Title: AN IN-SITU EXPERIMENT ON THE EFFECTS OF ZOOPLANKTON GRAZING AND NUTRIENT REGENERATION ON THE PHYTOPLANKTON OF YAQUINA BAY, OREGON

Abstract approved: Herbert C. Curl, Jr.

Natural populations of phytoplankton from Yaquina Bay, Oregon, were incubated in large polyethylene bags in-situ, with and without the natural assemblage of zooplankton. Samples were taken daily for two weeks in April, 1974. The biomass of phytoplankton in the bag without grazers reached values two to three times the biomass of phytoplankton in the bag with grazers. Sixty-eight to 93% of the cells in the bag without zooplankton were *Thalassiosira fluviatilis*, while the major species in the bag with zooplankton were *T. fluviatilis*, *T. decipiens* and *Chaetoceros debilis*, no one of which ever accounted for more than 40% of the cells. Nitrate-nitrite became depleted two days earlier in the bag without grazers and urea and ammonia values were higher in the bag with the grazers. Primary productivity, per unit cell volume, was higher in the grazed bag following nutrient depletion,
suggesting some productivity enhancement by grazers. Physiological parameters indicate that the cells in the bag with zooplankton were richer in carbon, nitrogen and chlorophyll \( a \), a higher percentage of the chlorophyll was degraded to phaeophytin and carbon to nitrogen ratios were lower. Data obtained from the bag with zooplankton were similar in range to data from the bay. The results support the contention that zooplankton can have a large effect on phytoplankton in terms of biomass, species composition, productivity and physiological state.
An In-situ Experiment on the Effects of Zooplankton Grazing and Nutrient Regeneration on the Phytoplankton of Yaquina Bay, Oregon

by

Ellen Elizabeth Deason

A THESIS

submitted to

Oregon State University

in partial fulfillment of the requirements for the degree of

Master of Science

Completed 15 January 1975

Commencement June 1975
ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. Herbert Curl, Jr., who suggested this research topic and provided support for my efforts. Mike DeManche, Dr. Dale Pillsbury, Dr. John Nath, Dr. Charles Miller and Gene Ruff discussed the design of the incubation system with me and offered many useful ideas. Equipment was borrowed from Dr. Dale Pillsbury, Dr. Charles Miller, Dr. Andrew Carey and Dr. Robert Olsen. Jim Farrel, Ron Barrels, Sandy Moore and the crew of the R/V CAYUSE helped deploy and retrieve the gear. Chris Marlowe helped with the pumping operation, Chip Frey dove on the bags, and Ken Johnson provided knowledge on the workings of the bay. Joan Flynn guided me in zooplankton identification and Deneb Karentz aided in phytoplankton identification. Dr. Larry Small and Dr. Charles Miller gave much time to discussing results and editing the thesis. My grateful thanks to all these people.

My great appreciation goes to Greg McMurray, my partner in Newport, who helped me in more ways than I can list and most of all, never stopped smiling.

Most of all, I thank John Dickinson. He has given unselfishly of his time and energies at every step of the way. His confidence and support have meant even more to me than all the weights lifted, the dives in cold water and the hours of editing this thesis.
This project was supported by Sea Grant Project 061 through NOAA contract 2-35187 and by State of Oregon Sea Grant funds.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td>Description of Yaquina Bay Study Site</td>
<td>9</td>
</tr>
<tr>
<td><strong>MATERIALS AND METHODS</strong></td>
<td>12</td>
</tr>
<tr>
<td>Incubation System Design</td>
<td>12</td>
</tr>
<tr>
<td>Experimental Procedure</td>
<td>18</td>
</tr>
<tr>
<td>Sample Analysis Procedure</td>
<td>23</td>
</tr>
<tr>
<td><strong>RESULTS</strong></td>
<td>27</td>
</tr>
<tr>
<td>Environmental Parameters</td>
<td>27</td>
</tr>
<tr>
<td>Phytoplankton Biomass</td>
<td>31</td>
</tr>
<tr>
<td>Primary Productivity</td>
<td>34</td>
</tr>
<tr>
<td>Phytoplankton Physiological Parameters</td>
<td>37</td>
</tr>
<tr>
<td>Phytoplankton Species Composition</td>
<td>40</td>
</tr>
<tr>
<td><strong>DISCUSSION</strong></td>
<td>48</td>
</tr>
<tr>
<td>Nutrient Regime</td>
<td>48</td>
</tr>
<tr>
<td>Phytoplankton Biomass</td>
<td>49</td>
</tr>
<tr>
<td>Primary Productivity</td>
<td>52</td>
</tr>
<tr>
<td>Physiological State Parameters</td>
<td>54</td>
</tr>
<tr>
<td>Phytoplankton Species Composition</td>
<td>56</td>
</tr>
<tr>
<td>Suggestions for Further Research</td>
<td>59</td>
</tr>
<tr>
<td><strong>CONCLUSIONS</strong></td>
<td>61</td>
</tr>
<tr>
<td><strong>BIBLIOGRAPHY</strong></td>
<td>63</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>1</td>
<td>Map of Yaquina Bay</td>
</tr>
<tr>
<td>2</td>
<td>Incubation system design</td>
</tr>
<tr>
<td>3</td>
<td>Attachment of tube to vertical wire</td>
</tr>
<tr>
<td>4</td>
<td>Pumping system for filling bags</td>
</tr>
<tr>
<td>5</td>
<td>Daily insolation and tidal height at time of sample</td>
</tr>
<tr>
<td>6</td>
<td>Ammonia and nitrate - nitrite</td>
</tr>
<tr>
<td>7</td>
<td>Urea and phosphate</td>
</tr>
<tr>
<td>8</td>
<td>Particulate nitrogen, particulate carbon and chlorophyll a</td>
</tr>
<tr>
<td>9</td>
<td>Phytoplankton cell number and cell volume</td>
</tr>
<tr>
<td>10</td>
<td>Carbon-14 productivity per cubic meter per hour and per langley</td>
</tr>
<tr>
<td>11</td>
<td>Carbon specific productivity and cell volume specific productivity</td>
</tr>
<tr>
<td>12</td>
<td>Particulate nitrogen per cell volume, particulate carbon per cell volume, and carbon to nitrogen ratios</td>
</tr>
<tr>
<td>13</td>
<td>Chlorophyll a per cell volume and chlorophyll a per carbon</td>
</tr>
<tr>
<td>14</td>
<td>Productivity index and percentage of total phaeophytin and chlorophyll a present as phaeophytin</td>
</tr>
<tr>
<td>15</td>
<td>Frequency of cell numbers of phytoplankton species</td>
</tr>
<tr>
<td>16</td>
<td>Frequency of cell volume of phytoplankton species</td>
</tr>
</tbody>
</table>
Figure Captions, continued

17 Abundances of *Thalassiosira fluviatilis*, *Thalassiosira decipiens* and *Chaetoceros debilis* 45

18 Interaction of *Thalassiosira fluviatilis* and *Thalassiosira decipiens* 47

LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>100 Liter Zooplankton Addition on Day 2.</td>
<td>22</td>
</tr>
</tbody>
</table>
AN IN-SITU EXPERIMENT ON THE EFFECTS OF
ZOOPLANKTON GRAZING AND NUTRIENT
REGENERATION ON THE PHYTOPLANKTON OF
YAQUINA BAY, OREGON

INTRODUCTION

Much of the research concerning lower trophic level relationships in marine pelagic habitats has focused on the requirements of the herbivore consumers. Zooplankton grazing rates, grazing mechanisms and nutritional requirements have been emphasized. The predator-prey relationship must, obviously, influence the phytoplankton as well. A time series experiment measuring the state of a natural estuarine phytoplankton population, with and without zooplankton present, has been attempted in Yaquina Bay, Oregon. Large plastic containers suspended in the estuary were used to isolate the plankton populations, while maintaining environmental conditions as nearly natural as possible. This thesis reports the effects of zooplankton presence on the phytoplankton standing stock, primary productivity, physiological condition of the phytoplankton, and phytoplankton species composition.

