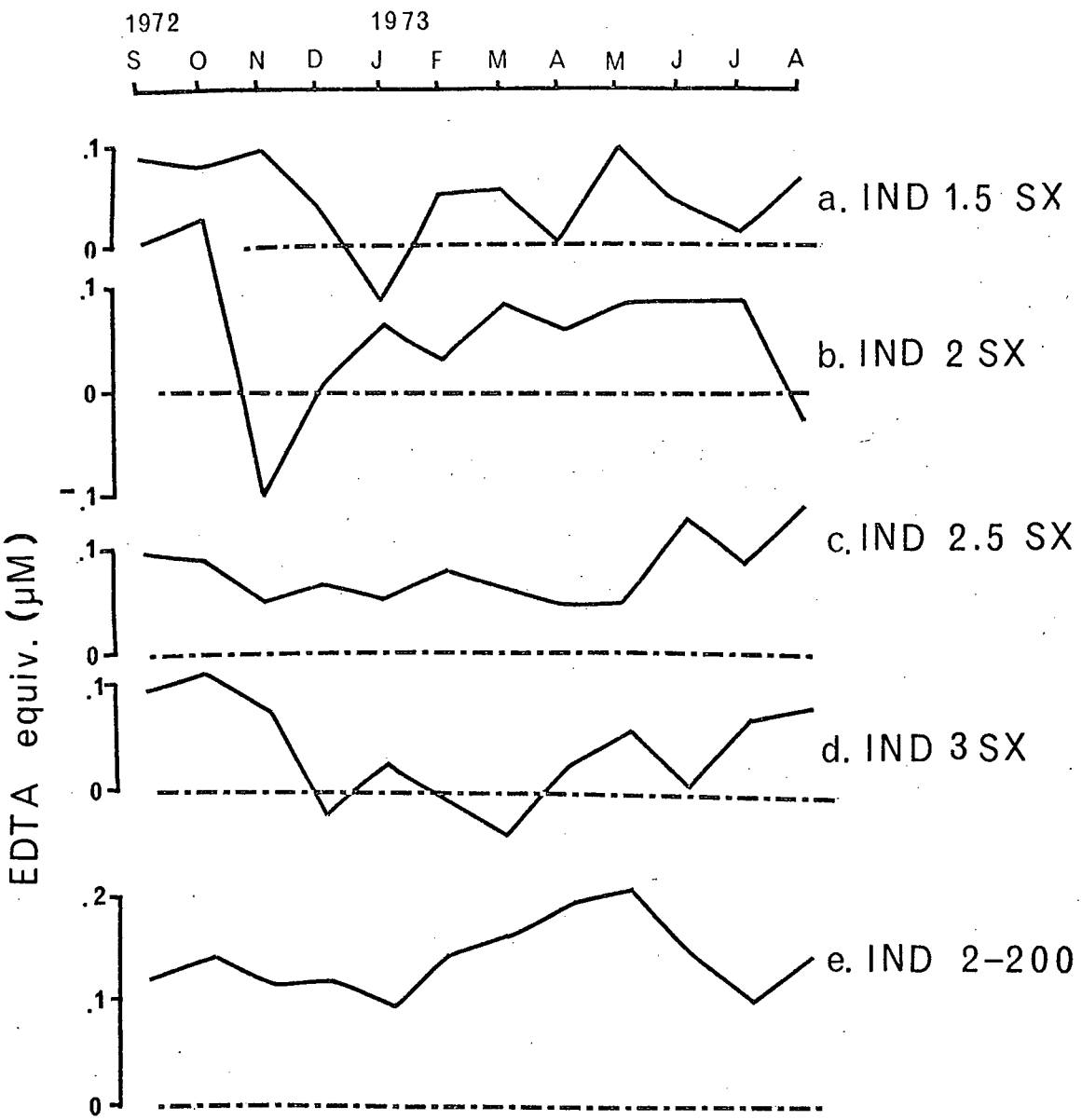

1972
1973
S O N D J F M A M J J A
EDTA equiv. (µM)
.1
0
a. IND 1.5 SX
.1
0
-.1
b. IND 2 SX
.1
0
c. IND 2.5 SX
.1
0
d. IND 3 SX
.2
.1
0
e. IND 2-200

Figure 18. Percent survival of the organism from the egg through the third nauplius as a function of the EDTA concentration in water which has been treated with ultra-violet light (●). Also shown is the percent survival obtained in untreated water (o), and the method of obtaining an EDTA equivalence is indicated by the arrows. Values shown are the means of the twelve months of the study (approximately 700 animals in total for each of the means) and standard deviations.

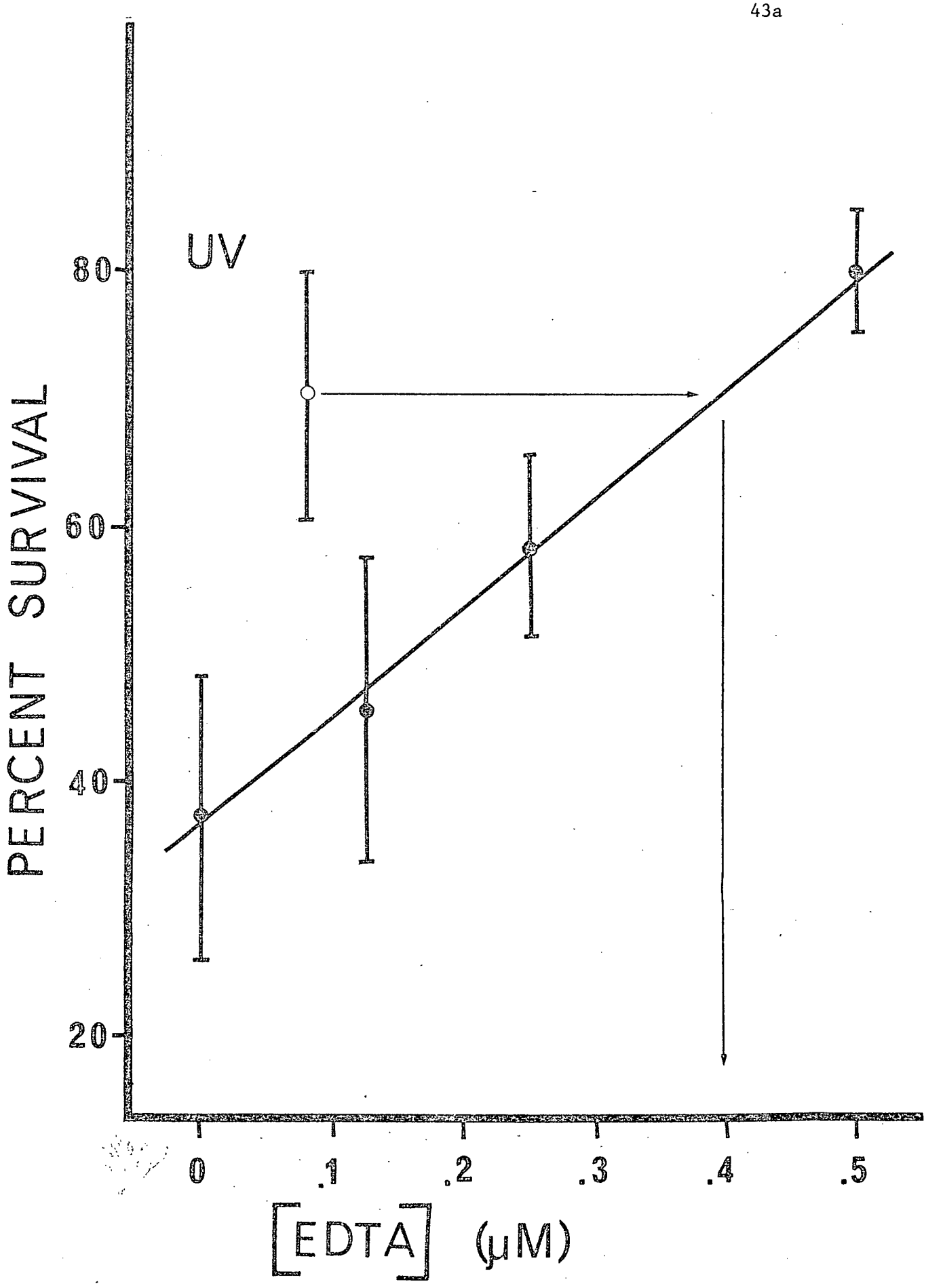

UV
PERCENT SURVIVAL
80
60
40
20
0
.1
.2
.3
.4
.5
[EDTA] (μM)

