ESTUARINE MICROPLANKTON ECOLOGY: AN EXPERIMENTAL APPROACH

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Abstract

The data presented in this thesis are the result of laboratory experiments, executed concurrently with a year-long field study.

Microcosms (20 l volume) were filled with mixtures of natural low and high salinity waters in order to give a salinity range from $\leq$ 5 ppt, 10 ppt, 18 ppt to $\geq$ 26 ppt. The mixed waters were enriched so as to simulate the entrainment of nutrient-rich, saline water in a salt wedge estuary. Nitrate-N (20 μg-at l^{-1}), phosphate-P (2 μg-at l^{-1}) and silicate-Si (50 μg-at l^{-1}) were added to the highest salinity microcosm, proportionally less to lower salinities. A distinct pattern of autotrophic and heterotrophic growth developed resembling natural events in the Fraser River estuary during the period between winter and late spring. Despite seasonal variability of the source waters, the simulated spring bloom was reproducible under constant laboratory conditions, thus allowing the continued performance of experiments. _Skeletonema_ _costatum_ and _Thalassiosira_ spp. were consistently dominant in the simulated bloom, as they are in the Strait of Georgia. The salinity values influenced the microplankton ecology with respect to phytoplankton species composition and heterotrophic activity.

In Light- and Dark-experiments nutrient uptake and growth kinetics of bacteria and algae were studied, as well as the role of heterotrophic microflagellates. The interactions of the microplankton were described in numerical models. In the

presence of high substrate concentrations (5 mg l^{-1} glucose = 2 mg l^{-1} glucose-C), the glucose cell quota of bacteria determined the timing of the heterotrophic bloom, while grazing parameters and the gross growth efficiency of the microflagellates determined the absolute numbers. With an ecological efficiency of 57% between the two trophic levels, the Dark-model represented a system which was neither substrate nor predator controlled, but something in between. The Light-model gave an estimate of nitrogen recycling by microflagellates.

In perturbation experiments the impact of high organic load (glucose), shading, Cu, a heavy metal mixture, and the herbicide 2,4-D on the microplankton populations was monitored. Naturally occurring perturbations had much greater impact on estuarine ecology than anthropogenic ones, even when pollutants were added at concentrations exceeding those in moderately polluted estuaries.

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I. <u>INTRODUCTION</u>

Estuaries are of great importance to biological oceanographers for two reasons; first, for their generally high biological productivity and second, because they are centres of human activity. Many of the world's largest metropolitan areas have developed near estuaries. Through their role in transportation, food production, waste disposal, and various recreational pursuits, estuaries are significant to human welfare. At the same time, commercial fisheries have been damaged and some completely destroyed by pollution; these include fish species which use the estuary as a migratory route (e.g. Pacific salmon) as well as those which breed in estuarine areas and use the estuary as nursing grounds for their young (e.g. herring, cod, flatfish species).

Since the mid-1960's, the need for a more comprehensive understanding of estuarine ecosystems has stimulated conferences and symposia summarizing the knowledge of the natural characteristics of estuaries (Lauff 1967), as well as stressing specific biological topics such as estuarine microbial ecology (Stevenson & Colwell 1973), physiological aspects (Vernberg 1975), estuarine processes and interactions (e.g. Cronin 1975; Wiley 1976 and 1978). The impact of pollutants on the flora and fauna and the problems associated with eutrophication are gaining increased consideration (Perkins 1974; Neilson & Cronin 1979). Most of the studies are largely descriptive, but the

investigation of processes is needed for a better understanding of the estuarine ecosystem. A comprehensive discussion of microbial ecology in a brackish water environment is presented by Rheinheimer (1977). Based on a field study in Kiel Bight (Baltic Sea), laboratory investigations and numerical modeling, the role of bacteria and fungi and their contribution to total production is evaluated.

Estuaries are among the most complicated aquatic ecosystems. They are transition zones and frontal regions between freshwater and marine environments. Their biological processes are intimately linked and influenced by extreme and rapid fluctuations of environmental conditions, among them salinity, light and temperature. Due to constant mixing and transportation of organisms away from an estuarine study area, the value of ecological surveys is very limited. Surveys can indicate only that changes are occurring. In order to study processes of growth, physiology and interaction of the estuarine biota different approaches are necessary.

Mathematical simulation models of varying complexities have been generated in an attempt to synthesize existing knowledge into a systematic and integrative scheme (Kremer & Nixon 1978; Hamilton & Macdonald 1979). Although these models are valuable in the interpretation of interactive components of the estuarine ecosystem, they suffer from the lack of realistic resolution (Vernberg et al. 1978). The natural frequency of the processes to be simulated has often been ignored during the data collection.

Elaborate continuous-flow apparatus are necessary in order to simulate tides or seasonal changes in river flow rates in a laboratory. A series of chemostats has been used in a study by Cooper & Copeland (1973). With their system it has been possible to investigate the responses of an estuarine plankton community to variations in hydrological factors and the impact of industrial effluent with changes in freshwater input rates. It still has to be proven whether complicated continuous flow apparatus will necessarily give more realistic results.

Some success in studying biological processes in a water column has been achieved in large scale 'mesocosms' (Grice & Reeve 1982). Up to 1500 tons of seawater have been captured and the primary, secondary and tertiary production have been followed under experimental conditions. In an estuary the same approach is restricted by the lack of physically stable mooring sites as well as spatial limitations in the presence of all other activities on a busy estuary, such as the Fraser River estuary in British Columbia.

An alternative approach has been adopted in the experiments of this thesis. The estuarine environment is simulated with respect to changes in salinity using containers which allow the study of the microplankton community. This reduces the size of the containers required from 'mesocosms' to 'microcosms' (c. 20 1). I define microcosms according to Leffler (1980) as small, living models of processes rather than models of specific natural ecosystems. Usual aquaria and single-species cultures are normally excluded. The changes in scale "between beakers

and bays" (Strickland 1967) require the alteration of important physical factors, e.g. turbulence and light. The consequences of these changes are poorly understood. Therefore, microcosms and natural ecosystems possess their own characteristic properties, and microcosms can serve as valid models of ecosystems only for properties that are common to both (Leffler 1980).

The research presented here is designed to answer four questions. First, does the natural cycle of events with respect to the microplankton in the Fraser River estuary differ with increasing salinity values? Second, is it possible to reproduce some characteristics of estuarine microplankton ecology (e.g. the diatom spring bloom) in laboratory scale microcosms? Third, if the answer to the second question is yes, can the simulated events be replicated with sufficient accuracy to allow for the performance of meaningful perturbation experiments? Fourth, is it possible to represent the events observed in the experimental microcosms by a mathematical model which would help to understand how the microcosm components interact?

Microplankton studies in the Strait of Georgia and the Fraser River estuary have previously been performed (e.g. Parsons et al. 1969; Stockner et al. 1979; Albright 1977, 1983b), but so far the effects of salinity variations have never been thoroughly examined (Harrison et al. 1983). Therefore, in order to answer the first question, a one year field survey in the Fraser River estuary was undertaken simultaneously with the laboratory experiments. The field sampling over a salinity

range from ≤ 5 ppt to ≥ 26 ppt is described in Chapter VI. The results of the field study are compared to laboratory experiments, and behavioural similarities are evaluated and discussed.

In answer to the second question, an estuarine salinity regime is simulated with respect to increasing entrainment of saline, nutrient-rich deep water, which permanently fertilizes the estuary. The collection of high and low salinity water, the mixing of the waters, and the initiation of the microcosms are described in Chapter II. The evolving pattern produced by growth differences of the biota is similar to that of a natural spring bloom (Chapter III).

Autotrophic and heterotrophic growth is studied in Dark- and Light-experiments. An attempt is made to isolate factors which influence production in the microcosms, to investigate interactions among the living components, and to evaluate the role of microflagellates as part of the estuarine plankton community.

In answer to the third question, the replicability of microcosms is studied (Chapter III). The aim is to prove that the pattern of events generated in the laboratory is reproducible despite physical, chemical and biological changes in the parent water masses collected at different seasons of the year. In this way, perturbations of the simulated phytoplankton bloom can be performed at any time.

Perturbation experiments help in determining which factors, natural or man-made, have the greatest impact in altering the

microplankton ecology of the simulated estuary (Chapter V).

In order to answer the fourth question, uptake and growth rates of phytoplankton and bacteria estimated in Dark- and Light-experiments are used as a basis for the mathematical simulations described in Chapter IV. Numerical modeling is thought to help in the interpretation of processes under investigation and in the identification of the important variables in the microcosms.

While a biological model such as the estuarine microcosm creates new data, a mathematical model based on these data is an inexpensive way to represent the processes observed, to provide better understanding of the same, to perform more extensive perturbations and to aid in the testing of hypotheses. Finally, it is intended that these results will assist in answering a larger question; what are the forcing functions for phytoplankton growth in an estuary and can these be perturbed by anthropogenic effects? This question is discussed in Chapter VII.

II. GENERAL METHODS

1. INTRODUCTION

The use of containers, ranging in size from small flasks to large plastic bags, is a relatively recent development in marine ecological research. Pioneering work has been done by Strickland & Terhune (1961). In a large floating plastic bag they were able to follow changes in the plankton population. Pilson & Nixon (1980) reviewed 21 representative marine micro- and mesocosms, summarized difficulties with scaling, natural and artificial light, turbulence, water exchange, predator exclusion and variability.

The size of containers chosen for the experiments reported in this thesis allows for the study of bacteria, phytoplankton and microflagellates. They hold sufficient water for frequent sampling over a three-week period and represent the upper limit which can be managed by one or two people. This size is a compromise between feasibility and minimal statistical requirements. The microcosms are run as batch cultures; the operating time is short. Constant physical factors and a constant initiation method improve replicability between microcosms and the reproducibility of experimental results between seasons.

An initial nutrient enrichment was thought to be necessary because of seasonal variability in the substrate of the source

waters. Low natural substrate levels in batch cultures may result in growth so small that significant differences of population changes are impossible to observe. The addition enhances already high nutrient levels, as well as ensuring growth of the microplankton population when levels are low. Consequently, experiments can be performed at all times of the year. The initial enrichment simulates the decreasing entrainment of nutrient-rich water in a salt wedge or highly stratified estuary.

A meaningful comparison of laboratory and field data requires the parameter measured to be the same. As such, the microplankton community is studied by enumeration of bacteria and microflagellates and by identification of the major phytoplankton species. The biomass of the latter is estimated by chlorophyll _a_ measurements. Nitrogen is considered to be the limiting factor for autotrophic growth (Antia _et al_. 1963; Ryther & Dunstan 1971); therefore, concentrations of nitrate plus nitrite have been measured. Glucose is used as the substrate in evaluating relative heterotrophic activity, because almost all bacteria can utilize glucose (Mandelstam _et al_. 1982). In the field, determination of dissolved monosaccharides gives an indication of a readily usable carbon and energy source for heterotrophs.

2. LABORATORY EXPERIMENTS

2.1 Experimental Set-up

In the experiments described below, Pyrex flasks (c. 20 l) were filled with a mixture of natural low and high salinity water. Low salinity surface water ($\leq$ 5 ppt) was collected from the banks of the Fraser River main arm c. 200-500 m upstream from Steveston harbor at high tide. The high salinity water was pumped from c. 20 m depth at the West Vancouver Laboratory of the Dept. of Fisheries and Oceans (Figure 1). On collection day the waters were mixed in order to give a salinity range from 2-5 ppt, 10 ppt, 18 ppt to 26-30 ppt (Figure 2). Four flasks represented one unit. A total of 12 flasks allowed for a control unit and two experimental units.

All experiments were performed at 12°C in a controlled environment chamber. The flasks were positioned in front of banks of four daylight fluorescent tubes, so that an irradiance of 200-250 μEinst m^{-2} s^{-1} was measured in the middle of the empty containers. The irradiance was saturating for diatom species and smaller dinoflagellates (Chan 1978). All experiments were performed on a 12:12 LD-cycle. The waters in all flasks were magnetically stirred and aerated with sulfuric acid (3%)-washed air. Nitrate-N (20 μg-at l^{-1}), phosphate-P (2 μg-at l^{-1}), and silicate-Si (50 μg-at l^{-1}) were added to the highest salinity flask, proportionally less to lower salinities. These additions were made to both control as well as experimental microcosms. Together with the initial nutrient
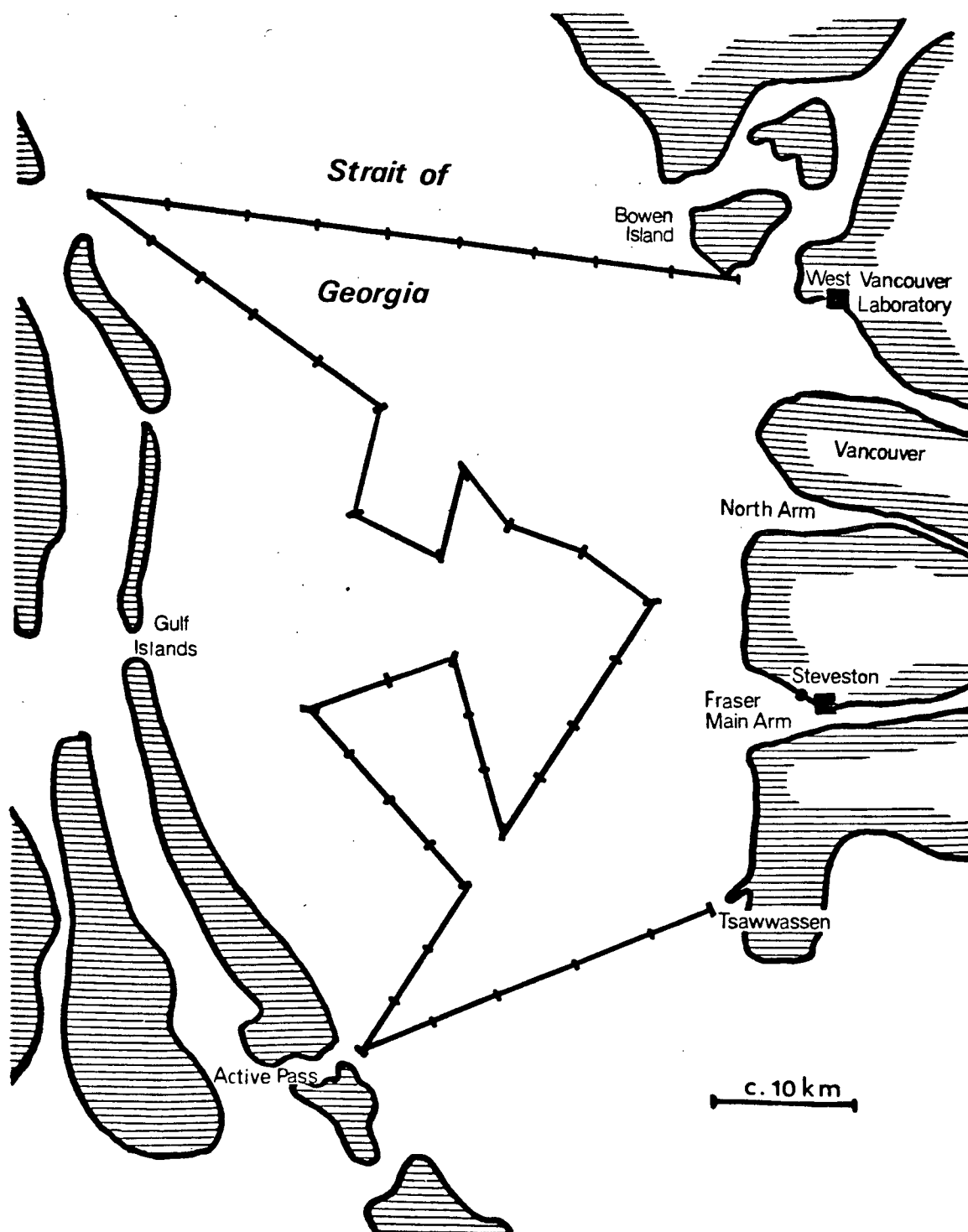
Figure 1 - Ship's course off the Fraser River mouth during sample collection.
■ Sampling sites for laboratory experiments.
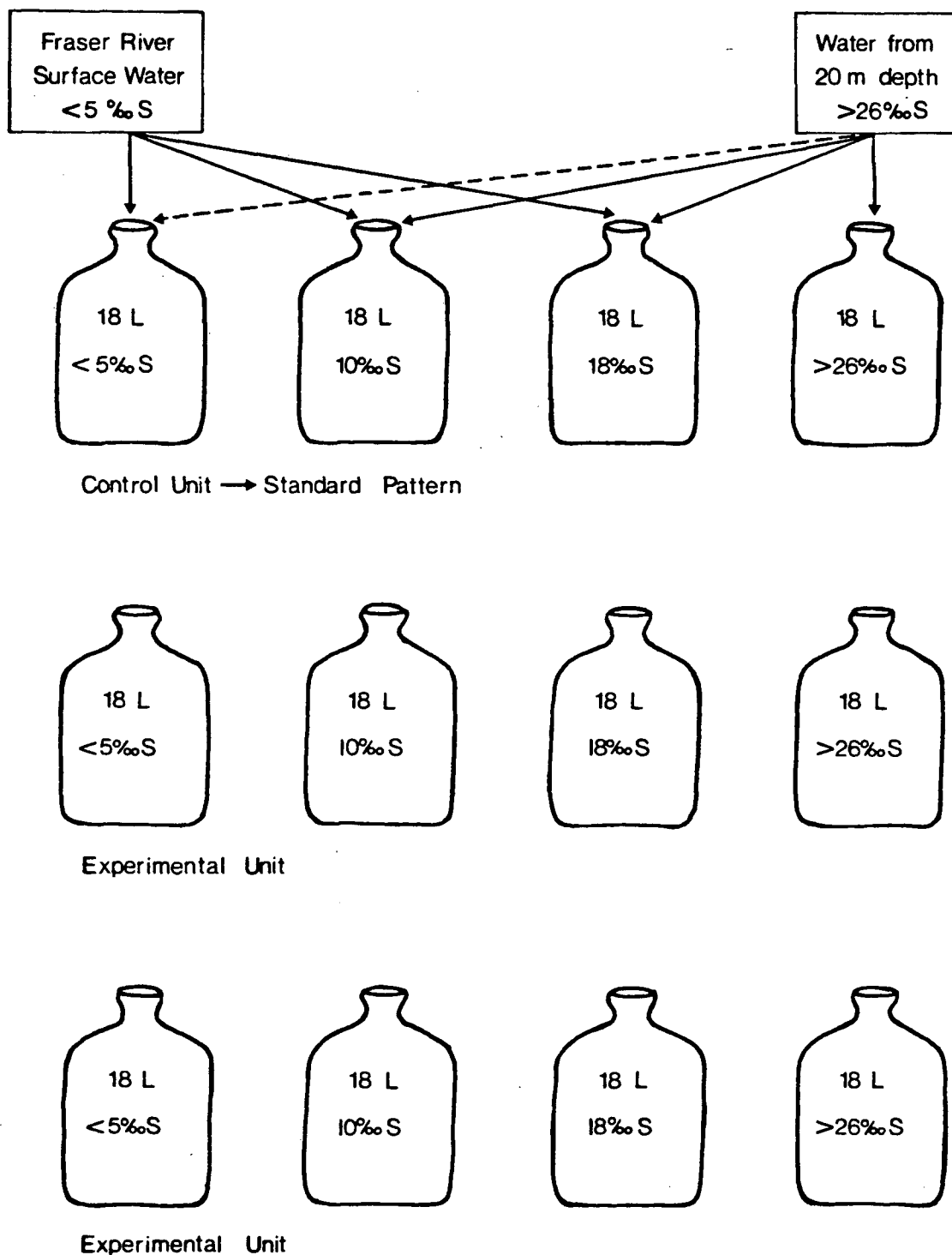
Figure 2 - Laboratory set-up; dashed line indicates occasional mixing of saline water with very low salinity river water.

enrichment, 1.2 μM EDTA+iron and 1 mg l^{-1} of glucose were added to all salinities in order to ensure non-limiting growth conditions for the microplankton under study.

2.2 Sampling Design

After collection and mixing of the low and high salinity waters on day 1, the biota was allowed to adjust to laboratory conditions overnight. Initial samples were taken on day 2 prior to nutrient addition and/or start of the experimental treatment, 24 h later and then every second day for two to three weeks. All samples were collected by suction from the flasks using Tygon tubing of 8 mm diameter. By the end of the experiment the initial volume of the microcosms was reduced by c. 33%.

Chlorophyll a, nitrate, bacteria and microzooplankton were sampled as described above. For the determination of nutrient uptake rates and growth rates of bacteria and phytoplankton, additional samples were taken at 12 h intervals during the exponential growth phase. Depending on the questions asked in experiments, plankton was sampled during the lag phase, during exponential growth, at the end of exponential growth and after nutrient exhaustion (senescence). The sampling of other parameters has been described in the appropriate chapters elsewhere.

2.3 Replication Experiments

Replication experiments focussed on two questions: what is the variability between duplicate flasks and how does the response pattern of the microcosms change with respect to seasonal changes in the water chemistry? Replication of microcosms with identical treatment was tested over a salinity range from ≤ 5 ppt to ≥ 26 ppt during different seasons of the year. The comparison focussed on variability in peak heights and on rates of increase and decrease of parameters measured. Plankton samples were compared for relative abundance of species.

Data generated in microcosms usually are in the form of a time series for each parameter measured. If the series from two identically prepared microcosms are compared, they may exhibit similar form, but be shifted in phase. When data are compared at any one instance, the analysis of the variance would likely infer that the two microcosms do not replicate. With appropriate statistical tests it is possible to eliminate the effects of phasing and compare the real time dynamics (Haque et al. 1980), something which was not done in the present thesis.

Seasonal variations in the response pattern of the microcosms were examined by comparing the 4-flask control units which were run concurrently with all experiments. The time series of the control units reflect the variability due to both seasonal changes in the chemistry of the different water masses and sample variation.

3. ANALYSIS OF SAMPLES

3.1 Nutrients

All samples collected in the laboratory and in the field were immediately filtered through glass fiber filters (Whatman 934-AH, 42.5 mm diameter), stored in Nalgene bottles and frozen to -20°C. Samples were stored for not more than three weeks and then quickly thawed before analysis. Automated determination for nitrate plus nitrite was done as described by Armstrong et al. (1967). The range of concentrations was 0-30 μg-at N l^{-1}; samples with higher concentrations were diluted with a known amount of deionized distilled water before analysis.

In one set of laboratory experiments, ammonium (0 to 8 μg-at N l^{-1}) was measured concurrently (Slawyk & MacIsaac 1972).

3.2 Pigments

The analysis of chlorophyll a and phaeophytin a was carried out according to the methods described by Strickland & Parsons (1972). In the field, 500 ml to 1000 ml of seawater was filtered, in laboratory experiments 200 ml to 500 ml, depending on the density of the phytoplankton bloom. To the last 100 ml of each sample 1 ml of magnesium carbonate suspension was added. The 0.45 μm filters (Millipore, 47 mm diameter) were stored in the dark in a desiccator and frozen to -20°C. Storage time prior to analysis did not exceed three weeks. The extraction was done in 90% acetone overnight. Following centrifugation, the clear supernatant liquid was pipetted into a 10 cm path

cuvette. The extinction was measured in a Perkin-Elmer Double Beam Spectrophotometer (Coleman Model 124 D) at 750 nm and 665 nm against a 90% acetone blank containing a dissolved Millipore filter. For determination of phaeophytin a, samples were acidified and remeasured after 5 min. The concentrations of chlorophyll a and phaeophytin a were calculated according to the equations given in Strickland & Parsons (1972).

3.3 Total Bacterial Numbers

Subsampling for enumeration of total bacterial numbers was always done first. Using an automatic pipette, 10 ml aliquots were placed into glass vials and immediately preserved with filtered (0.22 μm) formaldehyde (final concentration of 2%). Samples were stored in the dark at 5°C for no longer than ten days (Daley & Hobbie 1975). Bacteria were stained with acridine orange and counted on 0.2 μm Nuclepore filters (Hobbie et al. 1977). A microscope with an epifluorescent unit including a broad band blue excitation filter was used for enumeration. Ten fields and a total of at least 200 bacteria were counted.

Total numbers were calculated according to the equation

$$\frac{cells}{ml} = \bar{X} \frac{stained\ area\ of\ filter}{area\ of\ counting\ grid} \frac{1}{0.95 \times 2}$$

$\bar{X}$ = mean number of cells in 10 or more fields
2 = ml of sample filtered
0.95 = dilution factor after formaldehyde preservation
stained area of filter = $2.0 \times 10^8 \mu m^2$
area of counting grid = 9,200 μm^2

3.4 Dissolved Monosaccharides

Dissolved monosaccharides (MCHO) were analysed by the method described by Johnson & Sieburth (1977) and Johnson et al. (1981). The excess borohydride was destroyed with 0.05 ml of 0.7 N HCl instead of 0.36 N HCl. The higher acidity assured complete oxidation, thus improving monosaccharide recovery. Otherwise the outlined procedure was followed meticulously.

The three part analysis begins with a borohydride reduction to convert pentoses and hexoses to their sugar alcohols. The total alditols are then oxidized with periodate, to form two moles of formaldehyde per mole of monosaccharide. The formaldehyde is analysed spectrophotometrically with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH). The method is suitable for concentrations as low as 40 μg l^{-1} total monosaccharide carbon. Salinity has no effect on the test.

The exclusion of contamination from the sensitive MBTH assay was finally achieved without precombustion of the glassware. In all determinations glucose was used as the monosaccharide standard.

3.5 Heterotrophic Activity

The measurement of relative heterotrophic activity is based on the following considerations. If one assumes that V_{max} (maximum uptake velocity) changes with time but the value ($K_t + S_n$; K_t = transport constant at 1/2 maximum uptake rate, S_n = natural substrate concentration) does not, it means that bacterial populations become 'more active' or 'less active' (Parsons et al. 1977). According to Griffiths et al. (1977)

V_{max} is correlated with single substrate additions and gives a measure of relative microbial activity. The measurement must be related to two variables: (1), the number of bacteria and (2), the metabolic state of the bacteria. Since the number of bacteria is determined independently by counting, further variation in relative metabolic activity can be diagnosed as being due to the metabolic state of the bacteria.

Samples for heterotrophic activity measurement were processed immediately after subsampling on shipboard or in the controlled environment chamber. Duplicate dark bottles were filled with 100 ml aliquots and incubated with uniformly labelled D-[^{14}C(U)] glucose (specific activity 4.8 mCi mmol^{-1}, New England Nuclear). Only one concentration of substrate (165 μg glucose) was added and the net uptake of labelled solute measured. Field samples were incubated for two hours at ambient temperature ± 2°C; laboratory samples were incubated for one hour at 12°C. After filtering through 0.22 μm filters (Millipore, 25 mm diameter), filters were placed in scintillation vials filled with 10 ml of Aquasol-2 (New England Nuclear). A blank was filtered immediately after addition of ^{14}C-glucose to 100 ml of seawater. Samples were counted 24 h later in an Unilux III Liquid Scintillation System (Nuclear Chicago, Des Plaines, Ill., USA). A standard ^{14}C-source was used for efficiency correction. Given the specific activity of ^{14}C-glucose, the incubation time and sample size, the uptake of glucose in μg l^{-1} h^{-1} was calculated. This relative heterotrophic activity was then related to the total number of

bacteria in the sample, and expressed as glucose uptake in μg h^{-1} per 10^9 bacteria.

3.6 Phytoplankton And Microzooplankton

For analysis of field and laboratory plankton populations, 100-200 ml samples were preserved with 5-10 drops of Lugol's solution (200 g KI + 100 g I_2 in 2000 ml H_2O + 190 ml glacial CH_3COOH). Counting and identification was done in a Palmer-Malony chamber (volume 0.1 ml). A total of 400 cells in at least four fillings were counted. Heterotrophic and autotrophic flagellates were not separately enumerated. Based on 400 cells, the relative abundance of species in the sample was calculated.

Flagellates were grouped according to size, small ones of < 25 μm and large ones of > 25 μm length. To calculate cell volume, the dimensions of 20 cells were measured; the phytoplankters Skeletonema costatum and Thalassiosira spp. were assumed to be cylindrical.

In laboratory experiments live cell counts for growth rate estimates of phytoplankton were done by using a Fuchs-Rosenthal chamber (volume 3.2×10^{-3} ml). Live microzooplankton estimates were arrived at by employing the same chamber. It was assumed that the numbers of flagellates leaving the field of view was equal to the number swimming into view. Again green and colourless flagellates were not distinguished. The largest zooplankters in the microcosms were tintinnids. These were enumerated after preservation with a drop of Lugol's solution in a Palmer-Malony chamber under low magnification.

III. <u>THE STANDARD PATTERN</u>

1. <u>INTRODUCTION</u>

The 'captured' microplankton population is thought to represent a viable part of the estuarine ecosystem. Containment ensures that the biota can be monitored over a period of time and facilitates manipulations by the researcher. The initial nutrient enrichment results in a distinct pattern of autotrophic and heterotrophic growth. This standard pattern resembles a natural diatom spring bloom. Due to simulation of the estuarine salinity and nutrient regime, the total biomass will increase with higher salinities, while the sequence of heterotrophic and autotrophic growth remains the same. The salinity range, and seasonal and experimental variability influence the standard pattern as do abiotic factors due to the experimental set-up and so-called bottle effects. This has to be kept in mind when analyzing the results and comparing them to the field.

In order to investigate the processes which generate and govern the standard pattern, heterotrophic growth is studied in Dark-experiments. Further increases in organic substrate help to estimate bacterial growth rates and threshold concentrations for uptake. Phytoplankton growth rates and nitrogen cell quota are obtained from Light-experiments. The experimental data gathered in this way are used to test the numerical models as shown in Chapter IV.

The standard pattern represents a simulation of a phytoplankton bloom, which is initially characterized by a period of unlimited growth and high productivity. Under these conditions, the impact of natural and man-made perturbations on the microplankton population can be examined and separated from other possible factors which influence autotrophic and heterotrophic growth. The importance of the standard pattern will become apparent in perturbation experiments described in Chapter V, where it serves as a control. Deviations from the pattern will be used to show the impact of the imposed disturbances on the simulated bloom.

2. PATTERN OF EVENTS

The initial nutrient addition assured non-limiting growth conditions with respect to nitrogen, phosphate, silicate, iron and organic substrate. It created an environment similar to that preceding a natural phytoplankton spring bloom. Figure 3 shows a typical pattern of nitrogen and chlorophyll _a_ concentrations and bacterial and microflagellate numbers as found in the microcosm experiments. While absolute values differed, the pattern of events was similar at all salinities.

The bacterial population responded in between 24 and 48 h to the change in the environment and to the nutrient (glucose) addition. Numbers peaked in a first bloom between days 3 and 5, dropped sharply by day 7, and increased to a second heterotrophic bloom following the phytoplankton bloom. Autotrophic growth started after a lag phase of 3 - 5 days, and chlorophyll _a_ values peaked between days 7 and 11, depending on the size of the inoculum and the dominant algae. At day 7 nitrogen (nitrate and nitrite) was nearly exhausted and bacterial numbers reached a minimum. Increasing concentrations of phaeopigments by day 9 indicated decay of cells as nutrients became limiting (see Figure 13). An initial decrease in microflagellate numbers at intermediate salinities, 10 ppt and 18 ppt, was most likely due to the abrupt environmental changes which occurred when high and low salinity source waters were mixed. In all experiments, microflagellates seemed to follow the initial increase in bacterial biomass closely, while a
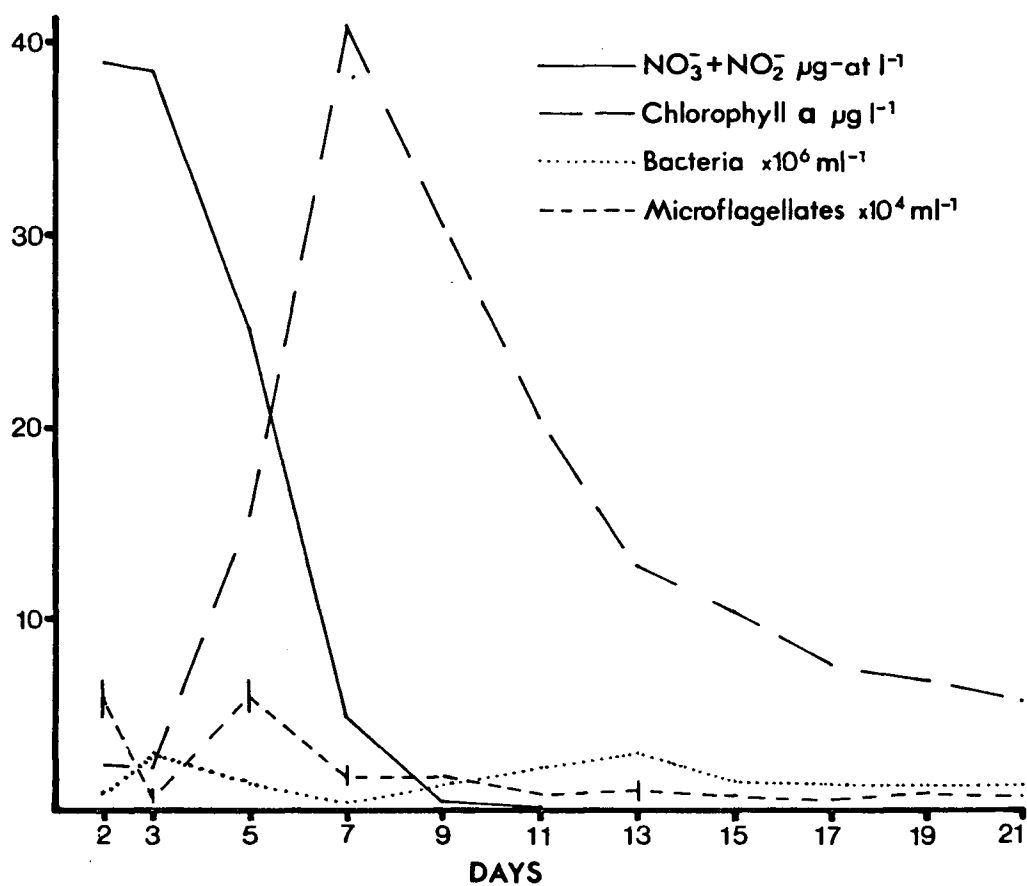
Figure 3 - Concentrations of nitrate and nitrite, chlorophyll a, bacteria and microflagellates in 18 ppt salinity microcosms. Mean values of six experiments are shown. For standard errors see Figures 4, 5, 6.

correlation with the second heterotrophic bloom was less striking. High numbers of tintinnids (30 to 80 ml^{-1}) were found by day 15.

The nutrient addition on day 2 increased the background level of nitrate. Bacteria and phytoplankton need nitrogen for protein synthesis, but both prefer to take up ammonium because it is less energy costly (Conway 1977; Mandelstam et al. 1982). Therefore, the initial concentration of ammonium (approx. up to 4 μg-at l^{-1}) was exhausted before the nitrate plus nitrite was taken up (cf. Figure 21). Nitrogen compounds excreted by the microzooplankton were assumed to be recycled immediately by bacteria and phytoplankton, until another substrate became limiting.

The model becomes unrealistic from day 7 on with respect to a natural bloom, because in the Fraser River estuary the biomass of herbivorous zooplankton is closely related to the primary productivity (Parsons 1979). These grazers, mainly copepods, were not observed in the microcosms. The largest grazers present were several species of tintinnids, which seem to forage on the microflagellate population (Spittler 1973; Heinbokel & Beers 1979).

3. <u>VARIABILITY</u>

Seasonal changes in the Fraser River are in general much greater than in the high salinity water collected from 20 m depth in Burrard Inlet. Temperature in the surface water of the former varies between 0°C in January and 19°C in August (Benedict <u>et</u> <u>al</u>. 1973), while in the deep saline water it varies only between 7 and 10°C during the year (Davidson 1979).

Nutrient concentrations showed a distinct seasonal cycle. Nitrogen (nitrate plus nitrite) values ranged from 4.1 μg-at l^{-1} to 19.6 μg-at l^{-1} in the river water. Lowest concentrations were found in September, highest in January. In the high salinity water, values ranged from 22.5 μg-at l^{-1} in Sept to 47.7 μg-at l^{-1} in April. Likewise, ammonium concentrations varied with the seasons, but were reported to be lower and less variable in magnitude (Shim 1977; Drinnan & Clark 1980).

Seasonal differences in the plankton community with respect to biomass and species composition caused considerable variability in the inoculum. Water collected during the winter months was dominated by microflagellates as described by Takahashi & Hoskins (1978) for winter conditions in Saanich Inlet. Accordingly, lowest chlorophyll <u>a</u> concentrations were found in waters during this period, while during the other seasons phytoplankton were more abundant, thus increasing the biomass of the inoculum.

Bacterial numbers were consistently higher in the brackish river water, fluctuating between 1.04x10^6 - 2.06x10^6 ml^{-1}, while

bacteria in the high salinity water varied between 0.19×10^6 – 1.03×10^6 ml^{-1}. The numbers represent total cell counts and no distinction was made between free or particle-attached bacteria. The proportion of the latter decreases with increasing salinity, and in the Strait of Georgia 85% of the bacteria are free-living (Bell & Albright 1981).

Beside variations in biomass, the microplankton community also experiences physiological changes during different seasons, e.g. photosynthetic rate increases with warming of the water in spring (Takahashi et al. 1973) and periods of maximum heterotrophic activity are found in summer and fall (Albright 1977).

Despite the many known and unknown sources of variation, the simulated pattern in the microcosms remains the same. The resulting chlorophyll a, bacteria and nitrogen curves (Figures 4, 5, and 6) represent data collected at different seasons of the year (winter, spring and fall), so that variability reflects seasonal differences in the response of the different waters as well as differences between the microcosms.