Most of the studies investigating the control of these factors have dealt with the influence of physical and chemical parameters such as nutrient supply and light levels. However, zooplankton can alter the nutrient regime by excretion, and it has long been suggested that
grazing is an important factor in determining phytoplankton biomass.

Riley and Bumpus (1946) attributed the inverse relationship between standing stock of phytoplankton and zooplankton to removal of phytoplankton by grazing. Riley's (1946) model of the phytoplankton populations on Georges Bank included a major effect due to the rate of grazing by zooplankton. Cushing (1958) postulated that algal production is usually controlled by grazing. McAllister et al. (1960) suggested that the phytoplankton crop at Ocean State "P" is reduced and controlled by grazing. Further study in large volume containers on shipboard failed to demonstrate cropping of plant growth by zooplankton on the order required to explain the observed biomass, but did show some differences between the cultures with and without zooplankton (McAllister, 1962). The plants in the cultures with zooplankton had greater mean doubling times for carbon and chlorophyll a. The chlorophyll concentration increased to a peak about 50 times the initial value in cultures without zooplankton and to about 10 times the initial value when zooplankton were present, as a result of cell removal by grazing. Smayda (1973) measured the grazing effects of the copepod Acartia clausi on the dynamics of the diatoms Skeletonema costatum, a major phytoplankter of Narragansett Bay, during a winter-spring bloom. This was done in conjunction with an evaluation of the effects of light, temperature and nutrients on the growth of the
diatom. Short term experiments were used to determine daily predation rates which were calculated to remove from 1.4 to 51.9% of the phytoplankton population. Smayda suggests that this grazing pressure contributed to the decline of both a winter and a spring bloom of *S. costatum*.

Grazer effects on primary productivity rates have not been measured in the marine environment. An enhancement effect was implied by a study on *Nitschia* cultures in which one-tenth of the cells were mechanically removed every three to four days (Riley, 1941). The total yield of cells in the culture was considerably greater than the yield in a culture without cell removal. Thus, the enhancement of production could be due to reduction of standing stock. Another mechanism by which grazers could affect production is increased nutrient supply by excretion. In fresh water, primary productivity was reportedly enhanced by the grazing of a herbivorous fish on phytoplankton assemblages in laboratory experiments (Cooper, 1973). The enhancement was directly proportional to increases in grazer biomass up to a certain point. Beyond that point, primary productivity was lower than in controls without grazers. Nutrient regeneration continued to increase with grazer biomass, but primary productivity declined, so Cooper attributes the enhancement to a reduction in standing stock and increased turnover rates rather than increased nutrient regeneration. An *in situ* field study with plastic enclosures
yielded similar results (John E. Korstad, personal communication). Enhancement of productivity was observed up to a certain grazer biomass for total phytoplankton, but not for small phytoplankton (\(<20 \mu m\)). It should be noted, however, that neither of these experiments takes carbon-specific productivity into account. It is possible that at the higher levels of grazer biomass, grazing pressure has reduced the phytoplankton population drastically. Since a population measure of productivity is related to biomass, this could result in a low productivity value while productivity per unit carbon may still be responding to increased nutrient regeneration.

The effect of grazing and excretion on the physiological state of the phytoplankton cells has not been thoroughly studied. McAllister (1962) found a higher chlorophyll \(a\) to carbon ratio in his non-grazed cultures at the end of the logarithmic growth period. The productivity index (photosynthetic rate per unit chlorophyll \(a\)) (McAllister, 1962) at ambient light tended to be greater in the cultures with zooplankton, but McAllister considered this to be a result of taxonomic differences caused by selective feeding.

The effect of grazing and excretion on species composition has not been studied directly in the marine environment. Laboratory grazing experiments have not used natural assemblages of phytoplankton and field studies have concentrated on size class selection or lumped measures of biomass such as chlorophyll or particulate
carbon. McAllister (1962) suggested that a decrease in inorganic silicate in the non-grazed culture indicated a shift to diatom production in the absence of grazing. Grazing effects by zooplankton on freshwater phytoplankton community composition were studied by isolating pond phytoplankton populations in small polyethylene bags (Porter, 1972). The total number of large cells (>30 μm) was not effected by lack of grazing, but the number of small cells (2-30 μm) was significantly greater in the bags without zooplankton. Flagellates, ciliates, large dinoflagellates and large chrysophytes were more numerous in the non-grazed bags.

The mechanisms by which zooplankton affect biomass, productivity, physiological state and species composition are grazing rate, selective grazing, and nutrient regeneration by excretion.

Grazing is dependent on the food concentration. Ingestion rate has been found to be proportional to food concentration up to a maximum value at which it remains constant (Adams and Steele, 1966; Parsons et al., 1967; McAllister, 1970, 1971; Frost, 1972) or decreases (Mullin, 1963; Haq, 1967) with further increases in food concentration.

Many copepods have been shown to discriminate when offered a choice of food organisms. This has been shown for mixed cultures (Harvey, 1937; Mullin, 1963; Petipa, 1965; Mullin et al., 1966; Mullin and Brooks, 1967) and natural phytoplankton (Parsons et al.,
Selection may be based on cell morphology or chemical content (Mullin, 1963) and many observations of selection for large cells have been made (Harvey, 1937; Mullin, 1963; Conover, 1966; Mullin and Brooks, 1967; Richman and Rogers, 1969; Martin, 1970). This could be due to active hunting for large cells or greater feeding efficiency on large cells. Frost (1972) found that as the size of the food particle increased, the numerical concentration of food at which *Calanus pacificus* reached maximal ingestion decreased. Size selection appears to be related to the food particle size distribution and concentration. Poulet (1973) found that *Pseudocalanus minutus* grazing on natural phytoplankton ate particles less than 39 μm more readily than larger particles when the small particles were more abundant. However, with decreased concentrations of the smaller particles, the copepod shifted to grazing on the larger size classes. Wilson (1973) fed *Acartia tonsa* with plastic beads of various sizes and observed grazing selection for the largest abundant beads.

Nutrient regeneration by zooplankton is potentially important to phytoplankton under nutrient limited conditions. A model often used to describe the dynamics of oligotrophic ocean waters is a two-layered euphotic zone divided by a pycnocline which restricts nutrient movement toward the surface mixed layer (Riley, 1965; Dugdale, 1967; Eppley et al., 1973). The specific growth rate of the
phytoplankton is nutrient limited above the pycnocline and light limited below. Zooplankton excretion could be expected to provide a major source of rate limiting nutrients to the phytoplankton in the mixed layer. Eppley et al. (1973) compared excretion rates with nitrogen-15 tracer assimilation studies and found that excretion provided 40 to 110% of the phytoplankton nitrogen assimilation requirements and 110 to 140% of the phosphorus requirement.