Table 3. Percent survival values in the ultra-violet series, with and without metal additions. Values are the percent of approximately sixty animals that survive until the third naupliar stage. Also shown are the EDTA equivalences which were determined, and the approximate slope of the line which relates survival of the organism to the EDTA concentration.

Month	Water	Metal Addition[1]	Untreated	Ultra-violet	Ultra-violet 0.125 µM EDTA	Ultra-violet 0.250 µM EDTA	Ultra-violet 0.500 µM EDTA	Approximate Slope	EDTA equivalence (µM)
May	2-200	none	73	34	48	55	76	.84	.45
June	2-200	none	64	27	36	49	71	.88	.47
July	2-200	none	54	21	37	51	75	1.08	.30
August	2-200	none	63	22	36	58	75	1.06	.38
September	2-200	none	52	34	40	48	76	.84	.38
	2-200	Mn 100	11	20		46	63	.86	--[2]
October	2-200	none	70	46	54	61	77	.62	.38
	2-200	Zn 8	43	34		45	48	.04	.19
	2-200	Mn 100	18	50		57	71	.42	--
	2-125	none	75	63	51	51	62	.00	--
November	2-200	none	71	34	50	73	83	.98	.33
	2-200	Zn 8	43	44		49	61	.34	.00
	2-200	Mn 50	55	58		54	59	.02	--
	2-125	none	80	50	51	67	83	.66	.45
	2-125	Mn 50	83	55		65	75	.40	.70*[3]
December	2-200	none	67	53	51	56	83	.60	.36
	2-200	Mn 100	60	37		59	80	.86	.27

... continued ...

... continued ...

December	2-125	none	79	50		58	74	.48	.54*
January	2-200	none	83	41	47	57	78	.74	.56*
	2-200	Zn 4	54	65		70	60	.00	--
	2-200	Mn 50	55	46		66	60	.28	.11
	2-200	Mn 50 Zn 4	80	53		53	58	.10	--
February	2-200	none	81	38	42	60	86	.96	.50
	2-200	Cu 1	69	40		59	59	.38	.58*
	2-200	Mn 100	23	11		45	58	.94	.09
	2-200	Mn 100 Cu 1	23	30		39	59	.58	--
March	2-200	none	74	56	55	65	80	.48	.41
	2-200	Cu 1	69	39		59	67	.56	.45
	2-200	Mn 50	52	57		76	79	.44	--
	2-200	Cu 2	60	47		48	53	.12	.60*
April	2-200	none	84	46	58	66	86	.80	.48
	2-200	Cu 1	71	32		57	74	.84	.46
	2-200	Cu 2	61	82		64	46	-.72	.28
	2-200	Cu 5	46	42		65	71	.70	.05
May	2-200	none	69	33	45	66	79	.92	.37
	2-200	Cu 1	67	36		62	74	.76	.38
	2-200	Cu 2	60	70		49	33	-.74	.13
	2-200	Cu 3	54	58		60	73	.30	--
	2-200	Cu 5	43	32		50	63	.62	.18

[1] Metal additions are µg/l.

[2] No intercept.

[3] Value obtained by extrapolation.

EDTA concentrations are very much larger than those at high EDTA concentrations. EDTA equivalence of untreated water (open circle) was determined from the intercept of the survival in untreated water with the line relating survival to EDTA concentration, as shown by the arrows in Figure 18 (in this case the mean EDTA equivalence is approximately 0.4 μM). The EDTA equivalences determined in this manner are shown as a function of time in Figure 19. During the first four months the values were erratic, and became stable in the following four months (September to December). During January the EDTA equivalence of the water reached a maximum level and then decreased through the subsequent months.

