PRELIMINARY EXPLORATION OF ESTUARINE ECOSYSTEM STRUCTURE
AT LOW TROPHIC LEVELS WITH
A CONTROLLED MICRO COSM

by
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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in
THE FACULTY OF GRADUATE STUDIES
(Department of Oceanography)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
December, 1985
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In a laboratory microcosm, two kinds of salinity gradients were created to simulate the process occurring in estuarine circulation with mixing sea water and fresh water. The behaviour of the freshwater and seawater ecosystem components in relation to the salinity gradients were investigated. Seven parameters were chosen as indicators of the different trophic levels in the ecosystem or of the environmental conditions. The interaction among the different trophic levels was reflected in the development pattern of phytoplankton, nutrients, bacteria, and nanozoooflagellates. The interaction among the ecosystem and environmental conditions were reflected in the difference between the different experiments with different salinity gradients and different ecosystem origin.

In stage I freshwater phytoplankton were tested on two different salinity gradients. The data showed that the freshwater biota could not pass through the salinity gradient. Most of them died or were inhibited during the mixing process. The autotrophic component in fresh water could no longer function as a autotrophic component but served as an organic substrate contributor. The inhibition of phytoplankton growth by the salinity gradient provided a condition in which the bacteria could concurrently develop in the ecosystem. In a homogenous condition with controlled flasks, the development of the autotrophic component was separated from heterotrophic
bacteria over time.

In stage II, a seawater ecosystem had a different response from that of fresh water. With an increasing salinity, the growth of phytoplankton could be limited at low salinities. This resulted in a delay of the maximum phytoplankton biomass and provided the first period of time for bacterial development before the bloom of autotrophic components. Thus bacteria formed a peak before phytoplankton developed. Seawater phytoplankton could, on the other hand, actively and quickly respond in their growth, on the salinity gradient. Thus the seawater autotrophic component may play the major role in primary production in a phytoplankton based estuarine ecosystem. The behavior of autotrophic components in both systems can have a strong effect on the rest of the components in the system; their changes could cause a great change in the whole system structure. Different developmental patterns of phytoplankton and bacteria in different experiments were explained with a conceptual diagram which summarizes the idea of energy states of an ecosystem and the function of phytoplankton and bacteria in ecosystem dynamics.

Temporal development patterns of the ecosystem components in our experiments may be extrapolated into spatial distributions if a body of water is moving seaward in an estuary. Thus a conceptual model is presented to explain the spatial distribution of many biologically important components which have often been reported in many field investigations of estuaries.
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Acknowledgement

This thesis would not have been possible without the help of many people. I am very grateful to my thesis supervisor, Dr. T. R. Parsons, for his encouragement, inspiration in the initiation and the freedom of exploration throughout the study. It is he who directed me in this field and I think his influence in thinking of ecosystems will be with me for many years to come.

I am grateful to Dr. P. J. Harrison for his constructive suggestions, helpful discussion as well as the kindness he showed to me during the past two years, and also for providing the use of his laboratory.

Special thanks are due to Dr. A. G. Lewis for his very helpful discussion and comments at the beginning of my laboratory work. Thanks are also due to Dr. F. J. R. Taylor, for his comments and allowing me to use his laboratory. Here, thanks are extended to Miss Judy Acreman for her help in my identification of species.

Much appreciation is extended to Dr. Carol Lalli for her hard work in improving my manuscript.

I am grateful to Dr. R.J. Andersen, who sat on my committee and for approval of my proposal and reading my thesis.

I like to thank Dr. P.H. LeBlond and Dr. R.W. Burling for their helpful discussion on the estuarine circulation.

My appreciation is also given to many fellows, faculty and secretaries at the Department of Oceanography for their kindness and the help they have given me whenever I needed it.

Special thanks to Miss Heather Dovey for her friendship and much assistance throughout the study and her assistance in typing this manuscript and in drawing the figures. Special thanks to Peter Thompson for his kind assistance during my use of Dr. Harrison's laboratory equipment and the helpful discussion during my experiment. Thanks are due to M.P. Storm for his assistance in measuring salinities for my experiments.

Many thanks to my fellow graduate students, for their friendship and general help which gave me a good environment in the past two years, and many thanks to students in Dr. P.J. Harrison's group for their general help.

To detail all of the support I have received is impossible. I would like to thank all my friends in Canada and in China for their continuous encouragement, care and help which directly and indirectly aided me in my two years of study.

I will always be grateful to my parents and my wife for their constant moral support and the confidence they placed in me; to them I dedicate this thesis.

Finally, I acknowledge that I was supported by the National Scholarship of The Peoples' Republic of China and an award from the International Development Research Centre of Canada.
1. Introduction

Estuarine ecosystems are a transition between fresh water and marine systems and, as such, are highly dynamic and relatively very complex. Within an estuary fresh water and sea water mix together to produce a density difference over space which drives the various estuarine circulation patterns. Pritchard (1955) classified four patterns, with the most typical being a partial mixing estuary which has the most typical circulation pattern associated with a distinctive spatial and temporal salinity gradient. Moreover, many biologically and ecologically important parameters which are composed of ecological conditions are related to this gradient. Therefore, there must be a conditional gradient along this transition for both source systems. This association provides a convenient way of using the conservative nature of salinity for identifying the dynamic spatial and temporal behaviour of the ecosystems along this transition zone from both extremes of salinity (0°/oo to 29°/oo). This dynamic gradient functions as a biological filter (Kennedy, 1984) most of the biological parameters react on this filter. Obviously, when fresh water flows into the estuary on the surface layer and causes a landward flow of sea water underneath, the ecosystem components which are suspended in both water bodies will be subject to the mixing process on the conditional gradient along the transition. Therefore, one may address a very broad
question of what is the effect of the salinity gradient, or in what ways does this conditional gradient act as a dynamic functional filter on either ecosystem by permitting or limiting the passage of ecological components from one type of environment to another? How do mixing processes and circulation patterns couple two very different ecosystems to form a hybrid ecosystem? This gradient filter may function as a converter, so one system's component may be changed into another kind of component (such as POC to DOC) when they pass through. This would result in a different distribution of energy and structure change in the ecosystem. The quality and quantities of the ecological components exported from the estuary may be different from those the estuary receives. It is interesting that while both marine and fresh water systems undergo extreme environmental changes the resulting hybrid estuarine systems are more productive than either of the original water bodies (Lauff, 1967). This is true even without man made enrichment from sewage or other added pollutants. Estuaries have been used as important nursery areas for many economically important fish species and this is a reflection of their high primary productivity.

An increasing demand for a comprehensive understanding of the structure, function, and dynamics of estuaries has stimulated a number of conferences and symposia summarizing knowledge of the natural characteristics of estuaries (Lauff, 1967), the interactions of these systems (Wiley, 1976), the comparison of the different processes in these estuarine
systems (Kennedy, 1982), the functions of estuaries (Kennedy, 1984), the dynamics of chemical nutrients (Neilson & Cronin, 1979). Microbial ecology and physiology as aspects of basic estuarine ecology also have received preliminary attention from Stevenson and Colwell (1973) and were included in a comprehensive discussion by Rheinheimer (1977). Recently, numerous papers about estuaries have appeared which increase our understanding and knowledge about this unique ecosystem. However, estuaries are very dynamic systems and it is proving difficult to construct an overall understanding about the system from studies dealing with specific aspects. Studies which treat estuaries as whole ecosystems or which look at whole processes may be more helpful in obtaining a better picture of the dynamic structure involved.

To fully understand the ecological structure of estuaries we have to focus our perspective at the scale within an estuary. Several studies have found a spatial pattern of most ecological parameters along an estuarine transition zone or salinity gradient (Foester, 1976; Helder, 1983; Palumbo and Ferguson, 1978; Seliger et al., 1981; Wright, 1978). Morris (1978) found strong heterotrophic activity at the turbidity maximum zone (or low salinity regions) (Allbright, 1977, 1983a, b,; Bent and Goulder 1981; Joint et al. 1982; Valdes et al. 1980, 1981; Wright, 1983) and maximization of the phytoplankton standing stock often appears at the mouth of an estuary (or the sea end of the gradient) (Parsons, 1969; Cadee 1978, 1984; Stockner et
Almost all other ecological parameters also show this type of spatial dynamic distribution (Ducklow, 1982; Kemp et al., 1982; Neilson et al., 1979; Peterson, 1975; Sharp et al., 1982; Cadee, 1984; Carpenter, 1985). So far, some attempts have been made to understand the mechanisms behind these phenomena either through mathematical modeling (Loern and Cheng, 1981; Parsons and Kessler, 1985; Peterson et al., 1984) or simulation in the laboratory (Margalef, 1967; Cooper et al., 1973; Spies & Parsons, 1984), but a clear picture is still lacking.

Ecosystem structure is the result of reactions within and between the physical system and ecosystem. First, the inherent (intrinsic) interrelationships among those ecosystem components in terms of material cycling and energy flow determine the covariance of those components among trophic levels. Second, the physical forcing field may arrange and modify the spatial and temporal position of ecological components within this field. A good understanding of the structure and dynamics of estuarine ecosystem functions requires knowledge of both physical mechanisms and ecological mechanisms.

In an estuarine flow, there are two major levels of ecological compartments contained in the water masses: 1). substances; including ions, inorganic nutrients, biological trace metals, dissolved organic material and particulate (detritus) materials, and 2). living organisms which mainly included phytoplankton, bacteria, flagellates, ciliates and
macrozooplankton (the latter are not included in our experiments). The concept about the structure and interactions of these ecological compartments has changed dramatically as a result of new evidence which confirms the important role of bacteria and flagellates in ecosystems (Sorokin, 1971, 1975). As early as 1963, Parsons pointed out that particulate organic carbon (POC) in the ocean amounts to about $2 \times 10^{16}$ g which is 5 times more than that of the phytoplankton biomass and 10 times less than that of dissolved organic carbon. So there is a very large bioenergy pool in the aquatic ecosystem. In estuaries, the (DOC+POC) is much higher than that in the ocean (Neilson et al., 1979; Billen et al., 1980; Wright, 1984; William, 1975; Turner, 1978). Bacteria have been known as users of DOC and POC for a long time. Bacteria are efficient scavengers of inorganic nutrients (Rhee, 1972; Parker, 1975), they can compete successfully with phytoplankton for inorganic phosphorus and other nutrients (Brown, 1975, 1981; Fredrickson, 1977; Saks, 1979; Vaccaro et al., 1969). Both bacterial growth rates and biomass can reach a significantly high quantity (Biddanda, 1985; Williams, 1981). But the position of bacteria in estuarine ecosystems only recently has been firmly established (Wright, 1985). They are at least as important as net plankton and large organisms. Bacteria play an equal role with phytoplankton in terms of energy flow and nutrient recycling (Sorokin, 1977; Sieburth et al., 1978; King et al., 1980). The biomass of the bacteria in the total system may approach that of the entire fauna (Bratbak, 1984, 1985; Fuhrman & Azam, 1982 Newell, 1981;
Watson, 1979). Since the turnover time of bacteria is much faster than that of macroorganism biomass, it follows that flux of energy and material is likely to be great (Marita, 1978). The very high conversion efficiencies in bacteria (50%-70%) (Calow, 1977) leaves a gap in remineralization of nutrients. Because direct excretion of inorganic nutrients apparently are low, this is not a major mode for remineralization (Williams, 1981). However many studies have demonstrated that probably 80 to 90% of nutrients in the euphotic zone of pelagic water are supplied by regeneration (Eppely and Peterson, 1979). This difference can be explained by an abundance of evidence that phagotrophic microflagellates are the main grazers of bacteria in the sea (Azam et al., 1983; Andersen et al. 1985; Caron and Goldman, 1985; Davis & Sieburth, 1984; Fenchel, 1982a, b, c; Goldman & Caron, 1985; Haas & Weebs, 1979). The grazing process opens a pathway for organic substances to be recycled back to inorganic form. A new and very important component in the ecosystem, "the microbial loop", is thus established. Following the discovery of this energy pathway, the distribution and state of the energy flow starting from photosynthesis in the ecosystem became a very interesting subject for further study and reconstruction. It has been estimated that 50% of carbon fixed by phytoplankton is immediately released as DOM (Thomas, 1971; Larsson & Hagstrom, 1982; Smith and Wiebe 1976). Bacteria use this energy source both from dissolved organic substances and detritus to create a large biomass flux and energy flow (Cobeney, 1982; Cole, et al. 1982; Lancelor, 1979).
Through this "microbial loop", part of the bacterial biomass serves to repackage energy into cells accessible to filter feeding zooplankton (Haas and Webb, 1979) and part of it is recycled back to inorganic substances (i.e. mineralized) (Caron and Goldman, 1985). Many investigations (Linley et al., 1983; Wright, 1984) have shown that estuaries support high populations of bacteria and high rates of bacterial activity have been found in this system for both inorganic nutrients and organic compounds (Faust, 1976; Faust & Conrell, 1977). So the microbial loop may play a more important role in this ecosystem.