Regeneration by zooplankton may also be important in coastal areas where spring phytoplankton blooms deplete nutrients. Harris (1959) estimated that 43, 66 and 77% of the phytoplankton nitrogen requirement in Long Island Sound was supplied by ammonia excretion in three consecutive years. DeManche (1975) estimated that the phytoplankton in Auke Bay, Alaska received at least 67% of their total nitrogen requirements from regenerated nitrogen during a two week period in June. In Narragansett Bay, Martin (1968) estimated that zooplankton excretion provided 2.5% of the phytoplankton's nitrogen requirements from late April through June and 181.7% from late August through November. Sixteen and nine-tenths percent of the phosphorus requirements were provided in the spring and 200% in the fall. Smayda (1973), also working in Narragansett Bay, found that zooplankton excretion could provide 2.6 to 78.6% of the daily nitrogen requirement for growth of the dominant phytoplankton species Skeletonema costatum during the winter and spring.
Incubations with nutrient additions indicated that ammonia availability was an important regulator of the growth of *S. costatum* in the bay. The highest excretion rates occurred from February through April, the lower rates in late spring probably contributed to the decline of a May pulse of *S. costatum*.

In addition to affecting specific aspects of phytoplankton production, the zooplankton-phytoplankton relationship appears to be a critical point of control in the marine ecosystem. Steele (1972) pointed out that 90% of plant production is eaten by herbivorous zooplankton and that the biomass of producers and herbivores is on the same order of magnitude in the sea. In contrast, usually less than 10% of terrestrial plant material is eaten while living and so nearly all enters a decomposer cycle. Hairston, Smith and Slobodkin (1960) postulated that herbivores are predator-limited rather than food-limited in a terrestrial system. Herbivores in the marine ecosystem appear to be more food-limited. Steele (1974) shows that the degree of stability, in the sense of whether a community will return to its original state after a disturbance, is not determined by the diversity of the community. Stability is, however, enhanced by a predator-prey relationship characterized by a density-dependent threshold response for feeding. In a simulation model of conditions in the North Sea, the interaction of zooplankton and phytoplankton appears to be a critical control point. The model gives realistic levels of the various
parameters so long as the zooplankton feeding threshold is not zero.

**Description of Yaquina Bay Study Site**

The area chosen for this study is located in Yaquina Bay, Oregon (Figure 1). Yaquina Bay is a drowned river valley fed by the Yaquina River and Elk Creek which have a 632 square kilometer drainage basin on the western slope of the coastal range (Goodwin *et al.*, 1970). Tides are mixed and semi-diurnal with an average range of 1.7 meters (Kulm and Byrne, 1967). Tidal currents measured during a period of minimal river flow in July had a maximum flood velocity of 0.67 m/sec and a maximum ebb velocity of 0.24 m/sec at Newport (Goodwin *et al.*, 1970). Considerable differences in velocity would be expected during times of maximal freshwater runoff. The tidal prism at Newport is $25.5 \times 10^6$ cubic meters and the cross sectional area of the bay at mean lower low water is 62% of that at mean higher high water (Goodwin *et al.*, 1970). Thus the lower bay is greatly influenced by coastal water.

According to the estuary classification system of Pritchard (1955), Kulm (1965) concluded that Yaquina Bay is well-mixed (less than 3% vertical salinity difference) from June to October and varies from well-mixed to partially-mixed (4 to 19% vertical salinity difference) from November to May. The volume of freshwater runoff is the crucial factor in the degree of mixing but a stratified condition
Figure 1. Map of Yaquina Bay.
is probably never reached due to the large average tidal range (Burt and McAllister, 1959).

The zooplankton of Yaquina Bay have been studied by Frolander (1964), Zimmerman (1972), Miller (1972), Frolander et al. (1973), and Johnson (1974). The zooplankton in the lower estuary are similar to those of the coastal ocean (Frolander et al., 1973). The annual cycle of species composition is a reflection of the annual cycle of surface currents along the coast. From March to August, surface currents are generally from the north. From September to January, surface currents are predominantly from the south (Bourke et al., 1971). Although many zooplankton species persist throughout the year in the estuary, the spring and summer months are dominated by forms with northern affinities while southern forms are also dominant in the winter months (Miller, 1972; Frolander et al., 1973).

The study site was located east of the small boat dock at the Marine Science Center (Figure 1). The lower bay seemed the logical experimental site since these experiments were done in April and zooplankton abundance is often higher in the lower part of the bay than in the upper part in the early spring (Frolander et al., 1973). Laboratory space was available at the Marine Science Center and its proximity allowed samples to be processed rapidly. Both hydraulic and electric power for pump operation were available at the dock, which also provided a stable sampling platform.
MATERIALS AND METHODS

Incubation System Design

A polyethelene incubation system was designed for the in situ grazing experiment. Polyethelene enclosures have been widely used to isolate water. In lakes, small diameter (0.5-2 meter) cylinders have been used by Goldman (1962), Kemmerer and Neuhold (1969), McLaren (1969), and Korstad (personal communication). Lund (1972) used a large diameter (45 meter) column and Porter (1972) used pillow-shaped bags about 0.5 cubic meters in volume. In salt water environments, spherical bags were used by McAllister et al. (1961) and Antia et al. (1963) and columns by Davies et al. (1974). Plastic columns are also being used in the Controlled Ecosystem Pollution Experiment (CEPEX) currently in progress.

Enclosing water in translucent polyethelene results in a small loss of available light and a slight warming of the water (Goldman, 1962). Circulation within the enclosure must surely be reduced, but the degree of reduction is unknown or unreported. Goldman (1962) suggests that lateral water movement could be transmitted through the thin, flexible walls by changes in pressure. Enclosures also prevent the lateral mixing of plankton populations, which, as Davies et al. (1974) point out, could be an important process in maintaining the general equilibrium of these populations in open water. Another
result of using plastic enclosures is the "wall effect" due to fouling on the surface of the bag. Growth of epiphytes and bacteria reduces light levels in the bag and may alter the nutrient regime. These problems are related to the size and shape of the enclosure through variations in the surface to volume ratio. In a long term experiment, these effects suggest the necessity of using a control which is also enclosed in plastic, rather than simply relying on open water measurements.

Preliminary work with plastic columns indicated that the water masses inside and outside the plastic enclosure had similar temperature and salinity structure. This was also found by Goldman (1962) and Davies et al. (1974). Davies et al. (1974) also found similar responses in the chlorophyll a levels in the bag and the outside water, with a short delay inside the bag. Primary production was lower inside the bag and influenced by fouling. Zooplankton caught in a 250 μm mesh net were more abundant in the bag, but the species composition was qualitatively similar to that found in the open water. There were significantly fewer predators inside the bag, which could account for the increase in the herbivorous zooplankton. These results indicate that plankton populations can be maintained in a plastic enclosure and that these populations can be a reasonable representation of populations in the natural marine environment. In addition, preliminary results from work under way in CEPEX, Saanich Inlet, British Columbia, indicate no effects of plastic containers on the feeding
behavior of herbivorous zooplankton (Michael R. Reeve, personal communication).

In designing a system for use in Yaquina Bay, several factors were considered. A major problem was the tidal effect. The maximum tidal range during the experimental period was 3.2 meters and the force of the tidal currents at the experimental site was considerable. The ebb flow was large due to runoff and was channeled past the Marine Science Center small boat dock where the system was anchored. The structure was shaped to present as small a surface area to the currents as possible and designed with considerable anchor weight. Wall effects were another important consideration in an experiment of two weeks duration and the surface to volume ratio was minimized to reduce their influence. Because of turbidity, the tidal currents and the near invisibility of the plastic when in water, underwater work was difficult. Therefore, the system was designed to be installed and sampled without underwater manipulation. Low cost was also a consideration.

The form (Figure 2), was essentially a cylindrical enclosure oriented horizontally in the water. It was made free-swinging so that current force would be largely exerted on one end. The ends and middle of the cylinder were clamped to a long rigid tube so that the resulting bags were large and ellipsoidal when filled with water, thus reducing the wall effects.
Figure 2. Incubation system design.