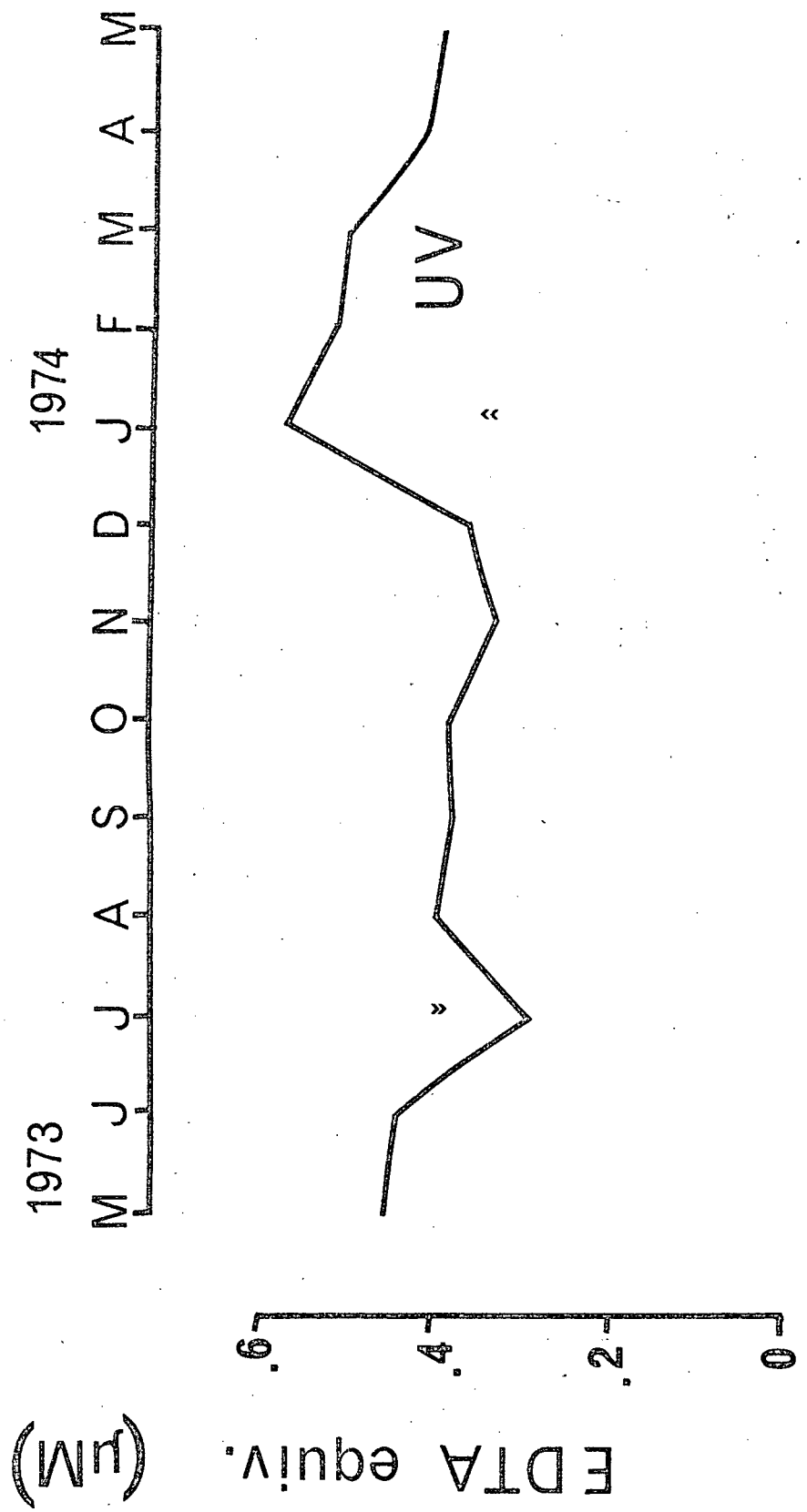
(d) Metal additions

Metals were added to ultra-violet treated and untreated seawater. The results from these series along with those for corresponding water with no metal additions are given in Table 3. The EDTA equivalence obtained in water with metal additions either did not change or decreased. The relationship between survival and increasing EDTA changes with some metals and not with others, depending both on the metal used and its concentration. The slope of the line which relates the survival of the organism through the prefeeding stages to the concentration of EDTA was different in water to which metals had been added (Table 3). Variations in slope are dependant both upon the metal used and its concentration. For example, in March, 1973, neither 1 μg/l copper or 50 μg/l manganese additions altered the slope, while 2 μg/l copper reduced the slope.

(e) Measurements on water before and after ultra-violet treatment

A series of measurements were made on water used in the laboratory

Figure 19. EDTA equivalence as determined in ultra-violet treated water as a function of time.


EDTA equiv. (µM)
6.
4.
2.
0
UV
1973
1974
M J J A S O N D J F M A M

experiments, before and after the treatment with ultra-violet light. These values are shown in Table 4. Measurements of the dissolved organic carbon concentrations are compared with the difference in percent survival of the organism between untreated and treated water (Figure 20).

(f) Chloroform extractions

Table 5 shows the values obtained for metal concentrations in water which had been extracted with chloroform. Also included are the metal concentrations in water which had not been treated in this manner, and the EDTA equivalence as determined with the ultra-violet bioassay series. Approximately one half of the dissolved manganese was removed by the extraction in the May sample, but not in the June sample. The other metals were only slightly affected by the extraction. The total amount of metals removed by the chloroform extraction does not coincide with the EDTA equivalence as determined by the bioassay of ultra-violet treated water.

Table 4. Levels (concentrations) of various chemical constituents of the culture water, before and after ultra-violet irradiation (October 1973).

Constituent	200 meter water		125 meter water	
	Before	After	Before	After
zinc (µg/l)	8.1	8.0	6.5	6.3
manganese (µg/l)	48.1	48.1	5.2	5.2
copper (µg/l)	1.2	1.2	0.8	0.8
nickel (µg/l)	1.3	1.3	1.2	1.2
cadmium (µg/l)	0.09	0.09	0.11	0.11
organic carbon (mg/l)	2.04	0.52	1.58	0.45
pH	7.81	7.80		
percent survival	70	46	75	63

Figure 20. The change in percent survival plotted against the change in the organic carbon content of the water which resulted from the ultra-violet treatment.

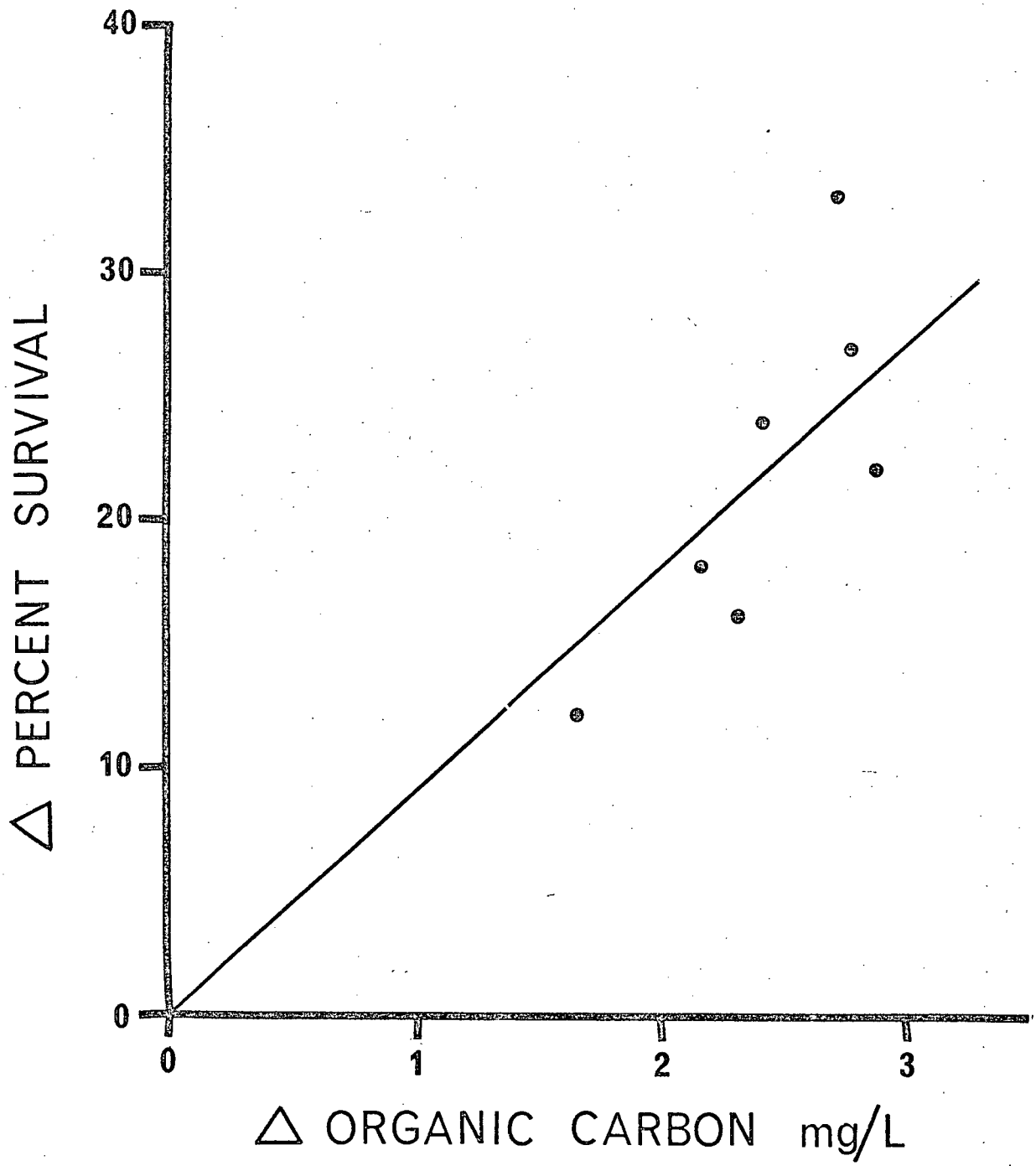

40
30
20
10
0
△ PERCENT SURVIVAL
0
1
2
3
△ ORGANIC CARBON mg/L

Table 5. Results from the chloroform extraction experiments, and the EDTA equivalence determined with the bioassay.