Because these two pathways of energy flow exist, they must have different functions for energy flow. The distributions of the energy in different pathways is a response of the ecosystem to the environmental conditions. The dynamic behaviour of these two components and the different response to the environmental conditions may reflect their functional differences and the significance of their coexistence. The time evolution difference of these two components in terms of their growth patterns which result from differences in adaptation capability, interacting, commensalism, competition and predation will reveal the interrelationship of the two pathways and their functional response to the environment. It is possible to make some realistic estimates of carbon flow through the two different pathways, provided that simultaneous measurements are made of each of the components of the standing stock in the same water column. This provides the basic concept in our experiment.
The questions addressed here are about some basic and essential processes in estuarine ecosystems:

1) What is the behaviour and response of some freshwater and seawater ecosystem components e.g., phytoplankton, flagellates, bacteria and nutrients to the salinity gradient and to the dynamic state of the estuarine environment in terms of the dilution process?

2) What are the dynamic interactions among these major compartments when they go through the salinity gradient at a constant dilution rate?

3) Do all the components behave in the same manner on the temporal salinity gradient as they did in the original environment?

4) What is the salinity effect on the growth of phytoplankton assemblages in both fresh water and sea water?

5) How can the difference in growth patterns of different components over time be extrapolated for spatial significance (in considering an estuarine circulation pattern)?

To attempt to answer these questions, I have to eliminate all other associated factors in nature such as: the horizontal light gradient; temperature difference; sediment influences, etc. These artificial conditions can be achieved in laboratory microcosm experiments and an apparatus was constructed to achieve the objective.

The purpose of this work was to make a preliminary investigation of the time evolution of growth patterns of two primary trophic levels along a temporal salinity gradient.
Estuarine ecosystems are such complicated systems encompassing so many features of other aquatic systems that laboratory experiments on a microcosm scale can not be a duplication of this system. The purpose here is not to imitate nature but, on the contrary, to simplify the situation in order to identify operational mechanisms and some major processes found in a wide variety of estuaries. For example, previous work has shown that low phytoplankton production at the low salinity segment of an estuary is due to the high turbidity and high light extinction rate. So we may ask the question: if the turbidity effect is eliminated, does that area become one of high production? If not, then the turbidity effect is only a subeffect which masks the real mechanism. In order to observe physiological effects (due to salinity or to other unknown factors) and physical effects (due to dilution rate and to time scale), two different temporal salinity gradients are simulated by constant entrainment of saline nutrient-rich deep water or dilution by fresh water over time within one microcosm; the experimental development and design are described in Chapter II. The evolution pattern and the structure produced by growth differences of the biota are described in Chapter III.

The immediate purpose of these experiments was to understand some of the interacting features of the biota, growth pattern arrangement and certain characteristics pertaining to the structure and functions of estuarine ecosystems which happened during the mixing of two very different water masses.
2. Experimental design

2.1. Preconsideration

The experimental model simulates the process in a two layer flow estuary with entrainment from the bottom (average tidal effect). It is assumed that the exchange of water between the estuary and sea takes place entirely by advection. Horizontal diffusion being negligible, the rate of flow can be calculated from a knowledge of the mean salinity of the inflow and outflow layers and freshwater influx (Pritchard, 1969). The salinity of the deep layer remains almost unchanged, so that only the upper layer is considered. It is assumed that the depth of the upper layer is constant at the steady state of the estuarine circulation. If the body of water at the head of the estuary is considered as one element which flows to the mouth of the estuary at average speed (see Fig. 1), the entrainment of high salinity seawater increases the salinity of the water within that element. As the requirement of continuity, mixed water flows out from that element. Thus there are two major processes happening in that body of water; salinity increases over time and the original water becomes diluted. Since only advection is considered and the whole circulation is in dynamic equilibrium, these two processes also happen over space. The temporal evolution of all the constituents contained in the water mass will correspond to the one spatial dimension. This means that in a body of water with entrainment of sea water at a certain dilution rate, the pattern of temporal evolution of the processes can reflect the spatial pattern of those processes in an estuarine system because all the parameters carried by
Figure 1. Flow diagram for the upper layer box of number $n$ in a two dimensional model of estuarine circulation with vertical mixing (advection only). $S$, salinity; $Q$, volume of flux rate; $S_v$, salinity at lower layer, $Q_v$, volume of flux from the bottom layer. Below: typical salinity profiles; $O$, low salinity end and $S$, high salinity end.
this moving body of water actually stay in a state of rest relative to their carrier. This provides us with the possibility of using a settled body of water in the laboratory to investigate the temporal evolution of desired biological features through constant entrainment of sea water to reproduce a salinity gradient and the dilution process. The temporal evolution pattern of some ecological component should reflect the spatial distribution of these components in the estuaries.

One point which is essential in the laboratory experiments is that there must be an equal starting point for both ecosystems in the estuary and in the laboratory. In laboratory conditions, the starting point of the whole primary production system is artificially controlled by operation of the light source. Light energy activates the photosynthetic process and the temporal evolution of the biological features begins. But in a real estuary, we are not sure that the head of the estuary (where we consider the processes to start) is the beginning of major phytoplankton development which drives the ecosystem. The analysis of an estuarine circulation pattern tells us that the seawater species of phytoplankton are carried into the estuary from the deeper water layer. This suggests that because of light limitation underneath the layer, these marine phytoplankton can not start photosynthetic growth until they reach the euphotic zone through entrainment if the estuary ecosystem is a sea water phytoplankton dominated system. The evolution of the components under our laboratory conditions will not entirely coincide with this estuary system.
On the other hand, what is the state of the freshwater ecosystem components upon reaching the estuary? What happens to them at the salinity gradient? Does this salinity gradient filter out phytoplankton components or other living components? The estuary may be actually a grave for some of freshwater components. The first experiment is designed to look at the response of the freshwater ecosystem when it encounters the salinity gradient.

A natural water column captured in a large container has been used to analyse some of ecological components; this is known as a "mesocosm" and they have been described by others (Grice & Reeve, 1982). This method has been suggested as an important tool in furthering our understanding of food chain transformation mechanisms (Parsons et al., 1978). For the purpose of microphytoplankton studies, the volume of water in an "Mesocosm" enclosure is unnecessarily large for these organisms, and too expensive and difficult to be manipulated. So, for this level of study, the size of the container is reduced to that of a "microcosm" which can be more easily manipulated and the conditions can more accurately be controlled at will. However, "batch culture" type microcosms are isolated water bodies which can not simulate the estuarine physical conditions and the ecological processes. On the other hand, chemostats have long been used as powerful tools in microbial ecology (Jannasch et al. 1974) and lately have been used in many studies of phytoplankton ecology. A series of chemostats connected in sequence has been used by Cooper and
Copeland (1973) for studying the growth of phytoplankton in a spatial salinity gradient created in the series of chemostat flasks. In an estuary, the physical gradient (e.g. gravitational gradient, etc.) is in equilibrium. However, for ecosystems, the estuary is a process tending towards equilibrium, like a continuous culture before reaching steady state. In terms of stability of an ecosystem, it may be briefly in a state of dampened harmonic oscillation. The salinity gradient is associated with a constant dilution rate, so the nature of the salinity gradient is common for the microbial ecosystem in both the estuarine field and the laboratory experimental conditions. Our results and interpolations are only valid in respect to the processes of admixture of sea and fresh water which is common in both systems (Leffler, 1980) and not to other estuarine properties (e.g. sedimentation, etc.).

2.2. Design of the Experiment

Since the experimental apparatus was designed to simulate import and export exchange characteristics and a salinity gradient, the "openness" of the system first had to be incorporated into the "batch culture". An estuarine ecosystem can be considered to be an "open" system for the upper water layer in which import of water of a very different character and export of mixed water are of paramount importance. An open continuous system would appear to resemble estuarine dynamics more closely (Cooper et al., 1973; Margalef, 1967). Based on this consideration, I adopted an approach between batch cultur
time around 6 days) was achieved in four, 6 l flasks by a constant input of sea water or fresh water and a constant output of mixed water over time. This established an increasing or decreasing salinity gradient over time in the flasks. The size of containers used for our experiments represented a suitable volume of water for studying bacteria, microflagellates and phytoplankton and the analysis could be managed by one person. Constant temperature and constant light intensity eliminated the effects of natural fluctuation. A 20 litre bottle was used as a reservoir to hold original unfiltered water or filtered water. These were covered with black plastic bags to simulate dark lower water layer in estuaries.

Every set of experiments was performed in two stages. With each stage having three units of two flasks each. In the first stage, I tested the fresh water ecosystem components. In unit one, the system was initiated with unfiltered fresh water and filtered sea water was allowed to run into the flask so that the salinity in the flask increased over time. In unit two, unfiltered fresh water was run into filtered sea water which was the initial water so that the salinity decreased over time. Unit three for both stage I and II are the same. Each of the flasks held original sea water and fresh water under constant environmental conditions to produce a "control culture". In stage two, using sea water as the original test water, the same procedure as that in stage one was followed to observe the seawater ecosystem behavior on the salinity gradient (see Table 1).

New medium was pumped in through Tygon tubing of 8 mm
diameter at a rate of 1 l/day (dilution rate of 0.16 day\(^{-1}\), residence time 6 days). One litre of mixed culture medium was pumped out before the new medium was pumped in. The outflow water was collected for analysis and subcultured at 24 h intervals. (Fig. 2)

Because the experiments were necessarily limited in size and scope, many parameters were not monitored. Only the most important and essential variables for each ecosystem component were measured as indicators of the dynamics of the growth pattern.

For heterotrophic bacteria, only the number and the average size of the bacteria were measured for biovolume concentration and biocarbon conversion. At the phagotrophic level, the number and the average size for non-pigmented microflagellates were determined to obtain the relative number and biomass. The biomass of the autotrophic level components was measured with the \textit{in vivo} autofluorescence method (Kiefer, 1973). The reason for choosing this method is that all the subsamples needed to be cultured further under "batch culture" conditions. Even though there are substantial variations per cell in this method for natural assemblages of phytoplankton with environmental conditions, control experiments have shown that the exponential increase of \textit{in vivo} fluorescence accurately reflects the exponential increase in cell abundance after the cultures have acclimated in a constant environment (Brand \textit{et al.}, 1981). Since in my experiments the general conditions were always the same, the community composition did not show much difference between experiments and the correlation between \textit{in vivo} fluorescence
Figure 2. Design of the experiments with three units.

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>Part I</th>
<th>Part II</th>
<th>Control</th>
</tr>
</thead>
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<tr>
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<tr>
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<td>filtered sea water (29 °/oo)</td>
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</tr>
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<tr>
<td></td>
<td>filtered fresh water (0 °/oo)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The procedures of the experiment in the two stages.
and extracted chlorophyll \( a \) was linear \( (r^2 = 0.98) \). For inorganic components, two kinds of nitrogenous nutrients were measured: the dynamics of \( \text{NO}_3^- \) concentration is a process of dilution and autotrophic utilization and the concentration of \( \text{NH}_4^+ \) is a result of utilization by phytoplankton and mineralization by bacteria; both are also affected by dilution.

2.3 Condition of Experiment

Natural sea water or fresh water were filtered with a 0.8 um Millipore filter to remove the phytoplankton seed populations and microflagellates but to let some fine particles and dissolved substances \(<0.45\) um) pass through. This retained the much of the original chemical properties of the water in order to investigate its properties in the salinity gradient.

The fresh water (0°/oo salinity) was collected from the bank of the Fraser River at New Westminster, approximately 32 km upstream from Steveston Harbour, at low tide. After collection, the water was stored for two days in a temperature controlled room where the experiment was performed for adaptation. The water was divided into two parts. One part was stored unfiltered and one part was filtered through 0.8 um AA Millipore filter. High salinity water was pumped from a depth of about 25 m and 200 m away from a dock at the West Vancouver laboratory of the Department of Fisheries and Oceans (Fig. 3). This water was covered with black plastic and immediately transported to a temperature controlled room (15°C). Some of the sea water also was filtered through 0.8 um Millipore filters. These two kinds of water were stored in a precleaned 20 l Pyrex flask as reservoir or in 6 l flasks as initial experimental water
under light until the experiment began. Intact sea water was also stored in dark reservoirs covered with black plastic. All experiments were performed at 15°C in a temperature controlled room. The experimental (6 liter) flasks were positioned in front of banks of four, cool white, fluorescent tubes of irradiance of 200-250 \( \text{uE} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) measured in the middle of the flask position. This is a saturating irradiance for diatom species and smaller dinoflagellates (Chan, 1978). The flasks were closed by caps permitting air exchange but preventing contamination. A magnetic stirrer was used to eliminate heterogeneity over space and sinking. All experiments were performed on a 12:12 LD cycle. Nitrate-N (20 ug\cdot at \text{l}^{-1}), phosphate-P (2 ug\cdot at \text{l}^{-1}) and silicate-Si (50 ug\cdot at \text{l}^{-1}) were added to the initial water. Initial nutrient enrichment was thought to be necessary for four reasons. First, to decrease the seasonal variability of both nutrient concentrations and major nutrient ratios in the source water. Second, to create conditions favourable for the growth of diatoms which have requirement for silicate. Third, to control the incubation time to approximately the same time for each experiment. Fourth, according to the Redfield ratio (e.g. 106 C : 16 N : 1 P by atoms), to make sure that nitrogen would be the limiting nutrient for autotrophic growth as is the case of natural marine environments (Antia et al., 1963; Ryther & Dunstan, 1971).