1. Fiberglass toroid buoy
2. Orange plastic cone
3. Flashing light
4. 6 ml clear polyethylene
5. 6.1 m fiberglass tube
6. 2 cm garden hose
7. Hose clamp
8. 50 lb. lead weight
9. 400 lb. concrete anchor
10. 3/16" cable
11. Marker buoy

Direction of ebb tide current
The translucent bags were made from a cylinder 10.7 meters long and 2 meters in diameter constructed from a flat piece of 6 ml polyethelene with a heat welded seam. The fiberglass support tube was 6.1 meters long and 5 cm in diameter with a 6 mm thick wall. This structural support was chosen because it is strong and flexible for its weight. Two 2 cm diameter garden hoses for sampling and filling the bags were fastened along the support tube and ran from the system to the dock. The plastic was fastened to the tube at the ends and center with hose clamps over strips of rubber to protect the plastic from tearing. To prevent the plastic from slipping out of the clamps and the clamps from slipping along the tube, they were placed on either side of a "collar," made by fastening styrofoam rings to the tube with fiberglass (Figure 3). Air vents were incorporated into the top of the bags by taping in the vent portion of beach balls with Monsanto clear plastic tape. The nozzle was clamped with a pinch clamp.

Floatation was provided by two fiberglass toroid buoys, which caused little shading of the bags. Because of a large amount of small boat traffic in the area, bright orange plastic cones and a flashing light were used as warning devices on the buoys. The system was anchored by three concrete weights totalling approximately 1200 pounds. One weight was attached directly to the bag and buoys, while the other two were connected in tandem with 3/16" cable and placed
1. 5 cm diameter tube
2. fiberglass "collar"
3. steel clamp
4. 50 lb. lead weight
5. thimble
6. shackle
7. wire clamp
8. 3/16" cable

Figure 3. Attachment of pole to vertical cable.
6 and 24 meters up bay. This prevented the bag and buoys from exerting any upward force on two of the three weights and was effective in keeping the system stationary in spite of the drag caused by the ebb tide.

The fiberglass tube, buoys and anchor weight were all attached to 3/16" cable long enough to allow for the highest tide. This left a large excess at low tide, so 50 lb. lead weights were used to keep the tube at a constant depth of 1.2 meters, with respect to the surface.

The anchor weights were installed approximately 12 meters east of the small boat dock at the OSU Marine Science Center in March, 1974, by the R/V PAUITE. They were left in the water while the tube and bag system was replaced for each experiment attempted. At low tide there was enough slack to flip the toroid buoys and clamp the tube system to the exposed wire, thus avoiding underwater work. The details of attachment are shown in Figure 3. The buoys were then righted and the tube and unfilled bags submerged to a depth of 1.2 meters. The bags were lashed to the tube until this point, but some air was inevitable trapped inside and this was released through the beach ball vents. The bags were checked periodically from underwater for tears, fouling and general condition.

**Experimental Procedure**

To test the effect of zooplankton on the natural phytoplankton
assemblage, the "phytoplankton bag" of the incubation system was filled with water from which zooplankton had been largely removed. The "zooplankton bag" was filled with water to which zooplankton from the bay were added. The experiment lasted two weeks and was sampled daily with the exception of two days. The bay was monitored for the same parameters in order to compare the bags to the natural environment.

The bags were installed on April 14, 1974. Two different pumps were used for filling the bags. One was a self-priming Jabsco centrifugal pump model 10490 with a neoprene impeller in a brass housing, which was run by the hydraulics of the R/V PAUITE. Water was collected by a 5 cm diameter PVC hose and organisms passed through the pump with little or no damage (Smith, 1975). When the PAUITE hydraulics were not available, a \( \frac{1}{4} \) horsepower Flotec Rotolator electric pump and 2 cm garden hose were used. In both cases the mouth of the intake hose was placed at a depth of approximately 1.2 meters.

The pumped water was filtered through a 120 \( \mu \)m mesh net to remove most of the zooplankton and caught in a 113 liter plastic garbage can. The garden hoses leading to the bags were gravity fed from the bottom of the garbage can (Figure 4). Zooplankton were added to the control bag by taking several net tows in the bay with a 120 \( \mu \)m mesh net and pouring the animals down the hose leading to the
1. small boat dock
2. hydraulic pump
3. 113 liter plastic garbage can
4. 120 μm mesh net
5. 2 cm garden hose
6. 5 cm PVC hose
7. plastic pipe

Figure 4. Pumping system for filling bags.
Zooplankton bag. A subsample of the addition was preserved. Care was taken to flush the hose with water after the zooplankton addition. This method did not include species which could avoid the net in the addition and delicate animals may have been damaged in the net.

Water was pumped into the bags for approximately 6½ hours on April 15 (day 1). In the morning the electric pump was used and in the afternoon the more powerful hydraulic pump was used. Underwater inspection revealed that the phytoplankton bags was well rounded, but that the zooplankton bag was only ¼ as inflated. On day 2, water was filtered and pumped into the zooplankton bag for 4 hours. However, the flow rate was low and the electric pump was unable to draw a sample from that bag. On day 4, divers discovered that some of the hose clamps had been crushing the garden hose and loosened them. Water was then pumped into the zooplankton bag for 12 hours. In an attempt to fill the bag to a level comparable with the phytoplankton bag, the water was not filtered and gravity fed, but pumped directly with the electric pump to the bag. In spite of the greater number of hours of pumping into the zooplankton bag, both bags received approximately half their water from an ebb tide and half from a flood tide. The zooplankton bag remained slightly smaller than the phytoplankton bag. Zooplankton additions were made on days 1, 2 and 5. The animals added were generally very small as is shown by the composition of the day 2 addition (Table 1). Oithona similis,
TABLE 1. 100 Liter Zooplankton Addition on Day 2.

<table>
<thead>
<tr>
<th>Species</th>
<th>number per 300 ml subsample</th>
<th>estimate of total number added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oithona similis female</td>
<td>93</td>
<td>30,970</td>
</tr>
<tr>
<td>Oithona similis male</td>
<td>8</td>
<td>2,665</td>
</tr>
<tr>
<td>Oithona similis immature</td>
<td>243</td>
<td>80,920</td>
</tr>
<tr>
<td>Pseudocalanus sp. female</td>
<td>1</td>
<td>333</td>
</tr>
<tr>
<td>Psuedocalanus sp. immature</td>
<td>46</td>
<td>15,320</td>
</tr>
<tr>
<td>Acartia clausi female</td>
<td>3</td>
<td>1,000</td>
</tr>
<tr>
<td>Acartia longeremis female</td>
<td>1</td>
<td>333</td>
</tr>
<tr>
<td>copepod nauplii</td>
<td>23</td>
<td>7,660</td>
</tr>
<tr>
<td>barnacle nauplii</td>
<td>155</td>
<td>38,415</td>
</tr>
<tr>
<td>harpacticoid copepods</td>
<td>5</td>
<td>1,665</td>
</tr>
<tr>
<td>crab zoea</td>
<td>1</td>
<td>333</td>
</tr>
<tr>
<td>Oikopleura</td>
<td>1</td>
<td>333</td>
</tr>
<tr>
<td>annelid larva</td>
<td>1</td>
<td>333</td>
</tr>
</tbody>
</table>

Pseudocalanus sp. copepodites and barnacle and copepod nauplii dominated the sample.