Sample	Treatment	Concentration (µg/l) Mn	Zn	Cu	Ni	Cd	Metals Removed (µM)	EDTA equivalence (µM)
May 200m.	none	20.2	4.5	1.0	1.0	0.11	0.22	0.45
	$CHCl_3$	10.3	2.2	1.0	1.0	0.10		
June 200m.	none	91.8	6.2	1.3	1.1	0.11	0.68	0.43
	$CHCl_3$	57.2	6.2	1.1	1.17	0.07		

DISCUSSION

(i) Field data

The water column in Indian Arm is stratified with low salinity water near the surface, and high salinity water from the Strait of Georgia, at depth. Intermediate to these two layers is a transition zone which fluctuates in depth and properties, depending upon the characteristics of the upper and lower layers. The major perturbations to this system are intrusions of water into the inlet from the Strait of Georgia (Gilmartin, 1962).

Two intrusions of high salinity water occurred during the period of the field study. The first of these was in January, 1973 (see Figures 5 and 6). This intrusion resulted in a partial breakdown of the stratification and was of a sufficiently dense nature to replace most of the deep water in the inlet. (Intrusions of this type, at this time of year, have been noted by Gilmartin, 1962; Gilfillan, 1970; Gardner, 1972; and Evans, 1973). A second intrusion occurred sometime in July, 1973. It was of a relatively minor nature (Lafond, personal communication) and is more apparent from the manganese data and biological data than from temperature and salinity values. (A January intrusion also occurred in 1974).

The January, 1973 intrusion is evident on the density profile (Figure 7). Subsequent to this intrusion there was a gradual decay of the density structure of the inlet. The July intrusion enhanced the rate of decrease of the density of the deep water. The decrease in density is the result of the estuarine circulation of the inlet. The levels of dissolved oxygen increased in January (Figure 8) while in July the oxygen present appeared to become mixed throughout the lower 100 meters of the water column. The gradual decreases in oxygen content of the deep water after the intrusions

are presumably the result of biological utilization.

In application to the study of the biological availability of metals, the January intrusion, being of relatively large magnitude, was expected to have a large effect on the organism-metal relationship. The July intrusion, on the other hand, does not result in the displacement of the deep water of the inlet, and should have had a lesser effect on the organism-metal relationship.

During this study the naupliar stages of Euchaeta japonica were found to be concentrated towards the bottom of the water column (see Figures 12 and 13). This has also been found in other studies (e.g., Pandyan, 1971; Evans, 1973). The naupliar stages were found in higher numbers, and higher in the water column, towards the mouth of the inlet (Figure 14). This is possibly the result of the nature of the water circulation in the inlet. Water entering the inlet may move up the inlet at mid depth for some distance. This could result in an area near the mouth where there is little exchange of water. The distribution of dissolved manganese exhibits a similar pattern, and substantiates this suggestion.

Reductions in numbers of both prefeeding and total naupliar stages (e.g., Figures 12 and 13) appear to be the result of the intrusions of water into the inlet. In January there was a large drop in the number of organisms present. During July, however, the organisms became more evenly distributed through the water column. Similar results were found by Evans (1973).

The results of the correlation analysis (Table 2) show strong positive correlations between the numbers of prefeeding naupliar stages and both salinity and density. Negative correlations are obtained with both temperature and dissolved oxygen. Regression analysis, however, shows that a linear relationship between prefeeding naupliar stages and each of these properties

does not exist. Cluster analysis suggests that the relationships which exist may be the result of the vertical distribution of these properties (Figure 15). In clustering of the variables, temperature and dissolved oxygen are shown to be relatively similar. Salinity and density are shown to be very similar (0.98) and become clustered with all of those properties which reach a maximum in the lower portion of the water column.

On the basis of Figure 9 the year was divided into three distinct periods. The first period (preintrusion) is typified by having nearly uniform salinity but a large temperature range. The second period of the year (postintrusion) is typified by having a wide range of salinities while the temperature of the water is relatively uniform. During this period the water stability is entirely contolled by the salinity structure. The third period of the year (intermediate) is the transition period between the postintrusion and preintrusion patterns. Vertical variations in both temperature and salinity are characteristic of this period, the stability of the water column being controlled by both of these features. Although these periods are referred to as being distinct, one must keep in mind that, except for the intrusion, they form a graded series.

Chemical properties of the deep water change as a function of time and hydrographic events. There are large changes in the concentration of dissolved manganese at two times during the study period (see Figure 10). The January intrusion resulted in the reduction of the high levels of manganese evident prior to the intrusion. After January, the manganese concentrations increased to very high levels (maximum concentration 131 µg/l) until July, when they again dropped. This is the result of the mixing of the lower 100 meters of the water column, which caused the manganese level to decrease, possibly the result of both redistribution and precipitation. Subsequent to

the July intrusion, manganese levels at the deeper depths again increased. The concentrating of manganese into the deep water may be the result of the formation of Mn (IV) oxides which are relatively insoluble. There is also some evidence of manganese existing in a complex with high molecular weight organic material from some of the laboratory studies.

Large molecular weight organic material present in seawater has been shown to be surface active (Riley, 1963) and able to bind transition metals (Barsdate, 1970). When chlorform is shaken in seawater it forms many small droplets, causing a large increase in surface area. This increases the interaction with the surface active material (Khailov and Finenko, 1970). When the chloroform is allowed to separate it draws the surface active organic material with it, forming a third layer which consists of the chloroform, some organic material, and a small amount of captured water.

The extraction of seawater with chloroform altered the concentrations of metals present. The results, shown in Table 5, indicate that in both samples approximately 50% of the manganese was removed by the extraction. No difference in copper concentration was evident in May while the difference found in June could easily have been the result of experimental error. Some of the zinc was removed by the extraction in May but none in June. There is a discrepancy between the amount of organic bound metals which were removed by the extraction and that which was estimated by the addition of EDTA in the bioassay. The results from these experiments can serve only as an indication of organic bound metals because insufficient controls were used.

Copper, zinc, cadmium, and nickel were also affected by the intrusions (e.g., Figure 11). Those changes which occurred at the intrusion were probably the result of a difference in the concentration of each metal in the intruding water and the water which it displaced. Increases in the amount of

particulate material and changes in the amount of copper associated with the particulate material may be the result of the amount and composition of such material in the intruding water. It is also possible that the intrusions caused the mixing of non-consolidated sedimentary material into the water column. If this is the case, the material may originate either in Indian Arm or in Burrard Inlet. This, however, requires further examination.

The correlation analysis showed a strong positive correlation between the concentration of manganese and the concentration of prefeeding naupliar stages (Table 2). This is probably the result of both being similarly affected by the movement of water within the inlet. Regression analysis showed that prefeeding naupliar stages and manganese were not linearly related. Strong correlations of prefeeding naupliar stages were obtained with particulate copper (positive) and particulate material (negative) during the postintrusion period. These probably resulted from the changes which occurred subsequent to the intrusion; that is, the settling out of suspended material and the increase in the concentration of prefeeding naupliar stages.

Three distinct clusters of variables were formed at the 50% similarity level in Figure 15. The variables within the cluster bounded by temperature and particulate copper were distributed such that the maximum values obtained were in near surface waters. The variables in the cluster bounded by salinity and sixth naupliar stage reached their maximum values in the deepest portion of the water column. The variables in the cluster, from zinc to particulate material, were distributed more or less evenly through the water column.