The replication of microcosms was examined in seven experiments at two different seasons; results are shown in Figures 7, 8, and 9. Nitrate and nitrite concentrations were not significantly different between replicates considering a precision of ± 1.14 μg-at l^{-1} in sample analysis. Bacterial counts were based on enumeration of ten microscopic fields and a total of at least 200 cells. According to Student's t statistic the relative error (a=0.05) of the mean (n=10) bacterial numbers
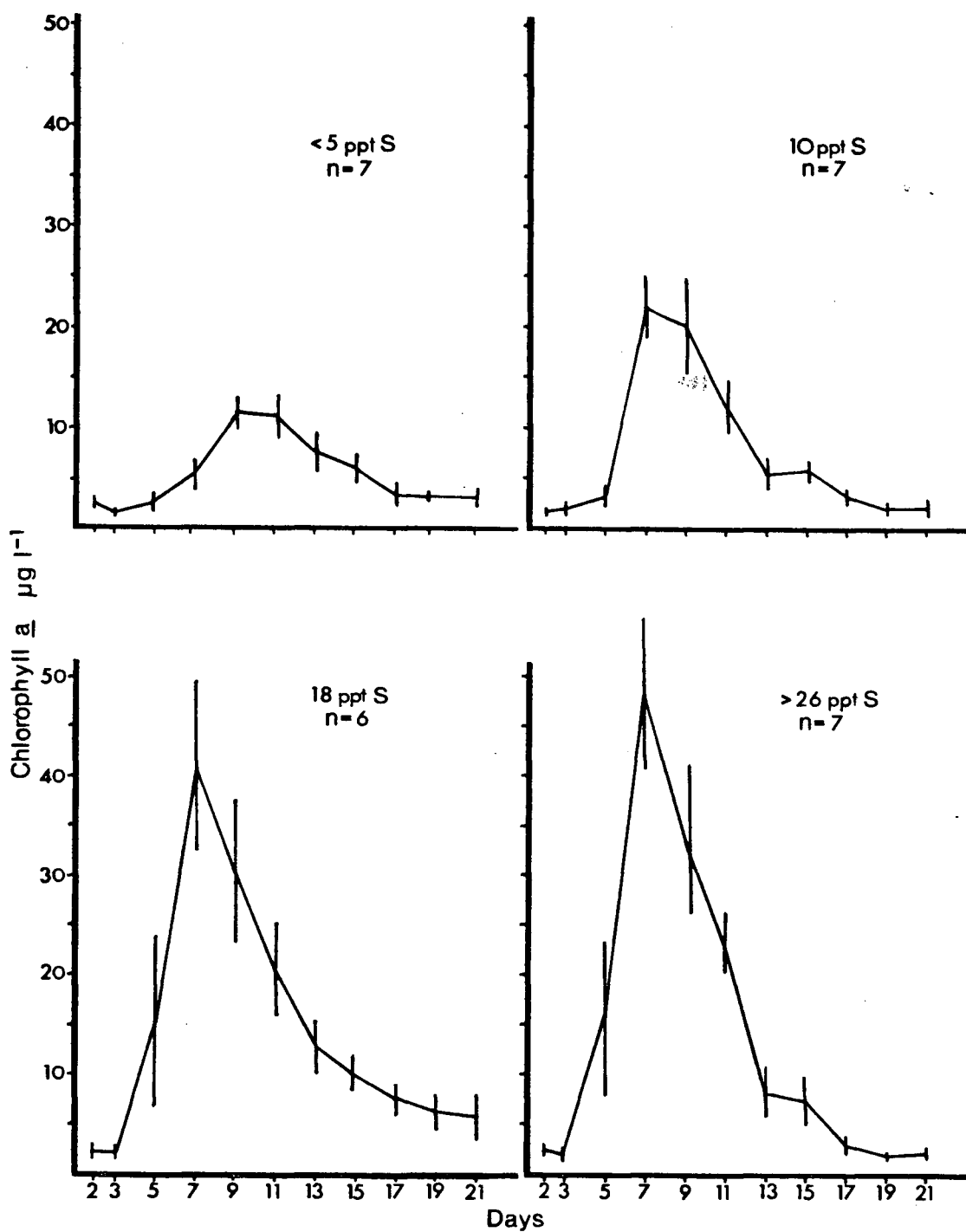
Figure 4 - The pattern of chlorophyll a concentrations; data collected at different seasons (winter, spring, fall). Bars indicate mean ± 1 S.E.; n=number of experiments.
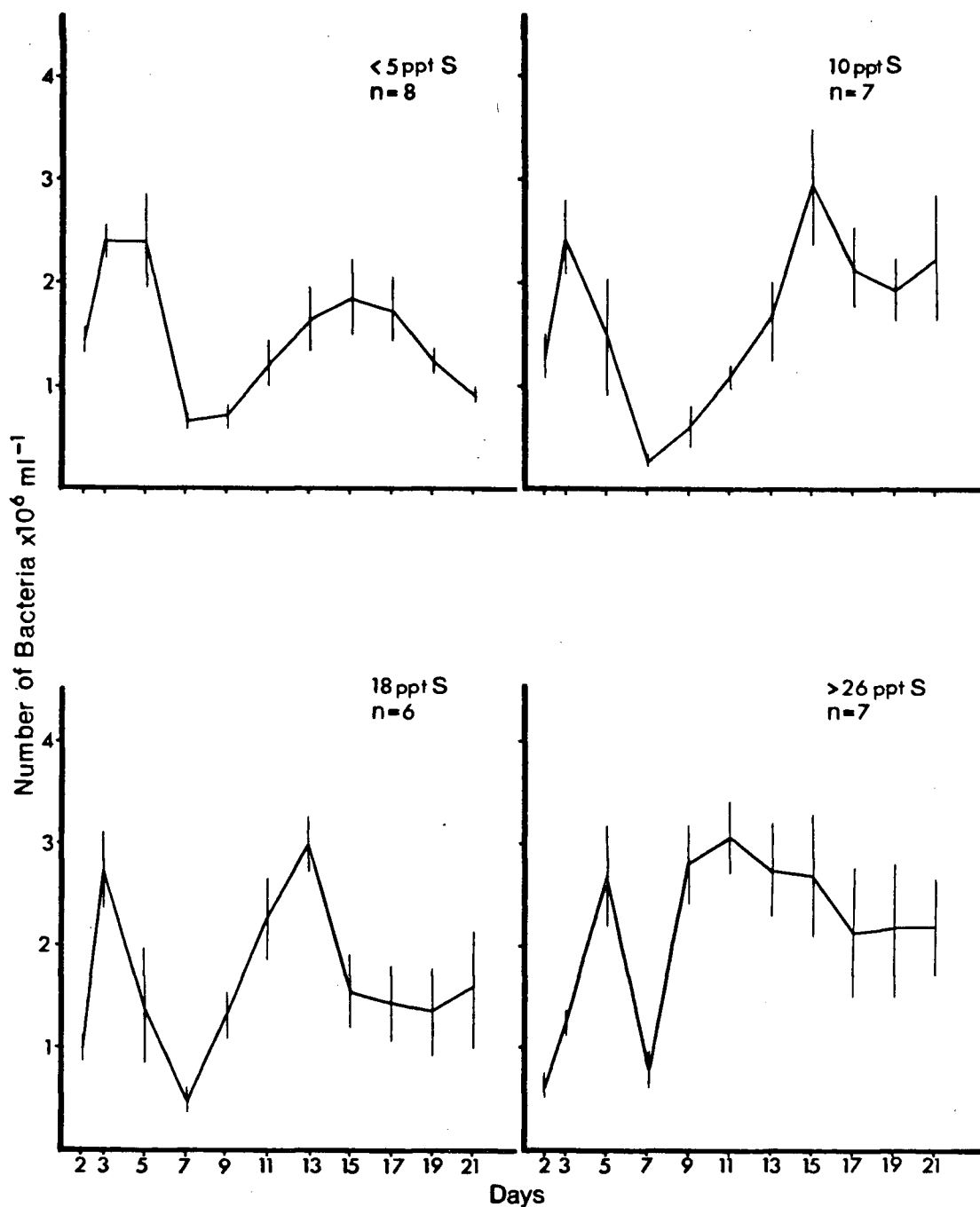
Figure 5 - The pattern of bacterial numbers; data collected at different seasons (winter, spring, fall). Bars indicate mean ± 1 S.E.; n=number of experiments.
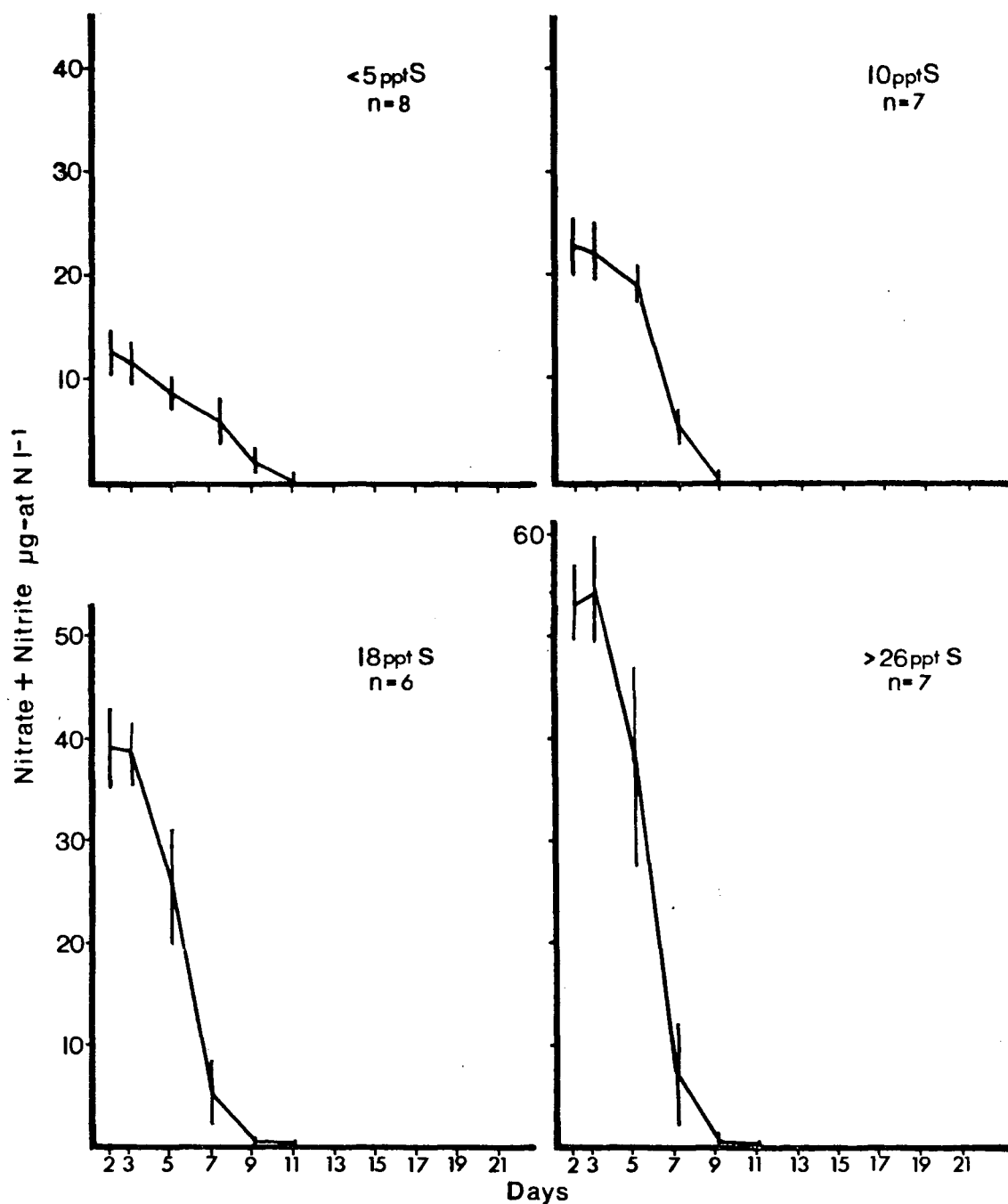
Figure 6 - The pattern of nitrate plus nitrite
concentrations; data collected at different seasons (winter,
spring, fall). Values on day 2 represent natural concentrations
plus additions.
Bars indicate mean ± 1 S.E.; n=number of experiments.
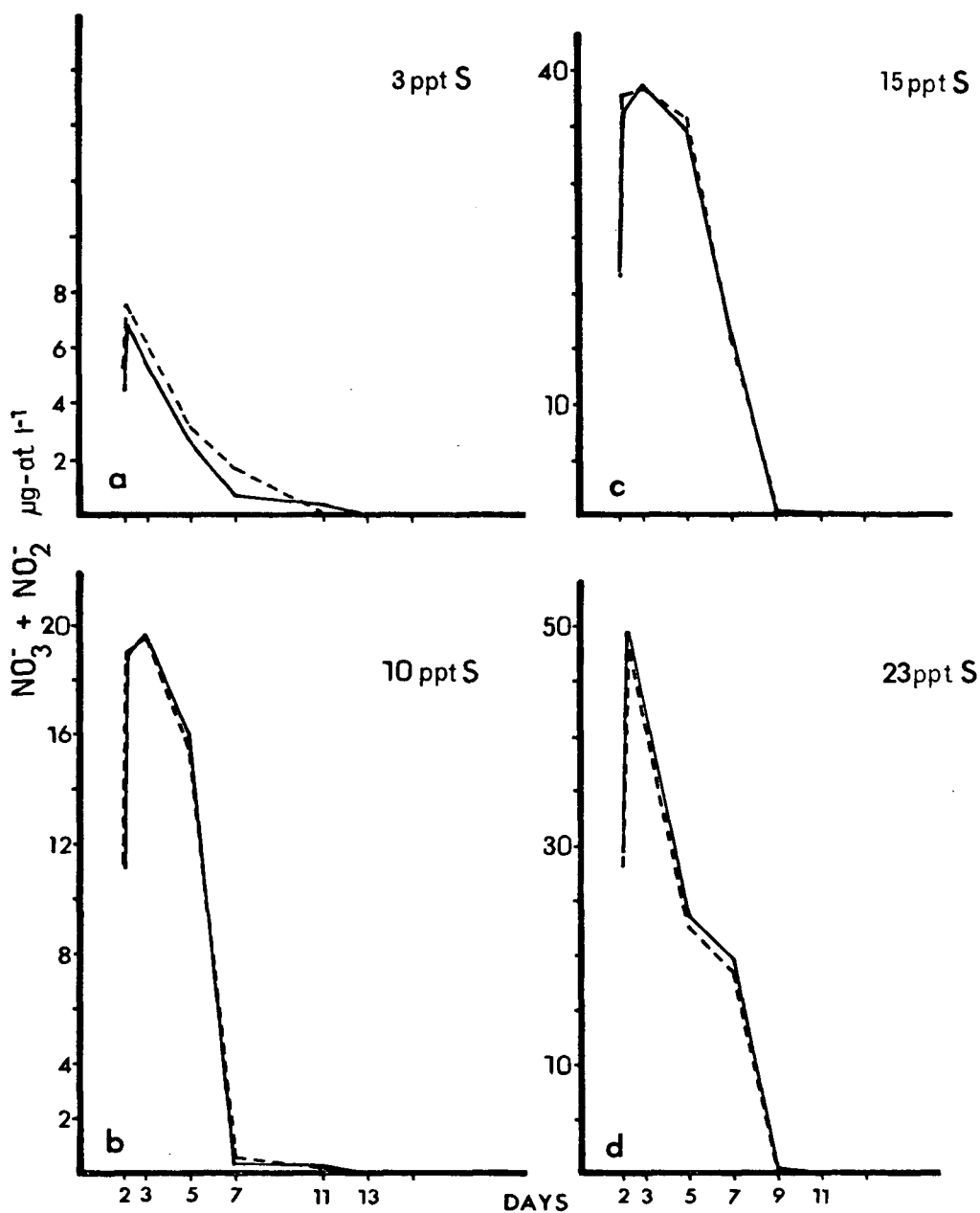
Figure 7 - Nitrate and nitrite concentrations in replication experiments: dashed and solid lines in (a) and (b) represent identical treatment; in (c) and (d), water of one microcosm (dashed) was filtered through a 116 μm net.
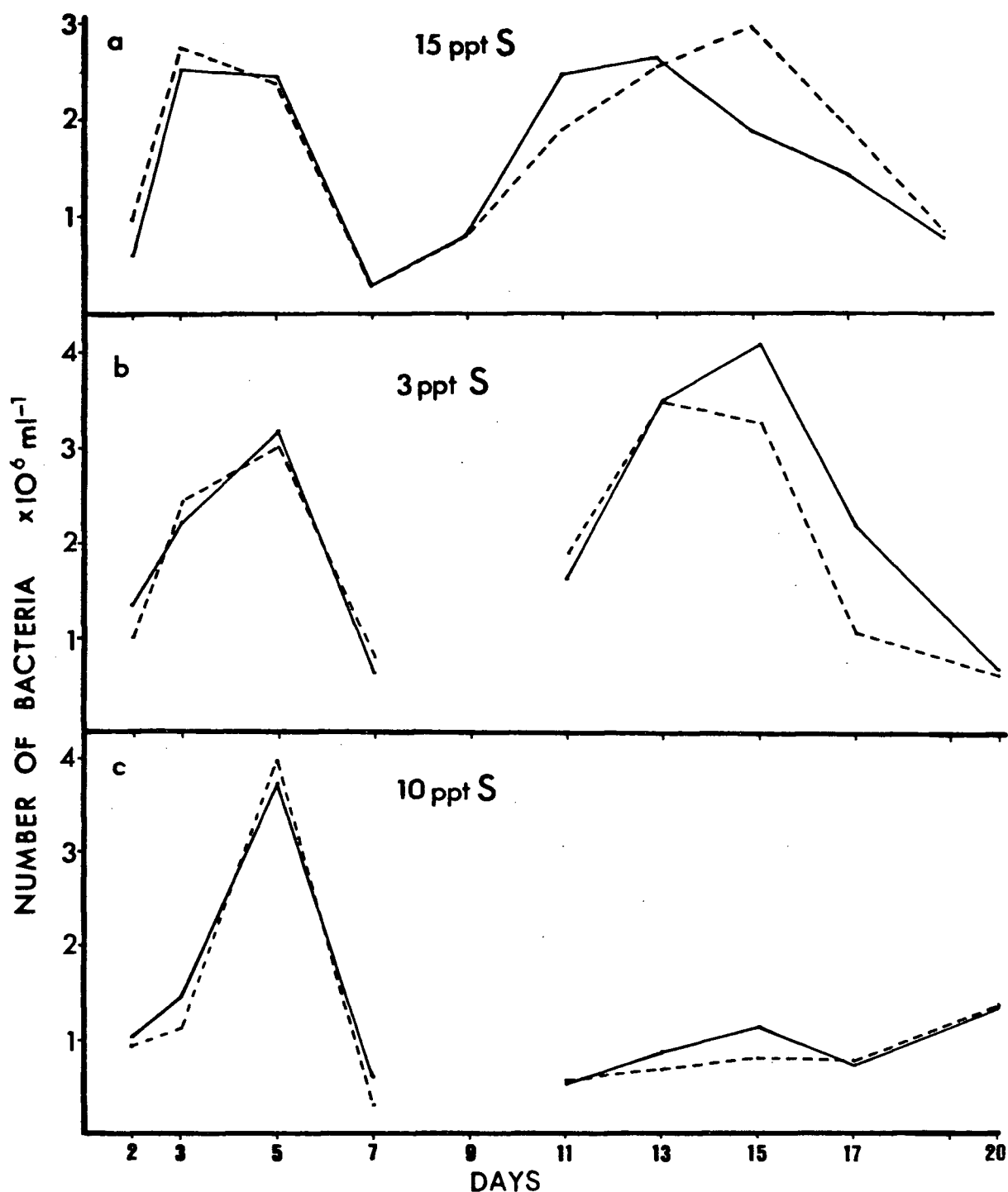
Figure 8 - Bacterial numbers in replication experiments:
dashed and solid lines in (b) and (c) represent identical
treatment, day 9 no samples; in (a), water of one microcosm
(dashed) was filtered through a 116 μm net.
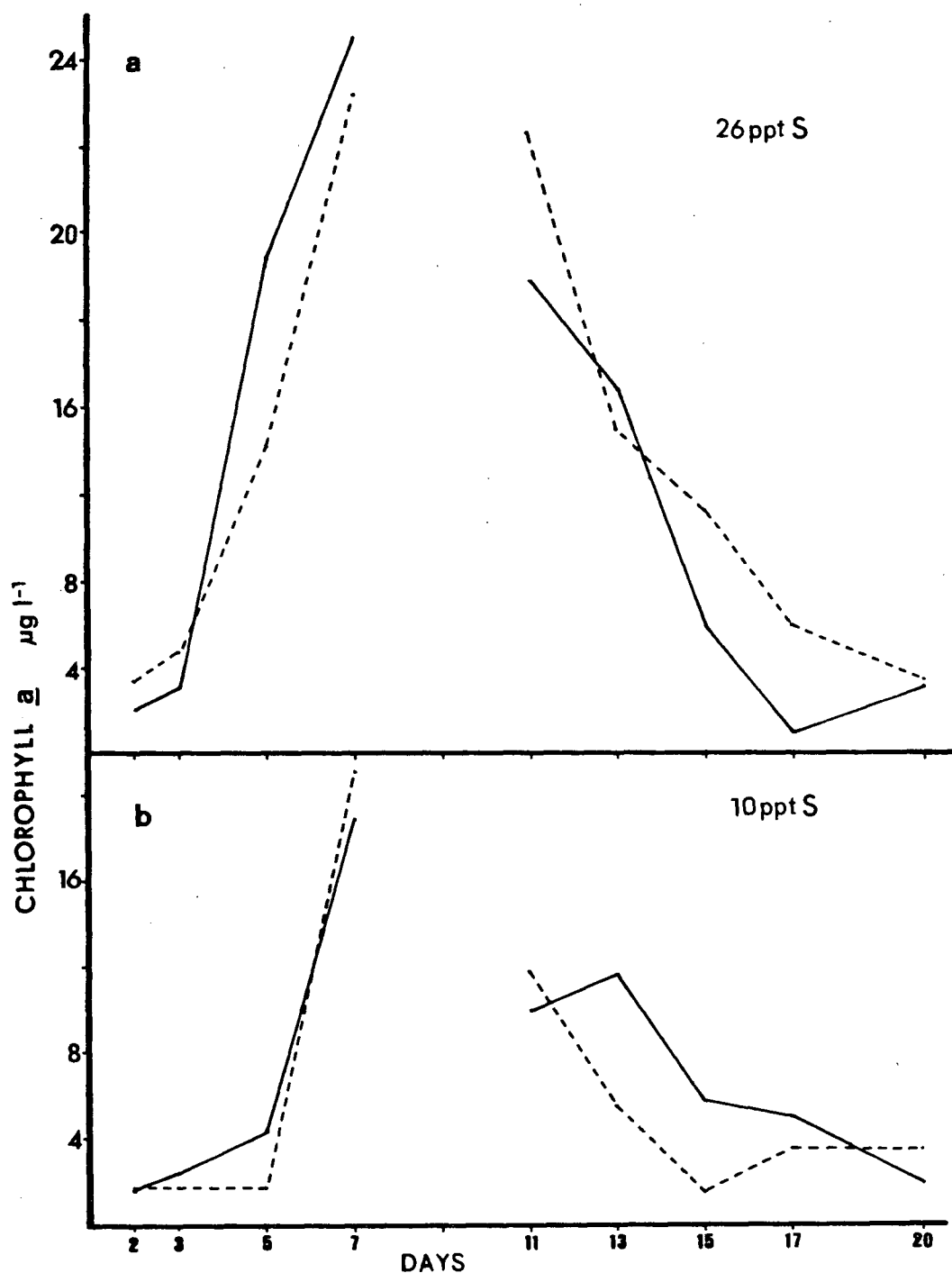
Figure 9 - Chlorophyll a concentrations in replication experiments: dashed and solid lines represent in (a) and (b) identical treatment; in (a), both microcosms with standard nutrient addition plus 5 mg l⁻¹ glucose; day 9 no samples.

was 15% (Venrick 1978). Variability was greatest in the chlorophyll a concentrations. The precision of the analysis was $\pm$ 1.51 μg l^{-1} for chlorophyll a values ranging from 0 - 10 μg l^{-1}. Since chlorophyll a concentrations at the height of the phytoplankton bloom exceeded this range up to seven-fold, dilution of the acetone extracts was necessary. Therefore, the precision was considerably lower in these samples.

4. EFFECTS OF SALINITY

The laboratory model simulates the estuarine ecosystem with respect to a salinity range. Therefore, this environmental factor is discussed separately from all the others which influence the microplankton ecology. Several properties are closely associated with salinity, among them nutrients, bacterial activity and phytoplankton species composition.

In the Fraser River estuary, nitrogen in the surface layer is supplied from entrainment of sea water (Tully & Dodimead 1957; Parsons et al. 1980) rather than by import from the river (Stockner et al. 1979). Nitrate data of the source waters (Fraser River and Burrard Inlet) confirmed the entrainment mechanism. Values in waters of 20 m depth always exceeded those found in the river surface waters.

Silicate is higher in the fresh water than in the sea water and substantial increase in concentrations has been observed during the beginning of the annual freshet (Parsons 1979). Another nutrient that appears to be higher in the Fraser River is vitamin B_{12} (Cattell 1973). Concentrations of organic carbon are closely associated with the decomposition of phytoplankton, but appreciable quantities are land-derived via runoff (Stephens et al. 1967). The availability of micronutrients (e.g. trace metals) is greatly influenced by changes in salinity (Cross & Sunda 1977). While nitrogen and consequently phytoplankton biomass increase with higher salinities, organic carbon and silicate show a reverse relationship.

According to Seki et al. (1969) the metabolism of bacteria is affected by salinity changes, and because of their slow growth rates in the natural environment due to low levels of organic substrate, bacteria need some time to adapt to a new salinity regime (Takahashi & Norton 1977). Valdes (1980) has shown in transplant experiments, that the majority of freshwater bacteria is killed when placed in marine water, but their marine counterparts remain viable in Fraser River water. The same author suggests that heterotrophic activity of the latter is even stimulated within a certain salinity range.

In microcosm experiments, higher relative heterotrophic activity was found at 10 ppt and 18 ppt salinity (Table 1), in those containers where high and low salinity source waters were mixed. It appears that the mixture provided better growth conditions for one or several bacterial strains of either natural population.

Table 1 - Relative heterotrophic activities at different salinities in duplicate laboratory experiments. Rates are based on uptake of ^{14}C-glucose and expressed as μg glucose h^{-1} per 10^9 bacteria.

≤ 5 ppt	10 ppt	18 ppt	≥ 26 ppt
8.56-17.63	57.35-91.33	51.82-65.98	27.68-42.85
($\bar{x}$13.10)	($\bar{x}$74.34)	($\bar{x}$58.90)	($\bar{x}$35.26)

In the microcosm blooms Skeletonema costatum and

Thalassiosira spp. were consistently dominant, as they are in the annual spring phytoplankton bloom in the Strait of Georgia. The dominant diatoms showed a distinct pattern under the different salinity regimes as shown in Figure 10. At ≤ 5 ppt, Thalassiosira spp. were dominant, while at 10 ppt, S. costatum always comprised more than 90% of the bloom. At ≥ 18 ppt, the relative abundances of both remained constant with S. costatum being dominant.

The increased abundance of S. costatum at 10 ppt, at relatively low but not the lowest salinity, has a correlation in a sediment assemblage analysis in Howe Sound, B.C. (Roelofs in press). Howe Sound is a high runoff, estuarine inlet in which the Squamish River at the head of the inlet markedly lowers the salinity of the surface waters. Mean monthly discharge of the Squamish River was greater in 1974 (i.e., lower salinity in the surface waters), when S. costatum was the dominant both in the phytoplankton (Stockner et al. 1977) and in all 24 sediment assemblages, than in 1973, when Thalassiosira spp. were dominant in the phytoplankton. Salinity values (0-20m) in both 1973 and 1974 did not fall below 10 ppt except at the mouth of the Squamish River (Stockner et al. 1977). Although a dominance of Thalassiosira spp. was never observed in the laboratory microcosms with ≥ 10 ppt salinity due to the weed-like growth of S. costatum in cultures, the results of the experiments might explain the dominance of S. costatum in an unusual year, such as 1974, when surface water salinity was markedly lower.

Although salinity is not the only controlling factor of the
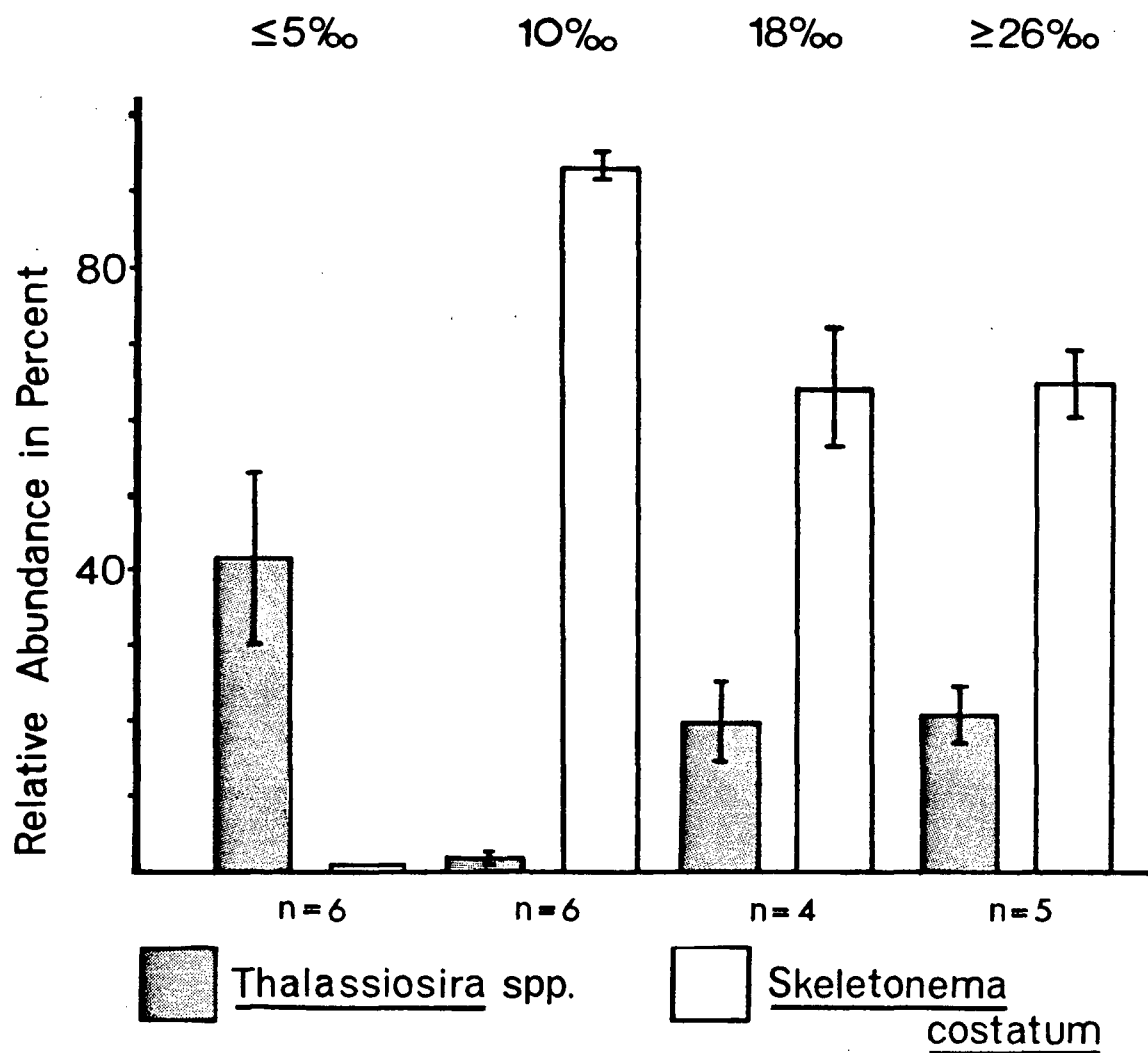
Figure 10 - Relative abundance of diatom species in
microcosm experiments at given salinities. Data were collected
at different seasons. Bars represent ± 1 S.E. of mean;
n=number of experiments.

abundance of S. costatum, these results demonstrate a consistent relationship in the microcosm and in the field studies (Roloefs & Spies unpubl. MS). Skeletonema spp. seem to thrive under brackish water conditions as noticed in Kiel Fjord, West Germany (Lenz 1977). The main reason might be the absence of competition in an extreme environment where fresh and salt water mix. A minimum of species abundance is reached at about 6 ppt salinity (Remane & Schlieper 1971).

The effect of salinity on phytoplankton species requires further study. Morphological changes in valve configuration have been observed in S. subsalsum (Paasche et al. 1975). The cell shapes of estuarine S. costatum are very variable, but it is not known whether there is a correlation with changes in salinity.

At ≤ 5 ppt, freshwater algae contributed considerably to the total biomass in the microcosms, but their numbers decreased rapidly in higher salinities. The abundance of microzooplankton was always greater in the river water than in the deep saline water. One explanation might be the occurrence of both coloured and colourless microflagellates in the surface water of the Fraser River, while at 20 m depth in Burrard Inlet photosynthetic flagellates might be light-limited and less abundant. Entrainment of deep saline water in the estuary mixes nutrients as well as colourless microflagellates into the surface layer, thus increasing the abundance of microzooplankton.

5. EFFECTS OF ABIOTIC FACTORS

5.1 Bottle Effects

Isolating natural waters in any kind of container will have effects on the growth of the biota, but very little is known of the causes and magnitude of these bottle effects. They are dependent on the volume to surface ratio of the container, decrease with increasing ratio, i.e. larger containers, and increase with increasing nutrient levels (Zobell 1943).

When working with natural microplankton, wall growth of bacteria cannot be prevented by any currently known method (personal communication, Dr H.W. Jannasch, Marine Biological Laboratory, Woods Hole). Marshall et al. (1971) have discussed bacterial attachment to container walls. Measurements by DiSalvo (1973) show that rapid irreversible attachment can occur within 1 - 2 minutes. Also, increased concentrations of dissolved organics, such as glucose in the microcosms, stimulate production of bacterial substances which mediate adhesion (Corpe 1974). Succession in colonization leads from bacteria to protozoa and diatoms. Harte et al. (1980) have successfully reduced visible surface growth by pouring the contents of their containers into clean ones on a weekly basis.

Because of the short duration of the experiments concerned with in this thesis, wall growth was not taken into consideration. With the decline of the phytoplankton bloom visible surface growth occurred in all containers. At this time initial glucose and nitrogen concentrations were exhausted and

wall growth was maintained by recycled nutrients.

Containment of natural water isolates the enclosed organisms, and, depending on the volume, even a short incubation can change the taxonomic composition and abundance of microplankton. Container volume, incubation time and nutrient regime may affect the course of changes within contained populations. Diatoms are generally more successful than flagellates (Smayda 1957; Venrick et al. 1977), a trend also observed in the microcosm experiments. Some species might get physically damaged during sampling procedure and mixing in the laboratory.

A reduction in species diversity can be explained on a theoretical level with the hypothesis of "contemporaneous disequilibrium" (Hutchinson 1961; Richerson et al. 1970). It states that a large number of species can coexist in a homogeneous environment consisting of many water parcels, if these parcels are prevented from coming into equilibrium due to a continuous input of perturbations. Thus, isolation from natural environmental fluctuations in containers should move populations towards equilibrium conditions and reduce species diversity (Venrick et al. 1977).

5.2 Constant Physical Factors

Constant physical factors in the laboratory reduce variability and improve replication. This is an advantage for statistical analysis, but makes any model less realistic because the natural environment is variable.

Artificial light has a different spectrum than sunlight and

a constant Light:Dark cycle eliminates seasonal changes in daylength, which is less important when only one event of a yearly cycle is studied. In the laboratory microcosms irradiances were relatively high, favouring diatoms and smaller size classes of cells (Chan 1978). Irradiances were assumed to be saturating even at the peak of the phytoplankton bloom. The initial drop in chlorophyll a concentrations may be caused by adaptation to higher light in the laboratory (Cosper 1982) and by sedimentation of large diatom species, in spite of mixing.

At all seasons water of the Fraser River was more turbid than the high salinity deep water. Sedimentation occurred despite constant stirring in low salinity microcosms. Large particles were removed by filtration of source waters through a $116-\mu m$-mesh Nitex screen without influencing bacteria numbers, nitrogen and chlorophyll a concentrations (see above Figures 7 and 8). With the decline of the phytoplankton bloom, particulate matter settled in all containers. Similar observations were made during shipboard tank experiments ($1 \ m^3$ volume) by Smetacek et al. (1980). According to these authors, settling of an increasing percentage of particulate matter was obviously caused by nitrogen deficiency. The sediment material had consistently higher C/N and C/Chl ratios when compared to the suspended matter, indicating the dependence of sinking rate on composition of particles.

The importance of mixing within enclosed water columns was observed in Controlled Ecosystem Pollution Experiments (CEPEX) at Saanich Inlet, B.C.. Unmixed containers consistently showed

rapid sedimentation of large-celled diatoms and their replacement by small flagellates (Thomas & Seibert 1977). Only brief daily stirring maintained larger phytoplankton cells; the N:P ratios were lower and more similar to the 'Redfield' ratio than in unstirred containers (Eppley et al. 1978). Mixing seemed to favour the growth of diatoms and increased chlorophyll a values (Oviatt 1981).

The limited air exchange in the microcosms was overcome by gentle air-bubbling, which ensured aerobic conditions. The constant temperature of 12°C was about the mean value which plankton experiences during spring in the Fraser River estuary (Benedict et al. 1973). Keeping physical factors constant facilitated the study of salinity effects in the estuarine laboratory model.

5.3 Nutrient Addition

The initial nutrient addition ensured that despite varying concentrations of macro-nutrients during the year, a basic supply of nitrogen, phosphate, silicate and organic substrate was available for heterotrophic and autotrophic growth. When background levels were high, as in winter and spring, they were boosted; at other seasons development of the standard pattern was ensured. Experiments without initial nutrient addition showed the same basic pattern but resulted in much lower biomass of bacteria as well as phytoplankton. An example is shown in Figure 11.

The height of the initial bacterial bloom was reduced by half without glucose being added. The residual heterotrophic
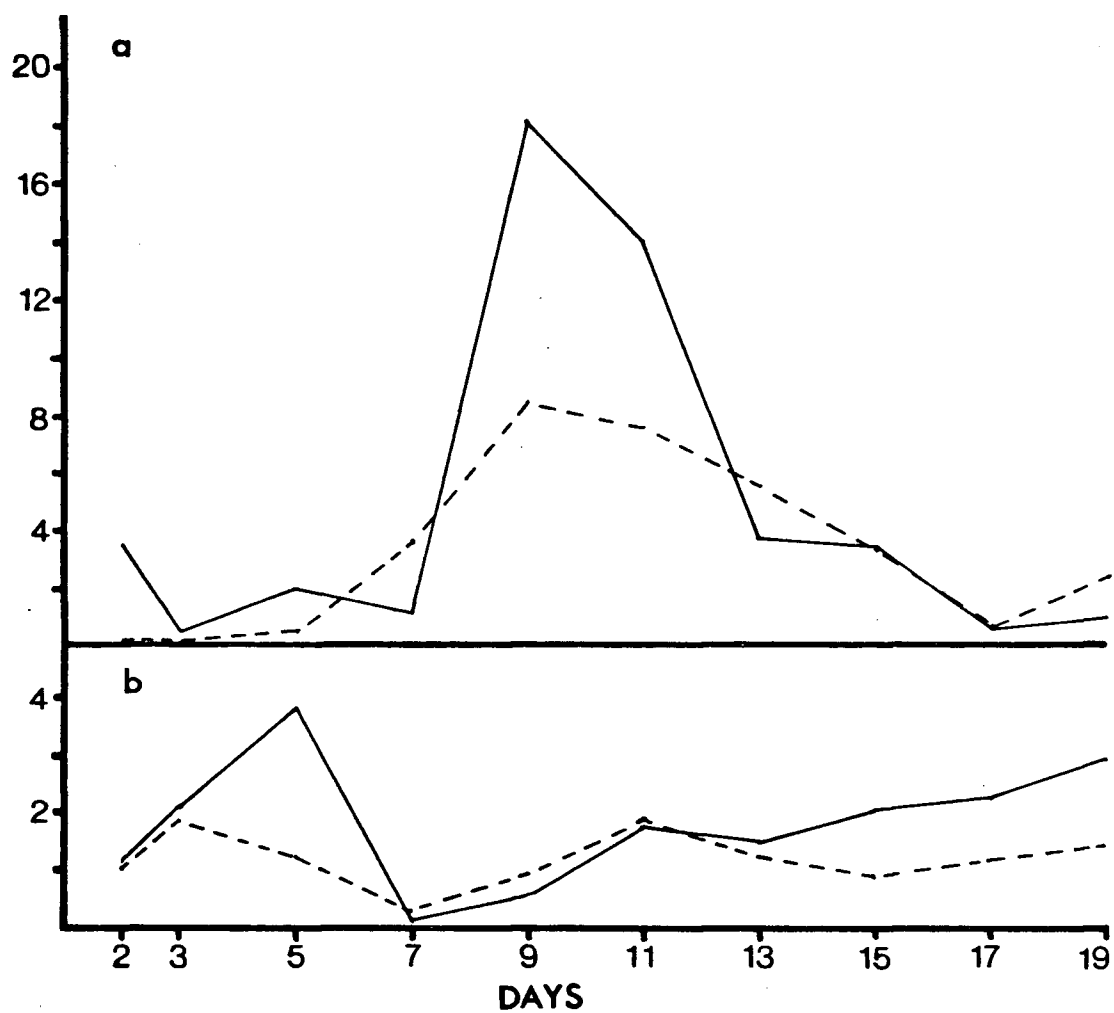
Figure 11 - Microcosm experiments with nutrient
addition=control (solid lines) and without addition (dashed
lines) at 8 ppt salinity; a - chlorophyll a concentrations
(μg l^{-1}), b - bacterial numbers (x10^6 ml^{-1}).

bloom was due to an increase in bacterial activity caused by the change in the environment, i.e. containment. In these microcosms, bacteria utilized the organic substrate present in the source waters. The glucose addition did not influence the second heterotrophic bloom which followed the decay of the phytoplankton, because the added amount was already exhausted in the first bloom.

EDTA+iron was necessary to counteract bottle effects. Iron is known to adhere to the wall of glass vessels and to precipitate in stored water, at which point it is slowly or no longer available to the organisms at all (Lewin & Chen 1971). The artificial chelator keeps iron in solution, so that algal growth is not limited by the lack of it. The addition of EDTA+iron was an important feature in the simulation of conditions preceding those of a natural diatom spring bloom.

6. INTERACTIONS OF THE BIOTA

6.1 Dark-experiments

To study heterotrophic growth without interference by autotrophs, microcosms were kept in the dark (black garbage bags). The initial addition of 1 mg l^{-1} glucose was increased to 5 mg l^{-1}, and in two microcosms even further to 5 mg l^{-1} on three consecutive days in order to enhance bacterial growth. Parallel to the dark-experiments, four microcosms with standard treatment were monitored. Beside the usual sampling intervals, samples were taken every 12 h between days 3 and 7. Concentrations of total dissolved monosaccharides, nitrate and nitrite, as well as ammonium were analysed. The absolute values of the latter might be affected by freezing and storage, as well as technical problems in the analysis of low salinity samples (Degobbis 1973; Eaton & Grant 1979). Therefore, the emphasis is on the general pattern, rather than absolute concentrations. The methods of analysis are described in Chapter II.

In the dark microcosms chlorophyll $\underline{a}$ concentrations never exceeded 5 μg l^{-1}. Initial total monosaccharides were 299 μg l^{-1} in the river water and 156 μg l^{-1} in the high salinity water. After addition of nutrients including 5 mg l^{-1} glucose, bacteria responded during the next 36 h with a doubling of numbers at $\leq$ 5 ppt, a five-fold increase at 10 ppt, six-fold at 18 ppt and nine-fold at $\geq$ 26 ppt salinity. In the lowest salinity, nitrogen was limiting bacterial growth (see Figure 21). By day 4 ammonium concentrations at $\leq$ 5 ppt and

10 ppt salinity were below detection. Nitrate and nitrite were exhausted by day 5.5 at $\leq$ 5 ppt but at 10 ppt concentrations of 2 - 3 μg-at l^{-1} were measured until the end of the experiment, while in the range of 10 to $\geq$ 26 ppt salinities, glucose became limiting by day 3.5 to 4.5.

The experimental set-up favoured aerobic bacteria which were able to utilize nitrogen for protein synthesis and carbon as substrate and energy source. The uniform morphological cell shapes during the first heterotrophic bloom indicated that only one or two species could respond to the sudden increase in substrate concentration and were able to outcompete all the others present in natural waters. This phenomenon is not uncommon in nature. The autochthonous or steady-state population consists of slowly growing cells, which are well adapted to the prevailing environmental conditions. A sudden change in these conditions, e.g. substrate influx, gives rise to an exponentially growing population (Brock 1971).

In the microcosms with 5 mg l^{-1} glucose addition on three consecutive days nitrogen seemed to be limiting before organic substrates at 10 ppt or became limiting at approx. the same time in the $\geq$ 26 ppt salinity container. With a total addition of 15 mg l^{-1} glucose the maximum increase in bacterial numbers was six-fold at 10 ppt, compared to five-fold after one addition, but increased from nine- to 23-fold at $\geq$ 26 ppt salinity in the presence of higher nitrate and nitrite concentrations. Figure 12 shows a composite picture of bacterial numbers, nitrogen and monosaccharide concentrations at 10 ppt salinity.
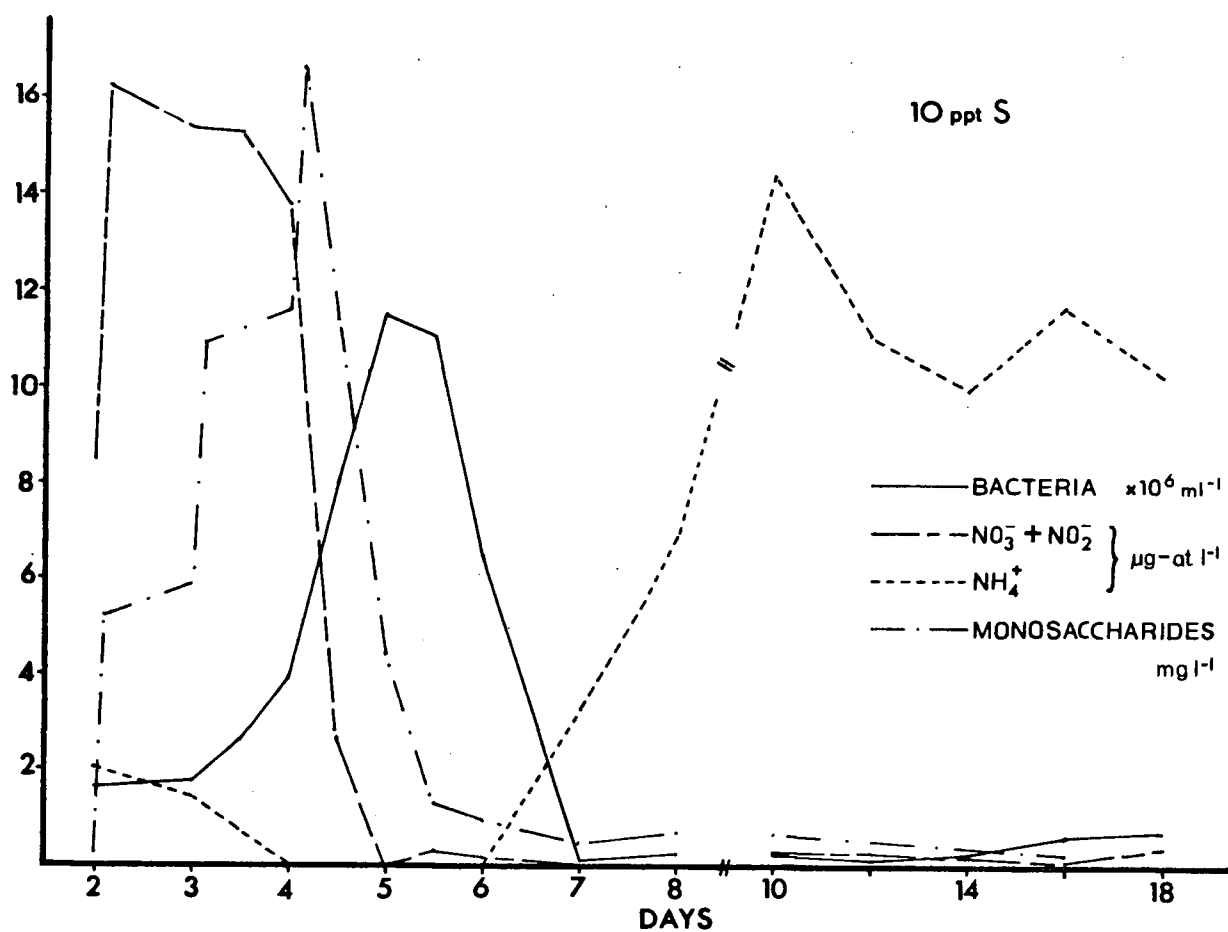
Figure 12 - Bacterial numbers, monosaccharide and nitrogen
concentrations in Dark-experiments at 10 ppt salinity; 5 mg l^{-1}
glucose was added on days 2, 3, and 4.

Microflagellate numbers peaked together with the bacterial bloom (cf. Figure 15). The rapid increase in ammonium beyond day 6 was due to excretion by microzooplankton and exhaustion of organic substrate below threshold levels and/or limitation by other essential nutrients. When bacterial numbers dropped, microflagellates decreased with approx. 1 - 2 days delay. Together with nutrient exhaustion, grazing might have prevented bacterial numbers from increasing higher than 11.5×10^6 ml^{-1} and contributed to their decline. Bacterial biomass remained low for another 7 days and increased only slightly towards the end of the experiment.

Heterotrophic bacteria are dependent on dissolved organic compounds for growth. Only a small fraction of the DOC in the natural environment has been identified (Williams 1975). In the Fraser River estuary values range from 1 - 6 mg C l^{-1} for most of the year (Albright 1977). Carbohydrates may account for 8 - 24% of the total DOC (Sieburth 1979). In nature (open sea) minimum threshold values for uptake of monosaccharides are approx. 50 μg C l^{-1}, which are much lower than critical values observed in laboratory cultures with pure bacteria (Jannasch 1970). This agrees with findings in the dark microcosms where threshold values for uptake were approx. 200 μg C l^{-1} monosaccharide (see Figure 12).

The utilization of soluble organic components by microorganisms may contribute a significant fraction to total production (Pomeroy 1974). Bacteria can rapidly assimilate the more labile portion (Azam & Hodson 1977). By following the

development of natural phytoplankton and dissolved carbohydrates in plastic tanks, Brockmann et al. (1979) found a significant positive correlation between glucose and diatoms, as well as between dissolved carbohydrates and phytoplankton biomass. During a natural bloom in the North Sea, glucose comprised more than 60% of the combined carbohydrate fraction (Ittekkot et al. 1981). Efficient heterotrophic utilization reduced substrate levels to ambient concentrations within a short time; the decrease was found to be in the same range as bacterial uptake (Brockmann et al. 1979).

Heterotrophic microflagellates in the size range 3 to 10 μm are effective bacteriovores (Haas & Webb 1979; Fenchel 1982b). Their normal nutritional mode is to ingest bacteria rather than dissolved organic matter or a combination thereof at least in the natural environment, where ambient substrate concentrations are low (Haas & Webb 1979). When exposed to unusually high concentrations, protozoa may be able to take up organic substrates directly (Droop 1974). In the Dark-microcosms direct uptake of glucose by microflagellates seemed to occur in addition to grazing of bacteria. No lag phase between a bacterial bloom and peak numbers of microflagellates is apparent (Figure 15), contrary to observations in field studies (Sorokin 1977; Fenchel 1982b).

6.2 Autotrophs And Heterotrophs

Some interactions between heterotrophs and autotrophs were seen in the microcosm experiments. The response of bacteria to allochthonous organic substrate (glucose) is shown in Figure 11 and in the Dark-experiments. Increased levels of dissolved monosaccharides (up to 685 μg MCHO-C l^{-1}) were observed during the decline of the simulated phytoplankton bloom as well as the coupling of primary production with increases in bacterial biomass (see Figures 3 and 13). Oxygen depletion was counteracted by air-bubbling. Photosynthesis caused pH changes of up to one unit during the phytoplankton bloom (Appendix 1). Competition between bacteria and algae could be observed and was studied in numerical simulations and perturbation experiments (Chapter IV and V).