Sampling was done between 1130 and 1200 PDT each day with the exception of day 4 when sampling was done at 1630. Water was pumped up through the garden hoses from the bags with a hand operated bilge pump (Guzzler model 400) except on day 2 when samples were obtained with the electric pump. Bay samples were pumped through garden hose from a depth of 1.2 meters. An initial ten liter sample was discarded to insure that the hose was clear. Ten more liters were pumped into a bucket from which bottles were filled immediately for each of the parameters measured.

Water was collected for pigment analyses, carbon and nitrogen
analysis, microscopic species enumeration, nutrient analyses, alkalinity determination and carbon-14 incubation. All samples except the carbon-14 incubation were covered with black polyethylene and taken to the Marine Science Center for processing. The carbon-14 incubation bottles were inoculated with carbon-14 and incubated at 1.2 meters for approximately two hours in a large mesh bag which hung from the small boat dock in an unshaded place. They were then placed in a light tight box and taken to the Marine Science Center lab. In addition, light data were available from a recording Eppley short-wave pyranometer at the Marine Science Center dock and tide data were obtained from a guage.

Sample Analysis Procedure

Nutrient samples were frozen and later analyzed for combined nitrate-nitrite, ammonia, urea, and phosphate with a Technicon AutoAnalyzer. The nitrate-nitrite method was that of Wood, Armstrong and Richard (1967) modified by Atlas et al. (1971). Sample to sample precision is reported as ± 2% (2σ). The automated ammonia method is that of Head (1970). With optimum machine operation the sample to sample precision for this method is reported to be ± 3.3% (2σ). Phosphate analysis was done by the method of Atlas et al. (1971), which is a modification of the procedure of Bernhardt and Wilhelms (1967). Precision is reported as ± 2% (2σ). Urea was
analyzed by the automated method of DeManche et al. (1973). Precision is reported as ± 2% (2). Pigment samples were filtered onto 0.8 μm Millipore membrane filters. The volume filtered varied between 454 ml and 1 liter. The filters were frozen in a dessicator until pigment determination was done by the method of Strickland and Parsons (1972) as modified by Glooshenko et al. (1972). Chlorophyll a concentrations were calculated from the equations of Richards as modified by Strickland and Parsons (1972). Phaeo-pigments were also calculated by the Strickland and Parsons (1972) procedure.

Particulate carbon and nitrogen samples were filtered through a Whatman GFC filter which was cut to a 13 mm diameter to fit a Swinney adaptor. The filters were placed in individual vials, dried in an oven overnight at 70°C, and stored until they were analyzed on a Carlo Erba Elemental Analyser model 1100.

A 250 ml sample was preserved with borax buffered formalin for species enumeration. Each sample was concentrated by settling in a 250 ml graduated cylinder for 2 days. The supernatant was aspirated off, leaving approximately 50 ml, which was again allowed to settle in a smaller graduated cylinder for 2 days. Supernatant was again drawn off, leaving 5 ml of concentrated plankton. After shaking the sample, 0.1 ml was pipetted into a Palmer cell. Cells were identified, enumerated and measured at 400 x magnification. Cells
were counted until the slope of the plot of "number of cells counted vs. number of species found" began to asymptotically approach zero. At least 100 cells were counted from each sample. Average cell volumes were calculated from the measurements and the geometry of each species. Cell numbers per liter and unit cell volume per liter were calculated for each species. Differences in species composition were evaluated using a chi-square test (Snedecor and Cochran, 1967).

Subsamples of the zooplankton additions were taken by dipping with a jar from the bucket in which they were collected. The zooplankton were identified to species and counted to give a rough idea of the composition of the additon.

Carbon-14 uptake incubations were filtered through a 0.8 μm Millipore membrane filter and put in a scintillation vial with 5 ml of Aquasol LSC scintillation cocktail. The vials were capped tightly and stored at room temperature. They were shaken periodically to help dissolve the filters. Samples were counted in triplicate on a Packard Tri-Carb model 3375 liquid scintillation spectrometer. Counting efficiency was determined for 20 samples by the internal spike method as recommended by Kobayashi and Maudsley (1969). A regression of those efficiencies on the automatic external standardization (AES) (r=0.88) was used to calculate efficiencies for the rest of the samples. The efficiencies were used to correct net counts per minute to disintegrations per minute. Disintegrations per minute were converted to mg
of carbon according to the equation of Strickland and Parsons (1972).

Carbonate alkalinitities for use in the equation were measured according to the method of Strickland and Parsons (1972).
RESULTS

Environmental Parameters

Daily integrated solar insolation (Figure 5a) varied from 238 to 615 langleys as spring storms moved through the area. Samples were taken at tidal heights which varied from 1.95 to -0.36 meters (Figure 5b). Both ebb and flood tidal periods were sampled during the experiment. The state of the tide affected the content of the bay sample and determined the exact sample position within the bags. On an ebb tide, the bay water was less dense than the water in the bags and so they tended to sink below the tubular support which was maintained at a fixed depth, thus samples were taken from near the top of the bag. On a flood tide, the bags rose to the water's surface and samples were pumped from the deeper portion of the bags.

Nutrient measurements (Figures 6 and 7) reflected plant growth processes during the experiment. The dynamics in the phytoplankton bag were similar to those expected from a large batch culture. In the phytoplankton bag, ammonia was reduced to a low level by day 4 and remained at low levels for the duration of the experiment (Figure 6a). Ammonia values were higher and more variable in the zooplankton bag, and the highest values were found in the bay. Initial nitrate-nitrite values were high (32 μg-at N liter^{-1}) (Figure 6b). In the phytoplankton bag, the values dropped rapidly and became negligible
Figure 5. a. Daily insolation. b. Tidal height at time of sample.
Figure 6. a. Ammonia, b. Nitrate-nitrite.
Figure 7. a. Urea. b. Phosphate
by day 9, while they were low only on day 11 in the zooplankton bag.

High concentrations of urea were found in the zooplankton bag (Figure 7a). The maximum urea value in the phytoplankton bag was 4.3 μg-at N liter⁻¹, while the zooplankton bag contained up to 29.1 μg-at N liter⁻¹. Phosphate values were consistently low and variable (Figure 7b). They were highest in the bay samples and showed a large drop in all samples on day 10.

**Phytoplankton Biomass**

Phytoplankton biomass, as indicated by chlorophyll a, particulate carbon, particulate nitrogen, cell count and cell volume, showed a much greater increase in the phytoplankton bag than in the zooplankton bag. Particulate nitrogen and carbon (Figure 8) started low and peaked in the phytoplankton bag on day 8 with a decrease on day 9. Zooplankton bag values were lower and more uniform. The biomass in the bay was less than in either bag. Chlorophyll a data showed the same general trends of increase and decrease (Figure 8c). There was, however, a greater similarity between bay and zooplankton bag values with a maximum of 12.0 mg Chl a m⁻³. The levels in the phytoplankton bag fluctuated dramatically and reached a maximum of 34.1 mg Chl a m⁻³ on day 10. Phytoplankton cell counts also followed a similar pattern (Figure 9a). The decrease in the phytoplankton bag occurred a day earlier (day 8) than with carbon, nitrogen
Figure 8.  a. Particulate nitrogen  b. Particulate carbon  c. Chlorophyll a
Figure 9. 

a. Phytoplankton cell number
b. Phytoplankton cell volume
and chlorophyll \textsubscript{a}. A maximum of $2.5 \times 10^7$ cells liter\textsuperscript{-1} was reached on day 10. Cell volume peaked on day 11 in both bags (Figure 9b).