(ii) Laboratory data

By running a series in which EDTA is added in addition to either

the copper enrichment or the ultra-violet treatment it is possible to increase the survival of the organism above that which occurs where the treatment is applied but no EDTA has been added. The amount of EDTA which is required to increase survival in the treatment to that which is obtained in untreated water (or with specific enrichments) is a measure of the relationship between the organism and the environmental water. What this amount of EDTA represents depends upon the series which is being considered. For a copper enrichment, the amount of EDTA which is required to increase survival of the organism to that attained in the unenriched condition is a measure of the toxic effect of the copper enrichment. For the sediment extract enrichments it is an approximation of the ability of the extracted material to reduce the toxic effect of the copper (e.g., Lewis et al., 1973). In the ultra-violet experiments it is a measure of the ability of the organic material, which was destroyed, to complex metals.

Experiments on the toxic effect of the copper enrichment provided indirect evidence of changes in the inter-relationships of the organic and metal components in natural systems. The toxic effect produced by a copper addition can be predicted to be constant. Changes in its effect may have been the result of alterations in the relationship between the organism and the metal-organic equilibrium in natural water.

The toxic effect of the addition of 5.4 µg/l of copper should be eliminated by the addition of an equimolar concentration of EDTA (approximately 0.11 µM). This was found to be the case during the fall of 1972 (preintrusion period, Figure 9), and for one other month (July, 1973). During this period the toxic effect of the copper addition was the result of the increase in copper concentration. However, during the period from January, 1973 until July, 1973 this was not the case, as the addition of a discrete

amount of copper resulted in a toxic effect equivalent to that of a greater amount of copper than was used. Figure 16 shows the method used to obtain the EDTA equivalence of the copper stress. Figure 17e shows the pattern of the toxic effect of copper as a function of time. The toxic effect of the copper enrichment began to increase in January and continued to increase until May (postintrusion period, Figure 9). From May until July the toxic effect decreased (intermediate period, Figure 9). There is at present no evidence for explaining this occurrence other than possibly the competition of metals.

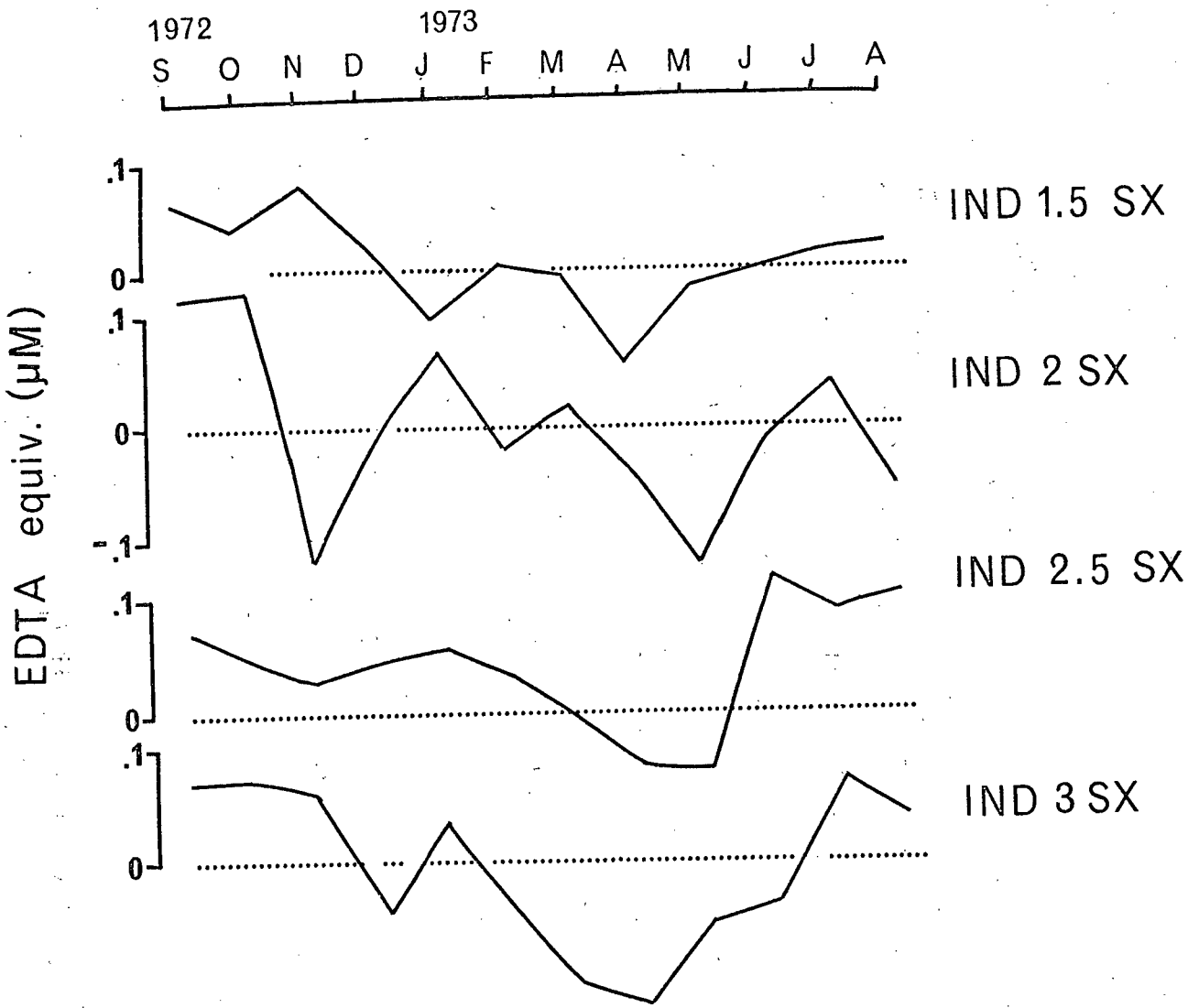
However, the toxic effect of the copper enrichment can be considered in the light of the ultra-violet experiments and the changes in hydrographic properties. At the time of the intrusion there is a displacement of the deep water of the inlet, resulting in a different set of chemical entities in deep water after the intrusion. It is believed that the intruding water may have had a relatively large concentration of organic material which could act to complex metals. The ability of this organic material may be such that it formed a complex with a metal which would be displaced when exposed to excess amounts of copper. The result of this displacement had an increasingly harmful effect during the months following January. This harmful effect was reduced in the months following May, suggesting that one of two mechanisms may be acting. First, the chemical composition and activity of the organic material may have changed in a manner which prevented the enhancement mechanism from becoming manifest. This could be the result of production of organic material in near surface layers which was then transported into the deep water. Second, the level of the toxic component might be reduced either by the effects of increased entrainment associated with increases in runoff, or by sorption onto a particle which settled out of the water column.

The mechanism of the increase in the toxic effect of a copper enrichment is not known at present and needs further examination. It is important to note that the changes in the toxic effect of the copper enrichment occur simultaneously to intrusions of water into Indian Arm. It is possible that the answer lies in the chemical changes which occur following an intrusion.

The ability of material extracted from sediments to reduce the toxic effects of a copper enrichment was different at each of the four locations studied. The noticeable changes as well as the differences between stations may be an indication that the material responsible for the activity of the extract is organic. One of the most noticeable features in Figure 17 is the large variability in the nature of sediment extracts from Ind-3. This station is relatively shallow and lies between the sites of entry of the two largest sources of freshwater and accompanying sediment load. The sediment extracts from Ind-2.5 show the least variability of the stations examined. There is a tendency evident at all of the stations with respect to the ability of the sediment extracts to reduce the toxic effect of copper enrichment. The ability of the sediment extract to reduce the toxic effect was lowest during the winter and highest in the late summer.