Every part of the experiment was run as one unit of two duplicates under exactly the same condition and the experiment
Figure 3. Sampling sites for laboratory experiments and surface salinity distribution in Fraser estuary and plum
was repeated at different seasons of the year. The purpose was not to study the variability between duplicate flasks and different seasons of the year, but try to ensure that relatively the same pattern of events occurred in the same experimental conditions and try to qualitatively compare the similarity of the evolution pattern between the two duplicate flasks. Thus while they may show some difference in quantitative data, the qualitative reaction should be the same. Haque et al. (1980) pointed out that some parameters in two identical microcosms 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 did not replicate. Calculation of autocorrelation functions or power spectrum functions, however, may eliminate the effects of phasing. In summary, the major interests here were not how much quantitative variance existed between the two same variables in duplicate flasks at any one instance, or in two experiments at different times of the year, but the qualitative feature of how the growth pattern dynamics and how much the different parameters may vary quantitatively at one instance due to interaction among them within one system.

Identical experiments was carried out at different times during of 1984-1985. Because of the complexity of estuarine conditions, any change in biologically important constituents may dominate the dynamic state of estuarine ecosystems. Seasonal variations in an estuary results in dramatic differences in species composition as well as quality and
quantity of biomass and trophic level interaction. Does the dynamic salinity gradient have the same effect on the ecosystem within the seasonal variation? Does water collected at different seasons which contain different seasonal species assemblages show more or less the same growth pattern being subjected to the same condition (conditional controlled chamber) which may or may not be quite different from their original conditions? The salinities of the original water collected at different times of the year are almost the same. Table 2 shows the salinity of the water used for the experiments at different times of the year.

Table 2. Salinity of initial waters collected at different times for the laboratory experiments.

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<tr>
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</table>

3. Methods of Analysis of samples

3.1 Nutrients

As major limiting nutrients, both nitrate and ammonium were measured. All samples collected in laboratory experiments were immediately filtered through precombusted (at 450°C for 24 h) glass fiber filters (Whatman 934-AH, 2.4 cm diameter),
stored in Nalgene bottles and frozen to -20°C. Stored samples were quickly thawed before analysis. Automated determination for nitrate plus nitrite was done as described by Armstrong et al. (1967) with Technicon Autoanalyzer II. The range of nitrate concentrations was 0-50 ug·at-N l⁻¹. Ammonium (0-4.5 ug·at-N l⁻¹) were measured concurrently (Parsons et al., 1984).

3.2 Total bacterial numbers, biovolumes and biomass

Aliquots of 10 ml were taken from each experimental flask for enumeration of total bacterial numbers. Subsamples were placed into glass vials and immediately fixed with 2.5 ml of filtered (0.22 um) formaldehyde (37%). Samples were stored in the dark at -20°C and quickly thawed before further treatment. Because of the attachment of bacteria to detritus and phytoplankton frustules and the formation of bacterial aggregates, the water samples of the bacteria must be pretreated in order to have a random distribution for counting purposes. The method for disaggregation of bacteria was taken from Velje (1984). Samples were incubated in 0.001 M tetrasodium pyrophosphate suspended in 0.44 M sodium chloride solution for 15 min and then treated with sonification at 100 W for 1 min (in my experiment). Then a one ml aliquot was taken from the treated sample water with an automatic pipette and put into 9 ml of filtered distilled water to get a final dilution of 10 times. Finally, 4 ml of diluted sample were filtered through Nuclepore polycarbonate membrane filters (pore size of 0.2 um and diameter 25 mm which had been prestained 24 h in 0.2% solution of Irgalan black BGL in 2% acetic acid); afterwards 0.4
ml acridine orange was added to stain the bacteria for 3-5 min and then the sample was filtered. Slides were made and counting was carried out with a Zeiss compound microscope equipped with an epifluorescent illumination system (Hobbie et al., 1977). Depending on the dispersive state of bacteria on the filter, 9-14 fields and a total of at least 200-400 bacteria were counted (Venrick, 1978). Total numbers were calculated according to the equation:

$$\text{Cells/ml} = \frac{\text{stained area of filter} \times X \times 10 \times 1.37}{\text{area of counting grid} \times 4}$$

$X = \text{mean number of cells of 9 or more fields,}$

$4 = \text{stained water on the filter membrane,}$

$10 = \text{dilution index,}$

$1.37 = \text{correction for added formaldehyde and tetrasodium pyrophosphate solution,}$

$\text{stained area of filter} = 2.0 \times 10^8 \ \mu m^2,$

$\text{area of counting grid} = 10,000 \ \mu m^2.$

For the biovolume of the bacteria, the length ($L$) and width ($W$) of the cell was taken as the average of 50 cells in each sample and the volume of cells was calculated as:

$$\text{Biovolume} = \frac{\pi}{4}W^2(L-W/3)$$

This formula applies to both rods ($L>W$) and cocci ($L=W$) (Bratbak, 1985). Bacteria carbon content was taken from the literature. $2.0 \times 10^{-13} \ g \ C \ cell^{-1}$ for bacteria was reported by Robinson et al. (1982) and average biovolume to specific carbon content of the cell is $2.4 \times 10^{-13} \ g \ C \ \mu m^{-3}$ (Bowden, 1977; Watson, 1979; Hagstrom, 1979); $5.6 \times 10^{-13} \ g \ C \ \mu m^{-3}$ was suggested by Bratbak (1985).
Biovolume/ml = bacteria number/ml x average volume

Bacteria carbon/ml = biovolume/ml x carbon conversion

3.3 Measurement of salinity and calculation of salinity gradient and nutrient dilution change

The initial salinities were measured in the physical oceanography laboratory in our department immediately after the collection of water. During the experiment, intermediate salinities for each day were calculated according to conservation and continuity of the salt using the following equation dealing with a two dimensional box model (Pritchard, 1969):

\[
S_i = \frac{V_1 \cdot S_{i-1} + V_2 \cdot S_0}{V_1 + V_2}
\]

\(V_1\) = volume of experimental water,
\(V_2\) = volume of water inflow from reservoir (1 litre),
\(S_i\) = salinity of that day in experimental water,
\(S_0\) = salinity of reservoir,
\(S_{i-1}\) = salinity of previous day,

The calculation was done with a small computer program.

The dynamic changes in nutrient concentration result from utilization by phytoplankton biota and bacteria and dilution effect by mixing with two different waters of different nutrient concentrations. For the pure dilution effect it was assumed that nutrient concentration is a conservative property and thus change was calculated as for salinity.

3.4 Microflagellates and phytoplankton

Many methods have been reported for enumeration and identification of microflagellates (Fenchel, 1975; Sorokin,
1979; Sherr et al., 1983 and Hewes, 1983). The method used here was direct counting using routine light microscopy at 40x magnification. 150 ml water samples were preserved with 5 drops of Lugol's solution (200 g KI + 100 g I\(_2\) in 2000 ml H\(_2\)O + 190 ml glacial CH\(_3\)COOH). Counting and identification were done in a inverted microscope base plate chamber (volume = 2 ml). Identification of heterotrophic and autotrophic microflagellates was according to the pink and brown colour. All the non-pigmented flagellates were not included in the counting, only the microzooflagellate size between 2-10 um were counted because those that are larger than this may have a higher trophic function (Fenchel, 1982b). Ciliates were found in the samples but the low number made it very difficult to obtain meaningful counts of this group. Ciliates were not counted, but their presence suggests they must play a important role in controlling flagellate numbers.

For analysis of phytoplankton only major dominant species were identified to genus or species, all other minor species were counted as one group. Because I considered the microcosm system based on trophic level, the species composition was not regarded as being of major importance.

3.5 Standing stock of phytoplankton

In vivo fluorescence was measured to give an indication of biomass of the phytoplankton assemblages in the cultures. Samples were held in 25 x 150 mm culture tube which were inserted into a Turner 10-000 fluorometer every day at a given time. The subsamples were then cultured in sequence at constant salinity and at the same condition and time period as in
experimental flasks. For the first experiment, chlorophyll a and in vivo fluorescence were measured simultaneously and then the correlation and regression between them calculated. The regression equation for in vivo fluorescence and the fluorescence with extracted chlorophyll a in 90 °/o acetone was

\[ Y = 0.70X + 1.73, \quad (r^2 = 0.98, \quad N = 6) \]

The regression equation for chlorophyll a (ug/l) and fluorescence (of extracted value) was:

\[ Y = 0.167X + 1.74, \quad (r^2 = 1.00, \quad N = 10) \]

according to these two equations chlorophyll a and biomass of phytoplankton can be calculated in the experiment. The ratio for Bacillariophyceae are not very different. (Kiefer, 1973; Parsons, 1969; Parsons et al., 1984).
4. Results

4.1 Experiment stage I

This stage of the experiment was designed to answer the question of what is the behavior of fresh water ecological components on the salinity gradient. Because the extrapolation of temporal evolution of an estuarine ecosystem to estuary spatial distribution is based on the precondition of the fresh water autotrophic components which stop functioning at the head of the estuary, the evolution curve is actually that of a sea water population of phytoplankton. So in this experiment sea water was filtered by a 0.8 um filter to remove the sea water phytoplankton population and microflagellates and then I used it either as dilution water or initial experimental water to create either an increasing salinity in part 1 or a decreasing salinity gradient in part 2. The response of fresh water components on these salinity gradients were investigated.

4.11 Increasing salinity gradient

In experiment part one, an increasing salinity gradient was maintained by adding filtered seawater (29⁰/o) to fresh water. The salinity change during the experimental period is shown in Fig. 4. An increasing temporal salinity gradient was clearly constructed in the Stage I, part 1. The resultant curve of phytoplankton and nutrients (NO⁻³, NH⁺₄) (Fig. 5a) shows the interaction between these two components on the salinity gradient as the combined effects of the salinity and the dilution. A small increase on day 1 and 2 was due to fresh water algal growth; the major species was Thalassiosira spp.. On
Figure 4. The temporal salinity pattern in all of the experiment stage I or II, part 1 with an increasing salinity gradient.
Figure 5a. The development pattern of autotrophic component and nutrients in the experiment stage I, part 1 during July, 1985.
Figure 5b. The development pattern of NH$_4^+$ in the experiment stage I, part 1 during July, 1985.
Figure 6a. The development pattern of autotrophic component and nutrients in the experiment stage I, part 1 during February, 1985.
Figure 6b. The development pattern of NH$_4^+$ in the experiment stage I, part 1 during February, 1985.
day 3, when the salinity increased to about 15°/oo, the growth of algae were depressed until day 6. Most of the fresh water diatoms died or were depressed when salinity reached 10°/oo (Blanc et al., 1969). Some of the diatoms showed very distinctive morphological changes and some other structural changes (such as a decrease in chloroplasts) and auxospores were formed within 48 h (Foester, 1971). The physiological state of these diatoms decreased with increasing salinity (Qasim, 1972; Paasche, 1975; Wetherell, 1961). An increase in in vivo fluorescence after day 6 was caused by a mixed population. At the beginning of growth, Thalassiosira spp. occurred and were then replaced by a small round diatom (unidentified). The decline of the latter population at day 16 may have been due to even higher salinity (>25°/oo). The salinity range in which the second group of diatoms grew was 15 to 25 °/oo and it appears therefore that the original freshwater was influenced by the saltwater. It may have been contaminated by spores of estuarine species which have an optimal growth salinity around 10-20°/oo (Blanc et al., 1969; Mahoney, 1979; Qasim, 1972; Paasche, 1975). The experiment carried out in February showed no increase of phytoplankton during the whole experimental time and also no decrease of nutrients (Fig. 6a). Ammonium which is frequently more rapidly taken up than nitrate decreased before the decrease of NO⁻₃ (Fig. 5b, 6b). Even though in the experiment carried out in February, 1985 there was no growth of phytoplankton, the concentration of NH⁺₄ still showed a decline during the first period of the experiment (Fig. 6b).
This indicated a consumption which was not due to the autotrophic component and was most likely due to free living bacteria. In this work, I found that *Thalassiosira* spp. appeared at the two ends of the salinity range. These two groups may belong to entirely two different species. Unfortunately they were not identified to the species level, but there was no connection between the two groups in the middle range of salinity. This dominant species is the major species in the annual spring phytoplankton bloom in the Strait of Georgia. In the Fraser River estuary nitrogen in the surface layer is supplied from seawater entrainment (Tully and Dodimead, 1957; Parsons *et al*., 1980). River water is rich in B₁₂ (Cattell, 1973) and micronutrients (Cross and Sunda, 1977). The inflow of nutrient rich water should stimulate the growth of phytoplankton. However, comparing the fresh water control (Fig. 14) with the experimental flask and considering the nutrient dynamics, the phytoplankton growth was depressed and delayed in the experimental flask by osmotic stress. Therefore, the dilution (i.e. the salinity gradient) functions as a filter essentially filtering out all the freshwater species.