Phytoplankton in the natural environment are always associated with bacteria, and there is evidence for mutual interaction. A positive correlation between the primary production of phytoplankton and bacterial abundance or microbial activity has been found (Es & Meyer-Reil 1982). The relationship can be masked by allochthonous inputs of organic matter. While the excretion of organic compounds by healthy phytoplankton is still debated (Hellebust 1974; Sharp 1977; Mague et al. 1980), senescent and dying algae are a major source of organic matter, as is grazing activity (sloppy feeding) by macrozooplankton (Eppley et al. 1981; Pfaffenhöfer et al. 1982). These compounds can readily be taken up by bacteria (Larsson & Hagström 1979; Wolter 1982), and may

determine the seasonal pattern of bacterial abundance (cf. Chapter VI). Microbial activity might better be correlated with the concentration of phaeopigments than with primary production (Fuhrman et al. 1980). In microcosm experiments, where chlorophyll a and phaeophytin a was measured, the increase in bacterial numbers to the second heterotrophic bloom seemed to follow the appearance of phaeopigments, rather than the height of the phytoplankton bloom (Figure 13).

Bacteria may inhibit algal growth by modifying the environment, e.g. depleting oxygen or changing the pH. The latter conditions are unimportant in the Fraser River estuary (Drinnan & Clark 1980). There are reports of bacteria and viruses that may cause algal cells to lyse (Cole 1982). Bacteria produce soluble substances which can inhibit or stimulate algal growth and may be of importance in the seasonal succession of phytoplankton species (Cole 1982). In the presence of high organic load bacteria can successfully compete with autotrophs for a limiting nutrient (see Chapter V).

In their function as decomposers, bacteria remineralize organic matter and replenish nutrients for phytoplankton growth. Bacteria can enhance algal growth by release of vitamins, e.g. vitamin B_{12} (Haines & Guillard 1974) and the poor growth of axenic algal cultures indicates that bacteria may have complex stimulatory effects.

Phytoplankton not only compete with bacteria for limiting nutrients, they may release antimicrobial substances (Sieburth 1968). In laboratory experiments S. costatum inhibited some
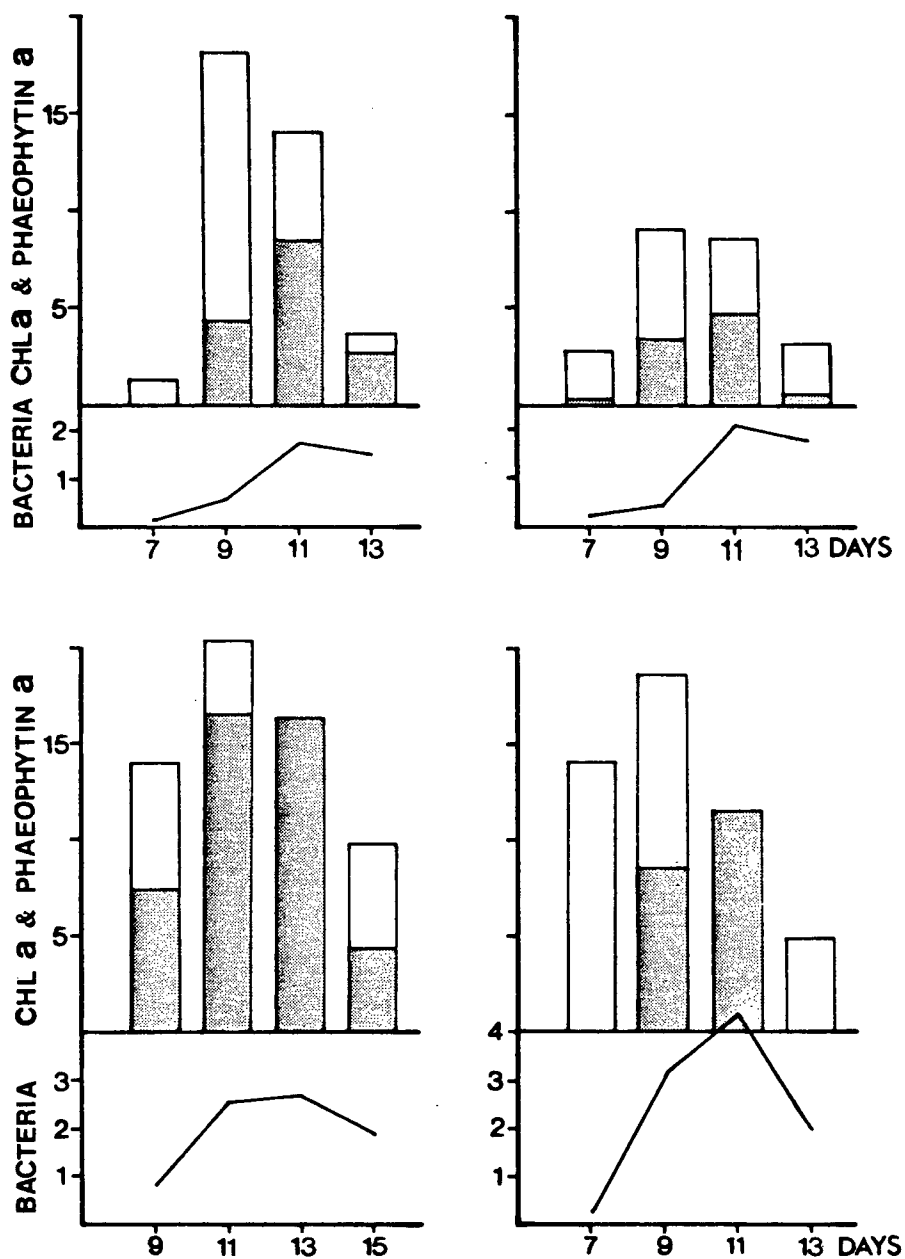
Figure 13 - Histograms show concentrations (μg l^{-1}) of chlorophyll a and phaeophytin a (shaded areas) during the phytoplankton bloom in four microcosm experiments. Solid line indicates bacterial numbers (x10^6 ml^{-1}).

strains of bacteria with the inhibitory action being strongest during the exponential growth phase (Kogure et al. 1979). In periods of intense photosynthesis the upward shift in pH may influence the bacterial activity and species composition (Cole 1982).

7. SUMMARY

The pattern of the simulated phytoplankton bloom was described in terms of changes in bacterial numbers, chlorophyll a and nitrogen concentrations, and microzooplankton abundance. The initial nutrient addition was followed by a first heterotrophic bloom which was terminated by exhaustion of glucose and microflagellate grazing. The heterotrophic bloom was succeeded by an increase in phytoplankton biomass. Exhaustion of nutrients terminated autotrophic growth. The decay of cells gave rise to a second heterotrophic bloom. Microzooplankton followed the changes in bacterial biomass.

Despite the seasonal variability of the source waters the standard pattern was reproducible. This way, perturbations of the simulated phytoplankton bloom could be performed at different times of the year.

The salinity values in the experiment influenced the microplankton ecology. Heterotrophic activity was highest at 10 ppt and 18 ppt salinity. Skeletonema costatum and Thalassiosira spp. had consistently been dominant in the simulated phytoplankton bloom. The increased abundance of S. costatum at relatively lower salinities (10 ppt) had a correlation to field studies.

Abiotic factors, such as bottle-effects, constant laboratory conditions and the initial nutrient addition influenced the biota of the microcosms and biased the course of events. Total consequences of the multiple effects resulting

from enclosure of water in containers were not known.

Dark-experiments facilitated the study of bacteria, their growth kinetics and nutrient uptake. The impact and role of microflagellates was discussed. Autotrophs and heterotrophs relate to each other in various ways; only some of their mutual interactions could be studied under the given experimental conditions.

IV. NUMERICAL MODELING

1. INTRODUCTION

Numerical modeling is used to describe the events within the laboratory microcosms in terms of the important processes and organisms. The model should show how the various components could be interacting and what effect the change of variables and parameters has. The development of the model will be in two steps: first, simulation of the Dark-microcosms by describing bacterial and microflagellate growth and interactions; second, simulation of Light-microcosms by describing autotrophic and heterotrophic growth and interactions. By starting off with the less complicated task, insights and results gained in the development of the Dark-model can be incorporated into the simulation of the more complicated Light-model.

The models are partly based on independent studies of processes and interactions in the laboratory microcosms. Some parameters and constants are taken from a numerical simulation by Fenchel (1982b), which describes interactions between bacteria and microflagellates in a natural ecosystem. Other data will be estimated from existing literature or have to be guessed. Due to constant laboratory conditions, the models will have no abiotic input variables. Processes are described in differential equations and are incorporated into FORTRAN-programmes (cf. Appendix 2). All rates in the equations are

expressed in units of hours. According to the sampling schedule of the microcosm experiments, the simulation starts on day 2 (24 h) when initial samples were taken and additions were made. The models are run for a minimum period of at least 300 h to cover the experimental observations. Values are printed out every 12 h. All simulation is done on the UBC Amdahl computer.

Criteria for the success of the models will be the closeness in timing and magnitude of events when the simulation run is compared to the experimental results in the estuarine microcosms.

2. MODELING OF DARK-MICROCOSMS

2.1 Variables, Parameters And Constants

Variables in the Dark-microcosms were bacteria (X), microflagellates (Y) and glucose (G). Based on results in Dark-experiments, glucose was assumed to be the growth-limiting substrate in the 10 ppt to $\geq$ 26 ppt salinity microcosms, while at $\leq$ 5 ppt nitrogen was the limiting factor (see Figure 21). Only the $\geq$ 10 ppt salinities were considered for the numerical simulation. The processes were characterized by Michaelis-Menton type functions. The changes of bacterial numbers (X; 10^6 ml^{-1}), microflagellates (Y; 10^3 ml^{-1}) and glucose concentrations (G; mg l^{-1}) over time (t) were expressed as

$$\frac{dX}{dt} = \frac{RM \cdot G}{GK+G} \cdot X - \frac{UM \cdot X}{XK+X} \cdot Y$$

$$\frac{dY}{dt} = \frac{UM \cdot X}{XK+X} \cdot Y \cdot EY - D \cdot Y$$

$$\frac{dG}{dt} = - \frac{RM \cdot G}{GK+G} \cdot X \cdot \frac{Q}{EX}$$

RM = maximum growth rate of bacteria
GK = half-saturation constant for glucose (mg l^{-1})
UM = maximum consumption rate of bacteria
XK = half-saturation constant for bacteria (10^6 ml^{-1})
D = death rate of flagellates
EY = gross growth efficiency of flagellates per bacterium
Q = glucose quota per bacterium (mg per 10^9 bacteria)
EX = growth efficiency of bacteria

Initial numbers of X and Y were determined in the laboratory microcosms; the values changed with the different salinity regimes. In Dark-experiments RM was calculated from the period of exponential growth. Rates of 0.05 at 10 ppt and 0.06 at 18 ppt and $\geq$ 26 ppt salinity were found. G, at the beginning of the simulation, was 5 mg l^{-1} glucose, due to a constant addition. GK was set to be 0.3 mg l^{-1}; values for all bacteria are between 0.2 and 0.8 mg l^{-1} (personal communication, Dr J.J.R. Campbell, Dept. of Microbiology, UBC). It was assumed that flagellates had a maximum consumption rate (UM) of 60 bacteria h^{-1}, a gross growth efficiency (EY) of 3×10^{-3} flagellates per bacterium, and that the half-saturation constant (XK) was 5×10^6 bacteria ml^{-1}. All these values (UM, EY, and XK) were experimentally found by Fenchel (1982a). The density-independent death rate (D=0.045) of microflagellates was taken from Fenchel's (1982b) model. Data of bacterial growth efficiency on glucose range from 0.5 to 0.95 in the literature (e.g. Fenchel & Blackburn 1979; Larsson & Hagström 1979; Bölter 1981). For the present simulation 0.8 was selected as given in Fenchel & Blackburn (1979). Ferguson & Rublee (1976) provide data of the carbon content of average-size marine microbes in coastal areas. Based on their findings, a glucose cell quota of 0.05 mg per 10^9 bacteria was calculated.

2.2 Simulation And Comparison With Original Data

A simulation run with the original parameters (RM=0.06; GK=0.3; UM=0.06; XK=5.0; EY=3.0; D=0.045; Q=0.05; EX=0.8), an addition of 5 mg l^{-1} glucose and initial bacteria and flagellate numbers as found in the $\geq$ 26 ppt salinity microcosm, is shown in Figure 14. The simulation did not fit any of the experimentally determined patterns in the salinity range of 10 ppt to $\geq$ 26 ppt which are presented in Figure 15. The original parameter set led to a model of unstable prey/predator oscillations, where the abundance of bacteria was not limited by substrate concentrations, but by grazing.

According to experimental data (Figure 15), bacterial numbers peaked between days 4 (72 h) and 5.5 (108 h) with maximum numbers ranging from 8.83×10^6 ml^{-1} to 10.02×10^6 ml^{-1}. Glucose exhaustion coincided with the peak of the bacterial bloom; therefore growth was limited by lack of organic substrate rather than by grazing. Limitation by glucose was demonstrated in microcosms with additions of up to 15 mg l^{-1}.

The increase in microflagellates seemed to parallel bacterial numbers and a peak was reached at approx. the same time and up to 24 h later. Microflagellates were counted at larger time intervals than bacteria. Also, due to the difficulty of live enumeration, the estimates of absolute numbers showed large variations in replicate counts. The exact timing of the flagellate bloom is consequently uncertain.

Probable reasons for the failure of the original simulation run include the following. In Figure 14, it was assumed that
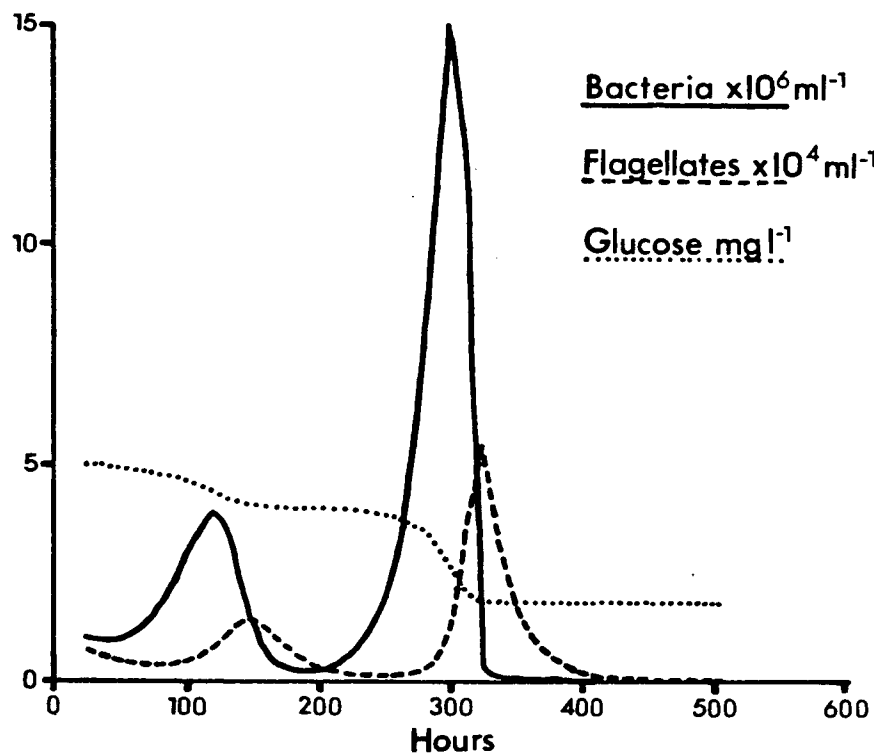
Figure 14 - Simulation run with original parameters
(≥ 26 ppt).   X=1.0   Y=7.0   G=5.0
RM=0.06   GK=0.3   UM=0.06   XK=5.0   EY=3.0   D=0.045   Q=0.05   EX=0.8
(see text, p. 57, for key to symbols)
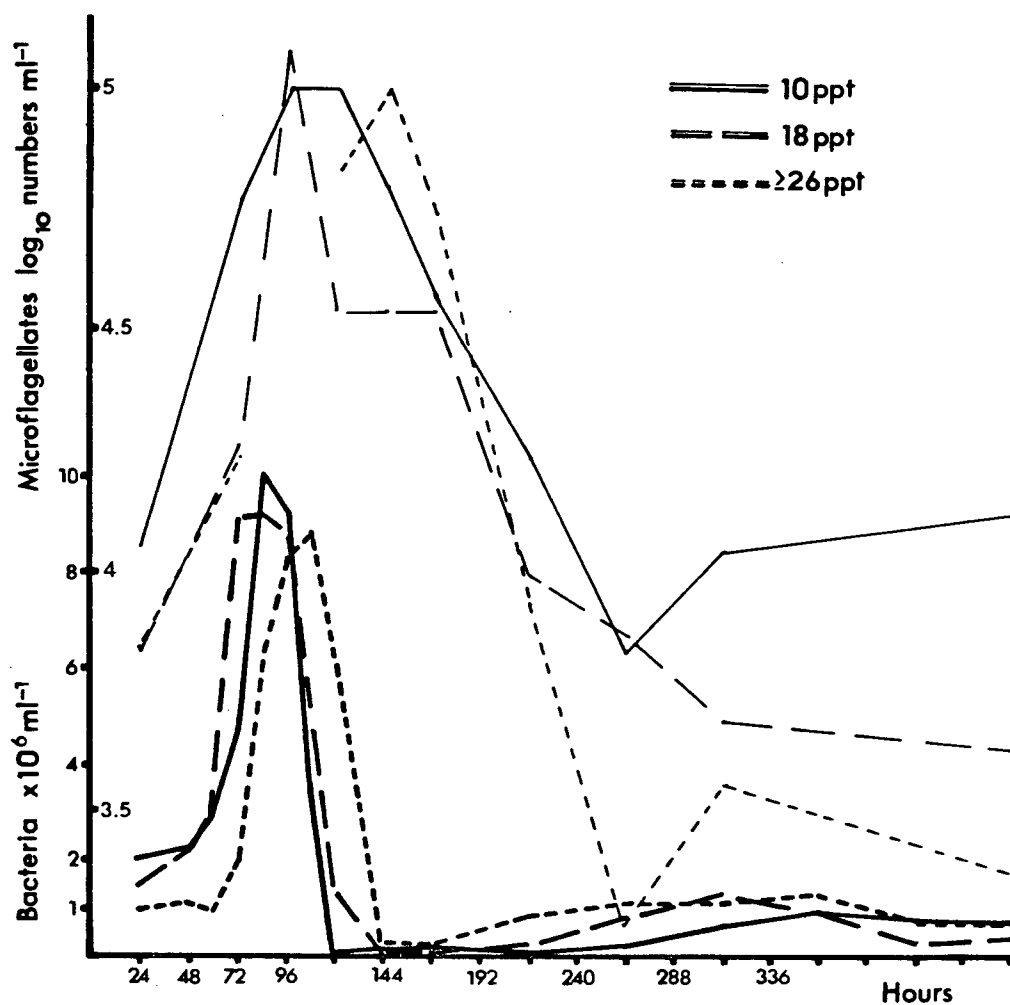
Figure 15 - Bacterial numbers (thick lines) and
microflagellates (thin lines) in Dark-experiments.

all of the initially present bacteria were growing by utilizing glucose. Activity measurements of marine bacteria indicate, however, that the majority of them are not active, but live in a state of dormancy (Wright 1978). In the eutrophic Kiel Bight (Western Baltic) the proportion of metabolically active microbes changes between 10 and 56% during the year (Hoppe 1978). Temperature and nutrient supply seem to be the main criteria for the bacterial activity state (Albright 1977; Hoppe 1978). An experimental temperature of 12°C and the fact that bacterial numbers were unchanged over 48 h (lag phase) is also consistent with the hypothesis that only a fraction of them was growing.

The calculated growth rate for bacteria was not corrected for grazing losses and therefore should be actually higher in the model.

The initial number of microflagellates was very likely lower. During enumeration colourless and coloured flagellates were not distinguished and probably not all were heterotrophs, i.e. bacteriovores. Considering the size of the flagellates in the Dark-microcosms, the majority of them was similar in volume to the smallest species studied by Fenchel (1982a).

The glucose cell quota calculation was based on average-size natural marine bacteria, which are growing in a substrate-limited environment. The simulation with the original parameters clearly showed that the bacterial glucose quota (Q) was too low, since the second bacterial bloom resulted in much higher numbers than observed and glucose was still not depleted. Enrichment of the growth medium with glucose resulted in bigger

bacteria (Mandelstam et al. 1982). A rough estimate of the
correct cell quota was calculated by dividing maximum observed
numbers, c. $10x10^6$ ml^{-1}, by 5 mg l^{-1} glucose taken up, which
gave a quota (Q) of 0.5 mg per 10^9 bacteria. In order to
account for grazing losses, the glucose quota (Q) had to be a
little lower than 0.5. If bacteria were bigger, fewer of them
had to be ingested in order to give an equivalent amount of
flagellate biomass, or, with the ingestion of one bacterium the
gross growth efficiency increased.

If there was indeed direct glucose uptake by
microflagellates as discussed in the previous chapter, it was
not included in the model run.

The variety of possibilities suggests that there might be
more than one set of parameters which mimics observations in the
Dark-microcosms. The aim was to find the one which seemed
biologically most reasonable. The change of one parameter very
likely would require adjustment of others to produce such a
result. Changes in the original parameters could be discussed
by listing them sequentially, or by starting with the final
result and describing the alterations which were needed to
achieve it. The latter approach was chosen in the subsequent
discussion in section 2.3.

2.3 <u>Simulation Of Different Salinity Microcosms</u>

In a series of simulation runs, initial conditions and grazing parameters were changed in order to increase total numbers in the first bacterial bloom and to convert all glucose into bacterial biomass. Figure 16 shows two of several possible models of the $\geq$ 26 ppt salinity Dark-microcosm. They differ in initial numbers of microflagellates (Y), maximum consumption rate of bacteria (UM) and proportionally adjusted gross growth efficiency (EY). In order to deplete the added 5 mg l^{-1} glucose, the cell quota (Q) was increased six-fold from 0.05 to 0.3, while bacterial growth efficiency (EX) and half-saturation constant (GK) for glucose remained unchanged with respect to the original simulation. The initial number of bacteria (X), which were growing at a rate of 0.06, was set at 20% of the observed value. Initial flagellate numbers (Y) were reduced with the death rate (D) unchanged. Flagellates were assumed to have a lower maximum consumption rate (UM) of 0.015 instead of 0.06 and to be less efficient grazers. The ratio of maximum consumption rate and half-saturation constant for bacteria (UM/XK) was decreased from 0.012 to 0.005, i.e. the flagellates cleared less volume per unit time. Due to the increase in individual bacterial biomass, the gross growth efficiency of flagellates (EY) was adjusted from 3.0 to 12.0 with respect to the decreased consumption rate (UM). Thus, the maximum carbon intake by flagellates was 1.5-fold higher. The flagellate bloom lagged 24 h behind the peak in bacterial numbers. Total flagellate numbers were acceptable, considering the variation in actual
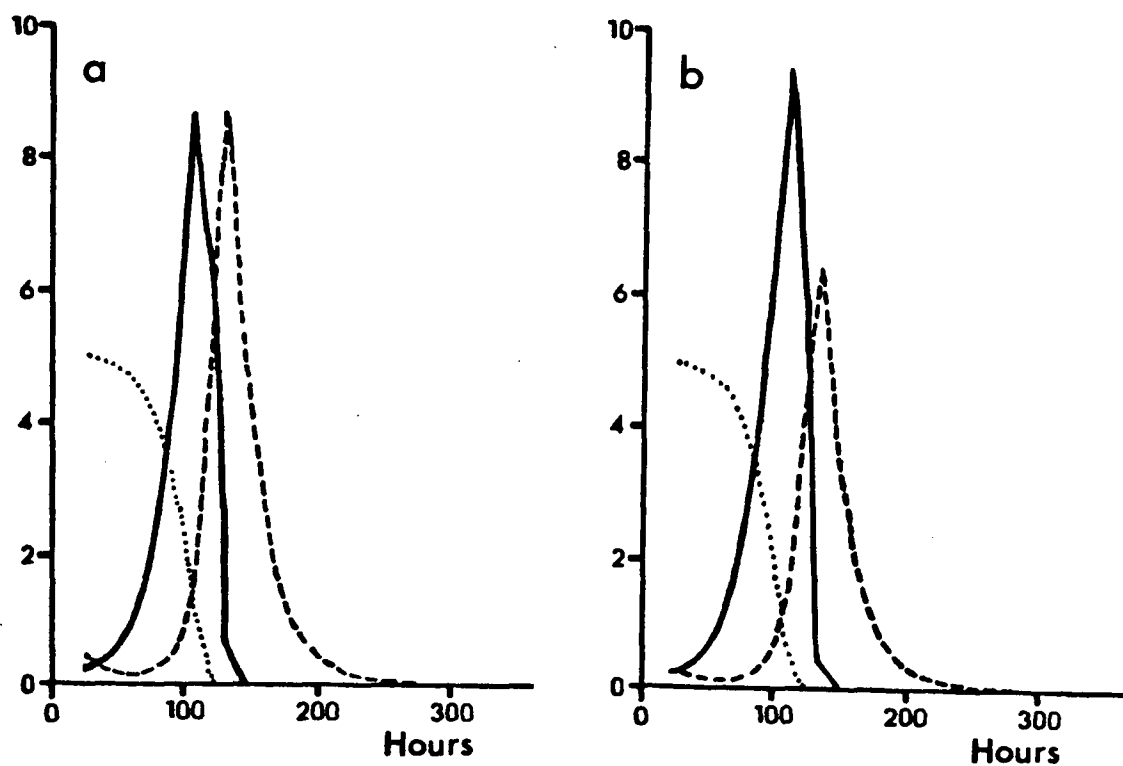
Bacteria x10⁶ml⁻¹

Flagellates x10⁴ml⁻¹

Glucose mg l⁻¹

Figure 16 - Two possible simulations of the ≥ 26 ppt
salinity Dark-microcosm. In both runs X=0.2; G=5.0; RM=0.06;
GK=0.3; XK=3.0; EX=0.8; Q=0.3; D=0.045.
a - Y=4.0; UM=0.015; EY=12.0.
b - Y=2.5; UM=0.020; EY=9.0.

counts. The possibility of direct glucose uptake could explain the higher numbers in the microcosm experiments.

Figure 17 shows simulation runs of the 10 ppt and 18 ppt salinity microcosms. At both salinities the bacterial bloom occurred earlier than at $\geq$ 26 ppt salinity and absolute numbers were higher. Several parameter changes were necessary in order to adjust the model. Activity measurements in the Fraser River estuary indicate that at intermediate salinities the proportion as well as the numbers of active bacteria increase (Valdes & Albright 1981). Initial bacterial numbers were indeed higher in the 10 ppt and 18 ppt salinity microcosms, therefore X had to be increased when the proportion of active bacteria was equal to that at $\geq$ 26 ppt salinity. Beside higher initial numbers, more bacteria (X) may actually grow in the microcosms. An increase of X to 0.6×10^6 ml^{-1} and further to 0.8×10^6 ml^{-1} was consequently justified. According to Valdes & Albright (1981) the mean bacterial volume in the estuary increases with decreasing salinity. To account for bigger bacteria, the glucose cell quota (Q) was set to 0.4. With an increasing amount of low salinity surface water, it was assumed that the proportion of heterotrophic microflagellates, which consume bacteria, decreased, while the proportion of photosynthetic ones became larger. Initial flagellate numbers (Y) were reduced to 0.1×10^4 ml^{-1} and 0.05×10^4 ml^{-1}.

Bacteria x10^6 ml^{-1}

Flagellates x10^4 ml^{-1}

Glucose mg l^{-1}
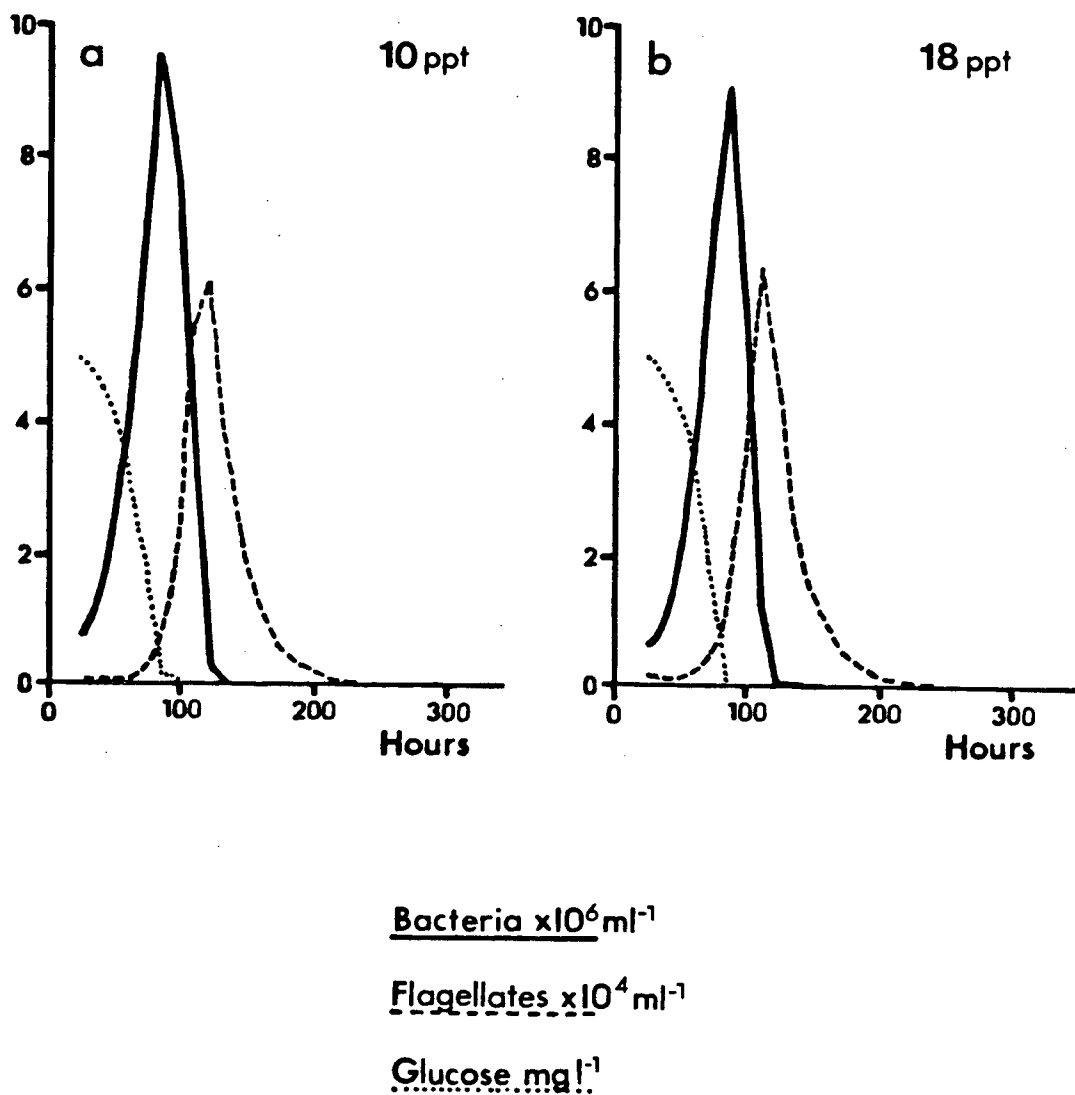
Figure 17 - Simulation runs of the 10 ppt and 18 ppt
salinity Dark-microcosms. In both runs G=5.0; GK=0.3; UM=0.015;
XK=3.0; EY=12.0; D=0.045; Q=0.4; EX=0.8.
a - X=0.8; Y=0.5; RM=0.05.
b - X=0.6; Y=1.0; RM=0.06.

2.4 Discussion Of Parameter Changes

According to experimental results, glucose exhaustion in the Dark-microcosms had to coincide with the bacterial bloom because glucose was identified as the growth limiting factor. With a constant initial substrate addition (G) and a fixed initial number of bacteria (X), the change in glucose concentrations was related to the growth rate (RM) of bacteria, the glucose cell quota (Q), the half-saturation constant for glucose (GK), and the growth efficiency (EX) of bacteria. A modest increase in the growth rate (RM) to account for grazing losses had little effect on both the timing of the bacterial bloom as well as absolute numbers and did not lead to glucose depletion. Changes in the half-saturation constant for glucose (GK) in the range of 0.2 to 0.8 mg l^{-1} did not affect bacterial dynamics significantly, because changes were effective only at low substrate concentrations. The greatest impact on glucose concentrations (G) was achieved by lowering the efficiency (EX) or increasing the cell quota (Q). But even with an efficiency (EX) as low as 0.5, glucose still did not limit bacterial growth. Only an increase in cell quota (Q), making bacteria bigger, caused substrate exhaustion together with the peak in bacterial numbers, which were, however, exceeding those found in the microcosms.

In order to simulate the Dark-microcosms with respect to absolute bacterial numbers and proper timing of the bloom, parameters concerning bacterial growth on glucose or those concerning grazing activity by flagellates could be altered.

Reasonable changes in growth rate (RM) were limited to a small increase in the value and therefore did not have much effect. Assuming that only a fraction of the microbial population was actually growing, i.e. by lowering the initial bacterial numbers (X), the timing of the bacterial bloom was adjusted as well as absolute numbers. When X was set at 5% of the observed value and the original glucose quota of 0.05 was not increased, absolute bacterial numbers exceeded those found experimentally more than four-fold and the bloom was too late. An increase in glucose quota (Q) to 0.3 resulted in lower bacterial numbers and by varying the initial value of bacteria (X), the timing of the bloom was changed; higher initial numbers (X) resulted in an earlier bloom, while lower ones delayed the bloom. The reduction of X in combination with a glucose quota (Q) of 0.3 without changes in grazing parameters resulted in too few flagellates.

In the original simulation, flagellates controlled bacterial numbers. With a lower grazing activity due to a smaller number of bacteria-ingesting flagellates (Y) or due to a lower maximum consumption rate (UM), bacterial numbers could be increased at the height of the bloom. A reduced maximum consumption rate (UM) without a change in the half-saturation constant for bacteria (XK) resulted in less efficient flagellate grazing, i.e. higher bacterial numbers and a delayed bloom. In addition, the time-lag between bacterial and microflagellate bloom became too long. Alterations of the half-saturation constant (XK) alone did not influence the timing of the

bacterial bloom significantly, but resulted in lower peak numbers. By decreasing the grazing activity without changing the gross growth efficiency (EY) of the flagellates, their total numbers were too low. The density independent death rate (D) of microflagellates was not altered at all, because there was no experimental value available.

By increasing the glucose cell quota (Q) six-fold, making bacteria bigger, and with no change in the maximum consumption rate (UM), the carbon uptake rate per flagellate would increase six-fold, resulting in far too large and too few flagellates. In order to simulate approx. flagellate numbers in the Dark-microcosms, fewer bacteria had to be ingested to give an equivalent amount of flagellate biomass. Consequently, the gross growth efficiency of flagellates (EY) had to be increased. With a glucose quota (Q) of 0.3 and a four-fold increase in EY, flagellates were 1.5 times as big in terms of glucose quota. Assuming a glucose quota (Q) of 0.3 and a bacterial efficiency (EX) of 0.8, an initial glucose concentration of 5 mg l^{-1} would yield a maximum number of bacteria of 13.3×10^6 ml^{-1}. Numbers observed in the Dark-microcosms were about 36% lower. In substrate-(glucose) controlled experiments, it appears that the glucose cell quota (Q) determines substrate limitation, and thus the timing of the bacterial bloom, while the gross growth efficiency (EY) determines the number of grazers and the height of the bloom.

In conclusion, the simulation of the events in the 10 ppt to ≥ 26 ppt salinity Dark-microcosms required changes of several

parameters of the original run. The glucose quota per bacterium had to be increased in order to convert all added glucose into bacterial biomass. Initial bacterial and flagellate numbers were lowered, because not all of the former were assumed to be active and growing, and probably not all of the latter were bacteriovores. The maximum consumption rate of bacteria had to be lowered and a decreased ratio of maximum consumption rate to the half-saturation constant made grazing less efficient. With flagellates grazing on bigger bacteria, the gross growth efficiency of the former had to be increased in order to give the proper number of grazers.

3.   MODELING OF LIGHT-MICROCOSMS

3.1 Variables, Parameters And Constants

Variables in the Light-microcosms were phytoplankton (P), nitrogen (N), bacteria (X), microflagellates (Y), and glucose (G). Autotrophic growth was expressed as changes in chlorophyll a concentrations.   Forms of nitrogen included ammonium, nitrate and nitrite; initial concentrations of all forms were added and expressed as a single variable N. Dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) were not measured in the microcosm experiments. Consequently, the uptake of DON and DOC other than glucose was not considered in the simulation, and neither were the release of organic substrates by phytoplankton or remineralisation processes by bacteria.   Thus, the model was restricted to the initial growth period.   Due to the lack of grazers, phytoplankton cells sank to the bottom of the container following nutrient depletion.   The loss of cells was not described.   Also not included in the model was the excretion of nitrogen compounds by microflagellates.

Based on results of the Light-experiments (standard pattern=1 mg l^{-1} glucose), it was assumed that bacterial growth was limited by glucose and phytoplankton growth by nitrogen in the $\leq$ 5ppt to $\geq$ 26 ppt salinity microcosms. Only in glucose perturbations and Dark-experiments, the addition of 5-15 mg l^{-1} of glucose resulted in nitrogen limitation of bacterial growth at low salinities. Heterotrophs grow on organic carbon, but in

addition require nitrogen compounds for protein synthesis. The uptake of both carbon and nitrogen was assumed to occur in a fixed proportion; thus carbon and nitrogen quotas for bacteria were considered to be constant. The growth efficiency on nitrogen was taken as 100% for bacteria (Azam et al. 1983) and phytoplankton. Algal cells were assumed to have constant nitrogen and chlorophyll a quota per cell during the entire experiment. The growth of photosynthetic microflagellates was ignored, because their total biomass was small in comparison to the biomass of the dominant algae.

The processes in the Light-microcosms were characterized by Michaelis-Menten type functions. The equations for changes in bacterial numbers (X), microflagellates (Y) and glucose (G) were the same as in the simulation of the Dark-microcosms (see section 2.1 in this Chapter). Equations representing changes in phytoplankton biomass (P) and changes in nitrogen concentrations (N) over time (t) were expressed as

$$\frac{dP}{dt} = \frac{SM \cdot N}{NK+N} \cdot P$$

$$\frac{dN}{dt} = -\frac{SM \cdot N}{NK+N} \cdot P \cdot \frac{QP}{QC} - \frac{RM \cdot G}{GK+G} \cdot X \cdot QN$$

SM = maximum growth rate of phytoplankton
NK = half-saturation constant for nitrogen (μg-at l^{-1})
QP = nitrogen quota per phytoplankton cell (pg-at)
QC = chlorophyll a quota per phytoplankton cell (pg)
RM = maximum growth rate of bacteria
GK = half-saturation constant for glucose (mg l^{-1})
QN = nitrogen quota per bacterium (μg-at per 10^9 bacteria)

Initial values of P, N, X, and Y were determined in the laboratory microcosms; the values changed with different salinity regimes. G, at the beginning of the simulation was 1 mg l^{-1} glucose (standard pattern), due to a constant addition. Parameters of bacterial and flagellate growth were taken from the Dark-microcosm simulation of the appropriate salinity value.

3.2 Simulation Of The 10 ppt Salinity Light-microcosm

In the 10 ppt salinity microcosms, a single diatom species, Skeletonema costatum, comprised more than 90% of the phytoplankton bloom, while at higher and lower salinities algal populations were more diverse.

The parameters describing bacterial and flagellate growth were the same as in the simulation run shown in Figure 16a. Based on data obtained from Dark-experiments, a nitrogen quota for bacteria (QN) of 2.0 μg-at per 10^9 bacteria was calculated. With a glucose quota of 0.4 mg (2.2 μM) per 10^9 bacteria, the glucose/N ratio per bacterial cell was 1.1, which was in agreement with the C/N ratio given in Fenchel & Blackburn (1979).

The calculation of the maximum growth rate (SM) of S. costatum was based on cell counts during the exponential growth phase. The value of 0.055 was equal to maximum rates reported by Smayda (1973) and Hitchcock (1980a) in high light intensity cultures at temperatures of 10 - 12°C, and was equal to the maximum growth rates of bacteria found in the Dark-microcosms. While phytoplankton and bacterial growth rates were equal, algae had a lag phase about three times as long as

bacteria. A combination of factors could be responsible for the delayed onset of phytoplankton growth. During the initial heterotrophic bloom, bacteria may have prevented algal growth by producing inhibitory substances (Cole 1982). Also, changes in environmental conditions, such as irradiance, photoperiod, osmotic changes, temperature and air-bubbling may have induced auxospore formation in the diatom population before vegetative growth resumed, as Drebes (1977) suggested.

For S. costatum Eppley et al. (1969) give a half-saturation constant (NK) of 0.5 μM for the uptake of nitrate. MacIsaac & Dugdale (1969) calculated a value of 1.0 for natural populations in coastal water, which is the same as in laboratory cultures. The higher value was used in the model. The chlorophyll a content per cell was calculated to be 0.5 pg in the 10 ppt salinity microcosm. The same value has been reported by Cosper (1982) for S. costatum at similar irradiances. The nitrogen quota was set at 3.0 pg (0.2 pg-at N) per cell (Cosper 1982). The resulting N/Chl ratio was 6; a value, equal to that given by Parsons et al. (1961) for nutrient sufficient cells.

A simulation run with bacterial and flagellate parameters as in Figure 16a, with proportionally adjusted initial bacterial (X) and flagellate numbers (Y), with an addition of 1 mg l^{-1} glucose (G) and with chlorophyll a (P) and nitrogen concentrations (N) as found in a 10 ppt salinity Light-microcosm, resulted in a premature phytoplankton bloom. Algal growth started without a lag phase resulting in early nitrogen depletion. Bacterial numbers matched those found

experimentally; relatively low total numbers resulted in a small population of heterotrophic flagellates. According to the model c. 90% of the microflagellates were photosynthetic.

While at the height of the phytoplankton bloom more than 90% of the algal cells were identified as S. costatum, their relative abundance at the beginning of the Light-experiments varied between 8.5% and 20%. Thus, the initial fraction of chlorophyll a originating from S. costatum cells was small. In order to simulate a bloom of this species, a reduction of the experimentally found chlorophyll a value seemed to be justified. When the initial value (P) was reduced to approx. 5%, chlorophyll a concentrations showed a sharp increase after a 2.5 day lag phase. In Figure 18, the simulation of a 10 ppt salinity Light-microcosm (X=0.95; P=0.075; N=19.66; SM=0.055; NK=1.0; QC=0.5; QP=0.2; QN=2.0) is shown and compared to experimental results of seven Light-experiments (standard pattern). The timing of the bloom and the chlorophyll a peak value were in agreement with results in the Light-microcosm. Because the sinking of phytoplankton cells or losses due to grazing were not included in the model, pigment values remained high. Nitrogen depletion coincided with the phytoplankton bloom.

Figure 18 - a - Model of 10 ppt salinity Light-microcosm.
b - Mean values of 7 experiments (standard pattern) at the same
salinity, but different seasons.

3.3 Simulation Of Other Salinities

The simulation of the 18 ppt and ≥ 26 ppt salinity microcosms is described and discussed together, because of behavioral similarities. The higher salinity microcosms differed from the 10 ppt one with respect to a shorter lag phase before the onset of phytoplankton growth, in their species composition and in higher nitrogen, consequently higher algal biomass. While at 10 ppt salinity, nutrient depletion coincided with the peak in chlorophyll a concentrations, i.e. the peak of the phytoplankton bloom, pigment values in the higher salinity microcosms increased by about 30% for another 24 h following nutrient depletion. Cells continued to divide and synthesize chlorophyll a by using nitrogen from internal cell pools.

The assumption of constant nitrogen and chlorophyll a quota obviously does not hold. Cell quotas vary with growth rate (Harrison et al. 1976; Goldman & McCarthy 1978) and decrease with increasing nutrient deficiency. When algal cell division continues following nutrient depletion, individual cells become progressively smaller. The use of constant quotas causes a discrepancy between the simulation run and the experimental data. When the exact chlorophyll a concentration is modeled, the nitrogen depletion is too late. With the nitrogen depletion properly timed, the chlorophyll a concentration is bound to be too high. In spite of this limitation, constant quotas were assumed for convenience sake.

At 18 ppt and ≥ 26 ppt salinity, the phytoplankton bloom was consistently dominated by S. costatum and Thalassiosira

spp.; they accounted for about 85% (by numbers) of all algal cells. The remaining 15% were made up by Chaetoceros spp., several pennate diatom forms and flagellates. S. costatum and Thalassiosira spp. seemed to occur in a fixed ratio of approx. 3:1. The latter species were not further distinguished, but cells were grouped into three different size classes. The smallest Thalassiosira cells had about twice the volume of one S. costatum cell. While the relative abundance of species at 18 ppt and ≥ 26 ppt salinity were the same, the proportion of large Thalassiosira cells in the highest salinity was double of that at 18 ppt.

Growth rate (SM) calculations were based on cell counts with no distinction between the different species. While S. costatum grew faster, the rate of growth of Thalassiosira spp. was slower, resulting in a mean maximum growth rate (SM) of all cells of 0.040 and 0.038 in the 18 ppt and ≥ 26 ppt salinity microcosms. The mean rate was lower than in the 10 ppt salinity experiments.