**Primary Productivity**

The primary productivity (Figure 10) was highest in the phytoplankton bag while the zooplankton bag had levels comparable to those found in the bay. Productivity per cubic meter (Figure 10a) and productivity on a light basis (Figure 10b) had patterns similar to those shown by the biomass indicators. Productivity peaked on day 6 and day 10 in the phytoplankton bag. The drastic drop in productivity on day 8 was coincident with the decrease in cell numbers and cell volume. The productivity in the zooplankton bag peaked on day 10 when normalized for light, but was always lower than that found in the phytoplankton bag.

When productivity was normalized for biomass, the trends became different before and after days 8 and 9. Carbon-specific productivity was calculated from carbon-14 data and particulate carbon values. Initially, carbon specific productivity was greater in the phytoplankton bag than in the zooplankton bag, and showed a large peak on day 5 (Figure 11a). However, after nitrate-nitrite was depleted in the phytoplankton bag, the normalized productivity values were comparable to those found in the zooplankton bag. When productivity is calculated on the basis of cell volume, the productivity in
Figure 10. Carbon-14 productivity.  

a. Per cubic meter per hour  
b. Per cubic meter per langley
Figure 11, a. Carbon specific productivity
   b. Cell volume specific productivity
the phytoplankton bag was lower than that in the zooplankton bag from day 8 through the duration of the experiment (Figure 11b).

**Phytoplankton Physiological Parameters**

The calculated physiological parameters show a definite change in the states of the populations in the phytoplankton and zooplankton bags around days 8 to 10. The amount of particulate nitrogen per unit cell volume and the amount of particulate carbon per unit cell volume showed almost identical patterns (Figures 12a and 12b). The cells in the zooplankton bag were always richer in carbon and nitrogen than those in the phytoplankton bag. Until day 8, they were about an order of magnitude richer.

The almost identical patterns of carbon and nitrogen content are reflected in the carbon to nitrogen (C/N) ratios. The C/N ratios dropped in both bags initially and then were constant until almost the end of the experiment (Figure 12c). The C/N ratio in the phytoplankton bag was maintained at 8 to 9.5 (μg C liter⁻¹/μg N liter⁻¹), while that in the zooplankton bag was at 6.25 to 7.5.

Chlorophyll a per unit cell volume (Figure 13a) had a similar pattern to that of carbon and nitrogen per cell volume. The values in the zooplankton bag were generally higher than those in the phytoplankton bag. In the phytoplankton bag there were two different levels of cellular chlorophyll: high before and low after the variations of
Figure 12. a. Particulate nitrogen per unit cell volume.
b. Particulate carbon per unit cell volume.
c. Carbon to nitrogen ratios.
Figure 13. a. Chlorophyll a per unit cell volume
b. Chlorophyll a per carbon
days 8 and 9. The highest values of chlorophyll per unit cell volume were found in the bay \( (8.75 \times 10^{-4} \text{mg Chl}_a/\mu \text{m}^3 \text{cell}) \).

Chlorophyll per particulate carbon fluctuated in the phytoplankton bag, while it was steady in the zooplankton bag and in the bay (Figure 13b). After day 10, however, the two experiments and the bay were quite similar. A productivity index \((\text{mg C/mg Chl}_a\text{-hr.})\) at ambient light was calculated from carbon-14 data and the chlorophyll data (Figure 14a). The patterns were similar to those of carbon specific productivity. Values were less than 3.0 mg C/mg Chl\(_a\)-hr except on day 9, when the drastic decrease in chlorophyll combined with the high carbon uptake on that day gave an index of 22.7 mg C/mg Chl\(_a\)-hr.

Phaeophytin is a form of degraded chlorophyll and the proportion of the total chlorophyll and phaeophytin which is present as phaeophytin indicates the degree of pigment degradation (Figure 14b). Values were generally higher in the zooplankton bag.

**Phytoplankton Species Composition**

The cumulative relative frequencies of the number of cells of the major species present indicate a great difference in species composition between the two bags and between the bags and the bay (Figure 15). The phytoplankton bag was dominated by *Thalassiosira fluviatilis* which accounted for 68 to 93% of the cells. In the
Figure 14.  

a. Productivity index  
b. Percent of total phaeophytin and chlorophyll a present as phaeophytin
Figure 15. Frequency of cell numbers of phytoplankton species
a. Phytoplankton bag b. Zooplankton bag c. Bay
zooplankton bag, *T. fluviatilis* was never more than 40% of the total cells and several other species were almost as important. When plotted on the basis of total cell volume of a species, the phytoplankton bag was dominated by *T. fluviatilis* and *T. decipiens*, while in the zooplankton bag, *T. decipiens* and *Chaetoceros debilis* were the most important species (Figure 16). In the bay, *T. fluviatilis* was an important constituent in samples taken at low tide, but *C. debilis* and *T. decipiens* dominated during high tide and flood tide.

The numerical abundance of *T. fluviatilis* was one to two orders of magnitude greater in the phytoplankton bag than in the zooplankton bag (Figure 17). *T. decipiens* was at least an order of magnitude more abundant in the phytoplankton bag until day 9. *C. debilis* was initially more abundant in the zooplankton bag than in the phytoplankton bag where it did not appear in the cell counts. From day 7 on, however, the levels were roughly comparable between the two bags.

The degree of difference in species composition of the two bags was examined using a chi-square test. A 2x4 contingency table was used with the cell numbers of *T. fluviatilis*, *T. decipiens*, *C. debilis* and lumped other species in the zooplankton and phytoplankton bags. The difference between bags was significant for all days tested: on day 4, $\chi^2 = 37.0$ (p < .005); on day 8, $\chi^2 = 482.5$ (p < .005); and on day 14, $\chi^2 = 30.4$ (p < .005).

An interesting interaction between the two species of
Figure 16. Frequency of cell volumes of phytoplankton species
a. Phytoplankton bag b. Zooplankton bag c. Bay
Figure 17. Abundance of a. *Thalassiosira fluviatilis*  
b. *Thalassiosira decipiens*  
c. *Chaetoceros debilis*
*Thalassiosira* is observed when the relative abundance with respect to cell volume is plotted with one species on an inverse scale (Figure 18). In the phytoplankton bag, there was a negative correlation between the patterns of the two species. They appear to have responded in an opposite fashion to the conditions in the bag. This relationship was uncoupled in the zooplankton bag.
Figure 18. Interaction of *Thalassiosira fluviatilis* and *Thalassiosira decipiens* a. Phytoplankton bag b. Zooplankton bag
DISCUSSION

Nutrient Regime

The differences in the nutrient concentrations between the two bags show the effects of zooplankton excretion (Figures 6 and 7). The levels of all forms of nitrogen were higher in the zooplankton bag than in the phytoplankton bag. The high concentrations of urea on days 4 and 7 clearly indicated the presence of zooplankton. Peaks of ammonia occurred on the same days as urea peaks in the zooplankton bag.

The presence of ammonia effectively inhibits the uptake of nitrate from a culture medium. Lundy (1974) found that nitrate uptake by phytoplankton in batch cultures began when concentrations of ammonia dropped below 2 μg-atoms liter⁻¹. The initial concentration of ammonia was below this level in both bags and nitrate was taken up immediately. While nitrate-nitrite became negligible in the phytoplankton bag on day 9, it was not depleted in the zooplankton bag until day 11. This difference may have been due to the urea and ammonia which were excreted in the zooplankton bag. The rise in nitrate-nitrite on the last two days of the experiment may indicate that bacterial wall effects were becoming important at this stage of the experiment. Although the expectation is that without resupply ammonia should have become depleted in the phytoplankton bag, it remained present at a low level throughout the experiment. However,
DeManche (personal communication) has found that freezing of nutrient samples may result in an increase in ammonia concentrations, which suggests that the persistence of ammonia was an artifact. Phosphate levels were low and did not appear to be different between the two bags.