Morris (1978) suggested that one mechanism of oxygen depletion in an estuary is due to a mass mortality of fresh water halophobic phytoplankton which results from the osmotic changes occurring in the low salinity region; the concommitant release of organic material and its assimilation by the heterotrophic microbes would cause decreased O₂. The effect of this process on the heterotrophic component will be discussed in the next section.
Figure 7. The development pattern of heterotrophic bacteria and nanozooflagellates in experiment stage I part 1 during July, 1985.

Figure 8. Growth of freshwater bacteria on plate media at different salinities.
Heterotrophic and phagotrophic component

Bacteria, as the lowest heterotrophic component in an ecosystem, utilize organic compounds and particulate organic material as energy sources. They exist in natural sea water in two forms; active or dormant bacterial cells. Active bacteria has a high $V_m$ for uptake of organic substrate (Parsons et al. 1977) and a very high growth rate (Ammerman, 1984; Hagstrom et al., 1984; Van-Wambeke, 1985; Landry, 1984; Hollibaugh, 1980). These properties combined with others provide the bacteria with the physiological capability to complement their ecological role. The bacterial numbers in our experiment are in the range $4 \times 10^5$ to $4 \times 10^6$ cells/ml, which is consistent with Watson's work ($1.5 \times 10^4$ to $6.29 \times 10^6$; Watson, 1979). Most of the cells are small-sized and cocci shaped at the beginning. Zooflagellates are the major consumers of bacteria in pelagic systems (Lighthart, 1969; Haas and Webb, 1979; Fenchel, 1982b). They appeared oval shaped and biflagellated. Most of them fall in the size range 2-10 μm. The identification of species was not thoroughly carried out but according to Kudo (1966), Fenchel (1982b) and Haas and Webb (1979), they are mostly Monadidae, Bodonidae and Amphimonadidae (Sherr and Sherr, 1982). They are very abundant in the aquatic environment; usually from 0.4 to $13 \times 10^3$ cells/ml in various parts of the world oceans (Sorokin, 1981). Their density is close to one. The size distribution is toward the small size fraction (2-4 μm) and the biomass distribution was more even (101.3-198.3 μg wet weight/ml) (Sorokin, 1981). These flagellates may grow at $1.5 \times 10^2$ -
2.2 x 10^2 cells/ml/h with doubling times of 9.7-18.2 h (Sherr & Sherr, 1982). These doubling times are quite high for natural waters, indicating microflagellates are capable of responding to the dynamics of bacterial populations under natural conditions at certain rate.

The qualitative interaction between bacteria and nanozooflagellates in trophic relations have been clearly reflected in these data (Fig. 7). The low counts (4 x 10^5 cells/ml) and small size of bacteria on day 1 are due to the high zooflagellate grazing (13.8 x 10^3 cells/ml) which is selective towards big bacteria at high concentrations (Fenchel, 1980a,b). This point may represent the lowest threshold of flagellate grazing on a substrate limited bacterial population. The slight increase in bacterial numbers on day 2 and day 6 may be due to enrichment of the bacteria from the inflow of saline water and the release of organic substrate by osmotic impact on freshwater organisms. In addition it resulted in the optimum salinity for bacteria as indicated in my laboratory work (Fig. 8). During the initial period, microzooflagellates declined sharply. The reason is not clear; one possible explanation is the increase in salinity may strongly affect the zooflagellate population or in a limited situation, a zooflagellate population can be strongly affected by dilution. Unfortunately, there is no work on the tolerance of zooflagellates to salinity. The second increase in bacteria population seems to be due to the release of extracellular organic products or some stimulating products by phytoplankton growth (Bell and Sakshaug,
1980). Because the standing stock of chlorophyll a was low compared with that of bacteria, it seems that the bacteria consumed a carbon source which may not have come entirely from phytoplankton. Even though we assume that growth of the autotrophic component under osmotic stress may have a high proportion of excreted organic products, there appears to be a gap in terms of the carbon budget.

The division of organic carbon among the three trophic levels indicates that under this condition, the heterotrophic component makes a very large contribution to the energy flow. The highest value of bacterial standing carbon was 314 ug C/l. The average size of the zooflagellates was ca. 4.1 um. If I assume that all cells are spherical then

\[ V = \frac{1}{6\pi D^3} = \frac{1}{6\pi (4.1)^3} = 36.1 \text{ um}^3/\text{cell} \]

the carbon conversion coefficient = \(0.75 \times 10^{-11}\) gC/cell for the 4.5 um diameter cells (Fenchel, 1982b). So the highest value for the zooflagellate carbon standing stock was 106 ugC/l (14.1 \(\times\) 10^3 cells/ml). The chlorophyll a maximum was 4.32 ugChla/l; if 30 is used as a C/chl conversion factor (Parsons, et al., 1969), the phytoplankton standing stock only accounts for 129.6 ugC/l; however the rate of all the three components' activities has not been measured. I have no idea of the energy flux in the system which would be a more meaningful index than just carbon standing stock. Nevertheless, the high growth rate of bacteria (Ammerman, 1984) and zooflagellates under certain conditions (Fenchel, 1982a) and the high grazing rate (20% of the bacteria population per day Fenchel, 1982b; and a
bacterial populations of $2 \times 10^6$ cells/ml/day to $7.5 \times 10^6$ cells/day in a $7 \times 10^6$ cells/ml population in an estuary; Wright, 1983), both suggest that these two components may have greater potential capability to contribute to the carbon energy flow in the whole ecosystem. Ciliates as a third higher level of trophic interaction (Banse, 1982) existed in the experimental medium, reaching a relatively high abundance (main species was Tintinnidae spp.). This implies a high level of transformation and in sharing the standing stock of carbon (Heinbokel, 1978). Thus the dynamic rate and physiological state probably contain more significant information for the revelation of the functional structure and energy pathways in the ecosystem.

These results suggest that fresh water phytoplankton cannot play the same role in an estuarine ecosystem as they did in the original water. They cannot pass through the salinity gradient without changing their role. Their ecological role is not as a primary producer, but an organic contributor.

Data analysis was facilitated by the use of a statistical software package, "SYSTAT", and an IBM personal computer. Correlation matrixes were calculated for each part of the experiment from 7 variables and 20 observations. The interrelationships between the different variables were assessed by comparing pairs of variables. The correlation coefficients between pairs of variables are shown in Table 3. The high correlation of some variables such as standing stocks of phytoplankton, NO$_3^-$, NH$_4^+$ and bacteria with time can be
Table 3. Correlation matrix obtained from the 7 variables X 20 observations in experiment stage I, part 1 during July, 1985, between time (day), Fluorescence (fluo), NO$_3^-$, NH$_4^+$, Flagellates (flg), Bacteria (bac), and salinity. Probability: * p<0.1, ** p < 0.05, *** p < 0.02, **** p < 0.01.

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Table 4. Correlation matrix obtained from the 7 variables X 20 observations in experiment stage I, part 2 during July, 1985, between time (day), Fluorescence (fluo), NO$_3^-$, NH$_4^+$, Flagellates (flg), Bacteria (bac), and salinity. Probability: * p<0.1, ** p < 0.05, *** p < 0.02, **** p < 0.01.

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easily explained by the time evolution of these components. However, if we consider a body of water moving seaward at the surface layer in an estuary, these correlations can be interpreted as occurring on a spatial scale since time is proportional to distance during the movement of the water. This assumes that: (1) the fresh water organisms stop functioning as autotrophic components at the head of the estuary, and (2) marine phytoplankton are the major autotrophic component in the transition zone, but only begin to function after reaching the euphotic zone.

Nitrate, nitrite and ammonium were negatively correlated with chlorophyll a because the phytoplankton remove nitrogenous nutrients to synthesize new biomass with nitrogen compounds being mainly incorporated into protein. Bacteria also take up nitrogen as a nutrient source, but such uptake may be limited to organic substrates which serve as the bacterial energy source. Heterotrophic bacteria were positively correlated with phytoplankton standing stock because at the beginning of the experiment, bacterial populations were limited by organic substrate. The organic products released during growth of phytoplankton are utilized by bacteria as the only source (Rheinheimer, 1977; Hoppe, 1978; Larsson and Hagstrom, 1979; Wolter, 1982). Microflagellates and bacteria were not significantly correlated, even though their trophic interactions are very clear on the covariation process. The cycles between these two components correspond very closely and often, having completed several interaction cycles during the experimental
Figure 9. The temporal salinity pattern in experiment stage I & II, art 2, with a decreasing salinity gradient.
Figure 10a. The development pattern of the autotrophic component and nutrients in Stage I, Part 2, with a decreasing salinity gradient in July, 1985.
Figure 10b. The development pattern of NH$_4^+$ concentration in Stage I, part 2 with a decreasing salinity gradient in July 1985.
Figure 11a. The development pattern of the autotrophic component and nutrients in Stage I, part 2 with a decreasing salinity gradient in February, 1985.
Figure 11b. The development pattern of NH$_4^+$ concentration in Stage I, Part 2 with a decreasing salinity gradient in February, 1985.
time, the correlations offset each other.

4.12 Decreasing salinity gradient

In the second part of the experiment, stage I, 5 l of filtered (0.8 um) seawater was used as the experimental water and fresh water was pumped into it. The fresh water ecological component contained in the inflow as a inoculum encountered a gradually decreasing salinity gradient (Fig. 9). The heterotrophic bacteria in the sea water also encountered a decreasing salinity gradient. Freshwater phytoplankton in culture did not start growing until day 6 (Fig. 10a, 11a). The increase in chlorophyll a started after day 6 (10°/oo on that day). The growth rate of phytoplankton increased as the salinity decreased. Calculated biomass (chlorophyll a) increased quickly reaching about 1.88 ug Chla/l at day 13-15 until the experiment was terminated. Phytoplankton carbon standing stock reached a maximum of 56.40 ug C/l (conversion factor 30 which is for healthy cells under rich nitrogen conditions (Antia et al. 1963). In my experiment the growth of phytoplankton cells was under the impact of salinity change. The condition was not a normal condition so this conversion value may contain a large deviation.

The experiment was carried out during February, 1985. It showed the same pattern except there was an in vivo fluorescence peak by day 2-3, which then decreased as the salinity decreased. This may have been caused by the growth of some seawater autotrophs which were less than 0.8 um and could be inhibited by a decreasing salinity.
Figure 12. The development pattern of bacteria and nanozooflagellates in Stage I, part 2 with a decreasing salinity gradient in July 1985.
Table 4 shows the correlation coefficient between 8 variables in the experiment part two. The correlation between most of the parameters can be explained by the development of phytoplankton, except zooflagellates. All the variables which are directly related to each other show a close correlation; for example, nutrients and phytoplankton, and, bacteria and phytoplankton are closely correlated, time relates to the ecological components because of the development course of the phytoplankton. Time and salinity correlated with each other because it is the requirement of the experiment. The nanozooflagellates were not correlated with other variables during given time course and this suggests that the trophic relation of nanozooflagellates is not closely and directly related to primary production in terms of trophic relationships.

Hobbie and Rublee (1977) demonstrated a close relationship between phytoplankton primary production and the potential bacterial uptake of organic substances. Recent research has confirmed this relationship. The development of phytoplankton in this microecosystem results in the production of phytoplankton biomass and provides dissolved organic substances which stimulate bacterial populations. The distributions of carbon and energy flow between these two pathways depends upon many factors. These include the species composition of the algal flora (Fogg et al., 1965; Wolter, 1982) and the physiological state of the population (Sharp, 1977); microflagellates also may have an effect (Wolter, 1982; Goldman et al., 1985). In our experiment, possibly all of these factors
affected the production of extracellular organic carbon (EOC). The production of EOC and its utilization as a bacterial food source is determined both by the standing stock of biomass and by the relative rate of release of different photosynthetic products. However, in this experiment the phytoplankton can not pass through the full spectrum of the salinity gradient, so the primary production contribution to standing carbon is very small (45.40 ug C/l). It is not likely that phytoplankton released EOC can sustain the bacterial requirement. So some other source of organic substrates must have been utilized. The interesting thing is that a close covariance was maintained between phytoplankton and bacteria.