Based on experimental data, an average chlorophyll a content per cell (QC) of 0.4 pg was calculated for the 18 ppt and ≥ 26 ppt salinities. As outlined above, the nitrogen quota showed large variation during the different phases of growth. A calculated value based on experimental data from the onset of exponential growth proved to be far too high, while values at a later phase were virtually zero. Consequently, quota had to be guessed and adjusted in order to give the right amount of chlorophyll a at the height of the phytoplankton bloom. At

18 ppt salinity a nitrogen quota (QP) of 0.25 pg-at per cell was most fitting, while at ≥ 26 ppt 0.3 pg-at was chosen. The half-saturation constant for nitrate (NK) remained unchanged with respect to the 10 ppt salinity simulation.

Initial chlorophyll a values (P) used in the models were about 30% of the original value. Bacterial and flagellate parameters were taken from the Dark-models shown in Figures 16a (26 ppt) and 17b (18 ppt). Initial bacterial (X) and flagellate numbers (Y) were adjusted proportionally, while the glucose addition was constant at 1 mg l⁻¹. In Figure 19 the simulation runs of one 18 ppt (X=0.6; Y=2.0; G=1.0; P=0.5; N=33.72; SM=0.04; NK=1.0; QC=0.4; QP=0.25; QN=2.0) and one ≥ 26 ppt Light-microcosm (X=0.2; Y=4.0; G=1.0; P=0.75; N=47.87; SM=0.04; NK=1.0; QC=0.4; QP=0.3; QN=2.0) are shown. The models are compared to mean values of bacterial, chlorophyll a and nitrogen concentrations of several experiments (standard pattern).

A simulation of the lowest (≤ 5 ppt) salinity Light-microcosm was hampered by the lack of data for bacterial and microflagellate growth. A Dark-model of this salinity was not considered because bacteria were limited by both nitrogen as well as glucose. At ≤ 5 ppt salinity, the phytoplankton bloom occurred later than at 10 ppt and was composed of small Thalassiosira spp. and green algae. The presence of the latter species was reflected in the high chlorophyll a quota (QC) of 1.2 pg. The mean maximum growth rate (SM) for all cells was 0.043. A simulation run with bacterial and flagellate parameters as in the 10 ppt salinity Dark-model, properly
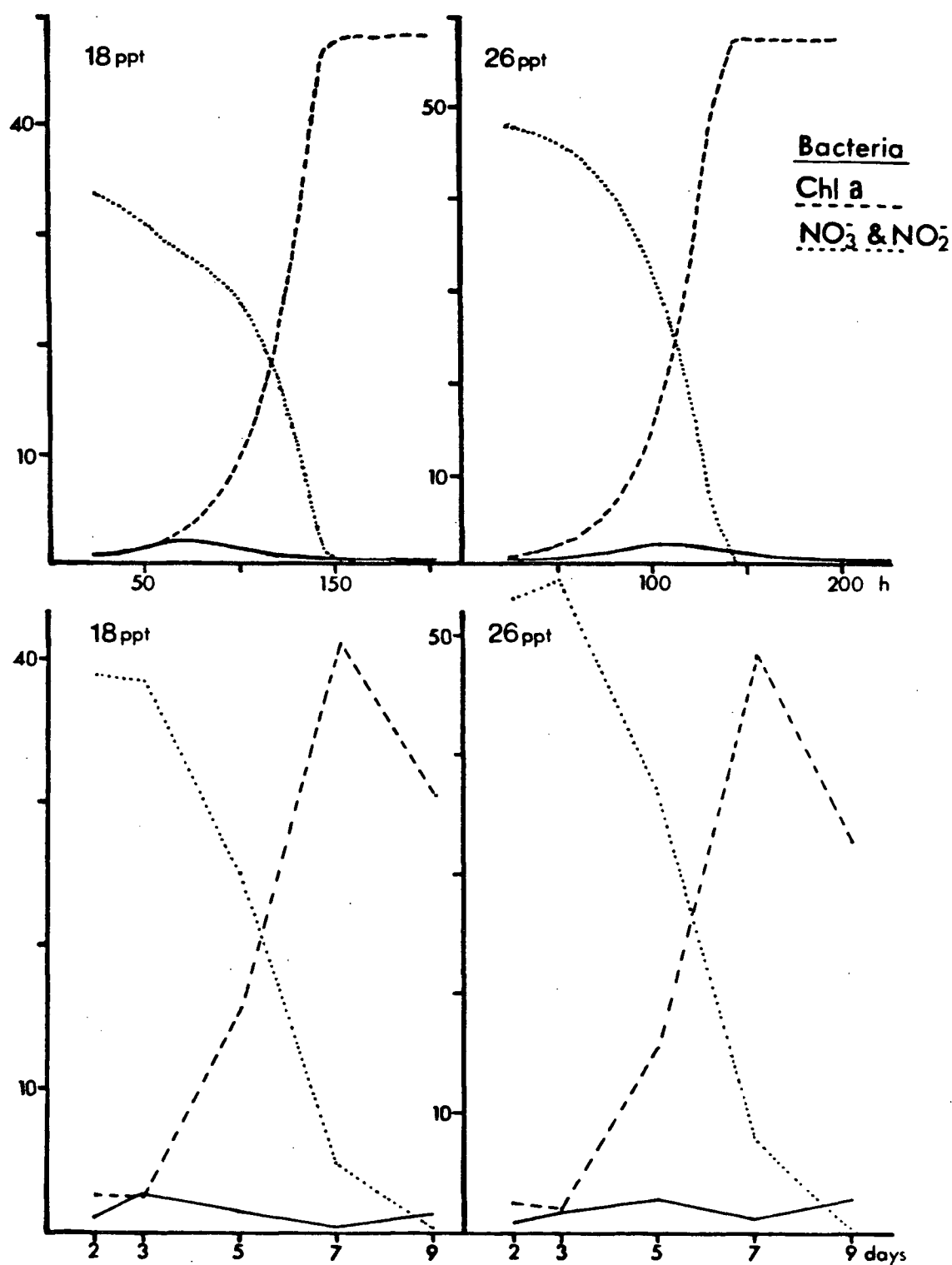
Figure 19 - Top - models of a 18 ppt and a ≥ 26 ppt salinity Light-microcosm. Bottom - mean values of 6 (18 ppt) and 7 (≥ 26 ppt) experiments at different seasons. (Bacteria x10^6 ml^{-1}; Chl a μg l^{-1}; NO$_3$ + NO$_2$ μg-at l^{-1})

adjusted initial bacterial (X) and flagellate numbers (Y), and an initial chlorophyll a concentration (P) of c. 7% of the experimentally found value, resulted in a model which simulated the ≤ 5 ppt salinity Light-microcosm well. But when this simulation was compared to the mean values of 7 experiments (standard pattern), the results were more divergent (Figure 20; X=1.0; Y=2.0; G=1.0; P=0.075; N=9.55; SM=0.043; NK=1.0; QC=1.2; QP=0.5; QN=1.8).

While at 10 ppt and higher salinities the simulation of one microcosm agreed well with experimental data collected at different seasons of the year, the ≤ 5 ppt salinity Light-model did not compare equally well with experimental data which included seasonal variability. This might be due to the lack of data for bacterial and flagellate growth at ≤ 5 ppt salinity or to a greater variation in the lowest salinity microcosms.

3.4 The Distribution Of Inorganic Nitrogen

Bacteria and phytoplankton need inorganic nitrogen for growth. In most microcosm experiments the uptake by one or the other could not be distinguished, because algal cells started to grow while the initial heterotrophic bloom was still in progress. In a few experiments, however, phytoplankton did not enter the exponential growth phase until bacterial numbers reached a minimum after the bloom. The nitrogen uptake due to bacteria ranged from between 3.5 and 4.7 μg-at l^{-1} in these microcosms, which was the same amount the Light-model predicted for simulations with 1 mg l^{-1} glucose (standard pattern) and nitrogen uptake was not significantly different over the
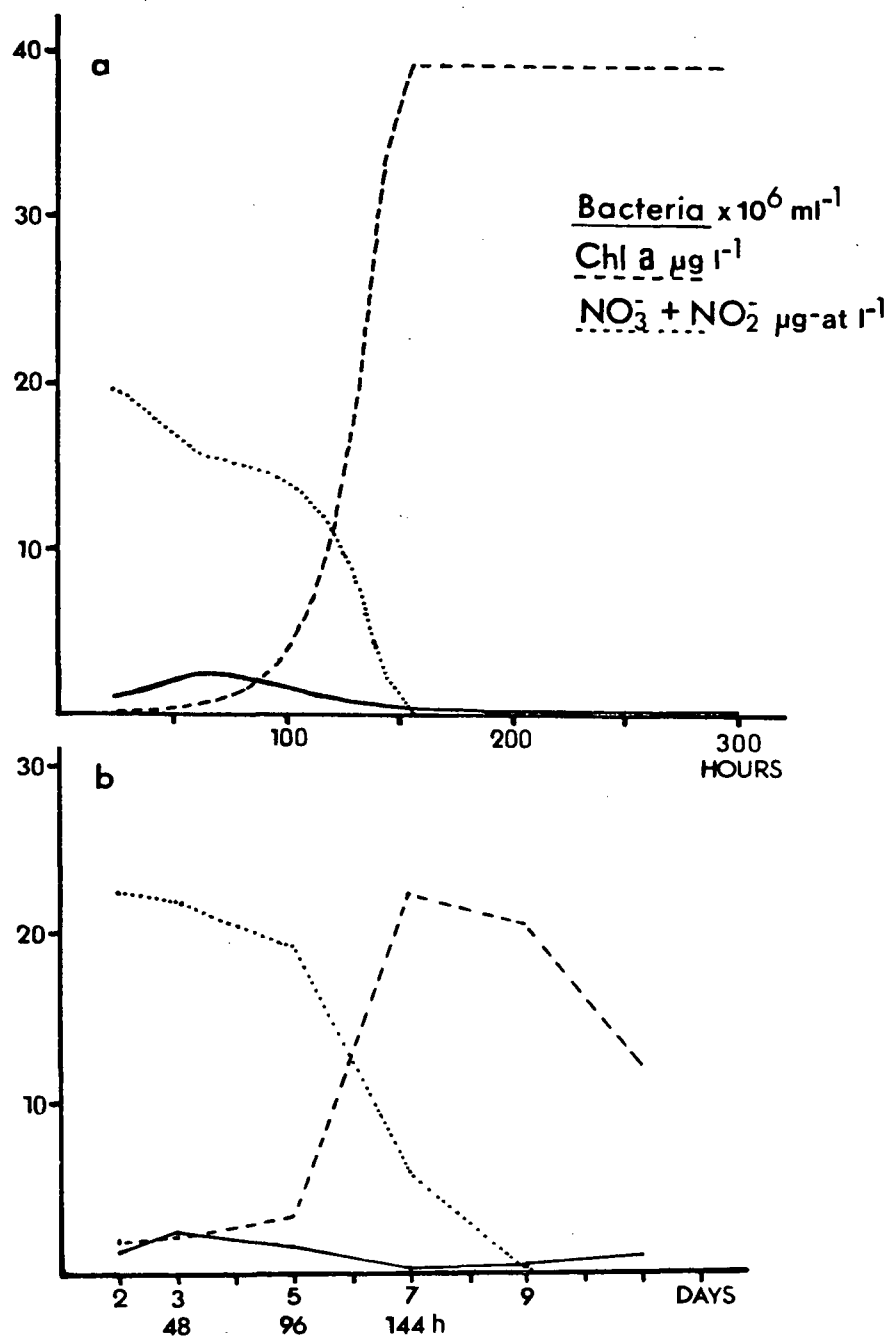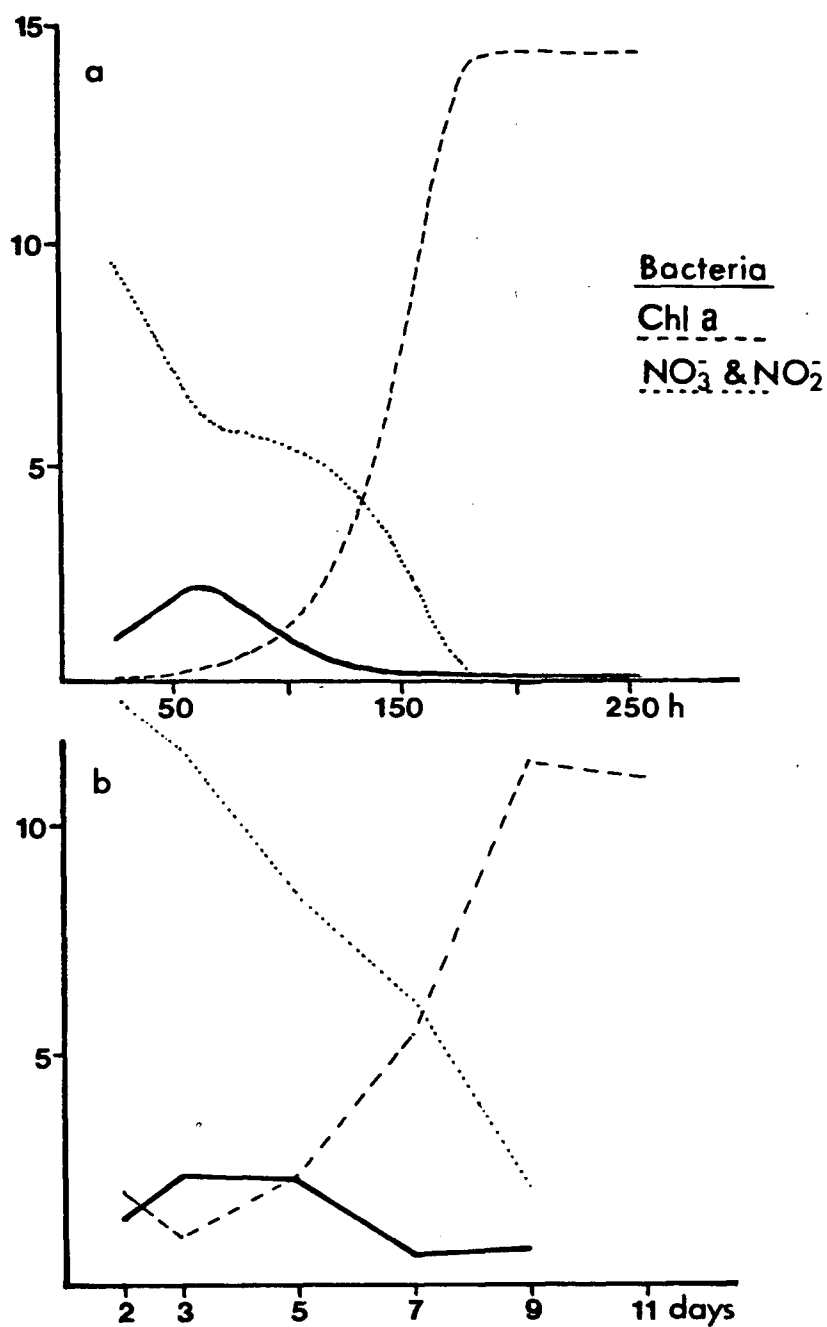
Figure 20 - a - Simulation of a ≤ 5 ppt salinity Light-
microcosm;
b - Mean values of 7 experiments (standard pattern) at the same
salinity, but at different seasons.
(Bacteria x10^6 ml^{-1}; Chl a μg l^{-1}; NO$_3$ + NO$_2$ μg-at l^{-1})

salinity range of ≤ 5 ppt to ≥ 26 ppt (3.6 - 5.3 μg-at N l^{-1}; $\bar{x}$=4.2). Due to the increase in nitrate plus nitrite concentrations with increasing salinity, bacterial uptake accounted for 38% of the total inorganic nitrogen at ≤ 5 ppt, but only for 11% at ≥ 26 ppt salinity.

In the following an attempt is made to balance inorganic nitrogen in order to evaluate the importance of nitrogen recycling which was not considered in the model. When bacterial growth was enhanced by increasing the glucose addition from 1 mg to 5 mg l^{-1}, with all other variables and parameters unchanged, the model predicted a five-fold nitrogen uptake by bacteria of approx. 20 μg-at N l^{-1}. The simulated increased bacterial uptake totally depleted nitrogen concentrations at ≤ 5 ppt and 10 ppt; no phytoplankton bloom could develop. At higher salinities, the remaining nitrogen provided for an algal bloom which was reduced by about 50% from the 1 mg level. Peak values of chlorophyll $\underline{a}$ were reached 24 h earlier, due to nitrogen exhaustion. The timing of the bacterial bloom was not different from the Dark-model, but numbers were lower at salinities where nitrogen became limiting.

The pattern predicted by the model differed from experimentally found data of glucose perturbations as described in Chapter V, section 3.1. In microcosms the increase in organic substrate to 5 mg l^{-1} glucose caused a delay and depression of the phytoplankton bloom at ≤ 5 ppt (see Figure 23), while the impact at higher salinities was less pronounced. The timing of the bloom was not altered at 18 ppt

and $\geq$ 26 ppt salinity, but peak values were slightly reduced (Figure 24).

The discrepancy between model prediction and experimental observations might be caused by two factors: the calculated nitrogen quota per 10^9 bacteria (QN) might be too high (i.e. the quota being different when bacteria are growing in high organic nutrient concentrations), or the model did not take into account nitrogen recycling by microzooplankton.

With a calculated nitrogen quota (QN) of 2.0 μg-at per 10^9 bacteria, the Light-model agreed well with bacterial uptake as observed in microcosms under standard conditions. The glucose/N ratio was equivalent to the average C/N ratio in the majority of bacteria (Fenchel & Blackburn 1979). However, when bacterial growth was enhanced by the addition of 5 mg l^{-1} glucose, the uptake of nitrogen ranged between 16 - 18 μg-at N l^{-1} in Dark-microcosms which was lower than predicted by the model (Figure 21). The discrepancy might be explained by actually lower nitrogen quota in the Dark-experiments or, if nitrogen quota were unchanged, that the missing 2-4 μg-at N l^{-1} were supplied by microzooplankton excretion, something not included in the simulation. Ammonium concentrations in Dark-microcosms showed a rapid increase during the decline of the heterotrophic bloom (Figure 21), while flagellate numbers reached a maximum at or shortly after the bloom.

The nitrogen quota per bacterial cell (QN) may be different when cells grow in rich medium, because uptake mechanisms for different substances are not completely independent (Fuhs et
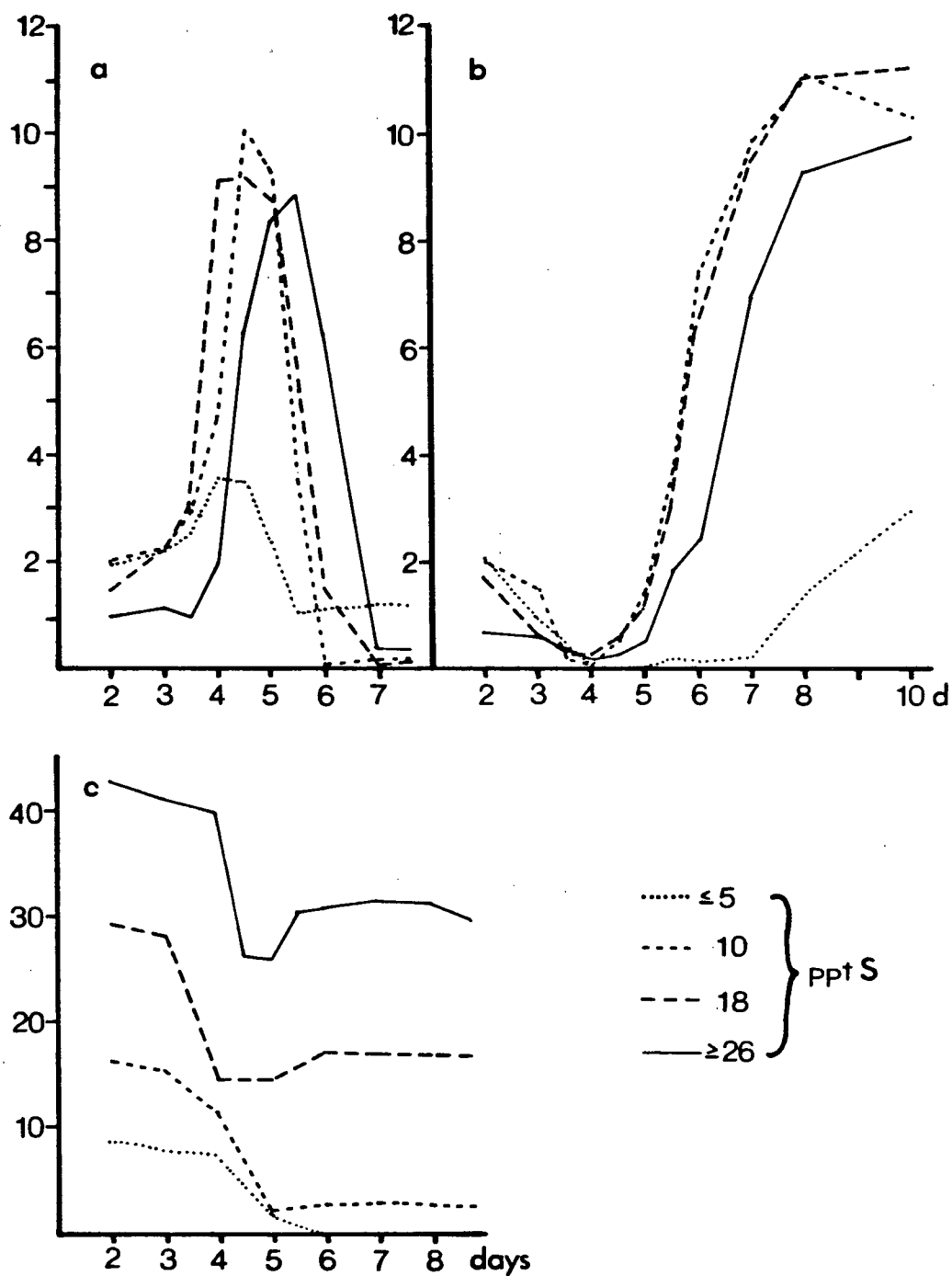
Figure 21 - Bacterial numbers x10^6 ml^{-1} (a), ammonium (b) and nitrate + nitrite (c) concentrations in µg-at l^{-1} in Dark-experiments at different salinities.

al. 1972). A variety of bacteria accumulate storage products,
e.g. PHB (Poly-β-hydroxy butyrate) in the presence of an excess
carbon or energy source (personal communication,
Dr H.W. Jannasch, Marine Biological Laboratory, Woods Hole). In
a model simulating glucose perturbation, a lower quota seemed to
be justified. In order to match the model and bacterial uptake
of 16 - 18 μg-at N l^{-1} as found in Dark-experiments, the quota
had to be lowered by 20-30%.

To conclude, with a nitrogen quota (QN) of 2.0 μg-at per
10^9 bacteria the Light-model compared favourably with
experimental results as found in Control-microcosms (standard
pattern = 1 mg l^{-1} glucose). After increasing the initial
amount of glucose to 5 mg l^{-1}, the model predicted a much lower
phytoplankton biomass than actually observed in glucose
perturbations. As discussed above, the nitrogen quota might
have been too high in the presence of large amounts of organic
substrate. With a cell quota (QN) reduced by 20% to 1.6 μg-at
per 10^9 bacteria, the model still predicted a reduction of the
phytoplankton bloom by 75% at 10 ppt and 25% at $\geq$ 26 ppt
salinity, while the addition of 5 mg l^{-1} glucose to Light-
microcosms did not result in a significant reduction at 10 ppt
and only a 16% reduction at the highest salinity. Values for
18 ppt salinity came in between these extremes. The discrepancy
between model and experimental observations can be attributed to
nutrient recycling by microflagellates, which was not included
in the simulation. While at $\geq$ 26 ppt salinity about 10% of the
bloom might be due to "regenerated production" (Dugdale &

Goering 1967), the proportion increases up to 66% at ≤ 5 ppt salinity. Not surprisingly, recycling of nutrients is of increasing importance at low ambient nitrogen concentrations. Recycling of nutrients could explain the smaller impact of glucose perturbations in the microcosms.

4. SUMMARY

The processes in Dark- and Light-microcosms were described in numerical models. The original data set based on experimental results, literature data and a model of bacteria and microflagellate interactions proposed by Fenchel (1982b) did not simulate the processes in Dark-microcosms at any of the given salinities. Several parameter changes were necessary to adjust the model to experimental observations, where bacterial cell quota and the gross growth efficiency of microflagellates had the dominant effect in determining the timing and absolute numbers in the bacterial bloom. There was no universal set of parameters fitting all salinities.

The Light-model, based on results of the Dark-simulation, experimental and literature data, was restricted to the initial phytoplankton growth phase, because neither recycling of nutrients nor any processes following the algal bloom were included. The Light-model agreed well with the processes observed in the 10 ppt to $\geq$ 26 ppt salinity microcosms, while at the lowest salinity the simulation was less close, probably due to the lack of proper data and greater seasonal variability in the experiments.

A budget of inorganic nitrogen revealed the partitioning of substrate between bacteria and algae. At low ambient nutrient concentrations bacterial uptake may account for a considerable proportion of total inorganic nitrogen. The importance of nutrient recycling by microzooplankton was demonstrated in the

discrepancy between the behavior of the Light-model when initial glucose concentrations were increased to 5 mg l^{-1} and the experimental results obtained in glucose-perturbed microcosms. In these experiments up to 66% of the phytoplankton bloom was attributed to regenerated nitrogen.

V. PERTURBATION EXPERIMENTS

1. INTRODUCTION

Estuaries generally are of high biological productivity and are therefore rather vulnerable to major changes in their balance. The impact of man's activities and perturbations is thus of great interest. The addition of pollutants (metals, oil, pesticides) have been shown to be harmful in numerous LD_{50} responses, yet, many estuaries have not changed appreciably despite these pollutants being present (e.g. Marshall 1982). In the Strait of Georgia the general level of productivity does not reflect pollution of this environment, either by poisons or enrichment (Parsons 1972; Harrison et al. 1983; Waldichuk 1983).

Despite pollution, estuaries are resilient, although in themselves highly variable environments. Tides, floods, erosion and sedimentation cause changes on a timescale from hours to centuries. It is hypothesized that natural and unnatural perturbations applied to an estuary do not result in a breakdown of the ecosystem, but may cause shifts in the food chain. The change in estuarine food chains, e.g. from an autotrophic to a heterotrophic system, might entail higher secondary and tertiary production under the latter (Parsons et al. 1981).

Obviously, the estuary itself cannot be manipulated to test the impact of various perturbations. For testing the hypothesis, a biological model of an estuary, consisting of a series of controlled aquatic microcosms, has been constructed.

Similar approaches to experiments on the benthic boundary layer (e.g. Zeitzschel & Davis 1978) and to pollution of freshwater microcosms (e.g. Taub & Crow 1978) have been reported. This thesis is novel in its approach to simulate estuarine salinity values and certain types of perturbations. The response of the microplankton to imposed perturbations might result in deviations from the standard pattern and this will then be taken as indication of a shift in the food chain of the estuary.

Some background data on the natural ecology of the microplankton of the Fraser River estuary are available. The area is generally dominated by a diatom spring bloom and by a diatom community of higher diversity during the summer (Stephens et al. 1969; Parsons et al. 1969; Shim 1976). Heterotrophic activity of bacterioplankton may dominate the microplankton during the winter months and constitute an important part of the community at other times of the year (Albright 1983a,b). Data on the water quality of the Fraser River have been reviewed by Drinnan & Clark (1980).

2. MATERIALS AND METHODS

The laboratory set-up, sampling design and analysis of samples were described in Chapter II. A total of 10 different perturbations over a salinity range from $\leq$ 5 ppt to $\geq$ 26 ppt were performed. With each perturbation experiment, a control, in form of a standard pattern, was run.

In perturbation experiments the glucose addition was increased from 1 mg l^{-1} (standard addition) to 5 mg l^{-1}, in order to simulate high organic load. Single additions were compared with repetitive ones on three consecutive days.

With neutral density screens, the incident light was reduced by 90 %. An irradiance of c. 22.5 μEinst m^{-2} s^{-1} was measured inside the empty and shaded flasks.

The impact of heavy metals was tested in perturbations including the addition of 30 μg l^{-1} of Cu as well as a heavy metal mixture (cf. Table 2) at concentrations of five and ten times the amount found in a moderately polluted estuary such as Narragansett Bay, Rhode Island, USA (Goldberg et al. 1977). The same approach employing the Multi-element-mix was used by Thomas et al. (1980) in a CEPEX study. Table 2 shows the composition and concentrations of the ten heavy metals in the original mixture. Since the standard addition of EDTA+iron would affect the availability of the heavy metals, changes in the experimental set-up were necessary. The EDTA+iron was replaced by addition of FeCl$_3$ in the same molar concentration in the experimental unit as well as in the control.

Table 2 – Composition of Multi-element-mix according to Thomas et al. (1980).

Metal	Compound	$\mu g\ l^{-1}$
As V	Na_2HAsO_4	5.0
Cd	$CdCl_2$	0.75
Cr	$K_2Cr_2O_7$	3.0
Cu	$CuSO_4 \cdot 5\ H_2O$	3.0
Hg	$HgCl_2$	0.15
Ni	$NiCl_2$	5.0
Pb	$PbCl_2$	3.0
Sb	$K(SbO)C_4H_4O_6 \cdot 1/2\ H_2O$	1.5
Se	SeO_2	1.5
Zn	$ZnCl_2$	5.0

In British Columbia, the herbicide 2,4-D is used in aerial spraying of woodlands. Application of the same in coastal areas might affect the water quality of estuaries and inlets. The herbicide 2,4-D, dichlorophenoxyacetic acid, mol. wt. 221.0, (D 2128-SIGMA, Chemical Company), was kindly supplied by Dr M.K. Upadhyaya of the Plant Science Department, UBC. The pure compound was used in the perturbation experiments rather than commercial formulations which contain other chemicals beside 2,4-D. The acid form of 2,4-D is only slightly soluble in water, at 25°C 0.09 g 100 ml^{-1}, but highly soluble in ethanol (Herbicide Handbook, Weed Science Society of America 1974). On

day 3 2,4-D was added to the microcosms resulting in final concentrations of 10^{-4} M and 10^{-3} M. The 2,4-D was dissolved in sub-samples of water from the microcosms. The 10^{-3} M concentration was mixed with 0.5 ml ethanol (95%) to assist dissolution, before being combined with the subsample and poured back into the microcosms. The ethanol in the highest 2,4-D concentration increased the total carbon added to 107.5 mg l^{-1} (96 mg C l^{-1} from 2,4-D and 11.5 mg C l^{-1} from ethanol), thus increasing the carbon source for bacterial growth.

3. RESULTS AND DISCUSSION

3.1 Effects Of Glucose

Productivity of an estuary is a result of balances between autotrophy and heterotrophy, and between import and export (Sibert & Naiman 1980). Estuarine circulation introduces nutrients from the ocean; rivers introduce organic matter, sediments and nutrients. These imports stimulate both autotrophic and heterotrophic growth.

Dissolved organic matter in estuaries includes large amounts of sugars (Seki et al. 1969) as well as small quantities of amino acids (e.g. Hobbie et al. 1968). While concentrations of the latter are low, measurements of flux rates indicate that the contribution to primary production might be as high as 10% (Hobbie et al. 1968; Billen et al. 1980). Heterotrophic populations have considerable potential for oxidizing small organic molecules. Within a period of 30 h, bacteria are capable of responding and adapting to a 100-fold increase in amino acid concentration (Williams & Gray 1970).

Glucose additions to microcosms of a salinity range from ≤ 5 ppt, 10 ppt, 18 ppt to ≥ 26 ppt resulted in a 25 to 75-fold increase in dissolved monosaccharide concentrations compared to natural levels found in the Fraser River estuary. The enrichment in organic carbon gave rise to higher bacterial numbers and led to lower chlorophyll a concentrations at the height of the simulated phytoplankton bloom. When 5 mg l^{-1} of glucose were added on day 2 together with inorganic nutrients,

the bacteria responded within 24 to 48 h with an exponential increase in numbers as shown in Figures 22 and 23. The fastest response was observed at intermediate salinities, 10 ppt and 18 ppt, while absolute numbers were highest at 18 ppt and $\geq$ 26 ppt. With three additions of 5 mg l^{-1} of glucose on three consecutive days (day 2, 3 and 4), a further increase in bacterial numbers was observed at salinities $\geq$ 10 ppt (Figure 22), while at $\leq$ 5 ppt absolute numbers were virtually the same after one and three additions (Figure 23).

In glucose-perturbed microcosms (see Figure 23), nutrients (NO_3 + NO_2) were exhausted 2 - 6 days earlier than in the controls. The higher organic substrate concentrations stimulated microbial growth, resulting in enhanced uptake of inorganic nitrogen by the bacteria. Beside an increase in metabolic activity, the stimulation was probably also due to a change in species composition, namely the rapid growth of a zymogenous microbial population. Nutrient concentrations in Figure 23 show the partitioning of substrate between heterotrophs and autotrophs. The initial decrease was due to bacterial uptake, the second due to assimilation by phytoplankton. The pattern was similar at all salinities.

Figures 23 and 24 show examples of chlorophyll a curves after one and multiple additions of 5 mg l^{-1} of glucose. With increasing concentrations of glucose the phytoplankton bloom was delayed and/or peak values of chlorophyll a reduced. At $\leq$ 5 ppt salinity the impact was greatest. One single addition caused a delay in the simulated phytoplankton bloom by two days and a
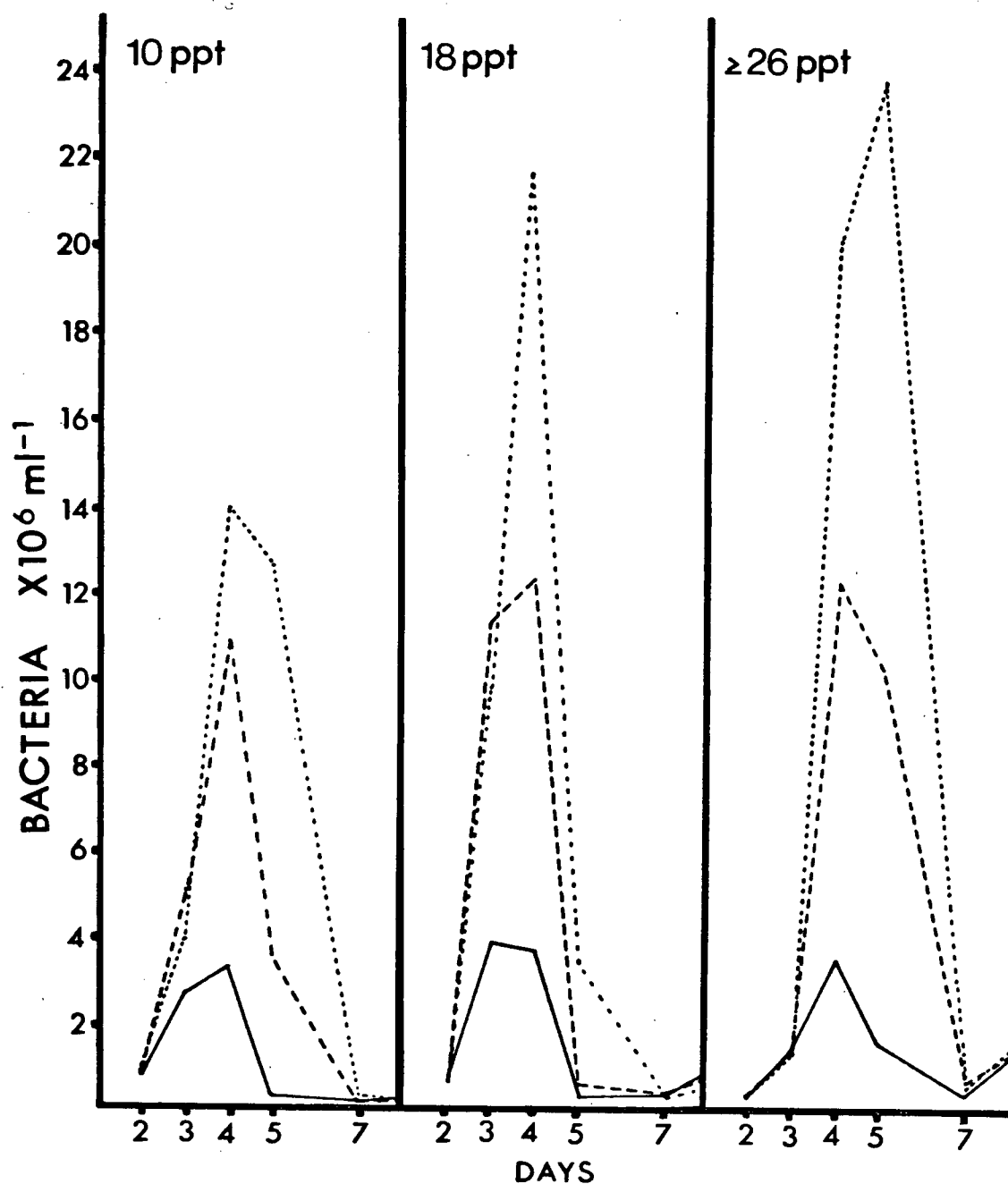
Figure 22 - Concentrations of bacteria in control (solid
line) microcosms and after one (dashed line) and three (dotted
line) additions of 5 mg l^{-1} of glucose.
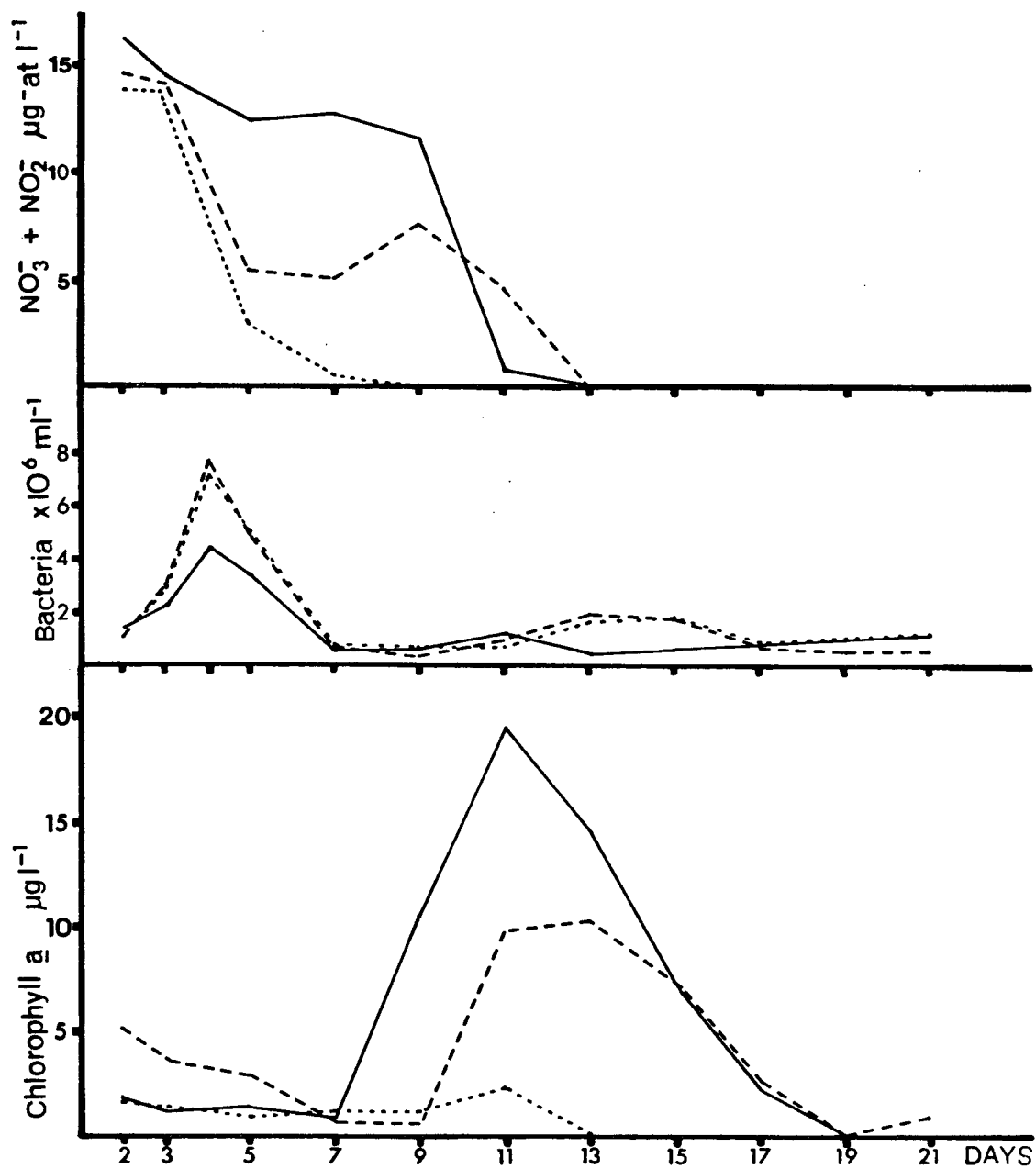
Figure 23 - Concentrations of nutrients, bacteria and chlorophyll a after glucose perturbation at ≤ 5 ppt; control (solid line), 5 mg l⁻¹ glucose on day 2 (dashed line), 5 mg l⁻¹ glucose on day 2, 3 and 4 (dotted line).
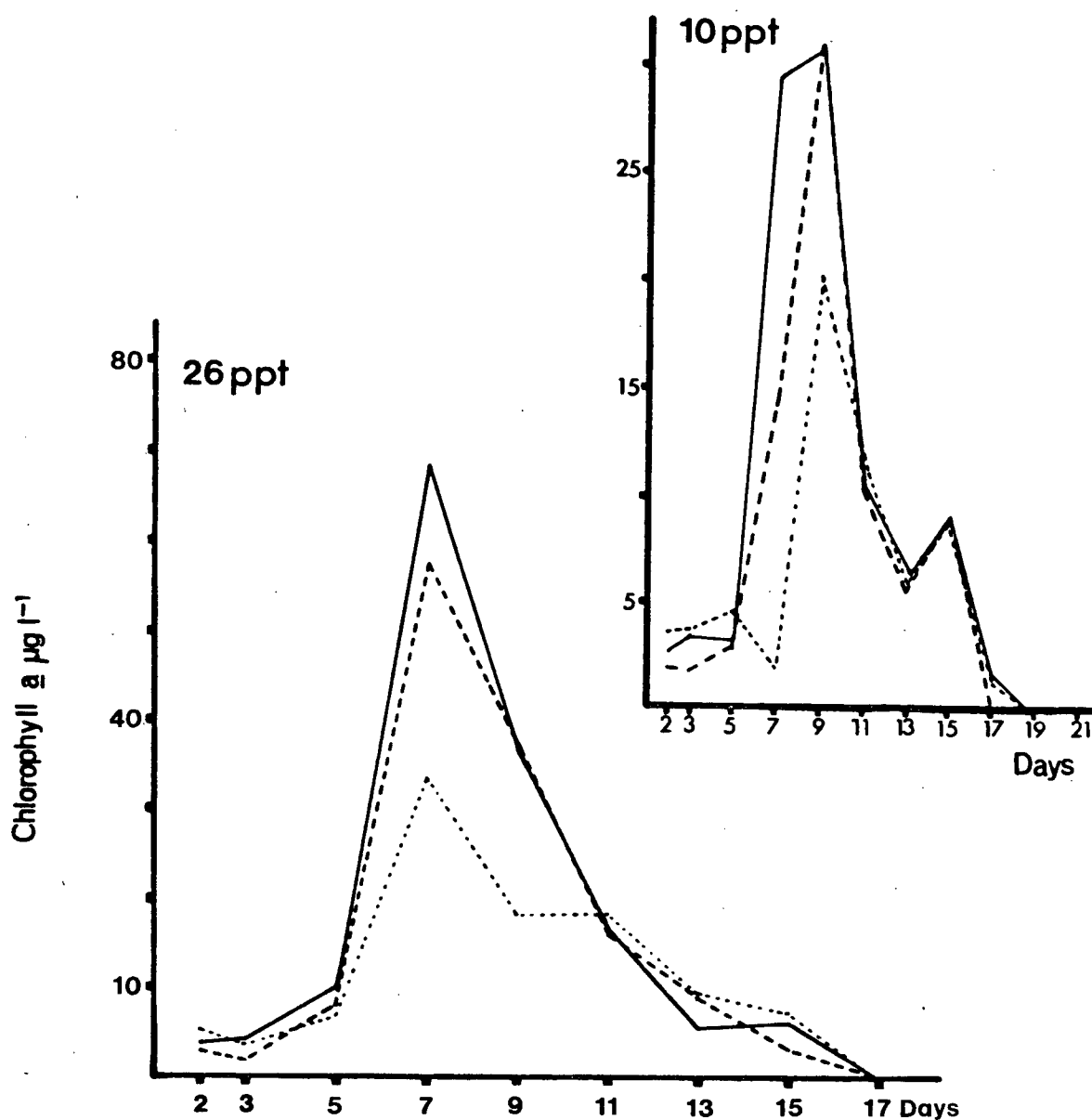
Figure 24 - Chlorophyll a concentrations at 10 ppt and
≥ 26 ppt salinity: control (solid line), 5 mg l⁻¹ of glucose on
day 2 (dashed line), 5 mg l⁻¹ of glucose on day 2, 3 and 4
(dotted line).
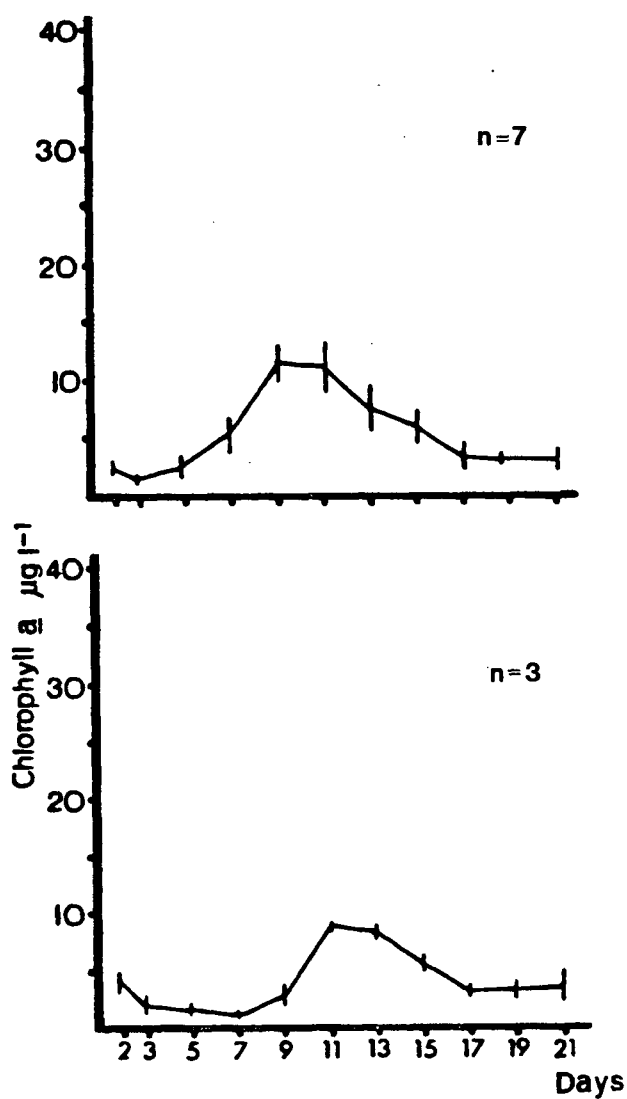
Figure 25 - Chlorophyll a concentrations in ≤ 5 ppt
salinity microcosms: a - standard pattern (control), b - Glucose
perturbation (5mg l⁻¹ on day 2). Bars indicate mean ± 1 S.E.;
n=number of experiments. Experiments were performed at
different seasons.

reduction in phytoplankton biomass. This delay was observed consistently at different seasons of the year (Figure 25), while the reduction of the bloom did not seem to be significant when mean chlorophyll a values included seasonal variability. After three additions of 5 mg l^{-1} glucose, phytoplankton growth was totally suppressed in the ≤ 5 ppt salinity microcosm (Figure 23).