The range of nutrient levels in the bay were similar to those found in the bags. Decreases in ammonia and nitrate-nitrite in the bay reflected the bloom which peaked on day 9.

**Phytoplankton Biomass**

The difference in the phytoplankton biomass reached in the bags showed the effect of removal by grazing in the zooplankton bag. The highest levels of particulate carbon and nitrogen in the phytoplankton bag were twice those in the zooplankton bag (Figure 8). Maxima of chlorophyll a and cell volumes in the phytoplankton bag were approximately three times those in the zooplankton bag (Figures 8 and 9). Cell numbers were greater by a factor of eight in the phytoplankton bag, but this was a misleading measure because of differences in species composition. These factors were not directly indicative of the amount of phytoplankton grazed by the zooplankton, since the effects were cumulative and there was no short-term non-grazed control for the zooplankton bag. The differences between the bags did indicate, however, the long term effects of biomass removal by
grazing.

Beside the differences in maximum biomass levels, the other striking differences between the two bags were the declines which occurred in the phytoplankton bag on day 8 or 9, which did not occur in the zooplankton bag. Phytoplankton cell number and volume decreased on day 8 with a coincident decline in productivity. The effects on particulate nitrogen, particulate carbon and chlorophyll a were delayed until day 9. These declines appear to be related to nitrate-nitrite levels in the phytoplankton bag which were low on day 8 and negligible on day 9. The light level was also low on days 8 and 9, but this condition would have affected both bags.

Lundy (1974) found evidence in laboratory batch cultures that chlorophyll a synthesis was tied closely with nitrogen assimilation. After the depletion of ammonia in his cultures, the exponential increase of chlorophyll a momentarily stopped and then resumed. Similar pauses were found in particulate carbon, nitrogen, and carbon-14 uptake. Lundy postulated that the pauses were a result of transfer from dependence on nitrate and ammonia together to total dependence on nitrate. In the case of my bag experiment, the declines occurred at the point of nitrate depletion and there was no clear nitrogen source to which uptake could have switched. Nevertheless, chlorophyll a and cell numbers and volume subsequently increased to their maximum values. A possible explanation for these new
increases after apparent nutrient depletion is that naupli could have entered the bag through the 120 μm mesh net. They could have excreted nitrogen which was taken up immediately and never reached a large concentration. However, it doesn't seem likely that such excretion could have supported the observed growth. Alternatively, the cells could have continued to grow by utilization of internal nitrogen pools. Carbon specific productivity and cell volume specific productivity were comparatively low in the phytoplankton bag after day 9 (Figure 11). Chlorophyll per unit cell volume in that bag appears to have two general levels, with a lower value after nutrient depletion (Figure 13a). These parameters seem to indicate that the observed growth in cell numbers and volume was based on lower carbon and nitrogen uptake. Perhaps the declines on days 8 and 9 were caused by the necessity for a change in physiological state, which may have taken the form of a transfer of constituents from the synthetic apparatus of cell to structural components. Another internal source of nitrogen for continued growth could have been soluble organic nitrogen compounds such as amino acids. Changes in species composition within the phytoplankton bag did not appear to be drastic enough to account for the decline and subsequent increase in biomass. There is also the possibility that the declines on day 9, or at least the magnitude of the declines, may have been a sampling artifact. Water was filtered from the same bottle for both pigment and
particulate carbon and nitrogen analyses, so the agreement between these separate factors does not preclude some sort of sampling error. No solid conclusions as to the cause of the observed pattern can be drawn from the available data, but in the presence of grazers in the zooplankton bag these variations did not occur.

The biomass levels in the bay compared well with those found in the zooplankton bag. A short lag in the timing of the maximum was observed in the bag. This is in agreement with the results of Davies et al. (1974) who found that chlorophyll distributions in their experimental bag were very similar to those outside with a time lag of two days. However, the apparent similarity of my results to theirs may have been due to tidal effects. The bloom in the bay appeared in the more saline high tide waters and the tides sampled were increasingly lower after day 9 of my experiment.

**Primary Productivity**

Hourly productivity per cubic meter or that normalized by light level was greater in the phytoplankton bag than in the zooplankton bag, except for the drop on day 8 (Figure 10). This result appears to be related to the dependency of productivity on the biomass present. However, there was no grazer enhancement as found by Cooper (1973) and by Korstad (personal communication) in the zooplankton bag when compared to the nutrient rich initial condition in the phytoplankton
bag even when the productivity was normalized for biomass (Figure 11). There was some indication of enhancement of productivity in the zooplankton bag when compared to the phytoplankton bag after nutrient depletion occurred. These values may or may not be significant, but the difference between normalized productivity and productivity per cubic meter definitely show the importance of measuring productivity on a biomass basis in a study of grazing effects. The results also indicate that conclusions about grazer effect on productivity will vary with the degree to which nutrients are limiting the phytoplankton population. No light can be shed on the mechanism of enhancement by these data, as both nutrient regeneration and removal of cells were taking place.

The patterns of productivity in the zooplankton bag at the start of sampling were different when normalized for carbon than when normalized for cell volume (Figure 11). The fact that the productivities were low on a carbon specific basis relative to the cell volume basis may indicate that the particulate carbon measurement included a significant amount of non-living carbon which would not have been included in cell counts. This carbon could have been detritus which was present in the water used for filling. It would thus have been present in the phytoplankton bag as well, but its significance would have been small because of the much greater level of cellular carbon. The C/N ratios did not show any indication of the detritus, probably
because it was fresh detritus which had not yet lost its nitrogen (H. Curl, personal communication). The non-living carbon could also have resulted from the presence of grazers. If zooplankton were fragmenting cells during the grazing process, they would have produced non-living particulate carbon in the zooplankton bag.

The productivity in the bay was similar to that in the zooplankton bag. Although productivity per cell volume was higher in the bay than in either of the bags, there was a similarity between the bay and the zooplankton bag in the time sequence of changes in this parameter. The degree to which this was an artifact from sampling at different stages of the tide is unknown.

Physiological State Parameters

Higher values of particulate nitrogen, particulate carbon and chlorophyll per unit cell volume in the zooplankton bag indicated that these cells were "richer" on a biomass basis (Figures 12 and 13a). The lower C/N ratio in the zooplankton bag also indicated a healthier population of cells when the grazers were present (Figure 12c).

The comparative richness of the cells in the zooplankton bag was probably due to the larger nutrient supply provided by grazer excretion. The differences between bags may also have been a result of the different species composition generated by grazing, if different species have different inherent C/N ratios and chlorophyll a
concentrations.

Lundy (1974) postulated that the C/N ratio is indicative of the "overall nitrogen sufficiency" of the population and noted that it responded sluggishly to rapid fluctuations in the population growth rate. Chlorophyll a, on the other hand, can be considered an indicator of the labile "synthetic machinery" of a phytoplankton population, and Lundy found that its concentration showed a quick response to changes in nitrogen assimilation. These generalizations are supported by the data from this experiment. The range of percentage change in C/N ratios in the phytoplankton bag was -15% to 10.7%. This reflected none of the fluctuations which were shown by the chlorophyll a values and by the chlorophyll a per unit cell volume (-96.0 to 2741.7% and -97.5 to 1300% percentage change, respectively).