Heterotrophic bacteria and microflagellates

Figure 12 shows the development of bacteria and microflagellates. Bacterial density at day 1 \((8.3 \times 10^5\) cells/ml) was higher comparing that in the stage I, part one. This level may represent an upper boundary of the organic substrate limitation because the initial water was filtered with a 0.8 um filter. Most of the bacterivorous microflagellates would have been eliminated from the medium. This would have released the grazing exploitation of bacteria by nanoflagellates (Gak et al., 1972; Fuhrman and Azam, 1980; Wright, 1984). With the inflow of fresh water inoculum, the zooflagellates would have occurred in a relatively higher bacteria medium. Thus the new microzooflagellate population may have grown very quickly. As the microflagellates increased, bacteria showed a slight decrease and then an increase to a small peak. During
this period, the bacteria may have grown actively due to the enrichment by organic substances resulting from mass mortality of fresh water organisms when they flow into the high salinity water (29°/oo). Some of the bacterial biomass may be transformed into zooflagellate biomass by grazing during one day. If we assume that bacterial biomass doubled during one day (Ammerman et al., 1984) from day 1 to day 2 under a certain organic substrate supply rate, then the bacteria population could have increased to 16 x 10^5 cells/ml. However only 6 x 10^5 cells/ml were present at day 2, suggesting that 10 x 10^5 cells/ml were removed from the population. If the bacteria/zooflagellate yield factor is taken as 100 B/F.(Laak et al.,1984), then 10 x 10^5 bacterial cells/ml may produce 10 x 10^3 zooflagellates/ml. This value is in agreement with my experimental data. The number of flagellates increased from 8.4 x 10^3 cells/ml on day 1 to 18.5 x 10^3 cells/ml on day 2. Thus the sudden increase of nanozooflagellates can be explained by the transformation of bacterial biomass into microzooflagellates. If this calculation is justifiable, its magnitude is similar to that observed by others. Kopylov and Moiseyev (1980) observed flagellate doubling times of 0.67 to 1.44 days with a consumption of 50% of the daily production of bacteria. Wright (1983) observed 7.5 x 10^6 cells/ml were removed per day by zooflagellates from a population of 7 x 10^6 cells/ml. This evidence indicates that the carbon flux through this pathway can be very fast and significant in proportion to other processes. But in the ecosystem because of the fast
Figure 13. The development pattern of the autotrophic component, nutrient and bacteria in a controlled system with original seawater during July, 1985.
Figure 14. The development pattern of the autotrophic component nutrient and bacteria in a controlled system with original freshwater during July, 1985.
uptake rate and great growth rate of bacteria, the bacteria can exhaust their substrate over long time periods and in general, maximum growth rates will only occur over short periods. These two components may limited to explore their potential capability over long time period. The results in the experiments is a combination of the effect of both factors (growth limitation and grazing). From day 6, the number of bacteria increased constantly and, coincidentally, the increase of bacteria was simultaneous with the increase in chlorophyll a (see Fig.10a). However, calculating the phytoplankton carbon biomass, it is also not likely that the carbon incorporated into bacterial biomass is totally from the release of photosynthetic products. The maximum bacterial biomass is 314 ug C/l and the maximum of phytoplankton biomass only reaches 46.40 ug C/l. There are two possible explanations: (1) that under stressed conditions, most of the photosynthetic products were released as organic substances and the inflow of fresh water also brought in some organic material, or, (2) phytoplankton may produce some other materials which can actually stimulate bacterial use of another source of DOC which may be unavailable in the absence of the stimulant released by phytoplankton. This may suggest that bacteria may be limited by some other required nutrient.

Microflagellates on day 2 reached $18.5 \times 10^3$ cells/ml and then declined to $9.4 \times 10^3$ cells/ml. This decreasing population shows a response to the increase of bacteria at day 4-5. This indicates that this flagellate population was food source limited and then was also removed by other causes. There
are two obvious causes for the decline: (1) a non-growing population may be strongly affected by dilution and (2) some large ciliates such as Tintinnidae spp. were common in the experiment. They may have been responsible for the decline in the flagellates (Spittler 1973; Stoecker, 1981). On day 15, as thenanozooflagellates increased, the bacteria decreased.

Summary of experiment Stage I

Both fresh water and sea water phytoplankton grew very well in the controlled flasks and exponential growth appeared at day 2. There was almost no lag phase; NO$_3$ decreased sharply to zero. The decline of biomass was caused by nutrient limitation (Fig. 13, 14). The species composition was in agreement with other research (Spies, 1984; Stockner, 1977). Dominant species in the fresh water and sea water are totally different. In the sea water control, almost 90% of the biomass is attributed to Skeletonema costatum. In fresh water, the major species was Thalassiosira spp. The maximum chlorophyll a in sea water and fresh water were quite different; this was mainly due to the initial nutrient concentrations being different (fresh water, 8.83 ug NO$_3$ /l; sea water, 24.19 ugNO$_3$ /l). However, both systems developed phytoplankton blooms and the development patterns were very similar. But the developing pattern of bacteria in the freshwater system showed an interesting difference during the first part of the experiment (e.g. during exponential growth phase of the phytoplankton) compared with that of the seawater system. The bacterial population showed a small increase, not completely inhibited by the growth of
autotrophs as they did in the seawater system, even though both of them were maximal after the degradation of phytoplankton. This may have been due to species composition being different in the two systems. Comparing experimental vessels with the control, it becomes obvious that the growth patterns were different. Not only was the biomass very low during the same time periods in part 1 and 2, but a very long lag period also appeared in both due to the salinity impact and to dilution process. The results in both parts 1 and 2 indicate that freshwater species can not continue growing or start growing at salinities higher than 10°/oo. It is safe to conclude that fresh water phytoplankton can not actively function on the salinity gradient as the primary autotrophic component; instead they may undergo lysis and release of cellular contents, or they may form resting spores or be predated. Fresh water species may still be cultured after some adaptations as, for example, in Wethell's work (1961). However, most partially mixed estuaries such as the Fraser River estuary have residence times of less than 10 days. Fresh water phytoplankton may quickly go through all the salinity concentrations and this sudden impact may inhibit species adaptation and germination and result in high mortality. This suggests that estuaries may function to convert most fresh water planktonic organisms (except heterotrophic bacteria) to heterotrophic utilisable components which then go through the bacterial pathway.

Data for heterotrophic components for two different
salinity gradients seems to suggest that bacteria populations are mainly controlled by two parameters: 1) by utilizable organic substrates (both concentrations and rates of production) and 2) by heterotrophic microflagellates. The activity of the heterotrophic component may not be as influenced by salinity as that of fresh water phytoplankton. This may be due to a close succession in growth between fresh water and sea water bacteria or because fresh water bacteria usually growing better in intermediate salinities (Spies, 1984).

The development and arrangement of the ecological components are very different in experimental systems (i.e. changing salinity) compared with the controlled systems (i.e. stable salinity). Controlled systems are a homogenous ecological system, in which the condition is a prime state containing the lowest system energy. It is a stable state for a system. This condition favours phytoplankton growth with a decline of bacteria during a phytoplankton bloom suggesting a slow down of energy and materials flowing through the heterotrophic pathway. In the control systems, bacteria did not show a co-increase with phytoplankton exponential growth as they did in the experimental vessels, but the maximum bacterial biomass shifted to the degradation phase of the phytoplankton (Fig. 13,14). This separation of two components over time provides the phytoplankton with the largest space and materials supply (i.e. econiche) in the system. This creates the largest input of light energy (external energy) into ecosystem (system energy). However, in experimental vessels, the salinity
gradient creates a heterogeneous condition over time. Phytoplankton growth was inhibited at some part of the gradient. This left some ecosystem space which was used by heterotrophic bacteria. As a result, the bacteria showed a coevolution with phytoplankton. These two different structures under different physical environmental conditions may reflect the functional response of an ecosystem to achieve the largest ecosystem efficiency.

The ecological significance of the different behaviour of phytoplankton and heterotrophic components on the salinity gradient (or a heterogeneous conditional gradient) suggests an ecological evolution of estuarine ecosystems. The phytoplankton cannot occupy a certain space in the gradient. However, if this space contains a high system energy (e.g. organic substances) the heterotrophic components may successfully utilize it. This is what happens at the head of many estuaries in which there is more organic input than in any other aquatic environment. The ecosystem should have a strategy to exploit this energy source. So, the heterotrophic component must play a more important role than in any other kind of aquatic environment.
4.2 Experiment Stage II

As was shown in experimental stage I, the fresh water autotrophic components are not the major functional autotrophic group on the salinity gradient in the estuarine ecosystem. Thus if estuarine systems remain a phytoplankton energy input pathway (e.g. phytoplankton based food chain), the sea water phytoplankton should play a dominant role in estuaries provided that the light intensity gradient (determined by sedimental loading and residence time coupled with estuarine circulation) permits the growth of the phytoplankton. The experiment stage II was designed to observe the behaviour of marine ecological components on a temporal salinity gradient. The original marine ecological components go along the gradient at a constant dilution rate simulating the process which happens to the sea water ecological components on the surface layer during estuarine mixing. In the process, marine ecosystem planktonic components are brought up to the euphotic layer by entrainment from below where it is dark. Simultaneously, a salinity gradient exists in the surface water over time and over distance. The experiments were also designed in three parts.

4.2.1 Increasing salinity gradient

In part 1, which was designed with an increasing salinity gradient, original sea water was pumped into lighted experimental vessels (volume, 61) from a covered reservoir to simulate the increasing salinity gradient over time which occurs in a partially mixed estuary. A sea water seed population was allowed to flow into a body of filtered fresh
Figure 15. The development pattern of phytoplankton and concentration of nutrients in stage II part 1 on an increasing salinity gradient during Aug. 1985.
Figure 16a. The development pattern of the autotrophic component and concentration of nutrients in stage II part 1 in an increasing salinity gradient during April 1985.
Figure 16b. The development pattern of NH₄⁺ in stage II part 1 in an increasing salinity gradient during April, 1985.
water in an increasing salinity gradient at a certain dilution rate \( \frac{D}{\text{day}} = 0.16 \) (Fig. 4) The evolution pattern of the autotrophic and nutrient \( (\text{NO}_3^- + \text{NO}_2^-) \) components are indicated in Fig. 15. During the first two days, the high chlorophyll \( a \) value was a result of some small fresh water autotrophic organisms \(<0.8 \text{ um}\) which grew because of the elimination of grazing by zooflagellates during filtration. This small chlorophyll \( a \) peak decreased to zero with the inflow of sea water. Several mechanisms may work in this process: osmotic stress, sedimentation due to a change in surface charge, and dilution by sea water containing low bacteria and high nanoflagellate concentrations. Chlorophyll \( a \) did not increase until day 5 (salinity 15°/oo). This prolonged lag phase compared with "control vessels" can be attributed to the inhibition effect of low salinity on the germination of seed populations and on the growth of the growing population as well as to the dilution rate. Figure 18 shows the log transformed data of chlorophyll \( a \) increasing at different constant salinities. The growth of marine phytoplankton was apparently inhibited at low salinity. As the salinity increased, toward an optimal condition, the growth rate became faster by day 5. The chlorophyll \( a \) standing stock increased exponentially thereafter. By this time, the salinity gradient spectrum was within the optimal range for marine phytoplankton growth, the gradient no longer sustained a heterogeneous condition so the whole system shifted to a phytoplankton priority system.

At the beginning, (Fig. 17) bacteria had a high density
Figure 17. The development pattern of bacteria and nanozooflagellates in stage II, part 1 with increasing salinity gradient during Aug., 1985.
Figure 18. Development of the autotrophic component in a subsample culture series at temporally constant salinity in stage II, part 1 during Aug. 1985.
which may be composed of high cyanobacteria as indicated by high chlorophyll a values. After day 2 it declined. Bacterial density reached its peak during the first period before phytoplankton started growing (by day 6). The maximum density appeared at day 4, then slowly decreased until the end of the experiment. Bacteria did not show a close correlation with the growth of phytoplankton after day 6 (see Table 3) as in all other experiments. But the pattern may be explainable by the covariation of the microflagellate evolution pattern (Fig. 17) and the inhibition effect before a full phytoplankton bloom as in the controlled experiment (Fig. 13). The lower density of nanozooflagellates is expected at the beginning because of the effect of filtration. Flagellates started growing from day 2 until day 6, when the first peak was reached. The exponential increase in the microflagellate population indicated a great bacterial biomass demand. To meet this demand, bacteria should also have undergone a great increase in biomass, indicating a high bacterial activity in the first period. The second increase of microflagellates from day 7 to the end of the experiment may be responsible for the lack of correlation of bacteria with phytoplankton growth. The increase of bacteria by utilization of the photosynthetic products released by phytoplankton may be offset by the nanozooflagellate grazing. The increase in bacterial density is indirectly reflected in the increase in nanoflagellate abundance. The diel and periodic release of organic products by phytoplankton (Burney et al., 1981, 1982; Eberlein, 1983; Hammer, 1983; Kaplan, 1982; Sournia,
Table 5. Correlation matrix obtained from the 6 variables X 12 observations in experiment stage II, part 1 during Aug., 1985, between time (day), Fluorescence (fluo), NO$_3$, Flagellates (flg), Bacteria (bac), and salinity. Probability: * p<0.1, ** p < 0.05, *** p < 0.02, **** p <0.01.