Table 3 indicates the composition of the plankton samples collected on day 9. At salinities of 10 ppt and higher, there virtually was no difference between the glucose-perturbed microcosms and the controls. At ≤ 5 ppt salinity the proportion of small flagellates (< 20 μm) was over 10 times greater than in the control microcosm. After glucose perturbation of 5 mg to a total of 15 mg l^{-1} they contributed nearly 50% of the plankton. The low chlorophyll a values in the perturbed microcosms (0.58 and 1.39 μg l^{-1}) suggested that the majority of flagellates were colourless. The chlorophyll a concentration was about 10% of the value in the control microcosm.

Algae and bacteria compete for organic substrates of low molecular weight, e.g. urea (Remsen et al. 1972). Evidence points to the success of bacteria, but opposite findings have been reported as well (Saks & Kahn 1979). The dominant diatom species, S. costatum and Thalassiosira, present in the microcosm experiments are not known to grow on glucose (Hellebust & Lewin 1977). Competition for inorganic substrates, such as phosphate and inorganic nitrogen compounds has been described and under certain conditions algal growth can be severely limited

(e.g. Rhee 1972; Thayer 1974).

Table 3 - Relative abundance of small flagellates, diatoms and green algae on day 9 in Control and after one and three initial glucose additions of 5 mg l^{-1}. Means ± S.E. (in brackets) are given; n=4.

SPECIES	CONTROL	5 mg l^{-1}	3x5 mg l^{-1}
≤ 5 ppt Salinity			
Diatoms & Green Algae	96.0(±1.63)	52.7(±3.02)	52.7(±1.50)
Small Flagellates	4.0(±1.63)	47.3(±3.02)	47.5(±1.50)
10 ppt Salinity			
Diatoms & Green Algae	98.8(±0.48)	99.5(±0.29)	97.5(±0.87)
Small Flagellates	1.2(±0.48)	0.5(±0.29)	2.5(±0.87)
18 ppt Salinity			
Diatoms & Green Algae	100.0	99.5(±0.50)	97.5(±0.48)
Small Flagellates	0.0	0.5(±0.50)	3.2(±0.48)
≥ 26 ppt Salinity			
Diatoms	99.2(±0.48)	99.5(±0.50)	96.8(±1.19)
Small Flagellates	0.8(±0.48)	0.5(±0.50)	1.5(±1.19)

In the Fraser River estuary low salinities are associated with low inorganic nutrient levels (Chapter VI). In the presence of high organic substrate concentrations bacteria may exhaust ambient inorganic nitrogen. Under these conditions

nutrient recycling by microzooplankton becomes increasingly important as shown in Chapter IV, section 3.4. Following the addition of 5 mg l^{-1} glucose, up to 66% of the biomass in the delayed and reduced phytoplankton bloom might be due to "regenerated production" (Dugdale & Goering 1967).

Heterotrophic and autotrophic competition for nitrate has been suggested by Parker et al. (1975) in a stratified estuary. In the presence of organic carbon-rich and nitrogen-deficient pulpmill effluent, heterotrophic growth was enhanced, while primary productivity was inhibited by lack of nitrogen. In spite of very low primary productivity, large standing crops of both zooplankton and fish indicated the persistance of a well developed food chain (Sibert & Brown 1975). Parsons et al. (1981) enriched CEPEX enclosures with glucose and studied the effects on the productivity of the system. Enhancement of heterotrophic growth caused depression of photosynthetic production by algae but resulted in higher secondary and tertiary production.

Competition between bacteria and phytoplankton for a limiting substrate was confirmed in the microcosm experiments. In the presence of high organic load and low nitrogen concentrations bacteria outcompete phytoplankton, causing a depression or complete suppression of autotrophs, as observed in the ≤ 5 ppt salinity microcosm (Figure 23).

Heterotrophic activity was measured on day 3, when bacteria were in the exponential growth phase. Due to substrate enrichment, glucose uptake rates were 1-2 orders of magnitude

higher than in samples from the Strait of Georgia at corresponding salinities (see Table 1 in Chapter III and Table 7 in Chapter VI). The highest uptake rates were found at intermediate salinities, 10 ppt and 18 ppt.

3.2 Effects Of Light

Among the environmental factors which influence photosynthesis of phytoplankton are light, nutrients and temperature. In the Fraser River estuary, temperature is limiting only near the surface during the winter and the spring (Takahashi et al. 1973). Nutrient limitation is important during the summer, while near surface light is the main limiting factor throughout the rest of the year.

During the annual freshet, May to June, the Fraser River carries a huge amount of silt. This silt load increases the turbidity of the water (higher k_T value), thus reduces the light available for photosynthesis. Dredging has the same effect, while the construction of dams in the river reduces the silt load; thus k_T becomes smaller.

The influence of light on the growth of natural plankton populations was studied at different salinities ($\leq$ 5 ppt to $\geq$ 26 ppt) at a constant temperature of 12°C. The actual amount of light received by the phytoplankton is not known because no measurement was taken in the water-filled microcosms, but an irradiance of c. 22.5 μEinst m^{-2} s^{-1} in the empty containers led to the assumption that light levels were indeed limiting.

Figure 26 shows the chlorophyll $\underline{a}$ curves at $\leq$ 5 ppt, 10 ppt, 18 ppt and $\geq$ 26 ppt salinities. The reduction of light
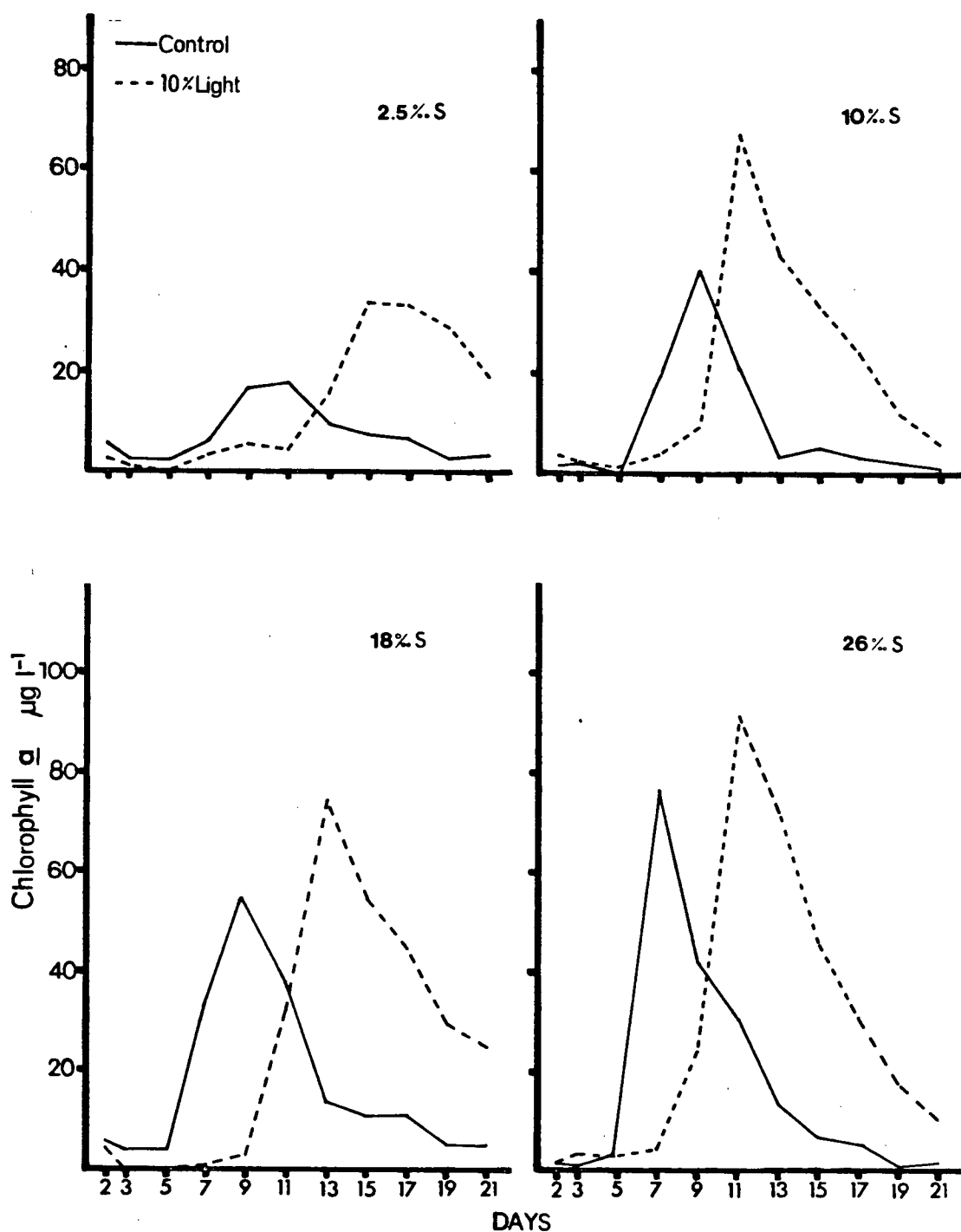
Figure 26 - Chlorophyll a concentrations of the control
(solid line) and 10%-light (dotted line) microcosms from ≤ 5 ppt
to ≥ 26 ppt salinities.

by 90% caused a delay of 2 - 6 days in the phytoplankton bloom.
The shortest delay (2 days) was observed at 10 ppt salinity and
the longest (6 days) at ≤ 5 ppt. At both 18 ppt and ≥ 26 ppt
salinity the delay was four days. Maximum chlorophyll $\underline{a}$
concentrations were always greater in the shaded microcosms.

Diel variations in the chlorophyll $\underline{a}$ content of marine
phytoplankton are well documented (Sournia 1974; Hitchcock
1980b). In order to eliminate these variations, sampling was
always done at the same time of the day between 10:00 and 12:00.
Various factors might have been responsible for the higher
chlorophyll $\underline{a}$ peak values in the shaded microcosms: the
variations in chlorophyll $\underline{a}$ content in different species growing
at different salinities, or higher chlorophyll $\underline{a}$ content per
cell as adaptation to low light (Beardall & Morris 1976). The
observed differences were mostly due to the latter.

Considering the species composition and succession of the
dominant diatoms, $\underline{Thalassiosira}$ spp. seemed to be inhibited
when the incident light was reduced by 90%. This effect was
most pronounced in the 18 ppt and ≥ 26 ppt salinity microcosms
where the phytoplankton bloom was always composed of the two
dominant species $\underline{Skeletonema}$ $\underline{costatum}$ and $\underline{Thalassiosira}$ spp..
Figure 27 shows the relative abundance of both species based on
a total of 400 cells counted. In the shaded microcosms
$\underline{S}$. $\underline{costatum}$ outgrew $\underline{Thalassiosira}$ spp.; the cell numbers of the
latter having been reduced by more than 30%. During enumeration
$\underline{Thalassiosira}$ spp. were grouped into three different size
classes (small cells of 1,889 μ^3 vol.; medium cells of 4,942 μ^3
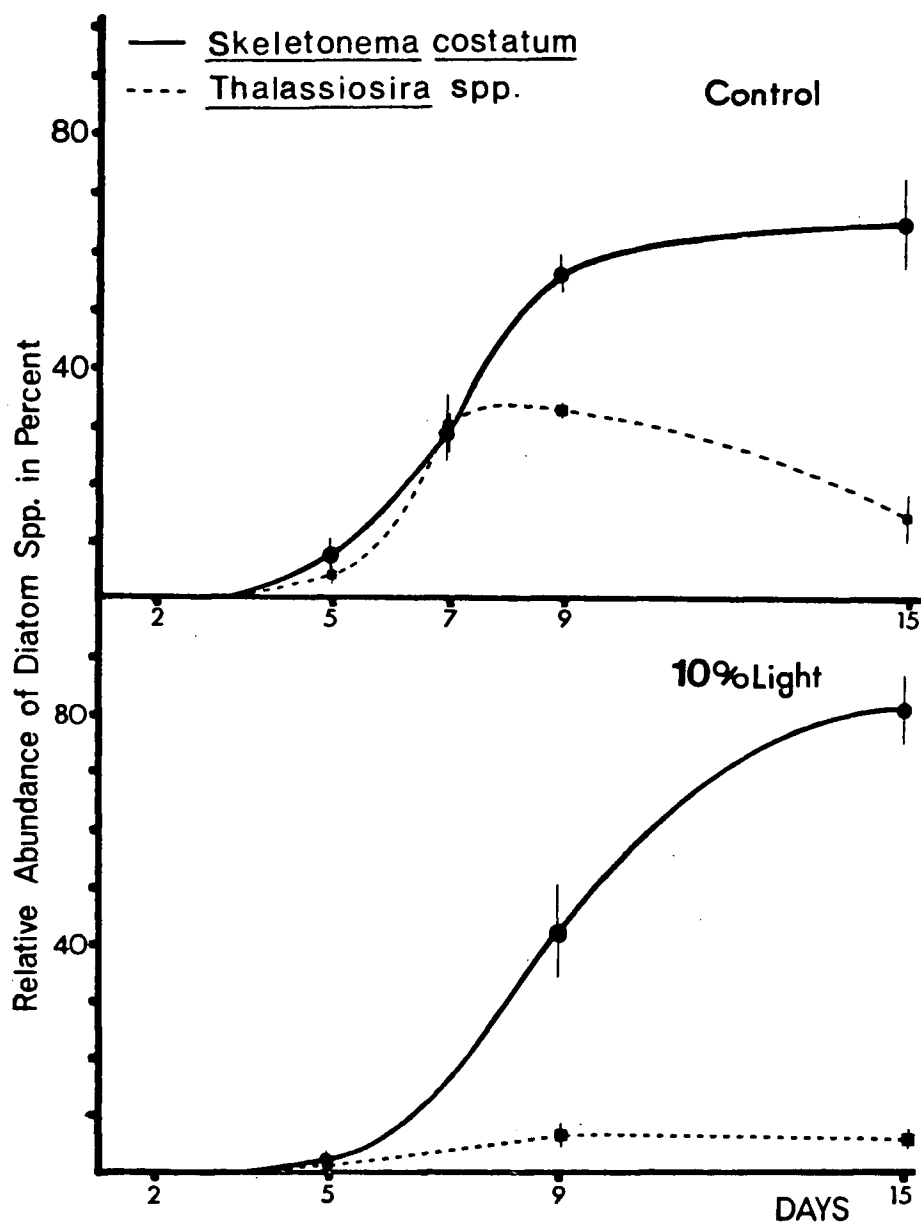
Figure 27 - Relative abundance of dominant diatom species
in control (c. 225 μEinst m^{-2} sec^{-1}) and 10% light
(c. 22.5 μEinst m^{-2} sec^{-1}) microcosms at salinities $\geq$ 18 ppt.
Bars indicate x $\pm$ S.E.; n = 2

vol.; large cells of 23,268 μ^3 vol.). While all size classes were found in the control microcosms, small Thalassiosira species dominated in the shaded containers while large cells were altogether absent. At ≤ 5 ppt salinity the dominant diatoms were Thalassiosira spp. in the control as well as in the low light microcosm, while at 10 ppt, S. costatum always comprised > 90% of the bloom species independent of variations in irradiances. This suggested that salinity was of greater importance than light.

The increased abundance of S. costatum under low light conditions is also seen in B.C. coastal waters, where in some years Thalassiosira spp., in other years S. costatum, is the first in the diatom spring bloom (Takahashi et al. 1973; Stockner et al. 1977). Stockner et al. (1977) noted that, largely as a result of lowered light levels in 1974, when S. costatum dominated both the phytoplankton and the sediment assemblages in Howe Sound (Roelofs in press) the spring bloom was later in 1974 than in 1973, when Thalassiosira spp. dominated the phytoplankton. Another example from the Fraser River area showed a Thalassiosira sp. dominant in the early spring bloom of 1966, while S. costatum was the early dominant in 1967; the light levels were lower in early 1967 than in 1966 (Takahashi et al. 1973). In years when less light was available than in 'normal' years, S. costatum outcompeted Thalassiosira spp..

The results of shading in the present experiments support the suggestion that irradiance plays a major role in the

determination of species succession and dominance in the spring bloom. Low irradiances seem to favour smaller species, while larger ones are eliminated. Effects of temperature have been excluded under the controlled experimental conditions at 12°C.

The effect of lower irradiances upon bacteria was not conclusive. The second heterotrophic bloom was delayed by two days at ≤ 5 ppt, 10 ppt and ≥ 26 ppt salinities, while at 18 ppt the timing did not differ significantly (cf. Appendix 3). No difference was observed in microflagellate numbers in either, control nor shaded microcosms because of the large variability in counts (cf. Appendix 3).

3.3 Effects Of Heavy Metals

A chemical interpretation of the results of the metal perturbation experiments is difficult because the identity and concentration of chelators, which determine the activity of the free metal ions, are not known in natural waters. In this empirical approach, however, the relative toxicities of various heavy metals can be investigated with respect to their ecological impact on natural estuarine microplankton populations.

In the presence of the standard addition of EDTA+iron, the five-fold concentration of the basic Multi-Element-mix (see Table 2, Section 2) had no significant effect on bacteria and microzooplankton numbers and chlorophyll a concentrations (cf. Appendix 4). However, plankton analysis revealed significant differences between the control and perturbed microcosms. At ≤ 5 ppt salinity, Thalassiosira spp., the dominant diatoms, were significantly reduced in numbers after heavy metal addition in favour of a greater proportion of small flagellates (< 20 μm). The results are presented in Table 4. At 10 ppt salinity, where S. costatum was dominant, no differences could be seen. At 18 ppt and ≥ 26 ppt salinity S. costatum seemed to grow better than Thalassiosira spp. in the presence of heavy metals, but only the results at 18 ppt salinity were significant (Table 5). The initial high values of small flagellates in the low and high salinity water is a seasonal effect. The water was collected in late January, 1982.

Table 4 : Relative abundance of dominant diatoms and small flagellates in control and heavy
metal perturbed microcosms. Means ± S.E. are given of a total of 400 cells
counted; n = 4.

	Species	Day 5	Day 11	Day 15
	2.5 ‰ S			
Control	Thalassiosira spp.	1.0(±0.71)	84.0(±0.41)	68.0(±3.03)
	Small Flagellates	99.0(±0.71)	1.8(±1.11)	10.0(±1.47)
5x ME-mix	Thalassiosira spp.	1.5(±0.64)	53.2(±3.54)	48.8(±4.35)
	Small Flagellates	98.2(±0.75)	30.0(±2.34)	28.2(±3.20)
	10 ‰ S			
Control	S. costatum	5.8(±2.32)	95.5(±1.55)	83.8(±2.17)
	Small Flagellates	94.0(±2.34)	1.8(±1.11)	13.0(±1.87)
5x ME-mix	S. costatum	1.5(±0.96)	91.5(±0.50)	78.0(±2.55)
	Small Flagellates	98.5(±0.96)	1.2(±0.63)	15.0(±3.24)

Table 5 : Relative abundance of dominant diatoms and small flagellates in control and heavy
metal perturbed microcosms. Means ± S.E. are given of a total of 400 cells counted;
n = 4.

	Species	Day 5	Day 7	Day 9	Day 15
	18 %. S				
	S. costatum	4.8(±3.45)	25.2(±5.26)	53.0(±8.03)	55.0(±2.61)
Control	Thalassiosira spp.	2.0(±0.91)	36.8(±4.25)	32.2(±4.68)	18.0(±2.27)
	Small Flagellates	91.0(±3.03)	34.2(±2.46)	9.2(±1.11)	21.2(±1.49)
	S. costatum	8.2(±2.39)	66.8(±4.92)	76.0(±1.08)	79.5(±2.22)
5x ME-mix	Thalassiosira spp.	2.0(±0.91)	10.0(±1.87)	12.8(±2.29)	5.2(±2.50)
	Small Flagellates	85.5(±2.99)	17.2(±3.09)	8.8(±1.80)	14.5(±2.33)

After the EDTA+iron was replaced by $FeCl_3$, the addition of the five- and ten-fold Multi-Element-mix or 30 μg l^{-1} Cu caused a delay in the phytoplankton bloom and a suppression of the chlorophyll a peak values at all salinities except in the 10 ppt microcosm. Here the timing of the chlorophyll a peak was not altered and the height not significantly reduced (Figure 28). Figures 29 and 30 show chlorophyll a curves at different salinities and with varying concentrations of heavy metals. When the phytoplankton bloom was delayed, nutrient exhaustion occurred later. Bacterial growth seemed to be stimulated following the addition of the ten-fold Multi-Element-mix as well as 30 μg l^{-1} Cu alone (cf. Appendix 5). This stimulation might have been due to the leakage of organic substrates through a damaged phytoplankton cell membrane (Bentley-Mowat & Reid 1977), or due to the excretion of organic compounds from cells under stress (Steemann Nielsen et al. 1969).

Without the artificial chelator EDTA, the effect of heavy metal perturbations on the species composition and succession was much more pronounced. The toxicity of the ME-mix was determined by Cu and Hg according to Hollibaugh et al. (1980). Besides the increase in small flagellates at ≤ 5 ppt salinity, a change in the dominant species was also observed. Instead of a bloom of the centric diatoms Thalassiosira spp. as in the control microcosm, the pennate diatoms such as Thalassionema spp. were dominant in the perturbed containers (cf. Appendix 5). After Cu additions Nitzschia spp. were found in greater numbers, which agrees with the findings of Thomas & Seibert (1977) during
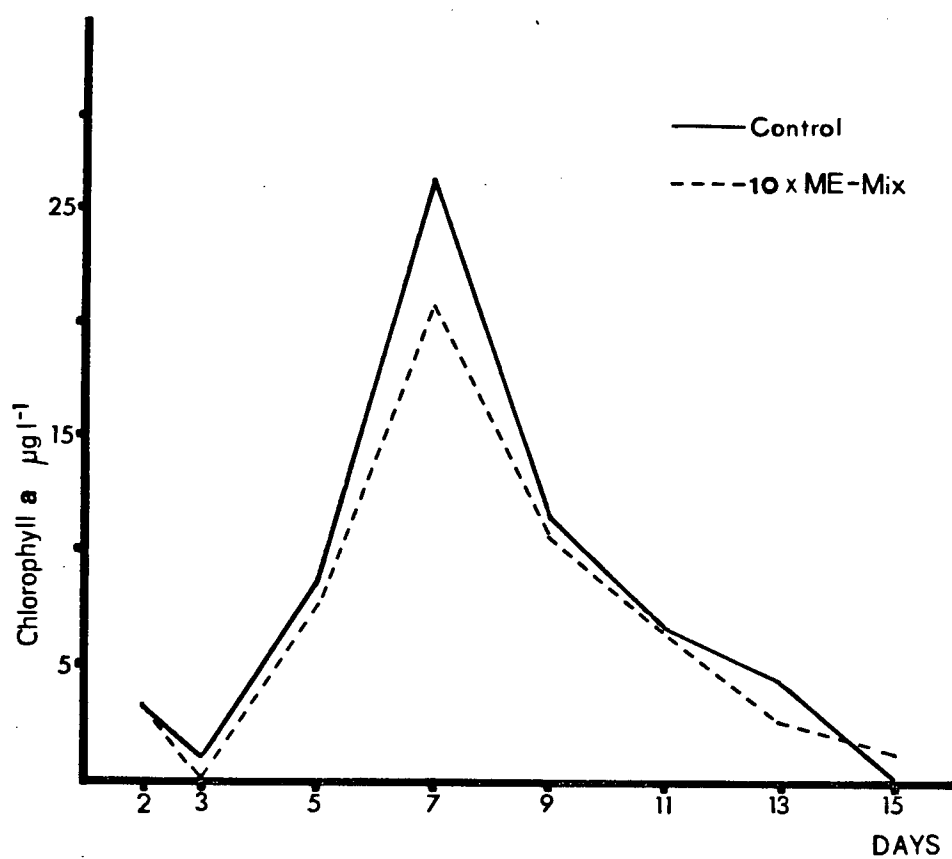
Figure 28 - Concentration of chlorophyll a in 10 ppt
microcosm after ten-fold Multi-Element-mix.
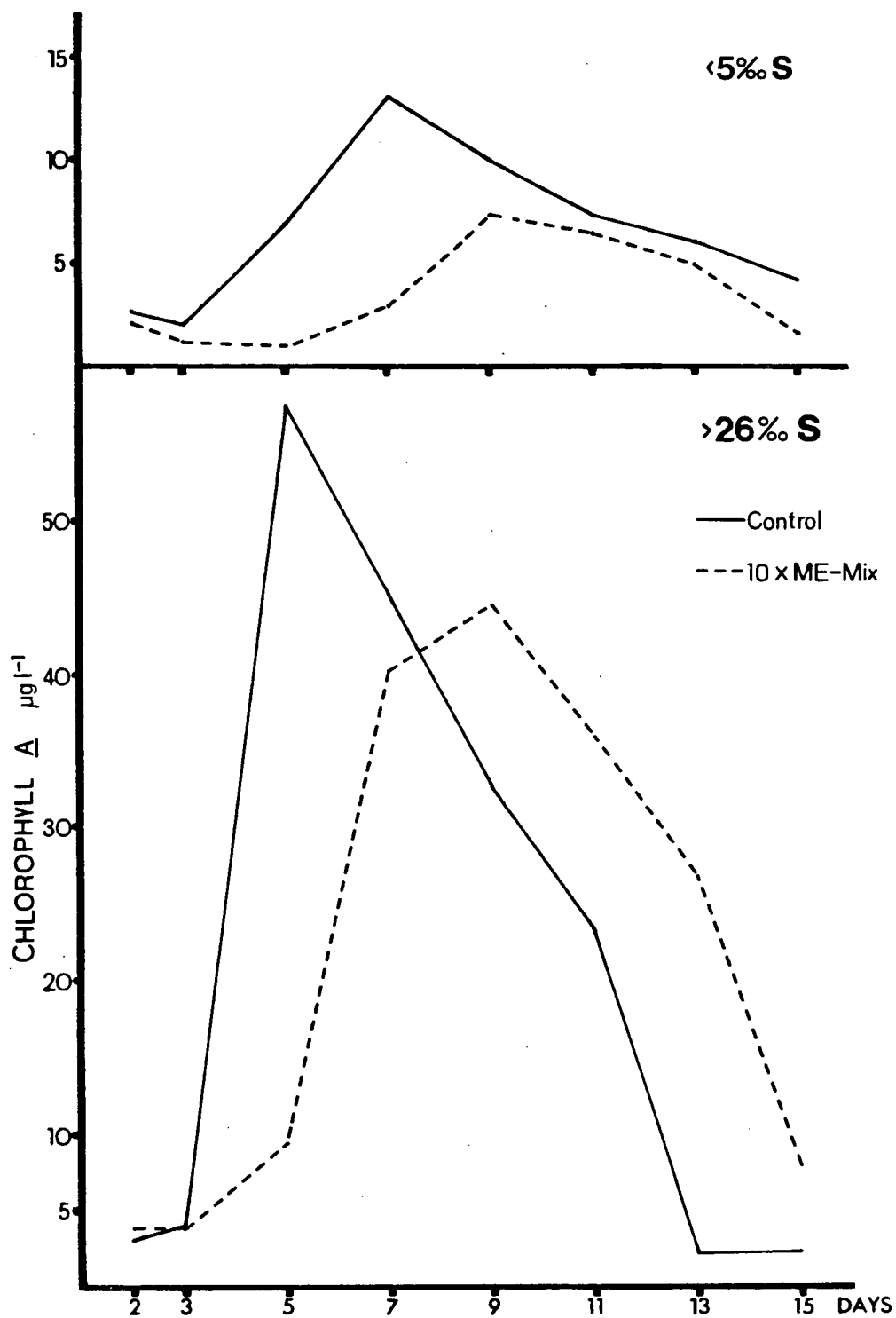
Figure 29 - Chlorophyll a curves at different salinities
after addition of Multi-Element-Mix.
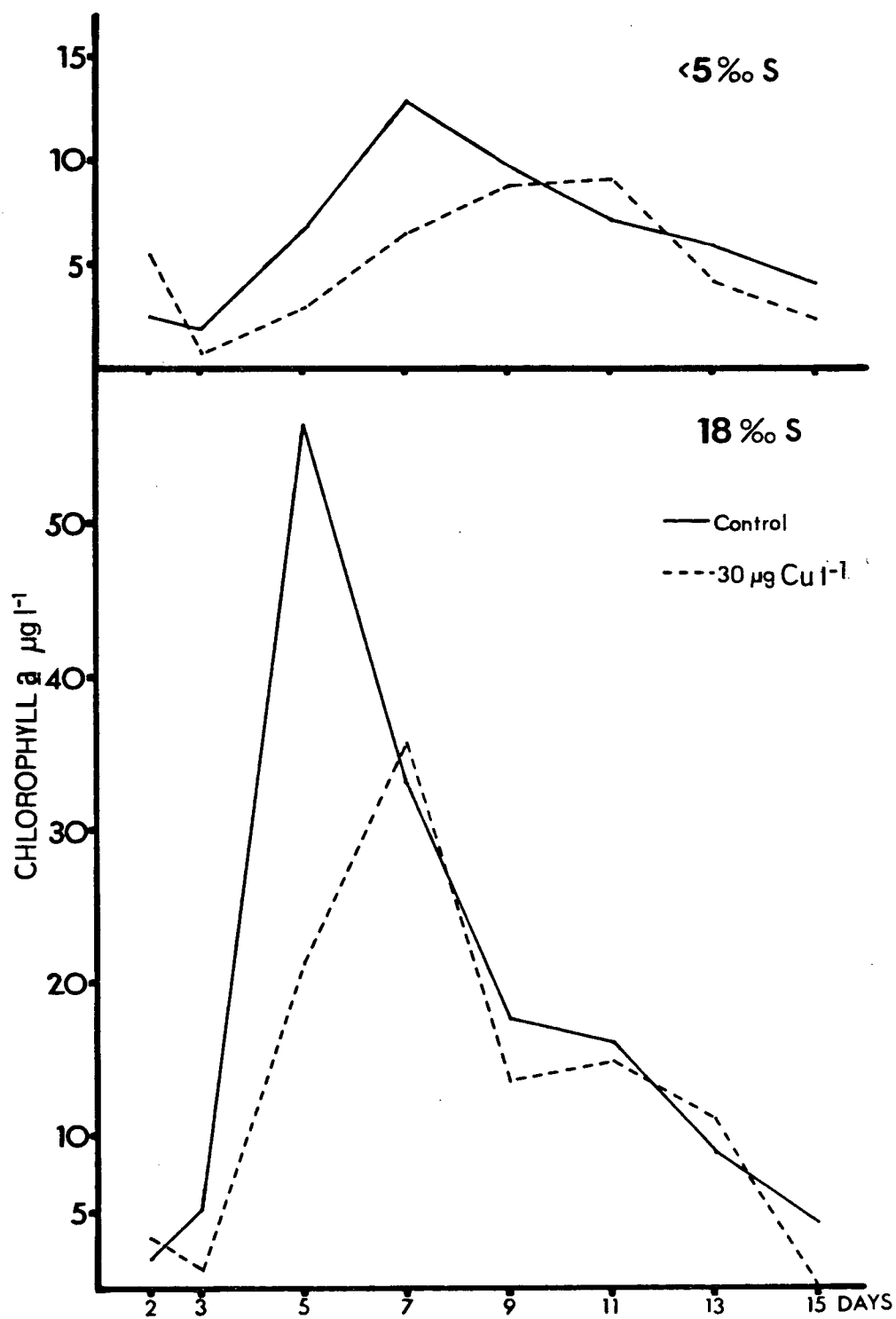
Figure 30 - Chlorophyll a curves at different salinities
after the addition of 30 μg l⁻¹ Cu.

a CEPEX study. Again, at 18 ppt and ≥ 26 ppt salinities
S. costatum grew better than Thalassiosira spp. in the presence
of heavy metals (cf. Appendix 5). At 10 ppt salinity, the
phytoplankton bloom was always composed of S. costatum, a
species which was least affected by metal additions. Morel et
al. (1978) tested the copper toxicity to S. costatum and found
it to be most resistant.

Thus, the occurrence and domination of S. costatum in
waters of c. 10 ppt salinity is an important buffer against the
destabilization of the estuarine ecosystem. This effect is not
observed with phytoplankton species that are more abundant at
lower and higher salinities, such as Thalassiosira spp..

3.4 Effects Of The Herbicide 2,4-D

The herbicide 2,4-D has been in use now for about 40 years
as a systemic herbicide to control broadleaf weeds, broadleaf
perennial herbaceous and woody plants, as well as aquatic weeds.
The effects of 2,4-D have been extensively studied in higher
plants. It causes increased and abnormal growth due to its
similarity to naturally occurring auxin, affects respiration,
depletes starch and sugar reserves and interferes with RNA, DNA
and protein synthesis; but the primary mode of action is still
not known. Concentrations of 10^{-4} - 10^{-3} M have been shown to
be inhibitory in higher plants (Ashton & Crafts 1981).

When sprayed from airplanes over large areas, rivers,
estuaries and inlets are not spared. According to the
recommendations in the B.C. FIELD CROP GUIDE 1983, 2,4-D may be
sprayed in solutions of 0.5 M maximum concentration. This

concentration results when the maximum recommended amount of 2,4-D ha^{-1} is dissolved in the smallest recommended volume. In running or highly mixed waters this initial concentration is immediately diluted. Stratification of 2,4-D with depth has been reported in stagnant waters where highest concentrations were found near the surface and phytotoxic activity showed little decrease for periods of several months (Wojtalik et al. 1971; Bovey & Young 1980). Degradation of 2,4-D depends on oxygen concentration and temperature. While the toxicity of 2,4-D on fish, including salmonids (Meehan et al. 1974), freshwater and estuarine invertebrates and freshwater algae have been studied, no such information has been assembled with respect to marine microplankton.

The herbicide 2,4-D was added on day 3 just before the start of the phytoplankton bloom. The addition of the 10^{-3} M solution caused a drop in pH in the lower salinity waters (2 units at ≤ 5 ppt; 1 unit at 10 ppt). In these microcosms the pH never rose above 7 during the entire experiment. At 18 ppt and ≥ 26 ppt salinity, the addition of 2,4-D did not change the pH because of the buffering qualities of seawater (cf. Appendix 1).

After the addition of 2,4-D at 10^{-4} M final concentration, the chlorophyll a curves in the ≤ 5 ppt and ≥ 26 ppt salinity microcosms were not significantly different from the controls (Figure 31). At 10 ppt (Figure 32) the chlorophyll a peak was delayed by 2 days but of the same peak height, while at 18 ppt the delay was less than 2 days and the peak height slightly
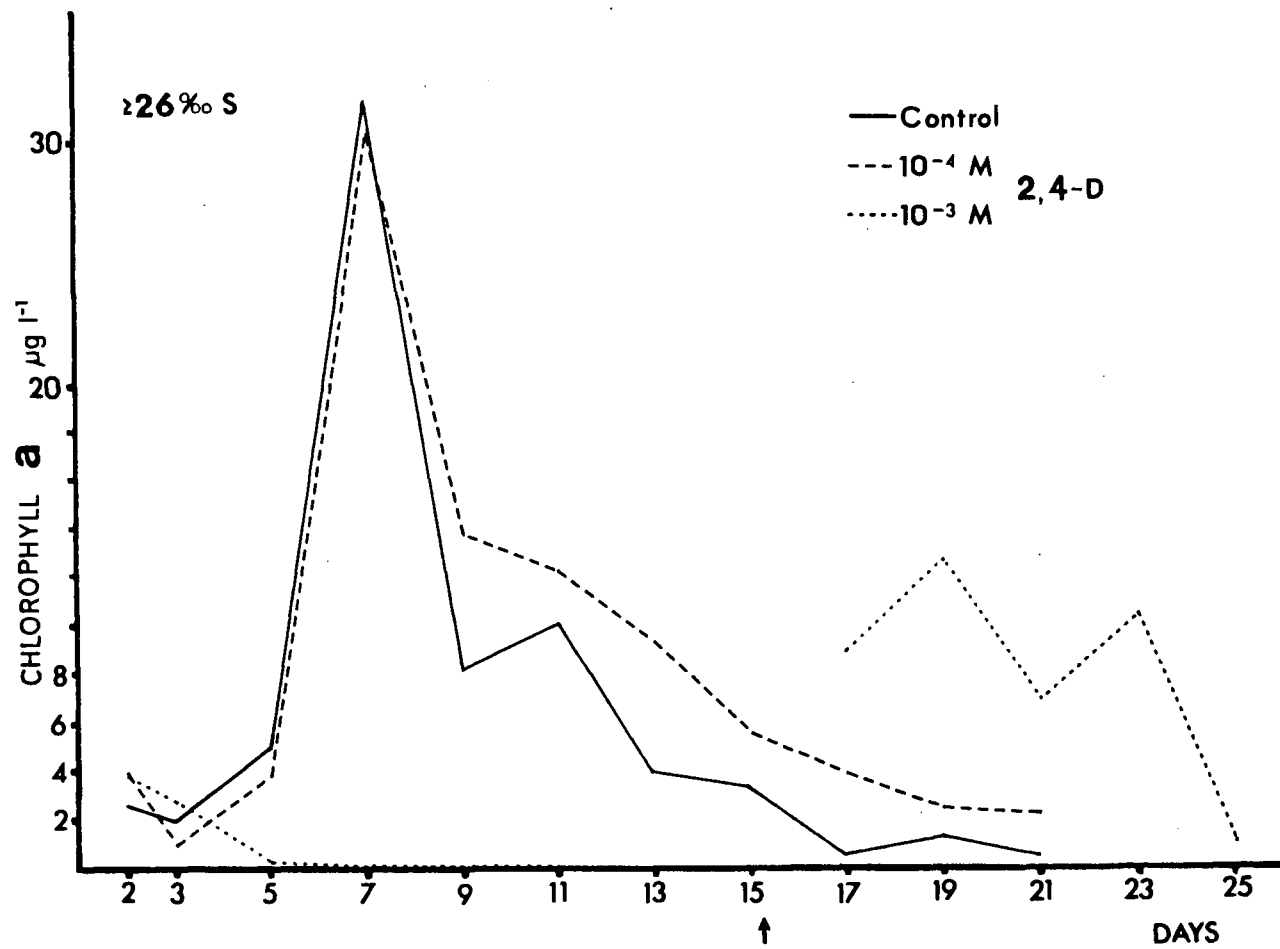
Figure 31 - Chlorophyll a concentrations in ≥ 26 ppt
salinity microcosms after addition of 2,4-D.
Arrow indicates second nutrient addition.
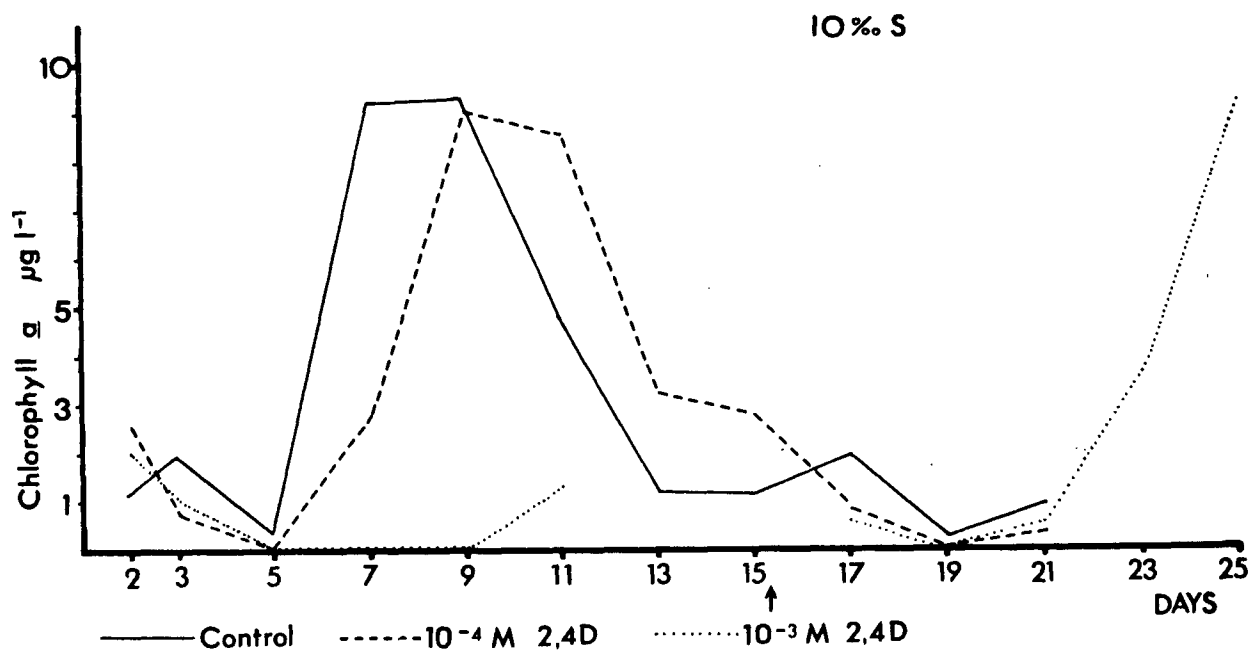
Figure 32 - Chlorophyll a concentrations in 10 ppt salinity
microcosms after addition of 2,4-D.
Arrow indicates second nutrient addition.

decreased. After addition of 2,4-D at 10^{-3} M final concentration no phytoplankton bloom occurred at any salinity until day 11.

On day 15 a second nutrient addition (same concentrations as in initial standard addition) was added to 3 microcosms ($\leq$ 5 ppt, 10 ppt and $\geq$ 26 ppt salinity) which previously had received 10^{-3} M 2,4-D. While chlorophyll a levels at 18 ppt (no second nutrient addition) remained low (c. 2 μg l^{-1}), a bloom of green flagellates started on day 21 in the $\leq$ 5 ppt and 10 ppt salinity microcosms (Figure 32). In the $\geq$ 26 ppt salinity container chlorophyll a values were over 8 μg l^{-1} on day 17, hovered around 10 μg l^{-1}, before dropping to 1 μg l^{-1} on day 25 (Figure 31). A visible difference in colour between the $\leq$ 5 ppt, the 10 ppt and the $\geq$ 26 ppt salinity microcosms was observed. The former two were green, while the latter yellowish-brown. In both low salinity containers the bloom was composed of green flagellates (Chlamydomonas sp., - identified and isolated as #440 by J.Acreman, curator of NEPCC) while at $\geq$ 26 ppt salinity green flagellates and diatoms were present.