The EDTA equivalences for the sediment extracts have been adjusted for variation in the toxic effect of the copper enrichment of the test water. The correction of the EDTA equivalences for this variation has been done in two manners. The first was by addition or subtraction of the difference between the observed EDTA equivalence of the copper enrichment's effect, and that theoretically expected, from the EDTA equivalence of the sediment extract as shown in Figure 21. The second was by multiplying the EDTA equivalence of the sediment extract by a factor which is the predicted EDTA equivalence of the copper enrichment divided by the observed. The first of the

Figure 21. EDTA equivalence of the sediment extracts corrected for variation in the toxic effect of the copper enrichment, as a function of time.


1972
1973
S O N D J F M A M J J A
EDTA equiv. (µM)
.1
0
-.1
IND 1.5 SX
IND 2 SX
IND 2.5 SX
IND 3 SX

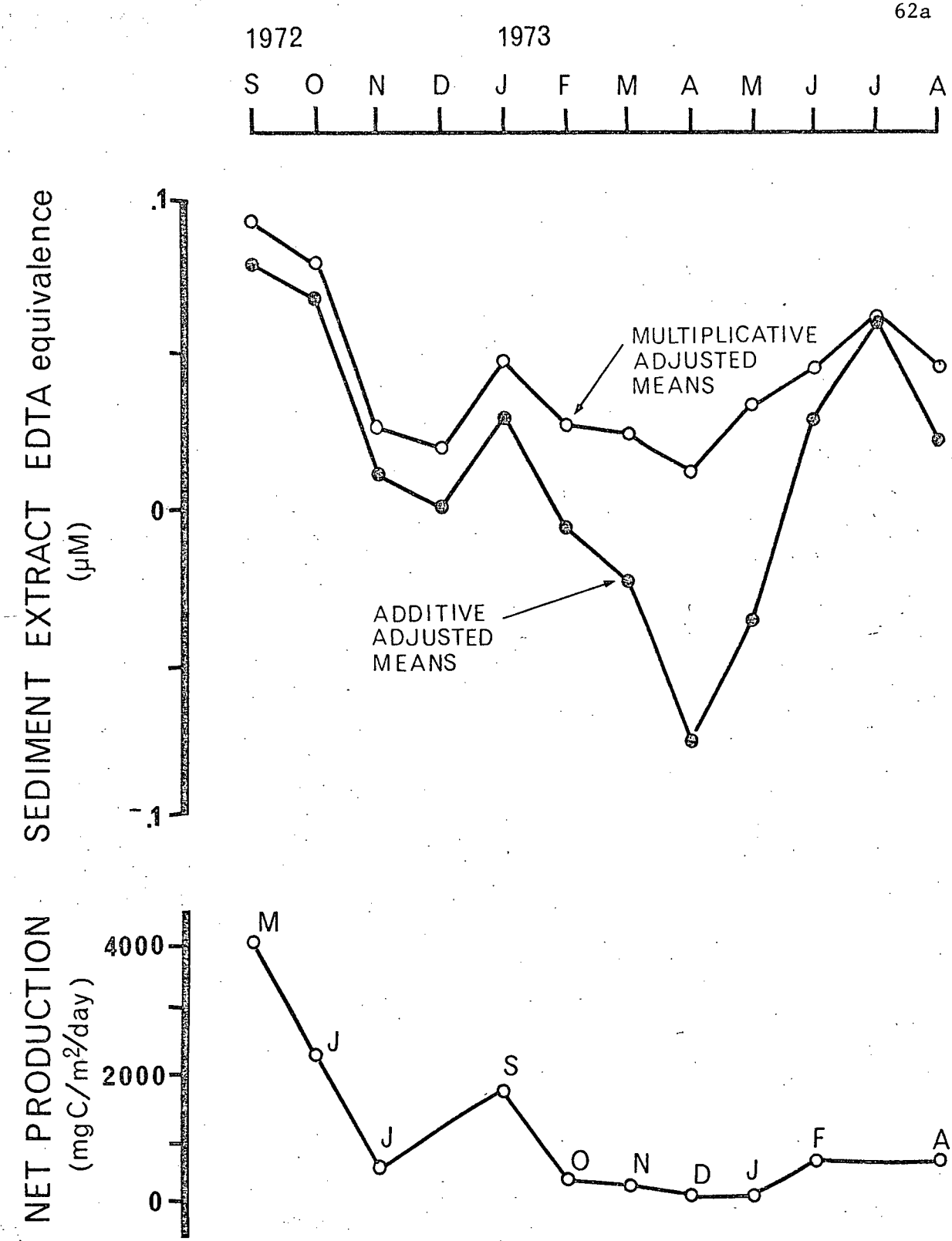
adjustments assumed that the change in the effect of copper altered only the intercept of the line relating survival of the organism to the concentration of EDTA, while the second assumed that the change in effect altered the slope of the line. It should be realized that both of these methods are extreme approximations, as the real correction factor would likely involve both height and slope adjustments.

In order to examine further the nature of sediment extracts, the means for all the stations of the corrected EDTA equivalences, adjusted in both manners described in the above, were plotted against time (Figure 22). The patterns which the two means exhibit are very similar which suggests that the corrections applied provided a reasonable approximation. The actual adjustment which should be applied would result in a line which would lie between the two shown in Figure 22.

Attempts were made to correlate both of the derived means with values given for net production given by Gilmartin (1964) for Indian Arm. If net production controls the input of organic material into the sediments, a time lag must be allowed for the movement of material from the surface waters into the sediments. When the data which Gilmartin (1964) gives were subjected to a four month shift, as shown in Figure 22, there was a positive and statistically significant correlation with mean EDTA equivalences of the sediment extracts ($r = 0.830$; $p = 0.003$ for additive adjusted means with net production, $r = 0.937$; $p = 0.0001$, for multiplicative adjusted means with net production).

The result is what would be expected on the basis of the suggestion by Gross et al. (1972) that organic material in sediments is derived from three sources; phytoplankton production, terrigenous input, and bacterial reworking of material from the first two sources. The data show that phytoplankton production is the primary source of organic material in the sediments

Figure 22. EDTA equivalence of the sediment extracts (transformed means) as a function of time and net production subjected to a four month shift (data for net production from Gilmartin, 1964).


1972
1973
S O N D J F M A M J J A
SEDIMENT EXTRACT EDTA equivalence (μM)
.1
0
-.1
MULTIPLICATIVE ADJUSTED MEANS
ADDITIVE ADJUSTED MEANS
NET PRODUCTION (mgC/m²/day)
4000
2000
0
M
J
J
S
O
N
D
J
F
A

of Indian Arm.

The use of ultra-violet light to destroy organic material was noted by Armstrong et al. (1966) as a decrease in the extinction coefficient of light at 2000-2500 Å. This band of wave lengths is characterictic of the presence of organic material. Other workers have also suggested that this was possible (e.g., Williams, 1968; Williams et al., 1969; Strickland, 1972). Hamilton and Carlucci (1966) suggested treatment of culture water with ultra-violet light to make it free of organic material. The apparatus used in the present study was similar to that used by Williams et al. (1969), and earlier by Anderson (1947; 1955).