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Table 6. Correlation matrix obtained from the 6 variables X 12 observations in experiment stage II, part 2 during Aug., 1985, between time (day), Fluorescence (fluo), NO$_3$, Flagellates (flg), Bacteria (bac), and salinity. Probability: * p<0.1, ** p < 0.05, *** p < 0.02, **** p <0.01.

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1974) may create situations in which bacteria are temporarily limited by substrate, so the exploitation by nanozooflagellates may show a net reduction effect.

Table 5 shows the correlation coefficient between the variables; bacteria did not show any significant correlation coefficient with fluorescence as in stage I experiment.

4.22 Decreasing salinity gradient

In part 2, the original sea water was held in experimental vessels and diluted with filtered fresh water. This resulted in a decreasing salinity gradient over time for the marine ecological microbial component and fresh water bacteria. The development of marine phytoplankton (Fig. 19) shows a two day lag phase, compared with the "controlled vessels". The extra one day lag may have been due to dilution ($D = 0.16 \text{ d}^{-1}$), and the perturbation of decreasing salinity may also affect the germination of seed populations. The optimal salinity for many seawater species, including Skeletonema was reported at a lower salinity range so the growth at the beginning may be slower relative to the dilution rate. The exponential growth phase started at day 3. Biomass increased very quickly and reached its peak by day 6. The depletion of nitrogen by day 6 stopped the phytoplankton biomass increased (Fig. 19) and the osmotic stress and dilution may have caused the decline of chl $a$ standing stock through the death and lysis of the cells. This in turn may have speeded up the bacterial activity. The inflow of fresh water introduced some nutrients (17.5 ug-at N to 6 l of water), which may have caused the prolonged plateau phase
Figure 19. The development pattern of the autotrophic component and concentration of nutrients in stage II, part 2 on a decreasing salinity gradient during Aug. 1985.
April 11, 1985.

II, Part 2: In a decreasing salinity gradient during component and concentration of nutrients in stage.

Figure 20A. The development pattern of the autotrophic.

Days

Relative Fluorescence

Nitrate (μg-at/l)
Figure 20b. The development pattern of $\text{NH}_4^+$ in stage II, part 2 on a decreasing salinity gradient during April, 1985.
observed during the Aug. 1985 experiment (Fig. 19), but the slow decline rate compared with the "control vessels" indicates that at least part of the biomass was in a chemostat state, if the nutrient flux was large enough as in the experiment carried out in April, in which fresh water contained much higher nutrients (Fig. 20a). The large biomass standing stock may be sustained in a temporally balanced state. This implies that, in the mouth of an estuary, certain conditions may exist briefly as in a natural chemostat environment which provides longer periods of high standing stock biomass; this may benefit the zooplankton larval feeding and may promote the carbon transport efficiency resulting in a high secondary production (Parsons et al., 1984).

Heterotrophic bacteria have a very strong ability to assimilate inorganic nutrients (Nicholas, 1963; Painter, 1970). With a sufficient source of organic carbon bacteria can successfully compete with the autotrophs for inorganic nutrients. Most of the organic products released during photosynthesis are carbohydrates so bacteria need nitrogenous nutrients for the synthesis of amino acids. The great amount of organic carbon released at the plateau phase may quickly be used by heterotrophic bacteria (Larson and Hagstrom, 1979; Martin, 1980; Wolter, 1982). This in turn speeds up the competition for inorganic nutrients and the death of phytoplankton. This chain reaction after exponential growth favours bacterial activity and increases the rate of materials recycling.

The evolution of nitrogen ($\text{NO}^-_3 + \text{NO}^-_2$) was not at first very tightly coupled with the growth of phytoplankton and
with the dilution. The decline of \((\text{NO}^{-3} + \text{NO}^{-2})\) was larger than the dilution effect. The bacterial growth during this period may have caused the decline of nitrogen nutrients but this uptake by bacteria seemed to be stopped by the depletion of available organic carbon sources. The continued decline in nitrogen concentration was obviously due to phytoplankton growth from day 3 to day 6. By day 6, nitrogenous nutrients \((\text{NO}^{-3} + \text{NO}^{-2})\) were depleted.

Some phytoplankton species were present in the experimental water, such as *Nitzschia* spp., *Thalassiosira* spp., *Ditylum* spp., *Chaetoceros* spp. and *Asterionella* spp., but all were rare. The major species was *Skeletonema costatum*, which became especially dominant at the end of the experiment. This may have been due to competition and dilution rate selection effects as shown by Harrison and Davis (1978).

Subsamples were taken every day and cultured in 25 x 150 mm borosilicate culture tubes in series in order to detect the growth or germination of these phytoplankton under a constant salinity in a batch culture state. Figure 22 indicates that the natural assemblage of phytoplankton had an optimal salinity of about \(15^\circ/oo\) in our experiments. This is consistent with the results of others which indicate that most coastal species of phytoplankton from different areas of the world show maximum growth at low salinities, \((15 - 25^\circ/oo)\) i.e. lower than normal sea water. (Brand, 1984, 1982; Qasim et al., 1972). *Skeletonema* is a common euryhaline planktonic diatom, optimal growth seems to occur at salinities of \((15-25\%)\) (Braarud, 1951;
Figure 21. The development pattern of bacteria and nanozooflagellates in stage II, part 2 on a decreasing salinity gradient during Aug., 1985.
Figure 22. Development of the autotrophic component in a series of subsample cultures at temporally constant salinity in stage II, part 2 during Aug. 1985.
Paasche, 1975; Brand, 1984), which is consistent with my data.

Figure 21 shows the coevolution curve of heterotrophic bacteria and nanozooflagellates. The higher nanoflagellate and lower bacterial biomass at the beginning was quite obviously consistent with that of other experiments. This may indicate the lowest threshold of grazing on bacteria by nanozooflagellates. Bacterial biomass reached its first peak before phytoplankton started growing but decreased sharply when the phytoplankton began exponential growth. This sharp decrease of bacteria was also shown in Spies' work (1984) and the data in the control vessel (Fig. 13, 14). These similar results in different studies at different times suggest that the results inevitably occur when favourable conditions lead to phytoplankton blooms. Low nanozooflagellate densities which occur during this period do not seem to be responsible for the reduction in bacteria.

The mutual control of biological activity by external metabolites is an important relationship between phytoplankton and bacteria. The specific interaction may be mediated through production of stimulative substances, the excretion of inhibitory substances, or through competition (Sieburth, 1959, 1968). Bell et al. (1974) reported a selective stimulation and inhibition of bacteria by Skeletonema costatum. Kogure (1979) confirmed this result with direct evidence that S. costatum has an inhibitory effect on Pseudomonas and Vibrio spp. and a stimulatory effect on Flavobacterium spp. during exponential phase. This antibacterial action basically depends upon the algal growth conditions. The strongest antibacterial activity was observed during the exponential
phase of algal growth. Martin (1980) showed that the bacteria population during the evolution may shift its composition with a succession of different generic groups to adapt to the different physiological states of phytoplankton (*Skeletonema costatum*). The dominant bacterial species during the exponential growth phase of phytoplankton and the beginning of the chl a plateau was different from that found during the phytoplankton death period. Martin found that Vibrio-like organisms are more important later, particularly when plankton mortality occurs. This is in agreement with work done by Sieburth (1968) and Simidu et al. (1971).

Considering the nutritional mode, the extracellular products of phytoplankton during exponential growth are mostly carbohydrates (Eberlein, et al., 1984). Bacteria which use these as their only carbon source must take up nitrogen. During this period, the nitrogen sources were not depleted. However, the bacteria which grow after the plateau phase have no ambient mineral nitrogen source and most likely utilize amino acids (Amano et al., 1982, Bright, 1983). This also suggests that a different nutritional mode may dictate different species groups.

In my experiment, bacteria growing during the first period were largely free-living while attached bacteria dominated after phytoplankton exponential growth. The latter were mostly attached to diatom frustules, and were big and rod shaped. In natural waters, free-living bacteria and attached bacteria may have different morphological properties and also may require
different nutritional modes of dissolved organic substrates (Fukami et al., 1985, 1983, 1981). The two peaks which developed in this part of the experiment may reflect this shift in composition. Phytoplankton may excrete inhibitory substances to decrease free-living bacteria which may be potential nutrient competitors and to stimulate another strain of bacterial growth. The fact that bacterial density decreased before nutrient depletion indicates that nutrient competition was not the direct mechanism for the bacterial decline before the phytoplankton bloom. Kogure (1979) showed that the addition of N and P did not influence the inhibitory effect and concluded that suppression of bacterial growth was not a result of competition.

He also found that higher and lower light intensities decreased the antibacterial activity. This may be due to the light inhibition and light limitation which reduce the growth of phytoplankton. This may explain why the inhibitory effect did not occur in the stage I experiment in which the growth of phytoplankton was inhibited by the salinity gradient. Thus there must exist a more complex dynamic state of interactions between bacteria and phytoplankton.

This phenomena indicates that under the conditions of an autotrophic bloom, heterotrophic pathways are shut down to give way to the utilization of mutually required nutrients for autotrophic production. Then the whole system becomes an autotrophic priority system.

What is the effect of this interaction on microflagellates?
There is no immediate answer to this question, since they are at a higher trophic level and depend on bacteria, they must be affected. The constant low level of nanozooflagellates until day 6 is quite different from other experiments. The decline of the bacterial population (Fig. 21) during first period may prevent the restoration of the nanozooflagellates.

The results in this experiment show the quite different responses of seawater phytoplankton populations to a salinity gradient. Seawater populations may have a wide range of adaptations to salinity but they can be inhibited on the low salinity range (<10°/oo). Subsample cultures show that below 10 o/oo of salinity, the seed population did not grow during the same period of time as in the experimental flasks. They cannot actively function or germinate in the low salinity portion of the gradient. This suggests that seawater populations can flourish only at the higher end of salinity spectrum.

The results in these two parts of the experiments suggest that bacteria occupy the first period of the experiment and phytoplankton occupy the last part. In other words, bacteria adapted to the low part of the salinity gradient, while phytoplankton adapted to the high part of salinity gradient. In extrapolating this result to an estuary, it means that the heterotrophic bacteria occupy the low salinity water at the head of the estuary, and phytoplankton standing stock appears greater at the mouth of the estuary. This is in agreement with many field observations (Albright, 1983; Bell et al., 1981; Wright,
Correlation calculation (Table 6) shows a higher significant coefficient between time, fluo, NO$_3^-$, and salinity. This is expected because of the development of the phytoplankton. However, the correlation between bacteria and phytoplankton did not appear in this part of the experiment.

The controls had two culture vessels containing original fresh water and sea water respectively. The evolution pattern of phytoplankton assemblages were similar in these two flasks (Fig. 23a,b). The major species of the chlorophyll maximum were 

*Skeletonema costatum* and *Thalassiosira* spp. in sea water and fresh water respectively. The maxima of biomass in these two flasks are quite different; this may result from the initial nitrate concentration difference. Sea water contains 35 ug-at N/l, while fresh water contains 17.5 ug-at N/l. The decline of standing stock after nutrient depletion is due to sinking to the bottom (even though I used a strong magnetic stirrer) and also due to degradation by heterotrophic bacteria (Fig. 13,14).
Figure 23a. The development pattern of the autotrophic component in a controlled system with original seawater during Aug. 1985.
Figure 23b. The development pattern of the autotrophic component in a controlled system with original freshwater during Aug. 1985.
Discussion

There is surprisingly little information on the ecosystem structure of fresh water entering the sea or of the ecological role of the fresh water phytoplankton component in an estuarine ecosystem. Some work has been reported on fresh water phytoplankton in estuaries and saline water environments (Blanc et al., 1968; Wetherell, 1961; Foester, 1973), but they focus more on the physiological state of these phytoplankton groups than on the ecological role in the estuarine ecosystem. Blanc et al. (1969) concluded that fresh water phytoplankton in diluted sea water (8°/oo - 26°/oo) were mostly dead or almost dead. Foester (1973) in a more detailed study, concluded that freshwater phytoplankton can survive in diluted saline waters. It is an important matter whether freshwater phytoplankton can still play an autotrophic role in estuarine ecosystems. Can they pass through the salinity gradient with some normal functions? If not, what is the effect of this disfunction of phytoplankton on the whole ecosystem structure. The results in my experiment (stage I) clearly demonstrated that freshwater phytoplankton can not actively function as an autotrophic component in an estuarine ecosystem. The living biomass contribution in experimental systems by freshwater phytoplankton was very small (Fig. 5a, 6a, 10a, 11a) compared with the production in the controlled systems (Fig. 13, 14). The activity of the freshwater phytoplankton was restricted in a narrow range of salinity. Along the whole salinity gradient,
most of the freshwater phytoplankton biomass was converted into the organic detritus pool. This change resulted in a structural change for the whole system which shifted the energy flow towards a heterotrophic pathway. Therefore the heterotrophic component became the major contribution in the fresh water component of the estuarine ecosystem.