The species composition was not significantly altered by 10^{-4} M 2,4-D at $\leq$ 5 ppt, 10 ppt and $\geq$ 26 ppt salinities, except for an apparent increase in numbers of Chaetoceros spp. at these salinities (cf. Appendix 6). However, at 18 ppt salinity numbers of the latter were significantly higher (25.0%±9.2) than in the controls (6.5%±5.0). The bloom induced after a second nutrient addition in the $\geq$ 26 ppt salinity microcosm was composed of Thalassiosira spp. (69.5%) and of green flagellates

(20.8%).

Bacterial numbers increased in the presence of 10^{-4} M and 10^{-3} M 2,4-D in microcosms, where pH was not altered. The higher numbers in 10^{-3} M 2,4-D might be partially attributed to the addition of ethanol which accounted for 10.7% of the carbon total (cf. Appendix 6).

According to studies of bacteria in the soil, some microbes are inhibited while others are stimulated by the presence of 2,4-D. Facultative anaerobes are more tolerant, gram negatives less inhibited than gram positive ones and 2,4-D is more toxic in acidic soils than in alkaline soils (Bovey & Young 1980). This might explain the reduced numbers of bacteria at $\leq$ 5 ppt and 10 ppt salinity where, in the presence of high 2,4-D concentrations, the pH was lowered by 1 to 2 units.

The disappearance of inorganic nitrogen in the containers with 10^{-3} M 2,4-D, where no phytoplankton bloom occurred, remains unexplained. Bacterial numbers were only slightly higher than in the controls. Heterotrophic activity was not measured, but counts of microzooplankton did not suggest higher grazing pressure in 2,4-D perturbed microcosms (cf. Appendix 6).

The effect of 2,4-D on phytoplankton has been variously reported in the literature. In laboratory bioassays of natural freshwater phytoplankton assemblages a stimulation of photosynthesis at 2 mg l^{-1} 2,4-D has been reported, while 10 mg l^{-1} and > 22 mg l^{-1} have been reported not to kill 18 genera of algae (Boyle 1980). This is in agreement with my results obtained in the estuarine microcosms, where 10^{-4} M

(22.1 mg l^{-1}) 2,4-D showed no significant alteration of chlorophyll $\underline{a}$ peak values. Poorman (1973) reported growth inhibition of <u>Euglena</u> <u>gracilis</u> after a 7 day exposure to 100 mg l^{-1} 2,4-D. In the present experiments 10^{-3} M (221 mg l^{-1}) 2,4-D prevented a phytoplankton bloom entirely. Observations after the second nutrient addition on day 15 seem to indicate that 2,4-D concentrations had been substantially lowered due to biological and chemical decomposition, to allow the growth of green flagellates and diatoms, especially <u>Thalassiosira</u> spp..

4. SUMMARY

An experimental approach was used to determine the factors, natural or man-made, which have the greatest impact on estuarine microplankton ecology. In microcosms filled with natural water of ≤ 5 ppt, 10 ppt, 18 ppt, and ≥ 26 ppt salinity, initial nutrient addition resulted in a simulated phytoplankton spring bloom. The influence of high organic load (glucose), shading, pollutants such as heavy metals and the herbicide 2,4-D on the microplankton populations were monitored.

The addition of glucose stimulated microbial growth, and at low inorganic nutrient concentrations, bacteria competed successfully with phytoplankton for the limiting substrate, thus causing a depression or total suppression of the simulated bloom. Low irradiances delayed the bloom and caused a change in the diatom species composition. Heavy metals, added as a mixture or Cu alone, altered phytoplankton growth only when their concentrations exceeded five to ten times those found in moderately polluted estuaries. At 10 ppt salinity even the highest test concentration employed did not significantly reduce the bloom, because the dominant diatom at this salinity was Skeletonema costatum, a species which is most resistant to heavy metal pollution. The herbicide 2,4-D had no significant impact on the phytoplankton bloom at 10^{-4} M concentration, but at 10^{-3} M the growth of algae was totally suppressed. Such high concentrations are unlikely to occur in the estuarine environment because of rapid mixing and dilution, and

decomposition of 2,4-D.

The impact of natural and man-made perturbations could be seen as an alteration of the species composition prior to changes in the timing and the magnitude of the simulated phytoplankton bloom. The greatest effect of perturbations was obtained in these experiments by manipulations which could be caused by natural events, such as shading from silt load or increases in organic substrate due to run-off. While these events could also be generated by man in an estuary, the heavy metal and herbicide perturbation, which should be regarded entirely as anthropogenic, caused less change in the phytoplankton bloom.

VI. FIELD STUDY IN THE FRASER RIVER ESTUARY

1. INTRODUCTION

Pritchard (1967) defined an estuary as a semi-enclosed coastal body of water with a free connection to the open sea and within which sea water is measurably diluted with fresh water derived from land drainage. Further classifications are based on geomorphological structures, the balance between freshwater inflow and evaporation, as well as estuarine circulation patterns. With respect to the latter, the Fraser River estuary is characterized by a tidally pulsed salt wedge in the lower reach of the river itself and a highly stratified 'plume' that extends over the central and southern part of the Strait of Georgia (LeBlond 1983). Recent reviews on the physical oceanography of the area are presented by Thomson (1981) and LeBlond (1983).

Freshwater runoff from the Fraser River determines the distribution of salinity, hence density in the estuary and their seasonal variations. Meltwater from snow and glaciers constitute more than two-thirds of the total runoff with a maximum in late May and early June. River flow and tidal amplitude determine the propagation of the salt wedge. The intrusion of salt water reaches furthest under low flow conditions, before being swept out again with the ebb tide. From the rivers's mouth, a shallow, brackish surface layer

(plume) spreads into the Strait of Georgia. Surface salinities vary between 0.5 ppt and 27 ppt during the annual cycle. Lowest values are found during the freshet in the vicinity of the mouth, while in winter, when runoff is small, salinities in the central and southern Strait of Georgia are uniformly high.

Entrainment of nutrient-rich deep water and runoff seem to enhance microplankton growth in the estuary (Parsons et al. 1970; Stockner et al. 1980; Albright 1983b), while the large sediment load of the plume water restricts light penetration, thus affecting photosynthesis. In a recent review by Harrison et al. (1983) the biological oceanography of the Strait of Georgia, including the Fraser River estuary, is summarized. Freshwater runoff causes large variations in salinity, but the effects on the microplankton community have yet to be examined.

In the present study biologically important factors are related to changes in salinity which result when fresh and salt water mix in the Fraser River estuary. The findings are compared to data from laboratory microcosms, in which a salinity range was simulated in order to study its influence on the microplankton community under experimental conditions.

2. SAMPLING IN THE FIELD

Surface water samples (1 m depth) were collected on a monthly basis from July 1981 to July 1982 in the southern part of the central Strait of Georgia. From May to July and in September sampling was done twice a month. Figure 1 shows the ship's course with its 42 fixed stations. It took approx. 18 h to visit all stations during one cruise. The cruises were part of an extensive STD-survey undertaken by Dr W.J. Emery (Dept. of Oceanography, UBC). The cruise track covered an area off the Fraser River mouth and provided the opportunity to take samples of different salinities according to the salinity values in the laboratory experiments. It did not, however, allow for the collection of $\leq$ 5 ppt, 10 ppt, 18 ppt, and $\geq$ 26 ppt salinity water during every month of the year, because the extent and direction of the Fraser River plume changes seasonally, as well as with tides and wind. During the winter months only higher salinities could be sampled, while in summer, during a period of highest run-off, only lower salinities up to 18 ppt were encountered.

At selected stations water samples were taken with a 5-1 Niskin bottle. On shipboard, temperature and salinity of the water were measured with a YSI Model 33 portable salinity-temperature-conductivity meter (Yellow Springs Instrument Co., Yellow Springs, Ohio, USA). If salinities were $\leq$ 5 ppt, 10 $\pm$ 1 ppt, 18 $\pm$ 1 ppt, or $\geq$ 26 ppt subsamples were taken for chlorophyll $\underline{a}$, nitrate and dissolved monosaccharide

determination, for total bacterial numbers, heterotrophic activity measurements, and phytoplankton identification. Usually triplicate samples were collected for each salinity value. For laboratory analysis, samples were immediately filtered, frozen and/or preserved. Subsamples for measurement of heterotrophic activity were processed on the ship. The analyses of all samples were done as described in Chapter II.

3. RESULTS AND DISCUSSION

3.1 Inorganic Nitrogen And Phytoplankton

Nitrogen, phosphate and silicate are essential macro-
nutrients for phytoplankton, especially diatoms. Among the
major inorganic nutrients, the only one to be supplied in
abundance by the Fraser River is silicate. Small amounts of
nitrogen and phosphorus are present as well (Drinnan & Clark
1980), but these elements are found in much larger amounts in
seawater which is entrained into the surface layer. During the
summer month it is believed that nitrogen becomes limiting
(Harrison et al. 1983).

By measuring ambient nitrate and nitrite concentrations in
the Fraser River estuary one would expect concentrations to
increase with increasing salinity. Field data in Figure 33 show
that this was indeed true for the greater part of the year. For
at least eight months, from October to May, the primary source
of nitrate and nitrite was deep saline water rather than land-
derived input. In the summer, from June to September, nitrogen
concentrations were low over the entire salinity range due to
increased uptake by microplankton. Under these conditions
nitrogen recycling by zooplankton and input from land (sewage)
becomes increasingly important in addition to entrainment.
Short periods of strong winds can also replenish nitrogen in the
euphotic zone, resulting in enhanced phytoplankton growth
(Takahashi et al. 1977).
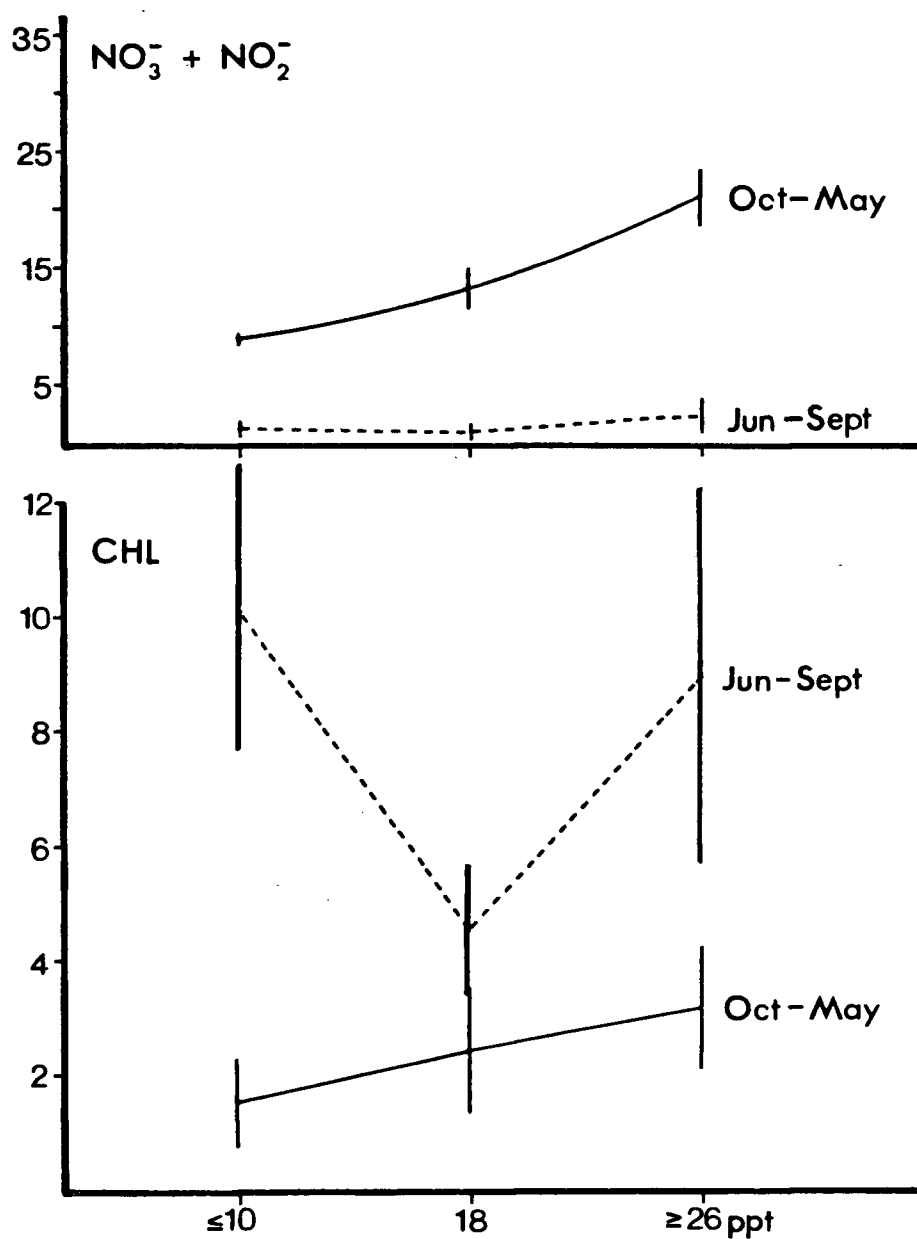
Figure 33 - Concentrations of nitrate + nitrite (μg-at l^{-1})
and chlorophyll $\underline{a}$ (μg l^{-1}) with respect to increasing salinities
at different seasons of the year.
Bars represent ± 1 S.E. of mean.

With respect to nitrogen concentrations, the nutrient addition to the laboratory microcosms simulated the range found in the Fraser River estuary during the greater part of the year. While in the laboratory experiments highest nitrogen concentrations, hence highest salinities, gave rise to the largest amount of phytoplankton biomass, the correlation in the field is masked by factors that were excluded from the microcosms. Most important among these being light limitation, grazing and patchiness. As shown in Figure 33, there seems to be a correlation between nitrogen (i.e. salinity) and chlorophyll a pigment values, when concentrations for the period October to May are compared. The values of both nitrogen and chlorophyll a showed an increase with salinity. Statistically, however, pigment concentrations were not significantly different over the salinity range. In the period from June to September greatest phytoplankton biomass coincided with very low nitrate and nitrite concentrations in the estuary. Under these conditions a large proportion was probably "regenerated production" (Dugdale & Goering 1967).

The cruise in mid-April 1982 coincided with the diatom spring bloom in the Strait of Georgia. The species composition of the plankton (26 ppt salinity) showed that in the 400 cells counted, only diatoms were present and of these, 93.8% were Skeletonema costatum and Thalassiosira spp. to the ratio of 3:2 respectively. While the former dominated on a quantitative basis, the latter contributed most to the biomass of the bloom because of its larger cell size. In all microcosm experiments

the simulated phytoplankton bloom was composed of the same two species assemblages, but under laboratory conditions $\underline{S}$. costatum did better than Thalassiosira spp., resulting in a 3:1 ratio at $\geq$ 18 ppt salinities. Throughout the summer, $\underline{S}$. costatum and Thalassiosira spp. maintained a strong presence, occasionally even forming another bloom (cf. Appendix 7).

The almost absolute dominance of $\underline{S}$. costatum at 10 ppt salinity as found in the simulated microcosm blooms was not seen in the field. During the natural spring bloom in the Fraser River estuary the cruise track did not allow for sufficient sampling of the lower salinities and the summer months were characterized by greater diversity in diatom species. In mid-June, 1982, small Thalassiosira spp. dominated the phytoplankton in the lowest salinity sample ($\leq$ 5 ppt) and three weeks later they accounted for about 70% relative abundance at $\leq$ 5 ppt and 10 ppt salinities. At the same time, $\underline{S}$. costatum was the dominant species at higher salinities. In the previous year (August 1981), $\underline{S}$. costatum formed a summer bloom at 18 ppt salinity, while over the entire sampling period its relative abundance at $\leq$ 5 ppt salinity did not exceed 6%. This finding agreed with results of microcosm experiments, where at the lowest salinity Thalassiosira spp. were always dominant in the laboratory blooms, while $\underline{S}$. costatum was found at 10 ppt and higher salinities. Chaetoceros spp. were of minor importance in microcosm experiments, but were bloom-forming species in the field during the summer months. Apparently, the laboratory manipulations resulted in a selection of a spring bloom diatom

assemblage, rather than conditions favourable to a summer bloom species like <u>Chaetoceros</u> spp. in the Fraser River estuary.

3.2 Dissolved Monosaccharide Concentrations

Total dissolved organic carbon (DOC) in the Strait of Georgia varies between 1.5 mg l^{-1} in winter and about 3.0 mg l^{-1} in summer (Parsons 1979). Dissolved monosaccharides (MCHO) may constitute 8-24% of the total DOC (Sieburth 1979), thus representing a considerable amount of readily available substrate for heterotrophs. Soluble carbohydrates, measured by the phenolsulfuric acid method of up to 3 mg l^{-1}, have been reported in the Nanaimo River estuary during July (Seki <u>et al.</u> 1969).

Throughout the field study in the Fraser River estuary, highest values of dissolved monosaccharides ranged between 500 and 620 μg MCHO-C l^{-1}. The precision of the analysis was ± 33 μg MCHO-C l^{-1} for values up to 500 μg l^{-1}. Concentrations did not show a distinct monthly maximum, but rather a correlation with salinity, i.e. distance from land and primary production. As shown in Figure 34a mean concentrations of dissolved MCHO-C were about double at low salinities during summer when compared to values at 18 ppt and ≥ 26 ppt salinities. Average concentrations at the highest salinity were comparable to values found in Narragansett Bay, R.I., during winter employing the same analytical method (Johnson & Sieburth 1977), while peak values at low salinities in the Fraser River estuary were significantly higher during the summer.

At the highest salinity (≥ 26 ppt), samples from all
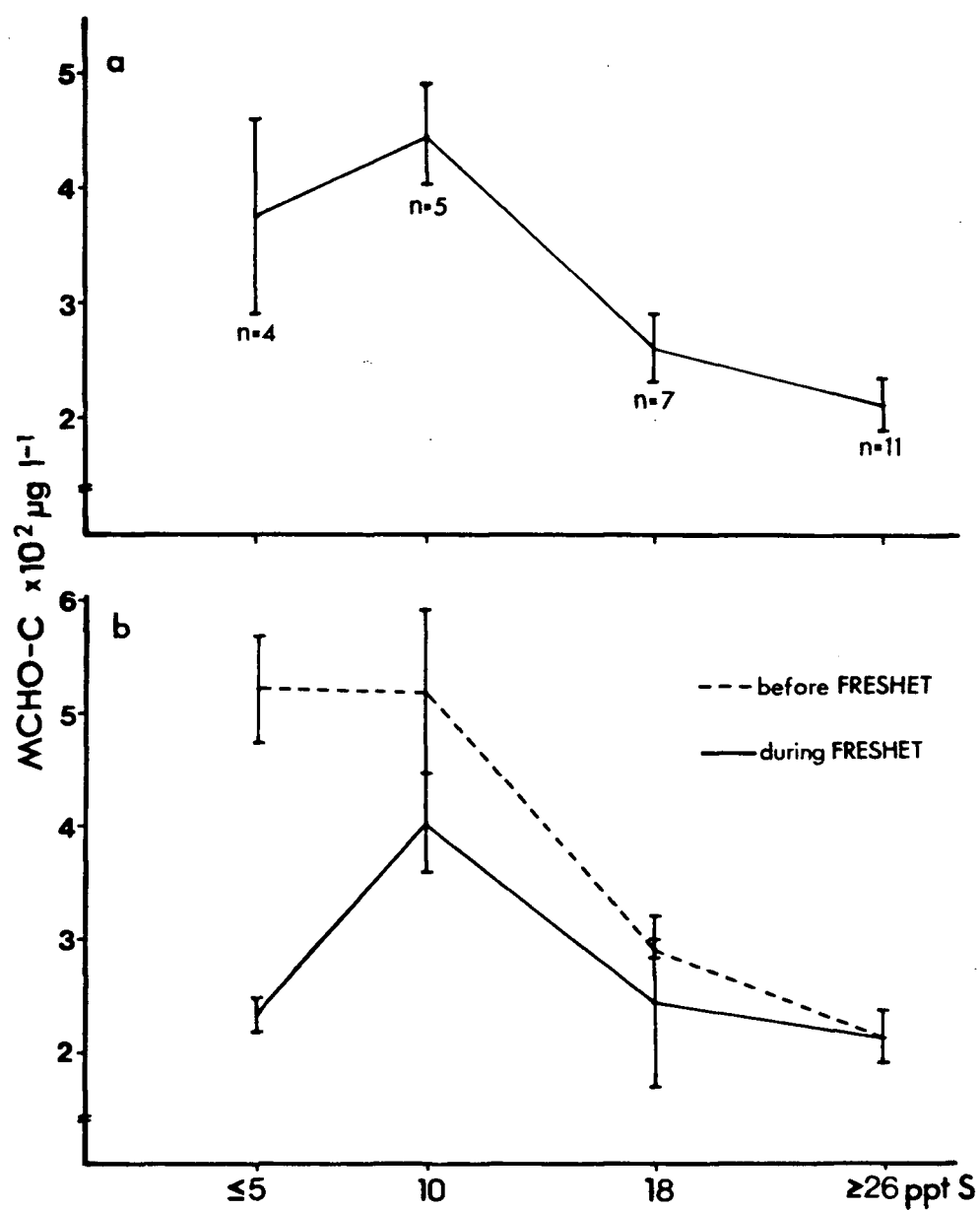
Figure 34 - Changes of dissolved monosaccharide concentrations with salinity. a - mean values of all cruise samples; b - mean values before and during the freshet; n=number of cruises.

seasons showed little variability (Figure 34), while at low salinities local hydrographic and biological factors caused considerable variation in the dissolved MCHO data. In Figure 34b the results of two cruises before and at the onset of the freshet are compared to data found during the period of highest runoff. Before the freshet, highest MCHO-C concentrations were associated with lowest salinities clearly indicating land derived input, while with increasing distance from land, i.e. higher salinities, concentrations dropped significantly. During the freshet, MCHO-C values were low at lowest salinity probably due to dilution. The maximum concentrations at 10 ppt and the large variability at 18 ppt salinity seemed to indicate that at this time dissolved monosaccharides were generated in situ rather than supplied by runoff. Before the onset of the freshet, chlorophyll a concentrations were low, while during the peak flow phytoplankton biomass increased about ten-fold suggesting a correlation between MCHO-C and higher primary production and grazing activities during this period of the year. While the main sources of dissolved monosaccharides seemed to vary with the seasons as does total DOC, the percentage of MCHO-C in the central and southern Strait of Georgia appeared to be constant throughout the year at approx. 14%.

In the laboratory control-experiments 1 mg l^{-1} glucose was added to all containers over the entire salinity range. While the total amount was about double the natural concentrations of dissolved monosaccharides at low salinities before the onset of

the freshet, the decrease of MCHO-C with increasing salinities was not simulated.

The presence of different microbial populations in the low and high salinity waters and during the different seasons, was reflected in the variable response to the substrate addition, as seen in the timing of the initial heterotrophic bloom and in absolute numbers at the peak (see Chapter III). While the initial addition of glucose was depleted during the first heterotrophic bloom, increased MCHO-C concentrations were found during the decline of the autotrophic bloom (up to 685 μg MCHO-C 1^{-1}). Extracellular dissolved monosaccharide to chlorophyll a ratios in the microcosms were 17 at $\leq$ 5 ppt, 28 at 10 ppt, 24 at 18 ppt, and again 28 at $\geq$ 26 ppt salinities. The smaller ratio at the lowest salinity might reflect the presence of green algae during the phytoplankton bloom, which have an intracellular carbohydrate to chlorophyll a ratio about 2/3 of that of Skeletonema costatum (ratio=21), the dominant species at higher salinities (Parsons et al. 1961). In the field, MCHO-C concentrations seemed to follow peaks in chlorophyll a with a 2 - 4 week time-lag (cf. Figure 35).

3.3 Bacterial Numbers

Bacterial biomass in estuaries is reported to vary with seasons and salinity (Albright 1977; Palumbo & Ferguson 1978; Ducklow & Kirchman 1983). During the one year sampling period in the Fraser River estuary a total of 75 samples were analysed for bacterial numbers. Table 6 shows significant seasonal differences in bacterial abundance. In the period between late

spring and summer bacterial biomass was two- to four-fold higher
than during the rest of the year.

Table 6 - Bacterial numbers ($\times 10^5$ ml^{-1}) at different
seasons of the year (mean ± 1 S.E.); n = number of cruises.

Salinity	29/30 Sept.-8 Febr.	13 April-16/17 Sept.
≤ 5 ppt	no samples	21.02 ±2.83 (n=4)
10 ppt	no samples	17.52 ±1.55 (n=5)
18 ppt	11.83 ±2.45 (n=3)	19.87 ±2.72 (n=6)
≥ 26 ppt	5.82 ±1.69 (n=6)	21.12 ±2.65 (n=6)

Numbers seemed to increase following the diatom spring bloom and
remained high during the summer month in the presence of larger
phytoplankton biomass (Figure 35).

Total bacterial numbers in the Fraser River estuary varied
between 1.8x10^5 ml^{-1} (lowest count) in December and
4.36x10^6 ml^{-1} (highest count) in August (cf. Appendix 2).
Numbers were lower than those found by Palumbo & Ferguson (1978)
in the shallow marsh estuary of the Newport River, N.C., but
were comparable to bacterial counts reported by Albright (1983a)
in three estuaries of the B.C. coast. While total bacterial
numbers of 75 samples varied little more than one order of
magnitude over the one year sampling period, the variation was

considerably lower than in counts given by Bell & Albright (1981). Employing the same enumeration technique their study reported numbers of more than 10^7 or even 10^8 bacteria ml^{-1}.

In laboratory experiments, after standard nutrient addition, bacterial numbers varied in the same range as in the field. Somewhat higher total numbers were found following the autotrophic bloom (highest count 5.4×10^6 ml^{-1}). Only in perturbation experiments, after the addition of 5-15 mg l^{-1} glucose, did bacterial numbers increase to a maximum of approx. 2.4×10^7 ml^{-1}.

In the Fraser River estuary a correlation between bacterial numbers and salinity was found only during fall and winter at 18 ppt and $\geq$ 26 ppt salinities, the only salinity values encountered during these months; total bacterial counts decreased with higher salinity (Table 6). Palumbo & Ferguson (1978) have explained the inverse linear distribution with salinity by conservative mixing of bacteria. In previous studies of the Fraser River estuary, decreasing bacterial numbers as measured by epifluorescence microscopy have been reported with increasing salinities (Bell & Albright 1981; Valdes & Albright 1981; Albright 1983a), but the results from the river and high salinity waters are statistically not significantly different and the variability of the samples is not indicated. According to results in Table 6 for the spring/summer period, the main phytoplankton growing seasons, total bacterial counts did not significantly differ over the entire salinity range of $\leq$ 5 ppt to $\geq$ 26 ppt.

The slight decrease in bacteria (Table 6) from ≤ 5 ppt to 10 ppt salinity during the period of greatest runoff might have been partially caused by dilution and/or sedimentation. The Fraser River carries a large silt load and the majority of bacterial cells seemed to be attached to particles (Bell & Albright 1981). Goulder (1976) found a positive correlation between attached cells and the number of particles in the Humber River. Attachment of bacteria may result in too low cell counts during epifluorescent enumeration because not all the cells might be visible, being hidden underneath particles. Chances of underestimation become progressively less with higher salinities. Increased sedimentation of particles occurs when fresh and salt water meet and the surface charge of particulates changes. In this process, particles as well as the attached bacteria are reduced in numbers. While sedimentation could explain the decrease in bacterial numbers during fall and winter and from ≤ 5 ppt to 10 ppt salinity in spring and summer, the subsequent increase in bacterial cells at higher salinities during the latter seasons must be due to other factors, such as increases in substrate concentrations resulting from primary production and possibly higher temperatures.

In laboratory experiments the second heterotrophic bloom followed the decline of the simulated phytoplankton bloom. A correlation between the increase in bacterial numbers and the presence of phaeopigments was suggested (cf. Figure 13), but a relationship between peak chlorophyll a concentrations and the subsequent increase in bacterial biomass could not be

established on a quantitative basis. The prevailing bacterial populations might have changed in the source waters during different seasons and the different natural populations might have needed varied times to adapt to changing experimental conditions.

3.4 Heterotrophic Activity

All measurements of relative heterotrophic activity were related to bacterial biomass in the sample and expressed as glucose uptake, μg h^{-1} per 10^9 bacteria. While bacterial biomass varied roughly one order of magnitude between the spring/summer and the fall/winter seasons, relative heterotrophic activities were also significantly different over the same periods of the year as shown in Table 7.

Table 7 – Relative heterotrophic activities (μg glucose h^{-1} per 10^9 bacteria) at different seasons of the year (mean ±1 S.E.); n = number of cruises

Salinity	29/30 Sept.-8 Febr.	13 April-16/17 Sept.
≤ 5 ppt	no samples	0.26 ±0.03 (n=4)
10 ppt	no samples	0.25 ±0.06 (n=5)
18 ppt	0.72 ±0.06 (n=3)	0.23 ±0.08 (n=5)
≥ 26 ppt	0.65 ±0.04 (n=6)	0.19 ±0.07 (n=5)

Relative heterotrophic activity was about three-fold higher during the fall and winter when bacteria were less abundant, than in the spring and summer. While heterotrophic activities showed significant seasonal variations, a correlation with salinity could not be established in the presence of seasonal and sample variation.

Among the factors which influence heterotrophic activity and growth, nutrients and temperature seemed to be the most important ones (Hoppe 1978). A study from Narragansett Bay, R.I., shows that seasonal changes in the water temperature were sufficient to select thermal and taxonomic types (Sieburth 1967). The different bacterial populations during the yearly cycle were found to have distinct growth maxima at a certain temperature. In the Fraser River estuary, colder temperatures seem to select bacterial populations with high heterotrophic activity (cf. Table 7 and Figure 35) while in summer populations heterotrophic activity seems to be lower. With sudden changes in organic substrate concentrations, yet another population might gain dominance (cf. Figure 35). It seems that glucose heterotrophic activity characterizes distinct bacterial populations, thriving at particular environmental conditions.

In laboratory experiments, in containers with a mixture of high and low salinity waters, higher heterotrophic activity was found at 10 ppt and 18 ppt salinities (cf. Chapter III, Table 1). Most likely, the mixture provided better growth conditions for a particular bacterial population present in the parent waters. With respect to heterotrophic activity, an

analogy between the laboratory results and observations in the estuary is purely speculative without further investigations.

Based on several transects through the Fraser River estuary, a stimulation of heterotrophic activities at intermediate salinities has been suggested by Valdes & Albright (1981) and Albright (1983a). These authors explain the stimulation by "a more nutritious milieu" in the estuary, which may be partially due to entrainment of deep saline water rich in nitrate and phosphate. But inorganic nutrients by themselves are of limited stimulating influence on heterotrophic bacteria; organic substrate is of greater importance (cf. Chapter III & IV; Seki et al. 1969). As shown in Figure 34, dissolved monosaccharide concentrations decrease with increasing salinity during the major part of the year. The higher heterotrophic activities at intermediate salinities might be due to various factors, e.g. one particular population of heterotrophs with preference for glucose uptake thriving under the given environmental conditions and changing conditions might favour a different population at any one time (Sieburth 1967).

3.5 Interactions Of Biotic And Abiotic Factors In The Estuary

Figure 35 summarizes seasonal variations in the microplankton, substrate concentrations, and temperature as observed during the one year field study in the Fraser River estuary. Because the timing of events at ≥ 10 ppt salinities did not differ, the data were lumped together and mean values were plotted. In the period from December to April, only ≥ 26 ppt salinities were encountered and depicted.
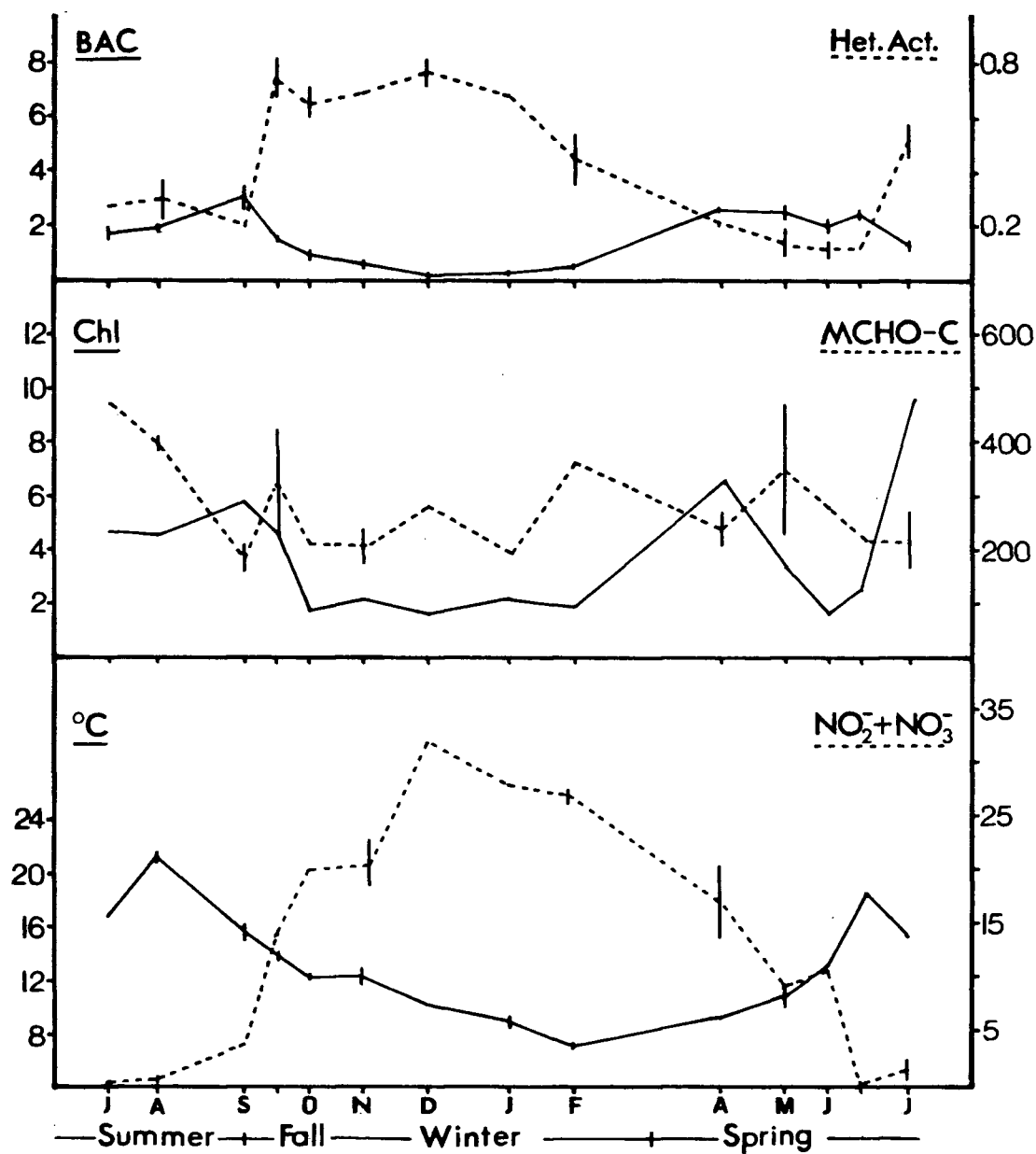
Figure 35 - Seasonal cycle of bacteria ($\times 10^6$ ml^{-1}), heterotrophic activity (μg glucose h^{-1} per 10^9 bacteria), chlorophyll $\underline{a}$ (μg l^{-1}), dissolved monosaccharides (μg MCHO-C l^{-1}), nitrate + nitrite (μg-at-N l^{-1}), and temperature ($^\circ$C).

As the Fraser River is located in temperate latitudes, seasonal changes have a major influence on the microplankton ecology. Additional variation is introduced by specific hydrological factors, e.g. the freshet. Temperature in surface waters varied between 7°C in February and 21°C in August. Low temperatures are shown to inhibit phytoplankton growth in early spring (Takahashi et al. 1973). The warming of the waters and increasing irradiances resulted in a diatom spring bloom in April, which was followed by a heterotrophic bloom in May (cf. Appendix 7). In plankton samples small flagellates increased in relative abundance to about 80%. With the onset of the freshet, chlorophyll a concentrations dropped further, because of dilution and light limitation in the silt-laden waters. River runoff and higher temperatures caused stratification, and another rise in chlorophyll a concentrations led to nitrate depletion at the height of the summer bloom. An increase in inorganic nitrogen in September resulted in a fall bloom, before temperatures dropped to winter values. Between October and February, chlorophyll a concentrations hovered around 2 μg l^{-1} while the plankton was dominated by small flagellates.

Temperature changes seemed to affect bacterial populations as well. At least three different dominant populations could be distinguished. In September the summer population with relative low heterotrophic activity was replaced by a winter population of low biomass but high heterotrophic activity. With the warming of the waters in spring, bacterial biomass increased

while heterotrophic activity decreased, marking the takeover by a different population. Yet another bacterial group seemed to be able to take advantage of increases in organic substrate in the presence of the summer autotroph bloom and high temperatures. There might have been many more distinct bacterial populations which could not be distinguished in the widly-spaced sampling pattern followed and the set of analyses performed. Increases in bacterial biomass seemed to coincide with higher primary production in fall and spring, while during the summer bloom bacterial numbers dropped, perphaps partially due to intense grazing. The low bacterial biomass in winter in the presence of large microzooplankton populations might also be explained by grazing pressure, while the high heterotrophic activity might be indicative of relatively high production.

Nitrate and nitrite concentrations showed an inverse relationship with chlorophyll a values, being high in the winter and low in the summer. Dissolved monosaccharide concentrations seemed to lag behind peaks in phytoplankton biomass by 2-4 weeks. But the correlation was obscured by large variability and very likely by the fast response of bacterial populations to increases in organic substrate (Brockmann et al. 1979).

4. SUMMARY

The results of a year long field survey in the Fraser River estuary have been presented. Surface water samples covering a salinity range from ≤ 5 ppt to ≥ 26 ppt were analyzed for nitrate and nitrite, dissolved monosaccharides, chlorophyll a, bacterial numbers, relative heterotrophic activity and microplankton species.

Seasonal and hydrographic changes in the estuary are shown to affect the microplankton ecology. Two major periods were distinguished, fall/winter and spring/summer, during which data differed significantly.

Throughout the greater part of the year, entrainment of deep saline water was identified as the major source of nitrogen. With respect to this process, the laboratory microcosms simulated field conditions, including the dominance of certain phytoplankton species found in both the natural and simulated spring bloom.

Dissolved monosaccharides were supplied either by runoff or autotrophic production. When supply by runoff dominated, concentrations decreased with distance from land, i.e. salinity, with in situ production dominant, highest values were found at intermediate salinities. An apparent increase in MCHO-C followed peaks in chlorophyll a concentrations in both the field and in laboratory microcosms.

Bacterial numbers varied between the same range in the field and in the laboratory after the standard nutrient

additions. In spring and summer, numbers in the field were significantly higher in the presence of enhanced primary production and numbers increased following the simulated bloom in the microcosms. A correlation with salinity was observed during the fall and the winter periods in the estuary.

Heterotrophic activities seemed to vary with changes in the seasonally dominant bacterial population. High values were found in winter associated with low biomass, while during the summer populations seemed to be more diverse. Higher relative heterotrophic activity in the field and in the laboratory might be indicative of a bacterial population best adapted to a prevailing environmental situation.

The seasonal cycle in the Fraser River estuary has been summarized with respect to the sample analyses performed. The interaction of abiotic and biotic factors resulted in a distinct pattern of microplankton succession.

VII. DISCUSSION

1. INTRODUCTION

More than 15 years ago Strickland (1967) advocated controlled environmental ecosystem experiments to fill the gap between the "super-naturalists" and the beaker-environmentalists in aquatic ecology. With new techniques, such as remote sensing from space, the former group acquired a powerful tool in studying large scale processes in the oceans, while the latter continued to reveal physiological responses of single or multiple species cultures to changing laboratory conditions. Controlled ecosystem research does not replace the need for culture experiments, nor does it make ocean surveys unnecessary (Parsons 1981). It does, however, allow greater species combinations to occur than can be obtained under normal culture procedures; thus, a combination of all three approaches might best promote one's understanding of marine aquatic ecosystems.

Wherever enclosures have been used in order to obtain an exact replica of nature, the results have been disappointing (Menzel & Steele 1978). The goal of microcosm studies is not to squeeze a natural ecosystem into containers of varying size and attempt to study 'the real world' in miniature; this goal is neither attainable or necessary (Oviatt et al. 1977). Microcosms are useful as biological models; the scales are different from the natural ecosystem, but natural events are

more closely simulated than in a single species culture (Parsons 1978).

The advantage of enclosures is largely in the study of interactions, rate processes over time, species assemblages and in the achievement of a mass balance for distribution of certain elements (Menzel & Steele 1978; Parsons 1981). Biological as well as mathematical models allow manipulations so that effects and limits of various forcing functions and biological coefficients may be better understood (Parsons 1978).

2. <u>METHODOLOGY</u>

In summarizing the experimental results of the CEPEX project, Menzel (1977) concluded that small-scale laboratory experiments with natural populations can be used with some confidence to predict events at the microplankton level in much larger systems. The aim of this thesis was to demonstrate the potential of microcosms in estuarine microplankton research and to evaluate their usefulness in testing the impact of natural and man-made perturbations.

2.1 <u>Spring Bloom Simulation</u>

A simulation of spring bloom conditions was chosen for two reasons: first, the diatom spring bloom marks the beginning of the annual growing season and determines the abundance of zooplankton, primarily herbivorous copepods which are the food of larval and juvenile fish later in the season (Parsons 1976). Any variation of the extent and timing of the spring bloom, as well as any change from a diatom based food chain to one based on bacteria and microzooplankton, might result in lower yield to the fishery (Greve & Parsons 1977). Second, resemblances between the dynamic behaviour of microcosms and that of natural systems have previously been shown in freshwater lake microcosms, where, following a well defined initiation procedure, a spring-like bloom occurred (Harte <u>et al</u>. 1980).

In microcosm experiments a sequence of natural conditions was simulated comparable to those in the field between winter

and late spring (cf. Chapters III and VI). Initially, the microplankton was dominated by small microflagellates, not unlike the winter populations (Takahashi & Hoskins 1978). The nutrient addition created an environment similar to that preceding the natural spring bloom, and laboratory conditions as described in Chapter II favoured the growth of diatoms. Despite changing species composition in the inoculum, the simulated bloom was always composed of Skeletonema costatum and Thalassiosira spp., a finding consistent with the natural diatom spring bloom of the Fraser River estuary (Takahashi et al. 1973; Shim 1976). In the laboratory as well as in the field, the decline in primary production was followed by an increase in bacterial numbers (Figures 13, 35) and microzooplankton abundance (Chapter III, Section 2; Chapter VI).

By choosing only one event for simulation, the diatom spring bloom, it is not postulated that other events are less important or not worthwhile studying or that the effects of the perturbations during the spring-like bloom are the same as throughout the year. The laboratory set-up and experimental conditions chosen were not suitable to simulate summer conditions when temperatures are higher, stratification is present and a different nutrient regime prevails – all factors favouring a different microplankton population (Chapter VI).

2.2 Simulation Of The Salinity Range

While the spring bloom simulation represents a study over time, the salinity range describes the spatial dimension in an estuary; it stands for increasing distance from the freshwater source. Due to estuarine circulation, tidal and wind mixing, salinity and temperature are very dynamic environmental properties and their representation in static containers and under constant laboratory conditions may seem inadequate. Since estuarine ecosystems are "open" systems, governed by import and export, Margalef (1967) and Cooper & Copeland (1973) considered continuous culture methodology more appropriate than batch type cultures because of the closer resemblance of the former to estuarine dynamics. The difference in approach is a matter of microcosm philosophy, as to whether the goal is a microecosystem a la nature, or a study-aid to investigate processes, interactions and species assemblages. While even the most sophisticated apparatus - still to be developed - will not give an exact replica of a natural estuary, the batch culture microcosms proved adequate for the purpose of this study in order to investigate the influence of salinity and associated factors on an estuarine microplankton community.

Due to entrainment of nutrient-rich deep water not only salinity increases, but inorganic nitrogen and phosphate as well. Many other environmental factors are either positively or negatively correlated with the increasing amount of seawater mixed into freshwater (cf. Chapters III and VI). Local conditions determine the indigenous plankton community and the

seasonal progression is regulated by changes in these conditions (Smayda 1980). Experimental and field observations show that phytoplankton species tolerate wide ranges of salinities (e.g. Braarud 1951; McLachlan 1961), therefore salinity per se is not considered to be a strong regulatory factor (Smayda 1980). However, in estuarine environments, Remane & Schlieper (1971) found a species minimum at 6 ppt, with increasing abundance of freshwater and marine species at lower and higher values, respectively.