Chemical measurements show that organic material present in natural waters was oxidized by ultra-violet irradiation (Table 4, Figure 19). The low levels of organic material which remained subsequent to the treatment of water may have been non-oxidized portions of the original material, or may have been the result of contamination from plastic materials used in the transfer and storage of the treated water. Other components of the seawater were apparently not altered by the treatment (Table 5). Armstrong et al. (1966) suggested that the pH of the water may be altered in proportion to the amount of organic material which is destroyed, as a result of increased concentrations of carbon dioxide, with the subsequent formation of bi-carbonate ions. Those measurements which were made during this study indicated that changes in the pH were minimal. This may be the result of aeration of the water during transfer of the water to the culture vessels.

The technique used in this thesis measured the complexing ability of the water in terms of the relationship between the organism and the water in which it was cultured, a measurement which can be made only in this way. The use of a bioassay in measuring complexing ability has some limitations.

Some attempts were made to determine if the estimates of complexing ability obtained by this method could be increased by the addition of small amounts of copper. Copper has a very high k_f and will enter into complexes displacing metals which may or may not be detrimental to the organism. If the metals which are displaced are non-toxic or there is a supply of free ligands then the measured complexing ability will increase. If, however, the metal which is displaced is as detrimental as copper, it will remain detrimental in ultra-violet treated water and the original estimate of the complexing ability of the water will not be increased.

The breakdown of organic material which is bound to metal ions will result in the release of the metal from the organic complex. This may change the relationship between the organism and the metal. The metal may have properties which cause it to be toxic to the organism in the ionic form (e.g., Steeman-Nielsen and Wuim-Anderson, 1970). Its release from the complex, in sufficient quantities, may be detrimental to the organism. If the organic material is responsible for making the metal available to the organism, either by increasing its solubility or by holding the metal in the form in which the organism requires it, the destruction of the organic material will result in the metal becoming unavailable to the organism. The release of metal ions into solution may also alter the relationship between metals, an area which needs much further examination (i.e., Table 2, results from copper series in April, 1974, where the addition of both 1 µg/l and 5 µg/l of copper caused a decrease in the intercept on the survival axis of the line relating percent survival to EDTA concentration, while 2 µg/l of copper resulted in a complete reversal of the slope).

The ability of a synthetic chelating agent to serve as a substitute for the destroyed organic material will depend largely upon the cause for the

decrease in survival. In the case of water from Indian Arm, EDTA has, where no metal additions have been made, served to replace that property which was lost from the water by ultra-violet irradiation. This is not, however, the case in waters where the metals are not sufficiently available to the organism (Lewis, unpublished).

The decrease in survival which occurs as a result of treatment of water with ultra-violet light is directly proportional to the amount of organic material which is destroyed (Figure 19). This indicates that there is a direct relationship between organism survival and the quantity of organic material which is present in natural seawater.

The EDTA equivalence of deep water, as determined with the ultra-violet treated series, changes as a function of time (Figure 19). During the year there is a relatively persistent decrease in the values obtained. The two deviations from this pattern occur at the time of intrusions of water into Indian Arm. In January, 1974 there is an increase in the EDTA equivalence from the low level of late winter to the maximum which was noted during the period of study. In July, 1973 there was a drop in the EDTA equivalence to the lowest value which was obtained. The intrusion is likely the controlling factor at these times. The intrusion of water into the inlet in January results in an exchange of the deep water for near surface water from the Strait of Georgia. In July the intrusion did not cause a displacement of the deep water from the inlet, but created some vertical mixing through the deeper portion of the water column. It is believed that this is the cause for the decrease in the EDTA equivalence at that time.

Gilmartin (1964) shows primary production in the inlet is highest in the early spring (e.g., Figure 22). Fogg (1958; 1966) and Lucas (1947; 1949; 1958) suggest that during periods of high productivity there is a release of

metabolites (e.g., glycollic acid, Shah and Fogg, 1973) by phytoplankton which increases the organic content of the water. Unfortunately, no information concerning organic carbon levels in the inlet are available during most of the year, although from the information which is available, it appears that the organic carbon content of the deep water decreased through the period from May until October.

The intrusion of near surface waters from the Strait of Georgia into the inlet, and increased productivity in the surface waters of the inlet during spring may both contribute towards increasing the organic carbon content of the deep water. During the period in which organic carbon levels decreased there was also a decrease in the EDTA equivalence of the water (Figure 19). It seems likely that both the intrusion and increased productivity are responsible for the high levels that the EDTA equivalence determined during the spring.

The ultra-violet EDTA series measures the relationship between the metals and the organism as a function of the amount of complexing which is present in the natural water. Because of differences in the biological properties of the various metals, the organisms respond to each in a characteristic manner. For example, copper is highly toxic when present in an ionic form (Pagenkopf et al., 1974), but relatively innocuous when present in an organic complex (Lewis et al., 1972; Lewis and Whitfield, 1974). Other metals may act in a similar manner or may be totally dissimilar. Some metals which may be bound to organic material seem to be beneficial when present in an ionic form (e.g., iron, Lewis unpublished) or may have little or no effect (e.g., manganese). Copper, zinc, and manganese were used in attempts at measuring total complexing ability and in attempts at understanding the relationship between the organism and various metals.

The concentration of EDTA which produced a survival in ultra-violet treated water equivalent to that which occurred in untreated water may not be a measure of all the complexing material present in natural waters. EDTA enters into stable complexes on a 1:1 molar basis with the majority of transition metals (Martell and Calvin, 1959), while natural organic material may be present in as great as 6:1 (molar) organic:metal complexes depending upon the nature of both the organic material and the metal. Additionally, it is possible that some organic material complexes metals but does not occupy all of the coordination sites on the metal and thus may not change the properties of the metal sufficiently to alter its biological effect. This complexing ability is impossible to measure at present and will likely remain so until chemical techniques are refined to allow this type of resolution.

The addition of any of the metals used (copper, zinc, and manganese) to water which was not treated with ultra-violet light always caused a decrease in survival. This relationship does not hold for water which has been treated with ultra-violet light, as manganese often increased the survival as did one concentration of copper (Table 3). Both manganese and zinc also altered the relationship of the survival of the organism to the concentration of EDTA (as evidenced by changes in slope). Survival in ultra-violet treated water is not changed by the addition of manganese when the EDTA concentration is zero, but is decreased at higher EDTA concentrations. This indicates that the metal is likely non-toxic as opposed to the effect of the addition of manganese to untreated water. Further evidence of this is shown in those experiments where manganese and copper were added together. The toxic effect of copper was reduced by the presence of manganese (see Table 3). Manganese is believed to alter the availability of a particular

metal by competing for the EDTA as its concentration increases. Zinc decreases the toxic effect which results from the ultra-violet treatment. The slope of the line relating organism survival to EDTA concentration under a zinc addition is essentially zero. This effect may be due to the interaction of the added zinc with the EDTA. Zinc itself has a low toxic effect on the organism and, in fact, might be slightly beneficial. However, it has a high stability constant (pk_s = 16.5) for the formation of a complex with EDTA. At low levels of EDTA, zinc acts in a manner which does not affect the organism. As the level of EDTA increases there is sufficient zinc present to allow 25% of the EDTA to exist in a complex with the added zinc. This relationship should develop in a linear fashion. It appears, however, that a break does occur. Possibly, as the EDTA concentration increases initially it is bound almost entirely to the added zinc (and other metals of higher pk_s). Above a certain limit this effect changes, in that the increase in EDTA concentration does not bind any further zinc. The EDTA then begins to bind metals which have lower stability constants, causing the survival of the organism to decrease proportionally. This may be a result of the binding of a particular metal which the organism requires.