Chlorophyll a per unit carbon (Figure 13b) has been found to be positively correlated to growth rates measured in steady state systems (Caperon and Meyer, 1972). Lundy (1974) found that chlorophyll a to carbon ratios also responded to growth rate changes in batch culture. The variations in the phytoplankton bag show the "boom and bust" nature of growth without grazers. Values in the zooplankton bag were more stable. McAllister (1962) found that chlorophyll a per unit carbon was greater in his non-grazed culture at the end of logarithmic growth. The comparatively large values of this parameter in the phytoplankton bag until day 9 are consistent
with his result. The differences between bags may have been due to the excreted ammonia and urea in the zooplankton bag. Eppley et al. (1971) found that the chlorophyll a : carbon ratios of log phase cells varied with the nitrogen source as nitrate > ammonium > urea.

The similarity of the patterns of chlorophyll a per unit carbon and carbon specific productivity (Figure 11a) show that the carbon production per unit chlorophyll was nearly constant. This was also indicated by the almost constant productivity index (Figure 14a), except on day 9 when the value is suspect.

Phaeophytin represents pigment which has been made non-functional by sun-bleaching, darkness, or by passing through a grazer's gut. The percent phaeophytin was generally higher in the zooplankton bag, presumably due to grazing activity (Figure 14b).

The values of the physiological parameters in the bay behaved independently of those in the bags, but the ranges of values were comparable. C/N ratios in the bay appear to have been influenced by the height of the tide at the start of sampling. Ebb tide values were uniformly high, while lower values were found with the higher tides. The pattern of chlorophyll a per unit cell volume in the bay was similar to the pattern of daily levels of insolation, with a lag.

**Phytoplankton Species Composition**

The difference in species composition between the two bags was
striking (Figures 15 and 16). There were no samples from the zooplankton bag early in the experiment with which to demonstrate initial similarity to the phytoplankton bag in species composition. In fact, *Chaetoceros debilis* may have been collected in the zooplankton net tows and included in the bag with the zooplankton addition, thus inflating its initial proportion in the population. However, by day 7, the abundance of *C. debilis* in the two bags was quite similar (Figure 17c) and it was the differences in abundance of the other species which accounted for the greater relative frequency of *C. debilis* in the zooplankton bag.

The greater abundance of *Thalassiosira fluviatilis* in the phytoplankton bag indicates that this species was being grazed in the zooplankton bag (Figure 17a). The degree of dominance as indicated by the proportions of species suggests that this species was being selectively grazed. *T. fluviatilis* is a small diatom (7.5 to 15 μm diameter in these samples) which has been reported from freshwater and from a polluted marine environment with a salinity range of 15% to 20% (Hasle, 1962). It sometimes forms summer blooms, particularly in places where water movement is minimal. This is consistent with the diatom's greater abundance in the fresher ebb tide water in the bay and also with its tendency to bloom in a bag of restricted water when unchecked by grazing. The abundance of *T. decipiens* indicates that it was also being grazed in the phytoplankton bag (Figure
It is a larger cell (15 to 50 μm in diameter) which forms chains. *C. debilis*, a spiny chain-former 25-45 μm in diameter, may not have been grazed, but the different starting conditions make interpretation difficult. The differences in abundances of species between bags should not be used as a quantitative measure of grazing as the effects of grazing were cumulative throughout the experiment.

The selective grazing on the small *T. fluviatilis* which caused the changes in species composition also created a change in size distribution. While some lab experiments, mainly with large zooplankton, have shown the selection of larger cells from a restricted size range, selection of smaller cells was found by Poulet (1973) and Porter (1972), using natural phytoplankton assemblages. Selection is limited by the size of grazer mouthparts and so the grazing of small cells is reasonable given the small size of zooplankton in the bag. *T. fluviatilis* was also abundant, which agrees with the findings of Poulet (1973) and Wilson (1973) that the species of copepods which they worked with fed on the most abundant size classes present.

The interaction between the two *Thalassiosira* species is indicated by the relative frequency of cell volumes (Figure 18). In the phytoplankton bag, these two species accounted for most of the cell volume and so it can be assumed that the effects of other species on the total volume were not biasing the result. This was not true in the zooplankton bag and may be the reason why the inverse relationship
broke down. In the phytoplankton bag, the peaks in *T. decipiens* relative abundance and the valleys of *T. fluviatilis* corresponded very closely to the peaks of daily insolation, with a slight time lag. This might indicate that *T. decipiens* is a "high light" species, while *T. fluviatilis* is a "low light" species.

**Suggestions for Further Research**

To determine the significance of the effects of zooplankton on phytoplankton in a more general sense, experiments such as this should be run at various time of year. In each case, the experiment would be different due to different initial conditions of phytoplankton and zooplankton species and different nutrient and hydrographic conditions in the bay.

Withdrawing phytoplankton controls from the zooplankton bag daily and incubating them to determine growth rates would provide information without cumulative effects and add more specific information on grazing rates and the ration received by zooplankton.

The design of the incubation enclosure used in this experiment worked well in that the parameters of the phytoplankton enclosed with grazers compared well with the range of "real world" values in the bay. For shorter experiments, however, somewhat less cumbersome equipment of a smaller size should be considered. Work during seasons with less runoff would probably not be so sensitive to tidal
currents.

The frequency of sampling and the number of parameters measured should not be decreased in an experiment of this type. The results of the experiment indicate large changes on the time scale of a day or less. Sampling at greater intervals or only at the termination of the experiment would have led to very different interpretations. This would probably be even more crucial in a smaller enclosure. Calculation of the large number of parameters seems necessary for a complete picture of the system. Using particulate carbon or particle counter data as the sole biomass indicator appears risky in bays and estuaries because of high levels of non-living detritus stirred up by tidal currents.

The results of this experiment indicate several areas which should be investigated further. Many of the mechanisms for the effects of grazing would be better examined under laboratory conditions than with further field work. The mechanisms of grazer enhancement of primary productivity, elucidation of the apparent interaction between Thalassiosira species, determining whether changes in physiological state are a direct result of grazing or caused indirectly through changes in species composition, and observations under controlled conditions of changes in species composition through selective grazing are topics which should be pursued.
CONCLUSIONS

1. Excretion by grazers provided a higher level of nutrients, particularly urea, in the zooplankton bag, while nutrient depletion occurred in the phytoplankton bag.

2. Grazing pressure in the zooplankton bag reduced the biomass 2-3 fold when compared to the phytoplankton bag. This indicates that grazing terms in models of primary production must be very fully evaluated. Moreover, they will be found to be complex, having multiple effects.

3. Productivity per cubic meter was higher in the phytoplankton bag. Carbon specific productivity rates were higher in the phytoplankton bag under non-nutrient-depleted conditions. Productivity on a cell volume basis indicated that non-living carbon may have been significant in the zooplankton bag and suggested a small productivity enhancement by grazers under nutrient depleted conditions.

4. Cells in the zooplankton bag were richer in carbon, nitrogen and chlorophyll a and C/N ratios were lower. These physiological differences were probably due to the greater availability of nutrients caused by grazer excretion. A higher percentage of the pigment in the zooplankton bag was in the form of phaeophytin a, as a result of grazing.

5. The species composition was different in the two bags, probably
due to selective grazing. The abundance of two of three major species was reduced by grazing in the zooplankton bag. Thus grazing may play an important role in seasonal succession of phytoplankton species. An interaction was observed between the two Thalassiosira species in the bags which suggests that their relative abundance is related to light levels.

6. The technique of isolating populations in plastic enclosures is a useful experimental tool. While the bags could not provide an exact analog of the bay, the range of values in the zooplankton bag was comparable to those found in the natural environment.
BIBLIOGRAPHY