The microcosms were specially suited to study the effect of salinity because other important factors, such as light and temperature, were kept constant. The different salinities were mixed from two source waters and the nutrient addition was well defined. Over the experimental salinity range, significant differences in species composition were found (Figure 10). In the lowest salinity microcosms, growth of S. costatum seemed to be inhibited, an observation consistent with the diminished abundance of this species in low salinity field samples (cf. Appendix 7). Paasche (1975) found the growth rate of an Oslofjord clone of S. costatum reduced by half at 4 ppt salinity and observed no growth at ≤ 2 ppt. Thus, salinity might affect phytoplankton species composition by competitive exclusion. The virtual domination of S. costatum at 10 ppt has a parallel in a sediment assemblage observed in Howe Sound, B.C. (Roelofs in press). Consequently, while the salinity range employed here differed from the more dynamic values found in nature, the results from the field and microcosm experiments complemented

each other and indicated that salinity might have a selective influence where extreme salinity fluctuations prevail.

Since distinct phytoplankton populations dominated different salinities, perturbations might have a different impact depending on the species present. Results of the perturbation experiments are discussed below (Section 4). It would be interesting to undertake a taxonomic study of bacteria in order to establish whether different populations grow in the different salinity microcosms, whether these match the field populations, how the zymogenous population differs from the autochthonous and whether the zymogenous species differ with the salinity values. Such a study might unravel the still largely unexplained increase in heterotrophic activities at intermediate salinities (Albright 1983b).

2.3 Replication And Reproducibility

By isolating a viable part of a natural ecosystem in laboratory microcosms, the variability and patchiness of nature due to the physical dissimilarity of water masses is excluded. Nevertheless, statistical replication is problematical in systems which tend towards random behaviour. However, despite the difficulties of replicability, microcosms have shown to be useful experimental tools in ecological research and pollution studies (Giesy, Jr. 1980).

The simulation of only one natural event, a careful mixing of high and low salinity source waters to enhance an even distribution of chemical and biological properties between

microcosms, and a well defined nutrient addition, resulted in good replication of duplicate experiments as shown in Chapter III. After all, the effect of perturbations is difficult to evaluate if the variance between replicates exceeds that between treatments. Effects of phasing between two identically treated microcosms were present, but were thought to be negligible in the short-term experiments (Figures 8 and 9). Therefore, no statistical tests were performed to eliminate the effects of phasing.

On a seasonal basis, the emphasis in the microcosm approach was not on statistical replication, but rather on obtaining reproducible results with respect to the standard pattern. Thus the impact of perturbations could be appraised relative to the standard pattern.

2.4 Analytical Methods

The sample analyses performed were thought to be important with respect to the emphasis of this thesis, but did not cover all analyses considered to be interesting and worthwhile studying, due to limitations on the absolute time available to a single researcher.

For nutrient measurements and in vitro chlorophyll a determination, standard methods as described by Strickland & Parsons (1972) were employed (cf. Chapter II). The extracted chlorophyll a was taken as an estimate of phytoplankton biomass. While under standard conditions and in the field, pigment analysis gives acceptable estimates, in perturbation experiments, chlorophyll a, as the only measure for biomass,

might lead to erroneous results. This became apparent in the shaded microcosms where the increased pigment concentration reflected a physiological response to the lower irradiance rather than an indication of a higher biomass.

Using epifluorescence microscopy as described by Hobbie et al. (1977), total bacterial numbers could be subject to underestimation: bacteria might be hidden underneath particles and very small ones might be missed altogether. Although, Zimmermann (1977) calculated that ca. 10% of the total bacteria could pass through 0.2 μm pore size Nuclepore filters, the same filters were used here.

The measurement of relative heterotrophic activity by uptake of small organic molecules describes only one aspect of microbial activity (Es & Meyer-Reill 1982) but allows for a comparison of heterotrophic activity between samples and seasons. Testing one substrate (glucose) might influence the results in favour of those bacteria preferring glucose as a carbon and energy source. The method is not suitable for the measurement of total activity (Hanson 1980) and does not allow for conclusions to be drawn concerning bacterial growth.

The dissolved monosaccharide assay described by Johnson & Sieburth (1977) is very sensitive and contamination in a routine laboratory can cause considerable problems. The three part analysis begins with a borohydride reduction to convert pentoses and hexoses to their sugar alcohols. The total alditols are then oxidized with periodate, to form two moles of formaldehyde per mole of monosaccharide. The formaldehyde is analysed

spectrophotometrically with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH).

The analysis includes sugar alcohols but not polysaccharides. With respect to the latter, it is unique among the tests for carbohydrates in seawater and it is superior to other methods in that calibration curves for equimolar concentrations of different carbohydrates are similar. The variation is only due to small differences in molecular weight between pentoses and hexoses. With respect to other organic compounds present in seawater, only serine interferes with the reaction, but at concentrations far exceeding those in seawater (Johnson & Sieburth 1977).

The enumeration of small flagellates was problematical. Live counts were tedious and had to be performed within a short time after sampling. Replicate counts showed large variability, because cells were moving around very fast. Coloured and colourless flagellates could not be distinguished under these circumstances. In preserved samples, a better fixation method may reduce deformation of cells and loss of flagella (van der Veer 1982). In order to distinguish autotrophic and heterotrophic microflagellates, the fluorochrome, fluorescein isothiocyanate (FITC) staining has been suggested as being superior to acridine orange (Sherr & Sherr 1983).

3. THE MERITS OF NUMERICAL MODELING

Numerical modeling was used to describe the processes within the Dark- and Light-microcosms with respect to the growth of microplankton as well as interactions between the two trophic levels, bacteria and heterotrophic flagellates. The models were used to test the validity of the measurements and the assumptions derived from these data. It was necessary to summarize all knowledge about the microcosms, as well as the underlying assumptions; the latter had to be clearly and quantitatively expressed. This process showed where understanding and data were missing. In spite of the rather simplistic modeling attempt, useful information was gained with respect to the processes within the microcosms.

3.1 The Dark-model

The original parameter set derived from experimental observations, literature data and Fenchel's (1982b) model, did not represent the events in the Dark-microcosms. By adjusting the parameters, it became apparent that the model's behaviour was more sensitive to some coefficients than to others. Reasonable changes in bacterial growth rate and half-saturation constants for bacteria and glucose had little influence on the model's output, while changes in the initial values of the state variables, bacteria and microflagellates, as well as bacterial efficiency and cell quota were of much greater importance. Reducing bacterial efficiency had a similar effect to an

increase in the cell quota, but the depletion of the initially added 5 mg l^{-1} glucose in combination with experimentally observed numbers of bacteria could not be achieved without an increase in the quota. The higher glucose cell quota of bacteria was most likely a response to substrate enrichment (Mandelstam et al. 1982; Es & Meyer-Reill 1982), and although high, represented a value not unknown in coastal areas (Azam et al. 1983).

The increase in glucose cell quota required changes in other parameters in order to develop a biologically reasonable model. Without adjustment of the maximum consumption rate and gross growth efficiency of the flagellates, their carbon intake would increase six-fold and would result in a six-fold increase in their biomass, with total numbers being far too low. Following a 75% reduction in consumption rate and a proportional increase in gross growth efficiency, the flagellates in the Dark-model were 1.5 times bigger in terms of their glucose quota compared to those in Fenchel's (1982b) model, while only 42% as efficient as grazers.

Fenchel's model of prey/predator cycles is based on a field study carried out during summer in shallow Limfjorden, Denmark, and on experimentally determined growth and consumption rates of heterotrophic flagellates. Bacterial numbers in the field fluctuated between 1.5-3x10^6 ml^{-1}. Assuming a constant bacterial growth rate, his model showed an inherent tendency for cyclical behaviour which could help to explain the relatively constant and low bacterial concentrations usually found in the

marine environment. However, Fenchel (1982b) suggests that the cycles were most likely initiated by a summer peak in primary production and that the amplitudes would tend to decrease over time. His model does not support such suggestions. On the contrary, the amplitudes tend to increase over time, due to the assumption of a constant bacterial growth rate. If the cycles are indeed initiated by an increase in organic substrate, his model does not account for such changes.

In Fenchel's model as well as in the Dark-model, bacterial growth rates were assumed to be constant. But the former model predicted prey/predator cycles, while the latter resulted in single bacterial and flagellate blooms. The dissimilarity was due to different initial conditions. While in Fenchel's model, all bacteria present were assumed to be growing, in the Dark-microcosms, only a small fraction was considered to be active and dividing. The substrate enrichment did not only result in bigger individual bacteria, but presumably caused changes in the bacterial population. Faster growing species were selected; thus flagellates were no longer able to maintain grazing control. The resulting numbers of bacteria were two to three-fold higher than those occurring in the field and in Fenchel's model. Similarly, not all flagellates initially present in the Dark-microcosms were heterotrophs, i.e. bacteriovores. Without the substantial reduction in the initial values of bacteria and microflagellates, the Dark-model led to prey/predator oscillations instead of the observed large single peaks.

In the presence of high substrate concentrations, the

glucose cell quota determined the timing of the bacterial bloom and grazing the peak numbers. The impact of grazing was regulated by the initial number of flagellates, the maximum consumption rate and the gross growth efficiency of the flagellates. While substrate concentrations were clearly of major importance, the Dark-model predicted the conversion of 57% of the bacterial carbon into flagellate biomass. With such high ecological efficiency, grazing was significant in the transfer of energy to higher trophic levels.

No universal parameter set fitted all salinities, indicating that the processes in the microcosms differed. But with minor adjustments in the glucose quota and in the initial value of bacterial and flagellate numbers, the Dark-model could be adapted to experimental observations at all salinities, such as differences in the lag phase of bacteria resulting in an up to 36 h shift in the timing of the bacterial bloom.

The Dark-model was useful in identifying the important coefficients and the nature of interactions between parameters. The dynamics described represented neither a classical prey/predator nor a totally substrate-controlled system, but rather something in between. The model was validated by simulation and comparison with respect to the bacterial dynamics, while the validity of flagellate dynamics could not be established, due to the lack of proper data. The model was insecure in some assumptions and did not lead to a single solution; further refinement was therefore abandoned.

3.2 The Light-model

The simulation of the Light-microcosms was restricted to the initial growth phase because the model ignored the release of organic substances by phytoplankton, the excretion of nitrogenous compounds by microflagellates and remineralization. The bacterial parameters arrived at in fitting the Dark-model were also found to be appropriate in the simulation of the Light-microcosms. Using the same parameters, total bacterial numbers, the timing of the first heterotrophic bloom and bacterial nitrogen uptake compared favourably with experimental results, at least for the $\geq$ 10 ppt salinities (Figures 18, 19).

Phytoplankton growth was simulated as a function of external nutrient concentrations, as previously done in many other models (e.g. Steele 1974; Winter et al. 1975). Because of the above mentioned assumption, the Light-model could not predict an increase in chlorophyll a following nutrient depletion. Only more sophisticated nutrient growth models, allowing for variable cell quotas and luxury consumption, would be able to do so. The Light-model, however, allowed one to budget inorganic nitrogen in control- and glucose-perturbed microcosms. By comparing the experimentally found results with those predicted by the model, it was possible to quantify the amount of phytoplankton biomass based on recycled nitrogen.

Johannes (1968) hypothesized that contrary to terrestrial ecosystems, where bacteria and fungi dominate nutrient regeneration processes, direct regeneration by bacteria in the marine environment constitutes only a minor fraction and under

certain conditions, bacteria and phytoplankton may even compete for inorganic nutrients. The latter has been confirmed in many studies (e.g. Rhee 1972; Parker et al. 1975; Parsons et al. 1981) as well as in this thesis (Chapters IV, V). Bacteria facilitate nutrient recycling by breaking down organic matter and by assimilating organic as well as inorganic substrates. They are themselves then consumed by bacteriovores and nutrients are recycled by excretion (e.g. ammonium and phosphate compounds). Recent findings of a more rapid recycling of particulate matter in nature than by microbial activity alone, support the concept that heterotrophic flagellates and microzooplankton play a major role in the remineralization in the sea (Harrison 1980; Mann 1982). Azam et al. (1983) suggest a 'microbial loop', including microzooplankton, to account for rapid nutrient recycling in the euphotic zone. Microheterotrophs seem to account for the largest fraction of regenerated nitrogen in many areas, up to 90% and more in coastal regions (e.g. Harrison 1978).

The excretion of microzooplankton has not been measured in the field because of the difficulty in separating the microplankton components (Harrison 1980). The microcosm experiments in combination with numerical modeling made it possible to indirectly assess the importance of recycling (Chapter IV). With exclusion of macrozooplankton, the discrepancy between phytoplankton biomass production as predicted by the Light-model and the actually observed value in the glucose-perturbations can be explained by microflagellate

excretion. Up to 66% of the phytoplankton biomass following the addition of 5 mg l^{-1} glucose might be attributed to "regenerated production" (Dugdale & Goering 1967), the percentage being highest in the presence of low nitrate and nitrite concentrations which agrees with findings in the field (Harrison 1980).

In the microcosm experiments, growth rates of bacteria and diatoms (_Skeletonema costatum_) were identical. A similar observation was made in a study of microbial plankton dynamics off Hawaii (Landry _et al_. unpubl. MS). The competitive advantage of bacteria over phytoplankton seems to be based on a faster response to environmental changes rather than to differences in growth rates of the dominant populations. The lag phase of bacteria in the microcosms was 1/3 of that of the algae. In the presence of a plentiful carbon source, bacteria might have already depleted a limiting nitrogen source before the diatoms entered the exponential growth phase.

4. PERTURBATIONS AND POLLUTANTS IN MICROCOSMS

In order to preserve the biological integrity of a valuable estuarine ecosystem, a better understanding of the natural ecology, as well as of the effects of perturbations is necessary. The purpose of this study was to evaluate the possible contribution microcosm research can make to this end. Results in Chapter V indicate that perturbations which could be caused by natural events or man's activity had much greater impact on the microplankton population than those of exclusively anthropogenic origin. With respect to the latter, the microcosm experiments reflect short-term observations; toxic effects attributable to long-term exposure are not seen, but these might be of importance, as well as the transfer of pollutants to higher trophic levels.

The initial nutrient enrichment of the natural waters in the microcosm experiments - certainly a perturbation in itself - enhanced the natural nutrient levels in a salt wedge estuary and facilitated the establishment of a reproducible pattern not unlike that of a natural diatom spring bloom. The perturbation by nutrient enrichment was thought to be necessary to set a 'base line' for further manipulations. The similarity between microcosm and natural events, e.g. the sequence of autotrophic and heterotrophic growth and the species composition, justified this approach.

4.1 Heterotrophic Populations

All perturbations performed affected the microplankton community. Glucose as well as any other organic compound, such as 2,4-D, were most likely the primary regulators of bacterial activity, as shown by the two order of magnitude higher glucose uptake following the 5 mg l^{-1} addition (Table 1, Chapter III) compared to field samples (Table 7, Chapter VI). Vaccaro (1969) postulated that as a result of organic enrichment a reduction of functional species occurs, while the total bacterial population increases. The great diversity in a natural microbial community enables bacteria to adapt to constantly changing nutritional environments (Lelong et al. 1980). Prevailing physical and substrate conditions select the most suitable species assemblage and subsequent changes in conditions determine the succession. In the presence of high organic concentrations, bacteria outcompete phytoplankton for a limiting nutrient because the former optimize the species composition more rapidly. Glucose addition increased bacterial production and resulted in a larger microflagellate population.

Parsons et al. (1981), in reporting on enrichment studies in CEPEX bags, suggested a direct energy transfer from bacteria to zooplankton. Size spectrum analysis did not indicate an intermediary step; higher secondary and tertiary production was observed. In the microcosms however, in the absence of net-zooplankton, grazing by microflagellates was important with respect to carbon transfer as well as recycling of nutrients (Chapter IV). A possible change in the food chain from an

autotrophic to a heterotrophic system was indicated in the lowest salinity microcosm, where flagellates accounted for about 50% of the plankton (Table 3). If, instead of a direct energy transfer from bacteria to zooplankton, microflagellates represent the link between the trophic levels, the yield of the system is very likely reduced.

Light might have a stimulatory effect on bacterial uptake of sugars and amino acids, but results do not seem to be conclusive (Es & Meyer-Reill 1982). A reduction in irradiance affected bacteria indirectly. The autotrophic bloom, thus the availability of organic substrates, was delayed and the second heterotrophic bloom appeared to be postponed. An increase in bacterial numbers following heavy metal addition has been observed in CEPEX experiments (Vaccaro et al. 1977) as well as in the microcosms. The stimulation of bacterial growth was most likely due to the release of organic substrate by phytoplankton under stress; a view favoured by the results of the CEPEX study (Vaccaro et al. 1977).

In CEPEX experiments heterotrophic activity was increased following copper additions but the values returned to pre-exposure levels after a few days (Menzel 1977); thus the method seems to have little merit as an indicator for pollution. Therefore, measurements of heterotrophic activity were not performed during metal-perturbations in the microcosms. However, bioassay experiments with proper controls and pollutant concentrations well above ambient may indicate whether tolerance has been developed and thus suggest if the population tested had

been exposed earlier to that pollutant (Menzel 1977).

4.2 Autotrophic Populations

Following glucose enrichment, competition of bacteria with algae for limiting nitrogen led to a depression, even total suppression, of the phytoplankton bloom (Figures 23, 24). The impact was reduced due to nutrient recycling by microzooplankton (Chapter IV).

Light reduction, whether caused by a natural silt-load or increased particulate matter from sewage or industrial effluents, might have a large impact on the estuarine phytoplankton bloom and consequently higher trophic levels. In shaded microcosms the algal bloom was delayed by 2-6 days. Light limitation reduces algal growth rates and a small inoculum of cells might be constantly flushed out of the estuary. A bloom may take much longer to develop and might occur eventually further seaward. The resulting mismatch of primary and secondary production with larval fish development might have deleterious implications for the fishery.

Light may also play a role in the determination of the species composition (Figure 27). Lower irradiances seemed to favour smaller species and cells, an important aspect in size-selected feeding of the dominant zooplankters.

In the microcosms, with heavy metals added, the centric diatoms Thalassiosira spp. were replaced by a population of pennates, and following copper additions, Nitzschia spp. seemed to be more abundant. Both observations agree with findings during the CEPEX experiments, where the selection of resistent

species resulted in a larger abundance of pennate diatoms, which might not be preferred by zooplankton (Thomas et al. 1977). Nitzschia spp. are known to excrete large amounts of organic substances (Steemann Nielsen & Wium-Andersen 1971) which may play a role in detoxification. While the actual reduction in algal biomass did not seem significant, changes in species dominance might have considerable implications. Algal species composition was shown to be a more sensitive indicator of metal perturbations than changes in biomass (Chapter V, Section 3). Copper additions are known to reduce photosynthesis (Thomas et al. 1977), but the effects will depend on the short-term pollution history of the original environment which will determine the sensitivity of a particular algal clone (Murphy & Belastock 1980).

The toxicity of metals, as shown in culture experiments with copper, is related to the ion activity rather than the total concentration. The ion activity in turn can be altered independently of total metal concentrations by variations in the amount of chelator and pH (Sunda & Guillard 1976). The pH in the Fraser River varies between 7 and 8, but might be as low as 6 (Drinnan & Clark 1980). The lower the pH, the smaller the copper ion activity and the effect of the heavy metal pollutant. Metal binding to the microcosm surfaces can also affect the apparent toxicity by lowering the total concentration of the ions in solution (Struempler 1973). Without knowing the chelator concentrations and in the presence of seasonally and biologically induced pH changes, the evaluation of heavy metal

perturbations becomes difficult, and the impact is better
assessed by using indicator species (Phillips 1977).

5. GENERAL CONCLUSIONS

The use of microcosms as experimental tools in estuarine research appears to be justified and is in keeping with earlier conclusions by (Menzel 1977) and Marshall & Mellinger (1980) who showed that small microcosms were a good representation of events in larger mesocosms, at least at the level of microplankton.

The experiments demonstrate that a careful initiation method results in good statistical replication of duplicate microcosms. Furthermore, the technique can be used to give reproducible results in terms of microplankton production over a salinity range from ≤ 5 ppt to ≥ 26 ppt despite seasonal variations in the parent water masses. The same laboratory conditions (constant light, temperature and nutrient addition) produce a constant response with respect to the microplankton pattern throughout the year. The diatom spring bloom generated in the laboratory, the so-called 'standard pattern', represents a realistic simulation of natural blooms as recorded in the Fraser River estuary.

Microcosms seem especially suited for physiological studies and ecological research, when the latter is verified by field observations or the former are examined in totally controlled laboratory culture experiments. In combination with numerical modeling, the validity of measurements and assumptions derived from the data can be tested. By representing the biological processes in the microcosms in mathematical models, important

coefficients can be identified and by changing the coefficients, their sensitivity with respect to the ecosystem processes can be evaluated.

Bacterial glucose quota and the gross growth efficiency of microflagellates are shown to have a dominant effect on the heterotrophic dynamics in the microcosms. The nature of interactions between microplankton can be identified and it is possible to estimate the ecological efficiency between trophic levels. Microcosm experiments, in combination with numerical modeling, make it possible to indirectly assess important processes, e.g. nitrogen recycling, which would be difficult or impossible to measure directly.

The experiments demonstrate that a technique can be used to assess the effects of natural and man-made perturbations. While people are largely concerned about the latter, nature actually controls the ecosystem behaviour more than man, in spite of the latter's pollutants. The greatest impact of a perturbation on the 'standard pattern' was obtained in these experiments by manipulations which could be caused by natural events, such as shading from silt load or increased organic substrate due to runoff. While these events could also be generated by man in an estuary, the heavy metal perturbation experiments, which should be regarded entirely as a simulated effect of man, caused less change in the 'standard pattern'. While nature seems to control the ecosystem, pollutants may still be important in other ways, i.e. in causing disease in man.

The use of microcosms in monitoring short-term effects has

been demonstrated. For the assessment of long-term changes caused by anthropogenic pollution other techniques might be superior.

BIBLIOGRAPHY

Albright, L.J. 1977. Heterotrophic bacterial dynamics in the lower Fraser River, its estuary and Georgia Strait, British Columbia, Canada. Mar. Biol. 39:203-11.

Albright, L.J. 1983a. Influence of river-ocean plumes upon bacterioplankton production of the Strait of Georgia, British Columbia. Mar. Ecol. Prog. Ser. 12:107-13.

Albright, L.J. 1983b. Heterotrophic bacterial biomasses, activities and productivities within the Fraser River plume. Can. J. Fish. Aquat. Sci. 40 (Suppl. 1):216-20.

Antia, N.J., C.D. McAllister, T.R. Parsons, K. Stephens and J.D.H. Strickland. 1963. Further measurements of primary production using a large-volume plastic sphere. Limnol. Oceanogr. 8:166-83.

Armstrong, F.A.J., C.R. Stearns and J.D.H. Strickland. 1967. The measurement of upwelling and subsequent biological processes by means of the Technicon Autoanalyzer and associated equipment. Deep-Sea Res. 14:381-89.

Ashton, F.M. and A.S. Crafts. 1981. Mode of Action of Herbicides. 2nd ed., J. Wiley & Sons, New York 525 p.

Azam, F. and R.E. Hodson. 1977. Size distribution and activity of marine microheterotrophs. Limnol. Oceanogr. 22:492-501.

Azam, F., T. Fenchel, J.G. Field, J.S. Gray, L.A. Meyer-Reil and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10:257-63.

Beardall, J. and I. Morris. 1976. The concepts of light intensity adaptation in marine phytoplankton: some experiments with Phaeodactylum tricornutum. Mar. Biol. 37:377-87.

Bell, C.R. and L.J. Albright. 1981. Attached and free-floating bacteria in the Fraser River estuary, British Columbia, Canada. Mar. Ecol. Prog. Ser. 6:317-27.

Benedict, A.H., K.J. Hall and F.A. Koch. 1973. A preliminary water quality survey of the lower Fraser River system. Tech. Rep. No. 2, Westwater Res. Center, Univ. B.C. 50 p.

Bentley-Mowat, J.A. and S.M. Reid. 1977. Survival of marine phytoplankton in high concentrations of heavy metals, and uptake of copper. J. Exp. Mar. Biol. Ecol. 26:249-64.

Billen, G., C. Joiris, J. Wijnant and G. Gillain. 1980. Concentration and microbiological utilization of small organic molecules in the Scheldt estuary, the Belgian coastal zone of the North Sea and the English Channel. Est. Coast. Mar. Sci. 11:279-94.

Bölter, M. 1981. DOC-turnover and microbial biomass production. Kieler Meeresforsch. Sonderh. 5:304-10.

Bovey, R.W. and A.L. Young. 1980. The Science of 2,4,5-T and Associated Phenoxy Herbicides. J. Wiley & Sons, New York 462 p.

Boyle, T.P. 1980. Effects of the aquatic herbicide 2,4-D DMA on the ecology of experimental ponds. Environ. Pollution (Series A) 21:35-49.

Braarud, T. 1951. Salinity as an ecological factor in marine phytoplankton. Physiologia Pl. 4:28-34.

Brock, T.D. 1971. Microbial growth rates in nature. Bacteriol. Rev. 35:39-58.

Brockmann, U.H., K. Eberlein, H.D. Junge, E. Maier-Reimer and D. Siebers. 1979. The development of a natural plankton population in an outdoor tank with nutrient-poor sea water. II. Changes in dissolved carbohydrates and amino acids. Mar. Ecol. Prog. Ser. 1:283-91.

Cattell, S.A. 1973. The seasonal cycle of vitamin B_{12} in the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. 30: 215-22.

Chan, A.T. 1978. Comparative physiological study of marine diatoms and dinoflagellates in relation to irradiance and cell size. I. Growth under continuous light. J. Phycol. 14:396-402.

Cole, J.J. 1982. Interactions between bacteria and algae in aquatic ecosystems. Ann. Rev. Ecol. Syst. 13:291-314.

Conway, H.L. 1977. Interactions of inorganic nitrogen in the uptake and assimilation by marine phytoplankton. Mar. Biol. 39:221-32.

Cooper, D.C. and B.J. Copeland. 1973. Responses of continuous-series estuarine microecosystems to point-source input variations. Ecological Monographs 43:213-36.

Corpe, W.A. 1974. Periphytic marine bacteria and the formation of microbial films on solid surfaces. In Effect of the Ocean Environment on Microbial Activities. (eds.) R.R. Colwell & R.Y. Morita. Univ. Park Press, p. 397-417.

Cosper, E. 1982. Effects of variations in light intensity on the efficiency of growth of Skeletonema costatum (Bacillariophyceae) in a cyclostat. J. Phycol. 18:360-68.

Cronin, L.E. 1975. Estuarine Research. Academic Press, New York. 738 p.

Cross, F.A. and W.G. Sunda. 1978. Relationship between bioavailability of trace metals and geochemical processes in estuaries. In Estuarine Interactions. (ed.) M.L. Wiley, p. 429-442. Academic Press, New York.

Daley, R.J. and J.E. Hobbie. 1975. Direct counts of aquatic bacteria by a modified epifluoresence technique. Limnol. Oceanogr. 20:875-82.

Davidson, L.W. 1979. On the physical oceanography of Burrard Inlet and Indian Arm, British Columbia. M.Sc. thesis, Univ. of British Columbia. 221 p.

Degobbis, D. 1973. On the storage of seawater samples for ammonia determination. Limnol. Oceanogr. 18:146-50.

DiSalvo, L.H. 1973. Contamination of surfaces by bacterial neuston. Limnol. Oceanogr. 18:165-8.

Drebes, G. 1977. Sexuality. In The Biology of Diatoms. (ed.) D. Werner, p. 250-83. Bot. Monogr. Vol. 13. Univ. of California Press, Berkeley.

Drinnan, R.W. and M.J.R. Clark. 1980. Fraser River estuary study: water quality. Ministry of Environment, Victoria, B.C. and Government of Canada, Ottawa. 160 p.

Droop, M.R. 1974. Heterotrophy of carbon. In Algal physiology and biochemistry. (ed.) W.D. Stewart. p. 530-39. Univ. of California Press, Berkeley.

Ducklow, H.W. and D.L. Kirchman. 1983. Bacterial dynamics and distribution during a spring diatom bloom in the Hudson River plume, USA. J. Plankton Res. 5:333-55.

Dugdale, R.C. and J.J. Goering. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. 12:196-206.

Eaton, A.D. and E.V. Grant. 1979. Sorption of ammonium by glass frits and filters: Implications of analyses of brackish and freshwater. Limnol. Oceanogr. 24:397-99.

Eppley, R.W., J.N. Rogers and J.J. McCarthy. 1969. Half saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. 14:912-20.

Eppley, R.W., P.Koeller and G.T. Wallace Jr. 1978. Stirring influences the phytoplankton species composition within enclosed columns of coastal water. J. Exp. Mar. Biol. Ecol. 32:219-39.

Eppley, R.W., S.G. Horrigan, J.A. Fuhrman, E.R. Brooks, C.C. Price and K. Sellner. 1981. Origins of dissolved organic matter in Southern California coastal waters: experiments on the role of zooplankton. Mar. Ecol. Prog. Ser. 6:149-59.

Es, F.B. van and L.A. Meyer-Reil. 1982. Biomass and metabolic activity of heterotrophic bacteria. In Advances in Microbial Ecology. Vol 6. (ed.) K.C. Marshall, p. 111-58. Plenum Press, New York.

Fenchel, T. 1982a. Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. Mar. Ecol. Prog. Ser. 8:225-31.

Fenchel, T. 1982b. Ecology of heterotrophic microflagellates. IV. Quantitative occurence and importance as bacterial consumers. Mar. Ecol. Prog. Ser. 9:35-42.

Fenchel, T. and T.H. Blackburn. 1979. Bacteria and Mineral Cycling. Academic Press, London, 225 p.

Ferguson, R.L. and P. Rublee. 1976. Contribution of bacteria to standing crop of coastal plankton. Limnol. Oceanogr. 21:141-45.

Field Crop Guide. 1983. To weed, disease, insect, bird and rodent control. Ministry of Agriculture and Food, Victoria, B.C. 142 p.

Fuhrman, J.A., J.W. Ammermann and F. Azam. 1980. Bacterioplankton in the coastal euphotic zone: distribution, activity and possible relationships with phytoplankton. Mar. Biol. 60:201-7.

Fuhs, G.W., S.D. Demmerle, E. Canelli and M. Chen. 1972. Characterization of phosphorus-limited algae (with reflections on the limiting-nutrient concept). In Nutrients and Eutrophication: the limiting-nutrient controversy. (ed.) G.E. Likens, p. 113-32. Am. Soc. Limnol. Oceanogr. Spec. Symp. 1.

Giesy, J.P. Jr (ed.) 1980. Microcosms in Ecological Research. DOE Symposium Series 52, CONF-781101. National Technical Information Service, Springfield, Va. 1110 p.

Goldberg, E.D., E. Gamble, J.J. Griffin and M. Koide. 1977. Pollution history of Narragansett Bay as recorded in its sediments. Estuar. Coast. Mar. Sci. 5:549-61.

Goldman, J.C. and J.J. McCarthy. 1978. Steady state growth and ammonium uptake of a fast-growing marine diatom. Limnol. Oceanogr. 23:695-730.

Goulder, R. 1976. Relationships between suspended solids and standing crops and activities of bacteria in an estuary during a neap-spring-neap tidal cycle. Oecologia 24:83-90.

Greve, W. and T.R. Parsons. 1977. Photosynthesis and fish production: hypothetical effects of climatic change and pollution. Helgoländer wiss. Meeresunters. 30:666-72.

Grice, G.D. and M.R. Reeve (eds.) 1982. In Marine Mesocosms. Springer Verlag, New York, 430 p.

Griffiths, R.P., S.S. Hayasaka, T.M. McNamara and R.Y. Morita. 1977. Comparison between two methods of assaying relative microbial activity in marine environments. Appl. Environ. Microbiol. 34: 801-5.

Haas, L.W. and K.L. Webb. 1979. Nutritional mode of several non-pigmented microflagellates from the York River estuary, Virginia. J. Exp. Mar. Biol. Ecol. 39:125-34.

Haines, K.C. and R.R.L. Guillard. 1974. Growth of vitamin B_{12} requiring marine diatoms with vitamin B_{12} producing marine bacteria. J. Phycol. 10:245-52.

Hamilton, P. and K.B. Macdonald (eds.) 1979. Estuarine and Wetland Processes with Emphasis on Modeling. Plenum Press, New York. 653 p.

Hanson, R.B. 1980. Measuring microbial activity to assess detrital decay and utilization. In Marine Benthic Dynamics. (eds.) K.R. Tenore & B.C. Coull, p. 347-56. Univ. of South Carolina, Columbia.

Haque, R., J.V. Nabholz and M.G. Ryon. 1980. Interlaboratory Evaluation of Microcosm Research: Proceedings of the workshop. EPA-600/9-80-019; ORNL/EIS-160. Nat. Tech. Information Service, Springfield, Virginia, 23 p.

Harrison, P.J., H.L. Conway and R.C. Dugdale. 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. I. Cellular chemical composition and steady-state growth kinetics of Skeletonema costatum. Mar. Biol. 35:177-86.

Harrison, P.J., J.D. Fulton, F.J.R. Taylor and T.R. Parsons. 1983. Review of the biological oceanography of the Strait of Georgia: pelagic environment. Can. J. Fish. Aquat. Sci. 40:1064-94.

Harrison, W.G. 1978. Experimental measurements of nitrogen remineralization in coastal waters. Limnol. Oceanogr. 23:684-94.

Harrison, W.G. 1980. Nutrient regeneration and primary production in the sea. In Primary Productivity in the Sea . (ed.) P.G. Falkowski, p. 433-60. Plenum Press, New York.

Harte, J., D. Levy, J. Rees and E. Saegebarth. 1980. Making microcosms an effective assessment tool. In Microcosms in Ecological Research. (ed.) J.P. Giesy, Jr. p. 105-37. DOE Symposium Series 52, CONF-781101. National Technical Information Service, Springfield, Va.

Heinbokel, J.F. and J.R. Beers. 1979. Studies on the functional role of tintinnids in the southern California Bight: Grazing impact of natural assemblages. Mar. Biol. 52:23-32.

Hellebust, J.A. 1974. Extracellular products. In Algal Physiology and Biochemistry. (ed.) W.D.P. Stewart, p. 838-63. Univ. of California Press, Berkeley.

Hellebust, J.A. and J. Lewin. 1977. Heterotrophic nutrition. In The Biology of Diatoms. (ed.) D. Werner, p. 169-97. Bot. Monogr. Vol. 13. Univ. of California Press, Berkeley.

Hitchcock, G.L. 1980a. Influence of temperature on the growth rate of Skeletonema costatum in response to variations in daily light intensity. Mar. Biol. 57:261-9.

Hitchcock, G.L. 1980b. Diel variation in chlorophyll a, carbohydrate and protein content of the marine diatom Skeletonema costatum. Mar. Biol. 57:271-78.

Hobbie, J.E., C.C. Crawford and K.L. Webb. 1968. Amino acid flux in an estuary. Science 159:1463-64.

Hobbie, J.E., R.J. Daley and S. Jasper. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33:1225-28.

Hollibaugh, J.T., D.L.R. Seibert and W.H. Thomas. 1980. A comparison of the acute toxicities of ten heavy metals to phytoplankton from Saanich Inlet, B.C., Canada. Est. Coast. Mar. Sci. 10:93-105.

Hoppe, H.-G. 1978. Relations between active bacteria and heterotrophic potential in the sea. Netherlands J. Sea Res. 12:78-98.

Hutchinson, G.E. 1961. The paradox of the plankton. Am. Nat. 95:137-45.

Ittekkot, V., U. Brockmann, W. Michaelis and E.T. Degens. 1981. Dissolved free and combined carbohydrates during a phytoplankton bloom in the northern North Sea. Mar. Ecol. Prog. Ser. 4:299-305.

Jannasch, H.W. 1970. Threshold concentrations of carbon sources limiting bacterial growth in seawater. In Organic Matter in Natural Waters. (ed.) D.W. Hood, p. 321-28. Institute of Marine Science, Univ. of Alaska, College.

Johannes, R.E. 1968. Nutrient regeneration in lakes and oceans. In Advances in Microbiology of the Sea, Vol. 1. (eds.) M.R. Droop & E.J. Ferguson Wood, p. 203-13. Academic Press, London.

Johnson, K.M. and J.McN. Sieburth. 1977. Dissolved carbohydrates in seawater. I, a precise spectrophotometric analysis for monosaccharides. Mar. Chem. 5:1-13.

Johnson, K.M., C.M. Burney and J.McN. Sieburth. 1981. Doubling the production and precision of the MBTH spectrophotometric assay for dissolved carbohydrates in seawater. Mar. Chem. 10:467-73.

Kogure, K., U. Simidu and N. Taga. 1979. Effect of Skeletonema costatum (Grev.) Cleve on the growth of marine bacteria. J. Exp. Mar. Biol. Ecol. 36:201-15.

Kremer J.N. and S.W. Nixon. 1978. A Coastal Marine Ecosystem: Simulation and Analysis. Springer-Verlag, Berlin. 217 p.

Landry, M.R., L.W. Haas and V.L. Fagerness, unpubl. MS. Dynamics of microbial plankton communities: experiments in Kaneohe Bay, Hawaii. School of Oceanography, Univ. of Washington, Seattle, WA. 15 p.

Larsson, U. and A. Hagström. 1979. Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. Mar. Biol. 52:199-206.

Lauff, G.H. (ed.) 1967. Estuaries. Am. Ass. Adv. Sci., Publ. 83, Washington, D.C. 757 p.

LeBlond, P. H. 1983. The Strait of Georgia: functional anatomy of a coastal sea. Can. J. Fish. Aquat. Sci. 40:1033-63.

Leffler, J.W. 1980. Microcosmology: Theoretical applications of biological models. In Microcosms in Ecological Research. (ed.) J.P. Giesy, p. 14-29. DOE Symposium Series 52, CONF-781101. National Technical Information Service, Springfield, Va.

Lelong, P.P., M.A. Bianchi and Y.P. Martin. 1980. Dynamique des populations planctoniques et bacterienne au cours d'une production experimentale de phytoplankton marin naturel. II. Structure et physiologie des populations et leurs interactions. Can. J. Microbiol. 26:297-307.

Lenz, J. 1977. Plankton populations. In Microbial Ecology of a Brackish Water Environment. (ed.) G. Rheinheimer. p. 79-89. Ecol. Studies 25, Springer-Verlag, Berlin.

Lewin, J. and C.H. Chen. 1971. Available iron: A limiting factor for marine phytoplankton. Limnol. Oceanogr. 16:670-75.

MacIsaac, J.J. and R.C. Dugdale. 1969. The kinetics of nitrate and ammonium uptake by natural population of marine phytoplankton. Deep-Sea Res. 16:47-57.

Mague, T.H., E. Friberg, D.J. Hughes and I. Morris. 1980. Extracellular release of carbon by marine phytoplankton: a physiological approach. Limnol. Oceanogr. 25:262-79.

Mandelstam, J., K. McQuillen and I. Dawes (eds.) 1982. Biochemistry of Bacterial Growth. Wiley, New York. 449 p.

Mann, K.H. 1982. Ecology of Coastal Waters. A systems approach. Univ. of California, Berkeley. 322 p.

Margalef, R. 1967. Laboratory analogues of estuarine plankton systems. In Estuaries. (ed.) G.H. Lauff, p. 515-21. Am. Ass. Adv. Sci., Publ. 83, Washington, D.C.

Marshall, H.G. 1982. The composition of phytoplankton within Chesapeake Bay plume and adjacent water off the Virginia coast, U.S.A.. Est. Coast. Shelf Sci. 15:29-43.

Marshall, J.S. and D.L. Mellinger. 1980. Dynamics of cadmium-stressed plankton communities. Can. J. Fish. Aquat. Sci. 37:403-414.

Marshall, K.C., R. Stout and R. Mitchell. 1971. Mechanisms of the initial events in the sorption of marine bacteria to surfaces. J. Gen. Microbiol. 68:337-48.

McLachlan, J. 1961. The effect of salinity on growth and chlorophyll content in representative classes of unicellular marine algae. Can. J. Microbiol. 7:399-406.

Meehan, W.R., L.A. Norris and H.S. Sears. 1974. Toxicity of various formulations of 2,4-D to salmonids in Southeast Alaska. J. Fish. Res. Bd. Can. 31:480-5.

Menzel, D.W. 1977. Summary of experimental results: controlled ecosystem pollution experiments. Bull. Mar. Sci. 27:142-45.

Menzel, D.W. and J.H. Steele. 1978. The application of plastic enclosures to the study of pelagic marine biota. Rapp. p. -v. Reun. Cons. int. Explor. Mer 173:7-12.

Meyer-Reil, L.-A. 1977. Bacterial growth rates and biomass production. In Microbial ecology of a brackish water environment. (ed.) G. Rheinheimer, p. 223-36. Springer-Verlag, Berlin.

Morel, N.M.L., J.G. Rueter and F.M.M. Morel. 1978. Copper toxicity to Skeletonema costatum (Bacillariophyceae). J. Phycol. 14:43-8.

Murphy, L.S. and R.A. Belastock. 1980. The effect of environmental origin on the response of marine diatoms to chemical stress. Limnol. Oceanogr. 25:160-5.

Neilson, B.J. and L.E. Cronin (eds.) 1979. Estuaries and Nutrients. Humana Press, Clifton , N.J. 643 p.

Oviatt, C.A. 1981. Effects of different mixing schedules on phytoplankton, zooplankton and nutrients in marine microcosms. Mar. Ecol. Prog. Ser. 4:57-67.

Oviatt, C.A., K.T. Perez and S.W. Nixon. 1977. Multivariate analysis of experimental marine ecosystems. Helgoländer wiss. Meeresunters. 30:30-46.

Paasche, E. 1975. The influence of salinity on the growth of some plankton diatoms from brackish water. Norw. J. Bot. 22:209-15.

Paasche, E., S. Johansson and D.L. Evensen. 1975. An effect of osmotic pressure on the valve morphology of the diatom Skeletonema subsalsum (A. Cleve) Bethge. Phycologia 14:205-11.

Palumbo, A.V. and R.L. Ferguson. 1978. Distribution of suspended bacteria in the Newport River estuary, N.C.. Est. Coast. Mar. Sci. 7:521-29.

Parker, R.R., J. Sibert and T.J. Brown. 1975. Inhibition of primary productivity through heterotrophic competition for nitrate in a stratified estuary. J. Fish. Res. Bd. Can. 32:72-7.

Parsons, T.R. 1972. Pollution problems in the Strait of Georgia. In Marine Pollution and Sealife. (ed.) M. Ruivo, p. 72-6. FAO, Fishing News (Books) LTD., Surrey, England.

Parsons, T.R. 1976. The IBP Strait of Georgia program. In Energy flow - its biological dimensions. The IBP in Canada: 1964-74. (eds.) T.W.M. Cameron & L.W. Billingsley, p. 267-74. Royal Society of Canada, Ottawa.

Parsons, T.R. 1978. Controlled ecosystem experiments. Rapp. P.-v. Reun. Cons. int. Explor. Mer 173:5-6.

Parsons, T.R. 1979. The Strait of Georgia Programme. In Marine Production Mechanisms. (ed.) M.J. Dunbar, p. 133-49. IBP 20. Cambridge Univ. Press.

Parsons, T.R. 1981. The use of controlled experimental ecosystems: a review. J. Oceanogr. Soc. Japan 37:294-8.

Parsons, T.R., K. Stephens and J.D.H. Strickland. 1961. On the chemical composition of eleven species of marine phytoplankters. J. Fish. Res. Bd. Can. 18:1001-16.

Parsons, T.R., K. Stephens and R.J. LeBrasseur. 1969. Production studies in the Strait of Georgia. Part I. Primary production under the Fraser River plume, Feb.-May 1967. J. Exp. Mar. Biol. Ecol. 3:27-38.

Parsons, T.R., R.J. LeBrasseur and W.E. Barraclough. 1970. Levels of production in the pelagic environment of the Strait of Georgia, B.C.: a review. J. Fish. Res. Bd. Can. 27:1251-64.

Parsons, T.R., L.J. Albright and J. Parslow. 1980. Is the Strait of Georgia becoming more eutrophic? Can. J. Fish. Aquati. Sci. 37:1043-47.

Parsons, T.R., L.J. Albright, F. Whitney, C.S. Wong and P.J. LeB. Williams. 1981. The effect of glucose on the productivity of seawater: an experimental approach using controlled aquatic ecosystems. Mar. Environ. Res. 4:229-42.

Parsons, T.R., W.H. Thomas, D. Seibert, J.R. Beers, P. Gillespie and C. Bawden. 1977. The effect of nutrient enrichment on the plankton community in enclosed water columns. Revue ges. Hydrobiol. 62:565-72.

Perkins, E.J. 1974. The Biology of Estuaries and Coastal Waters. Academic Press, London, 678 p.

Pfaffenhöfer, G.-A., J.R. Strickler and M. Alcaraz. 1982. Suspension-feeding by herbivorous calanoid copepods: a cinematographic study. Mar. Biol. 67:193-9

Phillips, D.J.H. 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments - a review. Environ. Pollut. 13:281-317.

Pilson, M.E.Q. and S.W. Nixon. 1980. Marine microcosms in ecological research. In Microcosms in Ecological Research. (ed.) J.P. Giesy, Jr. p. 724-41. DOE Symposium Series 52, CONF-781101. National Technical Information Service, Springfield, Va.

Pomeroy, L.R. 1974. The ocean's food web, a changing paradigm. BioScience 24:499-504.

Poorman, A.E. 1973. Effects of pesticides on Euglena gracilis. I. Growth studies. Bull. Environ. Contam. Toxicol. 10:25-8.

Pritchard, D.W. 1967. What is an estuary: physical viewpoint. In Estuaries. (ed.) G.H. Lauff, p. 3-5. Am. Ass. Adv. Sci., Publ. 83, Washington, D.C.