The addition of Cu 1 (1 µg/l Cu^{+2}) causes a consistent but not significant decrease in survival over the range of EDTA concentrations which were used. This is thought to be the result of two features of the system. The toxic effect of the copper addition is well known (e.g., Lewis et al., 1972) but is decreased by the increase of EDTA concentration (Lewis et al., 1972). The effect of increasing the EDTA concentration in treated seawater is to increase survival of the organism. When considered together it is apparent that the lines should be parallel because the binding of copper by EDTA has the highest stability constant of all the metals considered and in this

respect results in a pattern similar to that obtained with zinc.

From this it may be suggested the pattern of survival which results subsequent to a metal addition is dependant upon two distinct factors: the stability of the complex which is formed between the metal and the EDTA used as a standard, and; the effect of the metal in unenriched water. This is only a supposition at present and is an area which should be considered for further research, especially in light of the results obtained with increasing copper levels.

The survival of the organism in untreated water decreased as the result of copper additions, and can be shown to be directly proportional to the concentration of copper which was added (e.g., Table 3, May results for untreated water). In ultra-violet treated water this was not the case. The addition of Cu 1 (1 µg/l Cu^{+2}) did not change the relationship between the survival of the organism and the concentration of EDTA. If one considers the results found for untreated water, one would expect that the survival decrease obtained with increasing copper concentrations would be proportional to the amount of copper which was added. However, this is not the case. The addition of Cu 2 (2 µg/l Cu^{+2}) caused a significant increase in survival at low EDTA levels and a significant decrease in survival at high levels, when compared with ultra-violet treated seawater (e.g., Table 3, April and May, 1974). At higher concentrations of copper (Cu 3, Cu 5), increasing EDTA concentration again resulted in an increase in the survival of the organism, but survival was significantly lower through the entire range of EDTA concentrations when compared to ultra-violet water which was not enriched with copper. The mechanism which is responsible for these results is not known, but suggests a further regulatory mechanism of organic material which is present in natural waters.

SUMMARY

1. Major changes in the abundance of the prefeeding stages of Euchaeta japonica and in the concentrations of trace metals occur simultaneous to intrusions of water into Indian Arm.

2. Subsequent to winter intrusions there is an increase in the complexation capacity of the organic material present in the water.

3. The summer intrusion causes mixing of the properties measured through the deeper portion of the water column and results in a decrease in the complexing capacity of the water.

4. The toxic effect of a copper enrichment changes with time increasing from the predicted level at the time of the intrusion, reaching a spring maximum, and then decreasing to the predicted level in July.

5. The ability of material extracted from sediments to reduce copper toxicity changes through the year being highest in early fall and lowest in the late winter.

6. The activity of sediment extracts is related to the production of organic material in the nearsurface waters.

7. Laboratory experiments and measurements show that organic material present in natural waters is capable of controlling the availability of trace metals to Euchaeta japonica developmental stages. These include:

(i) Ultra-violet irradiation of seawater destroys the organic material which is present, but does not alter the other components of the water.

(ii) Decrease in survival of the organism results in water which has been treated with ultra-violet light, and the decrease is proportional to the amount of organic material which is destroyed.

(iii) Additions of several metals alter the relationship between the organism and the complexing ability of the water in experimental conditions.

8. The complex effect of organic material on the availability of metals prevents the prediction of the abundance of Euchaeta japonica prefeeding stages on the basis of one or more of the properties of the water.

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APPENDIX

Description of the ultra-violet lamp apparatus

The lamp used (Hanovia 189A-16) is a high intensity, 12 inch mercury arc under medium pressure in a clear fused quartz chamber. Clear fused quartz transmits the complete ultra-violet spectrum. This lamp operates at an input wattage of 1200. The following gives an indication of the output of the lamp; 13673-10140 Å (infra red) 48.68 watts, 5780-4045 Å (visible) 187.07 watts, 3660-3341 Å (near ultra-violet) 104.03 watts, 3130-2804 Å (medium ultra-violet) 117.01 watts, and 2753-2224 Å (far ultra-violet) 116.15 watts. The maximum output of this lamp is at 3660 Å.

The lamp has a stainless steel tube, 5/16" O.D. extending the length of the lamp assembly. This tube carries a flow of nitrogen gas (99% pure) to the bottom of the well, purging the inner well. Approximately 6 lbs/sq. in. positive pressure is maintained in the inner well to prevent the seepage of other materials into the lamp chamber. The nitrogen provides a neutral environment, permitting the lamp to operate at high temperatures without the oxidation of the external lamp parts. The flow of nitrogen into this chamber is controlled by a regulator on the tank which supplies the nitrogen. Pressure is maintained in the inner well with the aid of a needle valve mounted on the cover of the well head assembly. A line runs from this valve into an erlenmeyer flask containing water. This allows an indication of the amount of nitrogen which is passing through the inner well.

The entire well head assembly is constructed of corrosion resistant aluminum. The explosion proof junction box contains the high voltage terminals for the lamp connections. The well head assembly supports the inner and outer immersion wells. An inlet and an outlet in the well head assembly

allow the circulation of water as a coolant. A piece of tygon tubing is connected to the inlet in the well head assembly and the other end is anchored to a centering spring at the lower end of the inner well ensuring the delivery of water to the annular space at the base of the immersion well assembly.

Circulation of water between the immersion tubes at a rate of 1 to 2 gallons per minute and at 35 lbs/sq. in. transfers the heat produced by the lamp into the outlet flow. This water is first passed through a "Cuno" filter (5 micron porosity) to help prevent the formation of deposits between the immersion wells. The cooling system is safeguarded against failure by the inclusion of an automatic pressure switch arranged to extinguish the lamp if a water pressure failure occurs. The flow of water also serves to prevent the transfer of heat produced by the lamp to the sample being treated.

The immersion well assembly consists of two concentric tubes of Corning #7010 Vycor, a quartz like glass (96% silica). Vycor transmits ultraviolet light more readily than does either Corex or Pyrex, allowing 92% transmission at 3500 Å.

The power supply for the lamp is an oil immersed transformer providing stable voltage in the range of 200-240 volts and 7 amps. Additional portions of the electrical system include the following. A pressure reducing valve prevents water pressure in the immersion tubes from exceeding 37 lbs/sq. in. A solenoid valve is mounted on the water line before it enters the immersion wells, and is interconnected to the electrical supply in a manner which allows water flow to occur independant of the operation of the lamp. A pressure switch on the water outflow line responds to a pressure difference of 4 lbs/sq. in., shutting the lamp power off in the event of a pressure drop. Two pressure gauges are mounted near the inlet and outlet ports on the well head

assembly, allowing water pressure to be checked visually. The power supply to the lamp is wired through a timing clock. This allows the duration of the treatment of the water to be easily controlled. The entire assembly is mounted in a controlled environment chamber maintained at $8^{o}C$.

The ultra-violet lamp immersion tube assembly is suspended in a manner which allows it to sit within a 50 liter chromatographic chamber. This chamber is used for holding the seawater during treatment. The chamber is covered with four pieces of plate glass fitted in a manner which reduces the possibility of contamination. With the immersion tube in place the chamber holds 44 liters of seawater for treatment. Water is transferred to and from the treatment chamber by siphoning through a 3/4" I.D. nylon tube, although a tygon tube of the same diameter was used during the latter portion of the study.

Further details concerning the operational portions of the lamp can be obtained from Hanovia Lamp Division, Canrad Precision Industries, Newark, New Jersey.