Experiment stage II showed that the sea water phytoplankton can quickly function in a salinity gradient. This is probably because the evolution of phytoplankton has been in the same direction as the evolution of the salinity gradient from low to high over time and space. So the ecosystem is in a condition of always being close to a sea water state. The time series of biological change in the population of freshwater organisms in sea water (Stage I, Part 1) and the seawater organisms in the same gradient (Stage II, Part 1) together give a picture of what may occur in a real estuary (Fig. 5a, 6a; Fig. 15,16a). At the phytoplankton level, the sea water population is dominant in an estuarine system. This dominance gradually reaches its maximum at the high salinity end of the gradient. This salinity gradient creates a heterogeneous condition which clearly separates the phytoplankton niches over time. Marine species were inhibited at the low end of the salinity gradient (Fig. 15), as the gradient moves close to a species suitable range, phytoplankton grow very quickly and the biomass increases exponentially. The maximum development of biomass is almost the same as in the controlled vessels, but the delayed growth phase results from the salinity gradient. If we consider the same process happening in an element of water moving seaward on the surface layer...
of an estuary, the temporal evolution pattern of the ecological components in that element will become a spatial distribution pattern. This model may be a reasonable explanation of why estuarine ecosystems show this spatial distribution pattern. The fact that the increase of chl a along a salinity gradient reaches its maximum often at some distance from the mouth of the estuary is an evolving pattern which coincides with the spatial scale because of the movement of the water mass.

The dynamic pattern of nitrogenous nutrients in all our experimental systems was a function of evolution of phytoplankton and bacterial dynamics. This is to be expected because the nutritional relationship decided the covariance between living components and nutrients. That is, the growth process is a process of incorporating material into the living biomass of the components (phytoplankton and bacteria). Statistical calculations show a strong negative correlation between Chl a and NO$_3^-$, and a negative correlation between NO$_3^-$ and time which can be easily explained as a result of phytoplankton development over time. This kind of distribution of necessary nutrients along an estuarine salinity gradient is also shown in all works in which an increasing biomass of phytoplankton is found along an estuarine transition zone. In estuarine ecosystems, seawater phytoplankton and some estuarine populations are the major autotrophic components. Because the temporal evolution of the component from the head of the estuary coincided with spatial scale by continuous outflow, the increase of biomass along an estuary should be a standard phenomena as
the phytoplankton distribution spatial pattern and the primary conservative nutrients also have a corresponding spatial pattern. However, the dynamics of ammonium in the system has a quite different behaviour pattern. The dynamics of ammonium concentration is a combination of many processes which happen in the ecosystem. Phytoplankton and bacteria can utilize NH$_4^+$ as a nitrogen source. The assimilation of nitrate and ammonium is the major biological process by which these two components covert inorganic nitrogen to organic nitrogen, although their relative contribution to total nitrogen assimilation is extremely variable in time and space (Dugdale, 1967 McCarthy, 1972; Walsh et al., 1980). It is well known that in the presence of NH$_4^+$, unicellular algae preferentially take up this nutrient (Blasco and Conway, 1982; McCarthy & Eppley, 1972). The generation time of NH$_4^+$ and NO$_3^-$ is very different. This may result in quite different dynamic patterns. Bacteria can utilize amino acid as energy source and then be grazed by microflagellates which may directly release NH$_4^+$ (Adernson, 1985; Goldman and Caron 1985). It is interesting that in my experiments the dynamics of NH$_4^+$ showed different patterns with different system components but not with different salinity gradients (Fig. 5b, 6b, 16b and 20b). In the freshwater components testing experiment, the concentration of NH$_4^+$ shows a decrease from its initial value to a value close to zero in the first period of the experiment, before the increase of Chl a. There was no indication that this decrease was due to uptake by phytoplankton (this is most clear in the
Figure 24. The energetic dynamic state of an ecosystem in the energy level field.
experiment during April (Fig. 5b, 6b)). There was no decrease of NO$_3^-$ over the whole experimental period but the NH$_4^+$ still showed a decline over time. The heterotrophic bacteria production showed an increase of biomass which may possibly utilize NH$_4^+$ (Fig. 7). This may suggest that the bacteria which grow in the first period of the experiment were most likely at the expense at carbohydrate, and thus they need an extra nitrogenous nutrient to synthesize amino acids. In the experiments involving the seawater biota, the dynamics of the concentration of NH$_4^+$ are quite low but had increasing peaks after phytoplankton was degraded, regardless of the different salinity gradients. The NH$_4^+$ peaks may indicate the mineralization of phytoplankton detrital organic materials by the heterotrophic components. These two different patterns of NH$_4^+$ uptake may result from differences in the ratio of C/N in sea water and fresh water or a difference in the nature of the bacterial populations in either water (if we assume that the bacteria in both the initial waters had already reached maximum biomass as determined by some other factors). In experiment stage I the inflow of fresh water and sea water result in an osmotic stress which may cause the release of organic substrate from fresh water organisms. This addition of organic nutrients may stimulate the growth of bacteria. However, in the seawater system, the water may contain little carbohydrate because of the long history of utilization by bacteria in the aphotic zone. The sea water population had a higher initial cell concentration which may have resulted in
rapid uptake of NH$_4^+$ before the start of exponential growth.

The high metabolic rate and fast mineralization of heterotrophic components suggest that the system has a tendency to go back to the autotrophic priority system, that is when the internal energy is at its lowest level, and the system is dominated by inorganic substances and having the largest potential bioenergy carrying capacity. Therefore the whole system adjusts so that it is based on a low energy containing component which may be more stable.

From my experiment, the results clearly show that the behaviour of freshwater autotrophic component and seawater autotrophic component is totally different in the salinity gradient. In the real estuary, with one direction circulation (tide average) there is only an increasing salinity gradient. To fresh water components, this gradient works as a filter; it filters out most of the freshwater phytoplankton as they pass to high salinity and are converted into organic material. Some euryhaline phytoplankton may keep their function at intermediate ranges of the salinity gradient, but their contribution compared to the seawater population is very low. The seawater phytoplankton have a different behaviour. They can be inhibited at low salinities. However, the continual movement along the gradient moves them close to their optimal environment. So they function more actively as the outflow gradually provides more favourable conditions.

The most important feature of an ecosystem is the dynamics
of the phytoplankton production which must have a radical effect on the whole structure of the ecosystem. The most closely related ecological components to phytoplankton are the inorganic nutrient pool, the organic substance pool and the heterotrophic components. Because heterotrophic bacteria are a second level energy transformer, so their activity and production is primarily dependent on the availability of organic substance in the water. There are two different foundations based on these two components which decide different energy routes in the ecosystem.

In all our experimental systems it is shown that bacterial productivity is not affected by the salinity gradient as much as that of phytoplankton. For two different salinity gradients having two different heterotrophic components, biomass and growth of bacteria did not show a critical range of salinity as did the phytoplankton. This may be due to the presence of bacteria in both seawater and freshwater. And also either seawater or freshwater bacteria show a tolerance to a range of salinities. Their dynamics are much more tightly coupled with the amount and quality of organic substrates and the state of the autotrophic component.

If one considers the energy state of a ecosystem, the system can have two energy states, one is a high internal energy state which is thermodynamically unstable. There is a great amount of organic substances in the system. This system tends to release internal energy out of the system which goes back to an inorganic substance based system; this is a low internal energy
state and is more thermodynamically stable. Bacteria play a major role in this energy release process. Bacteria have a very high uptake rate ($V_m$) for both organic substances and inorganic mineral nutrients as well as a high growth rate ($u$) which can successfully compete over the autotrophic component when the energy state is high in the system. Also when the system energy state decreases to a certain level the bacteria become limited by the energy source. The physiological properties of the bacteria actually are consistent with their natural ecological role. The bacteria heterotrophic process is limited by the system's internal energy. The other state is a low internal energy state which is thermodynamically stable. This state can be excited by external energy through the photosynthetic process. As the light energy flows into the ecosystem, the energy state of the system increases to a certain level which is determined by the energy carrying capacity of the system; this capacity is mainly controlled by the limiting nutrients. This means that a high energy state system (living system) always needs energy input from outside of the ecosystem otherwise it will go back to ground state through a heterotrophic process (mainly bacteria). Between these two processes, heterotrophic processes are downhill processes; their potential may be greater than autotrophic processes which are uphill processes, because they need external energy support. This idea is summarized in a diagram (Fig. 24).

The physiological and energetic properties of bacteria provide bacteria the privilege of utilizing its energy source as
quickly and as completely as possible to accomplish its ecological role in an ecosystem, allowing the system to go back quickly to a stable state. The fast response mechanism is also reflected in the dormant nature of the bacterial population. When organic nutrients become limiting, bacteria do not immediately go through depletion as phytoplankton did, but go into a dormant stage which suggests that this storage nature provides a high background concentration of bacteria. This means that bacteria keep a certain storage biomass so they can quickly respond to the increase in energy state of the system. This process has the ability to let an ecosystem go back to a stable state as soon as possible. The whole system would tend to stay at a low internal energy state (phytoplankton priority systems) if there is no further energy input. Bacteria in this system state should be limited by organic substances. Further, there is no equilibrium between bacteria and microflagellates. This means that neither the amount of organic substrates can ever be effectively utilized, (so the system could not go back to stable state), nor can the microzooflagellates ever become fully developed due to their own feeding limitation on the bacterial food source. Implicit in this theory is that bacteria may have a low $K_m$ for organic material at low nutrient concentration (since the DOM concentration in seawater is low). Moreover, this $K_m$ can change with the changing of conditions (Azam et al., 1981; Vaccaro et al., 1967,1969). In a number of experiments in which substrate has been added to seawater (Parsons et al., 1977,1981) the bacteria showed a very high
$K_m$. This suggests that bacteria may shift the $K_m$ within the population from low to very high depending the substrate concentration so its growth rate can always catch up with the addition of organic substances. This means that the growth of bacteria can largely exceed the grazing of zooflagellates under high concentration of organic substrates. In my experiment, bacterial biomass always increased with the input of organic substances by phytoplankton growth. The microflagellates could not balance the growth of bacteria when the organic substance was not limited. The microflagellates may only offset the bacteria at a certain rate of consumption where bacterial growth is limited by organic nutrients. Some work indicates that the abundance of microflagellates is a function of bacterial cell numbers regardless of its physiological state (e.g. living or dormant). Therefore bactivorous flagellates can not limit the utilization of organic substances by bacteria and can not control the upper limit of the bacterial density. Bacterial concentration upper limit is decided only by availability of nutrients and a space density factor. After bacteria go into a limitation state, grazing decides the lower density limit, which is the grazing threshold of the microflagellate populations. Microflagellates are more an exploiter of bacteria than a limiter. The equilibrium between organic substances, growth of bacteria and grazing of microflagellates doesn't exist as suggested by some authors (Wright, 1978, 1982, 1984). This equilibrium being a mechanism which is against the actual function of bacteria in the ecosystem.
The properties of bacteria as an ecological functional requirement provide bacteria the ability of quickly responding to the addition of organic nutrients and it can often increase several fold within a 24 hour period (Wright, 1978, 1983). The dynamics of the bacteria is actually a reflection of the dynamics of the organic pool in the experiments. In the absence of phytoplankton during the first period of experiment stage I, II, part 1, the increase of bacteria should indicate the organic substance addition or resulting from the mixing of two kinds of water which provide a mutual benefit for the bacterial growth. This could be some micronutrients which may be short in one type of water but rich in the other. If we assume that before the experiment started the initial waters were both in an organic limited state for bacteria, the mixing process must then cause some transformation of organic substance to a labile source, or, if there is some labile organic source, its utilization must be limited by some other factors which could be released by mixing the two kinds of water.

In an isolated system, the organic source must be from the autotrophic component. Therefore bacteria depend on the autotrophic component which can entirely control the heterotrophic activity through the supply of substrate. However, in an estuarine ecosystem, the control of bacteria by phytoplankton is perturbed by allochthonous organic substance from the terrestrial environment. Therefore the bacteria may increase the importance of their role in the ecosystem due to
the independent course of substrate supply. They will play a much bigger role than in an autochthonous system in terms of energy flow and matter recycling. Their quantitative role depends on the ratio of available organic substances and the mineral nutrient.

The ecosystem structure may show three forms:

1. Phytoplankton priority systems.

In these systems, the conditions favour phytoplankton growth. Bacteria may be more or less limited by organic substances. The foundation of the system is that inorganic substances dominate. The quick growth of phytoplankton may inhibit any previous existing bacterial populations so that material and energy flow through the bacterial pathway is shut down. All the possible mineral nutrient pool goes to provide autotrophic productions as is shown in our experiment, stage II part 2 and all "control vessels" (Fig. 13, 14, 23a, b).