Remane, A. and C. Schlieper. 1971. Biology of Brackish Water. In H.-J. Elster & W. Ohle (eds.) 2nd ed. Die Binnengewasser Vol. XXV. E. Schweizbart'sche Verlagsbuchhandlung, Stuttgart. 372 p.

Remsen, C.C., E.J. Carpenter and B.W. Schroeder. 1972. Competition for urea among estuarine microcosms. Ecology 53:921-6.

Rhee, G-Y. 1972. Competition between an alga and an aquatic bacterium for phosphate. Limnol. Oceanogr. 17:505-14.

Rheinheimer, G. (ed.) 1977. Microbial Ecology of a Brackish Water Environment. Springer-Verlag, Berlin, 291 p.

Richerson, P., R. Armstrong and C.R. Goldman. 1970. Contemporaneous disequilibrium, a new hypothesis to explain the "Paradox of the plankton." Proc. nat. Acad. Sci. U.S.A., 67:1710-14.

Roelofs, A.K. 1983. Distributional patterns and variation of valve diameter of Paralia sulcata in surface sediments of southern British Columbia inlets. Est. Coast. Shelf Sci. in press.

Roelofs, A.K. and A. Spies, unpubl. MS. Light and salinity appear to control dominance of Skeletonema costatum in microcosms and in Howe Sound. Dept. of Botany, Univ. of B.C., Vancouver, 8 p.

Ryther, J.H. and W.M. Dunstan. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Sci. 171:1008-13.

Saks, N.M. and E.G. Kahn. 1979. Subtrate competition between a salt marsh diatom and a bacterial population. J. Phycol. 15:17-21.

Seki, H., K.V. Stephens and T.R. Parsons. 1969. The contribution of allochthonous bacteria and organic materials from a small river into a semi-enclosed sea. Arch. Hydrobiol. 66:37-47.

Sharp, J.H. 1977. Excretion of organic matter by marine phytoplankton: Do healthy cells do it? Limnol. Oceanogr. 22:381-99.

Sherr, B. and E. Sherr. 1983. Enumeration of heterotrophic microprotozoa by epifluorescence microscopy. Est. Coast. Shelf Sci. 16:1-7.

Shim, J.H. 1976. Distribution and taxonomy of planktonic marine diatoms in the Strait of Georgia, B.C. Ph.D. thesis, Dept. of Botany, Univ. of British Columbia. 222 p.

Shim, J.H. 1977. Distributional ecology of planktonic diatoms in the Strait of Georgia, B.C.. J. Oceanol. Soc. Korea 12:13-32.

Sibert, J. and T.J. Brown. 1975. Characteristics and potential significance of heterotrophic activity in a polluted fjord estuary. J. Exp. Mar. Biol. Ecol. 19:97-104.

Sibert, J.R. and R.J. Naiman. 1980. The role of detritus and the nature of estuarine ecosystems. In Marine Benthic Dynamics. (eds.) K.R. Tenore & B.C. Coull, p. 311-323. Univ. of South Carolina Press, Columbia.

Sieburth, J.McN. 1967. Seasonal selection of estuarine bacteria by water temperature. J. Exp. Mar. Biol. Ecol. 1:98-121.

Sieburth, J.McN. 1968. The influence of algal antibiosis on the ecology of marine microorganisms. In Advances in Microbiology of the Sea, Vol 1. (eds.) M.R. Droop & E.J. Ferguson Wood, p. 63-94. Academic Press, New York.

Sieburth, J.McN. (ed.) 1979. Exposed microbial habitats. In Sea microbes. p. 16-38. Oxford Univ. Press, New York.

Slawyk, G. and J.J. MacIsaac. 1972. Comparison of two automated ammonium methods in a region of coastal upwelling. Deep-Sea Res. 19:521-24.

Smayda, T.J. 1957. Phytoplankton studies in lower Narragansett Bay. Limnol. Oceanogr. 2:342-59.

Smayda, T.J. 1973. The growth of Skeletonema costatum during a
        winter-spring bloom in Narragansett Bay, Rhode Island.
        Norw. J. Bot. 20:219-247.

Smayda, T.J. 1980. Phytoplankton species succession. In The
        Physiological Ecology of Phytoplankton. (ed.) I. Morris,
        p. 493-570. Univ. of California Press, Berkeley.

Smetacek, V., B. von Bodungen, B. Knoppers, H. Neubert, F.
        Pollehne and B. Zeitzschel. 1980. Shipboard experiments
        on the effect of vertical mixing on natural plankton
        populations in the central Baltic Sea. Ophelia, Suppl.
        1:77-98.

Smith, W., V.R. Gibson and J.F. Grassle. 1982. Replication in
        controlled marine systems: presenting the evidence. In
        Marine Mesocosms: Biological and Chemical Research in
        Experimental Ecosystems. (eds.) G.D. Grice & M.R. Reeve,
        p. 217-25. Springer Verlag, New York.

Sorokin, Yu.I. 1977. The heterotrophic phase of plankton
        succession in the Japan Sea. Mar. Biol. 41:107-17.

Sournia, A. 1974. Circadian periodicities in natural populations
        of marine phytoplankton. Adv. Mar. Biol. 12:325-89.

Spittler, P. 1973. Feeding experiments with tintinnids. Oikos
        Suppl. 15:128-32.

Steele, J.H. 1974. The structure of marine ecosystems. Harvard
        Univ. Press, Cambridge, 128 p.

Steemann Nielsen, E. and S. Wium-Andersen. 1971. The influence
        of Cu on photosynthesis and growth in diatoms. Physiol.
        Plant. 24:480-84.

Stephens, R.W. Sheldon and T.R. Parsons. 1967. Seasonal
        variations in the availability of food for benthos in a
        coastal environment. Ecology 48:852-55.

Stephens, K., J.D. Fulton and O.D. Kennedy. 1969. Summary of
        biological oceanographic observations in the Strait of
        Georgia, 1965-68. Tech. Rep. No. 110, Fish. Res. Bd. Can.
        128 p.

Stevenson, L.H. and R.R. Colwell (eds.) 1973. Estuarine
        Microbial Ecology. Univ. of South Carolina, Columbia,
        536 p.

Stockner, J.G. and D.D. Cliff. 1977. Phytoplankton production
        and distribution in Howe Sound, British Columbia: a
        coastal marine embayment-fjord under stress. J. Fish.
        Res. Board Can. 34:907-17.

Stockner,J.G., D.D. Cliff and K.R.S. Shortreed. 1979. Phytoplankton ecology of the Strait of Georgia, British Columbia. J. Fish. Res. Board Can. 36:657-66.

Stockner, J.G., K.S. Shortreed and E.A. MacIsaac. 1980. Is the Strait of Georgia becoming more eutrophic? The benevolent Strait: reply. Can. J. Fish. Aquat. Sci. 37:1048-55.

Strickland, J.D.H. 1967. Between beakers and bays. New Scientist 33:276-78.

Strickland, J.D.H. and L.D.B. Terhune. 1961. The study of 'in-situ' marine photosynthesis using a large plastic bag. Limnol. Oceanogr. 6:93-6.

Strickland, J.D.H. and T.R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Fish. Res. Bd. Can. Bull. No. 167, 310 p.

Struempler, A.W. 1973. Adsorption characteristics of silver, lead, cadmium, zinc, and nickel on borosilicate glass, polyethylene, and polypropylene container surface. Analyt. Chem. 45:2251-54.

Sunda, W. and R.L. Guillard. 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. J. Mar. Res. 34:511-29.

Takahashi, M. and A.B. Norton. 1977. Seasonal changes in the microbial biomass in the Fraser River estuary, British Columbia. Arch. Hydrobiol. 79:133-43.

Takahashi, M. and K.D. Hoskins. 1978. Winter condition of marine plankton populations in Saanich Inlet, B.C., Canada. II. Micro-zooplankton. J. Exp. Mar. Biol. Ecol. 32:27-37.

Takahashi, M., K. Fujii and T.R. Parsons. 1973. Simulation study of phytoplankton photosynthesis and growth in the Fraser River estuary. Mar. Biol. 19:102-16.

Takahashi, M., D.L. Seibert and W.H. Thomas. 1977. Occasional blooms of phytoplankton during summer in Saanich Inlet, B.C., Canada. Deep-Sea Res. 24:775-80.

Taub, B.F. and M.E. Crow. 1978. Loss of a critical species in a model (laboratory) ecosystem. Verh. internat. Ver. theor. angew. Limnol. 20:1270-76.

Thayer, G.W. 1974. Identity and regulation of nutrients limiting phytoplankton production in the shallow estuaries near Beaufort, N.C.. Oecologia (Berlin) 14:75-92.

Thomas, W.H. and D.L.R. Seibert. 1977. Effects of copper on the dominance and the diversity of algae: Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:23-33.

Thomas, W.H., O. Holm-Hansen, D.L.R. Seibert, F. Azam, R. Hodson and M. Takahashi. 1977. Effects of copper on phytoplankton standing crop and productivity: controlled ecosystem pollution experiments. Bull. Mar. Sci. 27:34-43.

Thomas, W.H., J.T. Hollibaugh, D.L.R. Seibert and G.T. Wallace, Jr. 1980. Toxicity of a mixture of ten metals to phytoplankton. Mar. Ecol. Prog. Ser. 2:213-20.

Thomson, R.E. 1981. Oceanography of the British Columbia Coast. Can. Spec. Publ. Fish. Aquat. Sci. 56, 291 p.

Tully, J.P. and A.J. Dodimead. 1957. Properties of the water in the Strait of Georgia, British Columbia, and influencing factors. J. Fish. Res. Board Can. 14:241-319.

Vaccaro, R.F. 1969. The response of natural microbial populations in seawater to organic enrichment. Limnol. Oceanogr. 14:726-35.

Vaccaro, R.F., F. Azam and R.E. Hodson. 1977. Response of natural marine bacterial populations to copper: controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:17-22.

Valdes, J.M. 1980. Activities and biomasses of bacterioplankton within the Fraser River estuary. M.Sc. thesis, Simon Fraser Univ., Burnaby, B.C. Canada, p. 52.

Valdes, M. and L.J. Albright. 1981. Survival and heterotrophic activities of Fraser River and Strait of Georgia bacterioplankton within the Fraser River plume. Mar. Biol. 64:231-41.

Veer, J. van der, 1982. Simple and reliable methods for the fixation, mounting and staining of small and delicate marine plankton for light microscopic identification. Mar. Biol. 66:9-14.

Venrick, E.L. 1978. How many cells to count? In Phytoplankton manual. (ed.) A. Sournia, p. 167-80. UNESCO, Paris.

Venrick, E.L., J.R. Beers and J.F. Heinbokel. 1977. Possible consequences of containing microplankton for physiological rate measurements. J. Exp. Mar. Biol. Ecol. 26:55-76.

Vernberg, F.J. (ed.) 1975. Physiological Ecology of Estuarine Organisms. Univ. of South Carolina, Columbia, 397 p.

Vernberg, F.J., W. Kitchens, H. McKellar, K. Summers and R. Bonnell. 1978. The Dynamics of an Estuary as a Natural Ecosystem, II. U.S. Environ. Protection Agency, EPA-600/3-78-092, 76 p.

Waldichuk, M. 1983. Pollution in the Strait of Georgia: a review. Can. J. Fish. Aquat. Sci. 40:1142-67.

Weed Science Society of America. 1974. Herbicide Handbook. Weed Science Society, Champaign, Ill. 430 p.

Wiley, M. (ed.) 1976. Estuarine Processes. Vol. I, Academic Press, New York, 541 p.

Wiley, M. (ed.) 1978. Estuarine Interactions. Academic Press, New York, 603 p.

Williams, P.J.LeB. 1975. Biological and chemical aspects of dissolved organic material in sea water. In Chemical Oceanography. Vol.2 (eds.) J.P. Riley & G. Skirrow. 2nd edition, p. 301-63. Academic Press, London.

Williams, P.J.LeB. and R.W. Gray. 1970. Heterotrophic utilization of dissolved organic compounds in the sea. II. Observations on the responses of heterotrophic marine populations to abrupt increases in amino acid concentration. J. Mar. Biol. Ass. U.K. 50:871-81.

Winter, D.F., K. Banse and G.C. Anderson. 1975. The dynamics of phytoplankton blooms in Puget Sound, a fjord in the northwestern United States. Mar. Biol. 29:139-76.

Wojtalik, T.A., T.F. Hall and L.O. Hill. 1971. Monitoring ecological conditions associated with wide-scale applications of DMA 2,4-D to aquatic environments. Pestic. Monit. J. 4:184-203.

Wolter, K. 1982. Bacterial incorporation of organic substances released by natural phytoplankton populations. Mar. Ecol. Prog. Ser. 7:287-95.

Wright, R.T. 1978. Measurement and significance of specific activity in the heterotrophic bacteria of natural waters. Appl. Environ. Microbiol. 36:297-305.

Zeitzschel, B. and J.M. Davies. 1978. Benthic growth chambers. Rap. P.-v. Reun. Cons. int. Explor. Mer 173:31-42.

Zimmermann, R. 1977. Estimation of bacterial number and biomass by epifluorescence microscopy and scanning electron microscopy. In Microbial Ecology of a Brackish Water Environment. (ed.) G. Rheinheimer, p.103-20. Springer Verlag, Berlin.

Zobell, C.E. 1943. The effect of solid surfaces upon bacterial activity. J. Bacteriol. 46:39-59.

LIST OF APPENDICES

APPENDIX 1

pH - Measurements in Control-experiments and 2,4-D Perturbations

Day	5 ppt salinity				10 ppt salinity			
	C*	C**	10^{-4} M	10^{-3} M 2,4-D	C*	C**	10^{-4} M	10^{-3} M 2,4-D
2	6.39	7.65	7.71	7.73	6.88	7.73	7.73	7.75
3	6.59				6.91			
5	6.78	7.49	7.52	5.69	6.69	7.53	7.31	6.70
7	6.70	6.72	7.00	6.58	7.00	7.00	7.59	6.55
9	7.00	7.16	7.39	6.42	7.20	7.75	7.68	6.40
11	6.73	6.96	7.52	6.59	6.84	7.31	7.25	6.62
13	6.89	7.02	7.69	6.91	6.99	7.41	7.45	6.81
15	6.78	7.06	7.39	6.69	6.90	7.20	7.40	6.70
17	6.70	6.99	7.42	6.73	6.90	7.29	7.45	6.75
19	6.75	7.05	7.51	6.91	6.95	7.38	7.40	6.88
21		6.92	7.15	6.56		7.18	7.14	6.65
23				6.00				6.41
25				6.22				6.81

C* = Control-experiment (12.11.82-30.11.82)

C**= Control-experiment concurrent with 2,4-D perturbations

pH - Measurements in Control-experiments and 2,4-D Perturbations

Day	18 ppt salinity				≥26 ppt salinity			
	C*	C**	10^{-4} M	10^{-3} M 2,4-D	C*	C**	10^{-4} M	10^{-3} M 2,4-D
2	6.96	7.79	7.76	7.76	7.01	7.72	7.72	7.72
3	7.00				7.18			
5	7.10	7.34	7.60	7.31	7.32	7.60	7.66	7.30
7	7.25	8.21	8.29	7.15	7.95	8.41	8.16	7.20
9	8.00	7.61	8.20	7.25	8.12	8.19	7.91	7.20
11	7.90	7.37	8.28	7.40	8.00	7.95	7.71	7.39
13	7.98	6.71	7.78	7.10	7.82	7.55	7.57	7.25
15	7.60	7.55	7.97	7.49	7.55	7.81	7.77	7.58
17	7.35	7.63	7.76	7.49	7.35	7.72	7.69	7.87
19	7.15	7.53	7.84	7.58	7.25	7.70	7.79	8.05
21		7.22	7.40	7.15		7.39	7.47	7.81
23				6.71				7.00
25				6.85				7.00

C* = Control-experiments (12.11.82-30.11.82)

C**= Control-experiment concurrent with 2,4-D perturbations

APPENDIX 2

```
C      Program to Model Bacteria and Flagellates (Dark-model)
C      Initialize
       READ(5,20) X,Y,G
   20 FORMAT(10F10.4)
       READ(5,20) RM,GK,UM,XK,EY,D,Q,EX
       READ(5,20) TIME,DT
       NSTP=TIME/DT+.1
       NS12=12/DT+.1
       T=24
       WRITE(6,10)
   10 FORMAT(1X,4X,'RM',8X,'GK',8X,'UM',8X,'XK',8X,'EY',9X,'D',9X,'Q',8X,'EX')
       WRITE(6,40) RM,GK,UM,XK,EY,D,Q,EX
       WRITE(6,40) TIME,DT
   40 FORMAT(1X,8F10.3)
       WRITE(6,30) T,X,Y,G
       DO 500 I=1,NSTP
       R=RM*G/(GK+G)
       U=UM*X/(XK+X)
       DX=R*X-Y*U
       DY=Y*EY*U-D*Y
       DG=-R*X*Q/EX
       X=X+DX*DT
       Y=Y+DY*DT
       G=G+DG*DT
       T=I*DT+24
       J=I/NS12
       J=I-J*NS12
       IF(J.NE.0) GO TO 500
       WRITE(6,30) T,X,Y,G
   30 FORMAT(4F15.3)
  500 CONTINUE
       STOP
       END
```

```
C       PROGRAM TO MODEL LIGHT MICROCOSMS
C        INITIALIZE
        REAL N,NK
        READ(5,20) X,Y,G,P,N
20      FORMAT(10F10.4)
        READ(5,20) RM,GK,UM,XK,EY,D,Q,EX,SM,NK
        READ(5,20) QC,QP,QN
        READ(5,20) TIME,DT
        NSTP=TIME/DT+.1
        NS12=12/DT+.1
        T=24
        WRITE(6,10)
10      FORMAT(1X,4X,'RM',6X,'GK',6X,'UM',6X,'XK',6X,'EY',6X,'D',6X,'Q',6X,'EX',6X,'SM',6X,'NK',6X,'QC',6X,'OP',6X,'QN')
        WRITE(6,40) RM,GK,UM,XK,EY,D,Q,EX,SM,NK,QC,QP,QN
        WRITE(6,40) TIME,DT
40      FORMAT(1X,13F8.3)
        WRITE(6,30) T,X,Y,G,P,N
        DO 500 I=1,NSTP
        R=RM*G/(GK+G)
        U=UM*X/(XK+X)
        S=SM*N/(NK+N)
        DX=R*X-Y*U
        DY=Y*EY*U-D*Y
        DG=-R*X*Q/EX
        DP=S*P
        DN=-S*P*QP/QC-R*X*QN
        X=X+DX*DT
        Y=Y+DY*DT
        G=G+DG*DT
        P=P+DP*DT
        N=N+DN*DT
        T=I*DT+24
        J=I/NS12
        J=I-J*NS12
        IF(J.NE.0) GO TO 500
        WRITE(6,30) T,X,Y,G,P,N
30      FORMAT(6F15.3)
500     CONTINUE
        STOP
        END
```

APPENDIX 3

A – Bacterial numbers $(x10^6ml^{-1})$ in Control- and 10%-light-
   experiments

B – Microzooplankton counts (log numbers ml^{-1}) in Control- and
   10%-light-experiments

Day		2.5 ppt C	S	10 ppt C	S	18 ppt C	S	26 ppt Salinity C	S
2	A	1.31	1.42	1.16	1.11	0.65	0.82	0.48	0.58
3		3.18	3.20	4.24	4.64	4.04	4.49	1.10	2.90
5		0.89	0.89	0.22	0.36	0.21	0.42	2.40	1.01
7		0.96	0.75	0.20	0.34	0.28	0.25	0.54	0.39
9		0.91	0.94	1.21	0.87	1.27	1.46	4.02	1.68
11		2.21	1.10	1.64	1.71	1.13	2.12	2.94	3.06
13		1.60	1.65	0.99	2.31	2.73	2.29	0.90	0.81
15		1.93	1.38	1.52	0.76	1.18	1.12	0.80	0.88
17		1.98	1.48	1.14	0.88	0.54	0.65	0.69	1.23
19		1.53	1.37	1.79	1.41	0.86	0.62	1.07	1.38
21		1.07	1.24	1.91	1.19	0.40	0.76	1.45	1.50
2	B	4.97	4.99	4.94	4.65	4.57	4.54	3.94	4.00
3		5.18	4.99	4.86	4.76	4.05	4.21	4.21	4.05
5		5.03	4.81	4.14	4.24	4.44	4.48	4.24	4.35
7		4.78	4.81	3.94	4.14	4.03	3.40	4.60	3.10
9		4.60	4.81	4.00	3.88	4.24	3.49	4.27	4.21
11		4.21	4.30	3.94	3.88	3.88	4.00	4.21	4.44
15		4.51	4.27	4.00	4.00	3.70	3.88	4.00	3.94
17		4.14	4.60	4.00	3.94	3.40	3.40	3.49	3.94
19		4.05	4.63	3.40	3.70	3.40	3.57	3.27	3.40
21		4.48	4.48	4.10	4.18	3.40	3.88	3.49	3.57

C = Control (225 μEinst m^{-2} s^{-1})

S = 10%-light (22.5 μEinst m^{-2} s^{-1})

APPENDIX 4

A - Bacterial numbers ($\text{x}10^6\text{ml}^{-1}$) in Control- and 5xME-perturbation-experiments in presence of EDTA-Fe.

B - Chlorophyll _a_ (μg l^{-1}) in Control- and 5xME-perturbation-experiments in presence of EDTA-Fe.

Day		2.5 ppt		10 ppt		18 ppt		26 ppt Salinity	
		C	M	C	M	C	M	C	M
2	A	1.31	1.15	1.16	1.12	0.65	0.62	0.48	0.48
3		3.18	3.11	4.24	4.04	4.04	3.65	1.10	0.86
5		0.89	1.39	0.22	0.28	0.21	0.67	2.40	2.65
7		0.96	0.63	0.20	0.24	0.28	0.33	0.54	0.30
9		0.91	0.79	1.21	1.00	1.27	0.96	4.02	0.97
11		2.21	1.69	1.64	2.08	1.13	1.71	2.94	3.24
13		1.60	1.44	0.99	1.07	2.73	2.12	0.90	1.97
15		1.93	1.75	1.52	0.84	1.18	1.96	0.80	1.43
17		1.98	1.54	1.14	0.90	0.54	0.85	0.69	0.76
19		1.53	1.70	1.79	1.05	0.86	0.47	1.07	0.67
21		1.07	1.36	1.91	0.84	0.40	0.17	1.45	1.06
2	B	5.73	5.38	1.71	5.19	6.03	2.27	1.71	1.94
3		2.73	0.41	2.22	3.29	3.94	0.0	0.64	3.71
5		2.45	0.0	0.0	3.66	4.03	0.92	3.52	5.15
7		6.12	3.66	18.97	16.14	33.52	57.18	76.90	46.51
9		16.87	13.16	40.19	41.35	54.40	16.99	42.16	50.05
11		17.80	16.70	21.05	23.83	37.75	40.36	30.97	23.89
13		9.86	6.38	3.48	5.22	13.92	19.14	13.92	18.56
15		7.54	5.80	5.22	6.38	11.02	11.60	7.54	10.44
17		6.96	10.44	3.48	5.80	11.02	11.02	5.80	5.80
19		2.90	4.06	2.32	2.90	5.22	4.06	0.58	3.48
21		3.48	2.32	1.16	1.74	5.22	6.96	1.16	4.06

C = Control

M = 5xMulti-element-mix + EDTA-Fe

Microzooplankton counts (log numbers ml^{-1}) in Control- and
5xME-perturbation-experiments in presence of EDTA-Fe

Day	2.5 ppt		10 ppt		18 ppt		26 ppt Salinity	
	C	M	C	M	C	M	C	M
2	4.97	5.03	4.94	4.92	4.57	4.51	3.94	4.00
3	5.18	5.18	4.86	4.83	4.05	3.80	4.21	4.10
5	5.03	5.15	4.14	4.00	4.44	4.60	4.24	3.94
7	4.78	4.81	3.94	4.24	4.03	3.64	4.60	4.81
9	4.60	4.74	4.00	4.18	4.24	4.10	4.27	4.33
11	4.21	4.70	3.94	3.94	3.88	3.57	4.21	4.18
15	4.51	4.44	4.00	4.18	3.70	4.14	4.00	3.70
17	4.14	4.60	4.00	3.88	3.40	3.94	3.49	3.64
19	4.05	4.40	3.40	3.88	3.40	4.00	3.27	3.88
21	4.48	4.35	4.10	4.10	3.40	3.57	3.49	3.10

C = Control

M = 5xMulti-element-mix + EDTA-Fe

APPENDIX 5

Bacterial Numbers in Control- and Metal-Perturbation-experiments ($\times 10^6$ ml^{-1})

Day	2.7 ppt			10 ppt			18 ppt			26 ppt Salinity		
	C	Cu	M*	C	Cu	M**	C	Cu	M*	C	Cu	M**
2	1.39	1.75	1.74	1.28	1.35	1.29	1.26	1.67	1.12	0.61	0.62	0.62
3	2.49	2.20	1.72	2.08	2.45	1.50	1.98	2.04	1.68	1.15	1.25	0.88
5	3.56	4.91	5.03	1.03	1.94	4.42	3.72	5.44	4.06	4.13	10.74	5.83
7	0.66	0.91	1.05	0.30	0.84	0.31	1.18	1.52	1.17	1.62	1.64	3.33
9	0.29	0.42	0.66	0.42	0.64	0.45	1.75	1.92	1.97	2.64	2.61	2.94
11	0.45	0.44	0.68	1.11	0.59	1.37	1.83	1.76	1.52	1.65	2.43	0.84
13	0.74	0.44	0.70	2.33	0.64	1.28	2.79	1.73	1.48	3.97	2.39	0.58
15	0.60	0.60	0.46	2.38	2.17	0.81	1.84	1.00	1.68	3.99	2.47	0.35

In all microcosms EDTA-Fe was replaced by equimolar solution of $FeCl_3$.

C   = Control

Cu = 30 ug Cu l^{-1}

M* = 5xMulti-element-mix

M**=10xMulti-element-mix

Species composition in Control-experiments and after Metal-
perturbation (5xME) at ≤5 ppt salinity. EDTA-iron was replaced
by $FeCl_3$. Numbers represent relative abundance (%) at the
height of the phytoplankton bloom.

	control	5xME
Small flagellates	19.0	44.0
Thalassiosira spp.	70.0	21.0
Thalassionema spp.	6.0	30.0
S. costatum	3.0	
Freshwater species	<1.0	
Pennate diatoms	1.0	4.0

Species composition in Control-experiments and after copper addition (30ug 1^{-1}).
Numbers represent relative abundance (%); $FeCl_3$ instead of EDTA-iron addition.

Salinity 2.7 ppt

	Day 2	Day 5	Day 7	Day 9	Day 11
CONTROL					
Small flagellates	87.0	92.0	19.0	15.0	15.0
Thalassiosira spp.	3.0	5.0	70.0	80.0	74.0
Thalassionema spp.	2.0	1.0	6.0	3.5	8.5
S. costatum	6.0	<1.0	3.0		
Nitzschia spp.	1.0	1.0	1.0	1.0	2.0
COPPER					
Small Flagellates	87.5	63.0	85.5	40.0	13.0
Thalassiosira spp.	9.5	24.0	5.0	51.0	61.0
Thalassionema spp.	1.0	10.5	5.0	5.0	16.0
S. costatum			<1.0		
Nitzschia spp.	<1.0	1.0	<1.0	2.0	3.5

Salinity 10 ppt

	Day 2	Day 5	Day 7	Day 9
CONTROL				
Small flagellates	77.0	25.0	7.5	10.0
Thalassiosira spp.	7.0	2.0	1.0	1.0
Thalassionema spp.	2.0			
S. costatum	11.5	72.0	91.5	89.0
COPPER				
Small flagellates	80.5	26.0	6.0	19.0
Thalassiosira spp.	7.0	1.0		1.0
Thalassionema spp.	3.0			
S. costatum	8.5	73.5	94.0	80.5

Species composition in Control-experiments and after copper addition (30ug 1^{-1}).
Numbers represent relative abundance (%); $FeCl_3$ instead of EDTA-iron addition.

Salinity 18 ppt	Day 2	Day 5	Day 7	Day 9	Day 11
Small flagellates	68.0	23.5	8.0	8.0	5.0
Thalassiosira spp.	6.5	19.0	9.0	17.0	11.0
Thalassionema spp.	2.5				
S. costatum	20.0	54.5	82.0	71.5	78.5
Nitzschia spp.	1.5	1.5	1.0	2.0	4.0
COPPER					
small flagellates	62.5	46.5	11.0	17.0	
Thalassiosira spp.	10.0	5.0	11.0	6.0	
Thalassionema spp.	4.0				
S. costatum	18.0	47.0	76.5	71.0	
Nitzschia spp.	2.0	2.0	1.0	5.5	

Salinity 26 ppt					
CONTROL					
Small flagellates	68.0	20.5	20.5	15.0	18.5
Thalassiosira spp.	10.0	20.0	21.0	36.5	35.0
S. costatum	19.0	56.0	56.5	46.0	44.5
Nitzschia spp.	1.0	1.0	1.5	2.0	1.0
COPPER					
Small flagellates	63,0	29.0	9.0	10.0	
Thalassiosira spp.	12.5	9.0	6.0	8.0	
S. costatum	23.5	62.0	83.0	77.5	
Nitzschia spp.	1.0		2.5	5.0	

APPENDIX 6

Plankton composition after 2,4-D perturbations; numbers represent relative abundance of species (%).

Salinity 5 ppt

	Day 2: Control	10^{-4} M 2,4-D	10^{-3} M 2,4-D
Flagellates	80.0	69.0	81.2
Green algae	4.0	9.5	4.5
S. costatum	14.5	21.5	6.8
Thalassiosira spp.	1.0		0.5
Pennate diatoms	0.5		0.5

	Day 11:		Day 25:
Flagellates	6.8	10.5	96.0
Green algae	25.5	17.2	3.8
S. costatum	51.2	62.2	
Thalassiosira spp.	13.8	0.8	
Pennate diatoms	1.8	3.5	0.2

Salinity 10 ppt

	Day 2: Control	10^{-4} M 2,4-D	10^{-3} M 2,4-D
Flagellates	70.0	73.5	69.0
Green algae	11.5	4.0	11.0
S. costatum	16.0	20.0	17.0
Thalassiosira spp.	1.0	1.5	2.0
Chaetoceros spp.		0.5	
Pennate diatoms	1.5	0.5	1.0

	Day 7:	Day 9:	Day 25:
Flagellates	38.5	43.8	99.2
Green algae			0.5
S. costatum	58.8	44.8	
Thalassiosira spp.	0.5	3.8	
Chaetoceros spp.	1.2	6.0	
Pennate diatoms	1.0	1.7	0.2

Salinity 18 ppt

	Day 2: Control	10^{-4} M 2,4-D	10^{-3} M 2,4-D
Flagellates	73.0	67.0	62.5
Green algae	4.0	10.0	11.5
S. costatum	14.5	20.5	18.5
Thalassiosira spp.	3.0	2.0	1.0
Chaetoceros spp.			
Pennate diatoms	1.0	0.5	0.5

	Day 7:	Day 9:	Day 19:
Flagellates	26.5	16.5	86.8
Green algae	0.5		
S. costatum	58.2	38.5	1.2
Thalassiosira spp.	5.0	13.8	2.5
Chaetoceros spp.	6.5	25.0	0.2
Pennate diatoms	3.2	5.8	9.5

Salinity 26 ppt

	Day 2:		
Flagellates	71.0	60.0	61.0
Green algae	8.2	23.0	15.0
S. costatum	15.5	15.5	17.5
Thalassiosira spp.	2.0	1.0	2.0
Chaetoceros spp.			
Pennate diatoms	0.5	0.5	1.0

	Day 7:	Day 7:	Day 19:
Flagellates	35.2	48.2	20.8
Green algae			
S. costatum	43.0	22.0	0.8
Thalassiosira spp.	3.8	8.8	69.5
Chaetoceros spp.	5.2	12.5	1.0
Pennate diatoms	10.3	8.2	8.0

Bacterial numbers ($\times 10^6$/ml) in Control-microcosms and after addition of 2,4-D.

Day	5 ppt S			10 ppt S			18 ppt S			26 ppt S		
	C	10^{-4}	10^{-3}	C	10^{-4}	10^{-3}	C	10^{-4}	10^{-3}	C	10^{-4}	10^{-3}
2	1.46	1.27	1.19	0.98	1.37	1.24	1.20	1.22	1.17	1.25	1.23	1.04
3	2.01	1.82	2.51	1.89	2.22	2.69	2.18	2.28	2.60	1.71	1.56	1.93
5	3.49	3.96	2.85	3.29	4.87	3.87	2.62	3.60	5.15	4.83	5.25	6.20
7	0.56	0.56	2.56	0.12	0.24	1.80	0.53	0.26	1.87	1.15	1.11	2.46
9	0.74	0.43	1.51	0.19	0.43	1.62	2.11	3.12	2.65	4.67	7.58	4.13
11	1.58	0.67	0.63	1.13	2.11	1.46	2.33	4.61	0.62	4.44	5.75	2.50
13	1.93	0.70	0.28	2.29	1.81	2.14	3.49	1.97	2.82	1.66	3.00	2.42
15	2.75	0.98	0.71	3.19	1.60	1.51	1.13	0.75	2.23	1.00	3.09	1.67
17	3.06	1.59	1.14	2.31	1.36	1.35	1.80	1.06	1.43	1.14	1.08	1.61
19	1.69	1.74	1.10	1.29	0.66	0.86	2.58	0.99	1.47	2.17	1.35	2.97
21	1.16	1.97	1.35	1.51	0.25	1.76	3.27	0.84	1.84	2.19	0.78	3.08
23			1.31			1.60			1.45			1.52
25			1.51			2.63			1.31			1.13

C = Control
10^{-4} = 10^{-4} M 2,4-D
10^{-3} = 10^{-3} M 2,4-D

Microzooplankton counts (log numbers) in Control-microcosms and after addition of 2,4-D

Day	5 ppt S C	10^{-4}	10^{-3}	10 ppt S C	10^{-4}	10^{-3}	18 ppt S C	10^{-4}	10^{-3}	26 ppt S C	10^{-4}	10^{-3}
2		4.99			4.89						4.05	
3		5.49			4.69			4.65			4.24	
5	4.72	4.68	4.86	4.60	4.57	4.35	4.69	4.54	3.94	4.69	4.57	3.39
7	4.44	4.63	4.60	4.44	4.44	4.79	4.35	4.30	4.76	4.60	4.60	5.29
9	4.18	4.39	4.39	4.10	4.51	4.63	4.00	4.30	4.18	3.88	4.44	4.30
11	3.69	3.57	3.88	4.18	4.30	4.10	3.39	3.88	4.10	3.69	3.69	3.94
13	4.18	4.05	3.57	4.10	4.44	4.18	4.18	4.24	3.69	3.69	4.30	3.39
15	4.10	3.88	3.57	4.18	4.00	4.54	3.39	3.27	4.18	3.69	3.69	3.69
17	3.94	4.18	4.00	3.79	3.69	3.94	2.79	3.57	3.94	3.79	3.69	4.30
19	3.39	3.49	3.64	2.79	3.79	3.57	3.10	3.39	3.88	3.75	3.69	3.79
21	2.79	3.88	4.00	3.69	3.75	3.39	3.39	3.69	3.75	3.49	3.94	4.27
23			4.10			4.48			3.88			4.54
25			4.44			4.39			3.79			3.94

C = Control

10^{-4} = 10^{-4} M 2,4-D

10^{-3} = 10^{-3} M 2,4-D

Field Data from the Strait of Georgia (1981/1982)

Cruises (Date)	S (ppt)	T (°C)	Bacteria ($\times 10^6$/ml)	Het.Activ. (μg/h/10^9)	Chl (μg/1)	NO_2&NO_3 (μg-at/1)	MCHO-C (μg/1)
21.07.81	9.0	16.4	1.51	0.27	2.11	1.50	--
	9.8	16.8	1.81	--	3.76	0.51	470
10.08.81	18.0	20.0	4.36	0.12	9.26	0.48	517
	18.0	20.0	1.82	0.30	2.69	0.61	350
	17.5	20.0	1.81	0.24	2.97	0.28	283
	10.0	22.0	1.84	0.38	5.20	0.33	368
	10.0	22.0	2.08	0.48	9.86	0.66	455
	10.0	22.0	1.93	0.26	3.43	1.80	--
16.09.81	26.0	13.0	1.79	0.30	7.77	15.00	150
17.09.81	25.7	16.5	3.31	0.18	1.22	0.18	--
	27.0	16.5	3.32	0.24	9.28	b.d.	--
	26.8	16.0	4.02	0.08	4.87	b.d.	210
29.09.81	26.2	13.5	1.50	--	7.96	12.84	245
	26.8	14.0	1.45	--	6.95	15.38	234
	27.0	13.0	1.37	--	6.26	16.54	619
30.09.81	26.4	13.0	1.07	0.67	4.13	19.88	184
1.10.81	18.7	14.0	1.67	1.26	2.58	11.92	--
	18.9	14.0	1.53	0.38	3.27	11.78	--

Field Data from the Strait of Georgia (1981/1982)

Cruises (Date)	S (ppt)	T (°C)	Bacteria ($\times 10^6$/ml)	Het.Activ. ($\mu g/h/10^9$)	Chl ($\mu g/l$)	$NO_2\&NO_3$ ($\mu g\text{-}at/l$)	MCHO-C ($\mu g/l$)
15.10.81	19.0	12.3	1.48	0.58	1.76	16.10	331
	18.4	11.9	1.14	0.75	1.60	16.57	128
	17.5	12.2	1.19	0.60	1.37	15.47	--
	18.2	12.1	1.26	0.46	b.d.	15.77	299
	25.5	12.0	0.87	0.70	3.46	22.26	167
	26.5	12.0	0.59	--	1.30	26.28	--
10.11.81	25.5	14.8	0.57	--	1.67	21.47	--
	26.0	11.5	0.55	0.62	2.27	22.20	158
	26.0	12.3	0.34	0.64	2.81	23.88	79
11.11.81	18.0	11.4	0.68	0.63	2.25	17.36	279
	18.5	11.5	0.77	0.65	1.32	18.43	--
	18.0	12.0	0.80	0.94	2.25	19.50	252
7.12.81	26.8	10.1	0.18	0.89	1.18	32.16	--
	27.0	10.0	0.22	1.16	2.39	30.63	228
	27.0	10.0	0.25	0.26	1.32	32.51	332
12.01.82	27.0	8.7	0.28	0.94	2.37	28.58	131
	27.0	8.0	0.28	0.41	0.56	27.69	258
	27.0	10.0	0.21	--	3.48	27.90	195

Field Data from the Strait of Georgia (1981/1982)

Cruises (Date)	S (ppt)	T (^{o}C)	Bacteria ($\times 10^{6}$/ml)	Het.Activ. (μg/h/10^{9})	Chl (μg/l)	NO_2&NO_3 (μg-at/l)	MCHO-C (μg/l)
8.02.82	26.0	7.0	0.55	0.58	2.22	27.05	230
	25.0	6.9	0.41	--	1.67	27.26	270
	25.2	7.2	0.39	0.34	1.85	27.80	580
13.04.82	25.0	9.0	2.70	0.32	9.48	20.16	183
	25.0	9.0	2.63	0.26	9.30	18.14	302
	26.5	9.0	2.53	0.07	0.95	12.66	231
12.05.82	18.5	12.5	3.06	0.14	7.37	9.64	402
	27.0	12.0	2.41	0.04	2.92	10.56	150
	27.0	11.5	2.11	0.05	4.10	9.89	308
	27.0	12.0	2.20	0.04	3.80	10.82	18
13.05.82	10.0	9.0	2.24	0.22	1.62	7.82	589
	4.7	10.0	2.10	0.25	2.22	8.45	569
	18.2	11.1	2.96	0.14	2.50	9.92	192
1.06.82	18.5	14.0	2.02	--	1.51	10.69	217
	18.5	14.0	2.08	--	1.95	10.23	368
	26.0	13.0	2.19	0.06	3.06	12.03	--
	26.0	13.0	2.59	0.02	2.04	11.78	82
	26.0	13.0	2.53	0.10	1.35	12.29	144
	18.0	13.6	1.81	0.12	1.02	10.03	260

Field Data from the Strait of Georgia (1981/1982)

Cruises (Date)	S (ppt)	T (^{o}C)	Bacteria ($\times 10^6$/ml)	Het.Activ. (μg/h/10^9)	Chl (μg/1)	NO_2&NO_3 (μg-at/1)	MCHO-C (μg/1)
1.06.82	10.3	12.5	1.78	0.04	1.35	9.95	444
	9.3	12.0	1.55	0.06	0.97	9.82	--
	2.8	12.0	1.87	0.19	1.21	8.55	498
	1.5	12.0	2.07	0.11	1.67	9.27	398
	3.0	13.0	2.01	0.26	0.60	9.46	527
15.06.82	5.0	16.0	3.66	--	41.99	0.48	--
	18.8	18.5	2.89	--	2.02	0.23	221
	17.5	18.2	2.03	0.10	3.09	0.20	--
	5.1	20.2	2.25	0.34	2.09	2.21	263
	4.7	21.0	2.64	--	2.97	3.16	168
5.07.82	5.0	15.0	1.71	0.09	7.52	0.16	261
	10.8	15.5	1.82	0.16	11.20	1.39	400
	10.0	16.0	1.58	0.41	7.68	0.71	190
	10.1	16.5	1.24	0.49	8.93	0.46	367
	24.5	15.3	1.29	0.37	9.54	1.61	355
	25.0	15.1	0.73	0.36	10.95	0.42	125
	24.5	14.9	1.44	0.62	16.24	0.51	129
	4.8	15.1	1.26	0.41	24.50	5.49	230
	19.0	14.5	0.97	0.80	4.80	1.02	114
	17.2	14.5	1.35	0.37	9.84	4.72	134

Plankton Analyses of Cruise Samples: **5 ppt salinity**

	13.5.82	1.6.82	15.6.82	5.7.82
Small flagellates	77.5	78.5	34.5	23.5
Large flagellates		1.5	2.5	0.3
Green algae	12.0	7.0		2.0
Thalassiosira spp.	5.0	2.5	57.5	69.5
S. costatum	3.0	6.0	0.5	0.8
Pennate diatoms	2.5	3.5		0.2
Others		1.0	4.0	0.2
Dinoflagellates			1.0	2.0
Chaetoceros spp.				1.5

10 ppt salinity

	21.7.81	10.8.81	13.5.82	1.6.82	5.7.82
Small flagellates	60.8		86.5	73.5	21.8
Large flagellates				4.0	1.0
Green algae	14.7	8.2	7.0	7.0	
Thalassiosira spp.	8.8		1.5	1.5	74.0
S. costatum		15.1	2.5	9.3	
Chaetoceros spp.		58.1		0.5	0.5
Pennate diatoms		4.2	1.5	4.3	
others	1.0	2.2	1.0		1.5
Dinoflagellates	14.7	10.5			1.2
Tintinnids		1.7			

Plankton Analyses of Cruise Samples: 18 ppt salinity

	10.8.81	1.10.81	15.10.81	11.11.81	12.5.82	1.6.82
Small flagellates	1.7	75.0	71.0	66.0	82.5	79.5
Large lagellates		1.5		6.0	1.0	5.0
Green algae	1.9	7.5	15.0	9.5	1.0	0.5
Thalassiosira spp.	4.9	8.5	5.0	12.0	7.0	5.0
S. costatum	77.5				6.0	5.0
Chaetoceros spp.	11.2			2.0		1.0
Pennate diatoms		2.5	1.0	1.5	1.0	1.5
Others	1.0		4.0		1.5	2.5
Dinoflagellates	1.8					
Tintinnids		5.5	4.0	3.0		

	15.6.82	5.7.82
Small flagellates	54.5	15.5
Large flagellates	2.5	1.2
Green algae		
Thalassiosira spp.	16.5	5.0
S. costatum	15.0	70.0
Chaetoceros spp.	3.0	3.5
Pennate diatoms	1.5	3.2
Others	6.5	1.0
Dinoflagellates	0.5	0.5

Plankton Analysis of Cruise Samples: 26 ppt salinity

	16.9.81	29.9.81	15.10.81	10.11.81	7.12.81	12.1.82
Small flagellates	50.7	19.1	73.0	59.0	67.5	70.0
Large flagellates	7.5	1.5	2.5	5.0	1.5	6.5
Green algae	13.4	11.2	16.0	13.5	23.0	16.5
Thalassiosira spp.	5.0	11.8	6.5	13.5	7.0	5.0
S. costatum	11.0	8.4		2.0		
Chaetoceros spp.	2.5	37.4		2.0		
Pennate diatoms	6.0	9.7	1.0	4.0	1.0	
Others	0.4	0.9		1.0		
Tintinnids	3.5		1.0			1.5

	8.2.82	13.4.82	12.5.82	1.6.82	5.7.82
Small flagellates	94.5		91.8	48.5	10.0
Large flagellates	2.8		4.8	1.5	1.5
Green algae			2.2		
Thalassiosira spp.	1.8	41.5	0.5	2.0	5.0
S. costatum		56.8	0.5	23.0	52.0
Chaetoceros spp.				11.5	19.2
Pennate diatoms		1.8	0.2	12.5	11.8
Others				1.5	0.5