2. Phytoplankton and heterotrophic coevolution systems.

Provided bacteria are limited by their energy source, most of the organic nutrient must come from release of phytoplankton exudate during photosynthetic production. But if the growth of the autotrophic component is restricted for some other reason, the autotrophic component can not make full use of the system. This system has extra substrate for bacteria and so the bacteria can show a coevolution pattern. The inhibiting effect of phytoplankton on bacteria is not obvious. However, the use of released material from phytoplankton by bacteria is indicated in correlation calculations. These clearly show in
our experiment stage I and II (Fig. 7, 12) that the salinity gradient condition depressed the phytoplankton growth. So in this case the carbon budget showed two concurrent pathways.

3. Heterotrophic priority system.

The system at the beginning may have a high allochthonous organic substance addition, such as often happens in an estuary and some polluted water. Then the control of bacteria by the phytoplankton autotrophic component through energy supply is overruled by the addition of an allochthonous energy source. In this case, very strong metabolic growth of the bacteria under conditions of enough energy provide bacteria with the ability to dominate in the system. Most of the material goes into the heterotrophic pathway. The autotrophic production is then depressed, as shown by Parsons (1977, 1981) and Spies (1984). This is a homeostatic mechanism which occurs rapidly and eliminates the dissolved organic matter through bacterial biomass and mineralization. Then the system returns from a high energy state to a low energy state and provide available substance for external energy input to the system which is a phytoplankton priority state and more stable in terms of ecosystem structure.

The above discussion attempts to look at the interactions within an ecosystem. If we consider this cycle of energy in a time series of the same physical space, we have a succession of bacteria -- phytoplankton -- bacteria. However, at the population level, the two bacterial maxima may belong to different groups. In my experiments most of autotrophic
component development showed two distinctive periods. That is, the bacteria growing during the first period are quite different from that in the second period. As discussed in the previous sections, the first group of bacteria are most likely to utilize carbohydrate as an energy source and take up mineral nutrients during biosynthesis. This group of bacteria potentially compete for the inorganic nutrient pool with the phytoplankton, so they have a strong interaction with the autotrophic component. The decline of $\text{NH}_4^+$ in the experiment before phytoplankton growth may be due to this uptake. They may become a competitor with phytoplankton during photosynthesis because those bacteria are normally limited by an energy source in a natural system. The release of organic substance may allow them to grow and compete for the inorganic nutrient. Phytoplankton may inhibit this group of heterotrophic bacteria before or during exponential growth. The second group of bacteria appear during the degradation period. At this time all the available nutrients have been incorporated into organic substances. The depletion of nutrients causes the growth of autotrophic components to end. The system exhausts its autotrophic energy carrying capability. The bacteria living at the time are different from those in the first period. There are not enough nutrients left for them to take up. They are not competitors with phytoplankton. They most likely utilize degraded phytoplankton material which contains all the necessary biological elements, such as amino acids containing nitrogen or organic phosphorus compounds.
As the experiment shows, if the autotrophic component is restricted by some factors, the system can have two pathways for energy flow simultaneously. Because of the high growth rate and high adaptive ability, the bacteria may occupy many different environments which are not available to autotrophs, as long as there is enough internal system energy. In my results, bacteria can successfully occupy the salinity gradient range in which autotrophs are inhibited. Combining the experiment I, part 1 and stage II, part 1 (Fig. 5a & Fig. 15a), which is an increasing salinity gradient for fresh and seawater ecological components, it is apparent that the freshwater autotrophic component did not survive the salinity gradient. The seawater phytoplankton increased from the middle of this gradient but mainly occupy the high salinity end. However, the heterotrophic bacteria showed a potential to be active during first period of experiment before phytoplankton grow. This indicates that the salinity gradient creates a heterogenous condition to temporally separate these two ecological components along the salinity gradient. If this temporal distribution of two different components happens in a body of water moving in the surface layer of the estuary it can be expected to show a spatial distribution. Numerous studies have indicated that heterotrophic activity and bacterial numbers are great in the low salinity range of an estuary (Stevenson and Erkenbrecher, 1976; Wright, 1978; Wright et al., 1983). Wright (1983) concludes that there is a clear pattern of spatial distribution of bacteria in an estuary. Estuaries develop a bacterial
flora several fold higher in concentration than either the fresh water or sea water at either end of the estuary (Palumbo and Ferguson, 1978). The bacteria are only biologically active in the upper estuary. Wright (1978) indicated the specific activity of bacteria declined significantly in the transition to salt water. This is similar to the ecosystem structure over time shown in our experiments. Therefore the two phenomena, high phytoplankton biomass at the mouth and high bacteria population at the head, actually are related to each other and are a result of the dynamics of the whole system in response to the environmental conditions.

The parallel increase of biological energy flow through these two pathways seems obvious. However, under which conditions the standing stock of bacteria, microflagellates and phytoplankton can show a correlation is a combination of the interactions and interrelations among all ecosystem compartments. Therefore only by simultaneously measuring both relations between standing stock and flux rate can we estimate the functional role they play in the energy flow and carbon cycling in an ecosystem. That is, under what conditions does the autotrophic pathway slow down to give priority to heterotrophic growth as many workers have indicated in the field (Parker, 1975), in big containers of natural water (Parsons, 1982) and in laboratory cultures (Spies, 1984)? Also, under what conditions does heterotrophic production slow down? The inorganic environment generally favours an autotrophic pathway through the inhibitory effects of phytoplankton on bacteria (Lucas,
Considering the limitation of the residence time for biological production in an estuary, there should be a low phytoplankton biomass at low salinity and increasing amounts along the transition reaching a maximum at high salinity at the mouth of the estuary. If the ecosystem is a phytoplankton based system, at the head of the estuary, they may be not explored by phytoplankton. In a highly productive area this seems unreasonable. However, considering the behaviour of heterotrophic component, they show active behaviour all along the salinity gradient at least on the population level. Many conditions at the head of estuary indicate bacteria may fully utilize this space. The conditions which are favourable to heterotrophic bacteria are high organic nutrient supply by river discharge (includes all possible sources, e.g. release from marsh base, soil etc.) higher turbidity resulting in low light intensity, slow growth of phytoplankton i.e. low production of bacterial inhibitors and higher nutrient concentration as well as the physiological nature of bacteria such as high uptake rate of nutrients, high response speed to input of substances, high saturation coefficient and high growth rate provide bacteria with an advantage to overcome the time limitation by circulation.

In a real estuary, a temporal evolution of ecological components carried seaward in a moving water mass of the surface layer is always accompanied by an increasing salinity
gradient. For this process and on the microbial ecosystem level, the system in our experiment and in real estuary are in common. However, this common nature may be overridden in a real estuary by many factors (e.g. different circulation patterns, tidal effects, etc.). In an estuary more allochthonous organic substance may contribute than that in our experiment. For example, sewage outflow, landward counter current flow which may bring the sinking phytoplankton particles at the estuary mouth back to the head of the estuary to enrich maximum turbidity, and marsh based coastal release of organic as well as sedimentation and bottom effects all contribute to the estuarine trophodynamics. Most of these processes happen at the head of an estuary so the heterotrophic potential activity should be higher than in our simulated system at low salinity range.

Another discrepancy between our experiment and field studies is that in our experiment at the high salinity end, after phytoplankton degradation, the bacteria numbers increase greatly and most of them are big rods and are attached bacteria. This is well known and it is reasonable that a large amount of dead phytoplankton should provide more organic substance after the bloom. We should expect vigorous activity of attached bacteria in an estuary as I found in the last period of our experiment. In contrast, previous researchers have not found this zone. Bell and Albright (1981) found in the Fraser River estuary that the attached bacteria decreased along the salinity gradient. This may be a reasonable phenomenon in a real estuary. In the
estuary, when the water body reaches the frontal zone at the mouth, the flow speed slows down. The dead phytoplankton detritus will sink out of the upper layer as the flow speed decreases in the plume area (Lorenzen et al., 1983; Smetacek, 1985). The bacteria attached on the particulate material will also follow the host sinking out of the surface layer, but the free living bacteria would not sink. Thus the attached bacteria sink out of the surface layer. This sinking component can be carried landward to the head of the estuary (LeBlond, pers. comm.). This actually increases the number of attached bacteria in the low salinity range if this transportation process is shorter than the time for bacteria to complete the degradation or increase the necessary nutrients if the degradation has been completed during this transporting process. In our experiments we did not have this process. The attached bacterial population cannot sink out of the experimental flasks. However, we did find that after the growth plateau phase, the phytoplankton showed a great tendency to sediment. Even though we used a magnetic stirrer, still it was impossible to stop the sinking and a great amount of dead phytoplankton sedimented on the bottom. This also happened in Spies' work (1984). Therefore combining my experiment with the estuarine circulation, the spatial separation between heterotrophic production and phytoplankton production along the salinity gradient is a result of interaction in both ecosystem structure and natural physical structure.

In estuaries, a sea water phytoplankton based ecosystem
mostly occupies the high range of salinity gradient while the low range of salinity is occupied by heterotrophic bacteria. The heterotrophic population (bacteria) may be made up of both sea water and fresh water. These mixed populations have an adaptation for the whole salinity spectrum. Their evolutionary patterns are mainly decided by the state of organic substance pool. At limited concentrations of organic substance, bacteria are strongly affected by the physiological state of phytoplankton in terms of the amount of organic substrate released as well as the phytoplankton bloom in terms of its inhibition effect.

Considering the physical circulation and other factors in the physical field and the evolution of ecosystem components in an ecological space, and combining these two systems together, I propose a conceptual model in summary to demonstrate the structure of estuarine ecosystem and its arrangement in the physical space by the circulation process and the conditional gradient (Fig. 25).
Figure 25. The arrangement of ecosystem components in an estuarine circulation field.
Summary

1. In an estuary, the temporal development of an ecosystem in the upper layer of water moving seaward coincides with the spatial distribution due to the movement of the water mass. Therefore, certain spatial distributions may occur in an estuary as a result of this process.

2. Freshwater phytoplankton can not pass through a salinity gradient. Most of them die as the salinity increases, and consequently mass mortality may occur in an estuary. Freshwater phytoplankton can not function as autotrophic components in an estuarine ecosystem but they contribute to organic substances.

3. Seawater phytoplankton can quickly respond and grow in a salinity gradient and a maximum in biomass appears at the high end of the salinity gradient. As a result, autotrophic production in an estuary is primarily the result of marine species and maximum production occurs at some distance from the mouth of an estuary.

4. The energy flow through the microbial pathway becomes relatively important if the ecosystem has a high level of internal energy which can be created by allochthonous organic substances. The microbial pathway which is made up of bacteria and flagellates may have a great potential ability to contribute to the carbon energy flow and allow the whole ecosystem to return to a low energy state as soon as possible.
5. A salinity gradient does not seem to have a strong effect on bacterial growth. Microflagellates can affect the bacterial population dynamics. Bacteria may shift their species composition within a population to obtain a close succession and to adapt to different conditions.

6. The bacterial component is mainly decided by organic substances which allow growth to an upper limit, and they are then exploited by microzooflagellates and a lower limit is set by a feeding threshold. There is no dynamic equilibrium between these two processes when organic energy sources are not limited.

7. A hypothetical model of energy level field theory is proposed to explain the dynamic structure of the ecosystem and the function of autotrophic and heterotrophic components in an ecosystem.

8. Heterotrophic production occurs maximally near the mouth of the estuary and is the result of allochthonous substances as well as the decay of freshwater phytoplankton in the sea.

9. Heterotrophic production is more ubiquitous in the system, being made up of both freshwater and marine species. A bacterial maximum may be expected at the area of the phytoplankton bloom but also at certain depths as a result of sinking of organic materials.

10. The estuarine circulation transports materials along a salinity gradient to connect the ecosystem components of different trophic functions which are separated by the salinity gradient over space. This results in a complete cycling process.
In the mouth of an estuary, certain conditions may exist briefly as in a natural chemostat environment which provides longer periods of high standing stock biomass. This may benefit the zooplankton feeding and may promote the trophic efficiency resulting in high secondary production.
BIBLIOGRAPHY


Ferguson, R.L. 1981. Phytoplankton and bacterioplankton. In:


Fuhrman, J.A., and Azam, F. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in


Springer Verlag, New York, P. 430.


Hollibaugh, J.T., A.B. Carruthers, J.A. Fuhrman, and F. Azam,


Kiefer, D.A. 1973. The in vivo measurement of chlorophyll by


Larsson, U. and A. Hagstrom 1979. Phytoplankton exudate release


Distribution and activity of bacteria in the hardwaters of the Rhode River estuary, Maryland, U.S.A.


Microbial Ecology. Univ. of South Carolina, Columbia.


Williams, P.J. LeB. 1981. Microbial contribution to overall


